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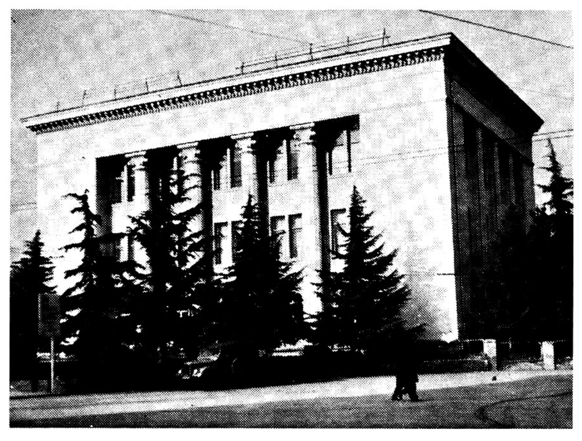
SPECIAL ISSUE



THIRD RUSSIAN—ITALIAN SYMPOSIUM ON CHROMATOGRAPHY

Organized by the Scientific Council on Chromatography of the AS U.S.S.R., P. G. Milikishivili Institute of Physical and Organic Chemistry of the AS Georgian S.S.R., the Georgian Scientific Research Institute for Scientific and Technical Information and Technical and Economic Investigations of the State Committee of the Ministerial Council of the Georgian S.S.R. for Science and Technology, and the Institute of Physics of the AS Georgian S.S.R., V. I. Lenin Georgian Polytechnical Institute, Scientific and Technological Instrumentation Society, Tbilisi (Georgian S.S.R.), October 26th—29th, 1970

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OPENING ADDRESS

With your permission I should like to offer cordial greetings to the participants of the Third Soviet–Italian Symposium on Chromatography, who have come to the city of Tbilisi.

A delegation of Italian scientists, consisting of Professors FRANCO POY, MARCO TARAMASSO, LUIGI BONIFORTI and GIANRICO CASTELLO, are attending our Symposium and we are glad to say to the Italian scientists: "Welcome to the land of Georgia".

This year, the Soviet Union has held an Italian culture week from October 15 and devoted to the further strengthening of Soviet–Italian friendship. In particular, the recent visit of representatives of the Italian people to Georgia, headed by the Vice-President of the Italian Syndicate of writers, senator FRANCO ANTONICELLI, has made its contribution to this cause.

For the many scientists and chemists of our country, working in the field of chromatography and adsorption and present here at this Symposium, this last week of October is also an Italian one.

The urgent scientific problems to be discussed at our Symposium on chromatography and the participation of leading Soviet and Italian scientists in this, make us confident that this Symposium will contribute to the development of chromatographic science and will help in the noble cause of strengthening further the development of scientific cooperation and friendship between the Soviet and Italian people.

Let us wish the participants of this Symposium success in their work.

G. V. TSITSISHVILI

INTRODUCTION

Chromatographic analysis is characteristically used for the study of sorption under dynamic conditions. TSWETT's method reduces essentially to the establishment of conditions for the formation of zones, determination of their sequence of displacement in a column or on a flat layer, and qualitative or quantitative analysis of these zones (solutions or gases) as they leave the column.

The establishment of the external conditions for the separation is at present more laborious, and less well resolved, than analysis of the products resulting from this separation. Nevertheless, modern chromatography does not aim merely at the analysis of complex mixtures. The method is so universal that it can now be used for the solution of more fundamental scientific problems, such as investigations of complex formation in solutions, studies of molecular interactions and of the surface magnitude and structure of adsorbents, investigations of molecular structures, determination of the molecular weights of polymers, and so on.

The advances in methods of investigation have led in turn to the development of new chromatographic apparatus. Major successes in this field have been achieved in gas chromatography, where it has been possible not only to produce improved equipment but also to transform the output information into a form suitable for direct input into computers and industrial process control systems. Equal successes in this direction may be expected in the domain of liquid chromatography, which deals with condensed systems.

The papers given at the third Soviet–Italian Symposium on Chromatography reflect almost all the trends in this field. Liquid ion-exchange chromatography features in the papers on the separation of rare, rare-earth, and transition elements on resins with complex formation or by displacement ion exchange. A similar separation mechanism is seen in oxidation–reduction chromatography, used in the realm of analytical chemistry.

The same section of the Symposium deals with the problems of investigating the molecular-weight distributions in polymers by thin-layer chromatography and the influence exerted by the nature and structure of sorbent surfaces on the distribution, and presents some new data on continuous preparative chromatography.

The application of natural mineral sorbents and zeolites and the investigation of their chromatographic properties make it possible to reveal the molecular interactions taking place in the course of chromatographic separations. A report on the regularities governing the retention on polar sorbents and on the interaction of polar substances with a non-polar liquid stationary phase stimulated considerable interest.

Some special problems, such as the specific features of flame-ionization (thermionic), helium, and other detectors, were presented in subsequent sessions of the Symposium. Here the lectures were of more practical character, devoted to the physico-chemical basis of the application of certain forms of chromatography.

The combined work of Soviet and Italian investigators, as well as the questions and discussions ranging over the entire realm of chromatography, proved as always extremely useful both from the point of view of development of chromatography as a science and for further strengthening of friendly scientific relationships between the two countries.

K. CHMUTOV

CHROM. 5223

AUTOMATION OF CHROMATOGRAPHY OF RADIOACTIVE SUBSTANCES

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SUMMARY

A technique for the automation of the registration of data of a chromatographic separation of radioactive substances is described whereby a device is used for delivery of information to a punched tape. The results of computer processing of the data are shown for an actual example—the study of the influence of nuclear radiation on isotopic exchange in the gaseous phase.

Studies of radioactive substances and the use of radioactive methods for the solution of different scientific problems have led to a wide use of radiochromatography. Along with any necessary apparatus modifications the processing of analysis data is improved by using methods of computing. It is noteworthy that sometimes an autonomous computing system is preferable with gas chromatography[1,2].

The system of automation of chromatographic separation of radioactive substances described in this paper implies that there is a Universal Computer available. It consists of two stages: the automatic recording of the data in a discrete form followed by computer processing.

The radioactivity of chromatographically separated substances is registered by a detector from which the pulses are transferred to an electronic counter. The use of a frequency meter as a registering device has a number of advantages in comparison with the devices used previously, *viz.*, it gives discreteness to the information obtained, direct digital registration, and the possibility of using the digital apparatus; the frequency meter sends a pulse "start printing".

A radiochromatographic device with the automatic recording of the analysis data by a printer is described in ref. 3.

The automatic recording of information stored in the frequency meter does not necessarily save an experimentalist from time-consuming work connected with data processing, as when computer processing is used, the information obtained must be presented in a form acceptable for the input device of the computer.

Fig. 1 shows a block diagram of a radiochromatograph in which teletype is used as the read-out device enabling us to make the information available on standard 17.5 mm punched tape as well as in the form of digital printing.

Each cell of the frequency meter counting decades is connected with a punching unit (teletype) through a system of inquest and control. This system consists of a

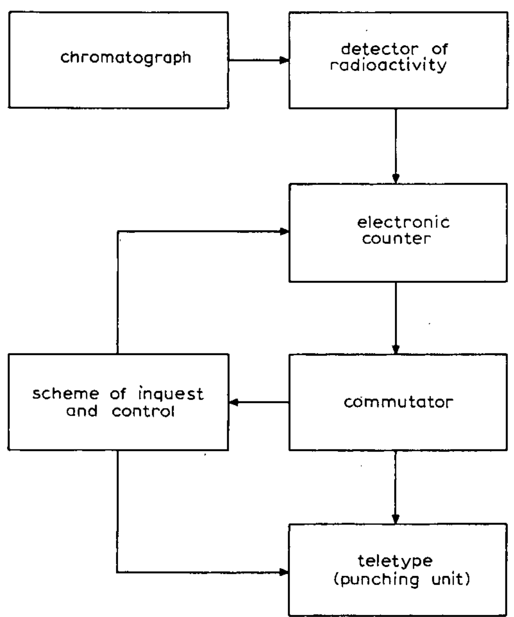

Fig. 1. Block diagram of a radiochromatograph in which teletype is used.

commutator, which is kinematically connected with the teletype and which probes each decade of the frequency meter, and a control system of the commutator for starting up the next cycle. Counting cells of all the frequency meter decades are connected with the commutator clamps. The commutator is made of a flat fixed ring, divided into $(n+1)$ equal parts, where n is the number of decades. Each of the first n parts has eight clamps used in the following way: the first clamp is for the starting signal of the teletype; the second for a synchronous pulse; the following four clamps are for passing through information from the frequency meter; the last two for the formation of the stop signal. All the clamps of the first n parts are fixed on the flat face on the side of the ring and are sensed by the main moving contact connected with the teletype shaft by a gear with a reduction coefficient of $1/(n+1)$.

The clamps for starting the investigation, for resetting of the frequency meter after punching and for starting a new cycle are fixed to the inner side of the ring and are sensed by an additional earthed moving contact. When the commutator is in operation the two moving contacts are synchronously probing either the clamps of the first n parts of the ring (the main contact) or the clamps of the last $(n+1)$-th part (the earthed contact) depending on the contact locations.

At the end of each counting cycle the electronic counting frequency meter produces a signal "print begins". This signal fires a thyratron T_1 (Fig. 2), at that moment the relay R1 is triggered and allows printing. When the contacts R_{K2} are switched off, the current flows through the tube T_3. The teletype electromagnetic circuit is closed and the armature is activated. When the commutator earthed contact approaches the clamp "A" the relay R2 is triggered, R_{K2} contacts close and the

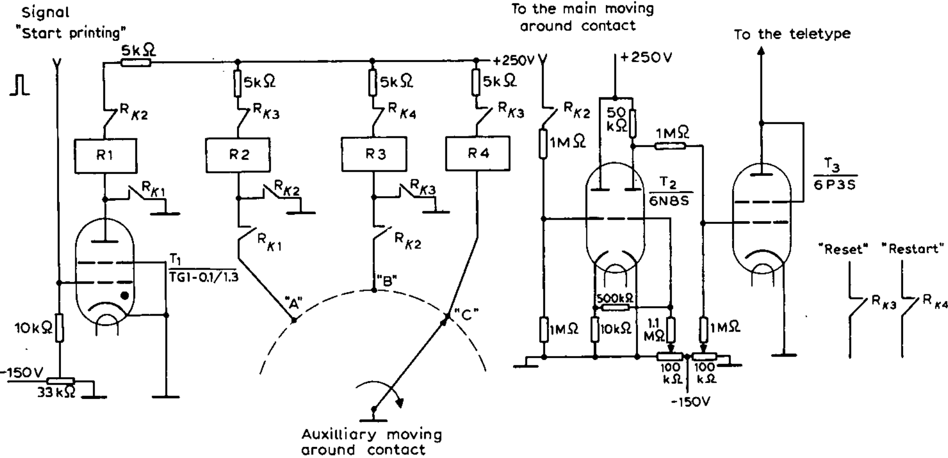

Fig. 2.

information from the main moving contact may be transferred to the tube T_2 grid, *i.e.* to the teletype electromagnet.

Voltage of +60V is applied to the first clamps of all n parts of the commutator. They are the starting clamps: when the positive signal is delivered to the input of tube T_2 the current in tube T_3 decreases and a currentless starting pulse is delivered to the teletype input.

The second clamps are freed, the voltage is not delivered to the tube T_2 input and T_3 is left in the current state. This is a current synchropulse (where current always flows).

The information from the frequency meter decades is delivered to clamps 3, 4, 5 and 6. This information is converted in such a way, that "zero" on the cell corresponds to +60V on the clamp and "unity" to zero voltage. In the first case the current does not flow through the tube T_3 (a currentless pulse) and in the second case the current does flow. The electromagnet having received the successive code of current and currentless pulses, the teletype punches and types. Each code group consists of seven pulses: "start", five pulses carrying information and "stop". Clamps 7 and 8 are vacant, that is similar to the delivery of the stop pulse.

The first clamp of the next section of the commutator then follows and the cycle is repeated; the relay of the information and the teletype punching from the second decade takes place etc.

When the earthed contact approaches "B" clamp, R3 relay operates, R2 relay switches off and simultaneously R_{K3} group of contacts performs "resetting". The tube T_3 returns to the current state. When the earthed contact approaches the clamp "C", R4 relay is triggered and a new counting cycle begins on the counting frequency meter.

The use of such a commutator instead of a selector[4] simplifies the control circuit and improves the system stability.

The punched tape obtained is put into the computer for processing of the experimental data.

When analysis is completed, successive discrete values $\{A_K\}$ are obtained.

The quantity A_K characterizes the radioactivity of the substance passing through the detector at the time t_K. The index "K" = 1, 2, 3, ..., N is determined by the expression

$$K = t_K/(\tau_1 + \tau_2) \quad (1)$$

where τ_1 is the time of counting; τ_2 is the time of information delivery from the counting cells; it is clear that the sum $(\tau_1 + \tau_2)$ is the duration of one cycle.

To determine the relative and absolute activities of each chromatographically separated component when a large number of identical analyses are carried out the method called a "flexible calibrating net" has been suggested[5].

This method is as follows. A calibration experiment is carried out with a non-radioactive mixture of all the possible components which can be determined under certain chromatographic conditions. At the same time the beginning and the end of the component elution are determined. Using expression (1) and the times of elution, the calibrating net of the boundaries studied is thus constructed. During processing of the main experimental data the preliminary calibrating net is corrected. The peak boundaries, in comparison with the calibration ones, are corrected, over different algorithms. As a result the boundary calibrating net is transformed to the corrected boundary net. The sequence of quantities $\{A_K\}$ is divided by this net into the peak areas and background regions. It should be noted that the broadening of peak boundaries due to the background does not affect the peak area, because during the subsequent processing the correction for background is provided.

The calculations of the absolute and relative activity of separated components are made by the formulae:

(1) Determination of the absolute activity σ_j

$$\sigma_j = \frac{1}{\eta}\left\{\frac{B_j}{1 - B_j\tau} - \Phi\left[n + \frac{\tau_2}{\tau_1}(n-1)\right]\right\}$$

where

$$B_j = \sum_{C_{jl}}^{C_{jr}} A_K + \frac{\tau_2}{2\tau_1}\sum_{C_{jl}}^{C_{jr}-1}(A_K + A_{K+1})$$

Φ is the counter background; η the efficiency of detection; τ the counter dead time; τ_1 the duration of each cycle of counting; τ_2 the duration of registration; C_{jl} is the peak left hand side boundary; C_{jr} is the peak right hand side boundary; n is the number of readings of A_K in the peak equal to $C_{jr} - C_{jl}$.

If a flow counter of internal volume V is used as the radioactivity detector and when the total gas flow rate through the counter is v, the calculated absolute activity has to be corrected by the factor v/V.

(2) Determination of relative activity θ_j

$$\theta_j = \frac{\sigma_j}{\Sigma\sigma_j} 100\%$$

Experimental studies of chemical effects of nuclear transformations[6] and studies of the effect of nuclear radiation on isotopic exchange[7] have been made by means of the technique described. Hydrogen (tritium), alkanes and olefines from C_1 to C_5 were separated chromatographically and determined radiometrically.

TABLE I

j	*Component*	*Boundary*	
		Left C°_{jl}	*Right* C°_{jr}
1	H_2	145*	166*
2	CH_4	166*	218*
3	C_2H_6	218*	265*
4	C_2H_4	265*	319
5	C_3H_8	319	426
6	C_3H_6	570	680
7	*iso*-C_4H_{10}	750	838*
8	*n*-C_4H_{10}	838*	902
9	C_2H_2	1302	1500
10	*iso*-C_4H_8	1630	1920*
11	*n*-C_4H_8	1920*	2180
12	C_5H_{12}	2500	2880

TABLE II

j	*Component*	*Boundary*	
		Left C_{jl}	*Right* C_{jr}
1	H_2	138	178
2	CH_4	178	208
3	C_2H_6	208	260
4	C_2H_4	260	319
5	C_3H_8	319	426
6	C_3H_6	570	680
7	*iso*-C_4H_{10}	750	816
8	*n*-C_4H_{10}	816	902
9	C_2H_2	1302	1500
10	*iso*-C_4H_8	1630	1880
11	*n*-C_4H_8	1880	2180
12	C_5H_{12}	2500	2880

TABLE III

j	*Component*	*Activity*	
		Absolute σ_j *(dis)*	*Relative* θ_j *(%)*
1	H_2	447 884 ± 684	96.016
2	CH_4	4 299 ± 65	0.921
3	C_2H_6	9 838 ± 99	2.109
4	C_2H_4	186 ± 14	0.039
5	C_3H_8	1 945 ± 44	0.416
6	C_3H_6	0	0.000
7	*iso*-C_4H_{10}	190 ± 14	0.041
8	*n*-C_4H_{10}	1 601 ± 40	0.343
9	C_2H_2	0	0.000
10	*iso*-C_4H_8	0	0.000
11	*n*-C_4H_8	401 ± 20	0.085
12	C_5H_{12}	121 ± 11	0.025

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The standard calibrating net of boundaries was determined from the routine experiment under the following conditions: the liquid phase was liquid paraffin, the solid support was diatomite from Zikeev quarry (T3K), granule size 0.25–0.5 mm; the column length was 8 m, the carrier gas was helium; the pressure at the inlet was 1.4 atm; the rate of the gas flux 60 cm^3/min; the column temperature was (100 $\pm$ 0.1) °C; the time taken for counting was 1.00 sec; the time of registration was 1.07 sec[3,5].

In the calibrating net of boundaries (Table I) the index * shows the boundaries corrected during the fundamental experiments. The other boundaries are considered to be constant.

Let us consider an example: an experimental study of the nuclear radiation effect on isotopic exchange in the system $C_2H_6 + T_2$ in the gaseous phase[7].

A quartz ampoule with a volume of 10 cm^3 was filled to a pressure of 300 mm Hg with the gas mixture C_2H_6(C_2H_6–98%, T_2–2%) and irradiated by the mixed radiation flux of the reactor (the neutron dose was $2.7 \cdot 10^{17}$ nvt).

The mixture of products formed in the cell as a result of isotopic exchange and radiolysis due to radiation was put into a radiochromatography apparatus and analysed. The experimental data were automatically recorded and then processed by a computer according to the technique described above.

As a result of correcting the boundaries over the corresponding ranges a net of corrected peak boundaries was obtained (Table II).

When radiochromatography was carried out under the above conditions, we obtained three thousand numbers characterizing the change of the recorded activity in time. Arrangements of numbers in accordance with Table II over peak and background regions and the following calculation of the absolute and relative activities gave some information about each separated component (see Table III, where the results of processing of the data obtained in the example considered are given).

In this paper the results of automation of a chromatographic separation of radioactive substances are given. The radiochromatograph is described as well as the device for information delivery to the punched tape. The computer processing of the data is shown for an actual example—the experimental study of the effect of nuclear radiation on isotopic exchange in the gaseous phase.

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CHROM. 5257

THE EFFECT OF THE GEOMETRICAL STRUCTURE AND THE SURFACE CHEMISTRY OF SILICA GELS ON SEPARATIONS IN LIQUID-SOLID CHROMATOGRAPHY

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SUMMARY

The effect of the geometrical structure of silica gels on retention volumes, band broadening and separation has been investigated. The retention times of nitrobenzene, aniline, diphenylamine and nitronaphthalene on hydroxylated and dehydroxylated silica gels have been measured. It has been shown that, for analytical purposes and taking into account the analysis time, it is preferable to use temperature programming of the column.

INTRODUCTION

Silica gels and alumina gels are widely used in liquid-solid chromatography[1,2]. The literature contains little information on the influence of geometrical structure and surface chemistry of hydroxylated and dehydroxylated silica gels on retention volumes and band broadening. The effect of the type of pores in the silica gels on the chromatographic separation of sterines was studied by KLEIN[3]. The influence of impurities in silica gels on retention volumes has been discussed by SPITZ[4].

The present work involves a study of the effect of the geometrical structure and the surface chemistry of silica gel on retention volumes, peak broadening and resolution.

EXPERIMENTAL

All measurements were made on equipment assembled in the laboratory. The detector was a UV spectrophotometer. Commercial silica gels were the adsorbents, the structural properties of which are given in Table I.

Hydroxylation of silica gels was carried out by boiling in distilled water for 2 days, and dehydroxylation by heating at 950° for 5–6 h. The particle sizes of the silica gels were 0.04–0.09 mm. *n*-Hexane was used as eluent. It was dried in a column filled with microporous silica gel.

TABLE I

STRUCTURAL PROPERTIES OF THE SILICA GELS USED

Silica gel	*Specific surface area, S (m^2/g)*	*Average pore radius (Å)*
C-3	260	82
C-4	540	14.8
KCK-2	368	64
KCK-2, 5	340	56
KCC-3	560	29
KCC-4	660	18.8
KCM-5	650	16.3
Silochrom C-80	80	—

RESULTS AND DISCUSSION

In order to study the effect of geometrical structure on retention volume (V_R) the dependence of V_R on the specific surface area (S) was obtained (Fig. 1). It can be seen from Fig. 1 that at surface areas greater than those specified, non-linear dependence of V_R on S is observed. The larger the molecules of adsorbate are the less is the deviation from linearity.

Nonlinearity of the dependence of V_R on S is connected with the "ultra-porosity effect" as in gas chromatography[5].

Fig. 2 shows the dependence of the selectivity criterion K_c (defined as the ratio of the difference of the corrected retention volumes to their sum for two neighbouring peaks) on the average pore radius. The selectivity of separation increases with

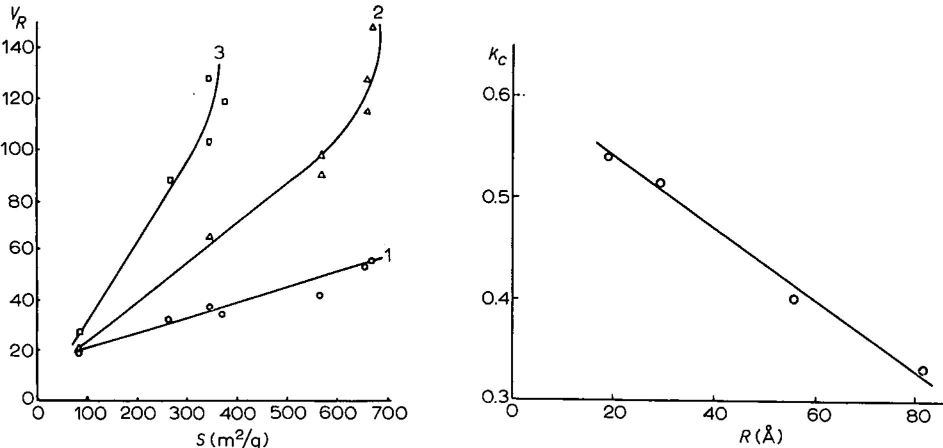


Fig. 1. Relation of retention volumes (V_R) to specific surface area (S) of silica gels. 1 = Benzene; 2 = naphthalene; 3 = phenanthrene. Temperature, 25°; column dimensions, 50 × 0.5 cm; flow rate of mobile phase (hexane), 0.85 ml/min.

Fig. 2. Relation of the selectivity criterion (K_c) (between benzene and naphthalene) to average pore radius (R). Conditions as in Fig. 1.

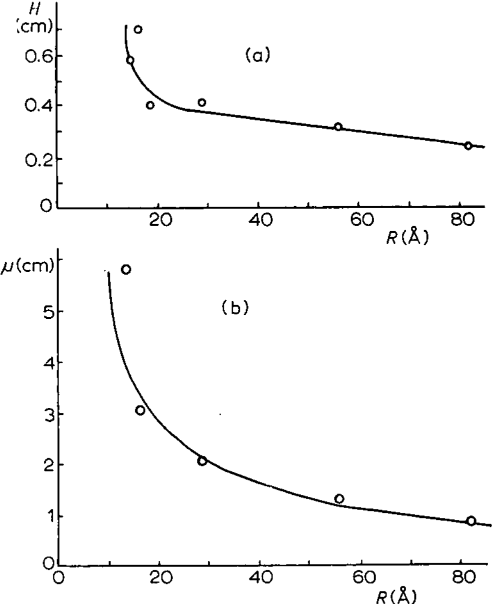


Fig. 3. Relation of: (a) H to R and (b) width of band (μ) to R. Conditions as in Fig. 1.

decrease of average pore radius. However with decreasing average pore radius the width of the band increases (Fig. 3).

The separation ability, estimated by analogy with gas chromatography[6] by the separation criterion K_1, depends on S to a smaller degree, since it is determined both by the effectivity and also by the selectivity of the separation (Fig. 4)[7].

For separation on silica gels with similar surface areas, but different average pore radii, larger retention times and better separations are observed with the more microporous silica gels (Fig. 5).

The dependence of the separation criterion K_1 and of the height equivalent to a theoretical plate on the velocity of the eluent is of interest in connection with slow mass-transfer in liquid–solid chromatography (Fig. 6a, b).

Fig. 6 shows that the effectivity of a column increases (*i.e.* the value of H decreases) with decrease of velocity, and that broadening occurs in the main as a

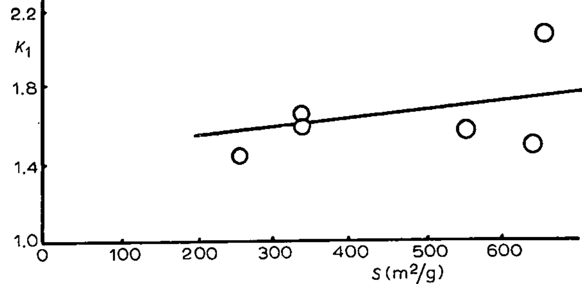
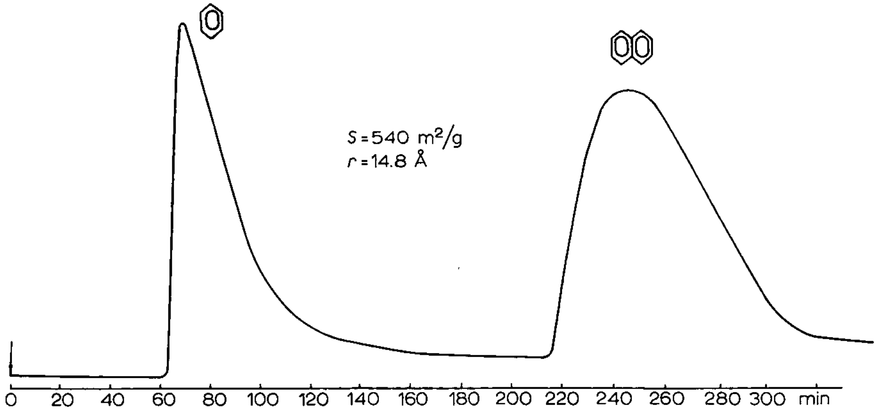


Fig. 4. Relation of the separation criterion (K_1) to S (between benzene and naphthalene).



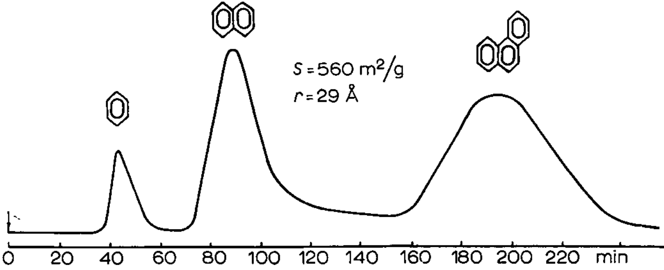


Fig. 5. Comparative chromatograms of benzene, naphthalene and phenanthrene obtained on silica gel column at equal specific surfaces and at various pore radius (shown on the chromatogram).

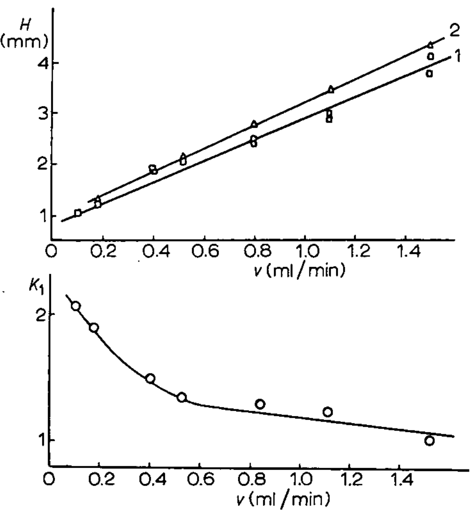


Fig. 6. (a) Relation of H to velocity of eluent: 1 = benzene; 2 = naphthalene. (b) Relation of the separation criterion (K_1) between benzene and naphthalene to velocity of eluent.

TABLE II

COMPARISON OF RETENTION TIMES ON HYDROXYLATED AND DEHYDROXYLATED SILOCHROM C-80
Eluent, hexane; flow rate, 0.85 ml/min.

Substances	*Adsorbent*	*Temperature (°C)*				
		60	*50*	*40*	*30*	*20*
Nitrobenzene	Dehydroxylated	27.3	29	34	36.5	40
	Hydroxylated	48	56.4	67	70	81
Aniline	Dehydroxylated	41	58	78.5	89	139
	Hydroxylated	210	264	332	342	—
Diphenylamine	Dehydroxylated	23	27	31	34.5	41.5
	Hydroxylated	60	82.4	102	108	152
Nitronaphthalene	Dehydroxylated	29.2	34	38.5	43	54
	Hydroxylated	53	65	81	—	120

result of kinetic factors. The separation ability of a column (the value of K_1) increases with decrease of velocity of the eluent (Fig. 6b). However, the decrease of velocity of the eluent leads to a great increase in analysis time. Taking the analysis time into account more macroporous silica gels are to be preferred for practical purposes. However, the nature of the substances to be analysed must always be taken into consideration.

In order to investigate the effect of the nature of the silica gel surface, retention volumes on hydroxylated and dehydroxylated silica gels were measured (Table II). All the substances given in Table II have considerably greater retention volumes on hydroxylated silica gels.

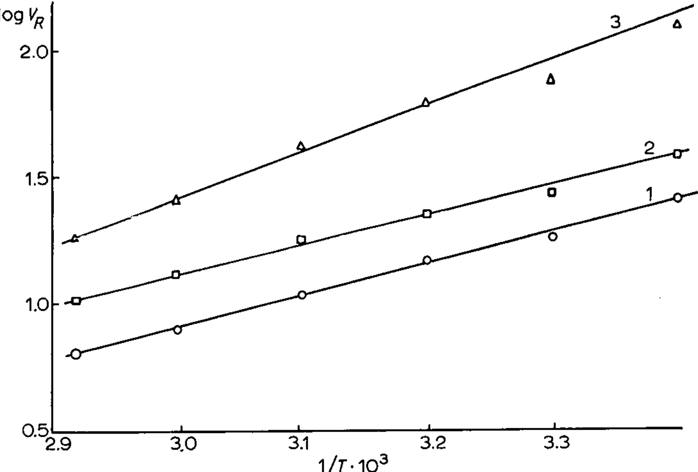


Fig. 7. Relation of log V_R to reciprocal of absolute temperature on a column containing dehydroxylated Silica Gel CX-80. 1 = diphenylamine; 2 = nitronaphthalene; 3 = aniline.

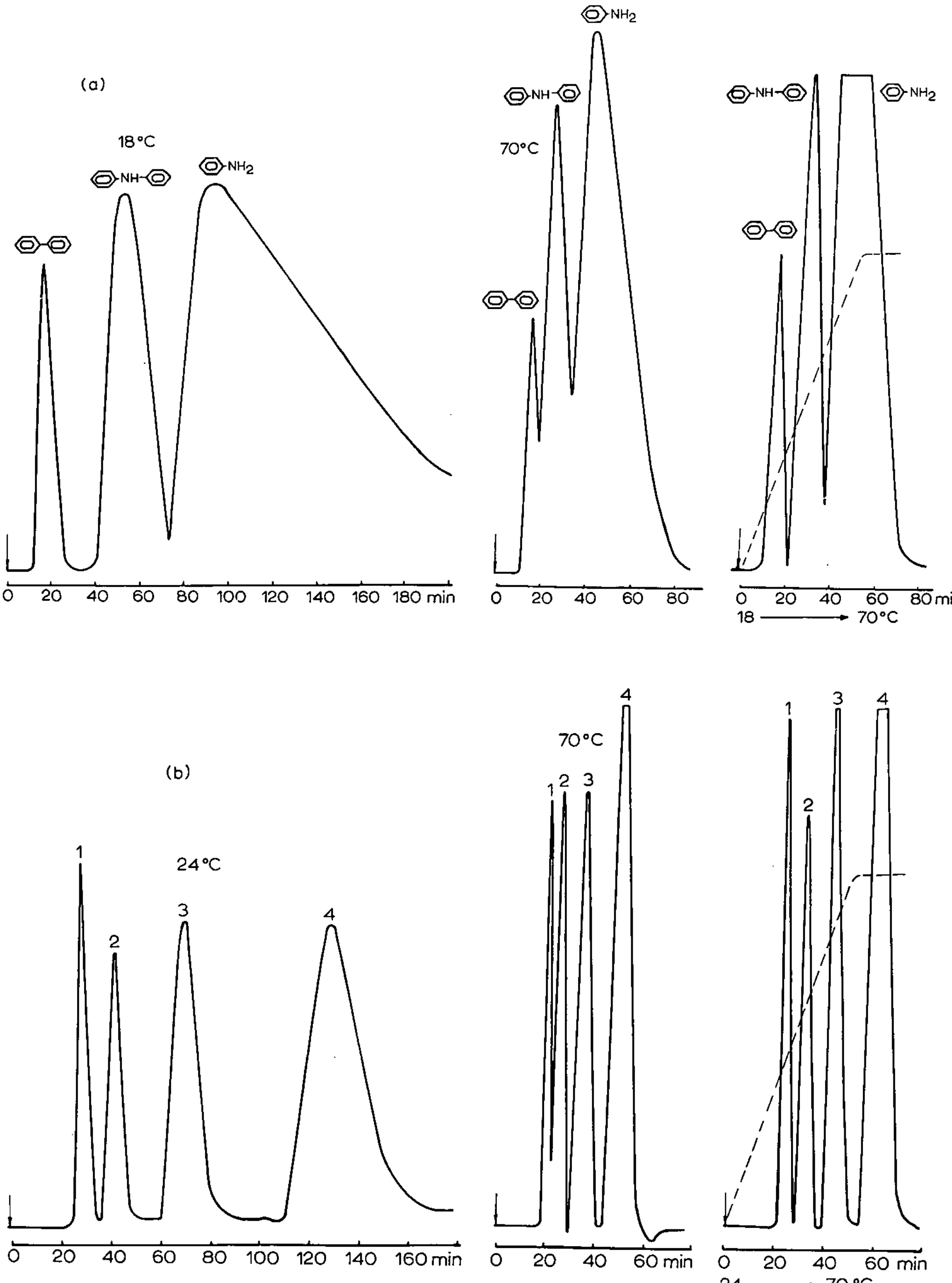


Fig. 8. Comparative separation. (a) Diphenyl, diphenylamine and aniline. Column dimensions, 50 × 0.5 cm; adsorbent, Silochrom G-80 + 5% KCl; flow rate of mobile phase (hexane), 0.85 ml/min. (b) 1 = benzene; 2 = naphthalene; 3 = phenanthrene; 4 = 1,2-benzanthracene. Temperature as shown on the chromatograms. Column dimensions, 50 × 0.5 cm; adsorbent, Silica Gel C-3; flow rate of mobile phase (hexane), 0.85 ml/min.

Fig. 7 shows the dependence of the logarithm of the retention volume on the reciprocal of the absolute temperature on dehydroxylated silica gel for a series of strongly polar substances. As in gas chromatography[5] this dependence is linear. From the slope of the lines, and by analogy with gas chromatographic measurements, it is possible to evaluate the energy of adsorption from solution (hexane). The energies of adsorption obtained from the diagrams are 8.6 kcal/mole for aniline, 6.0 kcal/mole for nitrobenzene and 5.7 kcal/mole for diphenylamine, respectively.

Fig. 7 shows that the retention volumes are very dependent on temperature and therefore programmed temperature of the column during the process of separation will be effective just as in gas chromatography.

The time of separation in liquid chromatography is longer and therefore the application of temperature programming means a considerable reduction in the time of separation and is of paramount significance (Fig. 8).

CONCLUSIONS

The effect of the geometrical structure and surface chemistry of silica gels on the degree of broadening and on the degree of separation in liquid chromatography has been investigated. From the results obtained it may be concluded that for analytical purposes, and taking into account the analysis time, it is preferable to use more macroporous silica gels (with $S \sim 80$–300 m^2/g and with average pore radius more than 80 Å).

Hydroxylation of the surface of silica gels leads to a large increase of retention volumes of polar substances, especially the amines.

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GAS CHROMATOGRAPHY ON MONOLAYERS

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SUMMARY

Densely adsorbed layers of polyethylene glycols of different molecular weights on graphitised carbon black and of polyarylate Ph-1 on macroporous silica gel were prepared. It has been found that monolayers of polyethylene glycols possess the property of specific molecular interaction and that the contribution of the energy of specific interaction to the total heat of adsorption increases with the decreasing molecular weight of the polyethylene glycol. The deposition of polyarylate as a thin adsorbed layer on to the surface of macroporous silica gel results in a decrease in the specific adsorption. In addition, the retention of *n*-alkanes and particularly of aromatic hydrocarbons on a polyarylate film is markedly decreased in relation to porous polyarylates and a number of other porous polymers.

INTRODUCTION

Modification of non-porous and wide-pore supports by means of adsorbed dense monolayers presents the possibility of obtaining quite homogeneous surfaces of different chemical composition with low energies of adsorption[1–8]. Furthermore, monolayer films held by adsorption forces on a large surface area of a strongly adsorbing support (adsorbent) are significantly more stable to heat than thick layers of these compounds on supports with small and weakly adsorbing surfaces such as are used in gas–liquid chromatography[1–3]. Further, exchange processes on the surfaces of monolayers take place much more rapidly than in the bulk of liquids[1].

Monolayers which are particularly stable to heat are obtained by chemically modifying the supports[1,8,9]. Graphitised carbon black[1–7] and wide porous silica gels[1,8] are usually used as the supports for the monolayers. If polymers or large organic molecules with various functional groups are used as the modifying compounds, then it is possible to obtain quite homogeneous specific adsorbents, selective for different classes of organic compounds.

In the present work dense layers of different polymers on graphitised carbon black and on macroporous silica gel were prepared and studied.

MONOLAYERS ON GRAPHITISED CARBON BLACKS

The influence of the molecular weight (M) of the polyethylene glycols (PEG) on the density and structure of the monolayer was investigated, and the adsorption of various compounds on the surface of PEG monolayers of different molecular weight was studied.

Channel carbon black, which had been heated at 3000° for 6 h in a current of argon and which had a specific surface area $s = 80$ m^2/g, was used as the support. PEGs with average molecular weights of 300, 3,000 and 15,000 were used. The adsorption isotherms of these polymers as determined from aqueous solutions were used to calculate the capacity of the monolayer, *i.e.*, the amount of polymer, a_m, required to cover the surface of the carbon black with a dense monolayer[10]. The PEG was deposited on the surface of the carbon black by adsorption from solution resulting in uniform coverage of the surface of the carbon black by the polymer[10]. The deposition of the polymer in amounts greater than the monolayer capacity was carried out by the usual method of depositing a liquid phase on a support as in gas–liquid chromatography[3]. The adsorption properties of the initial carbon black and of the modified samples were studied by means of gas chromatography using a Shimadzu GC-3A gas chromatograph. Before loading the column the samples were evacuated at 150° to about 10^{-3} torr (below the decomposition temperature of these polymers). The column temperatures were 75, 100, 130 and 150°. The compounds used as adsorbates were those whose molecules by their capability of molecular interaction belong to groups A, B and D according to the author's classification[1].

The dependence of the specific retention volumes V_g (ml/g of carbon black) of n-alkanes on the coverage of the surface of the carbon black by PEG is shown in Fig. 1. Molecules of n-alkanes belong to group A and are not capable of specific interaction with the functional groups of PEG. This dependence is also shown for benzene and diethyl ether in Fig. 1—these are molecules of group B which are capable of specific interaction with the terminal hydroxyl groups of PEG— as well as for n-alcohols—molecules of group D— which are capable of specific interaction with both the terminal hydroxyl groups of PEG and its ether groups. Molecules of group D are also capable of specific interaction with each other, forming associates.

The retention volumes of n-alkanes decrease sharply with increasing coverage of PEG up to a certain coverage, after which they remain unchanged. The sharp decrease in the retention volumes is explained by the lowering of the non-specific dispersion interaction with the adsorbent which is related to the smaller concentration of active centres on the surface as the carbon black is covered with polymer. The smaller concentration of active centres on the surface of the monolayer as compared with those on the support is connected first of all with the quite large van der Waals distance between the macromolecules when compared to that of the chemical bonds between the carbon atoms on the surface of the carbon black. This is also characteristic for adsorption on dense monolayers and on molecular crystals[11–13].

Molecules of alcohols may form hydrogen bonds with the functional groups of PEG. The increase in the contribution of the energy of the corresponding specific interaction to the total energy of adsorption in the region of low surface coverage of carbon black by PEG apparently predominates over the decrease in the dispersion interaction. Therefore, the retention volumes of alcohols, as can be seen from Fig. 1,

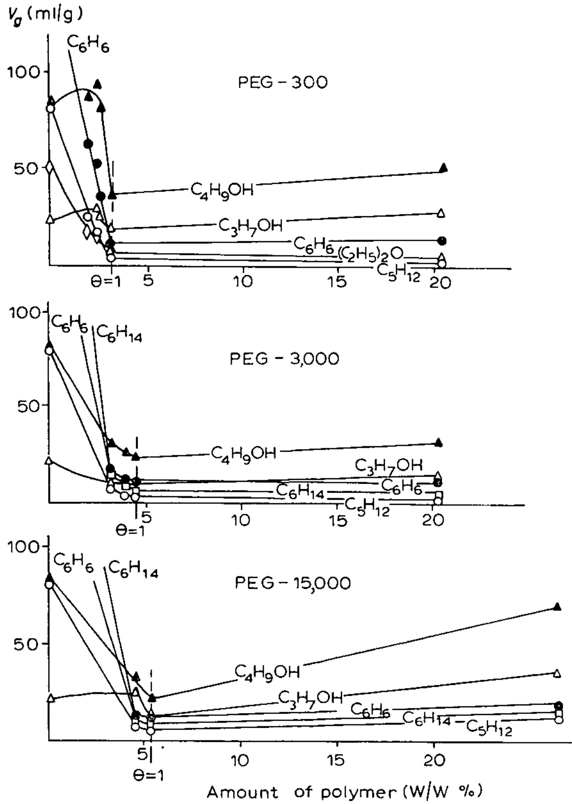

Fig. 1. Dependence of the retention volumes, V_g (ml/g), on the surface coverage of graphitised channel carbon black by polyethylene glycols of different molecular weights PEG-300, PEG-3000 and PEG-15000. The adsorbates are shown on the figure. The dashed line indicates the monolayer capacity determined from the isotherms of adsorption from solution.

increase with increasing coverage of carbon black by PEG-300 up to 2.3% PEG per unit weight of carbon black, which corresponds to a surface coverage of carbon black by polyethylene glycol of approximately 75%. If the amount of PEG is increased further, up to 3% PEG per unit weight of carbon black, *i.e.* the surface of the carbon black is practically completely covered with a dense monolayer of PEG, the retention volume of the alcohols decreases. It is evident that in this region the decrease in the dispersion interaction predominates over the increase in the specific interaction. With an even greater amount of PEG-300 on the surface of the carbon black, the retention volumes of the alcohols again increase and this is the result of the solution of the alcohols in the polymolecular film of PEG.

No increase in the retention volumes of alcohols with increasing surface coverage of carbon black has been observed for the larger macromolecules PEG-3000 and 15000. The packing of these macromolecules in the deposited layers is less orderly (and therefore some of the functional groups, capable of specific interaction with molecules of alcohols, are inaccessible or are oriented less favourably for the formation of hydrogen bonds) than in the case of adsorption of alcohols on the more

orderly packed layer of the smaller macromolecule PEG-300. In the case of diethyl ether and benzene the decrease in the energy of dispersion interaction predominates over the increase in specific interaction with increasing surface coverage of the carbon black by PEG, and is independent of its molecular weight.

A sharp break in the curves showing the dependence of the retention volumes on the surface coverage of carbon black by PEG is observed at approximately the same surface coverage of carbon black by polyethylene glycol both for compounds capable and incapable of specific interaction. These amounts are shown in Fig. 1 as vertical dashed lines and correspond to the formation of a sufficiently dense monolayer of macromolecules screening the surface of the carbon black. The capacities of the monolayers evaluated by different methods are compared in Table I. They are taken from the van der Waals dimensions of the monomer of the PEG macromolecule, from the adsorption isotherms determined for solutions and from the gas chromatographic data in Fig. 1. Only in the case of the low molecular weight polymer PEG-300

TABLE I

CAPACITIES OF DENSE MONOLAYERS OF PEG DEPOSITED ON GRAPHITISED CHANNEL CARBON BLACK

Modifying agent	*Capacity of dense monolayer (mg/m^2)*		
	Calculated from the van der Waals dimensions of the monomer unit of the macromolecule	*Determined from adsorption isotherms from solution data*	*Determined from gas chromatographic data*
PEG-300	0.44	0.43	0.42
PEG-3000	0.44	0.60	0.57
PEG-15000	0.44	0.74	0.73

does the capacity of the dense monolayer of the stretched-out macromolecule, calculated from the van der Waals dimensions of the monomer unit of the macromolecule, correspond to the capacity of this polymer layer determined from its adsorption isotherm from solutions. Apparently, among the PEG samples investigated, only PEG-300 molecules, with the lowest molecular weight, straighten out almost completely and pack densely on the surface of carbon black under the influence of the adsorption forces. In the case of PEG-3000 and particularly in the case of PEG-15000 complete stretching out of the macromolecules on the surface of carbon blacks does not occur. It is possible that these macromolecules are in fact not completely linear.

The macromolecules of these higher molecular weight polymers do not contact the surface of the carbon black with all their units and this is in agreement with other data[14]. In agreement with this is the fact that the concentration of ether groups and terminal hydroxyl functional groups of PEG, capable of specific interaction with adsorbate molecules on the surface of a dense monolayer, is greater in the case of PEG-300 than in the case of PEG-3000 and PEG-15000.

TABLE II

RETENTION VOLUMES, V_g (ml/g), AT 100° AND CORRESPONDING RELATIVE RETENTION VOLUMES, $V_g/V_{g(C_5H_{12})}$, ON DENSE MONOLAYERS OF PEG OF DIFFERENT MOLECULAR WEIGHT DEPOSITED ON GRAPHITISED CHANNEL CARBON BLACK

Adsorbate	*Carbon black*		*Monolayer*					
			PEG-300		*PEG-3000*		*PEG-15000*	
	V_g	$V_g/V_{g(C_5H_{12})}$	V_g	$V_g/V_{g(C_5H_{12})}$	V_g	$V_g/V_{g(C_5H_{12})}$	V_g	$V_g/V_{g(C_5H_{12})}$
n-C_5H_{12}	80	1.00	2.50	1.00	2.5	1.00	2.50	1.00
n-C_6H_{14}	253	3.17	5.30	2.12	5.35	2.14	5.38	2.14
C_6H_6	172.5	2.18	11.2	4.46	10.20	4.08	9.15	3.66
$(C_2H_5)_2O$	51.7	0.65	3.36	1.35	2.85	1.14	2.75	1.10
n-C_3H_7OH	23.2	0.18	14.6	5.81	10.45	4.18	8.10	3.40
n-C_4H_9OH	82	1.03	39.6	15.7	24.4	9.76	19.10	7.64

In accordance with this, on dense monolayers of PEG with different molecular weights the retention volumes of the n-alkanes which are adsorbed non-specifically are the same, but the retention volumes of compounds, capable of specific interaction with the functional groups of PEG, decrease with increasing molecular weight of the PEG. This is evident from Table II. It is also evident from Table II that PEG monolayers are particularly selective with respect to alcohols.

In order to evaluate the contribution of the energy of specific interaction of various molecules on monolayers of PEG to the total heat of adsorption it is convenient to compare the heats of adsorption of these molecules with those incapable of specific interaction (*viz.* n-alkanes)[1,7,15,16]. The dependence of the heats of adsorption on the total polarisability of molecules[7,16] belonging to groups B and D

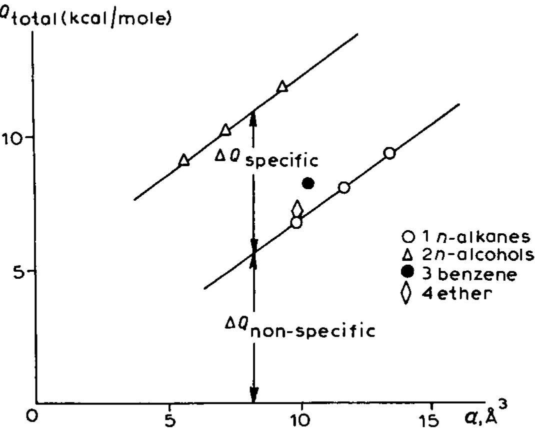

Fig. 2. Dependence of heats of adsorption, Q, at low surface coverage with a dense monolayer of PEG-300 (deposited on channel carbon black treated at 3000°), on the polarisability, α, of the adsorbate molecules. The contributions of the energy of non-specific interaction, $\Delta Q_{\text{non-specific}}$, and of the specific interaction, $\Delta Q_{\text{specific}}$, with the monolayer are indicated by arrows.

were therefore compared with the corresponding dependence for *n*-alkanes, molecules of group A (Fig. 2).

The contribution of the energy of specific interaction, $\Delta Q_{specific}$, was determined as the difference between the experimental heat of adsorption and the non-specific component which was found from the characteristic straight line which shows the dependence of the heats of adsorption of molecules of group A (*n*-alkanes), at low coverage, on the polarisability (Fig. 2 and Table III). On a dense monolayer of PEG-300 $\Delta Q_{specific}$ for alcohols is 5.5 kcal/mole, *i.e.* the energy of a hydrogen bond characteristic for alcohols. On increasing the molecular weight of the PEG the contribution of the specific interaction decreases for all compounds which are capable of specific interaction with the functional groups of PEG. This also shows that the concentration and availability of the functional groups on the surface of the monolayer decreases with increasing molecular weight of the deposited polymer and the orientation of these functional groups becomes less advantageous for specific interaction with molecules adsorbed from the gas phase.

It may be seen from Table III that the strongest specific interaction with the functional groups of PEG is observed for molecules of group D—for alcohols. At the same time the total energy of interaction of these molecules with a PEG monolayer is small. The large contribution of $\Delta Q_{specific}$ together with the comparatively small contribution $\Delta Q_{non\text{-}specific}$[7] permits the advantageous use of such a monolayer for the chromatographic separation of mixtures of alcohols. The separation of mixtures

TABLE III

APPROXIMATE VALUES OF HEATS OF ADSORPTION, Q, AND CONTRIBUTION OF ENERGY OF SPECIFIC INTERACTION, $\Delta Q_{specific}$, TO THEM, WHEN VARIOUS MOLECULES ARE ADSORBED ON THE SURFACE OF A DENSE MONOLAYER OF PEG DEPOSITED ON GRAPHITISED CHANNEL CARBON BLACK

Adsorbent	C_6H_6		$(C_2H_5)_2O$		n-C_3H_7OH		n-C_4H_9OH	
	Q	$\Delta Q_{spec.}$	Q	$\Delta Q_{spec.}$	Q	$\Delta Q_{spec.}$	Q	$\Delta Q_{spec.}$
PEG-300	8.3	1.3	7.1	0.3	10.3	5.6	12.0	5.6
PEG-3000	8.2	1.1	7.0	0.2	9.4	4.7	11.2	4.7
PEG-15000	8.4	0.9	7.1	0.0	8.9	3.6	10.9	3.6

of all alcohols from C_1 to C_4 is shown in Fig. 3. On channel carbon black (as distinct from graphitised thermal carbon black[1]) mixtures of the alcohols are not separated even at 100° and the peaks are very unsymmetrical. On samples modified by monolayers of PEG, a mixture of these same alcohols is separated even at 70°. In connection with this, the most effective columns are those filled with carbon black supporting a dense monolayer of PEG-300 which has the lowest molecular weight of the PEGs which were investigated.

As well as polyethylene glycol even lower molecular weight compounds, *e.g.* molecules of 2,4-dinitrophenylhydrazones[7], may be used as modifiers for carbon black. It is evident from Fig. 3 that dense monolayers of these compounds deposited on the surface of channel carbon black ($s = 80\ m^2/g$) are even more selective for alcohols than a PEG monolayer.

Thus, by modifying graphitised carbon black with polyethylene glycol of low

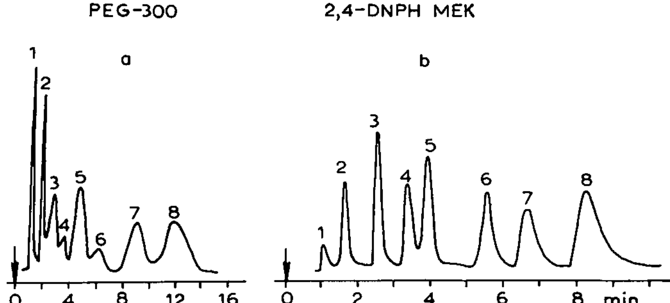

Fig. 3. Separation of a mixture of C_1–C_4 alcohols on monolayers deposited on channel carbon black: (a) on a monolayer of PEG-300 (t = 70°, l = 100 cm, d = 0.3 cm, flow rate of He = 38 ml/min); (b) on a monolayer of the 2,4-dinitrophenylhydrazone of methyl ethyl ketone (t = 70°, l = 100 cm, d = 0.3 cm, flow rate of He = 40 ml/min). (1) CH_3OH; (2) C_2H_5OH; (3) *iso*-C_3H_7OH; (4) *n*-C_3H_7OH; (5) *tert.*-C_4H_9OH; (6) *sec.*-C_4H_9OH; (7) *iso*-C_4H_9OH; (8) *n*-C_4H_9OH.

molecular weight by adsorption from an aqueous solution it is possible to form a dense monolayer consisting of straightened-out macromolecules oriented in a plane on the surface. Monolayers of PEG specifically adsorb molecules of groups B and D and may be used for the separation of alcohols at low temperatures. To improve the quality of such adsorbents it is necessary to use narrow fractions of linear macromolecules.

MODIFICATION OF SILICA GEL BY POLYARYLATE PH-1

As well as using graphitised carbon black which is a strong, but non-specific, adsorbing support for monolayers of molecules and macromolecules, specifically adsorbing adsorbents, in particular macroporous silica gel[1,7] may also be used. The molecules and polymers containing functional groups capable of forming hydrogen bonds with the silanol groups of the surface[17] must be held particularly firmly on the silica gels. An industrial macroporous silica gel of specific surface area $s = 17$ m^2/g and mean pore diameter about 500 Å (ref. 1) was used as a support for the adsorption film. The modifying polymer for the silica gel consisted of polyarylate Ph-1[18] of average molecular weight about 30 000. The macromolecule is made up of the monomer units (see scheme below) and consequently according to the author's classification[1] polymer Ph-1 belongs to adsorbents of type III *i.e.* it is a specific adsorbent carrying on its surface negative charges concentrated mainly on the oxygen atoms of carbonyl and ether groups.

Polymer Ph-I was deposited on the surface of the macroporous silica gel from a chloroform solution.

The specific surface area of the modified silica gel $s = 18\ m^2/g$ determined by low temperature adsorption of krypton[19] did not differ much from the surface of the initial silica gel ($s = 17\ m^2/g$) but was significantly greater than the specific surface area of the initial porous polyarylate Ph-I ($s = 3\ m^2/g$).

The average concentration of the polymer on the surface of the silica gel, determined by loss of weight during calcining of the modified sample at 800° in air, was 2.3 mg/m². This shows that a very thin adsorbed layer of Ph-I was formed on the surface of the silica gel. For uniform surface coverage its thickness cannot exceed 2–2.5 monolayers if we accept $a_m \simeq 0.9$–$1.0\ mg/m^2$ as the capacity of a monolayer, which value was obtained[7,20] for the adsorption of polystyrene on aerosil and on macroporous silica gels. a_m may be evaluated roughly from the geometric dimensions of the separate monomers of the macromolecule Ph-I. For flat orientation of the aromatic rings of the basic framework of the polymer, the area occupied by one monomer of Ph-I is approximately equal to 160 Å² and consequently $a_m \simeq 0.43$ mg/m² if the Ph-I macromolecules are completely unfolded when adsorbed on the surface of macroporous silica gel. This assumption is not likely to be true for such a rigid and difficultly soluble polymer as polyarylate Ph-I. Besides, on account of the presence of carbonyl groups in Ph-I it is possible that the orientation of the macromolecules in the monolayer is not flat and this must also lead to an increase in a_m.

The chromatographic investigation of these compounds was carried out using a Tswett-4 gas chromatograph provided with a flame ionisation detector. The samples were sieved and the fraction between 0.25–0.5 mm taken, dried *in vacuo* at 200°, loaded into the chromatographic column, 100 × 4 cm, and given a preliminary heating in a current of nitrogen at 200° until a stable zero line was obtained. Compounds whose molecules belong to groups A, B and D were used as adsorbates.

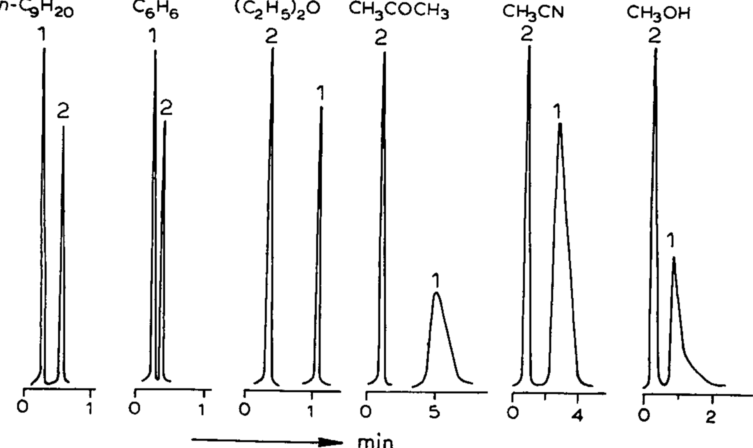

Fig. 4. Examples of chromatograms of a number of organic compounds on: (1) initial wide-pore silica gel; and (2) wide-pore silica gel modified with a thin layer of polyarylate. The adsorbates are shown on the figure. Experimental conditions: $t = 150°$; column dimensions 100 × 0.4 cm; flow rate of carrier gas (nitrogen) 40 ml/min; flame ionisation detector.

TABLE IV

RETENTION VOLUMES PER UNIT SURFACE AREA, V_S, AND HEATS OF ADSORPTION, Q, (AT LOW COVERAGES) ON POROUS POLYMER PH-1 AND ON A FILM OF PH-1 DEPOSITED ON MACROPOROUS SILICA GEL

Adsorbate	*Dipole moment (Debye units)*	*V_s (ml/m²) (150°)*			*Q (kcal/mole)*		
		Silica gel support	*Film Ph-1 on silica gel*	*Porous Ph-1*	*Silica gel support*	*Film Ph-1 on silica gel*	*Porous Ph-1*
Molecules of group A							
Hexane	0	—	0.08	3.42	7.0	12.3	13.5
Heptane	0	—	0.15	6.32	8.6	14.1	15.6
Octane	0	—	0.29	10.82	9.9	15.5	17.6
Nonane	0	0.16	0.54	—	11.4	17.0	19.6
Decane	0	0.23	0.99	—	13.0	—	—
Molecules of group B							
Benzene	0	0.13	0.19	21.6	8.3	12.4	15.2
Toluene	0.37	0.30	0.39	49.6	9.7	14.2	17.4
p-Xylene	0	0.65	0.79	—	11.2	15.9	19.6
Cumene	—	0.74	1.06	—	12.2	16.1	—
Diethyl ether	1.17	1.25	0.27	3.95	13.5	10.9	14.1
Dipropyl ether	1.77	2.71	0.67	9.62	15.2	—	—
Acetone	2.85	7.21	0.67	10.4	15.1	12.0	14.1
Nitromethane	3.54	0.83	0.27	25.3	10.1	11.7	13.7
Acetonitrile	3.94	2.38	0.48	14.2	12.2	9.4	12.8
Molecules of group D							
Methanol	1.67	1.23	0.25	3.56	—	—	10.5
Ethanol	1.70	—	0.41	6.59	—	—	12.6
n-Propanol	1.66	—	0.77	14.5	—	—	14.6
n-Butanol	1.66	—	2.04	35.6	—	—	16.6

It can be seen from Fig. 4 and Table IV that a deposit of a thin layer of Ph-1 on the surface of the silica gel leads to a noticeable increase in retention times, retention volumes per unit surface V_s and heats of adsorption of *n*-alkanes and aromatic hydrocarbons. On the other hand for ethers, ketones, acetonitrile and

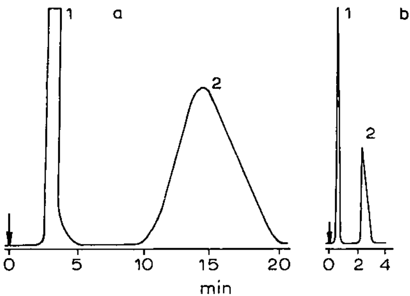

Fig. 5. Chromatograms of naphthalene on: (a) porous polyarylate Ph-1 at 200°; and on (b) silica gel modified by a thin layer of this polyarylate Ph-1, at 180°. The rest of the conditions are the same as in Fig. 4. Solvents: (1) benzene, (2) naphthalene.

alcohols which are all capable of forming strong hydrogen bonds with the silanol groups of silica gel, after modification of the silica gel surface with the polymer Ph-1, a sharp decrease in these values is observed. At the same time the adsorption properties of the polymer film, deposited on the silica gel, are substantially different from the properties of the porous Ph-1 polymer. It may be seen from Fig. 5 that naphthalene emerges from a column of silica gel, modified by a film of Ph-1 with a very narrow peak and much more rapidly than from a column of the porous polymer Ph-1, despite the somewhat lower experimental temperature and the quite large surface of the modified silica gel. This difference appears even greater on comparing

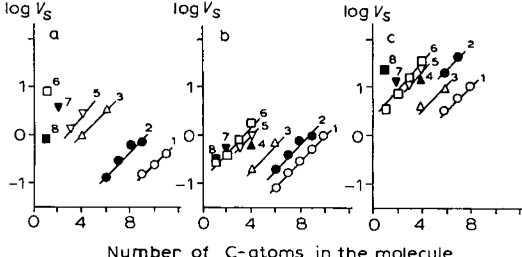

Fig. 6. Dependence of logarithm of retention volumes V_s at 150° of various adsorbates on the number of carbon atoms in the molecule for: (a) the initial silica gel; (b) silica gel modified with a thin layer of polyarylate Ph-1; and (c) the porous polyarylate Ph-1 itself. (1) *n*-Alkanes; (2) alkylbenzenes; (3) ethers; (4) ethyl acetate; (5) ketones; (6) *n*-alcohols; (7) acetonitrile; (8) nitromethane.

the retention volumes V_s referred to unit surface of the modified silica gel (Fig. 6 and Table IV).

The values of V_s on the porous polymer Ph-1, despite its small specific surface area as measured from adsorption of krypton, exceeds the value of V_s on silica gel modified by this polymer by one order of magnitude. Quite possibly the molecules of these adsorbates are capable of penetrating into the network of the porous polymer between its macromolecules which results in strong retention and high values of V_s and Q. High values of Q for *n*-alkanes are also characteristic of silica gel modified by a film of Ph-1. However, because of the fact that in this case the polymer was deposited as a very thin layer, the exchange processes (adsorption and desorption) take place much more quickly on the polymer film than in the porous polymer. The macromolecules of the polymer are probably arranged in a more orderly fashion on the surface of silica gel than in the network of the same porous polymer, being oriented to the surface of the support by their polar groups. This fact is demonstrated in particular by the smaller heats of adsorption of molecules of group B—diethyl ether, acetone and acetonitrile—on silica gel modified by a film of Ph-1, than on the same porous polymer Ph-1. Thus, the heats of adsorption of these adsorbates on a film of Ph-1 deposited on silica gel are less by 2–3 kcal/mole than the corresponding heats of adsorption on the porous polymer Ph-1. Thus a film of Ph-1 on silica gel is a less specific adsorbent with respect to these molecules of group B than the porous polymer Ph-1 itself. This demonstrates the fact that a film of Ph-1 is non-porous or at least significantly less porous in comparison to the polymer itself.

Thus, the adsorption properties of a thin layer of polyarylate Ph-1, deposited on silica gel, differ substantially both from the properties of the initial porous polymer Ph-1 and also from the properties of the support. Surfaces of silica gel modified by polyarylate Ph-1 sharply diminish its specificity and decrease the retention times and asymmetry of the chromatographic peaks of polar compounds: ethers, esters, ketones, nitriles and nitro-compounds. The use of polyarylate as a thin adsorption layer on silica gel permits a sharp reduction of the strong retention of *n*-alkanes and, in particular, of aromatic hydrocarbons characteristic of porous polyarylates and industrial porous polymers based on styrene and divinylbenzene. In addition to this, spreading of the chromatographic peaks is reduced.

CONCLUSION

The results presented here show that modification of adsorbent-supports which have the capability of different molecular interactions, by adsorption of dense monolayers of molecules and macromolecules, permits us to obtain new adsorbents with quite uniform surfaces, small energies of non-specific interaction, high specificity and selectivity, and sufficient thermal stability for numerous applications in gas chromatography.

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CHROM. 5256

ADSORBENTS FOR GAS–SOLID CHROMATOGRAPHY PREPARED BY EPITAXIAL MODIFICATION OF CLAY MINERALS WITH QUATERNARY AMMONIUM IONS

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SUMMARY

The epitaxial growth of a phase over a heterogeneous crystalline matrix is proposed in order to obtain homogeneous adsorbents able to show both steric effects and specific interactions.

The properties required by both phases in order to produce stable derivatives are examined. Examples are given of the epitaxial modification of mica-type layered silicates with N-alkylpyridinium and *n*-alkylammonium ions. Some unpublished and published results are reviewed for derivatives containing dimethyldioctadecyl-ammonium and trimethylhexadecylammonium ions.

The work of KISELEV AND YASHIN[1] demonstrates the great potentiality and, until now, far from complete exploitation that the gas–solid chromatography offers in difficult separations of geometric and steric isomers. Principally, the need is for new homogeneous and selective adsorbents.

Chemical modification of heterogeneous adsorbents both by coating surfaces with liquids and by grafting radicals to active centres, has been studied extensively. However such modification results in disordered surfaces and the peculiarity proper to a surface of rigid geometry is lost; in other words, the difference in adsorption enthalpy for isomeric forms disappears.

I should like to discuss the promising possibility offered by epitaxial growth of a phase over a heterogeneous crystal, in order to obtain homogeneous adsorbents able to show steric effects as well as specific interactions.

Epitaxial modification, *i.e.*, an ordered arrangement along the crystal axes, requires a suitable matrix with appropriate binding points (active groups or lattice vacancies) in order to establish either stable chemical or electrostatic bonds with the growing phase. On the other hand the radicals or ions, which are to be linked to the crystal faces, must be big enough so as to ensure complete coverage of the surface and to allow such a dense packing that free molecular rotation of the grafted groups is prevented.

A simple example of epitaxial modification is given by the organic derivatives of the clay minerals. Among the latter, the family of mica-type layer silicates are

of interest. They possess a sheetlike structure resulting from a lattice constituted of an octahedral layer containing either alumina or magnesia, sandwiched between two tetrahedral layers of silica[2]. A charge deficit within the lattice can be found as a result of isomorphous substitution by ions of lower valency. Electrical neutrality is then ensured by the presence of counter ions held on the basal surfaces; these ions are subject to displacement by means of ionic exchange reactions. The exchangeable cation concentration depends upon the nature of the mineral: consequently the "equivalent area", that is the surface available for each univalent cation, varies from infinite (*e.g.* for pyrophyllite) to 24 Å^2 (*e.g.* muscovite).

Let us look at an example: if a clay of large equivalent area (of about 100 Å^2, *e.g.* a montmorillonite) is exchanged with pyridinium ions, only about 1/4 of the available surface is covered. While the adsorbent obtained is heterogeneous, it nevertheless shows an interesting selectivity which resembles a sieve action: for instance 1,4-dimethylcyclohexane in the *trans* form is retained more than the *cis* isomer, the relative volatility being as high as $\alpha = 8$ at 150°; the first peak is symmetrical and the separation is of practical interest. The elution order of the xylenes is: *meta* then *para*, the reverse of that obtained with alkylammonium derivatives: both peaks are strongly asymmetrical and the retentivity depends on the sample amount.

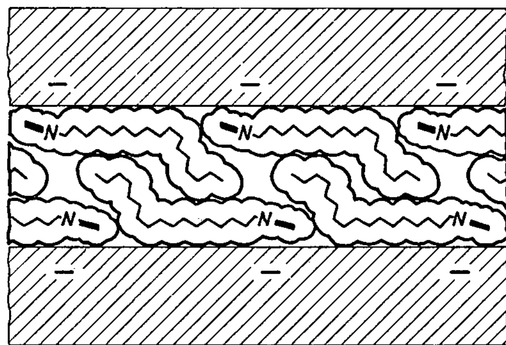

Fig. 1. Model for the arrangement of N-*n*-alkylpyridinium ions between silicate layers[3].

Now, if N-*n*-alkylpyridinium ions are used in place of the simple pyridinium ion, when the alkyl chain is 10 carbon atoms long, the above mentioned clay, with about 100 Å^2 of equivalent area, is completely covered since the alkyl chain lies flat on the silicate surface. Increasing the length of the chain results in the chain overlapping the aromatic ring[3], as shown in Fig. 1. The adsorbent thus obtained behaves like an homogeneous one: the selectivity towards the *cis–trans* forms of dimethylcyclohexane is lost; the xylenes elute in the order *para, meta,* with symmetric peaks (As = 1.2–1.5) and a relative volatility at 100°, α = 1.2–1.3. A new selectivity order also appears: ethylbenzene is retained more than *p*-xylene, contrary to what occurs with the alkylammonium derivatives. The efficiency of N-alkylpyridinium columns is low (HETP = 0.4–0.6 cm).

The structure of the *n*-alkylammonium derivatives has been studied in greater detail than that of any other organic cation[4]. The preferred arrangement of the alkyl chains, on a clay having a medium equivalent area, is the *trans–trans* configuration.

The bonding strength is high: in addition to coulombic forces, N–H···O hydrogen bridges are formed with the oxygen surface of the silicate sheet. The organic cations slope at an angle of 56° to the basal plane.

Unfortunately, from the gas chromatographic point of view derivatives of this kind behave homogeneously (As = 1.2) with highly charged silicates only (beidellites and vermiculites, equivalent area: 50–35 Å^2); besides the specificity is low (*m*- to *p*-xylene: relative volatility at 130°, α = 1.2).

Bulky cations as trimethylhexadecylammonium or dimethyldioctadecylammonium are convenient owing to both their basic character and their good surface covering power[5]. As the dimethyldioctadecylammonium ion is able to cover at least 200 Å^2 of surface, the complexes obtained with all the clay minerals belonging to the groups from hectorite to biotite (the equivalent area varying from 100 to 24 Å^2) behave as homogeneous adsorbents in gas chromatography: thus accurately prepared derivatives give symmetric peaks and do not require the addition of modifying liquids. Table I shows the most significant values obtained with representative members of each group. When an excess of dimethyldioctadecylammonium halide

TABLE I

PROPERTIES OF SOME REPRESENTATIVE DIMETHYL-DIOCTADECYLAMMONIUM DERIVATIVES OF MICA-TYPE LAYER SILICATES

Equivalent area (Å^2)	*Minerals*	*$(C_{18}H_{37})_2(CH_3)_2N^+$ derivatives*		
		C.E.C. (mequiv./1 g clay)	*X-ray basal spacing (Å)*	*meta–paraxylene relative volatility, 130°*
100	Hectorite, Hector	0.88	24.0	1.2
80–45	Montmorillonite, Sarighyuhski	1.18	25.8	1.2_5
	Montmorillonite, Pizhev	1.35	27.1	1.4
	Saponite, Groschlattengrun	1.19	29.4	1.4
	Nontronite, Andreasberg	0.83	29.6	1.5
	Beidellite II, Unterrupsroth	0.97	36.0	1.6
45–37	Vermiculite, Young River	1.08	33.3	1.6_5
	Vermiculite, South Africa	0.62	n.i.e.[b]	1.6
38–28	Illite, Fuzèrràdvàny	0.39	n.i.e.	1.3
28–23	Muscovite	n.d.[a]	n.i.e.	1.1
	Biotite from Don basin	0.14	n.i.e.	1.0_5

[a] n.d. = not determined.
[b] n.i.e. = no interlayer exchange.

solution is used for the exchange reaction, the X-ray basal spacing, for derivatives in the dry state, is 36–37 Å in every case. By removing the excess of the intercalated salt the basal spacing decreases to 24–26 Å with montmorillonitic minerals and to 29–37 Å with beidellitic and vermiculite minerals. This suggests a different arrangement of the organic cations depending on the different values of the equivalent area of the clay mineral used. The gas chromatographic effectiveness (for instance the

m- to *p*-xylene relative volatility) increases as the basal spacing increases, therefore it appears to be directly related to the packing density of the organic cations (see Table I). Thus the best results are obtained with beidellite and vermiculite type derivatives. With clay minerals having low equivalent areas ($\leqslant$ 37 Å^2) the exchange only occurs on the external basal surfaces, without any interlamellar penetration (*e.g.* as in the case of South African vermiculite and Fuzèrràdvàny illite). As the equivalent area decreases (24 Å^2 or less: biotite, mica) and when the counter ion is K^+, the exchange becomes incomplete and unsatisfactory gas chromatographic results are obtained, unless more sophisticated procedures in preparing the derivatives are adopted[6].

The specificity of "beidellite type" dimethyldioctadecylammonium derivatives towards molecules containing π-bonds is illustrated by the separation of cyclohexane, cyclohexene, 1,3-cyclohexadiene, 1,4-cyclohexadiene and benzene (Fig. 2).

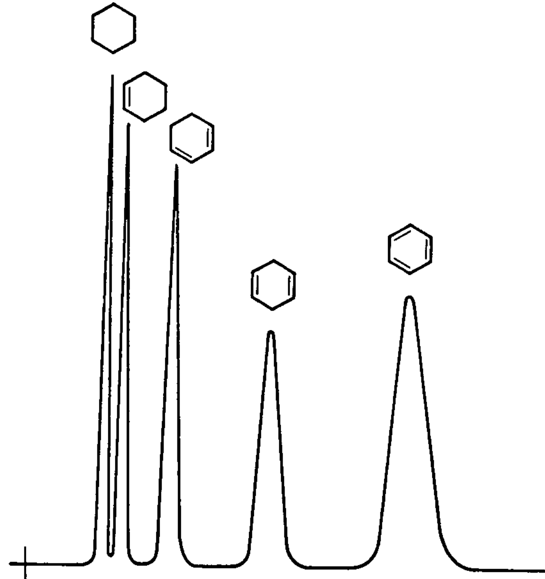

Fig. 2. Separation of cyclohexane; cyclohexene; 1,3-cyclohexadiene; 1,4-cyclohexadiene and benzene on dimethyldioctadecylammonium vermiculite (South Africa). Column length 1.9 m. Packing 15% wt. of organic derivative on Chromosorb P, 60–80 mesh. Temperature 95°; carrier gas H_2 at 60 ml/min.

The relative volatility value of 1.7–1.8 and the peak symmetry obtainable at 130° for the pair, *m*- and *p*-xylene, with the beidellite type derivatives are noticeably higher than that obtained with the known Bentone 34 (1.26 at 70°). Fast separations are obtained with derivatives which do not possess an interlayer phase, as shown in Fig. 3 (see also ref. 5). Further information concerning the separation of complex mixtures is given in ref. 7. The steric effects due to the epitaxial arrangement of the organic cations are evident in the separation of the trimethylbenzenes; these are eluted in the order 1,3,5-, 1,2,4- and 1,2,3- on pure alkylammonium halide while they are separated on beidellite type derivatives in the reverse order[5].

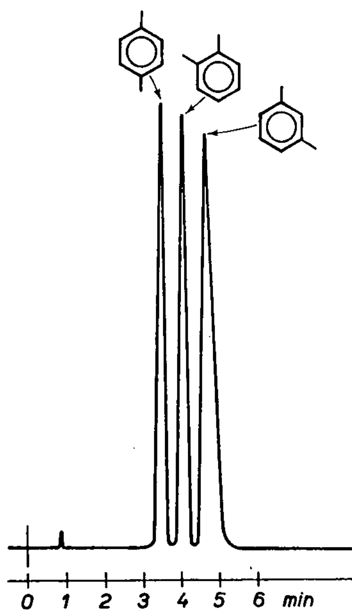

Fig. 3. Fast separation of xylene isomers on dimethyldioctadecylammonium illite (Fuzèrràdvàny). Temperature 140°. Other conditions as in Fig. 2.

In the range from 30 to 50° every dimethyldioctadecylammonium derivative shows a reversible characteristic transition. This was first noticed by gas chromatography, since the separation of *cis–trans* olefines does not occur below the transition temperature. The transition is never sharp, and annealing is necessary before reproducible behaviour is obtained with a differential scanning calorimeter. The results are tentatively explained in terms of hindered rotations around carbon–carbon bonds following an approach originally suggested by TEMPERLEY[8] for hydrocarbon-like polymers.

The entropy contribution for each such kink is compared to the total entropy change. It is found that 8–17 bonds are subjected to kinks whereas simple dimethyldioctadecylammonium chloride, in absence of clays, has 35 bonds subjected to torsion. One would conclude that it is not a matter of fusion in the usual meaning of the word: the disorder is limited to a section of the chain only.

From the gas chromatographic point of view, the transition does not influence the elution order, but only the column efficiency, following the sudden increase of peak asymmetry values from 1 to 2 and even higher values. At the same time the isotherm shape varies from sigmoid (Brunauer type) to the anti-Langmuir form, below the transition temperature, as is shown by the benzene isotherm at 42° on dimethyldioctadecylammonium vermiculite (South Africa) (see Fig. 4). Such isotherms represent a limiting case due to the absence of an interlayer organic phase. Fully exchanged clay minerals tend to approach linearity owing to the greater capacity. However in every case the linearity is limited to the higher temperatures and to the inferior portion of isotherm corresponding to the lower concentrations. In such a range, some evidence[7] suggests that both aromatic and saturated hydrocarbons are

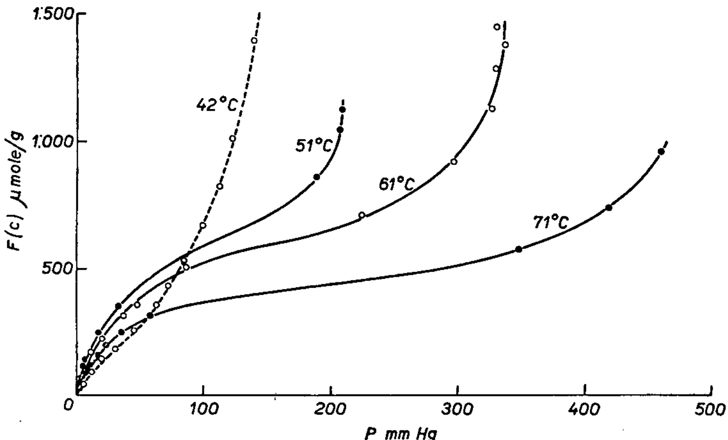

Fig. 4. Isotherms of benzene on dimethyldioctadecylammonium vermiculite (South Africa). Concentration, $F(c)$, of the adsorbate in the adsorbent as a function of the partial pressure P.

adsorbed on a homogeneous surface of paraffinic chains and that they maintain a great degree of translational freedom at the surface.

With regard to the surface arrangement of the dimethyldioctadecylammonium ions, a few plausible models can be suggested[7]. Fig. 5 shows two extreme situations such as arise with high and low equivalent area clays. The picture refers to the interlamellar space, but even on the outer surface, were the available space doubled, a

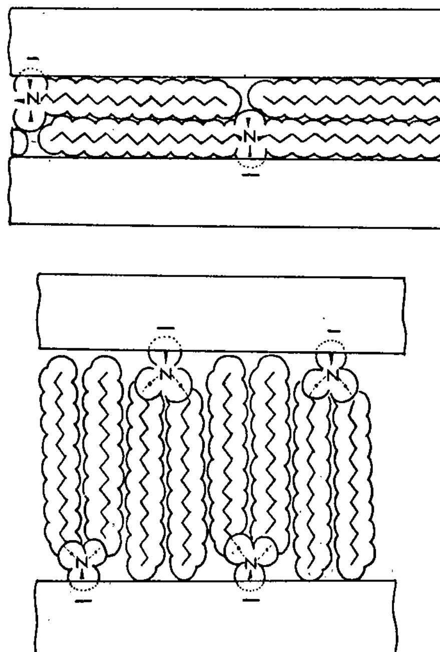

Fig. 5. Idealized models for the arrangement of dimethyldioctadecylammonium ions between the lamellae of (upper) a large and (lower) a small equivalent area clay mineral.

number of skew-chains are to be postulated with the most crowded clay surfaces.

Recent X-ray data indicate that the dimethyldioctadecylammonium ions penetrate between the lamellae with the long alkyl chains contacting the surface, when markedly underequivalent amounts of solution are used. As larger and larger amounts of organic molecules are grafted to the silicate, basal spacing increases progressively, thus suggesting that the alkyl chains are lifted from the surface. In addition, basal spacing measurements on a series of $R_2(CH_3)_2N^+$, in which the *n*-alkyl, R, varies from C_8 to C_{18}, suggest that the organic cation slopes at an angle of 60–70° to the surface (see Table II).

With a view to explaining the elution order of various hydrocarbons by means of the corresponding sequence of experimental and calculated heats of adsorption, an intermediate situation has been assumed for the arrangement of the alkylammonium ions. Adsorption energies on several positions have been evaluated and averaged[5]. Experimental isosteric heats of adsorption for *n*-paraffins fall within the range 0.2–0.5 kcal for all minerals studied. Theoretical values are seen to agree satisfactorily with the experimental ones. That the interaction with saturated hydrocarbons has been calculated correctly is again verified in the case of alkylbenzenes on hectorite; conversely results for aromatic compounds such as benzene, toluene and xylenes are very uncertain. In these cases the electrostatic interaction energies have been calculated without taking into account the polarisability anisotropy. As the information concerning the electric field intensity at various lattice positions is inadequate, the assumptions just made possibly have only a limited validity.

TABLE II

X-RAY DATA ON $R_2(CH_3)_2N^+$ BEIDELLITE (UNTERRUPSROTH) DERIVATIVES (EQUIVALENT AREA OF BEIDELLITE U.: 54 Å^2)

R = No. C	*Basal spacing (Å)*	*Spacing of organic interlayer (Å)*	*Height of the alkyl chain sloping at 70° to the surface (Å)*
8	22.7	13.3	14.5
10	21.4	12.0	16.5
12	29.0	19.6	18.5
14	32.0	22.6	20.5
16	29.0	19.6	22.5
18	36.0	26.6	24.5

From what has been said it may be inferred that organic derivatives of clay minerals, having better selectivity, can be obtained by increasing the surface concentration of the organic cations and the distance of the positive charge from the clay surface, as preliminary results with other alkylammonium ions appear to indicate. Thus for example, treatment of natural K-biotite with sodium tetraphenylboron to give Na-biotite results in an increased final content of trimethylhexadecylammonium ions, with a corresponding increase in the *m*-/*p*-xylene relative volatility to 2.2 at 130°.

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CHROM. 5218

INTERMOLECULAR INTERACTIONS IN GAS CHROMATOGRAPHIC SEPARATIONS ON ZEOLITES

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SUMMARY

Gas chromatography was used to determine the heats of adsorption of the hydrocarbon gases: methane, ethane, ethylene, propane, propylene, butane and also of carbon monoxide on type X zeolites containing the following cations: Na^{+}, Li^{+}, K^{+}, Rb^{+}, Cs^{+}, Ag^{+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+} and Cd^{2+}. It was found that type X zeolites containing silver and cadmium cations show specificity with respect to ethylene, propylene and carbon monoxide.

Chromatographic processes on solids are determined by intermolecular interactions taking place between the adsorbed substance and the adsorbent, as well as by the character of the diffusion of the substance caused by the specificity of the adsorption structure.

Zeolites are characterised by the presence of an orderly aluminosilicate framework leading to the formation of interconnected cavities identical in size and shape.

The possibility of modifying the zeolites by cation exchange allows their "fine structure" to be changed while maintaining a practically constant aluminosilicate framework.

Thus, the ionic forms of the same type of zeolite differ, in the main, according to the nature and position of the cations in the crystal lattice. Altogether this creates favourable conditions for the study of the character of adsorption interactions during the chromatographic separation of different substances on zeolites depending on the cation nature, assuming that the other parameters of the adsorbent remain constant.

The energy of the adsorption interaction may be approximately expressed for zeolites by the sum of the dispersion, orientation, polarisation and repulsive energies of interaction of the adsorbate molecules with the cations and atoms forming the framework[1,2].

In some cases adsorption interaction may lead to chemisorption and complex formation.

For adsorbents where concentrated positive charges are present, for example, in the case of adsorption on zeolites, when the molecules of absorbed substances are characterised by the presence of π-bonds, dipole and quadrupole moments, the role

of interactions of the type Φ_μ and Φ_θ (dipole and quadrupole interactions)[1,3] is especially important. In addition, changing from one cation form to another, interactions caused by the presence of chemically bound oxygen atoms in the zeolite framework should practically be unaffected.

We studied type X zeolites containing the following cations: Na^+, Li^+, K^+, Rb^+, Cs^+, Ag^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, and Cd^{2+}.

The specimens were obtained by means of repeated treatment of the original sodium zeolite forms with the corresponding aqueous solutions of the respective salts, in some cases the method of percolation was used (LiX). Pellets were prepared from powdered zeolites, grain size 15–30 mesh (0.5–1 mm), which after the appropriate thermal treatment (dehydration) were loaded into chromatographic columns.

Thermal activation of zeolites was carried out by two methods: (1) moderate activation consisting of heating the zeolites at 450° for 5 h, before loading into the column, followed by heating in the column at 300°; (2) deeper activation reached by heating of the zeolites in the column at 480° for 5 h, first with continuous pumping out and then in a flow of the carrier gas.

A mixture of C_1–C_4 hydrocarbon gases, carbon monoxide and hydrogen was used as a model mixture. Gas chromatography[4] was used to determine the heats of adsorption of the compounds mentioned. As in the chromatographic experiment we usually deal with a low adsorption space, the adsorption heats determined in such a way correspond, mainly, to the energies of interaction between the adsorbent and adsorbate.

Adsorption heats were determined over those temperature ranges of column heating, which correspond, as far as possible, to the most symmetrical separation curves, *i.e.* in such regions where almost complete adsorption equilibrium is established (Table I).

The data given in brackets were obtained for the specimens exposed to deep activation. The heats of adsorption of carbon monoxide and ethylene for cadmium zeolites have an approximate character due to the asymmetry of the peaks for these compounds on the chromatogram.

Heats of adsorption, *i.e.* energies of interaction of saturated hydrocarbons, characterised only by the presence of σ-bonds, with zeolites containing univalent cations increase with heavier cations and have the highest value on silver-containing specimens. That is proved by the fact that polarisibility of cations increases with the increase of number of electrons in an atom, and hence the dispersion interaction with adsorbed molecules of saturated hydrocarbons increases[5].

In the case of unsaturated compounds and carbon monoxide which are characterised by the presence of π-bonds, dipole and quadrupole moments, an opposite picture is observed, *viz.* interaction of these compounds is most pronounced with zeolites containing cations with small radii.

The data for the cesium form of the zeolite do not fit in with the general pattern for the other cations and this may be explained in the following way. Interaction of the compounds studied with the adsorption centres of a zeolite is caused not only by the presence of π-bonds, dipole and quadrupole moments in the molecules, but also by dispersion forces which are more pronounced on cesium zeolites.

The values of the differences between the heats of adsorption of methane and carbon monoxide as well as between ethane–ethylene and propane–propylene clearly

TABLE I

HEATS OF ADSORPTION (kcal/mole)

Zeolite	*Degree of substitution*	*25–80°* CH_4	*25–100°* CO	*60–160°* C_2H_6	*80–180°* C_2H_4	*100–180°* C_3H_8	*160–240°* C_3H_6	*160–240°* C_4H_{10}	$\Delta Q(CO-CH_4)$	$\Delta Q(C_2H_4-C_2H_6)$	$\Delta Q(C_3H_6-C_3H_8)$
LiNaX	91.0	4.1	9.0	5.7	10.7	7.7	14.1	9.5	+4.9	+5.0	+6.4
NaX	100	4.5	6.9	6.2	9.0	8.0	11.1	9.9	+2.4	+2.8	+3.1
KNaX	83.5	4.5	4.8	6.4	7.0	8.2	9.1	9.9	+0.3	+0.6	+0.9
RbNaX	54.0	5.0	4.8	6.6	6.8	8.3	8.9	10.5	−0.2	+0.2	+0.6
CsNaX	53.0	5.2	5.0	7.0	7.3	8.5	9.0	10.8	−0.2	+0.3	+0.5
AgNaX	90.0	5.8	—	8.3	—	9.9					
MgNaX	65	4.2	5.9	6.1	9.1	7.8	9.9	9.1	+1.7	+3.0	+2.1
		(4.2)	(6.1)	(6.3)	(9.4)	(8.0)	(10.9)	(9.7)	(+1.9)	(+3.1)	(+2.2)
CaNaX	90	4.1	7.9	6.2	10.9	7.2	13.9	9.2	+3.8	4.7	+6.7
		(4.8)	(9.3)	(6.9)	(28)	(8.9)			(+4.5)	(+21.1)	
SrNaX	95	5.5	7.8	7.5	13.0	9.6	14.9	11.3	+2.4	+4.5	+5.3
BaNaX	73	5.2	6.9	7.9	10.0	10.3	12.2	10.6	+1.7	+3.0	+1.9
CdNaX	88	4.7	10	7.1	27	8.0		10.3	+5.7	+19.9	

show what influence the nature of the cation has on the character of the interaction with molecules of carbon monoxide, ethylene and propylene and how specifically strong this interaction is for such cations as Na^+ and Li^+ which are characterised by a higher density of the positive charge.

Somewhat different behaviour is observed for zeolites with bivalent cations. For zeolites containing magnesium the heats of adsorption of all the compounds studied are greatly decreased in comparison with the data obtained on NaX. This may be caused both by the strongly hydrophilic character of this cation (the conditions of thermal activation are not sufficient for the complete dehydration of this form), and by the small extent of Na^+ exchange with Mg^{2+} in this specimen and by the possible destruction (partial) of the magnesium-containing zeolite[6].

In the case of Ca^{2+}, Sr^{2+} and Ba^{2+} substituted zeolites we observe an increase in the heats of adsorption of the saturated hydrocarbons with the increase in the number of electrons in the metal cations, together with a decrease in the heats of adsorption of the unsaturated hydrocarbons and carbon monoxide, the same as in the case of zeolites with univalent cations. The strontium form of zeolite does not obey this regularity, since an increase in the heats of adsorption is observed for all the olefines. It is thought that this property of the strontium form may be caused by formation of a separate phase, enriched with strontium[7], in this zeolite and by a decrease in the hydrophilic properties of the cation with its radius increase (*i.e.* by smaller screening of the cation by water molecules).

One sees from the data given in Table I that for zeolites with bivalent cations the influence of conditions of thermal activation on the values of adsorption heats is more pronounced. This influence depends to a great extent on the value of the energy of hydration of the cation. Hence the deeper thermal activation does not cause an appreciable increase in the heats of adsorption in the case of magnesium-containing zeolites, but with zeolites containing calcium it leads to an appreciable increase in the heats of adsorption of the C_1–C_4 hydrocarbon gases and carbon monoxide.

One should especially note the chromatographic properties of the silver and

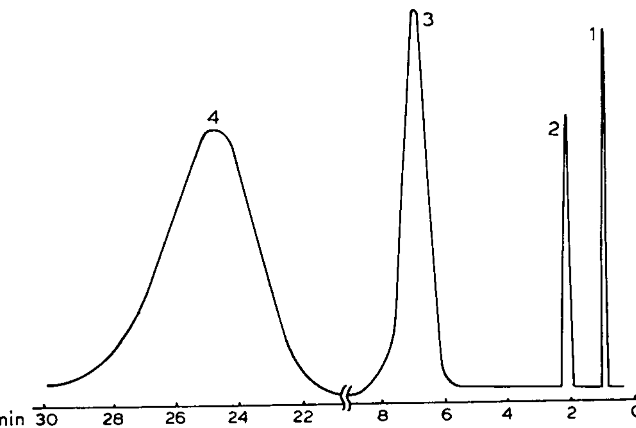

Fig. 1. Chromatogram of the mixture: 1 = methane; 2 = ethane, 3 = propane; 4 = butane. Adsorbent is a type X zeolite containing silver. Temperature of the column is 180°.

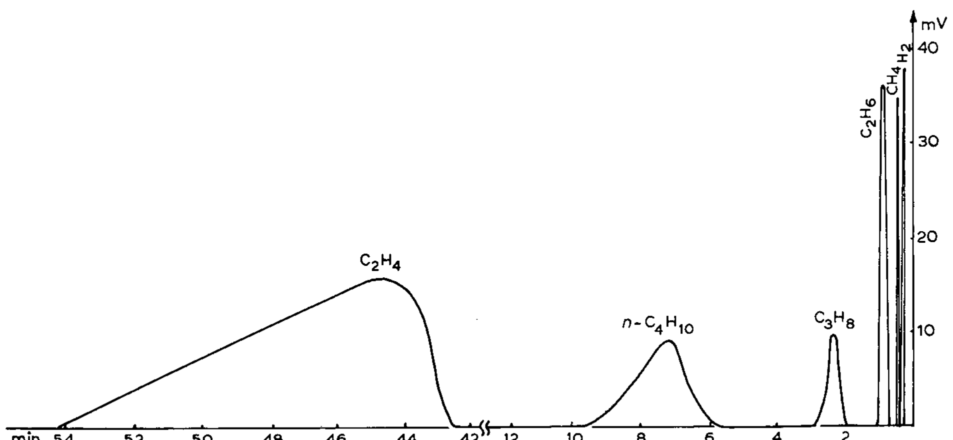

Fig. 2. Chromatogram of the mixture: hydrogen–methane–ethane–propane–butane–ethylene. Adsorbent is a cadmium-substituted zeolite. Temperature of the column is 200°.

cadmium zeolites. For instance, if on a NaX zeolite at a column temperature of 180°, there is the possibility of separating a mixture consisting of hydrogen, carbon monoxide (methane), ethane, ethylene, propane, butane and propylene, then hydrogen, carbon monoxide and unsaturated hydrocarbon can be strongly fixed on specimens containing silver, while the saturated hydrocarbons are easily eluted (Fig. 1), separating into different components[8].

A certain amount of specificity, not so sharply pronounced, is also shown by zeolites containing cadmium[9] with respect to the above mentioned components. However, if this property is already pronounced at a small degree of substitution of silver in the zeolite, it is only pronounced at a high degree of substitution of Na^+ by Cd^{2+} (85–90%). Thus with this form of zeolite propylene is strongly fixed at a column temperature of 200°, while all the saturated hydrocarbons involving butane

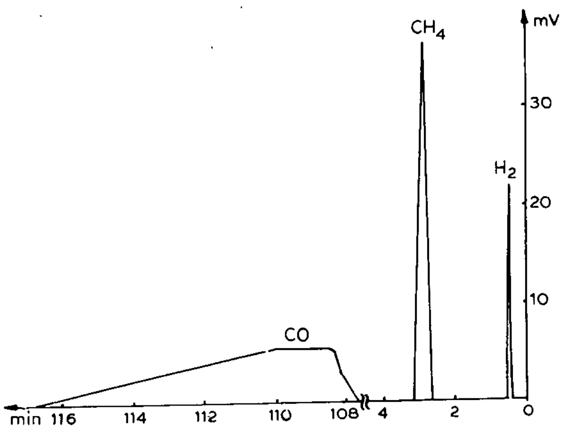

Fig. 3. Chromatogram of the mixture: hydrogen–methane–carbon monoxide on cadmium-substituted zeolite. Temperature of the column is 20°.

are eluted earlier than ethylene (Fig. 2) (it should be noted that under such circumstances, *i.e.* where the experiment is carried out with the zeolite in the silver form, ethylene is adsorbed very strongly).

This form of zeolite also shows a certain selectivity (Fig. 3) with respect to carbon monoxide. The retention time for this component increases sharply on cadmium-containing specimens. In addition, the symmetry of the peak corresponding to carbon monoxide on the chromatogram is sharply distorted.

Another characteristic feature of the cadmium zeolite is that at high column temperatures (300°) hydrogen, ethylene and to some extent carbon monoxide are practically not eluted.

Strong fixation of hydrogen on the zeolite containing silver was explained by the formation of the hydrogen zeolite form due to silver cation reduction by hydrogen[10,11].

$$Ag_2X + H_2 \rightarrow 2HX + 2Ag^0$$

Such a reaction takes place in the case when pure helium, without any admixture of oxygen, is used as carrier gas. When air is used as carrier gas the following reactions

$$4Ag^0 \cdot HX + O_2 \rightarrow 2Ag_2O + 4HX$$

can take place and further

$$Ag_2O \cdot HX + H_2 \rightarrow 2Ag^0 \cdot HX + H_2O$$

Indeed, in such cases we observe the formation of a large amount of water in the zeolite. The possibility of the existence of such reactions was also mentioned in RICKERT's paper[12].

However, the development of this reaction may be stopped, because, according to the existing opinion[13], when zeolite is heated silver atoms migrate to its outer surface, forming larger silver atom clusters, which cannot be completely oxidised and cannot be returned to their former positions.

In the case of cadmium-substituted zeolites, the development of reactions similar to those for silver is apparently characteristic, however, they take place only at high column temperatures (300°), while with the silver-substituted forms they take place readily at room temperature.

With carbon monoxide adsorption, on the basis of spectral data, one may assume a mechanism leading to complex formation[14,15] of this compound with the Ag and Cd cations. In the case of the zeolite containing silver, a more stable complex is most probably formed and the bond corresponds to a chemisorption process.

In the chromatographic separation of unsaturated hydrocarbons on these zeolites, complex formation with adsorbent cations apparently takes place. There are a number of data in the literature on the possibility of the formation of complexes between some metallic cations and olefines and acetylenes.

It is shown in our studies that these complexes are more stable for Ag^+ than for Cd^{2+}. Such a statement is confirmed by spectral studies made while investigating

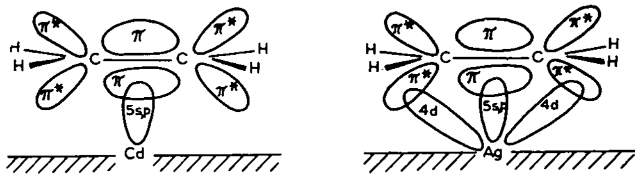

Fig. 4. Scheme of bond formation of ethylene molecules with cations Ag and Cd in a type X zeolite

ethylene adsorption on different cation-exchange forms of type Z zeolites[17].

In the case of AgX, it is possible that bond formation takes place due to overlapping of the filled π-orbital of ethylene with the vacant 5s,p orbital of a silver ion and by overlapping of the filled 4d orbital of the silver ion with a vacant π^* orbital of ethylene.

In the case of CdX interaction only proceeds as a result of the overlapping of π and 5s,p orbitals and the molecule may rotate freely around this bond.

Thus, from the aforesaid, one sees the importance of the nature of zeolite cations when going into details concerning the separate components of the energies of interparticle interaction in chromatographic processes.

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CHROMATOGRAPHIC PROPERTIES OF TYPE X ZEOLITES CONTAINING ALKALI METAL CATIONS

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SUMMARY

The chromatographic properties of type X zeolites containing cations of lithium, sodium, potassium, rubidium and cesium were studied for the separation of a model mixture of C_1–C_4 hydrocarbon gases and carbon monoxide. It was found that there is an inverse sequence of elution of methane and carbon monoxide in the course of the separation of this binary mixture depending on the nature of the zeolite cation. It was shown that lithium and sodium replaced forms of zeolites have a certain selectivity with respect to ethylene and propylene.

Zeolites are porous crystalline substances in which the negative charge of the aluminosilicate framework is compensated by the positive charge of cations. One of the important properties of zeolites is their pronounced capacity for cation exchange leading to a modification of these adsorbents without a practical change of the aluminosilicate framework structure.

Synthetic zeolites of the type CaA, NaX and CaX were used as adsorbents in gas chromatography in the sixties. Later, works appeared devoted to the possibility of the use of cation-exchange forms of zeolites in gas chromatography[1–8].

Studies of adsorption interactions have shown that zeolites have a definite specificity with respect to molecules of substances characterised by π-bonds, dipole and quadrupole moments[9].

It should be noted that the character of the interaction depends to an appreciable extent on the nature of the cation entering into the composition of the zeolite structure[9].

The aim of the present investigation was a study of the chromatographic properties of type X zeolites with different degrees of substitution of the sodium cations by lithium, potassium, rubidium and cesium cations; the separation of a model mixture of C_1–C_4 hydrocarbon gases, carbon monoxide and hydrogen was used as an example.

Cation-exchange forms were prepared from the sodium zeolites by means of their repeated treatment with an aqueous solution of the corresponding cation chloride or nitrate. Specimens with different contents of exchange cations were obtained depending on the number of treatments (see Table I).

Specimens 6 and 10 were obtained by the percolation technique. The stability of the zeolite crystalline structure in all synthesised specimens was controlled by the

TABLE I

DEGREE OF REPLACEMENT OF Na^+ BY OTHER CATIONS FOR SPECIMENS OF TYPE X ZEOLITES

Specimen	*Cation-exchange form*	*Degree of substitution (%)*	*Specimen*	*Cation-exchange form*	*Degree of substitution (%)*
1	NaX	0	8	KX	47.5
2	LiX	5	9	KX	83.5
3	LiX	22	10	KX	99.5
4	LiX	43	11	RbX	30
5	LiX	87	12	RbX	54
6	LiX	91	13	CsX	28
7	KX	26.4	14	CsX	53.5

corresponding Debyegrams. Photographs were taken in RKD chambers at non-filtered copper radiation on the URS-55 installation. The analysis of the Debyegrams has shown that all the specimens studied have a zeolite crystalline lattice.

The sorbents for loading into the chromatographic column were prepared in the following way. The zeolite powder was pressed into tablets without adding any binder, then these tablets were crushed, sieved and fractions with a grain size of 0.5–1 mm (15–30 mm) were taken for the tests. All specimens had been preliminarily dehydrated by heating at 450° for 4–5 h. After loading into the chromatographic column and before the work was begun, the zeolites were activated by heating them in a flow of the carrier gas at a temperature of 300°. In the case of the lithium-containing specimens, due to their highly hydrophilic properties the activation was also carried out at a temperature of 480°.

The chromatographic column was a spiral tube of stainless steel, 350 cm long, with an inner diameter of 0.4 cm. The temperature range of the column was from 25 to 260°. Since the chromatograph detector was of the thermochemical type, purified

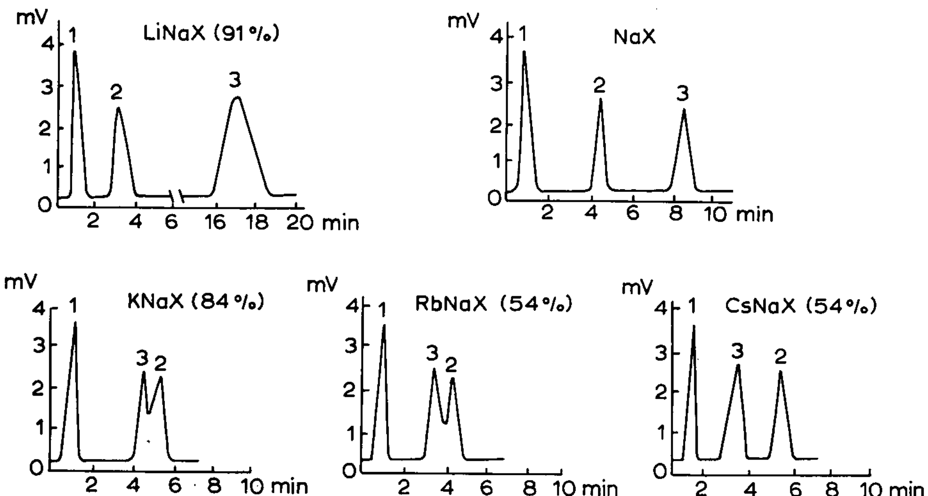

Fig. 1. Sequence of elution of methane–carbon monoxide on type X zeolites containing Li^+, Na^+, K^+, Rb^+, Cs^+: 1 = hydrogen; 2 = methane; 3 = carbon monoxide. Flow rate of carrier gas (air), 100 ml/min. Column temperature, 25°.

air was used as the carrier gas. The velocity of the carrier gas during the investigation of the separation properties of the zeolites was around 100 ml/min.

As a result of the studies it was shown that the character of the chromatographic separation of the binary mixture methane–carbon monoxide depends on the cation and the degree of its substitution in the zeolite. The presence of dipole and quadrupole moments is characteristic[10] of a molecule of carbon monoxide, and differs in this way from the methane molecule. Zeolites containing Na^+ and Li^+ display a certain selectivity with respect to carbon monoxide and methane is eluted earlier than carbon monoxide; furthermore a zeolite with a higher content of Li^+ retains carbon monoxide more than the Na^+ form (Fig. 1).

In the case of specimens with a high K^+ content, it is possible to perform a partial separation on them, but with the following sequence of elution: carbon monoxide–methane (Fig. 1).

On zeolites containing rubidium and cesium separation of this mixture proceeds with the following elution order: carbon monoxide–methane, even with small degrees of sodium ion substitution. However, a more complete separation of these components is observed with the cesium form. The values of the coefficient of selectivity K_s of methane and carbon monoxide are given for zeolites containing alkali metal cations in Table II.

TABLE II

THE SELECTIVITY COEFFICIENT K_s FOR METHANE–CARBON MONOXIDE MIXTURES ON VARIOUS ZEOLITES

Temp. (°C)	*NaX*	*LiX*					*KX*			*RbX*		*CsX*	
		5%	*22%*	*43%*	*87%*	*91%*	*26.4%*	*47.5%*	*83.5%*	*30%*	*54.0%*	*28%*	*53.5%*
25	0.42	0.37	0.37	0.37	0.60	0.72	0.024	−0.04	−0.08	−0.11	−0.14	−0.22	−0.27
40	0.34	0.29	0.25	0.29	0.51	0.63	−0.023	—	−0.09	−0.12	−0.16	−0.23	−0.27
60	0.23	0.22	0.19	0.21	0.41	0.52	−0.055	−0.07	−0.10	−0.16	−0.17		

As one can see from the data in Table II K_s of methane and carbon monoxide increases to a great extent with the transition from the specimen containing Na^+ to that with Li^+. However, in the case of forms with a low lithium content, K_s is less compared to the sodium form. This is probably due to the fact that the hydrophilic lithium cations having a small concentration occupy positions in the structure in which they are in a hydrated state and therefore their interaction with the molecules of the adsorbed substances is weakened. Regarding zeolites containing K^+, Rb^+ and Cs^+ an increase of the selectivity coefficient takes place both with the increase of the degree of their substitution and in the sequence mentioned. These cations are less hydrophilic and do not hydrate as much as Li^+.

Such behaviour may be explained as follows: electrostatic interactions caused by the existence of dipole and quadrupole moments of carbon monoxide molecules are more important for cations with small radii with a strong positive charge (Na^+, Li^+) than for larger cations K^+, Rb^+ and Cs^+[11].

In Table II one can also see the effect of the temperature of the chromatographic column on the degree of separation. Thus, an increase of the column temperature leads to an appreciable decrease in the values of the selectivity coefficients of the

methane–carbon monoxide mixture on zeolites containing lithium and particularly sodium. The value of the retention volume of carbon monoxide decreases more sharply than that of methane with an increase of the column temperature for all forms of cation-exchange zeolites. It is assumed that the intensity of the interactions of molecules with dipole and quadrupole moments with the zeolite is strongly disturbed with a temperature increase[12]. However in the case of sodium and lithium

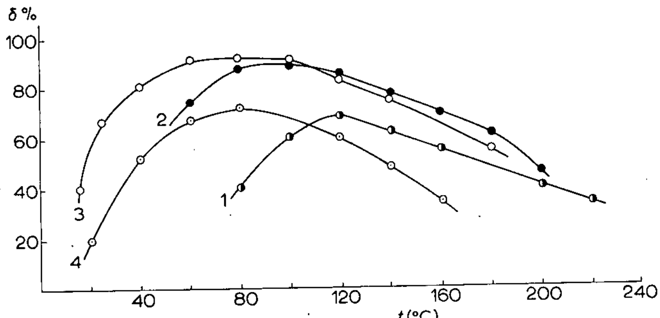

Fig. 2. Dependence of the separation extent (δ) for the mixture CH_4–CO on zeolites containing potassium on the column temperature. 1 = specimen 1; 2 = specimen 7; 3 = specimen 8; 4 = specimen 9.

specimens such a decrease in the values of the retention volumes for carbon monoxide does not lead to an inversion in the order of elution of the components. However, on a zeolite with a small potassium ion content (specimen 7) an inversion of the order of elution of carbon monoxide and methane takes place. Thus, at a column temperature of 25° the retention volume of carbon monoxide is larger than that of methane. With an increase of the column temperature to 40° the retention volumes of these components are equalised. A further increase of the column temperature leads to inversion of the sequence of methane and carbon monoxide elution and their separation takes place at 80°.

With specimens with a high potassium ion content as with Rb^+ and Cs^+, an

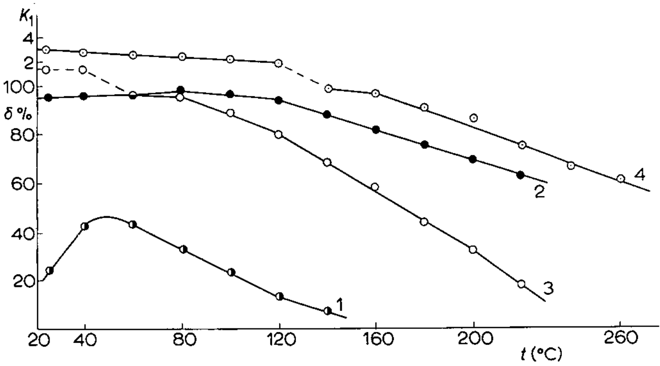

Fig. 3. Dependence of the separation extent (δ) for the mixture CH_4–CO on zeolites containing rubidium and cesium on the column temperature: 1 = specimen 11; 2 = specimen 12; 3 = specimen 13; 4 = specimen 14.

increase of column temperature leads to an increase in the values of the selectivity coefficient K_s associated with the reversibility of the methane and carbon monoxide elution order and with the weakening of direction of CO interaction with the adsorbent at increased temperatures. However, the increase in the value of the separation coefficient with the temperature increase is characterised by a certain maximum for zeolites containing K^+, Rb^+, Cs^+. Thus, curves of the separation coefficient (δ) plotted against temperature, on zeolites substituted with potassium, increase to a certain temperature limit, reach a maximum and then a decrease takes place (Fig. 2). The maximum of the curve, corresponding to the highest values of the separation coefficient, shifts on the graph from the right to the left into the lower temperature region with an increase in the potassium content in the zeolite. The same type of curve is characteristic for rubidium-substituted forms (Fig. 3), however, in the case of cesium specimens the maximum is less pronounced.

A decrease of the values of the separation coefficient at high temperatures may

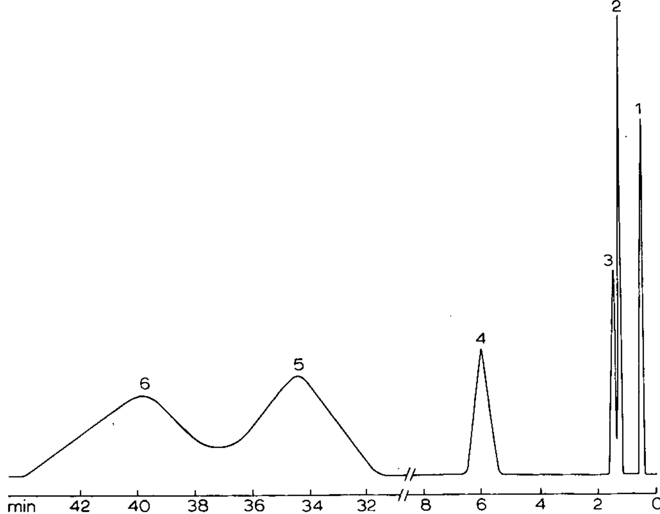

Fig. 4. Chromatogram of the mixture: 1 = hydrogen; 2 = methane; 3 = carbon monoxide; 4 = ethane; 5 = propane; 6 = ethylene. Adsorbent, NaX; column temperature, 100°.

be explained by a general weakening of the adsorption interaction of the chromatographed substances with the adsorbent surface.

The nature of a cation influences the character of the separation of hydrocarbon gases, in particular, the sequence of elution of separate pairs of components. Thus, on the zeolite NaX the following sequence of component elution takes place: hydrogen–methane–carbon monoxide–ethane–propane–ethylene. The column temperature was 80–100° (Fig. 4). With an increase of temperature to 120–140° there is no appreciable separation of propane and ethylene.

At temperatures of 160° and higher, the separation of this mixture is characterised by the inverse order of propane and ethylene elution (Fig. 5). As is seen from this chromatogram, butane is eluted earlier than propylene. An increase of the column heating temperature to 220° causes a considerable decrease in the propylene retention

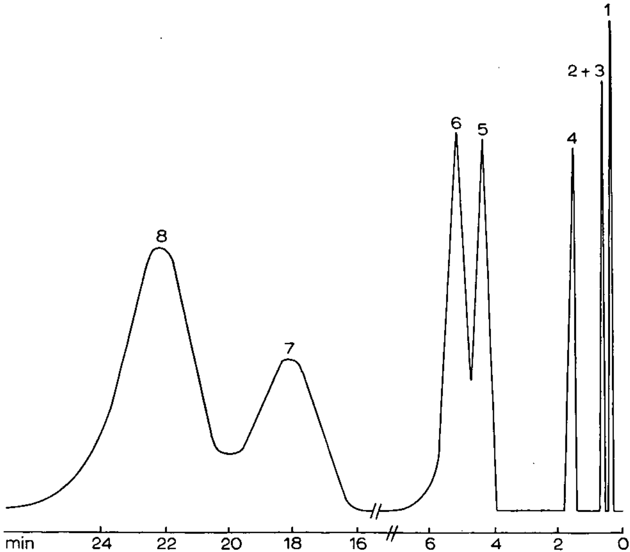

Fig. 5. Chromatogram of the mixture: 1 = hydrogen; 2 = methane; 3 = carbon monoxide; 4 = ethane; 5 = ethylene; 6 = propane; 7 = butane; 8 = propylene. Adsorbent, NaX; column temperature, 180°.

volume and it cannot be readily separated from butane. At higher column temperatures propylene is eluted earlier than butane, though the separation of these components does not take place. As to specimens containing lithium with a high degree of exchange of Na^+ by Li^+ (90%), then at any column temperature propane is eluted earlier than ethylene, and butane before propylene, *i.e.* there is no inversion of the

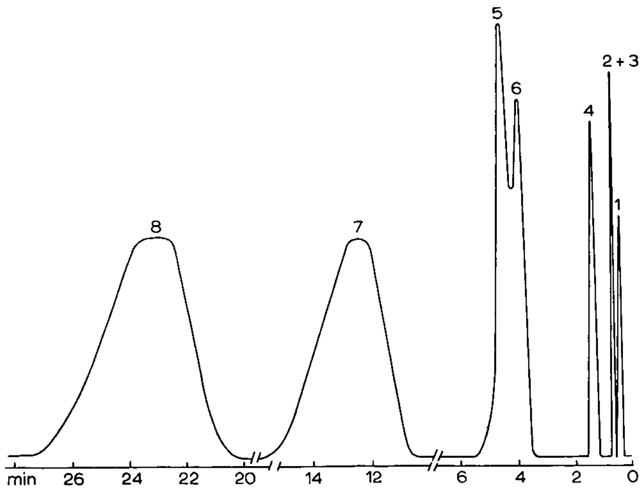

Fig. 6. Chromatogram of the mixture: 1 = hydrogen; 2 = methane; 3 = carbon monoxide; 4 = ethane; 5 = ethylene; 6 = propane; 7 = butane; 8 = propylene. Sorbent LiX (the degree of Na^+ substitution by Li^+ is 91%). Column temperature, 180°.

sequence of elution of these components depending on temperature (Fig. 6). Thus, it is seen from the data obtained that zeolites with metal cations having small radii (Na^+ and especially Li^+) display a strong adsorption capacity with respect to molecules of compounds characterised by π-bonds; this is reflected in an increase of the values of their retention volumes.

As to zeolites with heavier cations, such as K^+, Rb^+ and Cs^+, an unsaturated compound is eluted on them after the corresponding saturated compound with the same number of carbon atoms in the molecule. On such cation-exchange specimens, at any column temperature, one observes the following sequence of elution: methane–ethane–ethylene–propane–propylene–butane–butylene.

Studies of the influence of the nature of the cation on the character of chromatographic properties of the zeolite and the determination of the heats of adsorption of carbon monoxide and C_1–C_4 hydrocarbon gases allowed us to construct the selectivity series for these compounds with respect to the alkali metal cation (see Table III).

TABLE III

ADSORPTION ACTIVITY OF ZEOLITES DEPENDING ON THE CATION NATURE

Selectivity rows	
CH_4, C_2H_6, C_3H_8, C_4H_{10}	$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$
CO	$Li^+ > Na^+ > Cs^+ > K^+ > Rb^+$
C_2H_4	$Li^+ > Na^+ > Cs^+ > K^+ > Rb^+$
C_3H_6	$Li^+ > Na^+ > K^+ \sim Cs^+ > Rb^+$

As is seen from the above data for saturated hydrocarbons one observes an increase of selectivity with respect to a zeolite with the heavier cation entering its composition. This is caused by the fact that cation polarisibility increases with an increase of its radius and hence the role of dispersion forces, which mainly determine the interaction of saturated hydrocarbons, become more important.

For carbon monoxide and unsaturated hydrocarbons an opposite arrangement is characteristic. The position of cesium which does not fit this row may be explained in the following way: interaction of a molecule of these compounds with the zeolite is associated not only with the existence of π-electrons and the dipole moments in these molecules, but also with dispersion forces, which are displayed to a certain degree for the cesium form of the zeolite.

The work carried out permits some conclusions to be drawn about the important influence of the nature of the cation in type X zeolites on their separation properties.

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CHROM. 5266

MOLECULAR-STATISTICAL CALCULATION OF RETENTION VOLUMES IN GAS ADSORPTION CHROMATOGRAPHY

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SUMMARY

The retention volumes, V_R, for a series of hydrocarbons in gas adsorption chromatography on the basal face of graphite at zero surface coverage were calculated by a semi-emperical molecular–statistical method. The potential function of the interaction between a molecule and a surface is assumed to be equal to the sum of the potential functions of the interaction between atoms of the molecule and atoms of the adsorbent. The potential functions of the interaction between the C and H atoms of saturated hydrocarbon molecules and the C atoms of the graphite lattice (the atom–atom potential functions) were estimated by methods according to the theory of the intermolecular interactions and were corrected using experimental adsorption data for methane, ethane and propane on graphitized thermal carbon blacks. The calculated values of V_R for all the saturated hydrocarbon molecules considered (*n*-butane, isobutane, *n*-pentane, neopentane, cyclopentane, *n*-hexane, and cyclohexane) are in good agreement with the experimental values of V_R on graphitized thermal carbon blacks. The calculations of V_R for unsaturated and aromatic hydrocarbon molecules made it possible to estimate the difference between the potential functions of interaction of the C atoms of saturated, unsaturated, and aromatic molecules with graphite.

In the gas adsorption chromatography with small sample sizes the retention volume V_R depends on the structure and the surface area of the adsorbent, the structure of the molecules and the temperature. Therefore the interpretation of experimental data and the development of methods for the prediction of the retention volumes from the structure of the solid and the molecules is one of the main problems in the theory of adsorption chromatography[1]. To solve this problem it is necessary to use methods appertaining to statistical thermodynamics and the theory of the intermolecular interactions.

At low (zero) surface coverages the retention volume V_R per unit of surface area can be expressed through the partition function Q of the molecule by the equation[2–4]:

$$V_R = \frac{1}{A} \frac{Q-Q^0}{Q^0/V} \tag{1}$$

where A is the surface area of the solid, Q and Q^0 are the partition functions for the adsorbed molecule and molecule in the gas phase, respectively, V is the gas volume.

In the classical approximation for quasi-rigid molecules, *i.e.* for molecules which have no degrees of internal rotational freedom (for instance, molecules of methane, benzene)[2-4]

$$V_R = (1/8\pi^2 A) \int[\exp(-\Phi/kT)-1]\sin\vartheta \mathrm{d}x\mathrm{d}y\mathrm{d}z\mathrm{d}\vartheta\mathrm{d}\varphi\mathrm{d}\psi \qquad (2)$$

where Φ is the potential energy of the interaction between the molecule and the surface as a function of Cartesian coordinates of the molecule mass centre (x, y, z) and of the Euler's angles $(\vartheta, \varphi, \psi)$ which determine the orientation of the molecule with respect to the surface.

Most complex molecules have internal rotation. If comparatively stable configurations of the molecular-rotational isomers are formed during internal rotation (for instance, in the case of *n*-alkane molecules beginning with *n*-butane), the gas of such molecules can be considered as an equilibrium mixture of the rotational isomers of the molecule. In this approximation[5]

$$V_R = \sum_{i=1}^{m} x_i V_{R,i} \qquad (3)$$

where $V_{R,i}$ is the retention volume for the *i*th rotational isomer, x_i is the molal fraction of this isomer in the gas phase, m is the number of the rotational isomers.

If the potential barrier for the internal rotation of the molecule is considerably greater than the variation of the potential energy Φ of the interaction between the molecule and the surface during the internal rotation of the adsorbed molecule, and if the molecule does not form rotational isomers during internal rotation (for instance, molecules of ethane, propane, toluene) then in the first approximation the change in the internal rotation of the molecule during adsorption can be neglected. In this case for the calculation of V_R the molecule can be considered as quasi-rigid, *i.e.* eqn. 2 can be used[6].

Statistical eqns. 1–3 give the relation between V_R and the potential function Φ of the interaction between a molecule and a surface. Solely theoretical calculation of function Φ from the physico-chemical properties of the molecule and the surface of the solid or determination of this function from experimental values of V_R is a very difficult problem. This problem can be significantly simplified if we assume that the potential function Φ of the interaction between a molecule and an adsorbent is equal to the sum of the potential functions l of the interaction between atoms of the molecule and atoms of the solid, *i.e.*

$$\Phi = \sum_i \sum_j l_{ij} \qquad (4)$$

where l_{ij} is the potential function of the interaction between *i*th atom of the molecule and *j*th atom of the solid (the atom–atom potential function). This approximation is supported by the additivity of the heat of adsorption for linear and planar molecules. When the above approximation is used we must determine the atom–atom potential functions l_{ij} and summarize them taking into account the chemical and geometrical structure of the molecule and the surface.

In the first approximation the form of the atom–atom potential function can be chosen on the basis of the approximate theory of intermolecular interactions.

Buckingham's potential is, apparently, a good approximation to this function in the case of interaction between non-polar molecules and a surface:

$$l_{ij} = -C_{1ij}r_{ij}^{-6} - C_{2ij}r_{ij}^{-8} + B_{ij}e^{-r_{ij}/\varrho_{ij}} \quad (5)$$

where r_{ij} is the distance between two interacting atoms, C_1, C_2, and B and ϱ are parameters. In the first approximation these parameters can be estimated from the physico-chemical properties of the molecule and the solid using various approximate quantum-mechanical formulae and combination rules. Further they can be corrected using experimental values of V_R for some molecules of the same homologous series on a given adsorbent[3].

In this way values of V_R for a series of hydrocarbon molecules on the basal face of graphite were calculated. The experimental values of the Henry's constant K_1 determined at different temperatures for adsorption of methane, ethane and propane on thermal carbon blacks graphitized at approximately 3000° were used for the correction of the parameters of the potential functions $l_{C...C}$ and $l_{C...H}$ for the interaction of the C and H atoms of the saturated hydrocarbon molecules with the C atoms of the graphite lattice. The following equations were obtained for the corrected atom–atom potential functions:

$$l_{C...C} = -331r^{-6} - 513r^{-8} + 4.52 \cdot 10^4 \exp(-3.57r) \text{ kcal/mole} \quad (6)$$

$$l_{C...H} = -119r^{-6} - 227r^{-8} + 0.86 \cdot 10^4 \exp(-3.57r) \text{ kcal/mole} \quad (7)$$

where r is distance in Å. The corrected potential functions differ little from those estimated theoretically.

Using these atom–atom potential functions we calculated values of V_R for *n*-butane, isobutane, *n*-pentane, neopentane, cyclopentane, *n*-hexane and cyclohexane.

The calculated values of V_R for molecules of *n*-alkanes are compared with the corresponding experimental values on graphitized thermal carbon blacks in Fig. 1. The calculated values are in good agreement with the experimental ones for all six *n*-alkane molecules studied. The calculated and experimental values of V_R for the

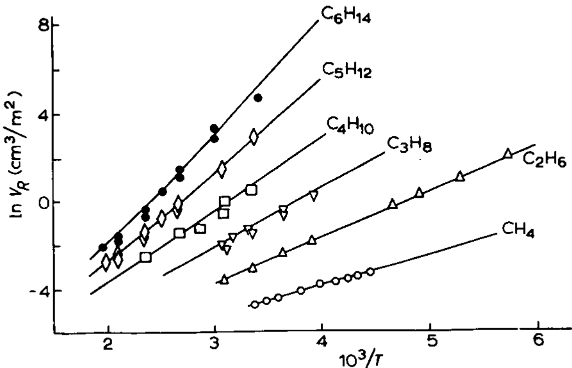

Fig. 1. Calculated (curves) and experimental (points) values of the retention volume as a function of the reciprocal of the absolute temperature for methane, ethane, propane, *n*-butane, *n*-pentane and *n*-hexane.

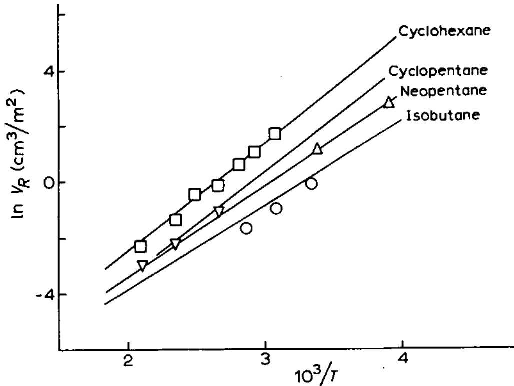

Fig. 2. Calculated (curves) and experimental (points) values of the retention volume as a function of the reciprocal of the absolute temperature for isobutane, neopentane, cyclopentane, and cyclohexane.

structural isomers of *n*-butane and *n*-pentane, *i.e.* for isobutane and neopentane, a well as for the alicyclic compounds—cyclopentane and cyclohexane—are shown in Fig. 2. They are in good agreement also.

So, our calculations showed that the retention volumes in the gas adsorption chromatography for saturated hydrocarbons can be calculated by the molecular-statistical method from the chemical and geometrical structure of the molecules and surface of the solid using the semiempirical atom–atom potential functions.

The state of the electrons of the carbon atoms in unsaturated and aromatic hydrocarbon molecules is somewhat different from that in saturated hydrocarbon molecules. Therefore the atom–atom potential function $l_{C...C}$ for the interaction of the carbon atoms of unsaturated and aromatic hydrocarbon molecules with the carbon atoms of the graphite lattice must be somewhat different from the potential function $l_{C...C}$, eqn. 6, of the interaction between the carbon atoms of saturated hydrocarbon molecules and the carbon atoms of the graphite lattice. To evaluate the magnitude of this effect, the experimental values of V_R for some molecules of

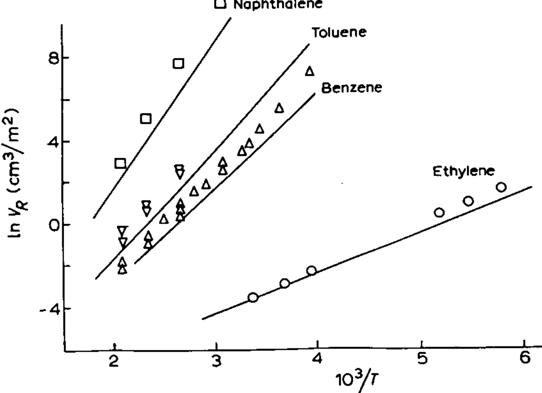

Fig. 3. Calculated (curves) and experimental (points) values of the retention volume as a function of the reciprocal of the absolute temperature for ethylene, benzene, toluene, and naphthalene.

unsaturated and aromatic hydrocarbons were compared with the values of V_R calculated using the same potential function for the interaction between carbon atoms as in the case of adsorption of saturated hydrocarbons. The results are shown in Fig. 3. The experimental values are somewhat greater than the calculated ones for all molecules. This divergence corresponds to the increase of the potential energy of the interaction between the carbon atom and the graphite surface by 0.08 kcal/mole (7%) in the case of unsaturated and aromatic molecules.

In principle, the atom–atom potential functions of the interaction of the carbon atoms of unsaturated and aromatic hydrocarbon molecules with the carbon atoms of the graphite lattice can be determined using experimental data for ethylene and benzene. Furthermore, using these functions the calculations of V_R for any other unsaturated and aromatic molecules can be made. The results apparently are also in good agreement with experimental data as in the case of saturated molecules. The verification of these assumptions is, however, prevented by the absence of the reliable experimental data on adsorption and chromatography of unsaturated and aromatic hydrocarbon molecules on graphitized carbon blacks over a wide range of temperatures. The difference between the potential functions $l_{C...C}$ for the carbon atoms in saturated, unsaturated and aromatic hydrocarbons is small. The above equations for the atom–atom potential functions can therefore be used for the approximate calculation of V_R for any hydrocarbon molecule on graphitised carbon blacks. The programs developed for the electronic computer permit such calculations to be made fairly quickly.

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CHROM. 5214

RETENTION BEHAVIOUR DURING SEPARATIONS ON POLAR SORBENTS CONTAINING ETHER GROUPS

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SUMMARY

The retention of various classes of compound on polar polymer sorbents containing ether functional groups was investigated and correlation was made between the retention values obtained and those obtained on non-polar polymeric sorbent and on polar stationary phases.

INTRODUCTION

As well as using non-polar polymeric sorbents in gas chromatography great use is made of polar sorbents—Porapacks R, N, S, T, and Chromosorbs 103–105 which are obtained by the polymerisation of styrene, divinylbenzene and polar monomers. By introducing monomers containing various functional groups into the polymerisation mixture it is possible to control the chemical properties of the surface of the sorbents obtained and thus to influence the nature of the intermolecular interactions of the surface of the sorbent with the components of the mixture being separated. The use of porous polymer sorbents containing polar functional groups in gas chromatography makes it possible to change the position of water and other polar compounds relative to non-polar ones and also to lessen the widening of the rear limit of the peak of a number of components—in particular amines, diamines, aldehydes.

Some data concerning the retention of polar molecules, water, unsaturated compounds on Porapacks R, N, S, T have been discussed in a number of works[1–5]. However, functional groups are not known to exist on the surface of these sorbents which makes it difficult to correlate the laws of retention of these components on Porapacks R, N, S, T with the nature of the functional groups on the surface. The development and study of polymer sorbents having certain chemical and functional composition must become of great practical and theoretical importance.

Comparative studies of the laws of retention of various classes of molecules on Polysorb-6 (styrene and *p*-divinylbenzene copolymer*) and Polysorbate-2**

* Polysorb-6 corresponds to Porapack Q, Chromosorb-102 and Polysorb-1.

** The authors' opinion is that polymer sorbents can be called "polysorbs" for convenience[16], but for polar sorbents it would be somewhat more useful to change this term so that it characterises the presence of the respective functional group. Thus polymer sorbents containing ether groups are called "polysorbates" by the authors.

(methyl acrylate and *p*-divinylbenzene copolymer) were carried out, and the correlation was studied between the values of retention obtained and the respective values, obtained in the case of dinonyl phthalate and polyethylene glycol (PEG)-2000 which were coated onto a teflon support—Polychrome-1.

The structure of the surface can be seen on the photographs taken by means of a scanning electronic microscope (Fig. 1) (see also ref. 9).

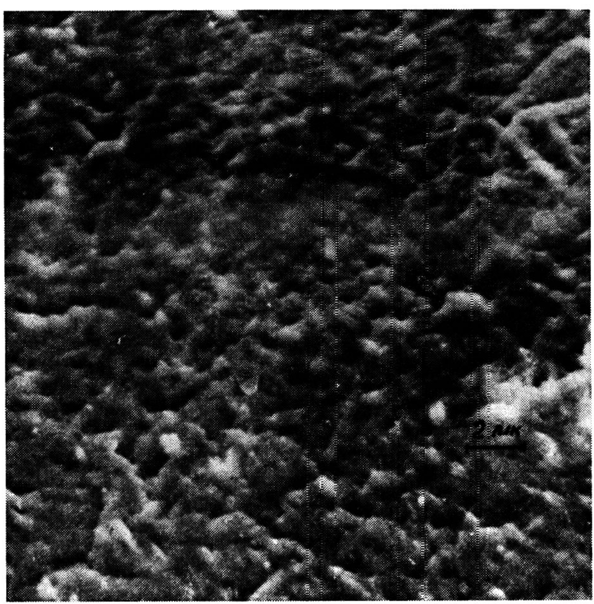

Fig. 1. Photograph (× 5000) of the surface structure of Polysorbate-2, taken by a scanning electron microscope (JSM-2 JEOL, Japan).

Gas chromatographic studies were carried out by means of an instrument having a column 1 m long and 4 mm in diameter. The operating temperature was 150°; the flow rate of the carrier gas (helium) was 30 ml/min, detection was by means of a katharometer. The principal differences in behaviour, during separation, of molecules having various bonds and functional groups on Polysorbate-2 are compared with that for the non-polar Polysorb-4.

THE DEPENDENCE OF RETENTION VOLUME ON BOILING POINT, ETC.

Polysorb-6

We have found a linear dependence between the corrected retention volume logarithm (log V_R) and the number of carbon atoms in the molecule for homologous series of: *n*-alkanes, aromatic hydrocarbons, *n*-alcohols, ketones and fatty acids[5]. For the homologous series of the above classes of compounds log V_R is also a linear function with respect to boiling points, standard entropy (Fig. 2), molecular weights of the compounds and to the general polarisability of molecules and this linearity holds for the whole range of temperature studied, 120°–180°.

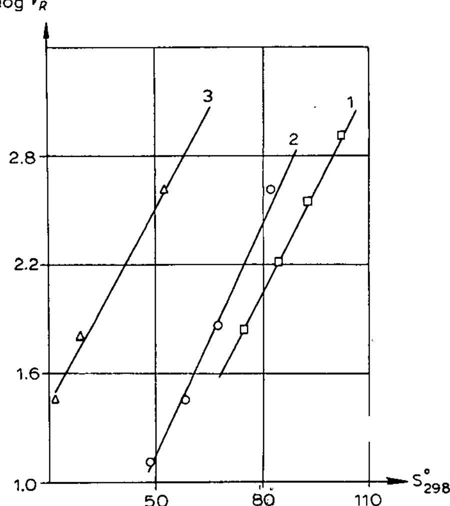

Fig. 2. Dependence of the log of the corrected retention volume upon the standard entropy of molecules on Polysorb-6. 1 = n-alkanes C_5–C_8; 2 = n-alcohols C_1–C_5; 3 = n-acids C_1–C_4.

Polysorbate-2

The dependence of log V_R on the number of carbon atoms (boiling point, molecular weight, standard entropy, general polarisability of molecules) in the molecules is linear for homologous series of n-alkanes but is non-linear for alcohols and acids (Fig. 3).

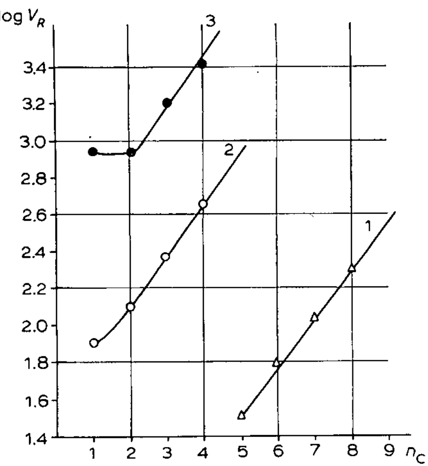

Fig. 3. Dependence of the log of the corrected retention volume upon the number of atoms of hydrogen in molecules on Polysorbate-2. 1 = n-alkanes C_5–C_8; 2 = n-alcohols C_1–C_5; 3 = n-acids C_1–C_4.

THE SEPARATION OF UNSATURATED COMPOUNDS

Polysorb-6

The local electronic structure of unsaturated compounds does not appear to provide any features which would contribute to their ability for specific interaction. The elution of unsaturated compounds occurs in accordance with their boiling points, and does not depend on the presence of the double bonds in the molecules (see Table I). This applies in the case of hydrocarbons as well as alcohols and acids.

TABLE I

RELATIVE RETENTION TIME OF UNSATURATED COMPOUNDS

t_R of *n*-pentane taken as a standard.

Sorbate	*b.p. (°C)*	*M.W.*	*t_R/t_R of n-pentane*			
			Poly-sorb-6	*Polysor-bate-2*	*10% of dinonyl phthalate on Poly-chrome-1*	*10% of PEG-2000 on Poly-chrome-1*
Pentane	36.1	72.1	1.0	1.0	1.0	1.0
Pentene-2	36.9	70.1	1.04	1.4	1.1	1.4
Pentadiene-1,3	44.1	68.1	1.2	2.3	2.6	2.6
Hexane	68.7	86.2	2.3	1.9	2.0	2.1
Hexene-1	63.5	84.2	2.2	2.3	2.1	2.4
Hexadiene-1,5	59.4	82.1	2.1	2.7	2.1	2.9
Cyclohexane	81.4	84.2	3.0	3.9	3.7	4.0
Cyclohexene	83.0	82.1	3.3	5.5	4.2	6.3
Benzene	80.1	78.1	2.9	8.8	5.1	13.3
Heptane	98.4	100.2	4.8	3.4	3.7	3.9
Heptene-3	95.6	98.2	4.6	4.2	3.9	4.3
Methylcyclohexane	100.9	98.2	5.6	5.2	5.2	5.0
Toluene	110.6	92.1	6.8	15.1	9.6	25.7
Propanol	97.2	60.1	1.05	7.1	2.8	24.1
Allyl alcohol	96–97	58.1	1.0	8.9	3.0	36.9
Propionic acid	141.1	74.1	3.9	49.5	—	—
Acrylic acid	141.0	72.1	3.8	69.1	—	—

Polysorbate-2

Separation depends on the local electronic structure of the unsaturated compounds. Their elution is determined by the presence and the number of unsaturated bonds in a molecule rather than by the boiling point. Aromatic hydrocarbons are retained much better than the respective cyclic compounds on this sorbent; this is particularly true in the case of unsaturated alcohols and acids (see Table I).

THE EFFECT OF DIPOLE MOMENT AND GENERAL POLARISABILITY

Polysorb-6

The molecular retention is independent of the value of the dipole moment for the series of compounds: pentane, diethyl ether, methanol, ethanol, water, acetone, acetonitrile.

The retention volumes increase with the general growth of the polarisability of the adsorbate molecules (Table II); the rapid elution of water (before methanol and pentane), is the result of the small value of the polarisability of the water molecules.

TABLE II

RELATIVE RETENTION TIME OF POLAR MOLECULES

t_R of *n*-pentane taken as a standard.

Sorbate	α (Å^3)	μ, D	t_R/t_R of *n*-pentane			
			Polysorb-6	*Polysorbate-2*	*10% of dinonyl phthalate on Polychrome-1*	*10% of PEG-2000 on Polychrome-1*
Water	1.49	1.84	0.12	3.5	1.1	41.1
Methanol	3.23	1.67	0.19	2.7	0.9	14.3
Ethanol	5.06	1.70	0.40	3.8	1.3	17.5
Acetonitrile	—	3.94	0.60	7.4	2.3	26.7
Acetone	6.32	2.73	0.69	3.7	1.6	8.7
Diethyl ether	9.02	1.17	0.87	1.7	1.2	2.5
n-Pentane	9.95	0	1.0	1.0	1.0	1.0

Polysorbate-2

The elution of the compounds: water, methanol, ethanol, acetonitrile, acetone, diethyl ether, pentane, is determined not only by the value of general molecular polarisability, but also by the value of molecular dipole moment (the retention time of pentane is less than that of acetone and acetonitrile). It is also influenced by the ability of the compounds to form hydrogen bonds with the polar-polymer surface: better retention of water and methanol than that of *n*-pentane is observed as compared to the retention on Polysorb-4.

THE RETENTION OF CHLOROMETHANES

Polysorb-6

The retention of chloromethanes is independent of the value of the dipole moment, and their elution from the Polysorb-6 column occurs in parallel with the increase of their molecular weights and boiling points (Table III).

Polysorbate-2

The retention of the chloromethanes depends on the value of the molecular dipole moment and on the ability of molecules to form hydrogen bonds. Chloroform

TABLE III

RELATIVE RETENTION TIME OF CHLORINATED METHANES

t_R of *n*-pentane taken as a standard.

Sorbate	*M.W.*	*b.p. (°C)*	*μ*	*p of saturated vapours at 150° (atm)*	*t_R/t_R of n-pentane*			
					Poly-sorb-6	*Poly-sorbate-2*	*10% of dinonyl phthalate on Poly-chrome-1*	*10% of PEG-2000 on Poly-chrome-1*
Dichloromethane	84.9	40.1	1.62	—	0.9	4.8	2.3	10.8
Chloroform	119.4	61.3	1.06	9.6	2.1	8.5	4.2	19.7
Carbon tetrachloride	153.8	76.8	0	5.8	3.1	6.5	4.1	9.1
n-Pentane	72.1	36.1	0	15.5	1.0	1.0	1.0	1.0

is retained better on Polysorbate-2 than the heavier and higher-boiling CCl_4, while retention time for methylene chloride is about 5 times greater than that of pentane (Table III).

THE RETENTION OF COMPOUNDS WITH SIMILAR BOILING POINTS

Polysorb-4

The retention of the substances with similar boiling points on porous styrene and divinylbenzene copolymers is practically independent of the value of molecular

TABLE IV

RELATIVE RETENTION TIME OF SUBSTANCES HAVING SIMILAR BOILING TEMPERATURES

t_R of *n*-pentane taken as a standard.

Sorbate	*b.p. (°C)*	*M.W.*	*μ*	*p of saturated vapours at 150° (atm)*	*t_R/t_R of n-pentane*			
					Poly-sorb-6	*Poly-sorbate-2*	*10% of dinonyl phthalate on Poly-chrome-1*	*10% of PEG-2000 on Poly-chrome-1*
Methanol	64.7	32.0	1.7	13.6	0.19	2.7	0.9	14.3
Tetrahydrofuran	64–66	72.1	—	—	2.3	6.5	3.6	8.9
Ethanol	78.4	46.1	1.68	9.7	0.4	4.2	1.3	17.5
Ethyl acetate	77.1	88.1	1.81	6.8	2.4	5.9	2.9	9.5
Carbon tetrachloride	76.8	153.8	0	5.8	3.1	6.5	4.1	9.1
Acetonitrile	81.6	41.05	3.94	—	0.6	7.4	2.3	26.7
Benzene	80.1	78.12	0	5.8	2.9	9.2	5.1	13.3
Cyclohexane	81.4	84.16	0	5.2	3.1	3.9	3.7	4.0
Water	100	18	1.84	—	0.12	3.8	1.1	41.1
Formic acid	100.7	46.03	1.67	—	0.4	28.5	—	—
Nitromethane	101	61.0	3.54	—	—	14.1	6.2	62.5
1-Dioxane	100.8	88.1	0	—	4.4	11.6	6.0	30.1
n-Heptane	98.4	100	0	3.6	4.8	3.4	3.7	3.9

dipole moment, the geometrical structure and the molecule's classification relating to groups (A, B, D) and their differing ability to perform molecular interactions; the retention value is primarily determined by the differences in the molecular weights of compounds and in the pressure of saturated vapours of the components at the separation temperature of 150° (Table IV).

Thus, ethanol (dipole moment = 1.68) elutes earlier than carbon tetrachloride ($\mu = 0$), and benzene (planar configuration) is retained on Polysorb-4 column for a shorter time than cyclohexane (mainly "chair" configuration); formic acid and *n*-propanol (molecules of the D-group capable of specific interaction with the sorbent surface), appear on the chromatogram much earlier than *n*-heptane (molecules of A-group, with σ-bonds).

Polysorbate-2

The retention of compounds with similar boiling points on Polysorbate-2 differs from that on Polysorb-6 and depends both on the dipole moment of the molecules being separated and on their ability to form hydrogen bonds with the active sites of the sorbent surface (Table IV). For example, water, *n*-propanol, nitromethane are retained on Polysorbate-2 better than *n*-heptane, in spite of their molecular weights being well below than that of *n*-heptane, while all the boiling points are close.

THE SEPARATION OF COMPOUNDS WITH CLOSE MOLECULAR WEIGHTS

Polysorb-6

The main factor which determines the separation of components having close molecular weights is the difference in the boiling points of the compounds and in the pressure of their saturated vapours; due to this *n*-butanol (b.p. 117.5° and $p = 2.6$ atm), for instance, is retained in the Polysorb-4 column better than diethyl ether (b.p. 35.6°, and $p = 16.8$ atm) (t_R *n*-butanol/t_R diethyl ether = 3.1). Tetrahydrofuran (b.p. 64–65°) is retained better than cyclopentane (b.p. 49.3°) and *n*-pentane better than furan (Table V).

Polysorbate-2

The retention of compounds with close molecular weights depends both on the dipole moment of the molecules being separated and on their ability to form hydrogen bonds with the active sites of the sorbent surface (Table V). Thus, *n*-butanol is retained much better than diethyl ether (t_R *n*-butanol/t_R ether = 7.8), and furan better than *n*-pentane.

The observed differences in the retention behaviour of components on these sorbents result from the changes in the chemical nature of the surface of the Polysorbate; *viz.* the transition from an aromatic polyhydrocarbon (Polysorb-6) to a sorbent containing ether functional groups (Polysorbate-2).

The results obtained show that Polysorbate-2 is characterised by specific molecular interactions and according to the classification of KISELEV AND YASHIN for types of sorbents, one containing ether functional groups should be regarded as a specific sorbent of type III[7].

The interaction of the components being separated with the surface of such

TABLE V

RELATIVE RETENTION TIME OF SUBSTANCES HAVING SIMILAR MOLECULAR WEIGHTS

t_R of *n*-pentane taken as a standard.

Sorbate	*M.W.*	*b.p. (°C)*	*μ*	*p of saturated vapours at 150° (atm)*	*t_R/t_R of n-pentane*			
					Polysorb-6	*Polysorbate-2*	*10% dinonyl phthalate on Polychrome-1*	*10% of PEG-2000 on Polychrome-1*
Hexane	86.2	68.7	0	—	2.3	2.4	2.0	2.1
Ethyl acetate	88.1	77.1	1.81	6.8	2.4	5.9	2.9	9.5
1,4-Dioxane	88.1	100.8	0	—	4.4	11.6	6.0	30.1
n-Amyl alcohol	88.1	138.0	1.65	—	5.6	26.7	9.1	85.4
Butyric acid	88.1	163.5	0.93	0.5	5.7	84.2	—	—
Furan	68.1	32.0	0.72	—	0.7	3.4	0.9	4.7
Diethyl ether	74.1	35.6	1.17	16.8	0.9	1.7	1.2	2.5
n-Pentane	72.1	36.1	0	15.6	1.0	1.0	1.0	1.0
Cyclopentane	70.1	49.3	0	—	1.4	2.3	2.0	—
Tetrahydrofuran	72.1	64–65	—	—	2.3	6.5	3.6	8.9
n-Butanol	74.1	117.5	1.63	2.6	2.8	13.2	5.9	4.7
Propionic acid	74.1	141.1	1.74	1.4	3.9	49.5	—	—
Acetone	58.1	56.2	2.85	11.5	0.7	3.7	1.6	8.7
Nitromethane	61.0	101.0	3.54	—	1.0	14.1	6.2	6
Acetic acid	60.1	118.1	1.70	2.4	1.0	28.0	—	—

sorbents depends essentially on the value of the dipole moment of the compounds, on their ability to form hydrogen bonds with the sorbent and on the strength of these bonds. In the case of Polysorbate-2 this can be demonstrated by the retention of fatty acids as compared to alcohols, both having a similar number of carbon atoms in the molecule. Far greater retention of formic acid is observed compared to methanol and of propionic acid as compared to propanol.

The ratio of the retention volumes of *n*-acids and *n*-alcohols having an equal number of atoms of carbon in molecules is given in Table VI. From Table VI it can be seen that the ratio of V_R of the acid to V_R of the alcohol decreases with an increasing number of carbon atoms in the respective molecules, which, probably, results from the lessening of the contribution of the specific interaction to the total

TABLE VI

RATIO OF THE RETAINED VOLUMES OF *n*-ACIDS AND *n*-ALCOHOLS HAVING EQUAL NUMBER OF CARBON ATOMS IN A MOLECULE

Components being separated	*Polysorb-6*	*Polysorbate-2*
1 $HCOOH/CH_3OH$	2.1	10.0
2 CH_3COOH/C_2H_5OH	2.3	7.4
3 C_2H_5COOH/C_3H_7OH	3.5	7.0
4 C_3H_7COOH/C_4H_9OH	2.0	5.8

TABLE VII

THE RETENTION OF STRUCTURAL ISOMERS

t_R of *n*-pentane taken as a standard.

Sorbate	*M.W.*	*b.p. (°C)*	*t_R/t_R of n-pentane*			
			Poly-sorb-6	*Poly-sorbate-2*	*10% of dinonyl phthalate on Poly-chrome-1*	*10% of PEG-2000 on Poly-chrome-1*
Pentane	72.1	36.1	1.0	1.0	1.0	1.0
Isopentane	72.1	27.9	0.8	0.8	0.8	0.9
Cyclopentane	70.1	49.3	1.4	2.3	2.0	2.0
Hexane	86.2	68.7	2.3	1.9	2.0	2.1
Cyclohexane	84.2	81.4	3.1	3.9	3.7	4.0
Hexene-1	84.2	66.5	2.2	2.3	2.1	2.4
Cyclohexene	82.1	83	3.3	5.5	4.2	6.3
Heptane	100.2	98.4	4.8	3.4	3.7	3.9
Methylcyclohexane	98.2	100.9	5.6	5.2	5.2	5.0
Octane	114.2	124.7	11.9	6.3	6.2	9.5
Isooctane	114.2	99.2	6.0	5.6	4.4	3.3
tert.-Butanol	74.1	82.8	1.2	4.5	2.2	10.4
sec.-Butanol	74.1	99.5	1.3	8.5	3.6	22.7
Isobutanol	74.1	107	2.2	10.0	4.5	2.7
n-Butanol	74.1	117.5	2.8	13.2	5.9	47.0

energy of molecular interaction—sorbate (group D molecule)–polar polymer sorbent—as the number of carbon atoms in the molecules of the alcohols and acids increases.

The retention of structural isomers was studied on the sorbents mentioned above (see Table VII).

Like Polysorb-6, Polysorbate-2 gives a more rapid elution of isomeric alkanes and better retention of cyclic hydrocarbons as compared to the respective *n*-hydrocarbons. Isomeric alcohols are characterised by better retention of molecules of normal structure compared to branched molecules, on Polysorbate-2, similar to that on Polysorb-6, *i.e.* the retention time of *n*-butanol is longer than that of isobutanol, *sec.*-butanol, and *tert.*-butanol, although the relative retention values of alcohols on Polysorbate-2 considerably exceed the respective values on Polysorb-6 (Table VII).

Fig. 4 shows the dependence of the relative retention time of C_4 isomeric alcohols on polymer sorbents upon their boiling point. The relationship is linear, but during chromatographic analysis on Polysorbate-2 the relative retention of alcohols and the slope of this relationship as compared to the respective relationship on Polysorb-6 increases.

These data indicate the specific interaction of alcohols with the ether functional groups of Polysorbate-2 and the growth of importance of hydrogen bonds between sorbate and Polysorbate-2 when the structure of the carbon chain in molecules of alcohols having .the same number of hydrogen atoms becomes less branched. The

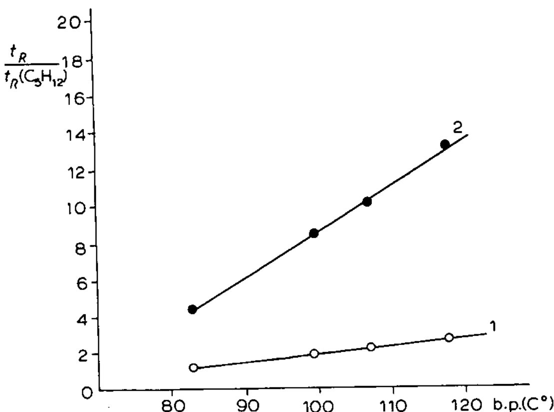

Fig. 4. Dependence of the relative retention time of isomeric C_4 alcohols upon the boiling point. (1) On Polysorb-6; (2) on Polysorbate-2.

similar values of the relative retention volumes of branched alcohols and alcohols of normal structure having similar boiling points, in particular *sec.*-butanol and *n*-propanol, *tert.*-butanol and isopropanol, during their separation on Polysorbate-2 proves the same point.

For instance, in the case of Polysorb-6 the ratio of the retention volumes V_R *sec.*-butanol/V_R *n*-propanol = 1.8; for Polysorbate-2 it is 1.2 and the ratio of the retention volumes V_R *tert.*-butanol/V_R isopropanol equals 1.7 and 1.1, respectively for Polysorb-6 and Polysorbate-2.

The peculiarities of the retention behaviour of components pointed out in the case of Polysorbate-2 can also be observed in the case of Porapack T[7]—the most polar of the known polymeric sorbents—the Porapacks.

A comparison was made between the retention values of various types of compounds on Polysorbate-2 and on the stationary liquid phases, dinonyl phthalate and PEG-2000, applied over an inert Teflon support—Polychrome-1[6]. Some similarity was observed in the retention of the components on the Polysorbate-2 and on these phases, although the values of the relative retention time of polar molecules on Polysorbate-2 exceed those for dinonyl phthalate, but are lower than for PEG-2000, which, probably, is the result of different concentrations in the functional groups in these sorbents.

Thus, methyl acrylate–divinylbenzene base polymer sorbent—Polysorbate-2—possesses separating properties which are similar to those of stationary polar phases.

The value of relative polarity found by the ROHRSCHNEIDER method[8] was 41% for Polysorbate-2, *i.e.* Polysorbate-2 is identical with stationary phases of average polarity and the value of its relative polarity somewhat exceeds that of Porapack T ($P = 34\%$).

Still more polar sorbents (polarity according to ROHRSCHNEIDER 60–90%) can be obtained by using other polar monomers as a base.

Thus polymer sorbents having various degrees of polarity according to the limits of ROHRSCHNEIDER scale for stationary phases can be developed.

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CHROM. 5216

ADSORPTION IN GAS-LIQUID CHROMATOGRAPHY

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SUMMARY

The equilibrium theory for relation values in gas-liquid chromatography was considered taking into account both the absorption of chromatography-volatile substances and adsorption at the stationary liquid phase/solid or stationary liquid phase/gas interfaces.

The methods of simultaneous determination of absorption and adsorption on the stationary liquid phase surface were suggested from experimental dependence of relation volume upon surface. Experimental data agree satisfactorily with obtained equations. It is necessary to take into consideration the adsorption at the stationary liquid phase/solid and stationary liquid phase/gas interfaces in order to perform the analytical and physico-chemical measurements.

During the last ten years some works have been published which were devoted to the processes occurring at the stationary liquid phase (SLP)/solid or SLP/gas interfaces in gas-liquid chromatography (GLC)[1-5]. This paper describes an attempt to consider the problem of absorption effect in general.

GLC has been intensively developed during the last twenty years, and at present it is the main technique used for the analysis of complex mixtures of volatile organic compounds. However, "pure" GLC, in which the chromatographic characteristics of the separated substances are determined by the properties of the SLP only, is seldom used in practice, since reversible (or irreversible) adsorption of the separated compound and that of the components of the mixture can occur at the SLP/solid support interface. These phenomena may cause large errors in the interpretation of the analytical and physicochemical experimental results. Therefore, development of GLC requires extensive study of the processes occurring at the SLP/solid or SLP/gas interfaces. It should be mentioned that the classical GLC theory does not take into account the multiphase nature of the real sorbents.

With an adequate amount of SLP on a solid support (Fig. 1), the sorbent in GLC can be regarded approximately as a combination of at least three phases capable of adsorption and absorption: (1) the SLP/gas surface, (2) the SLP/solid surface, and (3) the SLP. In general, the retention volume observed should be determined by a contribution from all three of these phases.

For the sorbent model being discussed the system of equations describing the equilibrium during chromatography of a single component, under the action of

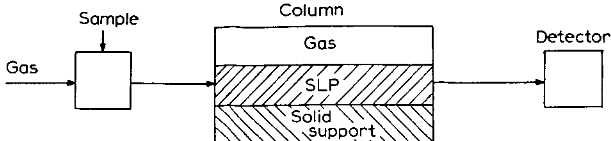

Fig. 1. Scheme of chromatographic device for gas–liquid chromatography.

longitudinal erosion factors, accounting for absorption in SLP and adsorption at the interface, can be written as:

$$u \frac{\partial c}{\partial x} + \varrho_g \frac{\partial c}{\partial t} + \varrho_{gl} \frac{\partial a_l}{\partial t} + \varrho_l \frac{\partial \alpha}{\partial t} + \varrho_{ls} \frac{\partial a_s}{\partial t} = D_x \frac{\partial^2 c}{\partial x^2} \tag{1}$$

$$a_l = \mathrm{f}_{gl}(c) \tag{2}$$

$$\alpha = \mathrm{f}_l(c) \tag{3}$$

$$a_s = \mathrm{f}\, l_s(a) \tag{4}$$

where

u = the linear velocity of the carrier gas;
x = the coordinate;
t = the time;
c = the bulk concentration of the compound in the gas phase;
α = the bulk concentration of the substance in SLP;
a_l = the surface concentration of the compound at the SLP/gas interface;
a_s = the surface concentration of the compound at the SLP/solid support interface;
ϱ_g = the fraction of gas phase in the cross-section of the column;
ϱ_l = the SLP portion in the cross-section of the column;
ϱ_{gl} = the ratio of the surface of SLP/solid support interface to the sorbent volume;
ϱ_{ls} = the ratio of the surface of SLP/solid support interface to sorbent volume;
D_x = the effective coefficient of longitudinal diffusion.

Eqns. 2 and 4 are the adsorption isotherms on the SLP and solid support surfaces, eqn. 3 is the dissolution isotherm in SLP. It differs from the ordinary equation (see for example ref. 6) by the presence of third and fifth terms, which account for the adsorption at the SLP/gas and SLP/solid support interfaces. An equation similar to eqn. 1 has been solved earlier[7].

The rate of motion of a peak of a chromatographic zone and consequently the retention volume, V_N, can be determined by solving eqns. 2 and 4.

$$V_N = \mathrm{f}_l' V_l + \mathrm{f}_{gl}' S_l + \mathrm{f}_l \cdot \mathrm{f}_{ls}' \cdot S_s \tag{5}$$

$$\mathrm{f}_{gl}' = \frac{\mathrm{d}a_l}{\mathrm{d}c}; \quad \mathrm{f}_{ls}' = \frac{\mathrm{d}a_s}{\mathrm{d}a}; \quad \mathrm{f}_l' = \frac{\mathrm{d}\alpha}{\mathrm{d}c}$$

where V_l is the volume of SLP in the column; S_l and S_s are respectively the SLP/gas and SLP/solid support interfaces in the column. Eqn. 5 is a general expression for

the model considered, where the whole surface of a support is covered with a macro-layer of the SLP. It takes into account the dissolution in SLP and adsorption at its interface boundaries.

If the adsorption and sorption isotherms are linear (*i.e.*, $f_{gl}' = K_{gl}$; $f_{ls}' = K_s$; $f_l = K_l$), then the retention volume equation should be:

$$V_N = K_l V_l + K_{gl} S_l + K_l K_s S_s. \tag{6}$$

From eqn. 6 K_l, K_{gl} and K_s can be found using the experimental relationships:

$$V_N = \psi(V_l) \tag{7}$$

$$S_l = \varphi(V_l) \tag{8}$$

The function 8 is non-linear.

For a graphical determination of the distribution factor it is expedient to use the equations:

$$\frac{V_N - \bar{V}_N}{V_l - \bar{V}_l} = K_{gl} \frac{S_l - \bar{S}_l}{V_l - \bar{V}_l} + K_l \tag{9}$$

$$V_N - K_{gl} S_l = K_l V_l + K_l K_s S_s \tag{10}$$

where $V_N = \bar{V}_N$ (V_l, S_l) is some reference point chosen in the low SLP content region.

Our experimental results and those reported elsewhere[8,9] are fitted well by eqns. 9 and 10.

Let us consider some conclusions resulting from eqns. 9 and 10 and use of the latter for analysing the experimental data.

Firstly the contribution of adsorption on the SLP interfaces to the retention volume can be considered. For example, Fig. 2 shows the relation of all three absorption processes in the SLP and adsorption at its interfaces for the system: the chromatographed compound–ethyl acetate; SLP–thiodipropionitrile; solid support–firebrick.

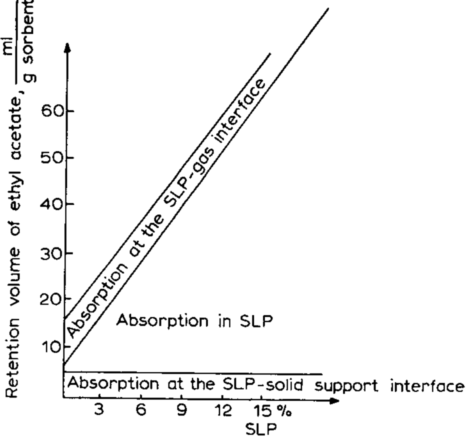

Fig. 2. Contribution of adsorption—at interface boundaries—to the retention volume for ethylene on β,β'-thiodipropionitrile.

TABLE I

CONTRIBUTION OF SOLID SUPPORT TO RETENTION VOLUME
Support, Chromosorb P.

Substance	K_l	K_{gl}	K_s	15% stearic acid on Chromosorb P						
				V_N	K_lV_l	$K_{gl}S_l$	$K_lK_sS_s$	$\frac{K_lV_l \cdot 100}{V_N}$	$\frac{K_{gl}S_l \cdot 100}{V_N}$	$\frac{K_lK_sS_s \cdot 100}{V_N}$
Benzene	150	2.5	0.025	187	157	9.9	20.1	84.0	5.3	10.7
Ethyl acetate	89.0	0.7	0.052	121	93.0	2.7	25.0	77.0	2.3	20.7
Hexyne-1	112.5	1.0	0.062	160	118	3.9	38.1	73.6	2.4	24.0

Table I shows the experimental data which also show the importance of the adsorption process on a solid support. It can be seen that adsorption on the surface of Chromosorb P may contribute (up to 24%) to the retention volume.

TABLE II

COMPARISON OF V_g VALUES USED FOR CALCULATION OF PHYSICAL AND CHEMICAL CHARACTERISTICS IN GLC FOR DIFFERENT COMPOUNDS ON β,β'-THIODIPROPIONITRILE

Compounds	Firebrick		Chromosorb W	
	V_g Our method	V_g Usual method	V_g Our method	V_g Usual method
n-Butyl ethyl ether	66	185	76	113
Propionic aldehyde	242	300	243	260
Benzene	384	425	372	389
Ethyl acetate	379	533	388	444
Acetone	393	450	400	414
Methyl ethyl ketone	718	849	714	753
1-Hexene	14	34	15	22
Cyclohexane	69	99	76	89

We have shown[10] that if adsorption is not taken into account the error in determining the physical and chemical characteristics of an SLP/gas system by a chromatographic method can be as much as 30–50% (see Table II).

TABLE III

HEATS OF SOLUTION AND ADSORPTION OF ALKANES AND ALKYNES ON APIEZON K COATED ON INZ-600

Substance	ΔH of solution (kcal/mole)	ΔH of adsorption (kcal/mole)	Heat of solution on squalane (kcal/mole) (literature data[12])
n-Hexane	5.8	4.55	6.21
Hexyne-1	5.2	10.9	—
n-Heptane	6.6	7.6	7.37
Heptyne-2	8.6	17.8	—

TABLE IV

COMPARISON OF CHROMATOGRAPHIC CHARACTERISTICS OF POLAR AND NON-POLAR COMPOUNDS ON DIFFERENT SUPPORTS UNDER GLC CONDITIONS (CONCENTRATION OF SQUALANE 10.65%)

Compounds	*Sterchamol* ($j = 0.91$)			*Chromosorb W* ($j = 0.84$)			*Chromosorb G* ($j = 0.90$)			*Porcelain*[a] ($j = 0.93$)			*Sodium chloride*[b] ($j = 0.91$)		
	Relative retention volume	*A*	V_g°	*Relative retention volume*	*A*	V_g°	*Relative retention volume*	*A*	V_g°	*Relative retention volume*	*A*	V_g°	*Relative retention volume*	*A*	V_g°
Cyclohexane	0.69	0.8	325	0.68	0.0	318	0.68	0.8	324	0.68	0.5	318	0.65	0.0	258
Isooctane	0.91	2.4	423	0.90	0.4	421	0.89	0.0	432	0.89	1.5	410	0.89	0.0	368
n-Hexane	0.37	0.8	176	0.37	0.0	168	0.37	0.0	171	0.37	0.6	163	0.35	0.0	147
Benzene	0.61	8.2	234	0.55	0.6	231	0.55	0.0	243	0.58	3.5	229	0.53	0.0	219
n-Heptane	1.00	2.6	475	1.00	0.4	485	1.00	0.0	477	1.00	1.5	473	1.00	0.0	410
Diethyl ether	0.75	—	—	0.20	3.1	78	0.12	0.0	58	0.80	—	—	0.10	0.0	41

[a] Concentration of squalane 5.65%.
[b] Concentration of squalane 1.00%.

Secondly, the possibility of new physico-chemical applications of gas chromatography follows from these three equations, namely measuring the adsorption of volatile compounds dissolved in SLP on the solid surface (for example, by determination of K_s). This possibility can be easily realised experimentally. The heats of solution of hydrocarbons in Apiezon K and adsorption on an Apiezon K–INZ-600 interface are shown in Table III. Thus it can be seen that for hydrocarbons the heats of adsorption considerably exceed those of solution.

Thirdly, eqns. 9 and 10 permit quantitative interpretation of such phenomena in GLC as zone asymmetry, irreproducibility of retention values, and in particular, their dependence upon the sample volumes.

Asymmetry of chromatographic zones is usually due to the solid support used. If for the compound analysed the adsorption isotherm at the SLP/solid support interface is non-linear and obeys the Freundlich equation

$$a_s = \alpha a^{\beta} \; (\beta < 1, \; \alpha = \text{constant}) \tag{11}$$

then the equation for the retention volume can be given in the following form (assuming that $K_{gl} \approx 0$)

$$V_N = K_l V_l + \frac{K_l S_s \alpha \beta}{c^{1-\beta}} \tag{12}$$

It follows from this equation that the retention volume increases with decreasing compound concentration in the gaseous phase, which explains a diffuse tail to the zone. Asymmetry of the latter is enhanced with increase in the value of $(1-\beta)$.

Eqn. 12 can be used also to interpret the empirical dependence of the retention volume on the sample volume q.

The equation for the relative volume, $V_{N(o)}$ describing this dependence (at $V_{N(st)} = K_{l(st)} \cdot V_l$) can be written as follows:

$$V_{N(o)} = \frac{K_l}{K_{l(\text{st})}} \left[1 + \frac{\varepsilon}{V_l^{\beta}} \left(\frac{K_{l(\text{st})}}{K_l} \right)^{1-\beta} \left(\frac{V_{N(o)}}{q} \right)^{1-\beta} \right] \tag{13}$$

where ε is a constant. The literature and our experimental data[8] are quantitatively described by eqn. 13.

Fourthly, eqns. 9 and 10 can be used to quantitatively estimate the adsorption properties of the solid supports under conditions of GLC and consequently the efficiency of the modified methods. It is expedient to characterise the adsorption properties of the solid supports by their retention volumes as the adsorption of the chromatographed compound on the surface per gram of the support covered with SLP[11].

As an example the contribution from adsorption of chromatographed compounds to the retention volume at the squalane–solid support interface for different support are given in Table IV.

Thus, adsorption phenomena are of great importance in the GLC and they should be taken into account in analytical, physical and chemical measurements.

Adsorption on the solid support surface can play a positive role by providing better separation of compounds with similar properties (for example, the organic mineral clays).

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CHROM. 5221

INTERACTIONS OF POLAR SOLUTES WITH NON-POLAR STATIONARY PHASES IN GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

The gas chromatographic solutions of polar substances in a non-polar stationary phase were studied. The semiempirical method of calculation of dispersion forces in such solutions is discussed. A comparison of gas chromatographic and static values of heats of solution shows that the concentration effect has great importance in the systems under study. The values of concentration effects were tabulated for the different chemical classes of substances.

The calculation of the thermodynamic functions of solution is the main problem in the theory of the selectivity of stationary phases in gas–liquid chromatography (GLC)[1]. The development of semiempirical methods of calculation of these values provides an opportunity for identifications to be made in gas chromatography. The heat of solution is one of two independent thermodynamic functions of solution which are used to describe the selectivity of stationary phases. Unlike the logarithm of the retention volume, the heat of solution can be calculated on the basis of additivity principles with sufficient accuracy. Microcharacteristics of atomic groups of solute molecules are used for such calculations[2]. The intermolecular forces in non-polar stationary phases consist of dispersion and induction forces. Therefore we have the simplest case for calculations using non-polar stationary phases.

In the case of non-polar substances as solutes and solvents, the heat of solution can be calculated as follows:

$$\Delta H_s = \Delta H_d - \Delta H_h \quad (1)$$

where

ΔH_s is the heat of solution;
ΔH_d is the heat of solution which represents the energy of the dispersion interaction;
ΔH_h is the heat of hole formation.

The last term of this equation is measured experimentally[2].

$$\Delta H_d = 0.3\, m \quad (2)$$

where m is the amount of branching of the main chain of the solute molecule (the

heat of hole formation is negligibly small for linear chain molecules in paraffin solvents).

$$D_i = \frac{\alpha_1 \alpha_2}{r^6},$$

$$i = \frac{\Delta H_{sp}}{\sum_p K_i D_i},$$

$$\Delta H_d = i \Sigma D_i K_i \qquad (3)$$

where

α_1 is the polarisability of the atomic group of the solute;

α_2 is the polarisability of the methylene group (non-polar solvent);

r is the sum of the van der Waals radii of the atomic group of the solute and of the solvent (for the solvent $r = 2$Å as for the methylene group);

ΔH_{sp} and $\sum_p K_i D_i$ refer to n-paraffins which have the same number of carbon atoms as the sum of heavy atoms of the solute (for example, the value of i for n-butane is used for the calculations of heats of solution of n-propyl chloride);

K_i is the coefficient of the intramolecular shielding of the atoms and of the atomic groups in the solute molecule[2].

The calculation of heats of solution should give sufficient exact data concerning non-polar isomeric substances; the resolution and identification of isomeric substances being one of the most difficult problems in gas chromatography. The investigation of the usefulness of this method of calculation for the solutions of polar substances in non-polar phases is reported in this paper.

The induction forces are induced on dissolving polar substances in non-polar solvents. A concentration effect[3] also occurs in such systems. The last effect is connected with the association of polar molecules in a non-polar environment. The heat of solution of aggregates of polar molecules is greater than the heat of solution of an individual molecule. Therefore, the induction forces and concentration effect lead to an increase in the heat of solution of polar substances in non-polar solvents as compared with the values calculated by eqns. 1–3.

Squalane is used for the experimental investigation of the solubility of polar substances. The chromatographic measurements of the heats of solution were made using "Varian-1860" and "Chrom-31" chromatographs. The temperature of the chromatographic column varied from 30 to 80°. The length of the column is 1.5 m. Squalane (0.25 g) is added to 10 ml silanised Chromosorb W. The standard deviation of the retention volumes is 0.4%; the standard deviation of the heats of solution is 0.1 kcal. A commonly used equation[4] is used for the calculation of the heats of solution from temperature relationships of specific retention volume.

The experimental and the calculated values, on the basis of eqns. 1–3, are listed in Table I. The calculated data show the value of the dispersion interactions. The difference between experimental and calculated heats of solution together with ΔH_h describes the sum of induction forces and the concentration effect (Δ). A comparison of Δ values shows that these values decrease in an homologous series. A constant value of Δ is established after the fourth to fifth members of a series.

Let us consider the possible reasons for the appearance of the Δ value in

TABLE I

COMPARISON OF CALCULATED (ΔH_d) AND EXPERIMENTAL (ΔH_s) HEATS OF SOLUTION OF DIFFERENT CHEMICAL CLASSES OF SUBSTANCES (kcal/mole)

Substance	$\Sigma K_i D_i$	ΔH_d	ΔH_h	ΔH_s	Δ
Allyl alcohol	13.4	4.0	—	7.25	3.25
n-Propanol	13.4	4.0	—	7.2	3.2
Isopropanol	13.3	4.0	0.3	6.65	2.9
tert.-Butanol	15.3	4.9	0.6	6.8	2.5
Nitromethane	17.4	5.25	0.2	8.0	1.95
Ethyl chloride	14.0	4.07	—	5.3	1.2
1-Chloropentane	21.6	7.35	—	8.5	1.1
1,2-Dichloroethane	18.2	5.45	—	6.3	0.85
1,1-Dichloroethane	18.2	5.45	0.3	6.05	0.9
1,1,2-Trichloroethane	22.5	7.22	0.3	7.7	0.8
Isobutyl chloride	18.9	6.05	0.3	6.55	0.8
sec.-Butyl chloride	19.0	6.08	0.3	6.45	0.7
tert.-Butyl chloride	18.5	5.95	0.6	5.25	−0.1
Carbon tetrachloride	23.2	7.4	0.6	7.0	0.2
Ethyl bromide	15.5	4.50	—	5.50	1.0
sec.-Butyl bromide	19.7	6.32	0.3	6.8	0.8
Acetone	14.4	4.35	0.2	5.05	0.9
Methyl ethyl ketone	17.0	5.45	0.2	6.1	0.85
Butyral	16.1	5.15	—	5.8	0.65
Acetonitrile	14.9	4.45	—	4.55	0.1
Ethyl acetate	20.4	6.55	0.2	6.25	−0.1
n-Propyl acetate	23.0	7.85	0.2	7.6	−0.05

squalane. The value of the induction forces is increased with the dipole moments of molecules. If the induction forces are the main tail of Δ, we will expect that nitriles and ketones have the greatest values of Δ. As a matter of fact the experimental data show that alcohols have the greatest values of Δ. Alcohols have a high ability to associate. Consequently, we can suppose that the discrepancies between the experimental and calculated heats of solution in squalane mainly refer to a concentration effect.

Some experiments on the measurement of the solubility have been carried out for a more detailed investigation of the nature of Δ. The solubility is measured as relation between the concentrations of the solute in liquid and gaseous phases, using chromatography for analysis of the gas phase[1]. Squalane (10 g) is placed in a glass saturator (60 ml) which is closed by a plastic cork. The cork has a hole. A gas sample is taken from the saturator through the hole in the cork. The Hamilton teflon syringe with fixed volume is used for sampling (volume is 0.025 ml). A measured sample of solute is placed in the saturator then the saturator is maintained at a constant temperature for 20–40 min. The gas sample is then taken to "Chrom-31" gas chromatograph which is fitted with a flame ionisation detector and integrator. This chromatograph has a column coated with squalane and a temperature of 70°. Specific retention volumes are calculated from static measurements for comparison with the chromatographic data. The heats of solution in the static measurements are calculated from the temperature dependence of specific retention volumes. The average error of the measurements of the heats of solution by the static method is *ca.* 0.2 kcal. The measurements are made within a temperature interval 20–60° and a concentration interval of 0.05–1.5%.

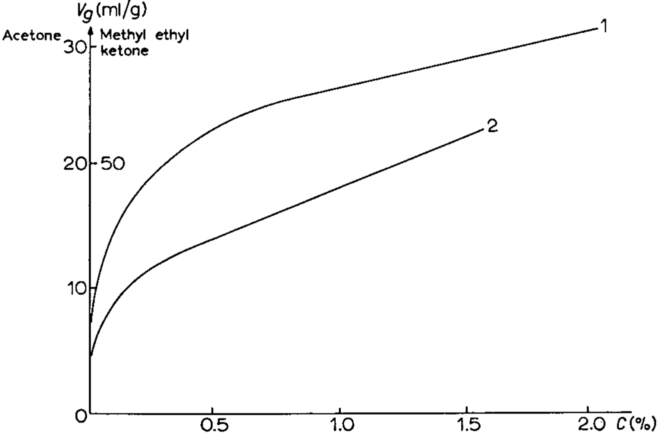

Fig. 1. Relationship between V_g of acetone (2), methyl ethyl ketone (1) and concentration of solution in squalane (%).

The concentration relationship of the specific retention volume is shown in Fig. 1. These data were obtained for acetone and methyl ethyl ketone at 50°. It is clear that the solubility of polar solutes in a non-polar solvent increases with concentration. This increase is greater for low concentrations and for acetone. The latter fact explains the effect of a decreasing Δ value in an homologous series. The values of the heats of solution of acetone and methyl ethyl ketone in squalane are listed in Table II. The heat of solution is a logarithmic function of the specific retention volume, therefore the increase of the heats of solution with concentration is smaller than for specific retention volume.

The comparison of chromatographic and static heats of solution shows that the average effective concentration of gas chromatographic solutions is *ca.* 0.1%. The retention data of benzene and hexane are independent of concentration.

Consequently the retention data and the results of the static measurements show that the Δ value depends mainly on the concentration effect.

The comparison of the data in Table I allows one to write the following series

TABLE II

COMPARISON OF CHROMATOGRAPHIC AND STATIC HEATS OF SOLUTION OF ACETONE AND OF METHYL ETHYL KETONE IN SQUALANE (kcal/mole)

Substance	*Method*	ΔH_s	*Concentration (%)*
Acetone	Chrom.	5.05	
	Static	4.6	0.007
		5.4	0.2
Methyl ethyl ketone	Chrom.	6.1	
	Static	5.8	0.01
		6.2	0.03
		6.6	0.09

TABLE III

VALUES OF THE CONCENTRATION EFFECT (Δ) FOR DIFFERENT CLASSES OF CHEMICAL SUBSTANCES (kcal/mole)

Chemical class	
Alcohols	2.5–3.2
Aliphatic nitro-compounds	2.0
Chloroalkanes	0.7–1.1
Bromoalkanes	0.6–0.8
Ketones	0.8
Aldehydes	0.7

of chemical classes of substances on the basis of their ability to associate: alcohols, nitro-compounds, halogen compounds, ketones, aldehydes, nitriles, ethers. The concentration effect is very small for nitriles and ethers. The concentration effect of alcohols depends on the shielding of the hydroxyl groups by hydrocarbon radicals: it decreases from *n*-alcohols to *tert.*-alcohols. The changes in the values of Δ for other isomeric compounds are very small. For example, the values of Δ of the chloroalkanes are changed only by 0.1–0.2 kcal. The concentration effect is negligible when the polar atomic groups are shielded. For example, carbon tetrachloride and *tert.*-butyl chloride have very small Δ values.

The experimental results obtained allows one to draw some conclusions concerning the calculation of the heats of solution of polar solutes in non-polar stationary phases. The value of the concentration effect decreases rapidly in the first members of an homologous series and is then stabilised. The possible Σ values of different chemical classes of substances are listed in Table III. These values allow the calculation of heats of solution for polar solutes in non-polar solvents in GLC.

Naturally, a change in the amount of stationary phase and in the length of the column varies the retention values of polar solutes in non-polar stationary phases. Therefore it is recommended that the stable experimental conditions are maintained for the comparison of calculated and experimental heats of solution of the substances under study.

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CHROM. 5222

METHODS FOR COATING THE STATIONARY LIQUID PHASE ON THE SOLID SUPPORT IN ANALYTICAL AND PHYSICO-CHEMICAL APPLICATIONS OF GAS–LIQUID CHROMATOGRAPHY

A CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF IMPURITIES

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SUMMARY

The method for coating the stationary phase on the solid support with the application of vacuum results in an improved spreading of the film, acceleration of internal diffusion, and hence a decrease in plate height.

Using as an example the measurement of retention volumes of some alcohols on squalane it has been shown that the addition of small amounts of a modifying agent insoluble in the stationary phase eliminates adsorption at the liquid–gas interface.

A method for multiple thermal enrichment of impurities has been developed which is based on an alternate action of the carrier gas stream and of the temperature field.

(1) COATING THE STATIONARY LIQUID PHASE ON THE SOLID SUPPORT

The selection of the method for coating the stationary liquid phase on the support and of the percentage loading of this phase g_u is of certain interest in connection with the analytical applications of gas–liquid chromatography (GLC) and of decisive importance in the determination of thermodynamic functions.

Of no lesser importance is a consideration of the methods for adding the liquid phase when thermodynamic studies are to be conducted. Investigations of this kind should furnish information as to the nature of the interaction between the stationary liquid and the solid support, and the sorbate–stationary phase.

Generally speaking, five possible types of sorption of the sorbate on sorbents in GLC may be distinguished, namely:

(1) solution in the stationary phase;
(2) adsorption at the solid support–gas phase interface;
(3) adsorption at the stationary phase–gas interface;
(4) adsorption at the stationary phase–solid support interface; and
(5) solubility in thin films of the stationary phase.

The subject of the present study is the selection of conditions under which all processes except the first one are reduced to minimum.

It is known[1,2], that the role of process (2) is reduced with an increase in g_u, and at a g_u of 3 to 5% this factor may be practically neglected. The contribution of process (4) (ref. 3) must be essentially lower than that of process (3). Adsorption from solutions is significantly less than that from gases since it is accompanied by a displacement of the solvent molecules.

Moreover the stationary phase may be a good solvent for the sorbate. This last effect however is only observed in some special cases. Thus only two basic processes have to be considered, namely (3) and (5), which in the case of sufficiently thin films cannot generally be viewed separately.

To ascertain the role of these processes and methods for their elimination, the values of specific retention volume V_g^T, depending on g_u, were measured for the following systems: hexane–squalane; ethanol–squalane; propanol–squalane. The hexane–squalane system was selected as the basic one since sufficiently reliable static measurements of the activity coefficients of the system are available.

Columns, 10–15 cm long and 3.8–4 mm I.D. were used; they were connected in series to a thermal conductivity detector. Celite 545, 60–80 mesh, was used as the solid support. The weight of the stationary phase in the column was determined to within 0.0001 g. The temperature of the column was kept constant to within 0.2°.

The size of the sample was chosen so that upon its further reduction the retention volume of the component of interest should not vary and would be independent of concentration. The retention volumes considered as true ones were those that corresponded to the horizontal portion of the retention time against peak area plot (Fig. 1). The size of the injected liquid sample did not exceed 0.1 μl, and that of the gas sample 0.05 cm³.

In the course of determining the retention times, t_R, the flow rate was periodically checked (after each measurement). The retention time was calculated as the average of 10 to 15 measurements.

The formation of a film, evident from hysteresis effects in capillary conden-

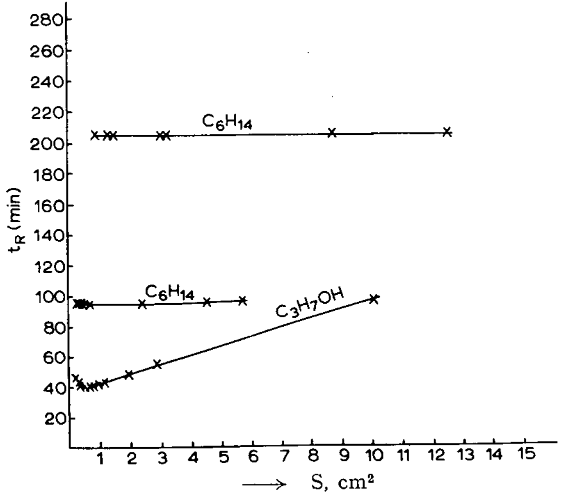

Fig. 1. Plot of retention time as a function of the peak area, S.

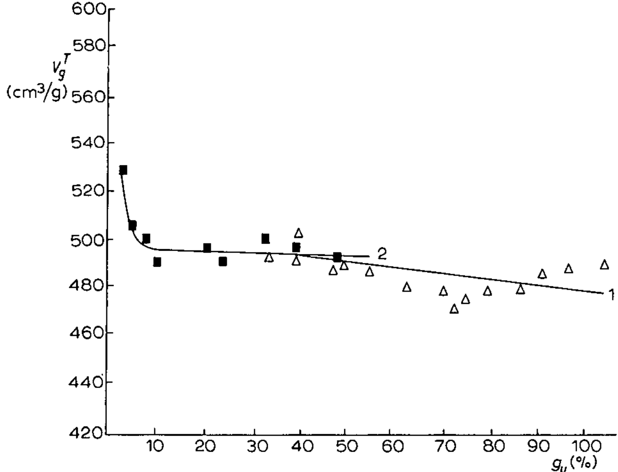

Fig. 2. Plot of specific retention volume of hexane on squalane as a function of percent liquid coating. Curve 1 = liquid phase coated under vacuum conditions; curve 2 = vacuum treatment of sorbents prepared by the usual procedure.

sation, can be prevented by the presence of air in the capillary pores, though the presence of air may hinder the complete filling of the support. It was therefore of interest to carry out the addition of the stationary phase under vacuum. To do this, a measured volume of the liquid was coated on a support previously subjected to vacuum (p = 0.01 mm) (Fig. 2, curve 1). The experiments showed that practically the same results could be achieved by subjecting the sorbent prepared by the usual method to vacuum (Fig. 2, curve 2). As will be seen from curves 1 and 2 of Fig. 2, a practically constant value of $V_g{}^T$ is observed over a rather wide range of liquid phase load. It should be noted that when effecting the coating under vacuum higher values of g_u (up to 100%) can be achieved than by the usual procedure (up to 80%).

We have also measured $V_g{}^T$ as a function of g_u (Fig. 3) for ethanol (curve 1) and propanol (curve 2) on squalane using the vacuum coating procedure.

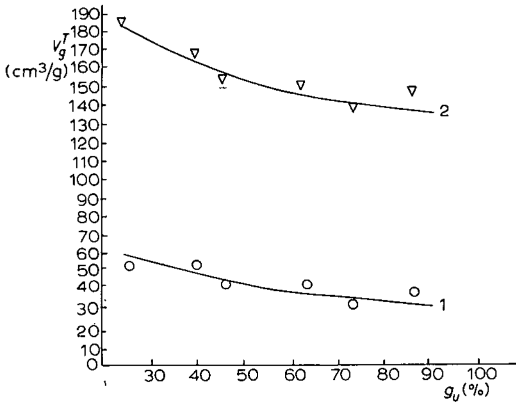

Fig. 3. Plot of specific retention volume of ethanol (curve 1) and propanol (curve 2) as a function of percent of squalane coating on Celite.

In order to characterise the difference in the state of the stationary phases when using the two different methods of coating considered here we made a study of the broadening of the hexane peak as a function of g_u and v_a (flow rate).

From the log (μv_a) — log v_a relationship (μ is the half-width of the peak) the position of the external and internal diffusion regions may be determined. It has been found that in the case of the usual coating procedure the region of internal diffusion begins at 28% of liquid phase and when vacuum coating is used, external diffusion is the controlling process up to 46%, and only above 95% can internal diffusion be considered as the controlling factor.

Thus the use of the vacuum coating procedure results in a reduction of peak broadening due to a more uniform distribution of the liquid. This fact confirms the suggestion made above concerning the pattern of the liquid phase distribution and the nature of its interaction with the adsorbate. This more uniform distribution, however, has been the reason why we have not achieved any decisive progress in attempting to minimize the role of the additional effects. Therefore we have investigated the possibility of eliminating these effects by modifying the sorbent.

The addition of a very small amount of a polar substance, practically insoluble in a non-polar stationary phase, can eliminate adsorption of the sorbate at the surface of the stationary phase (if it occurs) as a result of adsorption of the modifier at the stationary phase–gas interface.

We have investigated the effect of modification with the following six systems: (1) liquid phase squalane, modifier polyethylene glycol 300, sorbate hexane; (2) squalane, polyethylene glycol 300, ethanol; (3) squalane, polyethylene glycol 300, propanol;

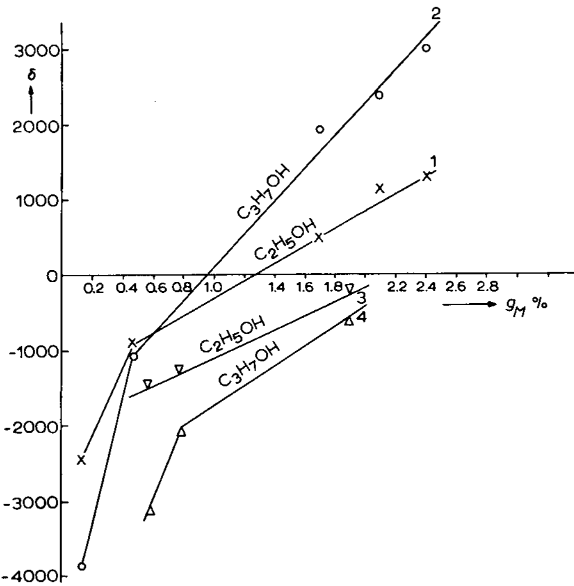

Fig. 4. The value of δ as a function of the amount of modifying agent added. δ is the difference between specific retention volumes of alcohol on squalane with and without modifying agent (PEG) addition (per unit weight of PEG).

(4) squalane, polyethylene glycol 1000, hexane; (5) squalane, polyethylene glycol 1000, ethanol; (6) squalane, polyethylene glycol 1000, propanol.

The experiments have revealed a significant decrease in V_g^T of the alcohols as a result of the modification. Fig. 4 shows a plot of the specific values of the change in V_g^T referred to 1 g of the modifier per 1 g of squalane ($\delta = \Delta V_g^T \cdot 100/g_M$) depending on the percentage of the modifier (the percentage of the modifier, g_M, is calculated depending on the weight of squalane).

It will be seen that in the case of the alcohols with low values of g_M a distinct decrease in V_g^T takes place, which because of the solubility of the alcohols in the modifier changes to an increase in V_g^T with high values of g_M (curves 1, 2). Experiments with a solid modifier (polyethylene glycol 100) showed a marked decrease in V_g^T with high values of g_M as well (curves 3, 4).

In the case of hexane no decrease in V_g^T is observed, while the increase, probably due to adsorption on the modifier, somewhat exceeds the experimental errors.

The difference observed in the behaviour of alcohols and hexane as far as modification is concerned suggests that in the case of hexane the increase in V_g^T is due to additional solution in thin films, and in the case of alcohols, to adsorption.

(2) CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF IMPURITIES

Despite the fact that a large number of thermal, adsorption, and chromatographic methods for concentrating and determining impurities are available the problem cannot be considered as solved. The availability of detecting devices with a fast response such as the flame ionisation detector enables one to envisage the possibility of increasing the sensitivity by repeatedly concentrating the impurity and transferring it to an extremely small volume. At the same time in the usual preliminary thermal concentration method only a concentration of Γ_0/Γ times is achieved in a single enrichment (Γ being the Henry coefficient in the zone of heating, and Γ_0 the Henry coefficient outside the zone of heating).

In the heat-dynamic and the chromatographic versions, concentration is limited by stationary band broadening. To reduce this broadening the use of large temperature gradients is necessary, which sharply deteriorates the resolution.

The broadening of the band hindering thermal enrichment can be diminished by discarding the stationary regime of the process and by decreasing the time spent for broadening.

In the method described here a number of acts of thermal enrichment are achieved on a single column. Broadening, particularly that in the last stages, is reduced by decreasing the time during which it can occur.

The test mixture is injected on to a column with an adsorbent and occupies a length of the bed approximating to the length of the furnace. The furnace which will heat to a certain constant temperature is subsequently made to move along the bed. Then either the carrier gas or the mixture to be analysed is swept through the bed. Upon leaving the heated portion the band width naturally decreases Γ_0/Γ times. This cycle of moving the furnace and sweeping with gas can be repeated.

If there were no broadening, enrichment (O) would be expressed by the formula

$$O = \left(\frac{\Gamma_0}{\Gamma}\right)^n \tag{1}$$

(where n is the number of acts of enrichment).

Broadening, however, restricts the magnitude of O to a certain limit (O_T) beyond which no further increase in n is justified.

Let τ be the time of residence of the band in the furnace. Then the band width μ, regardless of the initial width (if it is small enough) will be

$$\mu = 4\Gamma\sqrt{\left(\frac{\bar{D}}{\Gamma}\right)\tau} \tag{2}$$

where $\bar{D}$ is the effective longitudinal diffusion coefficient. $(\bar{D}/\Gamma)$ characterises a certain average value of this ratio at a time τ. If for the sake of simplicity it is assumed that the temperature rises in a linear fashion ($T = T_0 + \gamma t$) and the temperature dependence of D is neglected, then

$$\bar{\Gamma} = \frac{1}{t}\int_0^t \Gamma \mathrm{d}t \tag{3}$$

Since

$$\Gamma = \Gamma_0 \mathrm{e}^{-\sigma t}$$

where

$$\sigma = \frac{Q\gamma}{RT^2}$$

then

$$\bar{\Gamma} = \frac{\Gamma_0}{\sigma\tau}(1-\mathrm{e}^{-\sigma\tau})$$

and for sufficiently large values of τ,

$$\bar{\Gamma} = \frac{\Gamma_0}{\sigma\tau}$$

$$\mu = 4\Gamma\tau\sqrt{\frac{D\sigma}{\Gamma_0}} \tag{4}$$

and $O_T = \mu_0/\mu$. Here $\mu_0 = L\Gamma_0$ is the volume of the mixture injected, referred to unit area. Then

$$O_T = \frac{L\Gamma_0^{\frac{3}{2}}}{4\Gamma\tau\sqrt{D\sigma}} \tag{5}$$

It may be seen then that the method can achieve comparatively high enrichment. The result of the enrichment, however, is still inferior to that achieved with two acts (10^6) by the usual method.

It might appear that the method proposed here is of no essential interest since it only corresponds to two stages of the usual thermal enrichment. Such a view, however, is not justified. If the Γ_0 to Γ ratio is high enough then in the usual thermal

method of enrichment the same difficulties caused by broadening will arise and will not permit the second stage to be realised.

An experimental illustration of the method for two cases is now given. In the first case, the Henry coefficient is low, and it is therefore possible to realise a large number of enrichment stages. In the second case Γ is large, and enrichment equivalent to a small number of stages is achieved.

Enrichment achieved in the two cases is shown in Table I.

TABLE I

COMPARISON OF EXPERIMENTAL AND CALCULATED ENRICHMENT VALUES

Number of stage	*Chromosorb with PPMS concentration of propane 10^{-2} % (vol.)*		*SKT carbon concentration of propane $1.2 \cdot 10^{-6}$ % (vol.)*	
	Experimental enrichment	*Calculated enrichment*	*Experimental enrichment*	*Calculated enrichment*
I	4	4	$4 \cdot 10^3$	$4 \cdot 10^3$
II	14	16	10^4	$1.6 \cdot 10^7$
III	40	64		

The first case consists in propane enrichment on Chromosorb with 20% (by weight) of polyphenyl methyl silicone oil (PPMS). It is seen that the results of enrichment at all of the three stages closely approximate those calculated by the formula $O = (\Gamma_0/\Gamma)^n$.

The second case represents sorption of propane on SKT carbon. Here it can be seen that by the second stage poorer enrichment than that calculated is obtained, the same formula being used for calculation.

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CHROM. 5220

SOME NON-ANALYTICAL APPLICATIONS OF HIGH-PRESSURE GAS CHROMATOGRAPHY

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SUMMARY

Second interaction virial coefficients of more than sixty hydrocarbons and other organic compounds with nitrogen, argon and carbon dioxide have been defined by gas chromatography. The method of calculation of mixed second virial coefficients on the basis of retention indices measured at two pressures has been suggested.

For such cases when the dissolution of the carrier gas in the stationary liquid is noticeable, the interpolation method of mixed second virial coefficients definition has been suggested, including the use of two substances with molecular structure similar with the structure of the substances to be investigated as standards.

One of the most important applications of gas–liquid chromatography is the determination of the physical properties of gases and liquids. GOLDUP *et al.*[1] were the first to show the possibility of applying gas chromatography to the investigation of imperfections in gas mixtures. The change of retention corresponding to a change in the nature of the carrier gas was assigned to the gas imperfection. EVERETT[2,3] and YOUNG *et al.*[4–15] have calculated the second interaction virial coefficients and solute activity coefficients at infinite dilution on the basis of experimental net retention volumes measured at different pressures and with different carrier gases. The correlation between the solute distribution coefficients and the compressibility coefficient has been reported by KOBAYASHI *et al.*[16–23].

The purpose of this investigation is the chromatographic determination of the second mixed virial coefficients of interaction between the solute and carrier gas on the basis of chromatographic data obtained using packed and open tubular columns.

A more precise relationship for the specific retention volume calculation with the carrier gas compressibility correction may be written as

$$V_g = \frac{t_R - t_0}{g} \cdot v_a \cdot \frac{273.16}{T_p} \cdot (P_0 - P_{H_2O}) \cdot \frac{Z_p}{Z_0} \cdot \frac{J_3^2}{P_0} \quad (1)$$

where t_R and t_0 are the retention times of the solute investigated and the nonsorbing gas; g is the weight of the stationary liquid in the column; v_a is the carrier gas flow rate at the column outlet; T_p is the flow-meter temperature; P_{H_2O} is the saturated

vapour pressure at T_p; Z_p is the gas compressibility coefficient at column temperature and at pressure $P = P_0 \cdot J_2^3$, which is defined as $(PV/RT) = (B_{22}/RT)P_0J_4^5$; Z_0 is the carrier gas compressibility coefficient at the ambient temperature and the pressure P_0; V is the carrier gas molar volume, R is the gas constant, B_{22} is the second virial coefficient of the carrier gas, the pressure drop correction factor, in accordance with EVERETT's designation, $J_n^m = n/m \cdot [(P_i/P_0)^m - 1]/[(P_i/P_0)^n - 1]$; P_i is the inlet column pressure.

The specific retention volume is connected with the second mixed virial coefficient according to EVERETT[2,3] and YOUNG *et al.*[4-15] by the equation:

$$\ln V_g = \ln V_g^0 + \frac{2B_{12} - V_i^\infty}{RT} P_0 \cdot J_3^4 + \lambda \left(1 - \frac{\partial \ln \gamma^\infty}{\partial x}\right) P_0 \cdot J_3^4 \quad (2)$$

where V_g is the specific retention volume at the average column pressure $P_0 \cdot J_3^4$; V_g^0 is the specific retention volume of the same solute at the pressure extrapolated to zero; B_{12} is the second mixed virial coefficient of interaction of solute and carrier gas; V_i^∞ is the solute partial molar volume at infinite dilution; λ is the carrier gas molar solubility in the stationary liquid; $\partial \ln \gamma^\infty / \partial x$ corresponds to the alteration in the solute activity coefficient resulting from a change in the dissolved carrier gas molar fraction.

The log specific retention volume dependence on the average pressure is a straight line the slope of which is connected with the B_{12} value (if the carrier gas solubility in the stationary liquid is neglected). The distorting influence of the third virial coefficient is noticeable at higher pressures.

As shown in ref. 9, the use of different carrier gases permits one to carry out more precise extrapolation to zero pressure because the V_g^0 value is independent of the nature of the mobile phase and corresponds to the point of crossing of the straight lines which corresponds to the same solution but different carrier gas.

The direct second mixed virial coefficients calculation may be found according to the equation

$$-B_{12} = \frac{1.15\, RT\, \Delta \log_{10} V_g}{\Delta P} - 0.5\, V_i^0 \quad (3)$$

where $\Delta \log_{10} V_g$ is the difference of the logarithms of the solute specific retention volumes, corresponding to a pressure difference ΔP; V_i^0 is the molar volume of the liquid solute. Here $V_i^\infty = V_i^0$ is allowed. The error connected with this last assumption is about $\pm$ 3 cm³/mole according to GAINEY AND YOUNG[10].

The experiments were carried out with a gas chromatograph equipped with a flame ionisation detector.

A column, 150 cm long with an inner diameter of 3 mm, was packed with Chromosorb W coated with 30% of dinonyl phthalate. The oven temperature was controlled within $\pm$ 0.2°; the argon, nitrogen or carbon dioxide carrier gas flow rate was measured with an accuracy up to $\pm$ 0.5%.

As shown in Fig. 1, the deviation from the linear relationship of the retention volume logarithm *versus* the average pressure is very small and equal approximately to 0.005 log units for the hydrocarbons and other solutes investigated.

The calculated second mixed virial coefficient values are summarised in Table I which also shows B_{12} values reported in the literature for comparison.

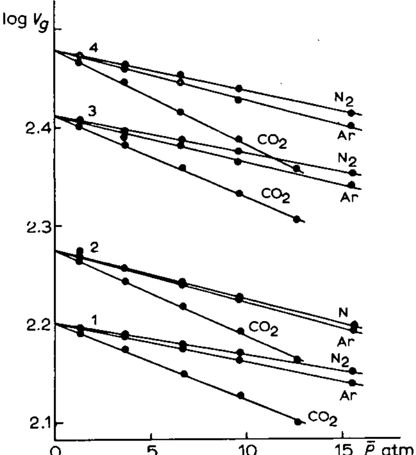

Fig. 1. Logarithm of the specific retention volume (at 80°) plotted *versus* the average column pressure of nitrogen, argon and carbon dioxide. 1 = dioxan, 2 = *n*-octane; 3 = toluene; 4 = butyl acetate.

TABLE I

EXPERIMENTAL SECOND INTERACTION VIRIAL COEFFICIENTS OF SOLUTE–CARRIER GAS MIXTURES ($-B_{12}$ cm^3/mole)

No.	*Solute*	*Carrier gas*					
		CO_2	*Ar*			N_2	
		80°	*50°*	*50°*[8, 13, 25]	*80°*	*80°*	*80°*[8,10,11]
1	*n*-Pentane	76	82	83 ± 20	68	60	49 ± 10
2	*n*-Hexane	147	106	107 ± 20	81	69	65 ± 10
3	*n*-Heptane	177	136	—	97	81	80 ± 10
4	*n*-Octane	227	160	—	122	98	95 ± 10
5	*n*-Nonane	249	181	—	146	117	—
6	*n*-Decane	—	—	—	—	130	—
7	Cyclohexane	163	102	—	86	63	50 ± 30
8	2,2,4-Trimethylpentane	147	128	—	87	47	—
9	Benzene	216	117	90 ± 10	105	74	72 ± 10
10	Toluene	235	135	—	120	94	—
11	Styrene	300	169	—	148	117	—
12	Pyridine	207	115	—	95	42	—
13	Nitromethane	188	91	—	62	41	—
14	Nitroethane	247	125	—	97	71	—
15	Chloroform	169	60	—	59	12	—
16	Carbon tetrachloride	154	115	—	67	24	—
17	Ethyl alcohol	95	118	—	46	10	—
18	Isopropyl alcohol	127	80	—	51	—	—
19	Acetone	129	—	—	58	—	—
20	Methyl ethyl ketone	184	86	—	65	36	—
21	Dioxan	136	124	—	86	67	—
22	Butyl acetate	260	138	—	119	79	—

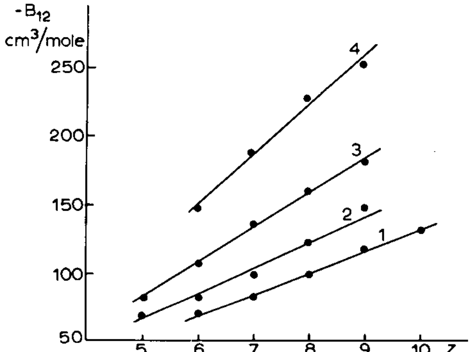

Fig. 2. Mixed second virial coefficient plotted *versus* *n*-paraffin carbon number. Carrier gases: 1 = nitrogen, 80°; 2 = argon, 80°; 3 = argon, 50°; 4 = carbon dioxide, 80°.

The relationship of the B_{12} values of *n*-paraffins *versus* carbon number is approximately a straight line (see Fig. 2).

It must be pointed out that an error of 1% taking place in the measurement of retention under our experimental conditions leads to a 10% error in the calculated value of the second virial coefficient. The error in B_{12} determination mainly depends on the experimental errors and the carrier gas solubility in the stationary liquid (the third term in the right part of eqn. 2).

In order to determine the B_{12} of a complex mixture of components when it is

TABLE II

SECOND INTERACTION VIRIAL COEFFICIENTS OF GASOLINE HYDROCARBONS–ARGON MIXTURES AT 25° CALCULATED ON THE BASIS OF PRESSURE DEPENDENCE OF RETENTION INDEX

No.	*Hydrocarbons*	$-B_{12}$ (*cm*^{3}/*mole*)	$-B_{12}$ (*cm*^{3}/*mole*)[25]
1	2,2-Dimethylbutane	108	115 ± 20
2	Cyclopentane	108	
3	2,3-Dimethylbutane	112	
4	2-Methylpentane	115	125 ± 20
5	3-Methylpentane	116	
6	Methylcyclopentane	127	
7	2,2-Dimethylpentane	128	
8	2,4-Dimethylpentane	130	
9	2,2,3-Trimethylbutane	127	
10	Benzene	112	
11	3,3-Dimethylpentane	132	
12	Cyclohexane	130	
13	1,1-Dimethylcyclopentane	134	
14	2-Methylhexane	137	
15	2,3-Dimethylpentane	135	
16	3-Methylhexane	138	
17	*cis*-1,3-Dimethylcyclopentane	136	
18	*trans*-1,3-Dimethylcyclopentane	136	
19	*trans*-1,2-Dimethylcyclopentane	136	

necessary to use open tubular columns, we attempted to measure B_{12} on the basis of retention indices because their values depend less on changes in the experimental conditions than the specific retention.

It may be shown that the change of the retention index due to a pressure change is

$$\frac{\Delta I}{\Delta P} = \frac{(I_1 - 100\,z)\,(\beta_{(z+1)} - \beta_z) - 100\,(\beta_x - \beta_z)}{-b_2} \tag{4}$$

where $\Delta P = P_2 - P_1$, $\Delta I = I_2 - I_1$, and I_1 and I_2 are the solute retention indices measured at the column pressure values of P_1 and P_2;

$$\beta_i = \frac{2B_{12i} - V_{1i}^{\infty}}{2.3\,RT};\; i = x,\, z,\, z+1;\; b_2 = \frac{\log_{10} V_{g(z+1)}}{\log_{10} V_{gz}}$$

is the logarithm of the relative retention of neighbouring n-paraffins at P_2, B_{12i} is the second mixed virial coefficient of interaction of i-th solute with the carrier gas. Eqn. 4 permits B_{12} values of solutes to be determined on the basis of known B_{12} values for n-paraffins.

The data obtained on an open tubular column coated with 1-octadecene when a gasoline fraction was separated were used for the calculation[24]. The $-B_{12}$ values in an argon media for n-pentane and n-hexane at 25° used corresponded to 98 and 124 cm^3/mol[25]. The second mixed virial coefficients calculated according to eqn. 4 are summarised in Table II, where for comparison the two isoparaffin data extracted from the literature are also given.

When the carrier gas solution in the stationary liquid is allowed for, the effective second virial coefficient values may be obtained from eqn. 2:

$$B_{\text{eff.}} = B_{12} + 0.5\,\lambda \left(1 - \frac{\partial \ln \gamma^{\infty}}{\partial x}\right) RT \tag{5}$$

The absolute value of $B_{\text{eff.}}$ is much less than the virial coefficient obtained with the same solute and same carrier gas but with a stationary liquid of very small solubilising power for the carrier gas. Therefore it is advisable that the virial coefficient calculation for a series of solutes with similar molecular structure be carried out on the basis of the known B_{12} values of two solutes of above group, because in this case the possibility of almost neglecting the effect of the third term of eqn. 3 occurs.

It is possible to calculate the second virial coefficients of solute–carbon dioxide mixtures on the basis of retention data of aromatics obtained with an open tubular column coated with polyethylene glycol 400 (pressure—up to 30 atm) using the B_{12} values of benzene and styrene as standards. The calculation was carried out according to equation

$$\frac{\Delta G}{\Delta P} = \frac{\beta_x - \beta_1 - G\,(\beta_2 - \beta_1)}{\log_{10}\,(t'_{R2}/t'_{R1})} \tag{6}$$

where

$$\Delta G = G_2 - G_1,\; \Delta P = P_2 - P_1,\; G = \frac{\log_{10} t'_{Rx} - \log_{10} t'_{R1}}{\log_{10} t'_{R2} - \log_{10} t'_{R1}},$$ β and t'_R

indices correspond to the solute investigated (x) and standards (1 and 2), the value G_1 corresponds to the pressure P_1 and G_2 and $\log_{10}(t'_{R2}/t'_{R1})$ to the pressure P_2,

TABLE III

SECOND INTERACTION VIRIAL COEFFICIENTS DETERMINED BY THE INTERPOLATION METHOD (eqn. 6)

Standard B_{12} values: Carbon dioxide carrier gas, benzene = 216 cm^3/mole, styrene = 300 cm^3/mole; argon carrier gas, benzene = 105 cm^3/mole, styrene = 148 cm^3/mole.

No.	*Solute*	*Ar*	*CO_2*	*No.*	*Solute*	*Ar*	*CO_2*
		$-B_{12}$ *(cm^3/mole)*	$-B_{12}$ *(cm^3/mol)*			$-B_{12}$ *(cm^3/mole)*	$-B_{12}$ *(cm^3/mole)*
1	Toluene	123	248	17	1,4-Diethylbenzene	168	342
2	Ethylbenzene	138	271	18	*n*-Butylbenzene	168	345
3	*p*-Xylene	143	284	19	1,3-Dimethyl-5-ethylbenzene	173	348
4	*m*-Xylene	141	282	20	1-Methyl-2-propylbenzene	171	327
5	Isopropylbenzene	148	286	21	1,2,3-Trimethylbenzene	165	335
6	*o*-Xylene	146	289	22	1,4-Dimethyl-2-ethylbenzene	174	348
7	*n*-Propylbenzene	152	292	23	1,3-Dimethyl-4-ethylbenzene	174	350
8	1-Methyl-3-ethylbenzene	154	309	24	1,2-Dimethyl-4-ethylbenzene	177	354
9	Iso-butylbenzene	159		25	1,3-Dimethyl-2-ethylbenzene	167	352
10	1,3,5-Trimethylbenzene	160	325	26	1,2-Dimethyl-3-ethylbenzene	168	349
11	*sec.*-Butylbenzene	160	312	27	1,2,4,5-Tetramethylbenzene	183	376
12	1-Methyl-2-ethylbenzene	158	310	28	1,2,3,5-Tetramethylbenzene	183	380
13	1,2,4-Trimethylbenzene	156	340	29	1,2,3,4-Tetramethylbenzene	178	386
14	1-Methyl-3-propylbenzene	167	328				
15	1-Methyl-2-isopropylbenzene	168	329				
16	1-Methyl-4-propylbenzene	169	318				

$t'_R = t_R - t_0$. The B_{12} values obtained are summarized in Table III. A similar calculation provided on the basis of retention indices gave results which differ from the literature data when they are compared with those measured above.

The relative $\partial \ln \gamma^\infty / \partial x$ value may be determined on the basis of the experimental data if one of the solutes investigated is used as a standard. If $\Delta B = B_{\text{eff.}} - B_{12}$, it is evident that

$$\Delta B_{\text{rel.}} = \left(1 - \frac{\partial \ln \gamma^\infty}{\partial x}\right)_{\text{rel.}} \tag{7}$$

The comparative evaluation of the effect of the solubility of the different carrier gases on the solute retention may be found in a similar way.

The values obtained for the second virial coefficients were used in order to make corrections for the gas phase imperfection during the investigation of the thermodynamics of the solution of various organic compounds in polar and nonpolar solvents.

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CHROM. 5219

GAS CHROMATOGRAPHY FROM THE STANDPOINT OF THE THEORY OF SETS

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SUMMARY

The relationship between chromatographic variables assuming a numbered set of rational values must be expressed in the form of Diophantic (undetermined) equations solved in rational numbers and having an infinite set of solutions (linear dependence, fractional linear dependence) as discussed in this paper. Chromatographic relationships satisfying this requirement are presented.

In recent years gas chromatography has developed at a remarkable rate. At present it is showing some tendency towards arranging the vast body of experimental and theoretical data available in some sort of system and verifying, in more detail the basic relationships and placing them on a firmer basis. When trying to prove the theory a particularly important role can be played by the methods of the theory of sets. Thus, it is desirable to use the theory of sets to establish and verify chromatographic relationships.

A set is a collection of elements possessing a certain common property. The sets can contain a finite number of members or an unlimited large infinite number of members. Two sets are considered equivalent if their elements can be placed in a mutually univocal correspondence. Numbered sets are equivalent to a set of numbers of a natural series or to a set of rational numbers, that is, all the members of a numbered set can be enumerated. Unnumbered sets are infinite sets of a higher power than the numbered ones.

Unnumbered sets are equivalent to a set of irrational numbers. The type of relationship between two physico-chemical (chromatographic) variables depends on the power of sets of values of variables. The power of sets of values of variables depends on the physico-chemical nature of the variable. Thus, *e.g.*, energy and related

values, because of the rule governing the quantification energy, must assume a numbered set of values. The values which are rational functions of energy values also assume a numbered set of values. A rational function is the ratio of polynomials with whole number coefficients. To find the power of a set of values of a variable, one should use the most general equations including this variable. In the thermodynamic equation $\Delta F = \Delta H - T\Delta S$, the energy values ΔF and ΔH assume the role of a numbered set of values, from which it follows that T and ΔS also must have a numbered set of values.

The partition coefficient depends on a change of free energy as shown by the following equation

$$\Delta F = RT \ln k$$

In this relation ΔF and T assume a numbered set of values, and $\ln k$ must also assume a numbered set of values.

The Kováts retention index is a fractional linear function of the logarithms of partition coefficients; the Kováts indices thus become a numbered set of values.

In the above examples the variables assuming a numbered set of values form particular types of rational functions (linear and fractional linear functions). As other examples of variables assuming a numbered set of values, time and space coordinates, and the number of carbon atoms in molecules of substances belonging to a homologous series may also be cited.

Concentration is an example of a variable assuming a unnumbered set of values. To demonstrate that concentration has an unnumbered set of values the equivalence of the sets of concentration values and of the real (irrational) numbers must be established.

Let us consider a flat square lattice, in the nodes of which the atoms or molecules of two kinds (A and B) are situated. By using the Cantor diagonal method all the nodes of the lattice can be enumerated. Let us now write the sequence of the numbers of the nodes: if there is an A atom in a node the number of the node in the sequence must be replaced by zero, while if there is an atom B in the node, the number is replaced by unity. In this way each pattern of atoms (or molecules) will be represented as a sequence of unities and zeros. A set consisting of a combination of zeros and unities corresponds to the whole set of concentration values. The collection of unities and zeros may be considered as a record of a real number in the dual system of calculus. The set of real numbers is unnumbered, hence the set of concentration values is unnumbered too.

It may be shown by the Diophantine analysis method[1] that, assuming a numbered set of values, the following relationships should exist between the variables:

Uni-univocal relations:

Non-uniform coordinates:

$$y = \frac{ax + b}{cx + d} \quad (1)$$

Uniform coordinates:

$$x_1 = ax_2 + b \quad (2)$$

Non-univocal relations:

$$y_1 = \frac{ax^2 + d}{bx^2 + c} \tag{3}$$

$$y_2 = \frac{fx + m}{bx^2 + c} \tag{4}$$

The relations are given with an accuracy to within projective transformations of variables.

The univocal relations between a numbered rational (x) and a real (y) variable are of the form: $y = e^x$ or $y = e^{Z(x)}$, where $Z(x)$ is a fractional linear function.

Eqns. 1–4 are Diophantine equations solved in rational numbers.

THE TEMPERATURE RELATIONSHIP

Since log V, log k, I, ΔI assume numbered sets of values, and the temperature dependence of these values is univariant, the temperature dependence of the logarithmic retention values (R) must appear as follows:

$$\log R = \frac{aT + b}{cT + d} \qquad \text{(Antoine's equation)}$$

This is confirmed in practice in chromatography.

RELATIONSHIPS IN HOMOLOGOUS SERIES

As the carbon number in molecules belonging to a homologous series of solutes assumes a numbered set of values, log $V(n)$ must be of the form:

$$\log V = \frac{an + b}{cn + d}$$

With $c = 0$ this dependence acquires a more usual form: $\log V = An + B$.

In recent years, several workers have found deviations from the simple linear relationship in homologous series. From the standpoint of the theory of sets a more general relation of the first type (eqn. 1) should be valid.

One of the authors here (YU.N.B.)[2] has made a study of the dependence of the difference of retention indices on polar and nonpolar phases (ΔI) in homologous series of solutes on the carbon number and found that this dependence follows relation 1.

PIEROTTI[3] and subsequently other workers have shown that the dependence of log k on the carbon number in homologous series of solvents or stationary phases is described by a fractional linear function of the number of carbon atoms in the solvent molecules.

The relationship between uniform quantities assuming a numbered set of values (*e.g.* logarithms of retention values of two solutes on several stationary phases, or logarithms of the retention values of a series of solutes on two stationary phases)

according to eqn. 2 must be a linear one. The methods of the theory of sets enable one to predict the type of relationship between the retention values and the physical properties, as well as between the heat and entropy of solution of substances in a sequence of solvents.

As an example of non-univocal relationships the equation describing the shape of a chromatographic peak may be considered. The vapour of the substance is passed through a tube filled with the sorbent. A system of coordinates moving along with the flow of the vapour of the substance is chosen. Let us assume that the continuous flow of the vapour of the substance, entering the column, consists of a set of narrow bands (zones) adjoining each other. The type of equation describing the shape of a narrow zone inside the column and the shape of the concentration front may be found. SCHAY[4] examined the shape of this zone by using labelled substances. Carbon dioxide was passed through a column packed with silica gel. A sample of radioactive carbon dioxide was injected into the column and registered at the column outlet by a radioactivity counter. In a common frontal experiment no separate zones, constituting the flow of vapour, are discerned, and only the concentration front is observed. If it is assumed that both ln c and t (flow coordinate) form a numbered set of values, then the shape of the front may be described by the following equation:

$$\ln c = \frac{bt^2 + C}{at^2 + d} \qquad \text{(S-shaped curve)}$$

while the shape of a narrow zone of vapour of the substance may be described by

$$\ln c = \frac{bt^2 + C}{ft} \qquad \text{(bell-shaped curve with a maximum)}$$

In elution analysis only a single narrow zone of concentrations is injected into the column, and this zone, after having passed through the column, is registered by the detector. Therefore the shape of the peak in elution analysis must be described by the last equation. This equation describes asymmetrical peaks.

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CHROM. 5215

A STUDY OF VARIABLE DIAMETER COLUMNS IN PREPARATIVE GAS CHROMATOGRAPHY

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SUMMARY

The operation of the variable diameter columns in the preparative gas chromatography has been studied. It has been shown that such columns are more efficient than those of constant diameter. To explain this effect the influence of the carrier gas flow rate and pressure upon the HETP and criterion of separation has been studied.

INTRODUCTION

In a great number of reports[1-6] concerning preparative gas chromatography, attention has been drawn to the increase of the height equivalent per theoretical plate (HETP) with an increase of column diameter and exactly the same unit load (*i.e.* the same sample volume per unit of cross-section or per cubic centimetre of sorbent) arising from the additional enlargement of the chromatographic band. Consequently, finding out methods for maintaining the efficiency of a separation after increasing the column diameters can be considered as one of the main tasks in preparative gas chromatography.

In considering this problem, a number of scientists[2,7-10] have made use of different redistributors (baffles, rings etc.), which made it possible to reduce the expansion of the chromatographic bands to some extent and, thereby, to increase the efficiency of the preparative columns. The results, obtained by PHILLIPS[11], of using columns of variable diameters in liquid chromatography, were taken as a basis. We decided to examine the possibility of using similar columns to increase the efficiency of the separation in preparative gas chromatography. Columns of such a type were used by WALKER[12] and FRISONE[9] for micropreparative separations as well as for defining mixtures. For instance, WALKER used columns of variable dimensions ranging from 100 to 600 cm in length and from 0.3 to 0.9 cm in diameter. In WALKER's work[12] attention is drawn to the great advantage of such columns when compared to columns of constant diameter.

Nevertheless, VERZELE[13] after having carried out an experiment with a great number of columns of variable diameters, achieved no advantage.

Up to now there is no common opinion in the literature about the possibility of increasing the efficiency of separation by such a method.

It was assumed that changes in the diameter of sections along the length of a sorbent would naturally cause changes in the distribution of the pressure of the carrier gas flow along the whole length of the column as well.

It is known[14–20] that pressure changes in a column greatly affect the efficiency of a separation, but data concerning this question are on the whole related to the analytical version. That is why FOWLIS *et al.*[17] proposed to work with a higher pressure than atmospheric at the inlet of the column. They carried out their experiments using an analytical column of 180 cm in length and 0.4 cm in diameter.

EXPERIMENTAL

We studied a series of columns of variable diameters and of approximately the same length (480–560 cm) containing sorbents of different volumes (1000–2200 cm^3). The columns under test were made up from sections 100–120 cm in length and 0.6, 1.2 or 2.6 cm in diameter.

The diameters of the sections in the columns decreased along the length from the inlet to the outlet. The list of columns of variable diameters, which have been studied by us is given in Table I.

TABLE I

SORBENT VOLUME (V, cm^3), MEAN CROSS-SECTIONAL AREA ($\bar{S}$, cm^2), VOLUME OF SAMPLE BEING INTRODUCED (V, ml) AND QUANTITIES CHARACTERISING THE CARRIER GAS FLOW RESISTANCE FOR COLUMNS OF VARIABLE DIAMETER

Dimensions of columns of variable diameter (size in cm)	V *(cm^3)*	$\bar{S}$ *(cm^2)*	V *(ml)*	$\bar{a}_0$ *(cm/sec)*	ΔP	j
500 × 1.2	550	1.13	0.08	0.74	1.06	0.64
480 × 2.6	2170	5.31	0.3	1.57	0.55	0.774
(120 × 2.6) + (400 × 1.2)	990	2.09	0.13	4.62	1.26	0.59
(240 × 2.6) + (300 × 1.2)	1530	2.99	0.17	3.68	1.13	0.62
(360 × 2.6) + (200 × 1.2)	1850	3.82	0.22	3.05	1.10	0.62
(360 × 2.6) + (200 × 0.6)	1680	3.51	0.20	3.99	1.83	0.49
(240 × 2.6) + (300 × 0.6)	1290	2.52	0.14	5.29	2.12	0.45

All the experimental data were obtained using a PACh V-04 preparative gas chromatograph constructed with a detector of the katharometer type for obtaining data on thermal conductivity. In all cases the temperature of thermostatic control was kept at 64°.

The columns were filled with TZK adsorbent (diatomite from the Zikeevskii Quarry) treated with a 2% solution of sodium carbonate and modified with 5% of liquid paraffin, the particle size of the sorbent being 0.2–0.4 mm. Hydrogen was used as carrier gas. A light distillate of straight-run gasoline of constant composition, received from the Novo-Gorkovskii refinery, was used as the test mixture. All the characteristics of separation were related to the peaks of *n*-pentane and isopentane

and the same specific volume of the sample related to 1 cm^3 of sorbent (Table I) was used in each case. The volume velocity of the carrier gas was measured at the column outlet with a flowmeter.

In order to compare columns of variable diameter, the dependence of the height equivalent per theoretical plate (HETP or H cm) and resolution value ($K_1 \simeq 0.5\ R$) on the mean linear velocity of the carrier gas flow have been studied.

The values under consideration were calculated according to well-known formulae.

In order to calculate the mean linear velocity of the carrier gas flow, the mean cross-sectional area of columns of variable diameter have been calculated according to the following formula:

$$\bar{S} = \frac{\sum\limits_{i=1}^{n} S_i L_i}{\sum\limits_{i=1}^{n} L_i} \qquad (1)$$

where S_i = the cross-sectional area and L_i = the length of every section.

The mean linear flow velocity at the outlet of the column was calculated according to the formula:

$$\bar{a}_o = v_o / \bar{S} \qquad (2)$$

where $\bar{a}_o$ = the mean linear flow velocity; and v_o = the measured volume velocity of the flow.

At the outset of our examination of columns of variable diameter, we expected a better efficiency on such columns as compared with the columns having just the same diameter as has been stated above, but the experimental data did corroborate this supposition.

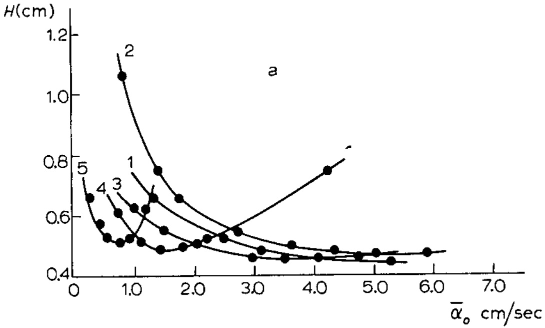
Unexpectedly, after having achieved the velocity corresponding to the minimum of the function $H(\bar{a}_o)$, when using columns of variable diameters (see the plots), any subsequent increase in velocity at the outlet did not give any increase in the efficiency, *i.e.* there was a plateau of function $H(\bar{a}_o)$, where the efficiency did not depend on the flow velocity, moreover, the minimum of the curve $H(\bar{a}_o)$ shifted to the region of large velocities. A similar plateau of velocities for the function $H(\bar{a}_o)$ has not been observed in this region of velocities (Fig. 1a) for columns of constant diameter.

This phenomenon is of great value in that it allows one, while not decreasing the efficiency of preparative chromatographic columns, to work with large velocities, and thus to increase the productivity of such a column by diminishing the time of separation.

If we consider the van Deemter equation with the correction for the pressure drop in the column:

$$H = A + B'/\bar{a} + C_j \bar{a} \qquad (3)$$

then the presence of plateau velocities for the function $H(\bar{a}_o)$ may be explained by a decrease in the C_j term in the above equation corresponding to a decrease in the j correction which, as can be seen from Table I, is smaller for columns of variable



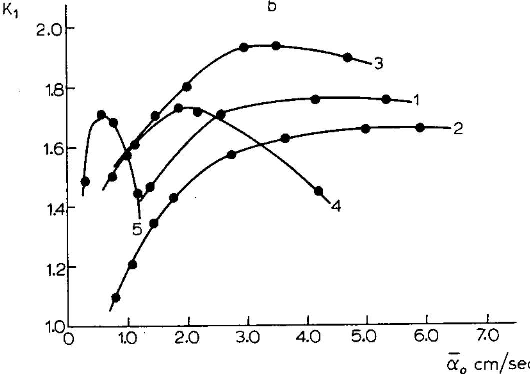


Fig. 1. Dependence of (a) height (H) equivalent per theoretical plate and (b) resolution value (K_1) on mean linear velocity ($\bar{a}_0$) of the carrier gas flow for columns of variable diameter with a constant ratio of the diameters of the sections. Columns sizes in cm: 1 = (240 × 2.6) + (300 × 1.2); 2 = (120 × 2.6) + (400 × 1.2); 3 = (360 × 2.6) + (200 × 1.2); 4 = (480 × 2.6); 5 = (500 × 1.2).

diameters than for columns of constant diameters. As the James–Martin correction for compressibility only accounts for the final pressure, it would be of great interest to consider the value of local velocity and local pressure along the length of a column of variable diameter. KEULEMANS[16] gives a formula whereby it is possible to calculate pressure at any point of a column of constant diameter:

$$P_x^2 = P_i^2 - X/L\,(P_i^2 - P_o^2) \tag{4}$$

where P_x = the pressure at any point; X = the distance from the beginning of the column to the point under consideration; L = the total column length; and P_i and P_o = the pressure at the inlet and outlet, respectively.

But this formula cannot be used for columns of variable diameters.

Using DARSI'S[21] equation:

$$V = -\frac{KS}{\eta} \cdot \frac{\mathrm{d}P}{\mathrm{d}X} \tag{5}$$

we have derived a formula from which the pressure can be calculated for the junction

point of the sections and then at any point in such a column:

$$P_c^2 = \frac{P_i^2 \cdot \frac{S_1}{l_1} + P_o^2 \cdot \frac{S_2}{l_2}}{\frac{S_1}{l_1} + \frac{S_2}{l_2}} \tag{6}$$

where P_c = the pressure at the junction of the two columns; P_i and P_o = the pressure at the inlet and outlet of columns of various diameters, and S_1; l_1; S_2; l_2 = the respective cross-sectional areas and lengths of the first and the second sections.

For calculating local velocities (α_x) of the carrier gas flow at any point in a column of various diameters a well known correlation has been used:

$$\alpha_x P_x S_x = \alpha_o P_o S_o \tag{7}$$

The alteration of local pressures and local velocities at different points in

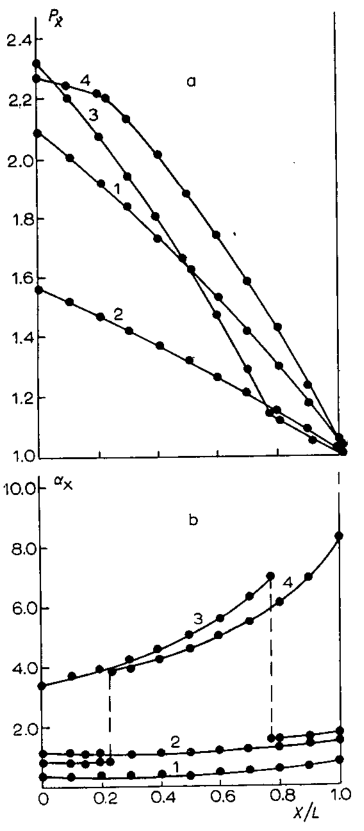


Fig. 2. Distribution of local (a) pressure (P_x) and (b) linear velocity (α_x) of carrier gas flow in columns of variable diameter. (X/L = ratio of the distance from the beginning of the column to any point X to the total length). Sizes of columns in cm: 1 = (500 × 1.2); 2 = (480 × 2.6); 3 = (400 × 1.2) + (120 × 2.6); 4 = (120 × 2.6) + (400 × 1.2).

columns of constant and variable diameters, which have been calculated according to the above formulas (4), (6) and (7), are shown in Figs. 2a and b.

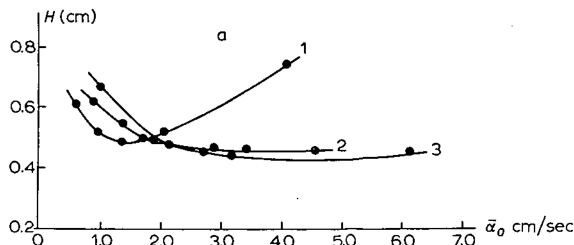
Such alterations in local pressures and velocities have been obtained for all the columns considered.

Let us consider the comparison of the efficiency and the resolution value for columns of variable diameter and for columns of constant diameter.

Figs. 1a and b show correlations for the efficiency and the resolution value which are the functions of the mean linear flow velocity of the carrier gas for columns of variable diameters, where the ratio of the lengths of the sections as well as the total sorbent volume are altered while the ratio of their diameters is maintained constant. Fig. 1a shows that the function $H(\bar{a}_0)$, for columns of variable diameter, has a plateau of velocities where the efficiency does not depend upon the velocity and is the same in this region for all the columns under consideration, whereas the resolution value decreases with the decrease in diameter at the end of the first section length (Fig. 1b).

The results given above have been fully corroborated when a series of columns of various diameters but having a different ratio of section diameters ($d_k = 2.6$ cm and $d_k = 0.6$ cm) was studied.

In addition, a series of columns of variable diameter, where the total length of the bed and the ratio of the section lengths have been constant, but where the ratio of their diameters and the total volume of sorbent have been altered, have been considered. The efficiency of such variable diameter columns was found to be the



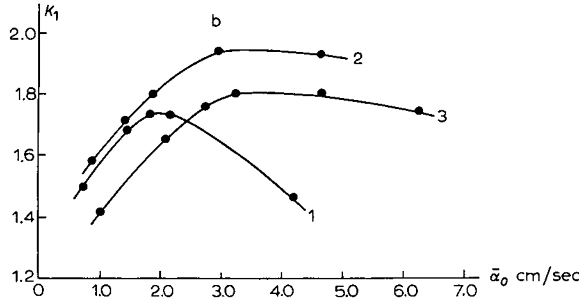


Fig. 3. Dependence of (a) height (H) equivalent per theoretical plate and (b) resolution value (K_1) on mean linear velocity ($\bar{a}_0$) of the carrier gas flow for variable diameter columns having different ratios of section diameter. Sizes of columns in cm: 1 = (480 × 2.6); 2 = (360 × 2.6) + (200 × 1.2); 3 = (360 × 2.6) + (200 × 0.6).

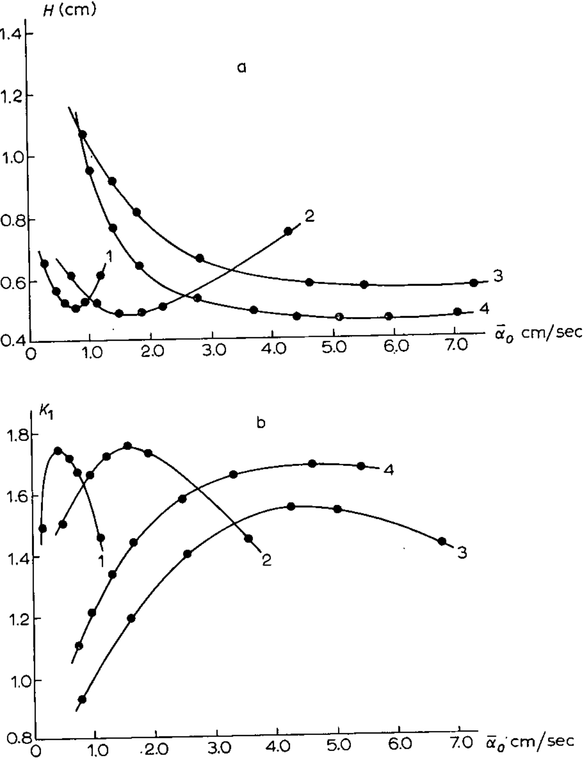


Fig. 4. Dependence of (a) height (H) equivalent per theoretical plate and (b) resolution value (K_1) on mean linear velocity ($\bar{a}_0$) of the carrier gas flow for variable diameter columns the sections being added in different orders in the chromatographic scheme. Sizes of columns in cm: 1 = (500 × 2.6); 2 = (480 × 2.6); 3 = (400 × 1.2) + (120 × 2.6); 4 = (120 × 2.6) + (400 × 1.2).

same for all columns (Fig. 3a), but the resolution value decreases with the decrease of the diameter of the final section (Fig. 3b).

Finally, columns of variable diameter having section lengths and diameters with the same ratio but only differing by the order in which the sections are introduced into the gas scheme of the chromatograph have been considered. From Figs. 4a and b it can be seen that there is a plateau of velocities for the function H ($\bar{a}_0$), where the efficiency does not depend upon the velocity, in both cases. Moreover, such a column is more efficient when the diameters of the sections decrease from the inlet to the outlet. Such a column also has a larger resolution value (Fig. 4b).

With regard to the plots of local pressure and velocity for all the columns of variable diameter (Figs. 2a and b) it may be assumed that formation of a plateau of velocities for the function H ($\bar{a}_0$), where the efficiency does not depend on the flow velocity of the carrier gas at the outlet of the column, is due to the sharp alteration in pressure at the junction of the sections created by the alteration of their diameters at these points. Furthermore, the larger the segment of the column, where the local velocity is left constant, the larger its efficiency and the resolution value.

When the ratio of the section lengths is the same, the ratio of their diameters

only affects the resolution value and has no effect on the efficiency of the columns (Figs. 3a and b).

In order to confirm previously quoted assumptions concerning the effect of pressure on the action of variable diameter columns additional experiments were carried out by introducing a gas resistor—a thin adjustable valve (throttle) at the outlet of the column.

To avoid the effect of the pressure drop along the column, an experiment was carried out on a short 120 cm × 2.6 cm column. Plots of efficiency against linear velocity of the carrier gas flow were obtained. The velocity was measured at atmospheric pressure after the throttle. The following columns were studied: (1) without the throttle; (2) with the throttle having a constant setting for the resistor; (3) with constant pressure at the outlet of the column.

On comparing the experimental data obtained, given in Figs. 5a and b, it may be seen that columns working under additional pressure at the outlet, are more

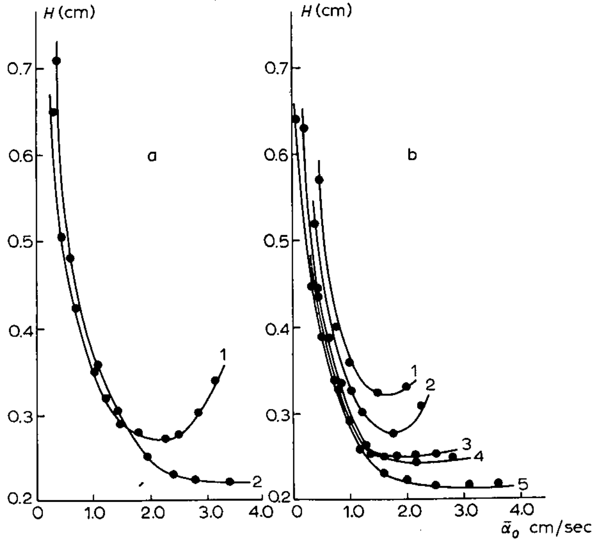


Fig. 5. Dependence of height (H) equivalent per theoretical plate upon linear velocity of carrier gas flow (α_0) for columns of constant diameter (120 × 2.6 cm): (a) 1 = without throttle; 2 = with throttle; (b) with constant pressure at the outlet; 1 = 1.5 atm; 2 = 1.75 atm; 3 = 2.75 atm; 4 = 2.25 atm; 5 = 2.5 atm.

efficient than columns working at atmospheric pressure. Furthermore, there is an optimum outlet pressure at which the column has its greatest efficiency. The pressure in this case is 1.5 kg/cm^2 higher than atmospheric (see Fig. 5b curve 5).

The results obtained on the 120 cm × 2.6 cm column were fully corroborated on the longer 480 cm × 2.6 cm columns. From Figs. 6a and b it may be seen that the column outlet pressure affects the efficiency as well as the resolution value of the given mixture.

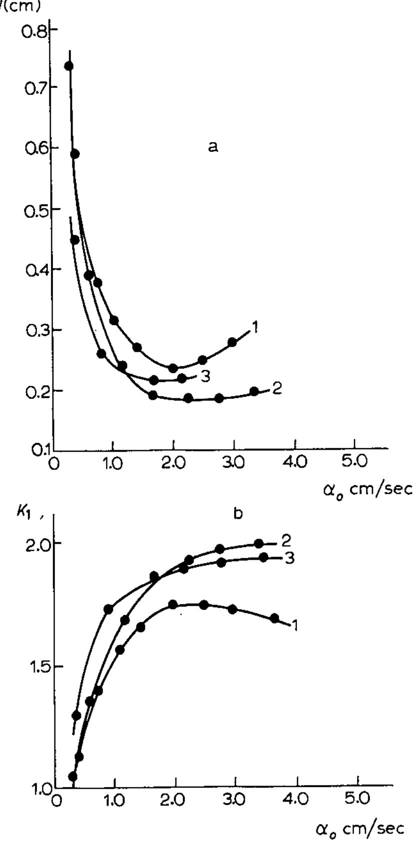


Fig. 6. Dependence of (a) height (H) equivalent per theoretical plate and (b) resolution value (K_1) on linear velocity of the carrier gas flow (a_0) for columns of constant diameter (480 × 2.6 cm): 1 = without throttle; 2 = with throttle set at a constant resistance; 3 = with constant pressure at the outlet.

CONCLUSIONS

1. A study of the effect of the carrier gas flow velocity and pressure upon the efficiency of columns of variable diameters has been carried out.

2. It was shown that when columns of variable diameter, as distinct from ordinary ones, are used the plot of the function H ($\bar{a}_0$) after reaching the minimum value for H value hardly changes on subsequent increase of the flow velocity ($\bar{a}_0$), in the region of velocities examined.

3. The distribution of local pressure and velocity along a column of variable diameter has been studied and a formula for calculating pressure at the junction of two sections has been derived.

4. It was shown that columns of large diameter acting at a higher outlet pressure than atmospheric are more efficient than similar columns working at atmospheric pressure. Moreover, there is an optimum value for the outlet pressure at which the efficiency of columns with a throttle is greatest.

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ANALYSIS OF TRACES OF CONTAMINANTS IN BREATHING OXYGEN WITH A HELIUM DETECTOR AND AN ELECTRON CAPTURE DETECTOR

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SUMMARY

A method is described which allows the determination of a few parts per billion of impurities in breathing oxygen for pilots and for therapeutic purposes. Fixed gases and chlorinated solvents are analysed by gas chromatography and detected with a helium detector and an electron capture detector, respectively. The analysis time is about 15 min.

The method can be applied to the analysis of air pollutants and to the determination of the purity of gases.

Very high purity is required for therapeutic oxygen for breathing and for liquid oxygen used for pilots of aircrafts and for the atmosphere in space capsules. Very pure oxygen must be used in these cases, as it has to be breathed by people that are in both critical health and environmental conditions.

The values for the threshold limits of contaminants in air[1–3] reported in Table I have to be drastically reduced, because of the possible synergistic effect of various contaminants, especially in unusual conditions. In this case, low concentrations of several impurities may have an effect greater than a higher concentration of a single contaminant.

For this reason, the limits allowed by the regulations are very small in comparison with the data reported in Table I. The maximum allowed concentrations of contaminants in aviator breathing liquid oxygen[4,5] are given in Table II.

Analysis of these contaminants has been carried out previously by several methods, and standard procedures have been developed. The combustible gases (methane and other hydrocarbons) were analysed by a flame ionisation detector, by means of direct injection of several ml of gas. The inert gases were analysed with a thermal conductivity detector, after concentration from several litres of oxygen by means of a cold trap[6]. The chlorinated compounds were analysed by IR analysis, using cells with an optical path[7,8] of 10 m.

All these systems have the disadvantage that several instruments are necessary for a complete analysis, and that the concentration of the impurities from a large volume of oxygen is very time consuming.

Gas chromatography provides a rapid separation and a sensitive means of

TABLE I

THRESHOLD LIMIT VALUES FOR CONTAMINANTS IN AIR

Contaminant	*Italy (p.p.m.)*	*U.S.S.R. (mg/m³)*	*U.S.A.*	
			(p.p.m.)	*(mg/m³)*
CO	50	20	50	55
CO_2	5000	—	5000	—
CH_4	—	—	1000	—
Hydrocarbons	500	100	500	—
Nitrogen oxides	5	5	5	9
CCl_4	10	20	10	65
$CHCl_3$	50 max.	—	50	240
$CHCl{=}CCl_2$	100	10	150	520
$CH_2Cl{-}CH_2Cl$	50	10	50	—
CH_2Cl_2	250	50	500	—

detection without need of concentration, if a helium detector (HeD)[9,10] and an electron capture detector (ECD) are used to measure the compounds separated.

Fig. 1 shows the practical arrangement of the analytical system. For complete separation three parallel columns were used:

(1) A Porapak Q column, 100–120 mesh, $\frac{1}{8}$ in. diameter and 3 m length, operated at room temperature with a helium flow of 60 ml/min, allowed the separation of CH_4, CO_2, N_2O, C_2H_2, C_2H_4, C_2H_6, C_3H_6 and C_3H_8, which were detected by a HeD.

(2) A molecular sieve column, Linde 5A, $\frac{1}{8}$ in. diameter and 6 m length, heated at 60°, with a helium flow of 60 ml/min, and connected to another HeD, separated H_2, N_2, CH_4 and CO.

TABLE II

MAXIMUM ALLOWED CONCENTRATION VALUES (IN P.P.M.) OF CONTAMINANTS IN BREATHING OXYGEN FOR PILOTS[4,5]

Contaminant	*Concentration*
CO	5
CO_2	5
CH_4	25
C_2H_2	0.05
C_2H_4	0.2
C_2H_6	2
Other hydrocarbons	1
N_2O	1
Cl solvents	0.1

(3) A column filled with 20% Apiezon L on Chromosorb W 80/100, $\frac{1}{8}$ in. diameter and 1.5 m length, heated at 60° and connected to an ECD, allowed the analysis of chlorinated hydrocarbons. The nitrogen flow was 20 ml/min.

The detectors and electrometers that were used were manufactured by Varian Aerograph, and the arrangement shown in Fig. 1 was obtained by partial modification

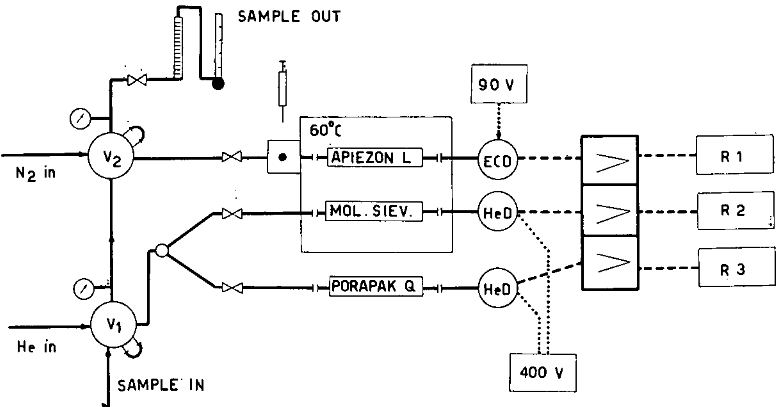

Fig. 1. Schematic drawing of the apparatus. V = sampling valves, R = recorders.

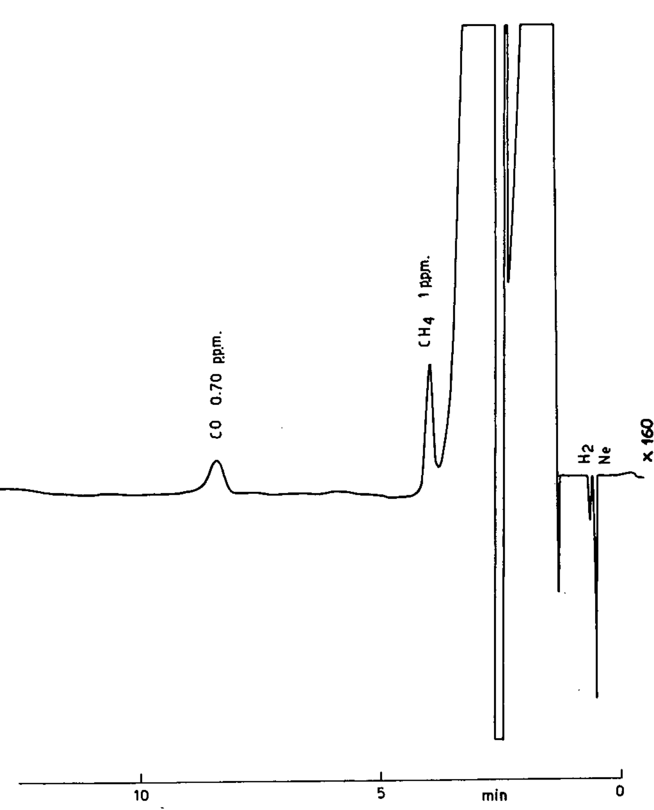

Fig. 2. Analysis on molecular sieves column; sensitivity 16×10^{-9} A/mV.

of a Trace Analyser Mod. 1532 by adding a third column with an injector and an ECD to the standard dual column system.

As helium detectors need very pure helium as carrier gas[10], a molecular sieve trap held at the temperature of liquid nitrogen was used for purification and was installed before the gas sample valve No. 1. The nitrogen for ECD was purified with a molecular sieve trap at room temperature to remove moisture and CO_2.

The two gas sampling valves, of the rotary type, were connected in series to the oxygen flow, and two precision gauges were used to monitor the actual pressure in the two sample loops. For proper quantitative analysis, a knowledge of the sample pressure is required. It is essential that the oxygen first flows through the valve connected to the helium detectors, because traces of helium that contaminate the oxygen flow when the valve is operated do not disturb the ECD. The reverse arrangement would introduce a certain amount of nitrogen into the oxygen flow that would be detected by the HeD.

The sample was introduced into the apparatus from a flow of oxygen with a pressure of 1 atm, and a volume of 1 ml was injected. Figs. 2–4 show different analyses on molecular sieve and Porapak columns. The sensitivity to impurities is very satisfactory.

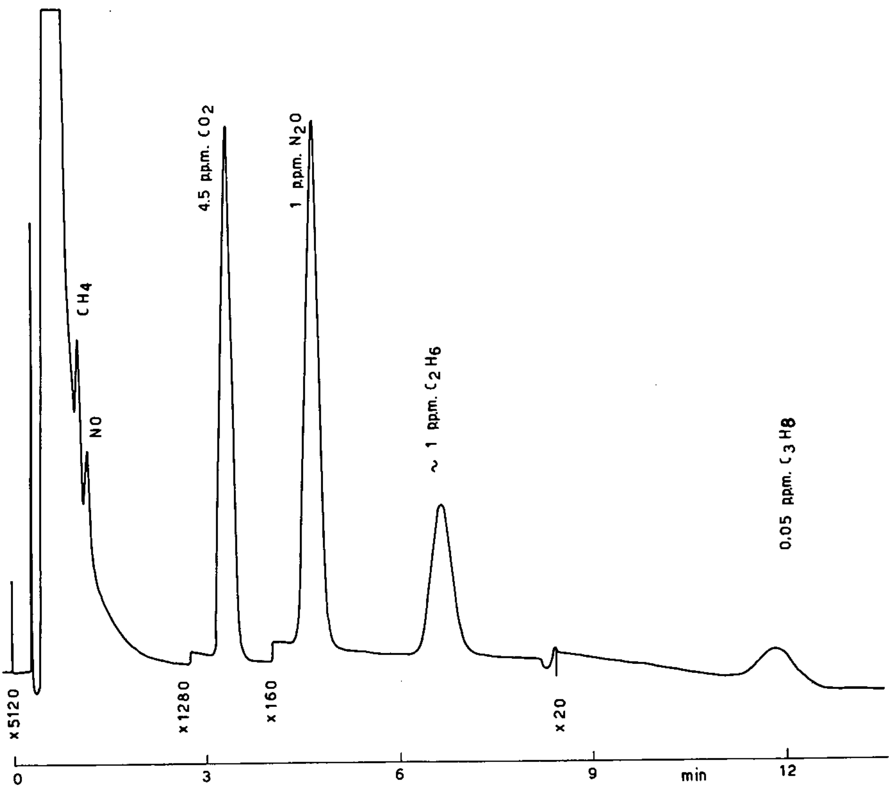

Fig. 3. Analysis on Porapak Q column. Standard mixture containing CO_2, N_2O, ethane and propane.

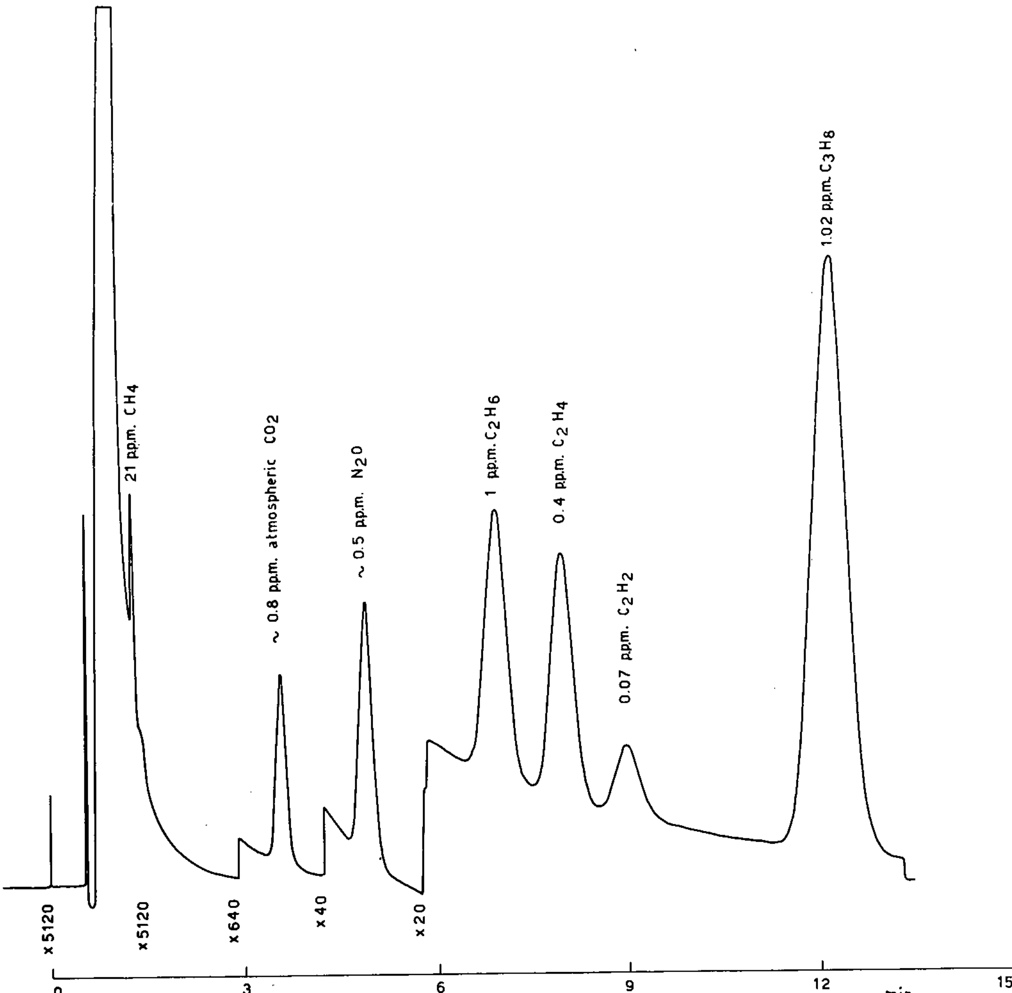

Fig. 4. Analysis on Porapak Q column. Standard mixture containing CH_4, N_2O, ethane, ethylene, acetylene and propane.

For calibration, standard mixtures, containing known amounts of contaminants diluted in oxygen, were used. This allowed an every day check on the performance of the instrument. The preparation of these mixtures was difficult, and the concentration changed over several weeks from the initial preparation, due to adsorption of contaminants on the inner surface of the vessel[11-13]. For this reason the composition of standard mixtures was tested by comparison with an absolute calibration, made with an exponential dilution flask[14,15]. Several calibration curves are reported in Fig. 5. The dilution flask was used both with pure helium and with the oxygen flow, to take into account during the calibration the effects on sensitivity due to the tailing of the oxygen peak. In fact, if the tail of the main peak is very long, it is impossible to reduce the attenuation of the amplifier sufficiently to detect the

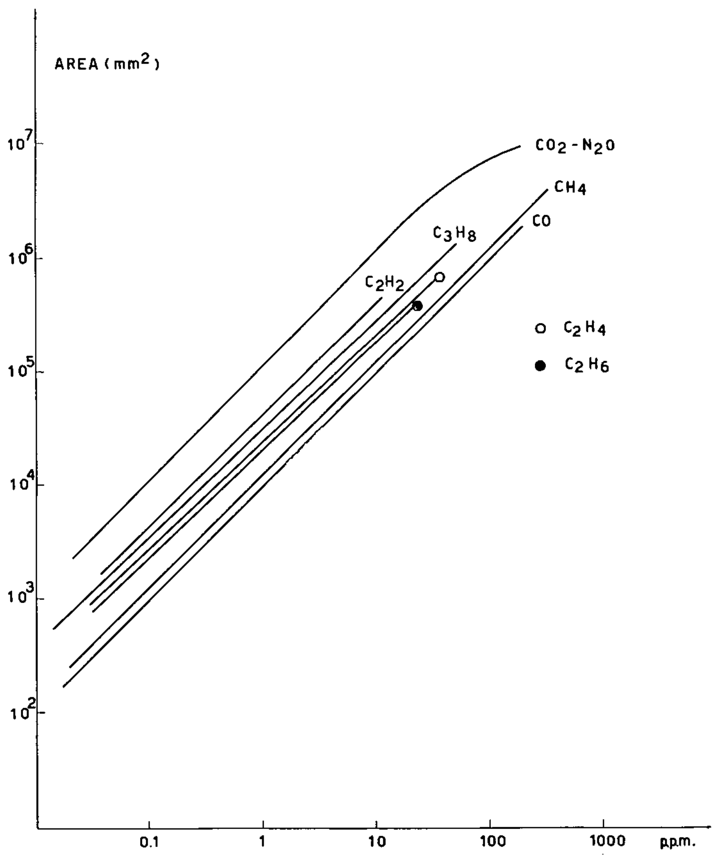

Fig. 5. Calibration curves obtained with the exponential dilution flask. Volume of the sample, 1 ml at atmospheric pressure; peak areas determined at maximum sensitivity (10^{-10} A/mV).

impurities, and a high sensitivity obtained during the calibration with the helium flow, does not mean that a similar sensitivity would be reached when the sample is mainly composed of oxygen. The true sensitivity of the instrument to a certain gas therefore depends on various factors: ionisation efficiency, sharpness of the peak, background and signal noise.

The sensitivity of helium detector to contaminants is given in Table III. Absolute values in coulombs per mole are reported. The observed current corresponds to an ionisation efficiency of 1.57–13.50% if one supposes that the ionisation of all the molecules of the eluted compounds gives 96 500 C per mole. Of course, the values reported in Table III do not represent the real efficiency of ionisation, as the collecting efficiency of the electrodes must be taken into account. Notwithstanding this, the sensitivity is very high, if one considers that the ionisation efficiency of a flame ionisation detector is about 0.0005% for hydrocarbons[16]. For practical purposes, the sensitivity can be expressed as mm^2 of peak area for a concentration of 1 p.p.m. of contaminant per ml of sample.

TABLE III

SENSITIVITY OF HELIUM DETECTOR TO CONTAMINANTS

	Response factor (C/mole)	*Ionization efficiency (%)*	*Peak area (mm^2/p.p.m.·ml)*
CH_4	1 510	1.57	12×10^3
C_2H_6	2 600	2.70	20.8×10^3
C_2H_4	2 780	2.88	23×10^3
C_2H_2	5 200	5.40	41.5×10^3
C_3H_8	3 800	3.95	30.6×10^3
CO	1 200	1.57	12×10^3
CO_2	13 200	13.70	105.5×10^3
N_2O	13 000	13.50	103.8×10^3

The separation of the chlorinated hydrocarbons was carried out on an Apiezon L column, as their separation on a Porapak column, though possible, was too long and not satisfactory. As the ECD tolerates a certain bleeding from the column, especially if the column is packed with a hydrocarbon stationary phase, gas–liquid chromatography was more convenient than gas–solid or gas–gel chromatography.

Fig. 6 shows the separation of chlorinated compounds in oxygen and in air. The sensitivity to the chlorinated compounds is given in Table IV, and it is interesting

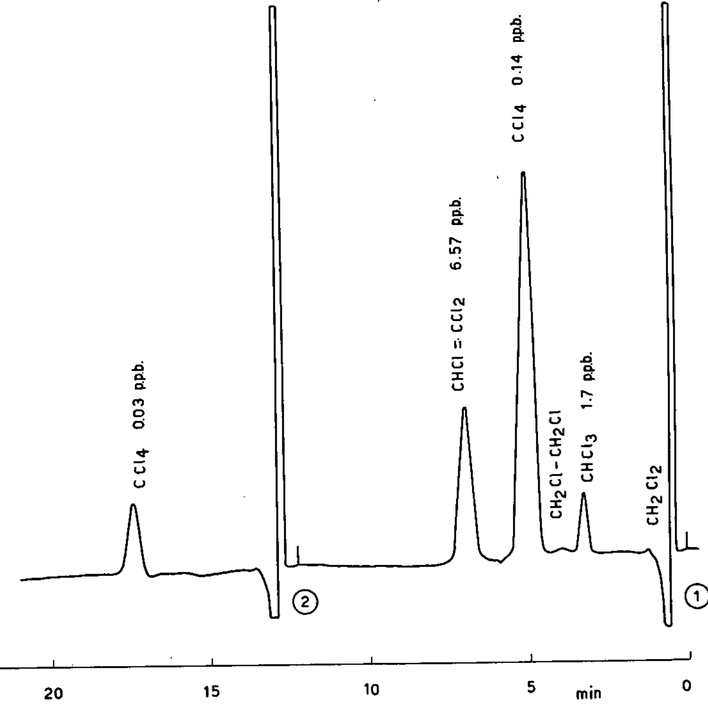

Fig. 6. Analysis of chlorinated compounds on an Apiezon L column with ECD. (1) in oxygen; (2) in air.

TABLE IV

SENSITIVITY OF THE ECD TO CHLORINATED SOLVENTS

On the recorder 1 mV full scale deflection, speed 20 in./h, a peak area of 1 mm^2 is equivalent to 0.56×10^{-10} C at an attenuation of $\times$ 20.

Contaminant	$C \cdot mole^{-1}$
CCl_4	3.00×10^5
$CHCl{=}CCl_2$	4.39×10^3
$CHCl_3$	3.28×10^3
$CH_2Cl{-}CH_2Cl$	3.53
CH_2Cl_2	2.84

to observe that the sensitivity is proportional to the number of chlorine atoms in the molecule. The calibration for the chlorinated compounds, due to the difficulties in preparation of a gaseous standard[13], was accomplished by injecting liquid standards with known concentrations of chlorinated compounds in pure *n*-hexane. As ECD is not so sensitive to contamination from air as the HeD, a conventional injector was installed between the gas sampling valve No. 2 and the column.

The sensitivity of the system to all the gases analysed is shown in Table V. The amount of contaminant that produces a peak area of about 50 mm^2 at the highest sensitivity is given. The values refer to a sample volume of 1 ml at atmospheric pressure. An increase of sensitivity may be achieved by using a greater sample volume and/or a higher pressure. Attention must be paid to the increase of the oxygen tail when increasing the quantity of sample. If the methane peak is overlapped by this tail, a still better separation can be obtained by using a longer molecular sieve column, but the time of analysis increases.

With the arrangement described, the whole analysis of a sample of oxygen for breathing or therapeutic use can be completed within 15 min. No interval is needed between the elution of the last peak (for example propane) and the introduction of

TABLE V

PRACTICAL LIMIT OF DETECTION (PEAK AREA ABOUT 50 mm^2) AT MAXIMUM SENSITIVITY FOR 1 ML OF SAMPLE AT ATMOSPHERIC PRESSURE

Contaminant	*Detection limit (p.p.b.)*
CH_4	4
C_2H_6	2
C_2H_4	2
C_2H_2	1
C_3H_8	1
CO	5
CO_2	0.4
N_2O	0.4
CCl_4	0.01
$CHCl{=}CCl_2$	1
$CHCl_3$	1
$CH_2Cl{-}CH_2Cl$	1000
CH_2Cl_2	1000

a new sample, but if the oxygen contains a lot of moisture, water eluting with a retention time of about 1 h from the Porapak column will saturate the corresponding HeD. It is convenient to make three analyses during the shortest possible interval and, when water begins to elute, to wait for a complete restoration of the initial baseline level.

The sensitivity data reported in Table V are very satisfactory if compared with the purity required by the regulations. The proposed method is therefore sufficiently sensitive and rapid for routine analysis of contaminants in breathing oxygen. It can obviously be applied to the analysis of contaminants in air or other gases, by proper selection of the column lengths and temperatures.

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CHROM. 5269

CORRELATIONS BETWEEN MOLECULAR STRUCTURE AND PHYSICAL PROPERTIES OF ALKYL IODIDES AND THEIR RETENTION INDICES

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SUMMARY

All the isomers of alkyl iodides with one to six carbon atoms were analysed by gas chromatography, and the retention indices relative to 1-iodoalkanes were calculated.

The relationship between molecular structure, iodine atom position, boiling point, density, molecular volume and retention index was investigated.

Correlations between the retention index and the boiling point were found, and a pronounced effect of the molecular volume was also observed for branched compounds with iodine substituted on the tertiary carbon atom or in the α position.

In these compounds the increase of molecular volume tends to compensate the decrease in the boiling point, giving very similar values of the retention index.

INTRODUCTION

The correlations between structure, physical properties and gas chromatographic (GC) retention data have considerable importance. In fact, if these relationships are known, the identification of unknown compounds can be made on the basis of the analytical parameters, without resorting to comparison with pure samples, which are often difficult to find.

The GC behaviour of several series of compounds was studied by us with different columns under carefully controlled conditions. The results were compared in order to obtain a general relationship. Here we shall describe the results obtained with all the isomeric alkyl iodides with 1–6 carbon atoms, and some longer straight-chain compounds, and the observed correlations between molecular structure, boiling point, molecular volume and retention index of these compounds.

EXPERIMENTAL

Many of the products analysed were prepared by synthesis[1].

The boiling points, at standard pressure, were determined by distillation and by the SIWOLOBOFF method[2]. When the determination of boiling points was not possible at atmospheric pressure, due to thermal decomposition, and reliable data

were not available in the literature, the values were calculated from the boiling points at reduced pressure by means of the equation

$$\Delta t = \frac{(273.1 + t)(2.8808 - \log p)}{\varphi + 0.15(2.8808 - \log p)} \qquad (1)$$

where Δt = °C to be added to the observed boiling point.
t = the observed boiling point.
$\log p$ = the logarithm of the observed pressure in torr.
φ = the entropy of vaporisation at 760 torr.

According to HASS AND NEWTON[3] the entropies of vaporisation were taken as equal to those of hydrocarbons with the same structure. Even if this assumption is not fully correct, the comparison between the values calculated in this manner, the boiling points determined at standard pressure, and the literature data was satisfactory in all the cases when these three values were available. The difference was never greater than 1°. The values obtained by means of eqn. 1 are indicated by* in Table I.

TABLE I

PHYSICAL CONSTANTS AND RETENTION INDICES OF ANALYSED COMPOUNDS

No.	*Compound*	T_b *(°C) at 760 torr*	*Density at 20° (g/ml)*	*Molecular volume (cm³)*	I_{ni}
1	Iodomethane	42.5	2.28	62.3	100
2	Iodoethane	72	1.93	80.81	200
3	1-Iodopropane	102.45	1.74	97.69	300
4	2-Iodopropane	89.45	1.70	99.99	245
5	1-Iodobutane	130	1.60	114.39	400
6	2-Iodobutane	120	1.598	114.54	359.5
7	1-Iodopentane	155.7*,[a]	1.51	131.16	500
8	2-Iodopentane	144.5	1.53	129.44	444
9	3-Iodopentane	145.5	1.52	130.30	458
10	1-Iodohexane	177	1.44	147.27	600
11	2-Iodohexane	165.6*	1.427	148.61	540
12	3-Iodohexane	167	1.45	146.25	540.5
13	1-Iodoheptane	198*	1.37	165.04	700
14	2-Iodoheptane	186.2*	1.38	163.85	636
15	3-Iodoheptane	187.4*	1.38	163.85	633.5
16	4-Iodoheptane	185	1.39	162.76	619.5
17	1-Iodo-2-methylpropane	120	1.61	113.68	360
18	2-Iodo-2-methylpropane	91.6*	1.55	118.08	271
19	1-Iodo-2-methylbutane	148	1.53	129.44	467
20	1-Iodo-3-methylbutane	147	1.51	131.16	451
21	2-Iodo-2-methylbutane	124.5	1.49	132.92	445
22	2-Iodo-3-methylbutane	141	1.503	131.77	444
23	1-Iodo-2-methylpentane	168	1.44	147.26	556
24	1-Iodo-3-methylpentane	170	1.46	145.24	561
25	1-Iodo-4-methylpentane	173.2	1.43	148.30	559
26	2-Iodo-2-methylpentane	142	1.41	150.40	538
27	2-Iodo-3-methylpentane	160*	1.45	146.25	541.5
28	2-Iodo-4-methylpentane	160	1.47	144.26	537
29	3-Iodo-2-methylpentane	147	1.31	161.88	536
30	3-Iodo-3-methylpentane	147	1.37	154.79	542

[a] Values marked * are extrapolated from low pressure data by means of eqn. 1.

The GC analyses were made on tricresyl phosphate columns, with a 15% loading on Chromosorb W 80–110 mesh, silanised with DMCS, using columns with I.D. of 2 mm, and length ranging from 1 to 3 m.

A Varian Aerograph gas chromatograph A-600 equipped with an electron capture detector was used. This instrument had an "all glass" arrangement to prevent decomposition of alkyl iodides during the analysis, and the injection of samples was made directly on to the column.

The retention values were calculated as adjusted retention times, by subtracting the gas hold-up time from the retention time of every substance.

RESULTS AND DISCUSSION

The compounds analysed, together with their physical constants and the retention indices relative to 1-iodoalkanes (I_{ni}) are listed in Table I. The use of the I_{ni} instead of the retention indices relative to normal paraffins (I_{np}) (ref. 4) is very convenient when analysing products with an electron capture detector, which is insensitive to saturated hydrocarbons[1,5].

The I_{ni} can easily be used instead of the I_{np}, due to the fact that the plots of log t_a as a function of the number of carbon atoms are parallel for *n*-paraffins and for 1-iodoalkanes, with the same column length and at the same temperature[5]. This parallel behaviour gives a linear relationship between the I_{ni} and the I_{np}.

TABLE II

COEFFICIENTS OF THE EQUATION $I_{np} = A_0 + A_1 \cdot I_{ni}$

	120-cm column			*300-cm column*	
	60 °C	*100 °C*	*120 °C*	*100 °C*	*120 °C*
A_0	573.23	573.53	561.99	579.56	564.12
A_1	0.9928	1.0208	1.0519	1.0135	1.0371

Table II gives the coefficients of the equation $I_{np} = A_0 + A_1 \cdot I_{ni}$ obtained by the least squares method from the values of I_{ni} and I_{np} for different analytical parameters.

The differences between the values of the angular coefficient A_1 are so small, and this coefficient is so close to 1, that for practical purposes one can calculate the I_{np} of every compound simply by adding A_0 to the value of I_{ni}. The A_0 can easily be determined for any column and analytical condition, by simultaneous injection of a few *n*-paraffins and 1-iodoalkanes.

An important characteristic of the I_{ni} is that δI_{ni} (*i.e.* the difference between the I_{ni} of the 1-iodoalkane and the I_{ni} of a branched isomer) is exactly the same as the δI_{np} (ref. 4) and can therefore be compared with the values of δI_{np} given by some authors for paraffins and other compounds[4,6].

Table II reports the boiling points at standard pressure for *n*-paraffins, 1-iodoalkanes and 2-iodoalkanes. As can be seen from the values of ΔT_b that decrease with

TABLE III

BOILING POINTS (T_b) OF n-PARAFFINS, 1-IODOALKANES AND 2-IODOALKANES WITH DIFFERENT NUMBER OF CARBON ATOMS (n) AND ΔT_b, THE DIFFERENCE BETWEEN THE T_b OF TWO MEMBERS OF HOMOLOGOUS SERIES

n	*n-Paraffins*		*1-Iodoalkanes*		*2-Iodoalkanes*		T_b (1-iodo-)– T_b (2-iodo-)
	T_b	ΔT_b	T_b	ΔT_b	T_b	ΔT_b	
1	−161.5	—	42.5	—	—	—	—
2	− 88.6	72.9	72.0	29.5	—	—	—
3	− 42.1	46.5	102.4	30.4	89.5	—	12.9
4	− 0.5	41.6	130	27.6	120	30.5	10
5	36.1	36.6	155.7	25.7	144.5	24.5	11.2
6	68.7	32.6	177	21.3	165.6	21.1	11.4
7	98.4	29.7	198	21	186.2	20.6	11.8
8	125.7	27.3	224	24	210	23.8	12
9	150.8	25.1	243	21	234.2	24.2	9
10	174.1	23.3	261	18	250.5	16.3	10.5
11	195.3	21.2					
12	214.8	19.5					
13	234	19.2					
14	252.5	18.5					

an increase in the number of carbon atoms, the increment of boiling point due to the addition of a methylene group is smaller when the length of the chain increases.

The ΔT_b have very similar values for 1-iodoalkanes and for 2-iodoalkanes and the differences between the T_b for these two series of compounds (last column of Table III) are practically constant, within the limits of experimental error.

Fig. 1 shows that the general behaviour of the retention indices closely follows the behaviour of the boiling points and that the retention times and the boiling points are higher when the iodine is in the first position, in comparison to the corresponding values when iodine is substituted on a carbon atom inside the chain.

The ratio $\delta I_{ni}/\delta T_b$ for 2-iodo- and 3-iodoalkanes is reported in Table IV.

TABLE IV

RELATIONSHIP BETWEEN THE DIFFERENCE OF THE BOILING POINTS OF ISOMERS (δT_b) AND THE DIFFERENCES IN THEIR RETENTION INDICES (δI_{ni})

The δ values are calculated from the 1-iodoalkane.

	δI_{ni}	δT_b	$\delta I_{ni}/\delta T_b$
2-Iodopropane	55	13	4.23
2-Iodobutane	40.5	10	4.05
2-Iodopentane	66	11.2	5.89
2-Iodohexane	60	11.4	5.26
2-Iodoheptane	64	11.8	5.42
3-Iodopentane	42	9	4.66
3-Iodohexane	59.5	10	5.95
3-Iodoheptane	66.5	10.6	6.27
4-Iodoheptane	81.5	13	6.27

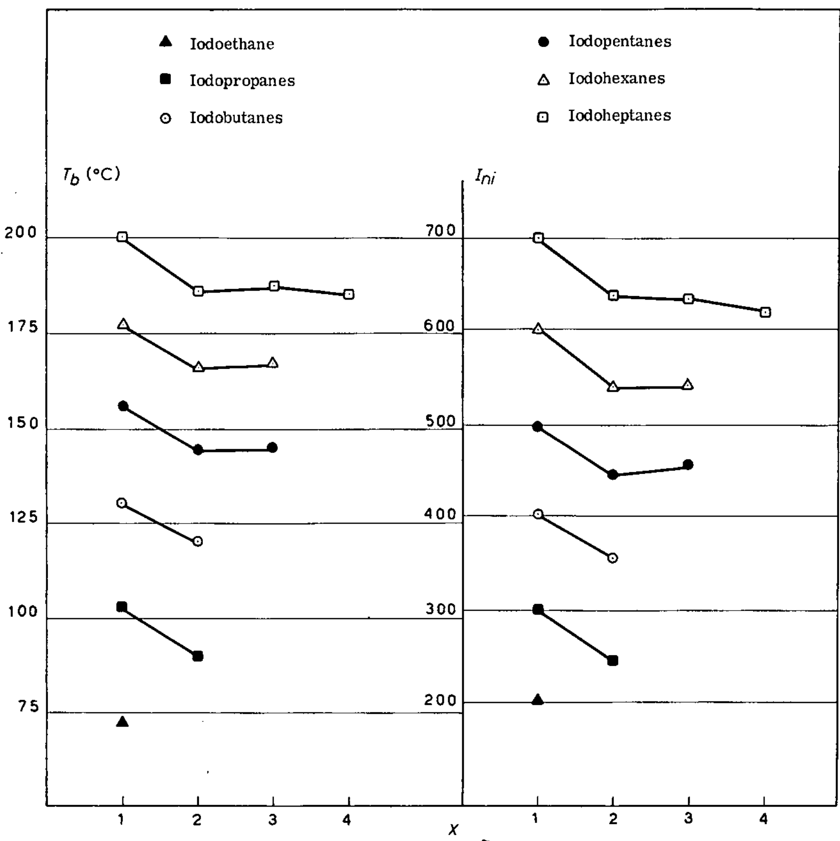

Fig. 1. Boiling point (T_b) and retention index (I_{ni}) of straight chain iodoalkanes as a function of the position of the iodine atom.

KOVATS[4] found that this ratio is approximately equal to 5, and the relationship was partially verified for straight-chain alkanes[6].

In this case the relationship seems to be verified for 2-iodoalkanes (an error of $\pm$ 0.3 units may be expected due to uncertainty in some boiling points) and this fact allows the calculation of the boiling points of higher homologues from the retention data. For 3-iodoalkanes the values of $\delta I_{ni}/\delta T_b$ show a slight increase with the number of C atoms in the molecule.

Molecular volumes for straight-chain iodoalkanes with 5, 6 and 7 carbon atoms have variations in the same sense as the boiling points and retention indices.

The molecular volumes given in Table I are obtained from the molecular weights divided by the density at 20°. Due to the dependence on temperature, these values are not valid at the temperature of analysis (100°) but it can be assumed that the increment is roughly proportional for all compounds and that the values are sufficiently exact for an indicative comparison.

When the iodoalkanes have a branched carbon chain, both retention indices and boiling points decrease with respect to the corresponding values for straight-chain compounds.

TABLE V

RELATIONSHIP BETWEEN THE δI_{ni} AND THE δT_b OF 1-IODO-X-METHYLALKANES

	δI_{ni}	δT_b	$\delta I_{ni}/\delta T_b$
1-Iodo-1-methylpropane	40.5	10	4.05
1-Iodo-2-methylpropane	40	10	4.00
1-Iodo-1-methylbutane	66	11.2	5.89
1-Iodo-2-methylbutane	33	7.7	4.28
1-Iodo-3-methylbutane	49	8.7	5.63
1-Iodo-1-methylpentane	60	11.4	5.26
1-Iodo-2-methylpentane	44	9	4.89
1-Iodo-3-methylpentane	39	7	5.53
1-Iodo-4-methylpentane	41	7.3	5.85

The values of δI_{ni}, δT_b and the ratio $\delta I_{ni}/\delta T_b$ for 1-iodo-X-methylalkanes, where X indicates the position of the methyl group, are reported in Table V. The values of $\delta I_{ni}/\delta T_b$ differ more than in the case of straight-chain compounds, but, as can be seen from Fig. 2, the general behaviour of the retention indices still follows the behaviour of the boiling points.

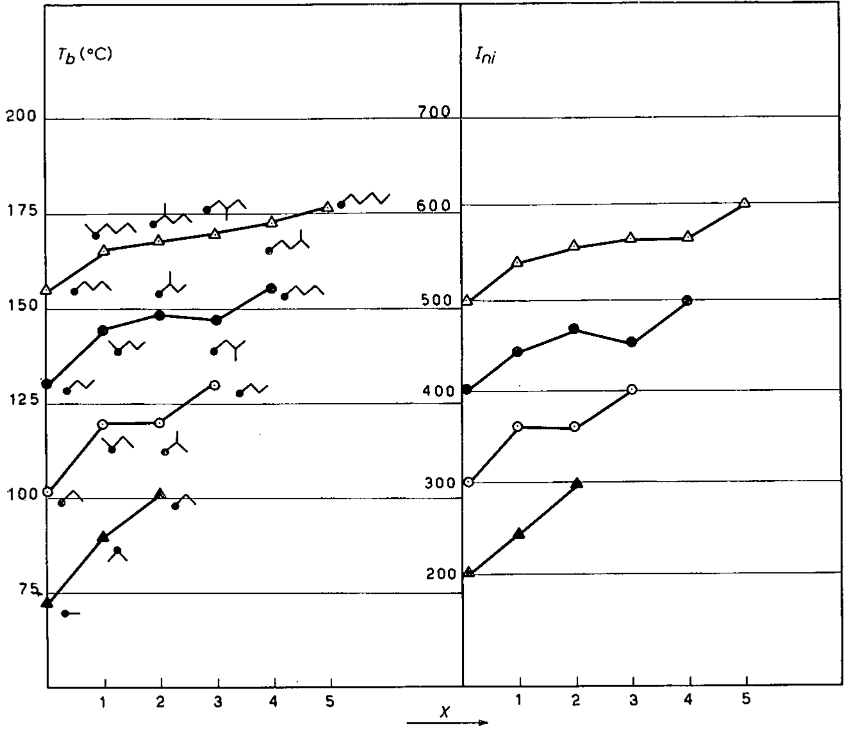

Fig. 2. Boiling point (T_b) and retention index (I_{ni}) of 1-iodo-X-methylalkanes, as a function of the value of X (position of the methyl group in the molecule).

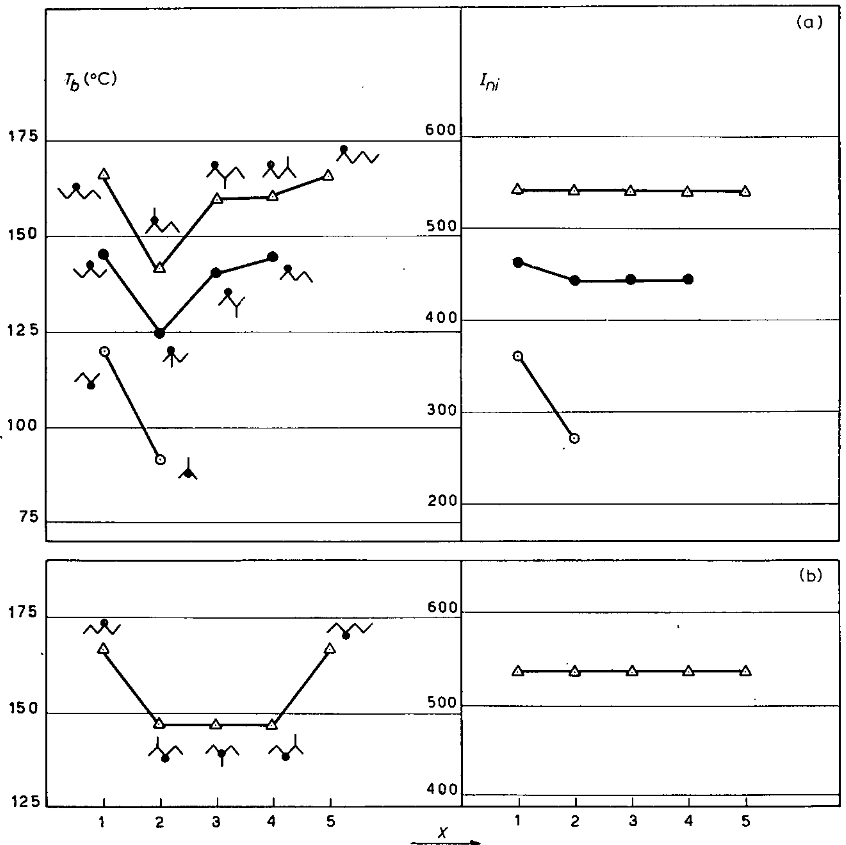

Fig. 3. Boiling point (T_b) and retention index (I_{ni}) of 2-iodo-X-methylalkanes (a) and 3-iodo-X-methylpentane (b), as a function of the position of the methyl group.

It must be mentioned that in Table V and in Fig. 2 and in all the following tables and figures, standard chemical nomenclature is not followed completely, in order to emphasise the fact that a compound belongs to a group with particular characteristics. For example, 2-iodohexane may be considered as 1-iodo-1-methylpentane, in order to compare its properties with those of similar products. For this reason, the straight-chain compounds with one less carbon atom are also reported in Fig. 3.

The behaviour of the 2-iodo- and 3-iodo-X-methylalkanes (see Table VI and Fig. 3) is quite different. The retention indices for isomeric compounds are very similar, but a sharp decrease is shown by the boiling points when both the iodine and the methyl groups are substituted along the chain. The minimum value is reached when the iodine and methyl group are on the same carbon atom in the 2-iodo-2-methylalkanes.

When the iodine atom is substituted in position 3, the methyl group in position 2 or 3 causes a sharp decrease of the boiling point without practically changing the retention index. It is very interesting to observe that the compounds which show this phenomenon and have a very low value of $\delta I_{ni}/\delta T_b$, such as 2-iodo-2-methyl-

TABLE VI

RELATIONSHIP BETWEEN THE δI_{ni} AND THE δT_b OF 2-IODO- AND 3-IODO-X-METHYLALKANES

	δI_{ni}	δT_b	$\delta I_{ni}/\delta T_b$
2-Iodo-1-methylpropane	40.5	10	4.05
2-Iodo-2-methylpropane	129	38.4	3.47
2-Iodo-1-methylbutane	42	9	4.66
2-Iodo-2-methylbutane	55	31.2	1.76
2-Iodo-3-methylbutane	56	16.7	3.35
2-Iodo-4-methylbutane	66	11.2	5.89
2-Iodo-1-methylpentane	59.5	10	5.95
2-Iodo-2-methylpentane	62	43	1.44
2-Iodo-3-methylpentane	53.5	17	3.15
2-Iodo-4-methylpentane	63	17	3.70
2-Iodo-5-methylpentane	60	11.4	5.26
3-Iodo-1-methylpentane	59.5	10	5.95
3-Iodo-2-methylpentane	64	30	2.13
3-Iodo-3-methylpentane	58	30	1.93

butane, 2-iodo-2-methylpentane, 3-iodo-2-methylpentane and 3-iodo-3-methylpentane, have molar volumes greater than those of the isomeric 1-iodoalkane (see Table I). In the case of 2-iodo-2-methyl and 3-iodo-3-methyl compounds this may be due to the steric hindrance of an iodine atom and three methyl groups on the same carbon atom.

In the case of 3-iodo-2-methylpentane a similar steric hindrance is given by two methyls on a carbon atom and an iodine atom plus an ethyl group on the next position. However, 2-iodo-3-methylpentane does not show the same behaviour notwithstanding the α position of the iodine atom with respect to a tertiary carbon atom.

From the point of view of the GC separation, it is clear that the increase of molar volume tends to compensate the decrease of boiling point, and the retention indices of isomeric compounds do not show such a large dependence on the structure. The phenomenon is probably complex, but it is interesting to observe that, by em-

TABLE VII

RELATIONSHIP BETWEEN THE δI_{ni} AND THE δT_b OF X-IODO-2-METHYLALKANES

	δI_{ni}	δT_b	$\delta I_{ni}/\delta T_b$
1-Iodo-2-methylbutane	33	7.7	4.28
2-Iodo-2-methylbutane	55	31.2	1.76
3-Iodo-2-methylbutane	56	16.7	3.35
4-Iodo-2-methylbutane	49	8.7	5.63
1-Iodo-2-methylpentane	44	9	4.89
2-Iodo-2-methylpentane	62	43	1.44
3-Iodo-2-methylpentane	64	30	2.13
4-Iodo-2-methylpentane	63	17	3.70
5-Iodo-2-methylpentane	41	7.3	5.85

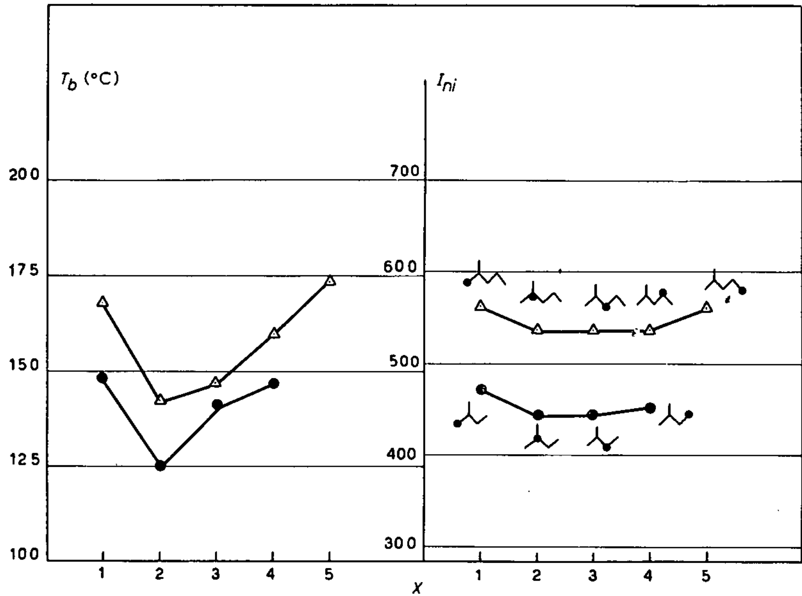

Fig. 4. Boiling point (T_b) and retention index (I_{ni}) of X-iodo-2-methylalkanes as a function of the position of the iodine atom.

pirically multiplying the boiling points by the molecular volume, values roughly proportional to the retention indices are obtained.

The retention index of 2-iodo-2-methylpropane decreases, according to its boiling point, and gives a higher value of $\delta I_{ni}/\delta T_b$. This behaviour may be due to its tetrahedral compact structure.

Table VII and Fig. 4 show the behaviour of compounds with a methyl group in position 2 and the iodine atom in different positions along the chain. It can be seen that the boiling points and the retention indices increase, when iodine is at the extremities of the chain and that boiling points reach their lowest value when the methyl group and the iodine atom are substituted on the same carbon atom or in an α position.

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CHROM. 5309

EVALUATION OF DYNAMIC GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF ADSORPTION AND SOLUTION ISOTHERMS

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SUMMARY

Various dynamic gas chromatographic methods for the determination of distribution isotherms in gas–solid and gas–liquid–solid systems are compared. The influence of the column length, the carrier gas velocity and the nature of the carrier gas is discussed. Isotherms of different shapes are studied. An equation for the calculation of distribution isotherms from gas chromatographic data is derived which applies not only at low mole fractions but also at higher mole fractions.

(1) INTRODUCTION

Various chromatographic methods for the determination of distribution isotherms have been described in the literature[1–46]. Sometimes gas chromatographic (GC) methods are the only means of obtaining these data under the given experimental circumstances. A common feature of these methods is speed. The accuracy can be high but it will depend on the method used as well as on the operating conditions due to the systematic error, which is introduced by the kinetic nature of the chromatographic process. No comparison of the various dynamic methods has been made on the basis of accuracy so far, mainly owing to the lack of suitable reference methods. In this paper non-equilibrium GC methods for the determination of distribution isotherms are compared with an equilibrium GC method. Although in principle the classical static volumetric and gravimetric methods for determining distribution isotherms can be used as references[8,9,13,18,20,41,42,45], these methods, when compared with the equilibrium GC method, have the disadvantages of being time consuming, having smaller temperature range and being less precise.

(2) THEORY

(2.1) The transport function in non-linear chromatography

Distribution equilibrium data can be obtained from the transport function, which describes the concentration as a function of place and time. The transport function can be derived from the mass balance in a column segment of length dz by

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combining the resulting mass balance equation with an equation relating the concentration in the fixed bed and fluid stream:

$$\frac{\partial\langle c_i^m\rangle}{\partial t} + \frac{(1-\varepsilon_m)}{\varepsilon_m}\cdot\frac{\partial\langle c_i^s\rangle}{\partial t} = -\frac{\partial\langle v\cdot c_i^m\rangle}{\partial z} + D_{im}^*\cdot\frac{\partial^2\langle c_i^m\rangle}{\partial z^2} \tag{1a}$$

$$\langle c_i^s\rangle = F(\langle c_i^m\rangle) \tag{1b}$$

where:

z = length coordinate
t = time
c_i^s = concentration of component i in the stationary bed s which is considered to be homogeneous
c_i^m = concentration of component i in the fluid stream m
v = fluid velocity
$\langle\ \rangle$ = average over the corresponding cross-sectional area
ε_m = fraction of cross-sectional area occupied by the fluid stream
$1-\varepsilon_m$ = fraction of cross-sectional area occupied by the stationary bed
D_{im}^* = dispersion coefficient of component i in the mobile phase due to diffusion and convection.

An expression for the mass transfer between the fluid stream and the fixed bed can be used for eqn. 1b. In this case the transport function can be derived[47–50] if the mass transfer equation is linear which implies a linear distribution isotherm (linear chromatography).

For a non-linear distribution isotherm (non-linear chromatography) a rigorous simplification is necessary in order to achieve a solution of eqn. 1. It is assumed that:

$D_{im}^* = 0$;
$\langle c_i^s\rangle = f^*(\langle c_i^m\rangle)$ = distribution isotherm for the fixed bed and the fluid stream;
$\langle v\rangle$ is constant;

so that eqn. 1 reduces to:

$$\left(1 + \frac{1-\varepsilon_m}{\varepsilon_m}\cdot\frac{d\langle c_i^s\rangle}{d\langle c_i^m\rangle}\right)\cdot\frac{\partial\langle c_i^m\rangle}{\partial t} = -\langle v\rangle\cdot\frac{\partial\langle c_i^m\rangle}{\partial z} \tag{2}$$

in which $d\langle c_i^s\rangle/d\langle c_i^m\rangle$ is the first derivative of the distribution isotherm. Eqn. 2 can be solved[51–53] for the case when the sample enters the column in a very short time, resulting in an equation for the migration velocity u_c of a concentration $\langle c_i^m\rangle$:

$$\left(\frac{dz}{dt}\right)_c = u_c = \frac{\langle v\rangle}{1 + \frac{(1-\varepsilon_m)}{\varepsilon_m}\cdot\frac{d\langle c_i^s\rangle}{d\langle c_i^m\rangle}} \tag{3}$$

From eqn. 3 an expression which describes the residence time t_c of the concentration $\langle c_i^m\rangle$ in the column can be derived:

$$\int_0^{t_c} \mathrm{d}t = t_c = \int_0^L \frac{\mathrm{d}z}{u_c} = \frac{L}{u_c} = \frac{L}{\langle v \rangle} \cdot \left(1 + \frac{(1-\varepsilon_m)}{\varepsilon_m} \cdot \frac{\mathrm{d}\langle c_i^s \rangle}{\mathrm{d}\langle c_i^m \rangle}\right) \tag{4}$$

in which L = length of the column.

The output function (elution function) describes the concentration in the column effluent as a function of time. The output function is determined by eqn. 4 and the condition[18]:

$$\int_0^{\langle c_i^m \rangle_L^{\max}} \langle c_i^m \rangle_L \cdot \mathrm{d}t = Q_i/w. \tag{5}$$

in which $\langle c_i^m \rangle_L^{\max}$ = maximum concentration of component i in the fluid stream at the column exit ($z = L$)

Q_i = amount of component i injected into the column

w = flow rate.

Eqns. 4 and 5 predict an elution peak with a sharp top, a vertical flank and a flank following eqn. 4.

(2.2) The transport function in linear chromatography with higher mole fractions

In the case of higher mole fractions the fluid velocity is influenced by the mass exchange between fluid stream and stationary bed[4,54–63]. This case can be treated if eqn. 1 is simplified by assuming:

$D^*_{im} = 0$ and $\langle c_i^s \rangle = K_i^* \cdot \langle c_i^m \rangle$ = a linear distribution isotherm, resulting in:

$$(1 + \kappa_i^*) \cdot \frac{\partial \langle c_i^m \rangle}{\partial t} = -\left(\langle v \rangle \cdot \frac{\partial \langle c_i^m \rangle}{\partial z} + \langle c_i^m \rangle \cdot \frac{\partial \langle v \rangle}{\partial z}\right) \tag{6}$$

with $\kappa_i^* = K_i^* (1 - \varepsilon_m)/\varepsilon_m$ = capacity ratio and where $K_i^* = \langle c_i^s \rangle / \langle c_i^m \rangle$ = distribution coefficient of component i between the stationary bed (s) and the fluid stream (m).

Substituting the mole fraction $\langle x_i^m \rangle$ for the concentration an expression for the migration velocity u_x of a mole fraction $\langle x_i^m \rangle$ can be derived for the case when the sample enters the column in a very short time:

$$u_x = \frac{\langle v \rangle + \langle x_i^m \rangle \cdot \dfrac{\mathrm{d}\langle v \rangle}{\mathrm{d}\langle x_i^m \rangle}}{1 + \kappa_i^*} \tag{7}$$

If eqn. 7 is used for the sample as well as for the eluent, a solution can be found which describes the dependence of the fluid velocity on the mole fraction and the capacity ratio of the component:

$$\langle v \rangle = \frac{\langle v \rangle_0}{1 - \dfrac{\kappa_i^*}{1 + \kappa_i^*} \cdot \langle x_i^m \rangle} \tag{8}$$

where $\langle v \rangle_0$ = fluid velocity in absence of component i.

Substitution of eqn. 8 in eqn. 7 gives the final expression for the migration velocity of a mole fraction $\langle x_i^m \rangle$:

$$u_x = \frac{\langle v \rangle_0}{(1 + \overset{*}{\kappa_i}) \left(1 - \frac{\overset{*}{\kappa_i}}{1 + \overset{*}{\kappa_i}} \cdot \langle x_i^m \rangle\right)^2} \tag{9}$$

From eqn. 9 an expression for the residence time t_x of a mole fraction $\langle x_i^m \rangle$ in the column can be derived:

$$t_x = \frac{L}{u_x} = \frac{L}{\langle v \rangle} \cdot (1 + \overset{*}{\kappa_i}) \left(1 - \frac{\overset{*}{\kappa_i}}{1 + \overset{*}{\kappa_i}} \cdot \langle x_i^m \rangle\right)^2 \tag{10}$$

Eqn. 10 determines the output function at higher mole fractions of component i in the fluid stream. At low mole fractions eqn. 10 approaches eqn. 4 taking into consideration that for a linear distribution isotherm $d\langle c_i^s \rangle / d\langle c_i^m \rangle = \overset{*}{K_i}$. Equations[4,59] slightly different from eqn. 10 can be obtained as a result of a more simplified treatment of the problem.

(2.3) Influence of the heterogeneity of the stationary bed

In the theoretical model the stationary bed is assumed to be homogeneous. In practice however the stationary bed is far from homogeneous. A chromatographic column in general consists of a stationary bed of porous particles and a fluid flowing through the space between the particles. The porous particles which together with the column wall form the stationary bed consist of a solid matrix and pores filled with either mobile phase (gas or liquid) or stationary liquid or both. A packed column contains pores of two sizes: wide inter-particle pores in which flow occurs and narrow intra-particle pores in which no flow occurs. In the theoretical model the distribution of the sample between the fluid stream on the one hand and all of the stationary phases on the other hand is considered. Leaving out the bulk of solid matrix, which does not absorb the sample, the following stationary phases can take part in the distribution process:

(a) the stagnant part of the mobile fluid phase in the intra-particle pores
(b) the stationary liquid in the intra-particle pores;
(c) the surface of the solid matrix; and
(d) the interphase of the mobile fluid and stationary liquid.

Accordingly the total differential change $(1 - \varepsilon_m) \cdot d\langle c_i^s \rangle$ of the amount of a component in the stationary bed per unit of volume of the column equals the sum of the partial differential changes in the various stationary phases. By substituting the corresponding sum: $(\varepsilon_\alpha - \varepsilon_m) \cdot dc_i^\alpha + \varepsilon_\beta \cdot dc_i^\beta + a_\sigma \cdot dc_i^\sigma + a_\tau \cdot dc_i^\tau$ for $(1 - \varepsilon_m) \cdot d\langle c_i^s \rangle$ in eqns. 3 and 4 the migration velocity and residence time of a concentration c_i^α are described as functions of the equilibrium distribution isotherms for the various stationary phases and the mobile phase:

$$u_c = u_0 / \left(1 + \frac{\varepsilon_\beta}{\varepsilon_\alpha} \cdot \frac{dc_i^\beta}{dc_i^\alpha} + \frac{a_\sigma}{\varepsilon_\alpha} \cdot \frac{dc_i^\sigma}{dc_i^\alpha} + \frac{a_\tau}{\varepsilon_\alpha} \cdot \frac{dc_i^\tau}{dc_i^\alpha}\right) \tag{11}$$

$$t_c = t_{R_0} \cdot \left(1 + \frac{\varepsilon_\beta}{\varepsilon_\alpha} \cdot \frac{dc_i^\beta}{dc_i^\alpha} + \frac{a_\sigma}{\varepsilon_\alpha} \cdot \frac{dc_i^\sigma}{dc_i^\alpha} + \frac{a_\tau}{\varepsilon_\alpha} \cdot \frac{dc_i^\tau}{dc_i^\alpha}\right) \tag{12}$$

where:

$u_0 = \langle v \rangle \cdot \varepsilon_m / \varepsilon_\alpha$ = migration velocity of the mobile phase
ε_α = fraction of the column volume occupied by the mobile phase
ε_β = fraction of the column volume occupied by the stationary liquid
$c_i^\alpha = c_i^m$ = concentration of component i in the mobile phase α, which consists of the fluid stream and possibly a stagnant film
c_i^β = concentration of component i in the stationary liquid β
a_σ = ratio between the surface area of the solid matrix and the total volume of the column
c_i^σ = surface concentration of component i on the surface σ of the solid matrix
a_τ = ratio between the interphase area of the fluid phases and the total column volume
c_i^τ = surface concentration of component i in the interphase τ of the fluid phases
$t_{R_0} = L/u_0$ = retention time of the mobile phase.

Since the equilibrium concentrations in the different phases are considered the symbol $\langle\ \rangle$ for the average value may be omitted.

Often only one of the stationary phases takes up a significant part of the distributed sample. Therefore the complex distribution may be considered as a simple two phase distribution, which can be the distribution between either a stationary liquid phase or the solid surface of the matrix and the mobile phase. In this case only the corresponding terms of eqns. 11 and 12 are significant and the others may be neglected.

(3) CHARACTERIZATION OF THE GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF DISTRIBUTION ISOTHERMS

The distribution isotherm is determined point by point. It is assumed that the sample compound is distributed between a single stationary phase and the mobile phase. The concentration in the stationary phase is calculated[9,11,18] from an area on the recorder chart:

$$\overline{c_i^s} = \frac{A}{S \cdot V_s} \tag{13}$$

in which:

$\overline{c_i^s}$ = average equilibrium concentration in the stationary phase; an average value for the whole column is obtained because of the gas compressibility (see 5.2).
A = area on recorder chart according to Figs. 1 and 2.
S = sensitivity, defined as the area on the recorder chart divided by the corresponding amount of component i.
V_s = volume of the stationary phase.

The concentration in the mobile phase can be calculated from the recorder deflection:

$$\overline{c_i^m} = \frac{y \cdot \omega}{\overline{w} \cdot S} \tag{14}$$

in which:

$\overline{c_i^m}$ = average equilibrium concentration in the mobile phase; the average value for the whole column has to be calculated since the average concentration in the stationary phase has been obtained by eqn. 13
y = recorder deflexion
ω = recorder chart speed
$\overline{w}$ = average flow rate of carrier gas in the column.

The sensitivity S can be determined from the area of a chromatographic peak when the corresponding amount of component i is known, as well as from the recorder deflection obtained with a known mass flow of the component.

(3.1) Nonequilibrium methods

(3.1.1) Peak maxima method[26,33,45] (Fig. 1). A series of chromatograms, obtained by injecting various amounts of a component i, is produced in order to determine c_i^s as a function of c_i^m at constant temperature. If it is assumed that eqn. 4 can be applied to the locus of the peak maxima, c_i^s and c_i^m can be calculated from eqns. 13 and 14.

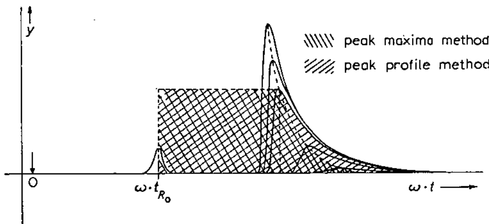

Fig. 1. Types of chromatograms used in the peak maxima and peak profile methods for the GC determination of distribution isotherms.

(3.1.2) Peak profile method[4,10,11–14,18,19,21,27–29,32,33,41–43,45,46] (Fig. 1). This method is similar to the peak maxima method, however the distribution isotherm is now determined from a single chromatogram. Either the peak flank at the front or at the rear, depending on which of the two is flatter, is assumed to be described by eqn. 4. The isotherm can then be calculated by means of eqns. 13 and 14 from points on the appropriate boundary.

(3.1.3) Step profile method [1–3,5,9,15,17,22,23,32,34,35,37–40,43] (Fig. 2). In this method carrier gas, containing the vapour of the component for which the distribution isotherm is to be determined, and pure carrier gas are alternately led into the column by means of a switching device. Switching results in the formation of positive or negative steplike concentration changes. Points on either the boundary resulting from the positive steplike concentration change or the boundary resulting from the negative steplike concentration change, depending on which of the two is flatter, are used to calculate the distribution isotherm, using eqns. 13 and 14 and assuming eqn. 4 to hold.

(3.1.4) The minor disturbance method[24, 25, 30, 36, 43, 44] (Fig. 2). Into the vapour stream of a component, as is used in (3.1.3) and (3.2), a very small amount of the component is injected. The resulting peak maxima at various levels of vapour content of the carrier gas are processed as in the peak maxima method (3.1.1). At high relative vapour pressures precautions must be taken to overcome the effect of condensation on the needle of the syringe.

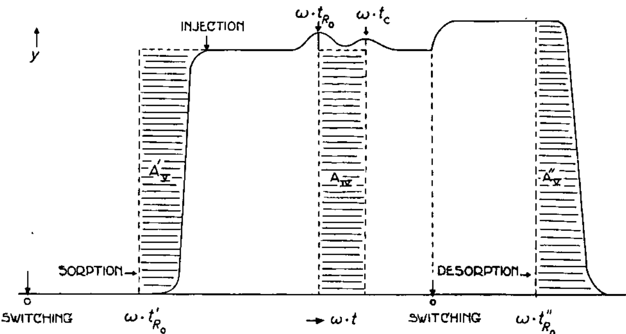

Fig. 2. Types of chromatograms used in the step profile, minor disturbance and equilibrium methods for the GC determination of distribution isotherms.

(3.2) Equilibrium method[6–9, 16, 20, 31, 43] (Fig. 2)

Just as in the step profile method carrier gas, containing vapour and pure carrier gas, are alternately led into the column until equilibrium is achieved, resulting in chromatograms of the type shown in Fig. 2. The areas A′ and A″ correspond to the amount which is sorbed and desorbed respectively. The area between the point of switching and $\omega \cdot t_{R_0}$ on the recorder corresponds to the amount of the vapour present in the mobile phase in the column. The retention times t_{R_0}' and t_{R_0}'' are determined with a non-retarded component. At the switching point for desorption a step is observed if a change in the flow rate occurs. By varying the vapour content of the carrier gas a series of chromatograms of the type shown in Fig. 2 is obtained. This can then be processed according to eqns. 13 and 14 to give the distribution isotherm.

(4) APPARATUS

(4.1) Construction

In order to perform the various dynamic GC methods on one and the same column under exactly the same experimental conditions the set-up as shown in Fig. 3 was devised.

The carrier gas flow is regulated by a pressure control valve (Fairchild Hiller Kendall 30) and a mass flow controller (Brooks, model 8743).

R_1 and R_2 are two matched restrictions consisting of stainless steel capillary tubing, 0.1 mm internal diameter and 5 m length.

The column C_l contains the liquid, the vapour of which is led to the column C_x in which the adsorption or solution of the vapour will be measured. The column C_l consists of a copper tube, 1 m length and 4 mm I.D., packed with a solid support (Chromosorb W, 120–130 μm) coated with the liquid to 30–40% w/w.

Either a thermostat (Becker 1452 P), when working at temperatures above 40°, or a cryostat (Haake T 21), when working at temperatures below 40°, is used to control the temperature of C_i, depending on the volatility of the vapour and the sensitivity of the detector. Temperature control is within 0.2°.

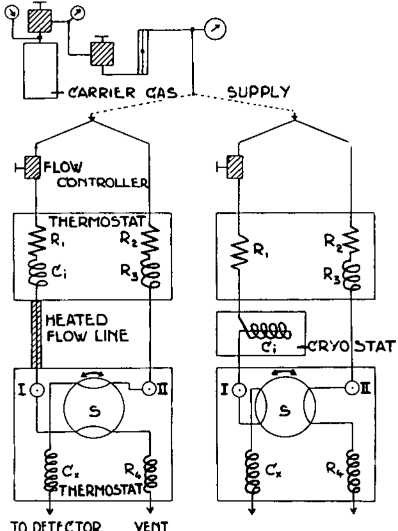

Fig. 3. Schematic diagram of the apparatus for measuring distribution isotherms by the various GC methods. When C_i is to be thermostatted the set-up on the left is used, when C_i is to be cryostatted the set-up on the right is used. R_1, R_2, R_3, R_4 = flow restrictors; C_i = evaporation column; I and II = injection ports; S = switch valve; C_x = sorption column.

R_3 is a restriction matching C_i. It consists of a packed column, like C_i but without the liquid coating.

The injection ports I and II can be heated up to 200°. It was found necessary to use special Teflon®-coated injection septa (Hamilton) as other materials invariably caused deactivation of adsorbents at the injection temperatures used (100–200°). A precision syringe (Hamilton) is used for the injection of the sample into the injection port.

The volume of the tubing from injection port I to C_x was estimated to be 0.15 ml and from injection port II to C_x 0.06 ml.

The switching valve is a high temperature sampling valve (Valco VSV-6-HT). Depending on which of the two possible positions it is in, either pure carrier gas or carrier gas loaded with vapour can be fed to column C_x.

C_x is the column containing either the adsorbent or the solvent, the latter coated on a granular (ideally inert) support, upon which or in which the distribution isotherm of the vapour, generated in C_i, is determined. The column is thermostatted by an air bath (Becker 1452 P) to within 0.2°.

R_4 is a restriction matching C_x and consists of a column, like C_x, packed with an inert granular material with the same particle diameter as that used in C_x.

A flame ionization detector (FID) of our own construction was used as sensor.

The signal is processed by an electrometer amplifier (Becker 2032-E) and recorded by a potentiometric recorder (Philips PR-2210).

Time measurements are done with a stopwatch with a precision of 0.1 sec.

(4.2) Operation

The following procedure is followed for the equilibrium method (3.2).

(1) The switching valve is set in the position where only carrier gas is admitted to C_X. The recorder deflection is adjusted to zero for this position.

(2) The switching valve is now set in the position where carrier gas loaded with vapour is fed to column C_X, in which the vapour is sorbed.

(3) After a constant recorder deflection is attained the valve is switched back to the first position and the vapour is desorbed from column C_X.

(4) The equilibrium concentrations of the vapour in the mobile phase (gas) and stationary phase (solvent or adsorbent surface) in column C_X are calculated from the recorded chromatograms (Fig. 2).

In practice perfect matching of the flow resistances, so that no change in flow through C_X occurs on switching, is difficult to achieve. However, matching is less critical when the detector response is proportional to the mass flow (FID) than when the response is proportional to the concentration (catharometer). A check on the effect of incomplete matching is made possible by the fact that the resulting areas of sorption and desorption should be equal. The difference between the areas of sorption and desorption was found to be less than 2 % in most experiments. In the case of methanol however the areas of sorption were significantly greater than the corresponding areas of desorption (see section 5.4). This effect is due to the higher non-linearity of the isotherm resulting in a tail which almost disappears in the base line but which represents a significant portion of the sorbed component. The standard deviations of the areas for sorption or desorption were found to be 1 % for $[(1 - \varepsilon_m)/\varepsilon_m] \cdot [d\langle c_i^s\rangle / d\langle c_i^m\rangle] \geqslant 0.5$.

Only a small correction for the volume (0.15 or 0.06 ml) of the tubing between the injection port and the column C_x, which in practice has a volume of 5–20 ml, has to be made. Cross leakage at the switching valve connections was found to be undetectable at first, but to have increased to about 1 % after regular use over a period of six months. The switching valve showed no memory.

The step profile method (3.1.3) is carried out analogously to the equilibrium method except for the interpretation, in which eqns. 13 and 14 are applied to points on the positive or negative steplike boundary. The peak methods (3.1.1 and 3.1.2) are performed with the switch in the position where pure carrier gas is fed into column C_X. The sample is then injected through injection port II. The minor disturbance method (3.1.4) is performed with the switch in the position where vapour containing carrier gas is fed into C_X. In this case injection port I is used.

The reproducibility of the FID characteristics from day to day is hampered by the varying ionization efficiency. In accordance with the literature[64] it was found that the sensitivity varies up to 10 % depending on the magnitude of the atmospheric pressure fluctuations. The FID is adjusted to maximum sensitivity with the aid of a signal resulting from feeding vapour containing carrier gas to C_X. First the air flow to the flame is set for maximum signal and then the hydrogen flow. It was found that the sensitivity S is practically independent of the flow rate of the carrier gas provided

the FID is operated within its linear range. The linear range of the FID is determined by plotting the logarithm of the recorder deflection at constant carrier gas flow rate *vs.* the inverse of the absolute temperature at which the vapour, fed into the carrier gas, is generated. The ordinate of the resulting graph is calibrated with vapour pressure values obtained from the literature. The end of the linear range is recognized by a deviation from linearity in the experimental graph. Two extreme cases are shown in Fig. 4, *viz.* dodecane for which the FID has a high sensitivity and carbon tetrachloride for which the FID has a low sensitivity. From graphs of various other components the end of the linear range of the FID was found to be practically the same as found from Fig 4. The linear range of the FID is thus seen to be independent of the component used.

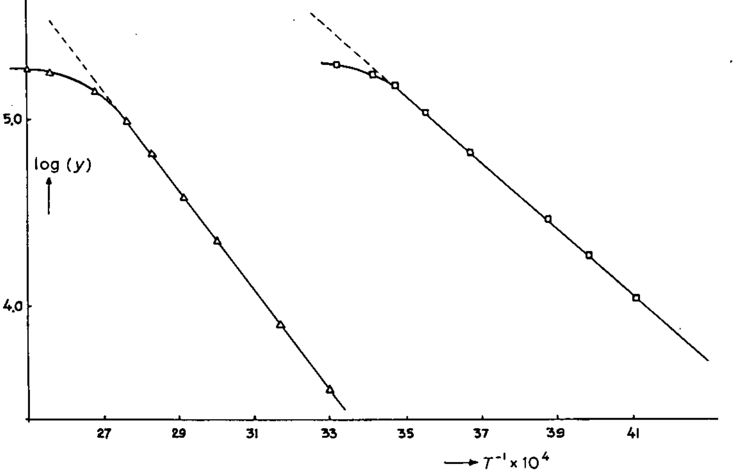

Fig. 4. Logarithmic plot of the detector signal (y in mV) *vs.* the inverse of the absolute temperature ($°K^{-1}$) of the evaporation column in Fig. 3. △, results obtained for dodecane, □, results obtained for carbon tetrachloride.

A more direct method of determining the linear range of the detector, made possible by the set-up used, is to compare the areas of sorption and desorption in the equilibrium method when matching is incomplete as shown in Fig. 2. If at a certain vapour content of the carrier gas the area for adsorption becomes significantly larger than the corresponding area for desorption the end of the linear range has been reached.

The upper limit of the range of the FID was found to be of the order of 10^{-8} A by both methods, thus giving a linear dynamic range of 10^{5}, the standard deviation of the noise being estimated at 4.10^{-13} A.

(5) EXPERIMENTAL RESULTS

The following notation is used to characterize the results of the different GC methods for the determination of distribution isotherms: I, peak profile method (■); II, peak maxima method (▲); III, step profile method (●); IV, minor disturbance method (×); V, equilibrium method (⊙).

Diatomaceous earth was used as adsorbent or support for the liquids (Chromosorb W® (Johns-Manville), non-acid-washed, particle diameter d_p 120–130 μm, spe-

cific surface area 2 m^2/g and Chromosorb G® (Johns-Manville), acid-washed, treated with dimethyldichlorosilane, particle diameter d_p 120–130 μm, specific surface area 0.3 m^2/g).

All the chemicals (Merck, Analar or B.D.H.) used were the purest grade available.

(5.1) Effect of the length of the column

The adsorption isotherms of acetone and methanol on Chromosorb W at 110° were determined by the various GC methods in columns of 50, 100 and 200 cm length and 4 mm I.D. The isotherms were determined in triplicate by each method on each column. The precision of the measurements in a given column by a given method was found to be better than 3 %. Examples of chromatograms obtained and used with the different methods are shown in Fig. 5. A systematic difference was found between the results obtained by the various GC methods. Typical examples are shown in Figs. 6a and b.

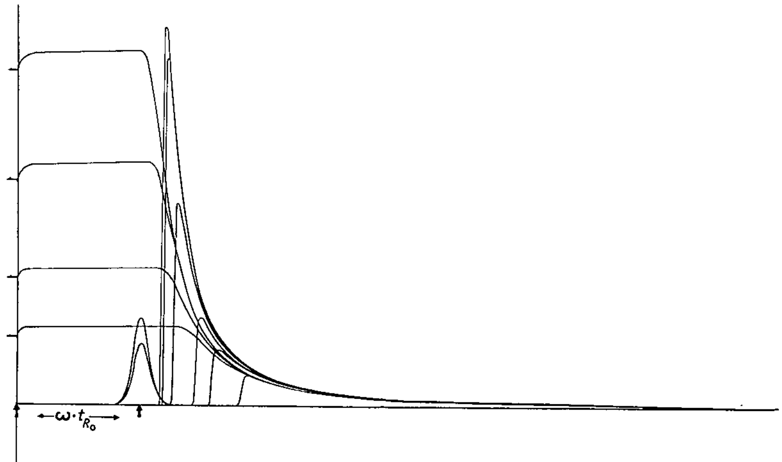

Fig. 5. Chromatograms obtained by the various GC methods for a convex distribution isotherm. Sample: methanol; column: 100 × 0.4 cm, packing 3.39 g Chromosorb W; temperature: 110°; carrier gas: nitrogen 1.25 cm sec^{-1}.

The deviations between equilibrium and non-equilibrium measurements can be explained by the kinetic nature of the chromatographic process. Diffusion, convective mixing and mass transfer will affect the results of non-equilibrium measurements to some extent.

As can be expected the results obtained by the equilibrium method are not affected by the length of the column, whereas the differences in the isotherms obtained by the different non-equilibrium methods diminishes slightly with increasing column length. The endpoint of the isotherms measured by the step profile method coincide with the equilibrium measurements since these values are determined in the same way in both methods.

The detector was calibrated according to Fig. 7 which shows a plot of the logarithm of the recorder deflection *vs.* the inverse of the absolute temperature at which the vapour was generated. The sensitivity S for the vapour in the FID can then be

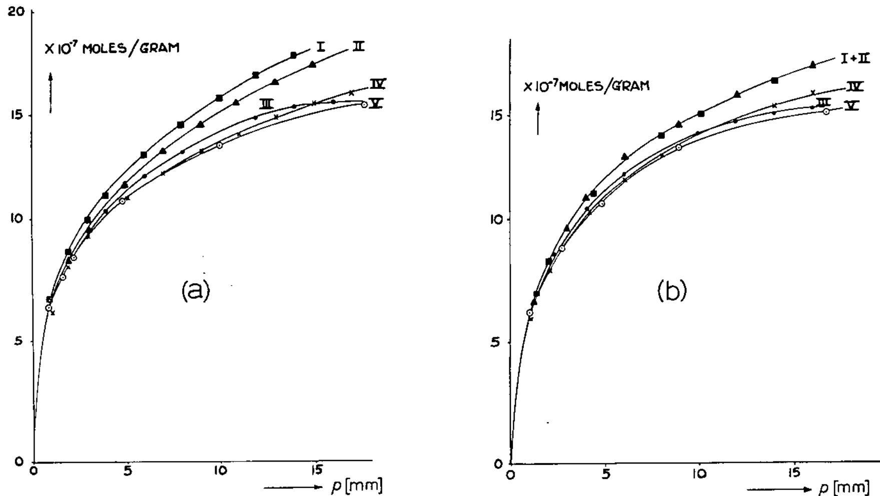

Fig. 6. Examples of convex distribution isotherms obtained by the various GC methods using different column lengths. Sample: acetone; system: Chromosorb W/nitrogen; temperature: 110°. (a) column length 50 cm; (b) column length 200 cm.

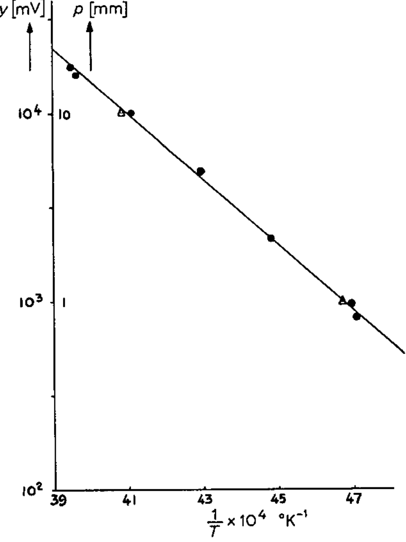

Fig. 7. Calibration of the detector: ● = detector response y (mV); △ = corresponding vapour pressures p (mm) of acetone from the literature[65].

found by calibration with vapour pressure values from the literature or by extrapolating to the boiling point at the pressure used.

(5.2) Effect of the velocity of the carrier gas and pressure drop across the column

First of all the effect of the carrier gas flow on the detector characteristics has to be considered. For the detector used (FID) it was found that the sensitivity S was independent of the flow rate providing the detector was operated within its linear

range. The linear range of the FID, when expressed in units of vapour pressure of a given component, decreases as the flow of the carrier gas increases. The latter effect limits the vapour pressure up to which the effect of the carrier gas velocity on the isotherm measurement can be investigated.

In deriving eqn. 4 the fluid velocity was assumed constant along the column. When the eluant is a gas, however, the fluid velocity increases with the length coordinate z because of the gas compressibility. The following expression can be derived for the velocity gradient in the column:

$$\langle v \rangle = \langle v \rangle_{z=0} \left[1 - \frac{z}{L} \left(1 - \frac{p_L^2}{p_0^2} \right) \right]^{-1/2} \qquad (15)$$

where $\langle v \rangle_{z=0}$ is the fluid velocity at the beginning ($z = 0$) of the column and p_0 and p_L are the pressures at the beginning and end of the column, respectively. As long as the velocity gradient remains small, eqn. 4 may be applied after substituting the average linear velocity of the mobile phase over the length of the column $\overline{\langle v \rangle}$ for $\langle v \rangle$.

In the stationary state, a concentration gradient occurs along the column because of the gas compressibility. The concentrations in the mobile and stationary phase obtained by the equilibrium method are therefore average values. From the equation[66,67] describing the pressure gradient expressions for the average concentration $\overline{c_i^m}$ in the mobile phase in the column can be derived:

$$\overline{c_i^m} = \frac{2}{3} \cdot \frac{1 - (p_L/p_0)^3}{1 - (p_L/p_0)^2} \cdot c_{i0}^m = \frac{2}{3} \cdot \frac{(p_0/p_L)^3 - 1}{(p_0/p_L)^2 - 1} \cdot c_{iL}^m \qquad (16)$$

where c_{i0}^m and c_{iL}^m are the concentrations at the beginning and end of the columns, respectively. The concentration c_{iL}^m can be calculated from the detector response, the concentration c_{i0}^m can be obtained by calibrating the evaporation column C_i (see Figs. 4 and 7). An analogous relation for the partial pressure p_i is obtained by replacing the concentration c_i^m in eqn. 16 by the corresponding partial pressure. The average concentration in the stationary phase in the column is calculated by means of eqn. 13 in which $A/S = Q_i^s$, *i.e.* the amount of sample contained in the stationary phase.

The average values found for the concentrations in the mobile and stationary phases are equilibrium data as long as the portion of the isotherm over which the concentrations are averaged can be considered as linear.

The systematic error introduced by the compressibility of the carrier gas is negligible in dynamic GC methods for the determination of distribution isotherms as long as the pressure ratio p_L/p_0 is nearly 1. In this work the pressure drop was 0.05–0.2 atm and the outlet pressure atmospheric.

The influence of the carrier gas velocity was investigated for the adsorption of acetone on Chromosorb W at 110° in a column of 1 m length and 4 mm diameter. In the velocity range of 0.5–4 cm/sec, the effect of the carrier gas velocity on the isotherms obtained by the various methods was not found to be significant.

(5.3) Effect of the nature of the carrier gas

When using helium instead of nitrogen as carrier gas similar results are obtained with respect to the scattering of the isotherms, the only difference being that the deviation between the peak maxima and peak profile method is somewhat increased due to the larger diffusion coefficient in helium.

The effect of the carrier gas on the distribution coefficient can be calculated[68,69] by means of the following equation:

$$\ln (K_i^{\mathrm{He}}/K_i^{\mathrm{N_2}}) = \frac{(1-x_i^m)^2}{RT} \cdot (\Delta B_{mm} - \Delta B_{mi}) \qquad (17)$$

in which:

K_i = the distribution constant in either helium or nitrogen,
x_i^m = the mole fraction of component i in the carrier gas,
ΔB_{mm} = the difference in second virial coefficients of the mobile phases nitrogen and helium,
ΔB_{mi} = the difference in second virial cross coefficients between the component i and the mobile phase nitrogen or helium.

In eqn. 17 it is assumed that the average pressure in the column when comparing the results in helium and nitrogen remains constant. Eqn. 17 was verified for the linear distribution of toluene and dodecane on Chromosorb W, coated with 2 % w/w squalane, at T = 100°. The following results were obtained:

$$(K^{\mathrm{He}}/K^{\mathrm{N_2}})_{\mathrm{toluene}} = 1.02 \pm 0.005,$$

$$(K^{\mathrm{He}}/K^{\mathrm{N_2}})_{\mathrm{dodecane}} = 1.04 \pm 0.005.$$

The sensitivity S of the FID was found to be about 10 % lower when helium was used as carrier gas instead of nitrogen under otherwise identical conditions. In contrast to the literature[70], it was found that the optimum flow settings for the hydrogen and air to the FID were practically unaffected when changing from nitrogen to helium as carrier gas, providing the FID was operated within its linear range.

(5.4) Effect of the shape of the isotherm

In Fig. 8 isotherms convex to the pressure axis, obtained by the various GC methods for acetone on Chromosorb W, coated with squalane, are shown. The total amount adsorbed on the solid surface and solved in the liquid is related to the mass of solid. From these results and Fig. 6 it can be concluded that increasing linearity of the isotherm brings an increasing relative agreement between the equilibrium and the peak maxima methods.

Chromatograms corresponding to a concave distribution isotherm are shown in Fig. 9. Convex and concave distribution isotherms as obtained for dodecane on Chromosorb W and Chromosorb W coated with squalane, respectively, by various GC methods are shown in Fig. 10.

It can be seen that the isotherms obtained by the nonequilibrium methods lie above the isotherms obtained by the equilibrium method in the case of a convex shape and below in the case of a concave shape. This can be explained by the influence of the kinetic effects.

Fig. 11 shows a chromatogram corresponding to an isotherm which is both convex and concave to the pressure axis (see also Fig. 12) for the case of adsorption of dodecanone-2 on silanized Chromosorb G. The profile methods are not practicable in this case. Comparing the sigmoid shaped isotherms obtained by the equilibrium and peak maxima methods for this case an alternating discrepancy can be seen on going from the convex to the concave part of the isotherm, resulting in a "straightening" of the isotherm obtained by the peak maxima method as compared with the isotherm obtained from the equilibrium method.

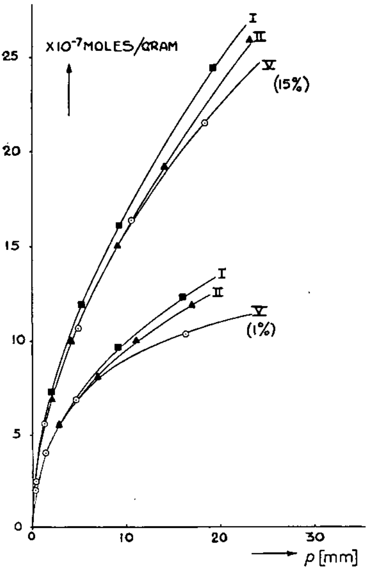

Fig. 8. Influence of the shape of convex isotherms on the results obtained by different GC methods. Sample: acetone; column: 100 × 0.4 cm, packing (1 %) (= 3.54 g Chromosorb W coated with 0.039 g squalane) and (15 %) (= 3.88 g Chromosorb W coated with 0.58 g squalane); carrier gas: nitrogen, 1.25 cm sec^{-1}; temperature 110°.

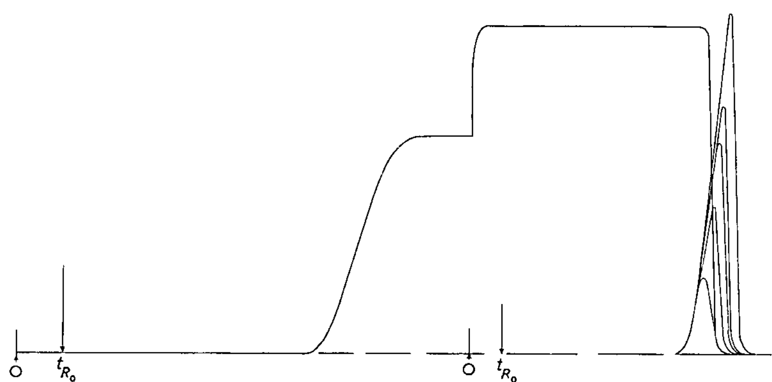

Fig. 9. Chromatograms corresponding to a concave distribution isotherm obtained by different GC methods. Conditions as in Fig. 10 (1 %).

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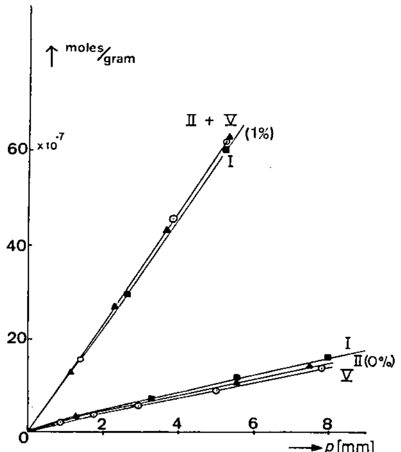

Fig. 10. Comparison of the results of different GC methods for a convex and a concave distribution isotherm. Sample: dodecane; column: 100 × 0.4 cm; packing: (0%) (= 3.55 g Chromosorb W) and (1%) (= 3.54 g Chromosorb W coated with 0.039 g squalane); carrier gas: nitrogen, 1.25 cm sec^{-1}; temperature 120°.

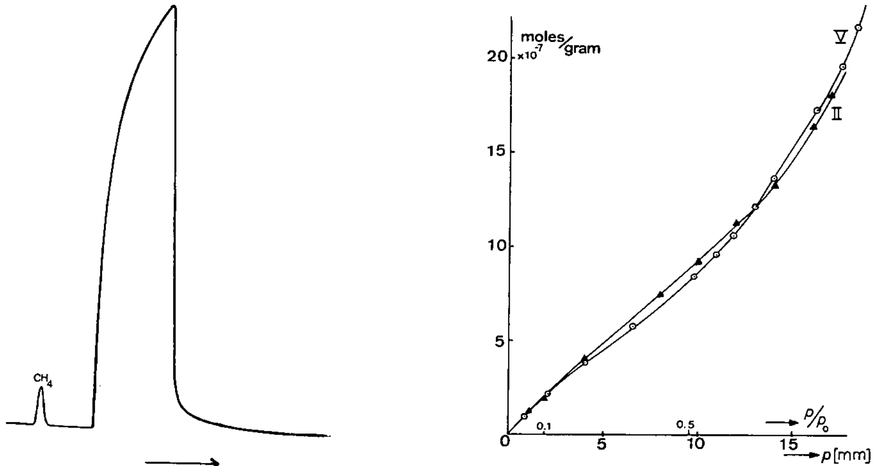

Fig. 11. Typical chromatogram corresponding to a sigmoid shaped distribution isotherm obtained by the peak maxima method. Sample: dodecanone-2; column: 100 × 0.4 cm; packing: 8.54 g Chromosorb G; carrier gas: nitrogen, 1.25 cm sec^{-1}; temperature 100°. The corresponding isotherm is shown in Fig. 12.

Fig. 12. Sigmoid shaped isotherm determined by the equilibrium and peak maxima methods. Conditions as in Fig. 11.

The determination of isotherms showing a strong convexity towards the pressure axis is systematically affected in all GC methods owing to the fact that the area under the tail cannot be accurately measured. In the equilibrium method an estimate of this error can be made by comparing the area obtained for desorption with the area of ad- or absorption. Both areas have to be equal. Only in the case of the adsorption of methanol on Chromosorb W was this systematic error found to be significant, as shown in Table I, decreasing relatively as the vapour pressure increases. All data are averages of three measurements. The standard deviation of the measurements

increased from about 1 % at low vapour pressures to about 5 % at high vapour pressures. It can be seen that the absolute difference of both areas remains constant.

TABLE I

ACCURACY OF THE MEASUREMENT OF THE AREA OF DESORPTION IN THE EQUILIBRIUM METHOD

Sample: methanol; column: 500 × 4 mm, nitrogen/Chromosorb W, 100°.

Area of adsorption (V sec)	*Area of desorption (V sec)*	p_i *(mm)*
31.5	24.0	1.55
48.5	41.1	3.9
60.1	53	8.0
70	65	18.2
79	72	33.3
90	84	66.6

(5.5) Effect of the number of theoretical plates of the inert tracer peak

When the column in which adsorption or solution is investigated does not have a sufficient number of theoretical plates for the inert component which is used to determine t_{R0}, the isotherms calculated by GC methods will be systematically affected. In this case the residence time $t_{\max}$ of the peak maximum will be smaller than the average residence time t_{R0}. A relation between $t_{\max}$ and t_{R0} can be derived mathematically[71].

$$(t_{\max})^2 = t_{R_0}^2 \cdot (1-N^{-1}) \tag{18}$$

N being the number of theoretical plates in the column.

All experiments were carried out with theoretical plate numbers greater than 300 for methane, which was used as inert tracer.

(5.6) Effect of the mole fraction in the mobile phase

The FID has a low sensitivity S for carbon tetrachloride. Due to this fact the upper linear range of the FID corresponds to a high vapour pressure of carbon tetrachloride (see Fig. 4). When used in a column containing Chromosorb W, coated with 15 % w/w squalane, the result is a linear isotherm; carbon tetrachloride is thus well suited to study the effect of the mole fraction. In Fig. 13 the isotherms shown are those obtained by the peak maxima and equilibrium methods. From the linear shape of the isotherm it can be concluded that in view of the results in preceding sections the isotherm from the peak maxima method should be identical with the isotherm obtained from the equilibrium method and that any discrepancy between the isotherms found by these two methods may be explained by eqn. 10 which after rearrangement reads:

$$\frac{t_x - t_{R0}}{t_{R_0}} = \overset{*}{\kappa_i} \cdot \left(1 - 2\langle x_i^m\rangle + \frac{\overset{*}{\kappa_i}}{1+\overset{*}{\kappa_i}} \cdot \langle x_i^m\rangle^2\right) \tag{19}$$

assuming $L/\langle v\rangle = t_{R0}$, that is $\varepsilon_\alpha = \varepsilon_m$.

The peak maxima method allows the determination of $(t_x - t_{R0})/t_{R0}$ as a function of the vapour pressure, from which κ_i^* values can be calculated from eqn. 19. The capacity ratio κ_i^* can also be determined as a function of the vapour pressure with the equilibrium method. In Fig. 14 the experimental values of κ_i^* obtained by both methods are compared. As can be seen the corrected values of the peak maxima method and the experimental values of the equilibrium method are in good agreement in the range of mole fractions studied ($<$ 0.1).

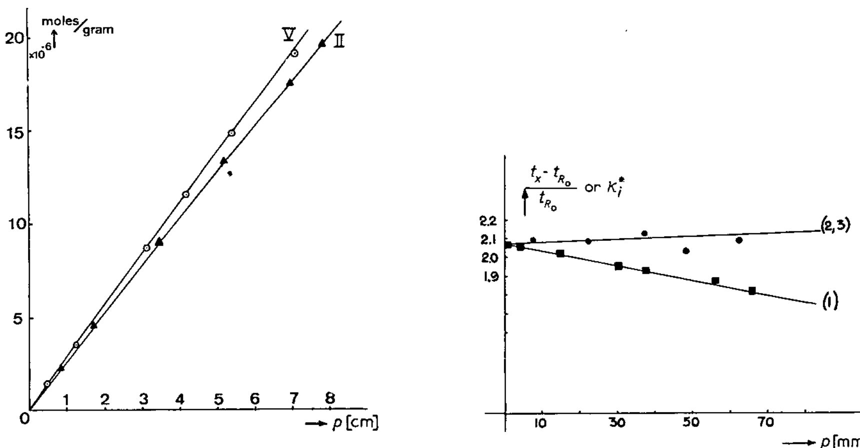

Fig. 13. Influence of the mole fraction on the isotherms obtained by the peak maxima method. Sample: carbon tetrachloride; column: 100 × 0.4 cm; packing: 3.88 g Chromosorb W coated with 0.58 g squalane; carrier gas: nitrogen, 1.25 cm sec^{-1}; temperature 100°.

Fig. 14. Correction for the influence of the mole fraction: (1) ■ = results of the peak maxima method; (2) ● = results of the equilibrium method; both corresponding to Fig. 13; (3) results of the peak maxima method corrected by means of eqn. 19.

A critical point in the equilibrium method is the determination of the retention time t_{R0} of the mobile phase. The retention time t_{R0} (see Fig. 2) in the sorption mode can be measured after equilibrium is attained *i.e.* when the recorder trace becomes constant. In the desorption mode, t_{R0} (see Fig. 2) cannot be measured since the flow rate changes slightly due to the change in flow resistance when the switch is set to the desorption position. Therefore it must be calculated according to eqn. 8 from the retention time t''_{R00} which is measured after desorption taking into account that $t_{R0}/t_{R00} = \overline{\langle v \rangle}_0/\overline{\langle v \rangle}$:

$$t''_{R_0} = t''_{R_{00}} \left(1 - \frac{\kappa_i^*}{\kappa_i^* + 1} \langle x_i^m \rangle \right) \tag{20}$$

According to eqns. 10 and 4 the mole fraction in the fluid stream and the curvature of the distribution isotherm affect the residence time $t_{\max}$ of the peak maximum and the peak shape either analogously or oppositely depending on whether the distribution isotherm is convex or concave. In order to estimate the effect of the de-

pendence of flow on the mole fraction and of the curvature of the isotherm, eqns. 4 and 10 may be combined to give:

$$t_{\max} = t_{R_0}\left(1 + \frac{1-\varepsilon_m}{\varepsilon_m}\,\frac{d\langle c_i^s\rangle}{d\langle c_i^m\rangle}\right)\cdot\left(1 - \frac{\kappa_i^*}{1+\kappa_i^*}\langle x_i^m\rangle\right)^2 \tag{21}$$

where $\langle c_i^m\rangle/\sum_i \langle c_i^m\rangle = \langle x_i^m\rangle$ and assuming $\varepsilon_\alpha = \varepsilon_m$.

Eqn. 21 is not exact since eqn. 10 is derived for a linear distribution isotherm. It is, however, a useful approximation which describes the combined effect of the mole fraction and curvature.

The combined effect of both phenomena on the results of the dynamic gas chromatographic methods for the determination of distribution isotherms can be demonstrated by changing the temperature. In general the slope and the curvature of the distribution isotherms decrease with temperature. Therefore an inversion of the peak asymmetry may be expected in the case of concave isotherms when the temperature is changed. At low temperature the effect of the curvature of the isotherm dominates whereas at high temperature the influence of the mole fraction on the fluid velocity dominates. An example of this "inversion" phenomenon is shown in Fig. 15. The correct slope of the isotherm can be estimated by means of eqn. 21. In agreement with expectations a concave isotherm is found which tends to become ilnear at high temperatures.

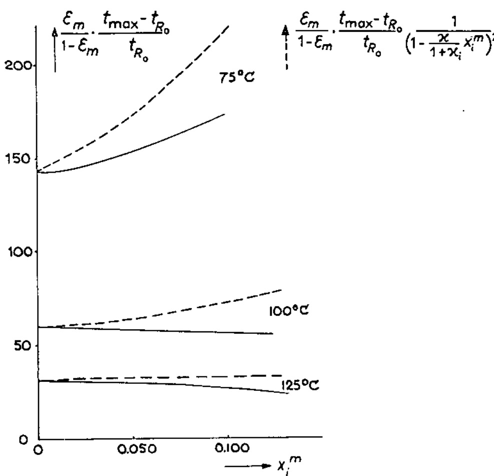

Fig. 15. Reversal of the peak asymmetry with the temperature: (———) results of the measurement; (– – – –) corrected according to eqn. 21. Sample: heptane; system: squalane/nitrogen; temperatures: 75, 100, 125°. By optimizing the flow rate of nitrogen, hydrogen and air the linear range of the FID was increased in order to make measurements with heptane up to 0.1 mole fraction possible.

(6) CONCLUSIONS

Dynamic GC methods allow a rapid determination of distribution isotherms in gas–solid and gas–solid–liquid systems over a wide temperature range.

The peak maxima and the peak profile methods can be carried out in a con-

ventional gas chromatography. The step profile, minor disturbance and equilibrium methods require a device for the preparation of a constant sample stream and a high temperature, non-absorbing, switching valve. In the case of the profile methods a certain section of the distribution isotherm is obtained in a single experiment, whereas with the other methods only a single point of the isotherm is obtained. Due to the kinetic nature of the chromatographic process the results obtained by the various dynamic GC methods differ somewhat. The highest accuracy may be expected for the equilibrium method. The greatest difference is found between the results of the peak profile method and the equilibrium method. Depending on the curvature of the isotherm the data obtained by the non-equilibrium methods are higher (convex shape) or lower (concave shape) than the data obtained by the equilibrium method. The most important phenomena influencing the accuracy in all the methods are the pressure drop across the column and the mass transfer, which affects the fluid velocity in the column and becomes significant at higher mole fractions. In the case of non-equilibrium methods the accuracy can be affected significantly by the kinetics (diffusion, convective mixing, mass transfer) of the chromatographic process and the influence of the sorption or desorption on the local temperature[72]. The relative error caused by the kinetics of the chromatographic process can be reduced by choosing conditions corresponding to a high number of theoretical plates for the linear chromatographic case. The pressure drop across the column should be kept as small as possible. For the interpretation of the elution curve an equation must be used which takes into account the change of the fluid velocity due to the mass exchange between the fluid stream and the stationary bed.

A limiting factor in improving the accuracy of the dynamic GC measurements of distribution isotherms is the accuracy of the determination of the detector sensitivity. In the case of volatile liquids it becomes especially difficult to inject an accurately known amount of sample. Therefore it is better to determine the vapour pressure, as described in 5.1, in order to determine the detector sensitivity.

Another factor which limits the accuracy of all dynamic GC methods for the determination of distribution isotherms is the measurement of the average flow rate $\overline{w} = \varepsilon_m V/t_{R0}$, V being the total volume of the column. The average flow rate in the column can be obtained by measuring the flow rate at the column exit and by converting it to the flow rate at average column pressure. Another method is to determine the volume of the mobile phase $\varepsilon_m V$ from the volumes of the column and the packing. In the equilibrium method the accuracy can be improved by making certain modifications, which also allow the determination of the simultaneous distribution isotherms of the components of a mixture. It is hoped to report on this subject in the near future.

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CHROM. 5358

CHROMATOGRAPHIE EN PHASE GAZEUSE RÉALISÉE SIMULTANÉMENT AVEC UNE PROGRAMMATION DE TEMPÉRATURE ET UNE PROGRAMMATION DU GRADIENT LONGITUDINAL NÉGATIF DE TEMPÉRATURE

THÉORIE DE LA RÉTENTION ET INFLUENCE DES PARAMÈTRES

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SUMMARY

Gas chromatography using simultaneously programmed temperature and a programmed longitudinal negative temperature gradient. Retention theory and influence of parameters

A new method for gas chromatography is described using a linear programmed temperature associated with a linear programmed longitudinal negative temperature gradient. As the gradient is negative, the temperatures at the column inlet are higher than those at the column outlet. The description of the apparatus is followed by an elaboration of the theory of retention of solutes. The role of various parameters is specified: column length, column temperature at the time of injection, rate of growth of temperature at column inlet and column outlet.

INTRODUCTION

Pour pallier l'inconvénient de la chromatographie à température programmée, dû au fait que la température au début de l'élution est choisie suffisamment basse pour permettre une bonne élution des solutés très volatils, et ce fait étant alors responsable d'un allongement du temps de rétention des derniers solutés, nous avions proposé plusieurs méthodes: la chromatographie à température programmée avec plusieurs fours indépendants[1,2], la chromatographie à température programmée couplée avec un gradient longitudinal de température établi[3,4], et la chromatographie utilisant simultanément une programmation de température et une programmation du gradient longitudinal positif de température[5].

La méthode préconisée dans cette publication est dérivée de la méthode précédemment citée, et elle est réalisée en travaillant simultanément avec une programmation de température de la colonne et une programmation du gradient longitudinal négatif de température. Nous conservons la convention suivant laquelle est appelé négatif un gradient correspondant à une température plus élevée à l'entrée de la colonne.

Afin de décomposer la difficulté, nous avons étudié préalablement la chromatographie avec programmation du gradient longitudinal de température, qu'il soit positif ou négatif[6,7], et nous avons pu ainsi vérifier la justesse des temps de rétention déterminés avec l'aide de la théorie élaborée.

Comme nous l'avions précisé précédemment[1,6,7], ces méthodes sont tout à fait différentes de la "Chromathermography" décrite par ZHUKHOVITSKII *et al.* [8] et dont la traduction française est "chromatographie à gradient de température".

Nous présenterons tout d'abord une théorie de la rétention des solutés élués, et les équations obtenues nous permettront ensuite de préciser le rôle joué par les paramètres: longueur de la colonne, température de la colonne à l'instant de l'injection, valeurs de la vitesse de l'élévation de la température à l'entrée et à la sortie de la colonne.

APPAREILLAGE ET CONDITIONS OPÉRATOIRES

Description du chromatographe

Chromatographe "Perkin-Elmer" F 7 équipé de catharomètres; colonne acier inoxydable, longueur 2 m, diamètre intérieur et extérieur 3 et 4 mm. Phase stationnaire: support Chromosorb G AW-DMCS 80/100 mesh; solvant 2.5 % caoutchouc silicone SE-52. Gaz vecteur, hélium, débit 25 cm^3/min. Solutés: alcanes normaux compris entre l'hexane et le dodécane.

Programmation du gradient longitudinal de température

Le gradient longitudinal de température le long de la colonne a été réalisé à l'aide d'un fil résistant "Rhodorsil"® (110 Ω, longueur 400 cm) enroulé autour de la colonne de telle sorte que le nombre de spires par unité de longueur de colonne varie le long de celle-ci selon une progression arithmétique. A l'entrée de la colonne, la longueur de fil chauffant enroulé par cm de colonne est égale à 3.5 cm, et la raison de la progression est égale à 0.012 cm de fil chauffant par cm de colonne, de telle sorte qu'il n'y a pas d'enroulement de fil chauffant à la sortie de la colonne.

La programmation linéaire du gradient longitudinal de température est réalisée en alimentant le fil chauffant avec une tension électrique dont la loi de croissance est déterminée expérimentalement.

Programmation linéaire de la température

Le système de chauffage prévu par le constructeur présente une inertie thermique considérable, et il existe un retard fort important entre la température affichée sur la programme et la température mesurée à l'intérieur de la colonne. Il est ainsi préférable d'enrouler autour de la colonne un fil résistant de façon régulière, et de l'alimenter sous une tension électrique dont la valeur varie selon une loi déterminée expérimentalement.

Réalisation des calculs

Les calculs ont été réalisés avec un ordinateur numérique IBM 1620, en utilisant la méthode de Runge et Kutta.

THÉORIE DE LA RÉTENTION DES SOLUTÉS

Plusieurs hypothèses sont formulées.

L'équilibre thermique est réalisé à chaque instant et à chaque endroit de la colonne.

L'élévation de la température résulte de l'action simultanée: de la programmation linéaire de la température de l'ensemble de la colonne, et de la programmation linéaire du gradient longitudinal positif de température le long de la colonne.

La vitesse linéaire du gaz vecteur est constante le long de la colonne en chromatographie isotherme.

En chromatographie isotherme, la vitesse de propagation du soluté caractérisé par son facteur de rétention R_F est:

$$\frac{\mathrm{d}l}{\mathrm{d}t} = V_{gv} \cdot R_F \tag{1}$$

dans laquelle V_{gv} est la vitesse linéaire du gaz vecteur, constante le long de la colonne selon la troisième hypothèse, et égale à la vitesse moyenne.

Le facteur de rétention étant égal au rapport du temps de rétention du gaz vecteur par le temps de rétention du soluté, l'équation 1 devient:

$$\frac{\mathrm{d}l}{\mathrm{d}t} = \frac{L_{\mathrm{exp}}}{t_{rT(\mathrm{exp})}} \tag{2}$$

L_{exp} étant la longueur de la colonne utilisée pour mesurer les temps de rétention $t_{rT(\mathrm{exp})}$ (2 m), $t_{rT(\mathrm{exp})}$ étant le temps de rétention du soluté déterminé de façon expérimentale à la température T avec la colonne de longueur L_{exp}.

Avec la méthode de chromatographie préconisée, la température au temps t à l'abscisse l le long de la colonne en choisissant l'entrée comme origine, peut être représentée par une différentielle totale:

$$\mathrm{d}T = \left(\frac{\partial T}{\partial t}\right)_l \cdot \mathrm{d}t + \left(\frac{\partial T}{\partial l}\right)_t \cdot \mathrm{d}l \tag{3}$$

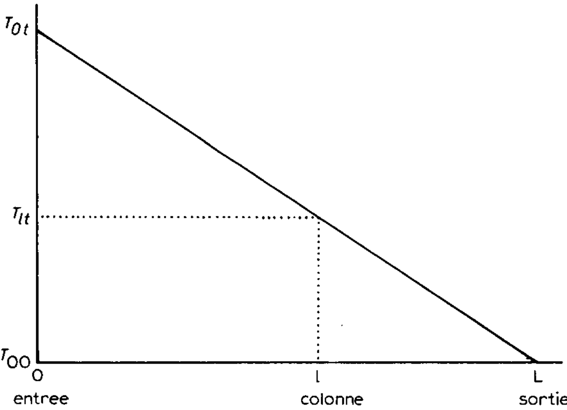

Fig. 1. Schéma représentant la variation de la température le long de la colonne.

Le profil de la température le long de la colonne varie avec le temps de la façon prévue par la seconde hypothèse, et nous l'avons représenté sur la Figure 1.

Nous appelons ainsi V_e et V_s la valeur constante de l'élévation de la température respectivement à l'entrée et à la sortie de la colonne. A l'instant où a lieu l'injection des solutés, choisi comme origine des temps, les vitesses V_e et V_s sont nulles, et la température en chaque point de la colonne est égale à T_{00}. Après l'injection, la vitesse V_e étant plus grande que la vitesse V_s, il se forme un gradient longitudinal de température.

Ainsi, la température T_{lt} au temps t et à l'abscisse l le long de la colonne, est égale à:

$$T_{lt} = T_{00} + \left(V_e + \frac{V_s - V_e}{L} \cdot l\right) \cdot t \qquad (4)$$

et les valeurs des dérivées partielles de l'équation 3 peuvent être calculées aisément:

$$\left(\frac{\partial T}{\partial t}\right)_l = V_e + (V_s - V_e) \cdot \frac{l}{L} \qquad (5)$$

$$\left(\frac{\partial T}{\partial}\right)_t = (V_s - V_e) \cdot \frac{t}{L} \qquad (6)$$

En remplaçant dans l'équation 3 les dérivées partielles par leurs valeurs citées dans les équations 5 et 6, ainsi que l'élément différentiel dt par sa valeur tirée de l'équation 2, on obtient l'équation différentielle fondamentale.

$$\frac{\mathrm{d}T}{\mathrm{d}l} = (V_s - V_e) \cdot \frac{l}{L} + V_e \frac{t_{rTL(\exp)}}{L_{\exp}} + (T_{lt} - T_{00}) \frac{V_s - V_e}{V_e \cdot L + (V_s - V_e) l} \qquad (7)$$

Remarquons que les longueurs L et $L_{\exp}$ peuvent être différentes. En effet $L_{\exp}$ est la longueur de la colonne choisie pour déterminer la loi de variation du temps de rétention $t_{rTL(\exp)}$ avec la température. L est la longueur de la colonne utilisée avec la méthode de chromatographie préconisée.

L'équation différentielle (7) peut être résolue numériquement avec l'ordinateur, en utilisant la loi expérimentale de la variation du temps de rétention $t_{rTL(\exp)}$ du soluté avec la température T. Il est ainsi possible de déterminer la température de rétention du soluté T_{Lt_r}, qui est la température à la sortie de la colonne au temps t_r.

Le temps de rétention t_r du soluté élué avec notre méthode peut être calculé aisément à l'aide de la relation:

$$t_r = \frac{T_{Lt_r} - T_{00}}{V_s} \qquad (8)$$

INFLUENCE DES PARAMÈTRES SUR LE TEMPS DE RÉTENTION DES SOLUTÉS

Les valeurs des temps de rétention calculées en utilisant l'équation 8 ont été comparées dans certains cas aux valeurs expérimentales, et une bonne concordance a pu être vérifiée entre elles.

Nous pouvons alors préciser l'influence sur le temps de rétention, présentée par les trois paramètres: vitesses d'élévation de la température à l'entrée V_e et à la sortie

V_s de la colonne, longueur de la colonne, température initiale T_{00}. Dans chaque cas, nous ferons varier un seul de ces paramètres à la fois, en maintenant les deux autres constants.

Influence des vitesses d'élévation de la température V_e et V_s

Nous avons représenté la variation du temps de rétention des alcanes normaux en fonction de la valeur donnée à la vitesse V_e, en maintenant les valeurs des autres paramètres constantes dans la Figure 2 avec $L = 2$ m, $T_{00} = 70°$, $V_s = 0.2°$/sec; et dans la Figure 3 avec $L = 2$ m, $T_{00} = 70°$, $V_s = 0.1°$/sec.

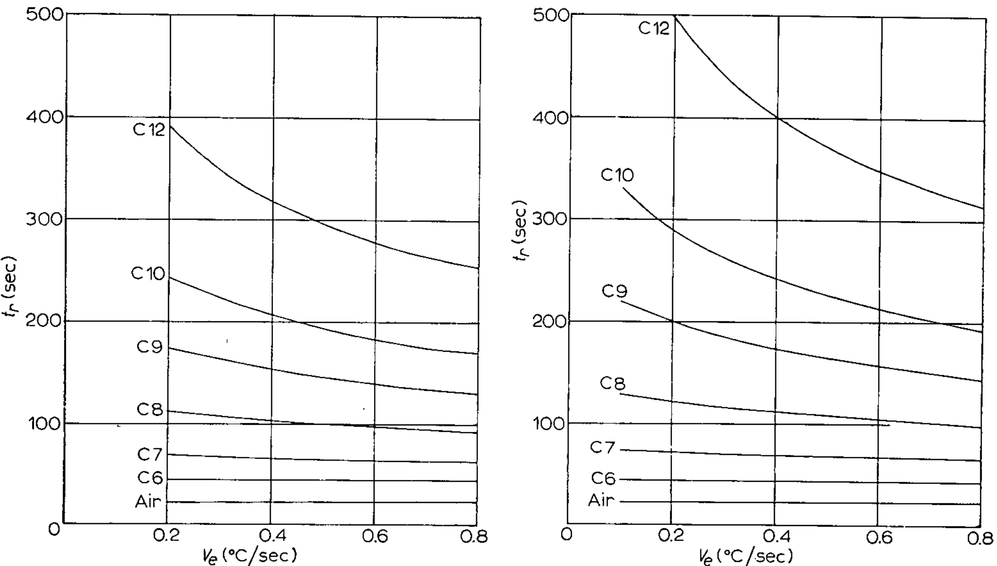

Fig. 2. Variation du temps de rétention des alcanes avec la valeur de la vitesse d'élévation de la température V_e à l'entrée de la colonne, avec $V_s = 0.2°$/sec, $T_{00} = 70°$, $L = 2$ m.

Fig. 3. Variation du temps de rétention des alcanes avec la valeur de la vitesse d'élévation de la température V_e à l'entrée de la colonne, avec $V_s = 0.1°$/sec, $T_{00} = 70°$, $L = 2$ m.

Ces deux figures permettent d'apprécier le gain de temps obtenu avec notre méthode vis à vis de la chromatographie à température programmée, puisque le temps de rétention obtenu avec cette dernière méthode apparait en Fig. 2 pour $V_e = V_s = 0.2°$/sec, et en Fig. 3 pour $V_e = V_s = 0.1°$/sec.

D'autre part plusieurs résultats apparaissent évidents: Le temps de rétention de l'air est constant, conformément à la troisième hypothèse. Le temps de rétention des solutés les plus volatils décroit très faiblement en fonction de la valeur donnée à V_e. Par contre, fait intéressant, le temps de rétention des solutés les moins volatils dans le mélange injecté, voient leurs temps de rétention décroitre de façon considérable lorsque V_e grandit.

Nous avons représenté sur la Figure 4, la variation du temps de rétention du *n*-décane avec la vitesse d'élévation V_s de la température à la sortie de la colonne, pour différentes valeurs de la différence des vitesses $V_e - V_s$ et en maintenant les deux autres paramètres constants: $L = 2$ m; $T_{00} = 70°$.

Sur la Figure 4, la courbe 1, pour laquelle $V_e = V_s$, correspond à la chromatographie à température programmée. Les trois autres courbes représentent la variation du temps de rétention obtenu avec notre méthode. Il est ainsi possible d'apprécier le gain de temps permis par notre méthode sur la chromatographie à température programmée, et la variation de ce gain de temps avec la valeur donnée aux deux autres paramètres: V_s d'une part, et $(V_e - V_s)$ d'autre part.

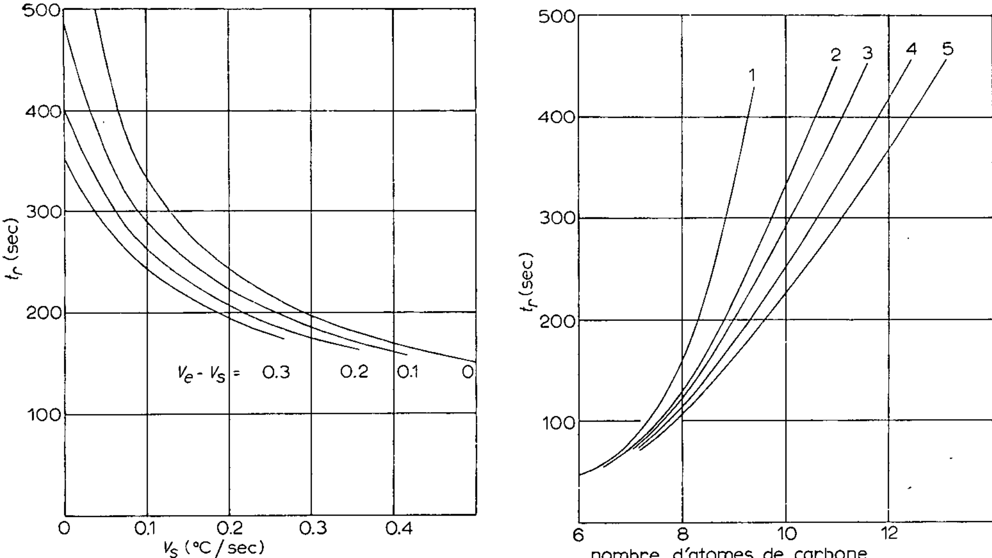

Fig. 4. Variation du temps de rétention du *n*-décane avec la valeur de la vitesse d'élévation de la température V_s à la sortie de la colonne, pour différentes valeurs de $V_e - V_s$. (1) $V_e - V_s = 0$; (2) $V_e - V_s = 0.1$; (3) $V_e - V_s = 0.2$; (4) $V_e - V_s = 0.3$. $T_{00} = 70°$ et $L = 2$ m.

Fig. 5. Variation du temps de rétention des alcanes avec leur nombre d'atomes de carbone, avec $L = 2$ m et $T_{00} = 70°$. (1) $V_s = V_e = 0$ (chromatographie isotherme à 70°); (2) $V_s = V_e = 0.1°$ per sec (chromatographie à température programmée); (3) $V_s = 0.1$, $V_e = 0.2$ (méthode préconisée); (4) $V_s = 0.1$, $V_e = 0.3$; (5) $V_s = 0.1$, $V_e = 0.4$.

Les Figures 2 et 3 laissent déjà prévoir un ressèrement des pics des solutés lorsque V_e croit. Nous avons précisé ce phénomène intéressant avec la Figure 5, où est réprésentés la variation du temps de rétention des alcanes normaux avec leur nombre d'atomes de carbone, en maintenant constants les valeurs: $L = 2$ m, $T_{00} = 70°$. La courbe 1 correspond à la chromatographie isotherme réalisée à 70°, et la courbe 2 à la chromatographie à température programmée dont la vitesse d'élévation de la température est égale à 0.1°/sec. Les trois autres courbes représentent la variation des temps de rétention des alcanes obtenus avec notre méthode en maintenant V_s égale à 0.1°/sec et avec trois valeurs différentes de V_e.

Nous constatons d'une part que le temps de rétention de l'hexane est identique pour toutes les chromatographies, et d'autre part que la pente des courbes obtenues est plus faible avec notre méthode que dans le cas de la chromatographie à température programmée.

Influence de la longueur de la colonne

Nous avons représenté sur la Figure 6 la variation du temps de rétention des alcanes avec la longueur de la colonne, en maintenant $V_e = 0.4°/\text{sec}$; $V_s = 0.2°/\text{sec}$; $T_{00} = 70°$.

Nous constatons la linéarité de la variation du temps de rétention de l'air avec la longueur de la colonne, et ceci résulte de la troisième hypothèse. Pour chaque alcane, la pente de la courbe obtenue décroit très vite lorsque L atteint des valeurs élevées, et ainsi la variation du temps de rétention avec la longueur de la colonne est moins rapide que la variation linéaire. D'autre part, la diminution de la valeur de la pente avec la longueur L est d'autant plus importante que la volatilité de l'alcane est plus faible. Ces deux conclusions rendent attrayante notre méthode de chromatographie, car il est possible de choisir une longueur de colonne suffisante pour permettre de séparer convenablement les premiers solutés, sans provoquer un allongement trop considérable du temps de rétention des derniers solutés élués du mélange.

Nous avons précisé ces conclusions en représentant sur la Figure 7, la variation du temps de rétention des alcanes avec leur nombre d'atomes de carbone, pour différentes valeurs de la longueur de la colonne, et en maintenant constantes les valeurs des autres paramètres: $T_{00} = 70°$; $V_e = 0.4°/\text{sec}$; $V_s = 0.2°/\text{sec}$. Nous constatons ainsi le résultat intéressant que les courbes obtenues sont approximativement des droites parallèles. Ainsi, un allongement de la colonne a pour effet d'agrandir tous les temps de rétention des différents solutés d'une même valeur constante. Cette valeur constante de l'allongement des temps de rétention correspondant à un allongement unitaire de la colonne, diminue d'ailleurs au fur et à mesure que croit la longueur de la colonne.

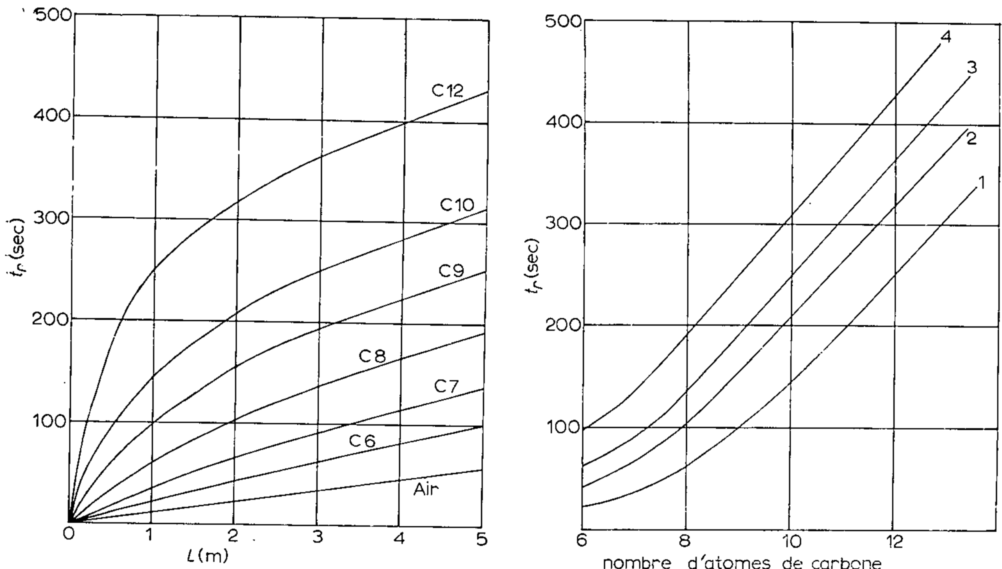

Fig. 6. Variation du temps de rétention des alcanes avec la longueur de la colonne. $T_{00} = 70°$, $V_e = 0.4°/\text{sec}$, $V_s = 0.2°/\text{sec}$.

Fig. 7. Variation du temps de rétention des alcanes avec leur nombre d'atomes de carbone. $T_{00} = 70°$, $V_e = 0.4°/\text{sec}$, $V_s = 0.2°/\text{sec}$. (1) $L = 1$ m; (2) $L = 2$ m; (3) $L = 3$ m; (4) $L = 4$ m.

Influence de la température initiale de la colonne

Sur la Figure 8 est représentée la variation du temps de rétention des alcanes avec la valeur donnée à la température initiale T_{00}, en maintenant constantes les valeurs des autres paramètres: $L = 2$ m; $V_e = 0.4°$/sec; $V_s = 0.2°$/sec. Nous constatons ainsi une décroissance très rapide du temps de rétention des alcanes avec la valeur donnée à la température T_{00}. Nous pouvons comparer sur la Figure 9 la variation du temps de rétention du n-décane lorsqu'il est élué d'une part en chromatographie isotherme à 70°, d'autre part avec la chromatographie à température programmée et avec une vitesse d'élévation de la température égale à 0.2°/sec et enfin avec la méthode préconisée et avec $V_e = 0.4°$/sec et $V_s = 0.2°$/sec. Nous constatons ainsi que l'influence du choix de la température initiale T_{00} sur la valeur du temps de rétention des solutés est bien moins important avec notre méthode.

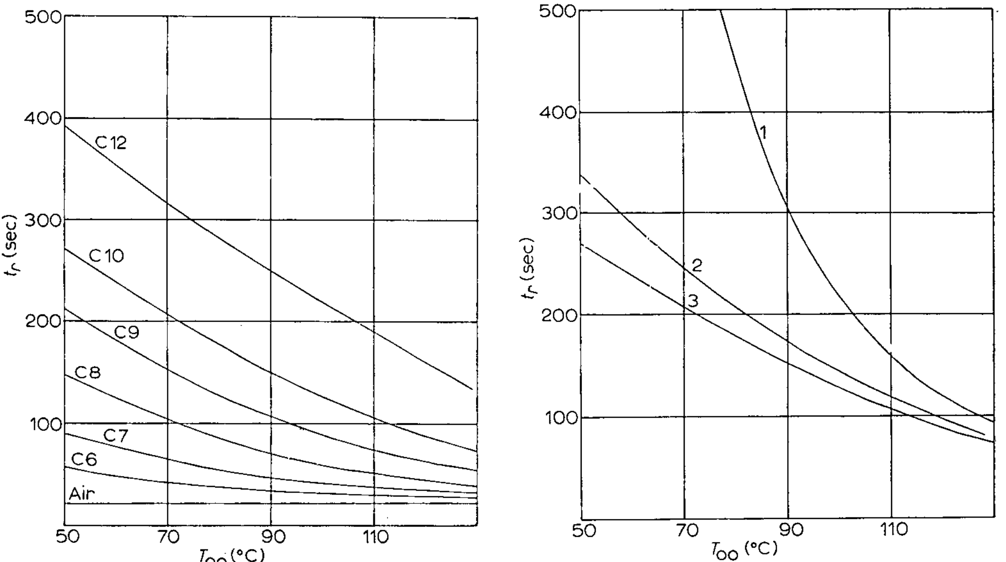

Fig. 8. Variation du temps de rétention des alcanes avec la valeur donnée à la température initiale T_{00}. $L = 2$ m, $V_e = 0.4°$/sec, $V_s = 0.2°$/sec.

Fig. 9. Variation du temps de rétention du n-décane avec la valeur donnée à la température T_{00}, lorsqu'il est élué avec une colonne de 2 m. (1) en chromatographie isotherme; (2) en chromatographie à température programmée avec une vitesse d'élévation de la température de 0.2°/sec; (3) avec la méthode préconisée et $V_s = 0.2°$/sec et $V_e = 0.4°$/sec.

CONCLUSIONS

La méthode nouvelle qu'est la chromatographie avec programmation simultanée de la température et du gradient longitudinal de température, a été présentée, et la théorie de la rétention élaborée permet de calculer des temps de rétention conformes à l'expérience.

L'influence de chacun des paramètres sur le temps de rétention des solutés a été examinée, qu'il s'agisse de la longueur de la colonne, de la valeur de la température

initiale, ou des valeurs des vitesses d'élévation de la température à l'entrée et à la sortie de la colonne.

Pour conclure en définissant les avantages offerts par cette méthode, nous pouvons résumer les faits suivants: la méthode préconisée permet de réduire le temps de rétention d'une façon plus importante que la chromatographie à température programmée, et ceci d'une façon particulièrement attrayante puisque le temps de rétention des premiers solutés élués n'est pas réduit, alors que la réduction est d'autant plus grande que la volatilité des solutés est plus faible. Ainsi, on obtient un ressèrement des pics des solutés qui ne porte pas préjudice à l'éfficacité de séparation des premiers solutés. De plus, un allongement de la longueur de la colonne provoque un supplément de rétention sensiblement égal pour tous les solutés.

RÉSUMÉ

Cette méthode nouvelle de chromatographie est réalisée en utilisant simultanément une programmation de la température de la colonne, et une programmation du gradient longitudinal de température. La valeur négative du gradient signifie que la température est plus élevée à l'entrée de la colonne qu'à la sortie. Après avoir décrit un appareillage proposé, la théorie de la rétention des alcanes normaux a été élaborée. La variation du temps de rétention a été précisée en fonction des paramètres: longueur de la colonne, température initiale de la colonne, vitesse d'élévation de la température à l'entrée et à la sortie de la colonne.

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CHROM. 5359

VARIATION DE L'INDICE DE RÉTENTION AVEC LA TEMPÉRATURE, DÉTERMINÉ EN CHROMATOGRAPHIE EN PHASE GAZEUSE DANS LE CAS DE SOLUTIONS IDÉALES

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SUMMARY

Variation of the retention index with temperature, determined in gas chromatography of ideal solutions.

Using known parameters it has been possible to determine the laws of variation of the standard enthalpy and entropy of evaporation of *n*-alkanes in three cases each corresponding to one of the following three simplifying hypotheses: (1) the enthalpy is constant; (2) the enthalpy decreases linearly with the temperature; or (3) the enthalpy decreases with the temperature according to the THEISEN relation. The general theory of the retention index is then elaborated for each case, assuming an ideal solution of the solutes in the solvent. The variation with temperature of the retention index for cyclohexane could be determined in each case, and the results obtained with the last method agree well with the experimental results.

INTRODUCTION

Il n'est pas nécessaire de rappeler l'intérêt provoqué par l'indice de rétention en chromatographie en phase gazeuse, car son emploi permet de classer de façon précise un soluté, et il constitue ainsi un outil précieux pour l'identification des constituants inconnus d'un mélange[1].

Il faut dire que l'indice de rétention a été introduit de façon empirique par KOVATS[2,3] il y a douze ans, et qu'il n'a été défini de façon théorique que tout récemment[4]. Cette étude thermodynamique de l'indice de rétention[4] a été réalisée d'ailleurs à température constante, en utilisant des solvants qualifiés de "polaires" et d'"apolaires", mais qui ne sont cependant pas susceptibles de former une solution idéale avec un quelconque soluté. D'autre part, la variation de la valeur de l'indice de rétention avec la température a été déterminée, soit de façon empirique, soit en admettant que les valeurs de l'enthalpie et de l'entropie de vaporisation sont indépendantes de la température[5-7].

La constance de l'enthalpie de vaporisation des solutés avec la température étant très loin de représenter la réalité, il nous a paru nécessaire d'élaborer une théorie générale de l'indice de rétention, qui tienne compte de cette variation de l'enthalpie et de l'entropie de solution avec la température. Pour ce faire, nous avons considéré

des solutions idéales, parce qu'elles permettent de simplifier quelque peu les difficultés, et aussi et surtout parce que les grandeurs thermodynamiques de vaporisation sont très bien connues pour un grand nombre de solutés.

PARTIE THÉORIQUE

Plusieurs hypothèses sont formulées:

(1) Les solutions liquides sont idéales, et les grandeurs thermodynamiques de solution sont égales aux grandeurs correspondantes de liquéfaction des solutés purs.

(2) Les grandeurs thermodynamiques sont les grandeurs standard, la pression à l'intérieur de la colonne étant peu supérieure à une atmosphère.

(3) Les solutions sont infiniment diluées, et la concentration du soluté est très faible dans la phase gazeuse.

(4) L'équilibre thermodynamique de l'interaction soluté–solvant est atteint.

(5) La vapeur du soluté et le gaz vecteur se comportent comme des gaz parfaits.

Détermination du temps de rétention d'un soluté

Le temps de rétention t_r d'un soluté élué à la température absolue T, est relié au coéfficient de partage k et au temps de rétention de l'air t_g, par la relation connue:

$$t_r - t_g = t_g \cdot k \cdot \frac{V_l}{V_g} \quad (1)$$

Le coéfficient de partage k est égal par définition au rapport de la concentration du soluté dans la phase liquide sur la concentration du soluté dans la phase gazeuse, cette concentration étant exprimée en poids de soluté par unité de volume de phase. On conclue aisément que le coéfficient de partage est égal au rapport de la molarité du soluté dans la phase liquide sur la molarité du soluté dans la phase gazeuse. Ce rapport peut s'écrire alors en utilisant les fractions molaires du soluté dans la phase liquide N_l et dans la phase gazeuse N_g:

$$k = \frac{N_l \cdot (n_l + n_l')}{N_g \cdot (n_g + n_g')} \quad (2)$$

La solution étant très diluée, et la concentration du soluté dans la phase gazeuse étant très faible, on peut négliger n_g devant n_g', et n_l devant n_l'. L'équation 2 devient alors:

$$k = \frac{N_l}{N_g} \cdot \frac{n_l'}{n_g'} \quad (3)$$

et le temps de rétention défini par l'équation 1 est égal à

$$t_r - t_g = t_g \cdot \frac{\mathrm{N}_l}{\mathrm{N}_g} \cdot \frac{N_l}{N_g} \quad (4)$$

D'autre part, la pression partielle du soluté peut s'exprimer soit en considérant

la solution infiniment diluée, soit en considérant la phase gazeuse qui se comporte comme un mélange de gaz parfaits. On obtient respectivement:

$$p = N_l \cdot v_{l\infty} \cdot p^0 \tag{5}$$

$$p = N_g \cdot P \tag{6}$$

Des relations 5 et 6, on tire évidemment, en se rappelant les deux premières hypothèses:

$$\frac{N_g}{N_l} = \frac{p^0 \cdot v_{l\infty}}{P} = p^0 \tag{7}$$

p^0 étant exprimé en atm.

Or, en utilisant la relation de Clapeyron, on trouve aisément:

$$\mathrm{Log_e} p^0 = -\frac{\Delta G_v{}^0}{R \cdot T} \tag{8}$$

Et l'équation 4 exprimant le temps de rétention du soluté peut alors s'écrire sous la forme classique:

$$t_r - t_g = t_g \cdot \frac{\mathrm{N}_l}{\mathrm{N}_g} \cdot \exp \frac{\Delta G_v{}^0}{R \cdot T} \tag{9}$$

ou sous la forme logarithmique:

$$\mathrm{Log_e}(t_r - t_g) = \mathrm{Log_e} t_g \frac{\mathrm{N}_l}{\mathrm{N}_g} + \frac{\Delta H_v{}^0}{R \cdot T} - \frac{\Delta S_v{}^0}{R} \tag{10}$$

Si l'on opère à débit massique de gaz vecteur constant, à toute température, le rapport t_g/N_g est constant.

Indice de rétention lorsque les grandeurs thermodynamiques de vaporisation ne varient pas avec la température

L'enthalpie et l'entropie standard de vaporisation des alcanes normaux sont dans ce cas indépendantes de la température, et ces grandeurs peuvent être représentées par une variation linéaire avec le nombre d'atomes de carbone n:

$$\Delta H_v{}^0 = \Delta H_0{}^0 + n \cdot \Delta h \tag{11}$$

$$\Delta S_v{}^0 = \Delta S_0{}^0 + n \cdot \Delta s \tag{12}$$

En remplaçant dans l'équation 10, les grandeurs définies par les équations 11 et 12, la relation exprimant le temps de rétention des alcanes normaux avec leur nombre d'atomes de carbone, devient:

$$\mathrm{Log_e}(t_r - t_g) = K + \frac{1}{R}\left(\frac{\Delta H_0{}^0}{T} - \Delta S_0{}^0\right) + \frac{n}{R}\left(\frac{\Delta h}{T} - \Delta s\right) \tag{13}$$

en posant:

$$K = \mathrm{Log_e} t_g \cdot \frac{\mathrm{N}_l}{\mathrm{N}_g} \tag{14}$$

Si un soluté i forme une solution idéale avec le solvant, son temps de rétention obtenu avec la même colonne à la température T est relié aux grandeurs thermodynamiques de vaporisation $\Delta H_{vi}{}^0$ et $\Delta S_{vi}{}^0$ de celui-ci.

$$\mathrm{Log_e}(t_{ri} - t_g) = K - \frac{\Delta S_{vi}{}^0}{R} + \frac{\Delta H_{vi}{}^0}{R \cdot T} \tag{15}$$

La valeur de l'indice de rétention I du soluté i est défini par l'intersection de la droite représentative de $\mathrm{Log_e}(t_r - t_g)$ avec n (équation 13 pour les alcanes), avec la droite parallèle à l'axe des n dont l'ordonnée est égale à $\mathrm{Log_e}(t_{ri} - t_g)$. On obtient ainsi en égalant les équations 13 et 15:

$$I = \frac{(\Delta H_{vi}{}^0 - T \cdot \Delta S_{vi}{}^0) - (\Delta H_0{}^0 - T \cdot \Delta S_0{}^0)}{\Delta h - T \cdot \Delta s} \tag{16}$$

L'équation 16 peut s'écrire simplement:

$$I = \frac{\Delta G_{vi}{}^0 - \Delta G_0{}^0}{\Delta g^0} \tag{16'}$$

On constate dans l'équation 16 que la variation de l'indice de rétention I avec la température est une fonction homographique. On peut remarquer aussi, comme le fit GIDDINGS[8], qu'il est possible dans ce cas de choisir pour les alcanes la valeur de l'entropie standard de vaporisation mesurée à leur température d'ébullition. On obtient alors pour Δs une valeur inférieure à 0.2 cal/°K ce qui peut permettre de négliger le terme $T \cdot \Delta s$ devant le terme Δh dans l'équation 16. Ce dernier résultat vérifie la variation sensiblement linéaire de l'indice de rétention avec la température qui a été observée expérimentalement[6,7].

Indice de rétention lorsque les grandeurs thermodynamiques de vaporisation varient linéairement avec la température

Il est très facile de constater que la constance avec la température, des grandeurs thermodynamiques de vaporisation ne correspond absolument pas à la réalité. En effet, l'enthalpie de vaporisation décroit avec la température et s'annule à la température critique du soluté. Cependant, dans le domaine des températures inférieures de 160°C à la température critique d'un soluté, on peut admettre avec une approximation convenable que l'enthalpie de vaporisation décroit de façon linéaire avec la température. Nous formulons l'hypothèse que la valeur de l'entropie standard de vaporisation décroit elle aussi de façon linéaire avec la température. On obtient pour les alcanes:

$$\Delta H_0{}^0 = A_0{}^0 + B_0{}^0 \cdot T \tag{17}$$

$$\Delta h = A + B \cdot T \tag{18}$$

$$\Delta S_0{}^0 = a_0{}^0 + b_0{}^0 \cdot T \tag{19}$$

$$\Delta s = a + b \cdot T \tag{20}$$

et pour le soluté i:

$$\Delta H_{vi}{}^0 = A_i + B_i \cdot T \quad (21)$$

$$\Delta S_{vi}{}^0 = a_i + b_i \cdot T \quad (22)$$

L'équation 10 appliquée aux alcanes normaux, devient:

$$\mathrm{Log_e}(t_r - t_g) = K + \frac{1}{R}\left(\frac{A_0{}^0}{T} + B_0{}^0 - a_0{}^0 - b^0 \cdot T\right) + \frac{n}{R}\left(\frac{A}{T} + B - a - b \cdot T\right) \quad (23)$$

et l'on constate alors que le temps de rétention varie de façon plus complexe avec la température que dans le cas précédent.

Le temps de rétention du soluté i s'écrit en utilisant la même équation[10]:

$$\mathrm{Log_e}(t_r - t_g) = K + \frac{B_i - a_i - b_i \cdot T}{R} + \frac{A_i}{R \cdot T} \quad (24)$$

et l'indice de rétention du soluté i devient:

$$I = \frac{A_i - A_0{}^0 + T(B_i - a_i - B_0{}^0 + a_0{}^0) + T^2 \cdot (b_0{}^0 - b_i)}{A + T \cdot (B - a) - T^2 \cdot b} \quad (25)$$

Indice de rétention dans le cas général

D'une façon générale, et dans tout le domaine de température où peuvent coèxister les phases liquide et vapeur du soluté, la variation linéaire de l'enthalpie de vaporisation avec la température ne convient pas tout à fait. En effet, elle ne correspond pas au fait que la décroissance de l'enthalpie de vaporisation devient de plus en plus rapide lorsque l'on approche de la température critique, et au fait que la pente de cette courbe tend vers l'infini à la température critique.

Une des meilleures représentations de la variation de l'enthalpie standard de vaporisation avec la température est celle de THEISEN[9] qui tient compte de la valeur nulle de l'enthalpie à la température critique T_c:

$$\Delta H_{vT}{}^0 = \Delta H_{vT_1}{}^0 \cdot \left[\frac{T_c - T}{T_c - T_1}\right]^{0.38} \quad (26)$$

Il n'existe pas de relation semblable pour représenter la variation de l'entropie standard de vaporisation avec la température, aussi nous avons admis que cette grandeur diminue de façon linéaire avec la température, comme le prévoient les équations 19 et 20:

$$\Delta S_v{}^0 = a_0{}^0 + b_0{}^0 \cdot T + n \cdot (a + b \cdot T) \quad (27)$$

Précisions tout d'abord la variation de $\Delta H_{vT_1}{}^0$ avec le nombre d'atomes de carbone n, dans le cas des alcanes normaux. Pour celà, choisissons pour chaque alcane la valeur de la température d'ébullition pour T_e dans l'équation 26. On constate d'autre part que l'enthalpie de vaporisation des alcanes normaux compris entre le pentane et le dodécane, déterminées à leur température d'ébullition normale, varie linéairement avec n, et l'équation 17 peut s'appliquer:

$$\Delta H_{vT_e}{}^0 = \Delta H_{0T_e}{}^0 + n \cdot \Delta h_{T_e} \quad (17')$$

De plus, les variations avec le nombre d'atomes de carbone de la température critique et de la température d'ébullition normale de chaque alcane, peuvent être représentées à l'aide de deux polynomes ne fluctuant pas entre les valeurs entières de n.

$$T_c = P(n) \qquad T_e = P'(n) \tag{28}$$

L'équation 26 devient alors pour les alcanes:

$$\Delta H_{vT}{}^0 = (\Delta H_{0T_e}{}^0 + n\cdot\Delta h_{T_e})\cdot\left[\frac{P(n) - T}{P(n) - P'(n)}\right]^{0.38} \tag{29}$$

et la relation entre le temps de rétention des alcanes et n devient:

$$\text{Log}_e(t_r - t_g) = K - \frac{a_0{}^0 + b_0{}^0\cdot T + n(a + b\cdot T)}{R} + \frac{\Delta H_{0T_e}{}^0 + n\cdot\Delta h_{T_e}}{R\cdot T} + \left[\frac{P(n) - T}{P(n) - P'(n)}\right]^{0.38} \tag{30}$$

Pour la substance i dont nous cherchons la valeur de l'indice de rétention I, nous obtenons en indiçant i à tous les coefficients définis précédemment:

$$\text{Log}_e(t_r - t_g) = K - \frac{a_i + b_i\cdot T}{R} + \frac{\Delta H_{iT_e}{}^0}{R\cdot T}\cdot\left[\frac{T_{ci} - T}{T_{ci} - T_{ei}}\right]^{0.38} \tag{31}$$

L'indice de rétention est alors défini en égalant les équations 30 et 31, et il est égal à la racine de l'équation 32:

$$T\cdot[a_0 + b_0\cdot T + \mathrm{I}\cdot(a + b\cdot T) - a_i - b_i\cdot T] =$$

$$(\Delta H_{T_e}{}^0 + I\cdot\Delta h_{T_e})\cdot\left[\frac{P(I) - T}{P(I) - P'(I)}\right]^{0.38} - \Delta H_{T_{ei}}{}^0\cdot\left[\frac{T_{ci} - T}{T_{ci} - T_{ei}}\right]^{0.38} \tag{32}$$

PARTIE EXPÉRIMENTALE

Chromatographe "Perkin-Elmer" F 7 avec catharomètres; colonne: longueur 2 m, diamètre intérieur 3 mm; liquide stationnaire: squalane à 4 %; support: Chromosorb G AW-DMCS 80/100 mesh; gaz vecteur: hélium, débit 30 cm³/min. Solutés: alcanes normaux, hexane, heptane, octane et nonane, et cyclohexane.

Les calculs ont été réalisés à l'aide d'un Ordinateur IBM 1620 modèle 2.

RÉSULTATS EXPÉRIMENTAUX

En utilisant des valeurs récentes[9] de l'enthalpie et de l'entropie standard de vaporisation des alcanes normaux, nous avons pu déterminer la variation de ces grandeurs thermodynamiques avec le nombre d'atomes de carbone, et éventuellement avec la température. Pareillement, il a été possible de déterminer la variation de l'enthalpie et de l'entropie standard de vaporisation du cyclohexane avec la température.

Nous étudierons successivement chacun des trois cas correspondant à une variation différente des grandeurs thermodynamiques de vaporisation avec la température.

Grandeurs thermodynamiques constantes

En admettant que les grandeurs thermodynamiques de vaporisation ne varient pas avec la température il a été possible de déterminer les valeurs de l'enthalpie et de l'entropie standard de vaporisation des solutés à une température égale pour chaque soluté à sa température d'ébullition normale[10]. Ces valeurs sont valables pour les alcanes normaux compris entre le butane et le dodécane.

TABLEAU I

VALEUR DES ENTHALPIES ET ENTROPIES DE VAPORISATION DES ALCANES ET DU CYCLOHEXANE EN CONSIDÉRANT QU'ELLES SONT CONSTANTES AVEC LA TEMPÉRATURE

$\Delta H_0^0 = 3340 \pm 100$	cal/mole	alcanes normaux
$\Delta h = 605 \pm 20$	cal/mole/n	alcanes normaux
$\Delta S_0^0 = 19.1 \pm 0.5$	cal/mole/°K	alcanes normaux
$\Delta s = 0.19 \pm 0.01$	cal/mol/°K/n	alcanes normaux
$\Delta H_{vi}^0 = 7140 \pm 100$	cal/mole	cyclohexane
$\Delta S_{vi}^0 = 20.2 \pm 0.5$	cal/mole/°K	cyclohexane

Les différentes valeurs regroupées dans le Tableau I coïncident assez bien avec les valeurs utilisées par GIDDINGS[8]. Nous devons dire cependant que l'utilisation de telles valeurs n'est pas convenable.

Grandeurs thermodynamiques variant linéairement avec la température

Dans le domaine de température situé à 160°C au-dessous de la température critique de chaque soluté, on peut admettre avec une bonne précision que les enthalpies de vaporisation décroissent de façon linéaire avec la température. Les valeurs expérimentales[9] nous ont permis de déterminer les variations des grandeurs thermodynamiques avec la température (Tableau II).

TABLEAU II

VALEURS DES ENTHALPIES ET ENTROPIES DE VAPORISATION DES ALCANES ET DU CYCLOHEXANE EN CONSIDÉRANT QU'ELLES VARIENT LINÉAIREMENT AVEC LA TEMPÉRATURE

$\Delta H_0^0 = (3040 \pm 40) - (9 \pm 0.2) \cdot T$ cal/mole	alcanes
$\Delta h = (1480 \pm 50) - (1 \pm 0.05) \cdot T$ cal/mol/n	alcanes
$\Delta S_0^0 = (26.7 \pm 0.4) - (0.045 \pm 0.002) \cdot T$ cal/mole/°K	alcanes
$\Delta s = (1.6 \pm 0.1)$ cal/mol/°K/n	alcanes
$\Delta H_{vi}^0 = (11900 \pm 500) - (13.5 \pm 0.5) \cdot T$ cal/mole	cyclohexane
$\Delta S_{vi}^0 = (37.2 \pm 0.5) - (0.045 \pm 0.002) \cdot T$ cal/mole/°K	—

N'ayant pu trouver les valeurs de l'entropie standard de vaporisation des alcanes à différentes températures, nous avons admis que ces grandeurs varient linéairement avec la température.

Grandeurs thermodynamiques dans le cas général

Nous avons admis pour les alcanes que l'entropie standard de vaporisation décroit linéairement avec la température comme dans le cas précédent, et que l'enthalpie standard de vaporisation décroit avec la température selon la relation de THEISEN[9]. Nous obtenons alors les valeurs citées dans le Tableau III:

TABLEAU III

VALEURS DES ENTHALPIES ET ENTROPIES DE VAPORISATION DES ALCANES ET DU CYCLOHEXANE DANS LE CAS GÉNÉRAL

$\Delta H_{vT_e}{}^0 = (3340 \pm 100) + (605 \pm 20)\cdot n$	alcanes
$\Delta S_v{}^0 = (26.7 \pm 0.4) - (0.045 \pm 0.002)\cdot T + (1.6 \pm 0.1)n$	
$\Delta H_{vi}{}^0 = (7140 \pm 100)\cdot\left[\dfrac{553 - T}{553 - 354.4}\right]^{0.38}$	cyclohexane
$\Delta S_{vi}{}^0 = (37.2 \pm 0.5) - (0.045 \pm 0.002)\cdot T$	cyclohexane

D'autre part, nous avons déterminé pour les alcanes normaux compris entre le pentane et le dodécane, les coéfficients des termes des polynômes $P(n)$ et $P'(n)$ qui représentent la variation de leur température critique et de leur température d'ébullition avec le nombre d'atomes de carbone, comme celà à été défini dans l'équation 28. Les polynômes choisis ne fluctuent pas, et ne possèdent ainsi pas d'extrémum entre les différentes valeurs de n. Pour chaque polynôme, nous avons calculé l'écart-type σ, et nous constatons ainsi que le polynôme de degré 4 est le plus satisfaisant pour la température d'ébullition, alors que pour la température critique le plus satisfaisant a le degré 5.

TABLEAU IV

DIFFÉRENTS POLYNOMES D'AFFITAGE DES COMBES "TEMPÉRATURE CRITIQUE" ET "TEMPÉRATURE D'EBULLITION" EN FONCTION DU NOMBRE D'ATOMES DE CARBONES

Degré			σ
2	T_e	$P'(n) = 121.2 + 42.69\cdot n - 1.005\cdot n^2$	$47.2\cdot 10^{-2}$
	T_c	$P(n) = 248.6 + 51.81\cdot n - 1.481\cdot n^2$	$100.9\cdot 10^{-2}$
3	T_e	$P'(n) = 91.94 + 54.05\cdot n - 2.401\cdot n^2 + 0.0547\cdot n^3$	$5.1\cdot 10^{-2}$
	T_c	$P(n) = 187 + 75.64\cdot n - 4.41\cdot n^2 + 0.1148\cdot n^3$	$19.47\cdot 10^{-2}$
4	T_e	$P'(n) = 77.18 + 61.71\cdot n - 3.84\cdot n^2 + 0.17\cdot n^3 - 0.0034\cdot n^4$	$4.16\cdot 10^{-2}$
	T_c	$P(n) = 153.4 + 93.11\cdot n - 7.69\cdot n^2 + 0.38\cdot n^3 - 7.81\cdot n^4$	$12.34\cdot 10^{-2}$
5	T_e	$P'(n) = 79.55 + 61\cdot n - 3.88\cdot n^2 + 0.2\cdot n^3 - 0.0067\cdot n^4 + 0.00011\cdot n^5$	$4.9\cdot 10^{-2}$
	T_c	$P(n) = 153.4 + 91.7\cdot n - 6.95\cdot n^2 + 0.24\cdot n^3 + 3.2\cdot 10^{-3}\cdot n^4 - 3.2\cdot 10^{-5}\cdot n^5$	$11.9\cdot 10^{-2}$
6	T_e	$P'(n) = 120.1 + 38.24\cdot n + 0.588\cdot n^2 - 0.0945\cdot n^3 - 0.0186\cdot n^4 + 0.00245\cdot n^5 - 0.000073\cdot n^6$	$4.53\cdot 10^{-2}$
	T_c	$P(n) = 197.4 + 68.87\cdot n - 3.257\cdot n^2 + 0.192\cdot n^3 - 4.07\cdot 10^{-2}\cdot n^4 + 3.95\cdot 10^{-3}\cdot n^5 - 1.23\cdot 10^{-4}\cdot n^6$	$14.47\cdot 10^{-2}$

Valeurs des indices de rétention

Nous avons calculé pour chacun des trois cas, les valeurs de l'indice de rétention du cyclohexane à différentes températures, en utilisant chaque fois l'équation convenable.

D'autre part, nous avons déterminé expérimentalement la valeur de l'indice de rétention aux mêmes températures en travaillant avec le squalane comme solvant. Puis, en utilisant ces valeurs des indices et des valeurs des coéfficients d'activité des alcanes et du cyclohexane dans le squalane[11] réunies dans le Tableau V, nous avons pu calculer les valeurs des indices de rétention qu'aurait le cyclohexane dans une solution idéale.

TABLEAU V

COÉFFICIENTS D'ACTIVITÉ DES SOLUTIONS DANS LE SQUALANE

	80°C	*105°C*
n-Hexane	0.66	0.65
n-Heptane	0.68	0.66
n-Octane	0.70	0.68
Cyclohexane	0.52	0.50

Nous avons alors réuni dans le Tableau VI les valeurs des indices de rétention du cyclohexane, obtenues experimentalement dans le squalane (colonne 2) les valeurs calculées à partir de ces valeurs dans une solution idéale (colonne 6), et les valeurs obtenues par le calcul en utilisant la première méthode (colonne 3), la seconde méthode (colonne 4) et la troisième méthode (colonne 5).

TABLEAU VI

INDICES DE RÉTENTION

T (°C)	*Exp.*	*Eqn. 1*	*Méthode 2*	*Méthode 3*	*Exp. idéale*
40	658	634.8	625	655.4	
50	660	635	627	657	
60	663	635.1	629	658.5	
70	667	635.3	631.5	660.1	
80	670	635.5	634	663.2	658
90	672	635.8	637	665	
100	673.5	636	640	668	662
110	676	636.3	643.4	669.5	665.5
130	680	636.7	651	674	
150	682.5	637	661	680.4	
160	683.2	637.5	667.7	683.5	

Nous constatons aisément sur le Tableau VI que les valeurs des indices de rétention obtenues en utilisant l'équation 30 correspondant à l'équation de THEISEN (colonne 5) sont celles qui s'approchent le plus des valeurs expérimentales exprimées dans le cas de la solution idéale (colonne 6), et ceci à toutes les températures.

CONCLUSIONS

Après avoir souligné l'intérèt que peut présenter l'emploi en chromatographie de la notion de solution idéale, de la même façon que dans la thermodynamique classique des solutions, nous avons pu déterminer des lois de variation des grandeurs thermodynamiques de vaporisation avec le nombre d'atomes de carbone des alcanes normaux. Ces lois ont été établies en utilisant des grandeurs expérimentales dans les trois cas: d'une part, dans un domaine très étroit de température pour lequel on peut admettre que ces grandeurs sont constantes; d'autre part, lorsque ces grandeurs décroissent linéairement avec la température, cette hypothèse étant valable dans le domaine des températures inférieures de 160°C à la température critique de tous les solutés considérés; et enfin, pour toutes les températures, en formulant l'hypothèse que l'enthalpie standard de vaporisation décroit avec la température selon la loi de THEISEN.

En utilisant ces lois obtenues dans chacun des trois cas, il a été possible de définir de façon plus précise des relations permettant le calcul des indices de rétention. Dans le troisième cas, la variation de la température critique et de la température d'ébullition avec le nombre d'atomes de carbone des alcanes, a été présentée sous forme de polynomes, et les valeurs obtenues pour l'indice de rétention sont celles qui approchent le mieux les valeurs expérimentales.

SYMBOLES UTILISÉS

ΔG_v^0, ΔH_v^0, ΔS_v^0: respectivement enthalpie libre standard, enthalpie standard, et entropie standard de vaporisation des alcanes normaux.

Δh, Δs, Δg: respectivement la contribution de chaque groupement $-CH_2-$ à l'enthalpie standard, l'entropie standard, l'enthalpie libre standard de vaporisation des alcanes normaux.

ΔH_0^0, ΔS_0^0, ΔG_0^0: respectivement la contribution des 2 H situés aux deux bouts de chaine, à l'enthalpie standard, l'entropie standard, l'enthalpie libre standard de vaporisation des alcanes normaux.

ΔH_{vi}^0, ΔS_{vi}^0, ΔG_{vi}^0: respectivement l'enthalpie standard, l'entropie standard, l'enthalpie libre standard de vaporisation du soluté i.

I: indice de rétention du soluté i.

k: coefficient de partage du soluté entre les deux phases.

n: nombre d'atomes de carbone des alcanes normaux.

N_l, N_g: respectivement fraction molaire du soluté dans la solution, et dans la phase gazeuse.

n_l, n_g: respectivement molarité du soluté dans la solution, et dans la phase gazeuse.

n_l': molarité du solvant dans la solution.

n_g': nombre de moles de gaz vecteur par litre de phase gazeuse.

N_l: nombre de moles de solvant situé dans la colonne.

N_g: nombre de moles de gaz vecteur situé dans la colonne.

P: pression de la phase gazeuse.

p: pression partielle du soluté.

p^0: tension de vapeur du soluté pur.

$\gamma_{l\infty}$: coéfficient d'activité du soluté infiniment dilué dans la solution.
T: température de la colonne (°Kelvin).
t_r: temps de rétention du soluté.
t_g: temps nécessaire au gaz vecteur pour traverser la colonne.
V_l, V_g: respectivement volume de la colonne occupé par le solvant, et par la phase gazeuse.

RÉSUMÉ

En utilisant des grandeurs expérimentales connues, il a été possible de déterminer des lois de variation de l'enthalpie et de l'entropie standard de vaporisation des alcanes normaux dans les trois cas correspondant chacun à l'une des trois hypothèses simplificatrices: l'enthalpie est constante, l'enthalpie décroit linéairement avec la température, et enfin l'enthalpie décroit avec la température selon la relation de THEISEN. La théorie générale de l'indice de rétention a été ensuite élaborée dans chacun de ces trois cas en considérant une solution idéale des solutés dans le solvant. La variation de l'indice de rétention du cyclohexane avec la température a pu être déterminée dans les trois cas, et les résultats obtenus avec le dernier cas s'accordent le mieux avec l'expérience.

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CALCULATION AND MEASUREMENT OF CONCENTRATIONS IN ISOTACHOPHORESIS

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SUMMARY

A set of equations is developed to describe isotachophoretic electrolyte systems in the steady state. pH measurements are done in capillaries and sucrose gradients to check the results of calculations based on these equations. The experimental values show good agreement with the theory.

INTRODUCTION

Isotachophoresis has already proved to be an electrophoretic method with a high resolving power[1,2]. The principle of this technique is that zones of ionogenic compounds, separated according to their mobilities and equilibrium constants, will move with the same speed. Their concentrations are then adjusted to the concentration of the first ion zone, called the leading electrolyte. The last ion zone is formed by the terminating electrolyte.

The theory of isotachophoresis has been dealt with by several authors. In a theoretical treatment of disc electrophoresis, ORNSTEIN[3] derived some equations for the separation of ions in the first isotachophoretic phase of this procedure. In this first phase, the discs are stacked upon each other and move with the same speed. In the second phase, they migrate according to the principles of zone electrophoresis. The equations are valid between pH 4 and 10, where the influence of protons and hydroxyl ions on the conductivity can be neglected.

SCHUMACHER AND STUDER[4] calculated pH increments in an isotachophoretic system for monovalent weak electrolytes, but where no buffer ions are taken into consideration. They attempted to verify this theory with experiments on paper. MARTIN AND EVERAERTS[5,7] calculated the pH of isotachophoretic systems for univalent ions including H^+ and OH^-. They found, like ORNSTEIN, a difference in pH between two consecutive zones. This pH shift varied from a few tenths of a pH unit to several units. BROUWER AND POSTEMA[6] described the very separation process by considering the ions before the steady state in isotachophoresis is reached (when all zones move with the same speed).

The purpose of this paper is to derive general equations that can be applied to

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most isotachophoretical systems. The concentrations of the ions in the moving zones will be calculated and the results will be compared to actual measurements.

THEORETICAL

Ion concentrations in isotachophoresis

Let us consider a system of two zones, which are moving according to isotachophoretic principles. The first zone contains A^-, ..., $A^{\alpha-}$ and the second B^-, ..., $B^{\beta-}$ ions. The general positive counter-ions are P^+, ..., $P^{\pi+}$ (Fig. 1). Choosing a

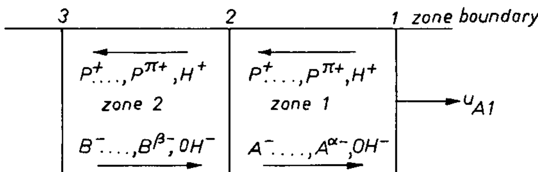


Fig. 1. Isotachophoretically moving zones of the ions A^-, ..., $A^{\alpha-}$ and B^-, ..., $B^{\beta-}$. P^+, ..., $P^{\pi+}$ is the buffering counterion.

particular concentration of A and a certain pH in zone 1, the concentration of P can be calculated from the equilibrium equations. The concentrations of B and P in the second zone will adjust to the concentrations in the first zone, in such a way that they will create a voltage gradient large enough to give the second zone the same speed as the first one. In order to calculate the concentrations of B and P in zone 2, one can assume the following conditions:

The balance of the electric current. The electric current is constant throughout the system. The current in zone 1 is the same as in zone 2.

The balance of mass. The concentration of the counter-ion within one zone is constant in the steady state. This means that the amount of P transported into a zone is equal to the amount that moves out of the zone.

The electroneutrality principle. The amounts of positive and negative charges are equal in the same zone.

Equilibrium equations. The acid and base equilibria determine the partial ion concentrations.

In the derivation of the following equations it is assumed that: (a) the diffusion effects are negligible; (b) the solutions are dilute, *i.e.*, no activity coefficients have to be included; (c) the area through which the current passes is constant; (d) the effect of electroendosmosis is negligible; (e) no hydrostatic flow exists.

The balance of electric current. For an explanation of the symbols used the reader is referred to the LIST OF SYMBOLS.

According to assumption (c) above, we have

$$I_1 = I_2 \qquad (1)$$

The electric conductivity in zone 1 is

$$\lambda_1 = c_{\mathrm{OH1}} m_{\mathrm{OH1}} z_{\mathrm{OH1}} + c_{\mathrm{H1}} m_{\mathrm{H1}} z_{\mathrm{H1}} + \sum_{i=0}^{\alpha} z_{\mathrm{A1}i} c_{\mathrm{A1}i} m_{\mathrm{A1}i} + \sum_{i=0}^{\pi} z_{\mathrm{P1}i} c_{\mathrm{P1}i} m_{\mathrm{P1}i}$$

$$= c_{\mathrm{OH1}} m_{\mathrm{OH1}} + c_{\mathrm{H1}} m_{\mathrm{H1}} + \sum_{i=0}^{\alpha} i c_{\mathrm{A1}i} m_{\mathrm{A1}i} + \sum_{i=0}^{\pi} i c_{\mathrm{P1}i} m_{\mathrm{P1}i} \qquad (2)$$

Ohms law gives

$$I_1 = G_1\lambda_1 \tag{3}$$

$$I_2 = G_2\lambda_2 \tag{4}$$

Substitution of (4) and (3) in (1) gives:

$$G_1\lambda_1 = G_2\lambda_2 \tag{5}$$

Substitution of (2) in (5) results in:

$$G_1(c_{\mathrm{OH1}}m_{\mathrm{OH1}} + c_{\mathrm{H1}}m_{\mathrm{H1}} + \sum_{i=0}^{\alpha} ic_{\mathrm{A1}i}m_{\mathrm{A1}i} + \sum_{i=0}^{\pi} ic_{\mathrm{P1}i}m_{\mathrm{P1}i}) =$$

$$G_2(c_{\mathrm{OH2}}m_{\mathrm{OH2}} + c_{\mathrm{H2}}m_{\mathrm{H2}} + \sum_{i=0}^{\beta} ic_{\mathrm{B2}i}m_{\mathrm{B2}i} + \sum_{i=0}^{\pi} ic_{\mathrm{P2}i}m_{\mathrm{P2}i}) \tag{6}$$

As the temperature in zone 1 is higher than in zone 2 (ref. 8), the net mobilities m_{P_1i} and m_{P_2i} will be different.

In an isotachophoretic system all zones have the same speed:

$$u_{\mathrm{A1}} = u_{\mathrm{B2}} \tag{7}$$

$$u_{\mathrm{A1}} = G_1 \cdot \sum_{i=0}^{\alpha} m_{\mathrm{A1}i} \cdot \frac{c_{\mathrm{A1}i}}{c^1{}_{\mathrm{A1}}} \tag{8}$$

Combination of (7) and (8):

$$\frac{G_2}{G_1} = \frac{c^1{}_{\mathrm{B2}}}{c^1{}_{\mathrm{A1}}} \cdot \frac{\sum_{i=0}^{\alpha} m_{\mathrm{A1}i}c_{\mathrm{A1}i}}{\sum_{i=0}^{\beta} m_{\mathrm{B2}i}c_{\mathrm{B2}i}} \tag{9}$$

Substituting (9) in (6) gives:

$$\frac{c^1{}_{\mathrm{A1}}}{c^1{}_{\mathrm{B2}}} = \frac{\sum_{i=0}^{\alpha} m_{\mathrm{A1}i}c_{\mathrm{A1}i}}{\sum_{i=0}^{\beta} m_{\mathrm{B2}i}c_{\mathrm{B2}i}} \cdot \frac{c_{\mathrm{OH2}}m_{\mathrm{OH2}} + c_{\mathrm{H2}}m_{\mathrm{H2}} + \sum_{i=0}^{\beta} im_{\mathrm{B2}i}c_{\mathrm{B2}i} + \sum_{i=0}^{\pi} im_{\mathrm{P2}i}c_{\mathrm{P2}i}}{c_{\mathrm{OH1}}m_{\mathrm{OH1}} + c_{\mathrm{H1}}m_{\mathrm{H1}} + \sum_{i=0}^{\alpha} im_{\mathrm{A1}i}c_{\mathrm{A1}i} + \sum_{i=0}^{\pi} im_{\mathrm{P1}i}c_{\mathrm{P1}i}} \tag{10}$$

This is the extended form of the KOHLRAUSCH[9] regulating function. It gives a relation between the ion concentration in the first and the second zone.

The balance of mass. Let us consider the total mass balance of the counterion P in zone 2. If the zone boundary 2 (Fig. 1) is moving forward with a speed u_{A1} and the total concentration of the counterion in zone 1 is $c^1{}_{\mathrm{P1}}$ then the mass transport of P into zone 2 due to this movement is $u_{\mathrm{A1}}c^1{}_{\mathrm{P1}}$

Furthermore, there is an electrophoretic transport of P-ions, which is equal to $G_1 \cdot \sum_{i=0}^{\pi} c_{\mathrm{P1}i}m_{\mathrm{P1}i}$.

The total mass transport of P into zone 2 is therefore:

$$u_{\mathrm{A1}}c^1{}_{\mathrm{P1}} + G_1 \sum_{i=0}^{\pi} c_{\mathrm{P1}i}m_{\mathrm{P1}i} \tag{11}$$

When the steady state is reached the mass transport of P through boundary 2 and 3 will be equal:

$$u_{A1}c^1{}_{P1} + G_1 \sum_{i=0}^{\pi} c_{P1i}m_{P1i} = u_{B2}c^1{}_{P2} + G_2 \sum_{i=0}^{\pi} c_{P2i}m_{P2i} \tag{12}$$

Insertion of (7) and (8) will result in:

$$c^1{}_{P1} - c^1{}_{P2} = -c^1{}_{A1} \cdot \frac{\sum_{i=0}^{\pi} m_{P1i}c_{P1i}}{\sum_{i=0}^{\alpha} m_{A1i}c_{A1i}} + c^1{}_{B2} \frac{\sum_{i=0}^{\pi} m_{P2i}c_{P2i}}{\sum_{i=0}^{\beta} m_{B2i}c_{B2i}} \tag{13}$$

The electroneutrality equations. The balance of charge for zone 1 is:

$$c_{OH1} + \sum_{i=0}^{\alpha} ic_{A1i} = c_{H1} + \sum_{i=0}^{\pi} ic_{P1i} \tag{14}$$

The charge balance for zone 2 is:

$$c_{OH2} + \sum_{i=0}^{\beta} ic_{B2i} = c_{H2} + \sum_{i=0}^{\pi} ic_{P2i} \tag{15}$$

Eqns. 14 and 15 will be different if we consider a system of positive ions A and B and negative ion P which is not the case for the eqns. 10 and 13:

$$c_{OH1} + \sum_{i=0}^{\pi} ic_{P1i} = c_{H1} + \sum_{i=0}^{\alpha} ic_{A1i} \tag{16}$$

$$c_{OH2} + \sum_{i=0}^{\pi} ic_{P2i} = c_{H2} + \sum_{i=0}^{\beta} ic_{B2i} \tag{17}$$

Equilibrium equations. From the equilibrium equations for A, B and P it is possible to derive the following equations:

$$c_{A1i} = \frac{c_{A10}}{(c_{H1})^i} \cdot \prod_{j=1}^{i} k_{Aj} \tag{18}$$

$$c_{B2i} = \frac{c_{B20}}{(c_{H2})^i} \cdot \prod_{j=1}^{i} k_{Bj} \tag{19}$$

$$c_{P1i} = \frac{c_{P10}(c_{H1})^i}{\prod_{j=1}^{i} k_{Pj}} \tag{20}$$

$$c_{P2i} = \frac{c_{P20} \cdot (c_{H2})^i}{\prod_{j=1}^{i} k_{Pj}} \tag{21}$$

Application of the equations to a system with a divalent terminating ion

Let us consider a system with a divalent, negatively charged, terminating ion and a monovalent, leading and buffering ion. The influence of protons and hydroxyl ions is neglected as a first approximation. Eqn. 10 will then take the form:

$$\frac{c^1_{A1}}{c^1_{B2}} = \frac{m_{A11}c_{A11}}{m_{B21}c_{B21} + m_{B22}c_{B22}} \cdot \frac{m_{B21}c_{B21} + 2m_{B22}c_{B22} + m_{P21}c_{P21}}{m_{A11} \cdot c_{A11} + m_{P11}c_{P11}} \quad (22)$$

By application of the electroneutrality principle:

$$c_{A11} = c_{P11} \quad (23)$$

$$c_{B21} + 2c_{B22} = c_{P21} \quad (24)$$

Substitution of (23) and (24) in (22) gives:

$$\frac{c^1_{A1}}{c^1_{B2}} = \frac{m_{A11}}{m_{A11} + m_{P11}} \cdot \frac{(m_{B21} + m_{P21})c_{B21} + 2(m_{B22} + m_{P21})c_{B22}}{m_{B21}c_{B21} + m_{B22}c_{B22}} \quad (25)$$

Combination of the mass balance (13) and electroneutrality rule (23), (24) results in:

$$c^1_{P1} - c^1_{P2} = -\frac{c^1_{A1} \cdot m_{P11}}{m_{A11}} + \frac{c^1_{B2}m_{P21}(c_{B21} + 2c_{B22})}{m_{B21} \cdot c_{B21} + m_{B22}c_{B22}} \quad (26)$$

The equilibrium equations are:

$$c_{A11} = \frac{(c^1_{A1} - c_{A11})}{c_{H1}} \cdot k_{A1} \quad (27)$$

$$c_{B21} = \frac{(c^1_{B2} - c_{B21} - c_{B22})}{c_{H2}} \cdot k_{B1} \quad (28)$$

$$c_{B22} = \frac{(c^1_{B2} - c_{B21} - c_{B22})}{c^2_{H2}} \cdot k_{B1} \cdot k_{B2} \quad (29)$$

$$c_{P11} = \frac{(c^1_{P1} - c_{P11})}{k_{P1}} \cdot c_{H1} \quad (30)$$

$$c_{P21} = \frac{(c^1_{P2} - c_{P21})}{k_{P1}} \cdot c_{H2} \quad (31)$$

If two of the nine parameters in the seven equations (25–31) are known, the other seven can be calculated. Thus if the pH and the total concentration of the leading ion are chosen, all other ion concentrations, including the pH in the terminating electrolyte, are fixed. Consequently we are able to calculate the net mobility[10] of the terminating ion, which is defined as

$$nm_B = \frac{\sum_{i=0}^{\beta} c_{B2i} \cdot m_{B2i}}{c^1_{B2}} \quad (32)$$

Polyvalent electrolyte systems

Dealing with systems of multivalent ions, where proton and hydroxyl ion-influence is not neglected, an even more nonlinear set of equations will be obtained than those described above. The use of data techniques is necessary to obtain all the roots.

With increasing nonlinearity, the equation will have an increasing number of roots, which are physically incorrect solutions for the system considered. The correct solution is obtained by rejecting all roots containing negative, imaginary and obviously unrealistic concentrations.

EXPERIMENTAL

Materials and methods

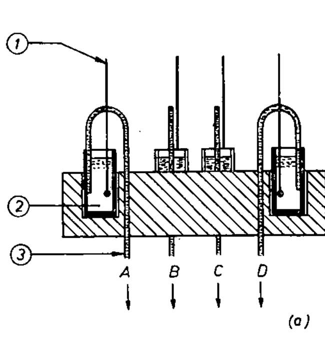
The equations derived in the preceding section can be checked by the determination of the pH changes between isotachophoretically moving zones. These measurements, and temperature measurements[8] by thermocouples, were done in a capillary tube apparatus. A second series of pH determinations was done on a preparative scale in a sucrose-gradient in a glass column. VESTERMARK[12] has already reported results of pH measurements in sucrose-gradients.

All the chemicals used are commercially available and of analytical grade.

The capillary apparatus

A set of six PTFE capillaries was mounted in a water-bath thermostat between two electrode compartments (Fig. 2). The inner/outer diameters of the tubes are 0.45/0.75 mm and the length is approximately 1.5 m. The cathode compartment consists of a perspex block, containing six reservoirs for the terminating electrolyte. The anode compartment is a reservoir for the leading electrolyte. A cellulose acetate membrane is placed over the latter and is stretched by an exactly-fitting polyethylene tube. This construction excludes hydrostatic flow and prevents endosmotic effects to a great extent.

A Baird-Atomic voltage supply, model 1512, was used. All measurements were



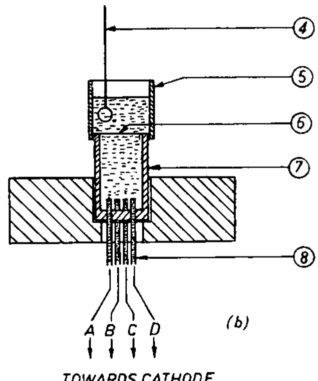


Fig. 2. The electrode compartments of the capillary apparatus. (a) Terminator block. 1 = cathode; 2 = reservoir for terminating electrolyte; 3 = capillary. (b) Leading electrolyte block. 4 = anode; 5 = polyethylene tube; 6 = membrane; 7 = compartment for leading electrolyte; 8 = capillary.

done with a constant voltage of 5 kV. In each experiment, one of the terminator-reservoirs was filled with 0.02 *M* picric acid to indicate how far the analysis had proceeded. The pH in each terminator-reservoir was increased with Tris to a value which was about 0.5 pH units below the pH of the leading electrolyte.

When an experiment was finished, the electrolyte from two capillaries was collected and its pH measured. Then the pH of the content of two other tubes and, finally, of all five tubes together, was determined. The results were averaged together with those of a second identical experiment. The standard deviation was in all cases less than 0.03 pH units.

The isotachophoresis apparatus used for measurement of the temperatures of the zones is basically the same as the one described by EVERAERTS AND VERHEGGEN[11], which has a thermocouple glued to the capillary wall to act as a heat detector.

The column apparatus

An isoelectric focusing column (LKB 8100) was used for measurements on a larger scale. Part of the platinum wire of the anode (Fig. 3) was removed to increase the migration distance. A constant flow of 10 ml of leading electrolyte per hour was maintained around the anode, to prevent the migration of protons and electrode products to the cathode. To prevent convection, the leading ion was introduced into the column in a sucrose gradient. The inner tube (Fig. 3), and the bottom part of the outer tube of the column, is filled with leading electrolyte in a 40 % sucrose solution. A sucrose gradient, from 40 to 10 %, is then layered on top of this solution. Finally, the terminating electrolyte is introduced. The temperature was kept at 25° by a water thermostat. Two power supplies (LKB 4471D) were connected in series and delivered

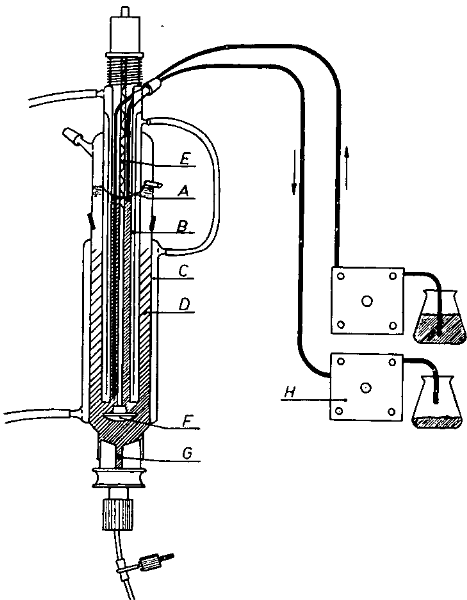


Fig. 3. Column apparatus. A = cathode; B = inner cooling jacket; C = outer cooling jacket; D = annular separation chamber; E = anode; F = valve; G = outlet; H = buffer circulation pump.

a constant voltage of 1600 V. The current decreased from 10 to 2 mA during the time of the analysis. The analysis time was 3 to 4 h, after which the electrolyte was pumped out of the column. Fractions of 2 ml were collected and the pH determined for each fraction. Fig. 4 shows the result of such an experiment.

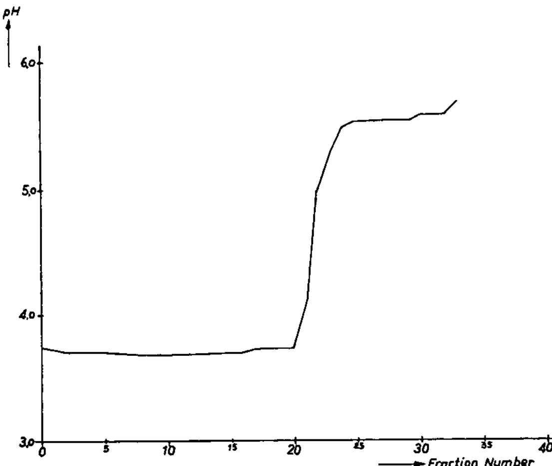


Fig. 4. The pH-change of the zones in an isotachophoretic separation of chloride and carbonate in a sucrose gradient. The buffering counterion is benzidine.

RESULTS

In the first series of experiments a solution of hydrochloric acid and histidine was used as the leading electrolyte. For the capillary experiments the concentrations were: 0.01 *M* HCl and 0.014 *M* histidine, which gave a pH of 5.75. In this system the pH of eleven weak acids, which were used as terminators, was determined. The temperature of the thermostatted bath was kept at 25°.

There is good agreement between the theoretical* and experimental values of the pH in the terminator zones, as can be seen in the first two columns of Table I.

The next two columns in Table I show the total concentrations of the terminating ion and the counter-ion in the terminator zone, as they were calculated from eqns. 10 and 13, respectively. The net mobilities are tabulated in the next column.

It is possible to calculate the electrical resistance in a zone from the partial ion concentrations. A plot of this parameter against the experimental recorder step-height of temperature measurement[7,8] results in Fig. 5. Theoretically, there should be a linear relation between the heat produced and the resistance, which in fact is the case for low resistances (Fig. 5). The deviation from the linearity at high temperatures can be explained by the fact that, in our calculation, the mobility dependence on temperature is not taken into account. Moreover the temperature measured by the thermocouple is not linear with respect to the temperature in the tube, because the heat transport by conductance, convection and radiation, changes with temperature.

The same histidine–HCl electrolyte system was used for the pH measurements

* All theoretical values were calculated with the GE 265 computer. All p*K* and mobility data are taken from literature (refs. 14–17).

TABLE I

THEORETICAL AND EXPERIMENTAL VALUES OF pH, CONCENTRATION, NET MOBILITY, RESISTANCE AND STEP HEIGHT IN AN ISOTACHOPHORETIC SYSTEM WITH HISTIDINE–HCl AS LEADING ELECTROLYTE

The experimental values are obtained from measurements in a capillary apparatus.

Ion species	$pH_{theor.}$	$pH_{exp.}$	$c^1{}_{B2}$	$c^1{}_{P2}$	nm_B	ρ (Ω *cm* $\times$ *10*^{3})	*h* (*mm*)
Chloride	5.75	5.75	0.0100	0.0145	78	1.12	0
Oxalate	5.76	5.78	0.0050	0.0143	72	1.20	20.1
Tartrate	5.79	5.80	0.0048	0.0141	62	1.38	47.2
Formate	5.79	5.81	0.0094	0.0139	56	1.50	51.5
Citrate	5.82	5.81	0.0043	0.0139	56	1.51	60.0
Succinate	5.85	5.81	0.0052	0.0138	52	1.61	68.0
Malonate	5.85	5.83	0.0055	0.0137	51	1.64	72.1
Acetate	5.89	5.87	0.0087	0.0132	39	2.03	109.4
α-Hydroxybutyrate	5.89	5.87	0.0085	0.0130	36	2.16	125.2
Phosphate	5.87	5.87	0.0077	0.0127	34	2.27	140.4
Carbonate	6.39	6.41	0.0089	0.0133	23	3.55	187.3
Diethylbarbiturate	6.88	6.74	0.0077	0.0121	6	11.80	—

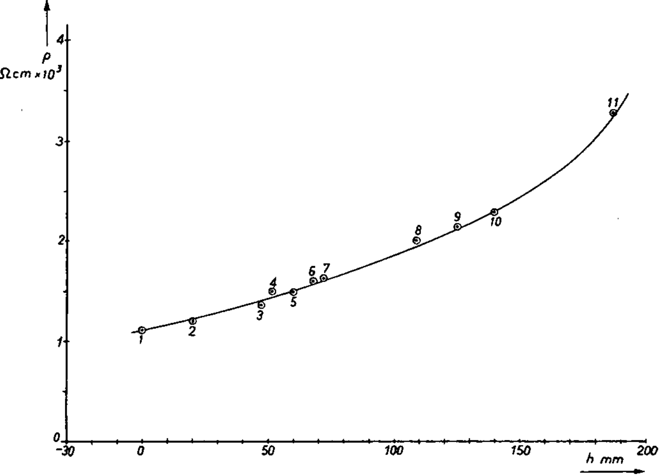


Fig. 5. The theoretical specific resistance of eleven isotachophoretically moving acid zones is plotted against the signal of a thermocouple which measures the temperature of these zones. 1 = chloride, 2 = oxalate, 3 = tartrate, 4 = formate, 5 = citrate, 6 = succinate, 7 = malonate, 8 = acetate, 9 = α-hydroxybutyrate, 10 = phosphate, 11 = carbonate.

in the glass column. For practical reasons the pH in the leading electrolyte was not the same in each experiment. The results are listed in the first three columns of Table II. Differences can be found between theoretical and experimental values for some acids. This could be due to diffusion and mixing effects during the sampling procedure. The influence of the original pH in the terminator solution on the pH of the isotachophoretically migrating terminator zone was studied in a few experiments. There was no difference in the experimental pH values as shown in Table II irrespective of whether histidine oxalate at pH = 5.1, or oxalic acid at pH = 1.95 was used as original

TABLE II

THEORETICAL AND EXPERIMENTAL VALUES OF THE pH IN TWO ISOTACHOPHORETIC SYSTEMS, ONE WITH HISTIDINE–HCl AND THE OTHER WITH BENZIDINE–HCl AS LEADING ELECTROLYTE

The experimental values were obtained from measurements in a sucrose gradient.

Ion species	*Leading electrolyte: histidine–HCl*			*Leading electrolyte: benzidine–HCl*		
	pH_{Cl}	$pH_{theor.}$	$pH_{exp.}$	pH_{Cl}	$pH_{theor.}$	$pH_{exp.}$
Oxalate	5.88	5.89	6.00	3.88	4.09	4.06
Formate	5.88	5.92	5.92	3.78	4.19	4.22
Succinate	6.10	6.16	6.21	3.70	4.36	4.34
Tartrate	6.11	6.13	6.19	3.88	4.12	4.12
Phosphate	5.93	6.04	6.14	3.72	3.87	4.10
Citrate	5.91	5.97	6.10	4.20	4.36	—
Malonate	5.85	5.94	5.90	3.81	3.89	4.00
Acetate	5.78	5.92	5.91	3.80	4.65	4.62
α-Hydroxybutyrate	5.86	5.98	6.05	3.70	4.58	4.28
Carbonate	5.89	6.43	6.40	3.70	5.52	5.52
Diethylbarbiturate	5.90	6.92	7.06	3.75	5.98	5.60

terminating electrolyte. Also, carbonate solutions at pH 9.1 and 7.0 gave the same pH values.

A second electrolyte system was used based on benzidine and hydrochloric acid. The reasons for the choice of benzidine were threefold. Firstly, the use of benzidine as a buffer ion enables the validity of the equations for a system with a divalent counter ion to be checked. Secondly, most of the weak acids which are used as terminators have their pH values within the buffering region of benzidine. Thirdly, because benzidine is a buffer at low pH, it can be used to prove that the equations are still valid even if a relatively large part of the current is carried by H^+.

On the other hand benzidine is not very stable, only slightly soluble in water and is poisonous. The pH values of the citrate and phosphate zones in the column could not be measured exactly, because large amounts of white precipitate were formed in the column, probably because of the higher ionic strength of these com-

TABLE III

THEORETICAL AND EXPERIMENTAL VALUES OF THE pH, CONCENTRATIONS AND NET MOBILITY IN AN ISOTACHOPHORETIC SYSTEM WITH BENZIDINE–HCl AS LEADING ELECTROLYTE.

The experimental values were obtained from measurements in a capillary apparatus.

Ion species	$pH_{theor.}$	$pH_{exp.}$	$c^1{}_{B2}$	$c^1{}_{P2}$	nm_B
Chloride	3.35	3.35	0.00100	0.0055	78
Oxalate	3.56	3.42	0.00682	0.00475	46
Formate	3.97	3.85	0.00999	0.00453	35
Succinate	3.65	3.62	0.00803	0.00434	35
Tartrate	3.75	3.75	0.00631	0.00429	33
Phosphate	3.60	3.70	0.00720	0.00417	32
Citrate	3.78	3.74	0.00676	0.00399	28
Malonate	4.16	4.00	0.00864	0.00375	21
Acetate	4.47	4.20	0.00958	0.00346	15
α-Hydroxybutyrate	4.44	4.19	0.00444	0.00330	14
Carbonate	5.36	4.80	0.00536	0.00303	4

ponents. Tables III and II show the theoretical and experimental pH values in the capillary and column system, respectively. There are differences between calculated and experimental values, which will be discussed below.

In the preceding two series of experiments, pH values were measured in the terminating zones, using the same pH in the leading electrolyte. In a third series of experiments the influence of the pH of the leading electrolyte (0.03 *M* acetate) on the pH of the terminator zone (cacodylate) was studied over a wider pH range. The pH of the leading electrolyte was varied by changing the counter ion (Tris) concentration. The results are plotted in Fig. 6. Three experimental points seem to fit the theoretical curve. In two cases there is a small deviation from the curve.

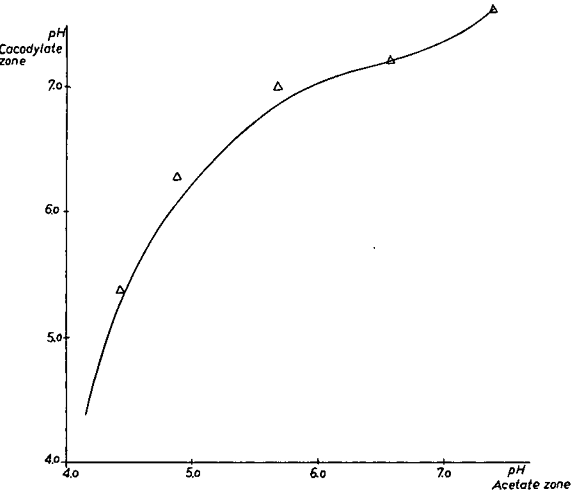


Fig. 6. The relation between the pH in the leading electrolyte and in the terminator zone for an isotachophoretic system. Acetate (0.03 *M*) is the leading ion, cacodylate is the terminator. The concentration of the counterion Tris determines the pH of the leading electrolyte. The $\triangle$-symbol is used for the experimental results; the curve being the one determined theoretically.

DISCUSSION

The purpose of this paper was to derive equations applicable to most isotachophoretic systems and to compare the results of these equations with experimental measurements. The results of the pH measurements seem to confirm the theory. However, one should bear in mind that only a limited number of electrolyte systems has been tested here. Small differences between calculated and measured pH values are probably due to variations in mobility and pK data taken from literature. Moreover, temperature differences between the leading electrolyte zone and the terminator zone should be taken into account. The original pH in the terminator solution is not decisive for the pH created in the isotachophoretically moving terminator zone.

Mobilities and pK values are the most important parameters for the separation of ions. When working in a pH range near the pK values ($\pm$ one pH unit) of the intermediate ions it is clear that small differences in pK values will influence the isotachophoretic separation more than small differences in the mobilities.

The pH chosen for the leading electrolyte is important for the separation of the sample ions. Fig. 7 shows clearly that phosphate and formate, for example, will move in separate zones, whatever pH between 7.5 and 5.75 is chosen in the leading electrolyte. Acetate and phosphate, however, are not separated or are very difficult to separate around pH 6.5. It also should be mentioned that the order of the net mobilities of two compounds (*e.g.* phosphate and acetate) can be changed by varying the pH of the leading electrolyte.

The results of the calculations and experiments, as described above, give the impression that the pH in an anionic system always rises from one zone to the next according to the net mobility in those zones. However, it is possible for the pH in two succeeding zones not to rise. In a system which contains weak and strong electrolytes with comparable mobilities, a pH drop can be expected.

Fig. 7 illustrates this effect. At pH 7.5, phosphate will move in front of picrate, but at a higher pH. This pH-drop might cause some difficulties in the separation, when the pH of the leading electrolyte is decreased to 7. When phosphate ions mix with the picrate ions owing to diffusion or convection effects, they experience a lower pH and consequently acquire a lower net mobility which in turn prevents them from migrating back into the phosphate zone.

This effect is disturbing when complex mixtures are separated with the aid of spacers[1,2,13]. If the spacers are strongly acidic and are used for separations of compounds with higher pK values (*e.g.* proteins), they cannot separate and will migrate together with these compounds in one zone.

Another case when the equations are not applicable is this: Let us consider an anionic system at a high pH, say 12. This pH will give a constant migration of hydroxyl ions from the terminator zone through the preceding zones. It is no longer

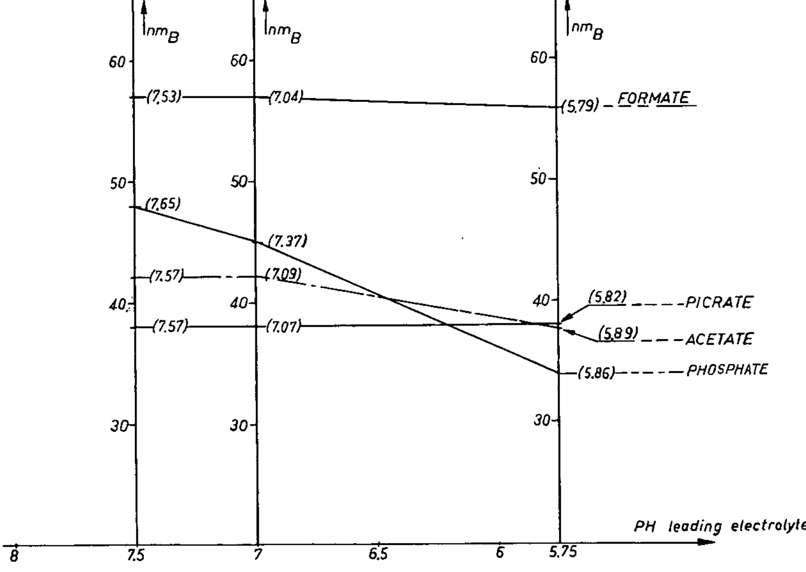


Fig. 7. The net mobilities of some acids are plotted against the pH of the leading electrolyte. Tris is the counterion at pH = 7.5 and pH = 7. Histidine is the counterion at pH = 5.75. The values in parenthesis show the pH of the zone of the ion species considered.

necessary then for the sample ion zones to follow each other directly with the same speed, since the hydroxyl ions will carry a considerable part of the current. It is difficult to predict at which pH the conditions for an isotachophoretic system are no longer fulfilled. The degree of isotachophoresis depends on the ratio of the current transported by hydroxyl ions and by the anions in question. As long as the conductivity of the hydroxyl ions is low (*e.g.* 1 %), compared to the conductivity of the sample ions, the equations can be applied. For cationic systems protons will give a corresponding effect at low pH's.

LIST OF SYMBOLS, INDICES AND ABBREVIATIONS

Symbols

c partial ion concentration (mol cm^{-3})
c^1 total concentration (mol cm^{-3})
G voltage drop (V cm^{-1})
h stepheight (mm)
I current (A)
i ionisation degree
j ionisation degree
k equilibrium constant
m mobility ($cm^2V^{-1}sec^{-1}$)
nm net mobility ($cm^2V^{-1}sec^{-1}$)
r resistance (Ω)
u velocity (cm/sec)
α, β, π maximal ionisation degrees for ion A, B and P
λ conductivity ($\Omega^{-1}cm^{-1}$)
ρ specific resistance (Ω cm)

Indices

A1*i* ion A in zone 1 with charge i
A1 ion A in zone 1
OH1 hydroxyl ion in zone 1

Abbreviations

$pH_{exp.}$ experimentally determined pH value
$pH_{theor.}$ theoretically determined pH value
PTFE polytetrafluoroethylene
Tris trishydroxymethylaminomethane

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CHROM. 5304

EVALUATION OF LINEAR GRADIENT LOADED COLUMNS IN TEMPERATURE PROGRAMMED GAS CHROMATOGRAPHY

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SUMMARY

The performance of gradient loaded columns in temperature programmed gas chromatography has been evaluated. It was found that the elution temperatures and elution times are independent of the type of gradient (positive, negative or no gradient). At optimal working conditions (small injection volume and optimal carrier gas flow) resolution is best with evenly loaded columns, intermediate for columns with negative gradient and worst for columns with positive gradient.

When deviating from optimal carrier gas velocity, resolution decreases much more for evenly loaded columns than for gradient columns. On increasing the injected volume, the decrease in resolution is much more pronounced for columns with positive gradient and no gradient than for columns with negative gradient. Thus, columns with negative gradient are superior to evenly loaded columns when large injection volumes are required.

It is suggested that columns with negative liquid load gradient could prove especially useful in preparative temperature programmed gas chromatography.

INTRODUCTION

The isothermal performance of chromatographic columns connected in series has been evaluated theoretically and practically[1,2]. Columns with various amounts of liquid phase along the column (Gradient Loaded Columns) can be considered as a special case of chromatographic columns in series.

The performance of columns with a two[3] and with a multiple[4] stepwise approximation to a continuous linear gradient and to an exponential gradient[14] have been described. Gradient loaded columns with decreasing liquid load (negative gradient) have been claimed to have advantages over uniformly loaded columns with the same amount of liquid phase; for example, a better resolution is obtained for solute-pairs of low or intermediate partition ratio[4,14] and a greater sample size can be injected without loss of resolution[5,14]. The finding that better resolution can be obtained in temperature programmed gas chromatography (TPGC) with a column that has been used for a considerable length of time by changing the direction of the gas flow[6], as

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well as the finding that such a column performs better than a new one[6], prompted us to evaluate the performance of gradient loaded columns in TPGC.

EXPERIMENTAL

5-g portions of Gas-Chrom Q, 80–100 mesh, were loaded with 10 %, 7 %, 5 % and 1 % Polymer EGSS-X (Applied Science Lab.) by the evaporation technique[7]. Three 1.30-m U-shaped columns were deactivated[8] and in order to fill them in a symmetrical manner, a glass wool plug was inserted 8 cm from the end of the column. (The first 8 cm of the column have to remain empty as they come in the flash heater zone.) The gradient column was prepared by sucking consecutively 1 g portions of 10 %, 7 %, 3 % and 1 % loaded stationary phase into the column. The column was further packed under pressure by gently tapping until the packing was exactly 8 cm from both ends of the column. The reference column was packed with a homogeneous mixture of 1 g of each of the packings, and an absolute reference column was packed with the same amount of liquid phase loaded uniformly (5 %). The columns were put in an F & M gas chromatograph, Model 402, purged with nitrogen and conditioned overnight in a nitrogen atmosphere at 220°, after which they were flushed out for a few hours at the same temperature with nitrogen (10 ml/min). The direction of flow for the gradient column was from the region of low liquid loading toward the region of high liquid loading, in order not to reduce the gradient. Temperature programmes were started at 130° (T_i) and the initial isothermal period (t_i) was 4 min in each case. A mixture of fatty acid methyl esters covering a wide range of boiling points (methyl myristate to methyl lignocerate) was used in order to determine the influence of flow rate, programming rate and injection volume upon the elution temperature, the resolution between critical pairs of compounds and other parameters for column performance.

The flow rate was calculated from the time interval between injection and elution of the solvent peak. This time interval was found to be inversely proportional to the flow rate. Elution temperatures were calculated from the elution times, and the resolution[9] and the relative peak dip[10] for the critical pair methyl stearate–methyl oleate were taken as a measure of the separating power of the column.

RESULTS

Influence of flow reversal on the performance of gradient loaded columns

As the columns were packed in a symmetrical way (the columns being symmetrical and the dead spaces at the beginning and at the end of the column being identical) flow reversal could be achieved by turning the column in the oven of the gas chromatograph, *i.e.* by connecting the end that had been connected with the detector with the injector, and *vice versa*.

The effect of the gas flow on the elution temperatures of methyl palmitate ($C_{16:0}$), methyl arachidate ($C_{20:0}$), methyl lignocerate ($C_{24:0}$) and methyl docosahexaenoate ($C_{22:6}$) for both positive and negative liquid phase gradients (programming rate of 4°/min) is presented in Fig. 1.

It is clear that elution temperatures are not dependent on the direction of flow in gradient loaded columns. This has been found to be true for programming rates between 0°/min (isothermal operation) and 10°/min.

The influence of programming rate upon elution temperature (at constant flow rate of 17 ml/min, measured at 1 atm and 25°) was also investigated, and again it was found that elution temperature is independent of the direction of flow. The influence of programming rate on the elution temperature of methyl stearate is shown in Fig. 2.

The influence of gas flow and gas flow reversal on resolution (R), relative peak dip (P_d), peak widths at half height ($w_{1/2}$), and distance between peak maxima (Δv)

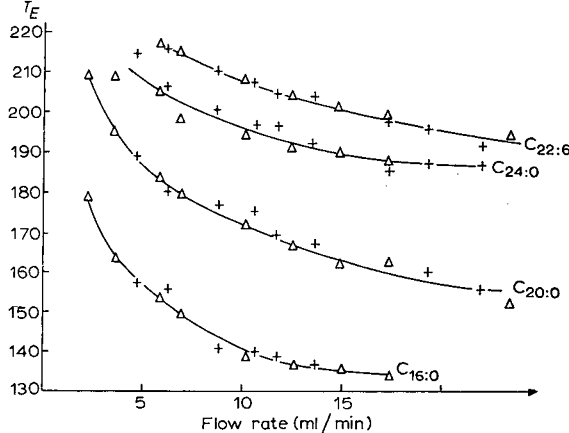

Fig. 1. Elution temperatures (°C) of methyl docosahexaenoate ($C_{22:6}$), methyl lignocerate ($C_{24:0}$), methyl arachidate ($C_{20:0}$) and methyl palmitate ($C_{16:0}$) at various flow rates for columns with positive (+) and negative (△) gradients. Working conditions: T_i = 130°; t_i = 4 min; programming rate = 4°/min.

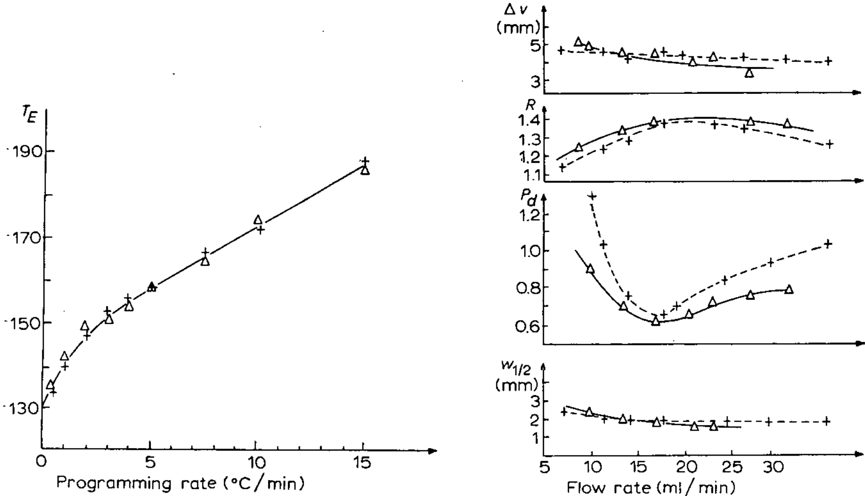

Fig. 2. Elution temperature (°C) of methyl stearate at various programming rates for columns with positive (+) and negative (△) gradients. Working conditions: T_i = 130°; t_i = 4 min; flow rate = 17 ml/min (p = 1 atm; T = 25°).

Fig. 3. Resolution (R), relative peak dip (P_d), peak width at half heigth ($w_{1/2}$) and distance between peak maxima (Δv) as a function of carrier gas flow for columns with positive (+) and negative (△) gradients. Working conditions: T_i = 130°; t_i = 4 min; β = 4°/min.

for the critical pair methyl stearate–methyl oleate was determined. The results are shown in Fig. 3. It can be seen that columns with decreasing gradient exhibit a better performance than columns with increasing gradients, especially when one does not

TABLE I

THE EFFECT OF THE INJECTION VOLUME UPON RESOLUTION BETWEEN $C_{18:0}$ AND $C_{18:1}$

Experimental conditions: $T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min; F = 17 ml/min.

Injection volume (μl)	*Positive gradient*	*Negative gradient*
0.5	1.26	1.38
1	1.23	1.38
1.5	1.21	1.30
2	1.18	1.28
3	1.07	1.18
4	0.87	1.13
6	—	1.11
8	—	1.09

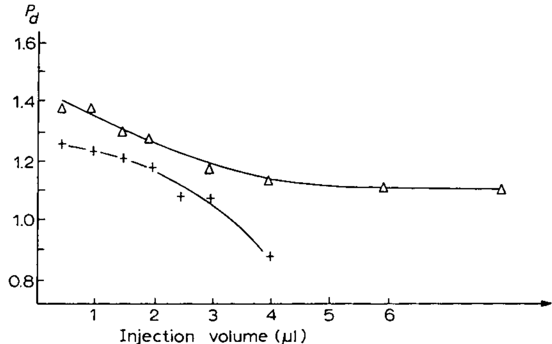

Fig. 4. Resolution as a function of injection volume for columns with positive (+) and negative (Δ) liquid load gradients. Working conditions: $T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min; $F = 17$ ml per min.

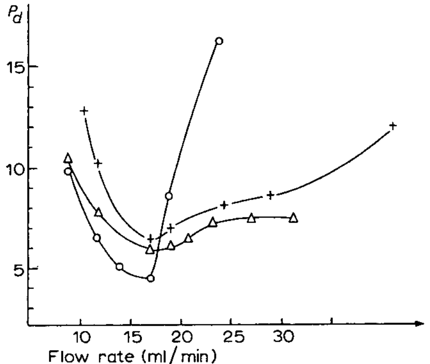

Fig. 5. Relative peak dip as a function of flow rate for columns with positive (+), negative (Δ) and no (○) gradient. Working conditions: $T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min; $F = 17$ ml/min

work at the optimal gas flow velocity (optimal with respect to the resolution of the critical pair under consideration).

The above effect is also found with variation of the injection volume: the more one deviates from the optimal (very small) injection volume, the greater the superiority of the column with a negative liquid load gradient.

The influence of the injection volume upon the resolution between methyl stearate and methyl oleate for columns with positive and negative liquid load gradients in TPGC is shown in Table I and presented graphically in Fig. 4.

As can be seen from Table I, the resolution is dependent in both cases on the injection volume. However, as in isothermal GC[5], columns with negative gradients perform much better than columns with positive gradients.

The comparison of gradient loaded columns to the columns loaded with a homogeneous mixture

The gas flow that gives optimal separation between methyl stearate and methyl palmitate (as judged from the relative peak dip factor) is independent of the nature of the column (positive, negative or no liquid phase gradient; Fig. 5).

Elution temperatures are also independent of the type of column. This is not the case, however, for the resolution between $C_{18:0}/C_{18:1}$. For the mixed column, the optimal resolution ($T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min) was 1.60, for the absolute reference column (5 %) 1.55 and for the gradient columns 1.39 (positive gradient) and 1.38 (negative gradient).

As in isothermal GC[11], the heterogeneity of the packing does not affect resolution significantly in TPGC. On the other hand, liquid load gradients in the column do decrease the resolution.

As to the deterioration of the resolution with increasing injection volumes, homogeneously packed columns appear to perform better than columns with a positive gradient, but not as well as columns with a negative gradient. Thus, the resolution when a 4-μl sample was injected was only 73 % of that when a 1-μl sample was injected for a positive gradient column, 82 % for a negative gradient column and 78 % for a homogeneously loaded column (with the same amount of liquid phase). Moreover, it was found by plotting the relative peak dip *vs.* the gas flow (Fig. 5) that deviations from the optimal flow affected the resolution much more for homogeneously loaded columns than for columns with either positive or negative gradient.

DISCUSSION

The finding that the elution temperature (or the retention time) is independent of the type of linear gradient in the column (positive, negative or no gradient), (but with the same amount of partitioning liquid) is contrary to the situation in isothermal GC where the retention times do depend on the type of gradient[12,14]. The theory for retention on gradient loaded columns in TPGC which confirms this finding will be published elsewhere.

The fact that the column with negative gradient, which has a larger variation in solute velocity than the column with positive gradient (compared to the latter one, the solute velocity is slower at the inlet (higher liquid loading) and faster at the outlet (lower liquid loading)) performs better than the column with positive gradient

is in accordance with the finding of GIDDINGS[13] that large increases in carrier gas velocity (thus in solute velocity) do not lead to significant loss in resolution.

As columns with negative gradients perform better in TPGC than homogeneously loaded columns when the injection volume is not very small, they will be especially useful when large volumes need to be injected as in temperature programmed preparative gas chromatography.

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CHROM. 5324

GAS CHROMATOGRAPHIC ANALYSIS OF LOW BOILING FATTY ACIDS IN BIOLOGICAL MEDIA*

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SUMMARY

Different types of Porapak packing material have been tested in order to find a suitable combination for the separation and determination of micro-quantities of formic, acetic, propionic, isobutyric, butyric, 3-methylbutyric and valeric acids present in biological media as intermediates and end-products in microbial fermentation. The best results were obtained with Porapak N (100–120 mesh). With a helium flow rate of 50 ml/min through an 85-cm stainless steel column with 2 mm I.D. and temperature programming between 165 and 215° at a rate of 4°/min, the seven acids can be completely separated in 15 min. 2-Methyl- and 3-methylbutyric acids cannot be separated.

Quantitative determination of formic acid is possible down to 200 ng by this method and the other six acids can be determined down to 20 ng. These sensitivities allow direct injection of the biological (aqueous) medium into the chromatograph, the only pretreatment being centrifuging and membrane filtration.

INTRODUCTION

Saturated fatty acids with 1–5 C-atoms occur as intermediates and end-products in different types of microbial fermentation and their separation and quantitative determination in fermentation broths is of great interest. However, in most cases only small amounts of these compounds are present (< 50 p.p.m.). Different types of sample preparation have been studied in order to increase the concentration of the acids in the final sample and to isolate them from water-soluble but non-volatile organic compounds in the fermentation broth.

Steam distillation has been found to give 4–5 fold dilution of the acids in the condensate, even when the sample is saturated with NaCl. Solvent extraction is rendered difficult by the unfavourable partition coefficients of these acids between water and organic solvents. In addition, the most favourable solvents, like ethyl acetate, methyl ethyl ketone and diisopropyl ether,[1] create a difficulty, when gas chromatographic (GC) analysis is to be used, by interfering with the retention volumes of the acids. However, gas chromatography must be considered as the most advan-

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tageous method for this purpose as it allows the separation of the acids and the quantitative determination of each of them. The difficulty of performing the separations and especially the quantitative determination of these volatile free fatty acids arises mainly from their relatively high boiling points and their adsorption on the solid support. Numerous techniques have been used to reduce or eliminate these problems. LEE AND BETHEA[2] reviewed the many combinations of liquid phases, liquid phase blends and supports reported up to 1968. Summarizing this, together with their own experience, they stated that satisfactory results could only be obtained on using a non-volatile acidic substance, such as H_3PO_4, trimer acid or isophthalic acid, as an additive to the liquid phase, and Teflon as solid support. Their best results were obtained with columns of 10 % FFAP + 0.2 % H_3PO_4 or 10 % trimer acid + 0.8 % dinonylnaphthalene disulfonic acid or 10 % ethofat 60/25 + 0.5 % isophthalic acid, all with 40–60 mesh Teflon as solid support. Unfortunately the concentrations of the solutions used for this work are not mentioned, but it seems that they were quite high.

In 1969, DOELLE[3] reported a method for the determination of some of the straight- and branched-chain saturated fatty acids with 1–7 C-atoms, using a column with 3.125 % PEGA on Chromosorb W-AW. He tested twelve other stationary phases including Carbowax 20M, polyethylene glycol 600, Apiezon L, DEGS, LAC-269 + H_3PO_4 and E310 which he did not find suitable for this problem. With his proposed column he could quantitatively determine formic acid down to 10 μg and the other acids down to 0.1 μg. This is already a good approach to the requirements of the biologists. However, the separation of formic and acetic acids was too poor for the simultaneous determination of these two acids.

It is surprising that very little has been reported on the use of porous polymers like Porapak for this problem. Waters Associates recommend Porapak Type Q or QS for the separation of C_1–C_4 fatty acids. MAHADEVAN AND STENROOS[4] found that the addition of H_3PO_4 was necessary because these acids could not be eluted from untreated Porapak Q. In the present paper a method for the determination of straight- and branched-chain C_1–C_5 fatty acids in biological media is reported, using a Porapak N column.

EXPERIMENTAL

Apparatus

Centrifuge, IEC International Centrifuge, model HRl.

Membrane filtration, Millipore A.H. microanalysis filter holder, XX50 025 00; Millipore filter, 0.22 μ.

Gas chromatograph, Hewlett Packard Model 7624 A; double column. Flame ionisation and thermal conductivity detector. 1 mV-Moseley Recorder.

Column, 85 cm long with an I.D. of 2 mm. The stainless steel column was packed with Porapak type Q (batch No. 689) or type QS (batch No. 787) or type N (batch No. 799); all 100–120 mesh, obtained from Waters Associates.

Chemicals

All acids used as standards were of the highest purity obtainable from Th. Schuchardt, Munich, Merck AG, Darmstadt (G.F.R.) and Carlo Erba, Milan (Italy).

Method

A sample of *ca.* 5 ml taken from the fermentation broth is immediately centrifuged for 20–30 min at 10000–15000 r.p.m. depending on the size of the cells present.

1 ml of the supernatant is then passed through a 0.22 μ millipore filter (pressure filtration to avoid losses), but this filtrate is rejected. Another 1 ml of the sample is then filtered, using the same filter, and this filtrate is used for the GLC analysis. During all these operations the sample is kept below 5°. About 3–5 μl are injected into the gas chromatograph, equipped with a Porapak N column. The instrument conditions were as follows.

Temperatures: injection port, 210°; flame ionisation detector, 230°; hot wire detector, 210°.

Gases: helium, 50 ml/min; hydrogen, 40 ml/min; air, 400 ml/min.

Bridge current: 195 mA.

Column temperature programme. Start: 165°; 2 min isothermal at 165°, followed by programming at 4°/min to 195° then 6–10 min isothermal at 195°. This temperature programme was used for all the chromatograms reported in this paper.

RESULTS AND DISCUSSION

Attention was paid to two problems in particular at the start of this study: first, the cleaning up of the broth sample, and secondly, the GC column used for the separation and determination of the acids. Since GC was to be used for the analyses of the acids in the broth it was thought that it would be absolutely necessary to separate the water-soluble non-volatile compounds (salts, amino acids etc.) before the injection in order to avoid drastic base line troubles. These could be caused by a kind of continuous pyrolysis in the injection port and at the top of the column. Two techniques envisaged for the cleaning-up, *viz.* steam distillation and solvent extraction, have, however, been found to be useless, as already mentioned above, when microquantities of these acids are to be determined. Our experience with directly injected samples from the fermentation of different yeasts and bacteria has shown that the phenomena of ghost peaks and base line instability only arise when there are cells or cell fragments remaining in the sample. This is difficult to avoid by centrifuging but very easy by filtration through a 0.22-μ membrane. After this two-step pretreatment, *viz.* centrifuging and membrane filtration, it was possible to use a Porapak N column for *ca.* 700 injections before the peak shape started to deteriorate. The problem of finding a suitable column packing material for these low boiling fatty acids was defined by the amount of each acid to be detected: between 300 and 500 ng of formic and at least 50 ng of the other C_2–C_5 acids per injection. No column described in the literature was found to be satisfactory for this purpose. The free acids produced visible tailing, whether or not the stationary phases had been blended with non-volatile acidic substances. This tailing may be negligible when macro amounts are injected but becomes a dominant factor with microquantities. Porous polymer beads have been used for several years as GLC column packing material and there is one type, Porapak Q, which is recommended by the manufacturer for low boiling fatty acids. Unlike MAHADEVAN AND STENROOS[4], we did not find that the addition of H_3PO_4 was necessary for the elution of the acids from this material. Very strong tailing still occurs but it is

not influenced by treatment with H_3PO_4. A chromatogram of acetic and propionic acids, 40 ng of each on Porapak Q, is shown in Fig. 1a. Fig. 1b shows the same chromatogram on the silylated material Porapak QS, and shows a remarkable

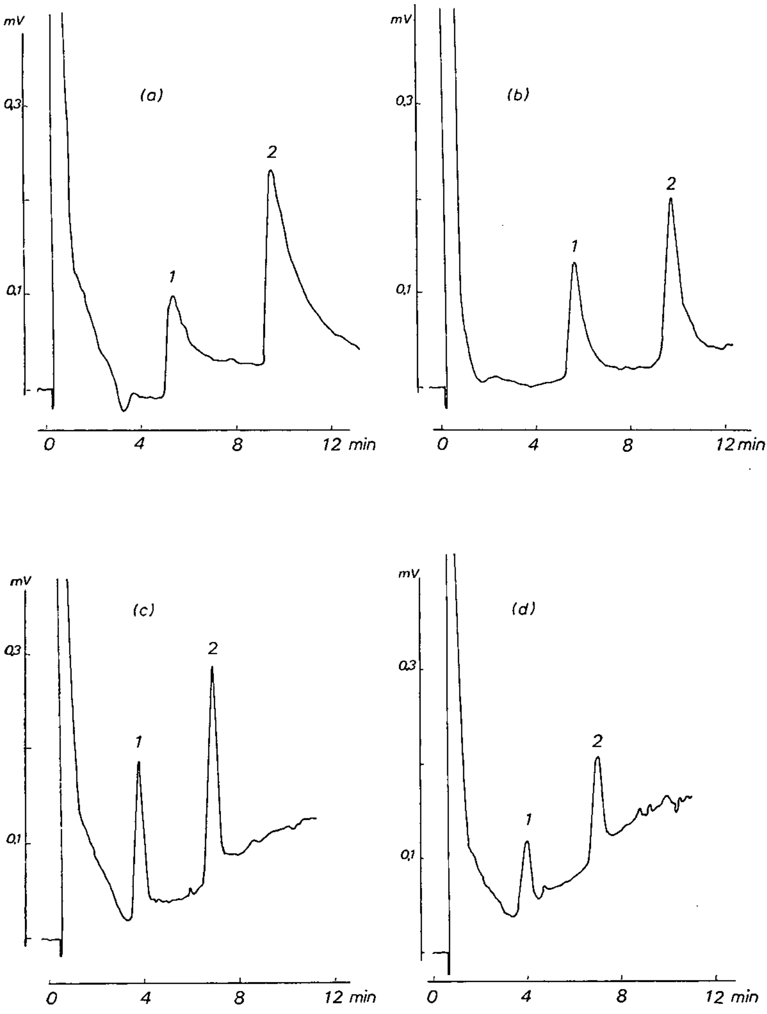

Fig. 1. Acetic (1) and propionic acid (2), on (a) Porapak Q, 40 ng each; (b) Porapak QS, 40 ng each; (c) Porapak N, 40 ng each; (d) Porapak N, 20 ng each.

improvement of the peak shape. Encouraged by these results we tested all the Porapak types which are available at present and found that Porapak N is the best material for the separation of C_1–C_5 fatty acids. In Figs. 1c and 1d the chromatograms of acetic and propionic acids, each at 40 and 20 ng, are shown respectively. The peaks are sufficiently symmetrical and narrow to allow peak height measurement to be used for quantitative analyses. The method is applicable down to 20 ng, except for formic acid,

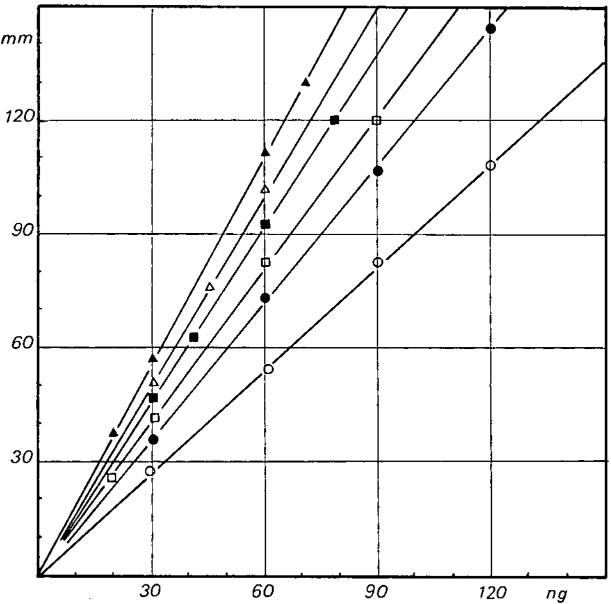

Fig. 2. Calibration curves for acetic (○—○), propionic (●—●), 2-methylpropionic (■—■), butyric (□—□), 3-methylbutyric (▲—▲) and valeric acid (△—△). Flame ionisation detector; range 1, attenuation 16. Size of injection sample 5 μl. Peak height in mm plotted against ng injected.

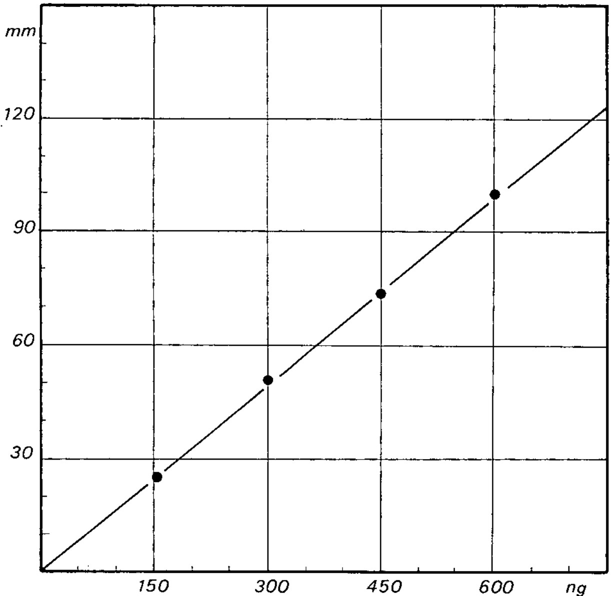

Fig. 3. Calibration curve for formic acid. Thermal conductivity detector: attenuation 1. Size of sample injected 5 μl. Peak height in mm plotted against ng injected.

where the limit is 200 ng. The standard calibration curves are given in Figs. 2 and 3. The lowest amounts detectable are *ca.* 100 ng for formic acid and 10 ng for the other six acids. This means that on injecting 5 μl, formic acid can be determined down to 40 p.p.m. and the other acids down to 4 p.p.m. (The difference in sensitivity between formic and the other acids is because formic acid gives no true signal with the FID so that a thermal conductivity detector must be used.) Fig. 4 shows the chromato-

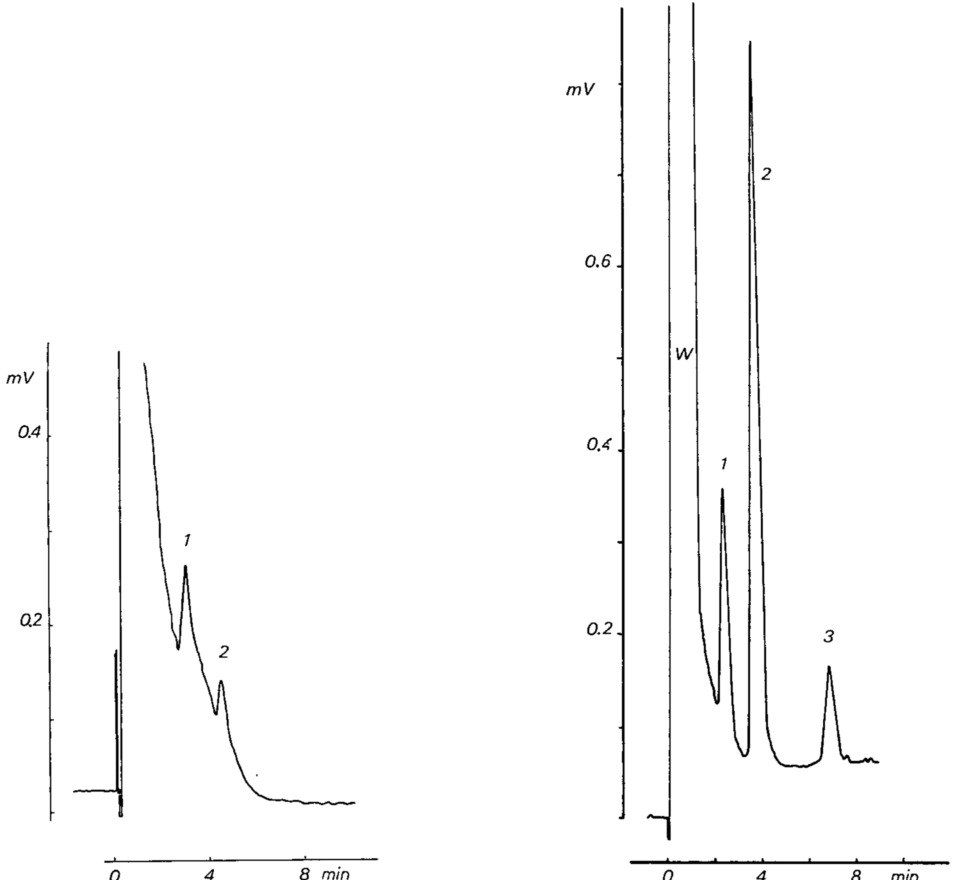

Fig. 4. 150 ng formic acid (1) + 90 ng acetic acid (2) on Porapak N. Sample size 5 μl. Thermal conductivity detector: attenuation 1.

Fig. 5. Separation of water (W) and formic (1), acetic (2) and propionic acids (3) on Porapak N. TC-detector.

gram of 150 ng of formic acid (Peak 1). The injection of pure water before starting an analysis, especially in the low concentration range, is essential to check that there are no acids retained on the column.

85 cm has been found to be the optimal column length, as with shorter columns the separation of water and formic acid becomes difficult. Fig. 5 shows the separation of water and formic, acetic and propionic acids and Fig. 6 the separation of acetic, propionic, butyric, 2-methylpropionic, 3-methylbutyric and valeric acids. 2-Methyl- and 3-methylbutyric acid cannot be separated, even with much longer columns.

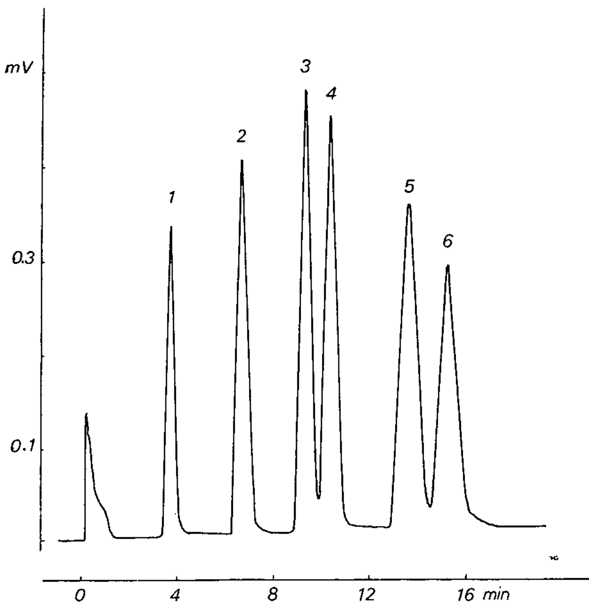

Fig. 6. Separation of acetic (1), propionic (2), 2-methylbutyric (3), butyric (4), 3-methylbutyric (5) and valeric acids (6) on Porapak N. Detector, FID.

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CHROM. 5301

SEPARATION OF SOME METHYL O-METHYL-D-XYLOFURANOSIDES BY GAS CHROMATOGRAPHY

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SUMMARY

The separation of the methyl furanosides of O-methyl-D-xyloses, which may be formed upon methanolysis of a methylated polysaccharide containing D-xylopyranose as a structural unit, is described. The order of elution of the compounds under investigation follows, with no exceptions, the rule generally accepted for methyl O-methyl-glycopyranosides.

INTRODUCTION

Methylation analysis, despite its known shortcomings, has been of utmost importance in the elucidation of the structure of polysaccharides. Gas chromatography (GC) solves many of the problems encountered when complex mixtures of carbohydrate derivatives formed upon methanolysis and/or hydrolysis of methylated polysaccharides are analyzed[1-4] and is virtually the major analytical technique in methylation analysis.

One of the groups of compounds most frequently used for GC analysis in the investigation of the structure of polysaccharides is the methyl glycosides which are obtained upon methanolysis. These substances are fairly stable and, except those of the methyl mono-O-methylhexosides, are sufficiently volatile to be injected directly. However, when a methanolyzate is analyzed, identification of the four stereoisomers (two methyl pyranosides and two methyl furanosides) which may be formed from certain of the sugar moieties on methanolysis of a fully methylated polysaccharide can create a problem.

Methanolysis of some of the O-methyl ethers of D-xylose has been studied by BISHOP AND COOPER[5] who identified and determined, by GC, the amounts of the individual glycosides formed after exhaustive methylation as methyl per-O-methyl-D-xylosides. This procedure, for obvious reasons, is not suitable for the analysis of the methanolyzate of a methylated polysaccharide, where it is necessary to have a method of separation and reasonable resolution for all the possible components in the mixture. Data useful for the identification of methyl O-methyl-D-xylopyranosides have been summarized by BISHOP[3] and ASPINALL[6]. As all the possible methyl O-methyl-D-xylofuranosides which may be produced upon methanolysis of a methylated polysaccharide containing D-xylopyranose as a structural unit are now known[7,8],

we now wish to present data helpful in the identification of these compounds in methylation analysis.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Research Chromatograph, Model 5750 G, with a dual column system and dual flame ionization detector was employed. Relative retention times were taken from the record of a Hewlett-Packard Integrator 3370 A.

Operating conditions

Gas chromatography was carried out on the following columns: (A) 6 ft. × 1/8 in. (O.D.), packed with 10 % Apiezon L on Gas-Chrom Z 80–100 mesh (Applied Science Laboratories), flow rate 32 ml/min, temperature programmed 100–290° (8°/min); (B) 6 ft. × 1/4 in. (O.D.), packed with 10 % Carbowax 20 M–terephthalic acid (Hewlett-Packard) on Chromaton N AW-DMCS 70–80 mesh (Lachema, Brno), flow rate 32 ml/min, temperature programmed 100–240° (8°/min); (C) 6 ft. × 1/4 in. (O.D.), packed with 10 % diethylene glycol succinate (Lachema, Brno) on Chromaton N AW-DMCS, flow rate 35 ml/min, temperature programmed 100–216° (8°/min); (D) 6 ft. × 1/8 in. (O.D.), packed with 3 % ECNSS-M (Applied Science Laboratories) on Chromaton N AW-DMCS, flow rate 25 ml/min, temperature programmed 100–210° (4°/min).

Derivatives

Methyl α- and β-D-xylofuranoside was made according to AUGESTAD AND BERNER[9]. Methyl 2,3,5-tri-O-methyl-D-xylofuranosides were made according to BISHOP AND COOPER[10]. Methyl 2-O-, 3-O-, and 2,3-di-O-methyl-D-xylofuranosides were made as described by KOVÁČ AND PETRÍKOVÁ[7,8]. The compounds were injected in methanol (2 % solution).

RESULTS AND DISCUSSION

The problems regarding the separation of the complex mixture of stereoisomers present in a methanolyzate of a methylated polysaccharide arise particularly when the polysaccharide subjected to methanolysis contains pentoses. BISHOP *et al.*[5,10,11] found that methyl furanosides, resulting from methanolysis, occur to a larger extent in the case of pentoses than in the case of hexoses. It is known that D-xylose occurs, as a structural unit of polysaccharides, exclusively in its pyranose form. Thus, methanolysis of such a polysaccharide results in the possible formation of methyl furanosides of 2-O-, 3-O-, and 2,3-di-O-methyl-D-xylose. Therefore, our attention was focussed mainly on the separation of these substances.

Identification of the products of methanolysis is aided by the application of certain generalizations concerning the pattern in which the components of the mixture are eluted from the column. Based on empirical data, BISHOP[3] concluded that in the case of fully methylated glycosides, furanosides precede pyranosides in the series of arabinosides, galactosides and fructosides, whereas in the series of xylosides and glucosides, the order of elution is reversed. Another important generalization was

TABLE I

RELATIVE RETENTION TIMES OF METHYL O-METHYL-D-XYLOFURANOSIDES

No.	*Compound*	*Column*			
		A	*B*	*C*	*D*
1	Me 2,3,4-tri-O-Me-β-D-xylopyranoside	1.00[a]	1.00[b]	1.00[c]	1.00[d]
2	Me 2,3,5-tri-O-Me-β-D-xylofuranoside	1.18	1.18	1.20	1.34
3	Me 2,3,5-tri-O-Me-α-D-xylofuranoside	1.32	1.29	1.29	1.54
4	Me 2,3-di-O-Me-β-D-xylofuranoside	1.55	1.55	1.69	2.34
5	Me 2,3-di-O-Me-α-D-xylofuranoside	1.66	1.66	1.79	2.62
6	Me 2-O-Me-β-D-xylofuranoside	1.85	1.96	2.15	3.36
7	Me 2-O-Me-α-D-xylofuranoside	1.87	2.35	2.87	5.08
8	3-O-Me-β-D-xylofuranoside	2.05	2.44	2.87	5.28
9	Me 3-O-Me-α-D-xylofuranoside	1.80	1.85	2.01	3.11
10	Me β-D-xylofuranoside				6.68
11	Me α-D-xylofuranoside				5.92

[a] 6.61 min.
[b] 6.64 min.
[c] 7.45 min.
[d] 3.49 min.

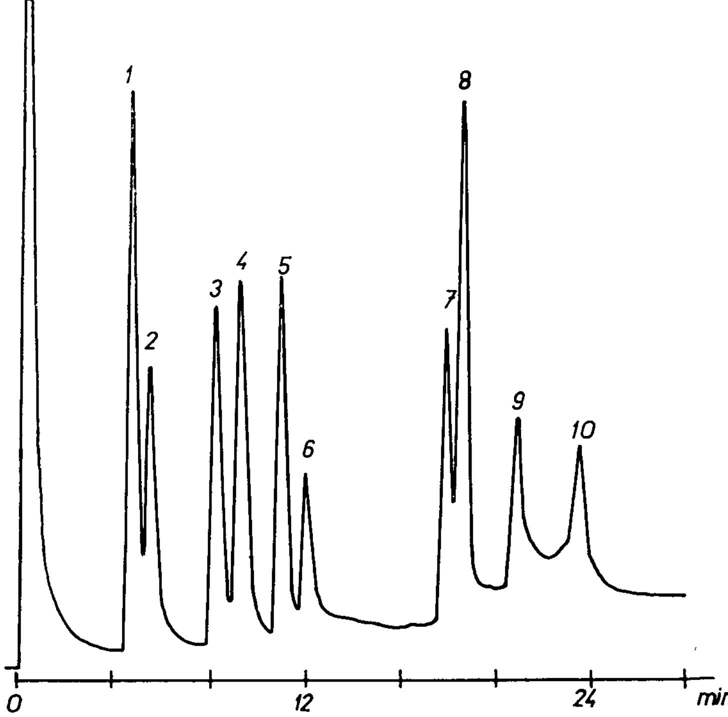

Fig. 1. Separation of methyl O-methyl-D-xylofuranosides. Column D. 1 = Methyl 2,3,5-tri-O-methyl-β-D-xylofuranoside; 2 = methyl 2,3,5-tri-O-methyl-α-D-xylofuranoside; 3 = methyl 2,3-di-O-methyl-β-D-xylofuranoside; 4 = methyl 2,3-di-O-methyl-α-D-xylofuranoside; 5 = methyl 3-O-methyl-α-D-xylofuranoside; 6 = methyl 2-O-methyl-β-D-xylofuranoside; 7 = methyl 2-O-methyl-α-D-xylofuranoside; 8 = methyl 3-O-methyl-β-D-xylofuranoside; 9 = methyl α-D-xylofuranoside; 10 = methyl β-D-xylofuranoside.

made for the series of partially methylated methylglycopyranosides. According to BISHOP[3], in the case of all the methyl O-methylglycopyranosides examined and on all liquid phases, that anomer in which the glycosidic methoxyl group is in a *cis* position relative to the methoxyl group at C-2 has the higher retention volume. When the C-2 methoxyl group is unsubstituted, the order in which the two anomers are eluted is reversed. At the time BISHOP wrote his review, and as far as the authors know at present, there are insufficient data to show whether this generalization is valid for methyl O-methylglycofuranosides as well.

In the work presented we tried to ascertain whether the same rule, as far as the order of elution of anomers is concerned, can be applied for the methyl O-methyl-D-xylofuranosides investigated. In Table I the retention times, on four columns of different polarity, relative to the retention time of methyl 2,3,4-tri-O-methyl-β-D-xylofuranoside are summarized. It follows from the presented data that BISHOP's generalization for the order in which methyl O-methylglycopyranosides are eluted can be extended to the methyl O-methyl-D-xylofuranosides under investigation.

Of the four liquid phases used for the separation of the methyl O-methyl-D-xylofuranosides investigated, ECNSS-M was found to be the most suitable (see Fig. 1), as both separation and resolution on the column D was the best. Here, even methyl D-xylofuranosides were resolved satisfactorily for quantitative work.

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CHROM. 5308

DETERMINATION OF TESTOSTERONE IN HUMAN URINE BY MEANS OF HORIZONTAL THIN-LAYER AND GAS-LIQUID CHROMATOGRAPHY

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SUMMARY

A method has been developed for the determination of testosterone in human urine by using enzymic hydrolysis, ether extraction, solvent partition and horizontal thin-layer chromatography to purify the urinary extracts, followed by gas-liquid chromatography or SACHS' colorimetric method. Comparison of the two methods of estimation of testosterone in the final extract shows that determination by gas-liquid chromatography is more reproducible than the colorimetric method when applied to the same eluates from the horizontal thin-layer chromatography. The reliability criteria studied show that this method is specific for routine analysis of testosterone in urine of normal men and normally menstruating women. Results of determinations on human urine are presented.

INTRODUCTION

It has always been accepted that testosterone (17β-hydroxyandrost-4-en-3-one) is the most potent of the naturally occurring androgens which it is possible to determine in tissue and biological fluids and thus can give useful information about the androgenic state of the subject. Although it is metabolised to 17-ketosteroids, the urinary estimation of these compounds is rarely useful in the assessment of human androgen production because the main precursors of the 17-ketosteroids are only weakly androgenic. Very recently it has been demonstrated, in rats and humans, that certain essential organs metabolise testosterone preferentially into 5α-dihydrotestosterone[1-3]. In some biological assay systems dihydrotestosterone is even more powerful as an androgen than testosterone itself. But, at present, methods for its determination have not been described. Therefore, the quantitation of testosterone still has a prominent role in the study of androgenic function. Many methods have been described to determine the urinary levels of this compound[4-13], but a lot of them show evident technical disadvantages, particularly for the separation of testosterone (Ts) from epitestosterone (Epi-Ts) which is not derived directly from the hormone[14]. In addition, the results obtained are not all in agreement[15,16].

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The present paper concerns a rather simple procedure for the determination of urinary testosterone. After enzymic hydrolysis and extraction with ether, the fraction containing Ts and Epi-Ts is separated and purified by horizontal thin-layer chromatography (HTLC). Further simple chromatographic partition provides a reasonable separation of the two epimers. Finally, the Ts was determined by gas–liquid chromatography (GLC) with a flame ionization detector (FID) and electronic integration or by a spectrophotometric colour reaction.

MATERIALS AND METHODS

Solvents and reagents

The solvents and reagents used were of analytical grade. The solvents were redistilled before use and tested by GLC. Silica Gel HF_{254} (Merck) was sieved and then washed before use with absolute ethanol. [4-^{14}C]Testosterone (S.A. 58.2 mCi per mmole) obtained from the Radiochemical Centre of Amersham, was used in tracer quantities as an internal standard and for estimating recoveries. Ketodase (β-glucuronidase, 500 U/ml) was obtained from Warner Chilcott.

Horizontal thin-layer chromatography

The Desaga outfit and the Desaga B.N.-Kammer for horizontal migration were employed. Glass plates, 20 $\times$ 20 cm, were prepared as previously described[17,18]. The urinary extracts were quantitatively transferred with acetone (0.2 ml, 3 times) to a thin-layer plate; spotting was approximately 1.5 cm from the lower edge and 2–2.5 cm from the side. The following solvent systems have been used: (a) benzene–ethyl acetate (60:40); (b) dichloromethane–ethyl acetate (90:10) and (c) benzene–ethyl acetate (80:20). The plates were developed, at room temperature, using horizontal migration, with a system of cooling by running water. After UV absorption, the area of silica gel containing the labelled Ts was loosened with a microspatula, aspirated into a sintered glass disc (1 cm diameter, No. 2 grade), eluted under positive pressure with 5 $\times$ 0.3 ml absolute ethanol and, finally, the eluate evaporated to dryness.

Gas–liquid chromatography

A Fractovap model D (Carlo Erba), glass column, gas chromatograph equipped with double flame ionization detector was used. Chromosorb W (80–100 mesh) was acid washed, silanised, and coated with 3 % SE-30; the packing was blown into the glass coiled columns 80 cm long and 2 mm I.D. Glass columns, of the same dimensions, but packed with silicone-treated Gas-Chrom P (100–120 mesh) coated with 1 % XE-60 were also employed. The samples were introduced into the glass vaporisation chamber by means of a Hamilton liquid microsyringe. The carrier gas was nitrogen (filtered through molecular sieve and with a flow-rate of 60 ml/min) and the flow rates of the flame gases were adjusted to achieve satisfactory sensitivity and base-line stability. The temperatures were 300° for the flash heater, 228° for the columns and 240° for the detectors. The peak areas were measured by triangulation and by an electronic decade counting system (max. count rate: 1500 c.p.s.) with a Keinzle (model D-11 E) digital printer, and by using a corresponding external standard run before and after each urinary sample.

Identification and determination of radioactivity

Radioactivity due to the ^{14}C was identified on thin-layer plates by autoradiography (with overnight exposure of the plates to Agfa-Gevaert no-screen X-ray films) or by using a Thin-layer Radio-Scanning apparatus (Desaga), with a ^{14}C counting efficiency of 15–18 %. Liquid scintillation counting (LSC) was done in 15 ml of scintillation solution (42 ml of Liquifluor in 1000 ml of redistilled toluene) by means of a Nuclear-Chicago Mark I Liquid Scintillation Spectrometer. The counting efficiency (mean: 89 % for carbon) for each sample was determined by reference to a calibration curve, which was plotted from a set of quenched standards by using an external ^{133}Ba source and a channels-ratio method.

Colour reaction for testosterone and its spectrophotometric determination

The SACHS' colour reaction[19] at 620 mμ was used. Absorption spectra were recorded in a Beckman D.U. model spectrophotometer.

Standard analytical procedure

A flow sheet of the method is shown in Fig. 1.

Hydrolysis and extraction. Samples of 100 ml of male and 200 ml of female urine from a 24 h collection were used for each determination. After enzymic hydrolysis

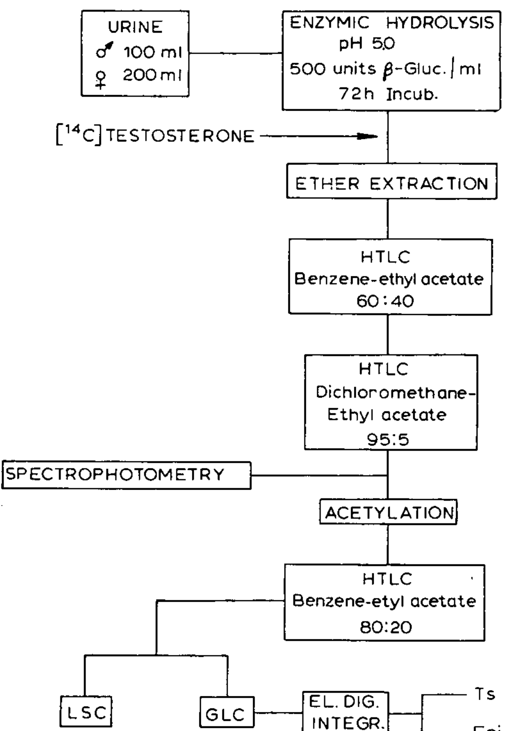

Fig. 1. Flow diagram of the method used for routine determination of urinary testosterone.

(urine adjusted to pH 5.0, addition of 500 U of ketodase per ml urine and, finally, incubation for 72 h at 37°), the urine was cooled and extracted three times with 1 vol of ether. The combined ether extracts were washed three times with 0.2 vol of 1 *N* NaOH and three times with 0.2 vol of distilled water. The ether extracts were dried with anhydrous Na_2SO_4 and evaporated to dryness on a water bath under nitrogen.

HTLC. The extracts were applied to the silica gel plates alongside a standard of Ts as a reference. Development was with solvent system (a). After detection by UV-light absorption of the reference Ts, the areas of the chromatogram corresponding to urinary Ts were then eluted with absolute ethanol. The eluates were evaporated, redissolved in acetone and rechromatographed in solvent system (b). Identification and elution of the steroid compound was repeated and the dry extract was acetylated by the addition of 0.2 ml of pyridine and 0.1 ml of acetic anhydride, the mixture being left at room temperature for 12 h. The reagents were removed under nitrogen on a water bath at 55° and the extract was rechromatographed using solvent system (c). The area corresponding to urinary Ts-acetate was eluted, then evaporated, redissolved in a known quantity of absolute ethanol and small portions were removed for LSC. The residual liquid was evaporated again and then subjected to GLC.

RESULTS AND DISCUSSION

Hydrolysis

Previously described methods of determining urinary Ts have used enzymic hydrolysis with a different concentration of β-glucuronidase at 37° for 48 h. In the present study incubation at 37° for 72 h with a concentration of 500 U/ml of ketodase

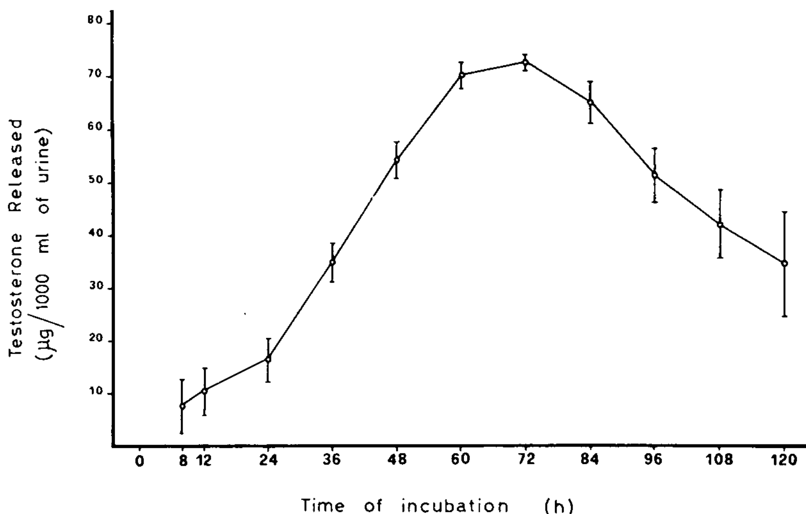

Fig. 2. Amount of testosterone released in relation to the time of incubation; constant enzyme activity. Urine samples were obtained from a pool of normal men. Each point of the curve represents the mean and the standard deviation of 5 determinations.

was found to give reproducible results. Using the same concentration of enzymic activity but with smaller or longer incubation periods, we obtained a considerable reduction in the amount of Ts released and a progressive increase in the standard deviation. This is evident from Fig. 2. The stability of Ts under our conditions of hydrolysis was confirmed by duplicate analyses. A 5 μg sample of authentic Ts was added to 100 ml of urine (100 ml of the same urine is the blank). In 7 experiments the mean amount of Ts recovered, as estimated by GLC, was 60.3 $\pm$ 6.5 % (S.D.).

Thin-layer chromatography

Fig. 3 shows the preliminary purification of Ts and Epi-Ts obtained with solvent system (a). Visualisation of the plates with ethanol–sulphuric acid (50:50), makes it possible to pick out different spots, some of these are visible even under UV light. The second HTLC in system (b) clearly separated Ts from Epi-Ts (Fig. 4). Finally,

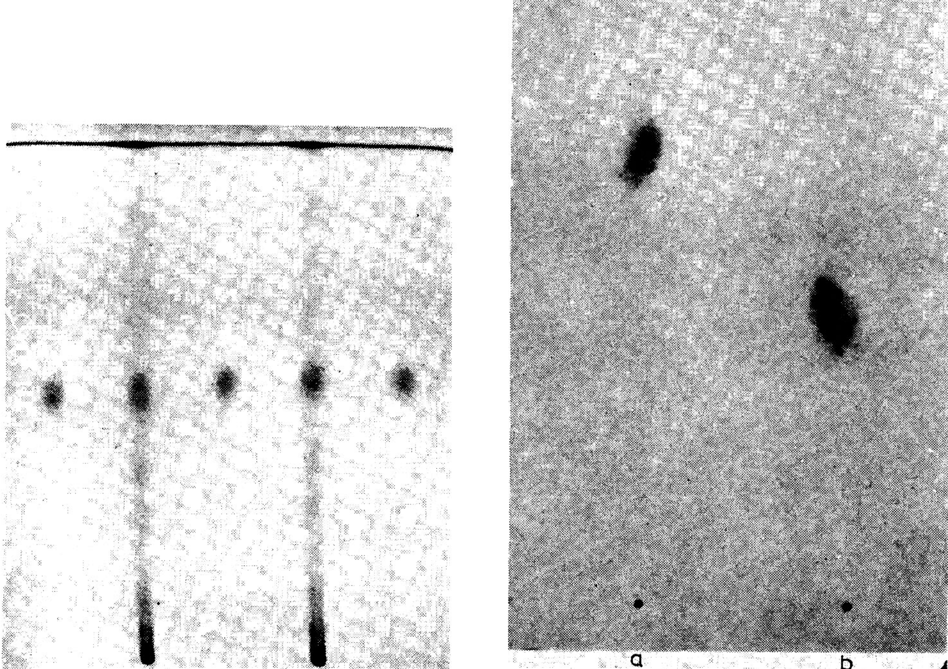

Fig. 3. Horizontal thin-layer chromatography (under UV light), in solvent system (a), of urinary extracts obtained from two normal men (scale: 3/1).

Fig. 4. Separation of testosterone (a) from epitestosterone (b). HTLC with continuous flow of solvent system (b) scale: 3/2).

Fig. 5 shows the separation of Ts-acetate in system (c); this was satisfactory for GC quantitation.

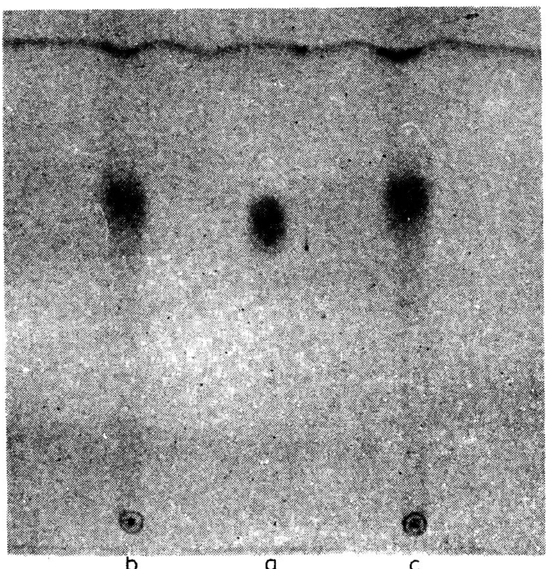

Fig. 5. HTLC (solvent system (c)) of authentic testosterone acetate (a) and two urinary extracts (b and c) of normal men, after acetylation (scale: 3/1).

Spectrophotometric analysis of testosterone

We used SACHS' colour reaction which is specific for Ts, Epi-Ts, androstenedione and progesterone. Under our conditions the reaction is reproducible over the range of 1.0 to 20.0 μg of Ts per 1.0 ml of solution; the coefficient of variation ranged from 3 to 5%. Fig. 6 shows the absorption spectra of the colour resulting from SACHS' reaction obtained by scanning a solution of authentic and urinary Ts.

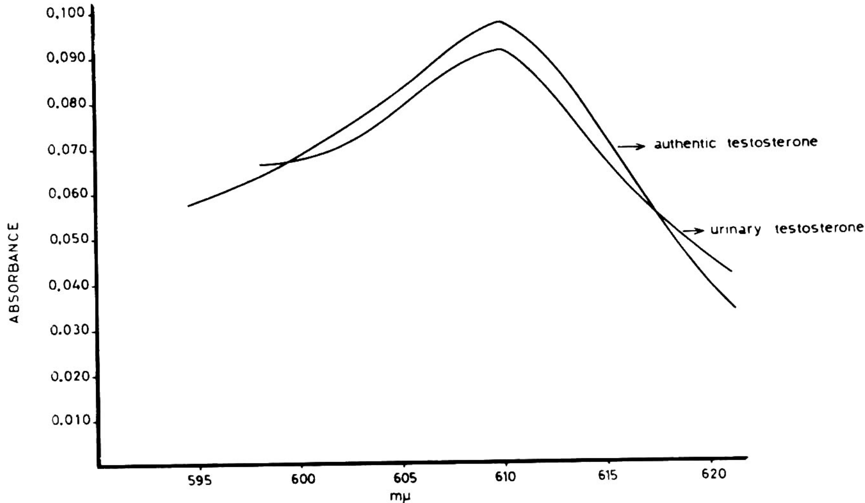

Fig. 6. Absorption spectra of the colour (SACHS' reaction) of authentic testosterone and of that obtained from a pool of normal male urine.

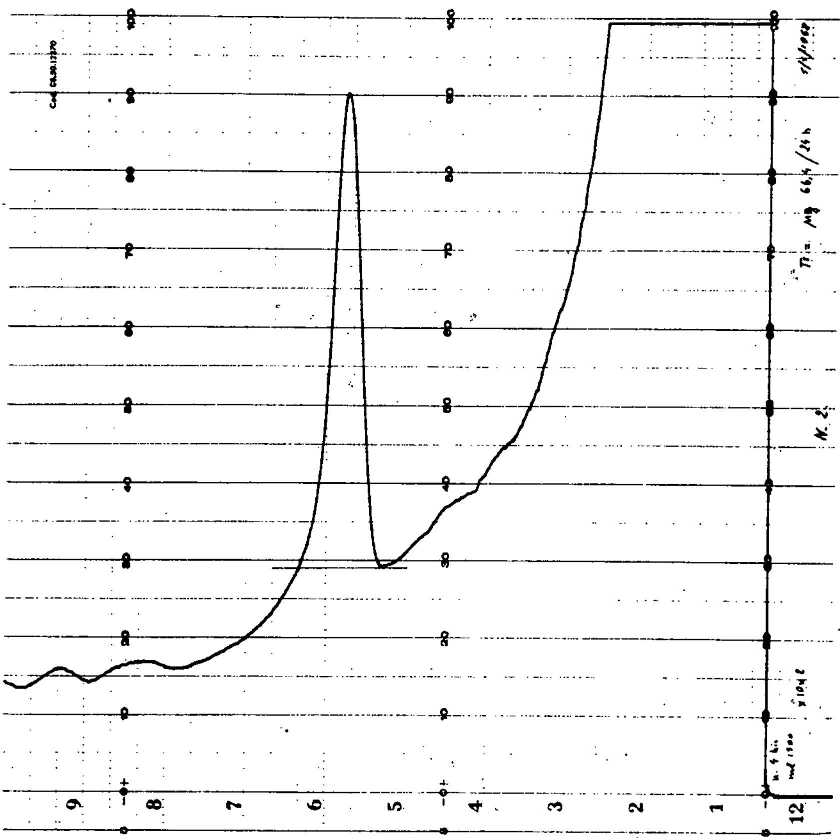

Fig. 7. Gas chromatographic analysis of a 3/10 testosterone urinary extract from normal men. Retention time, 10.2 min (att., × 10 × 2). Other conditions as described in the text.

Gas–liquid chromatographic analysis

Initially micro-columns coated with 1 % SE-30 were used; however the impurities were found to have a retention time (t_R) similar to that of Ts. A column coated with 3 % SE-30 provided better separations. Figs. 7 and 8 show representative gas chromatograms obtained from urinary extracts of normal men and normally menstruating women. In previous methods[4,9] the separation of Ts from Epi-Ts was not achieved. However, we have obtained this by using a continuous flow method in the HTLC (Fig. 4) and also with the GLC analysis. Figs. 9 and 10 show the separation of Ts from Epi-Ts both of a standard solution and of urinary extracts from normal men. The reproducibility of the injection technique and of the GLC has been tested by repeated analysis of standard Ts-acetate. The results are shown in Table I. It will be seen that the coefficient of variation is lower when the peak area is determined by electronic integration: 1.8–3.5 %. Fig. 11 shows the calibration curve based upon numerous replicate chromatograms which have been obtained by the analysis of known quantities of authentic Ts-acetate. There is satisfactory linearity between the mass of injected Ts expressed in integrator units and the mass calculated by the triangulation method, respectively.

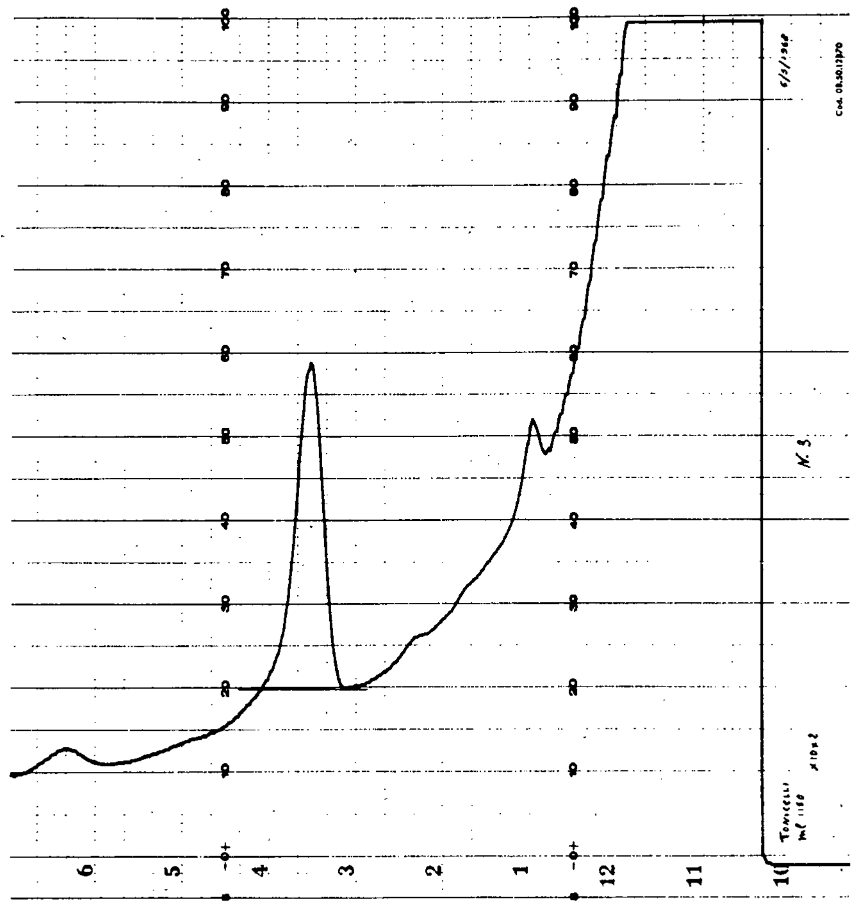

Fig. 8. Gas chromatographic analysis of 9/10 testosterone urinary extract from normally menstruating women in luteal phase. Retention time, 10.15 min (att., × 10 × 2). Other conditions as described in the text.

TABLE I

REPRODUCIBILITY OF GAS–LIQUID CHROMATOGRAPHY (INCLUDING LIQUID INJECTION OF PURE DERIVATIVE) USING A FLAME IONISATION DETECTOR AND ELECTRONIC DIGITAL INTEGRATOR

Chart speed: 1/2 cm/min.

	Authentic testosterone-acetate (μg)	
	0.010	*0.020*
Number of samples	15	20
Integration units		
Mean ± S.D.	32.328 ± 1.142	62.858 ± 1.136
Coeff. of variation	3.5%	1.8%
Peak areas (mm^2)		
Mean ± S.D.	542 ± 43	984 ± 47
Coeff. of variation	7.9%	4.7%

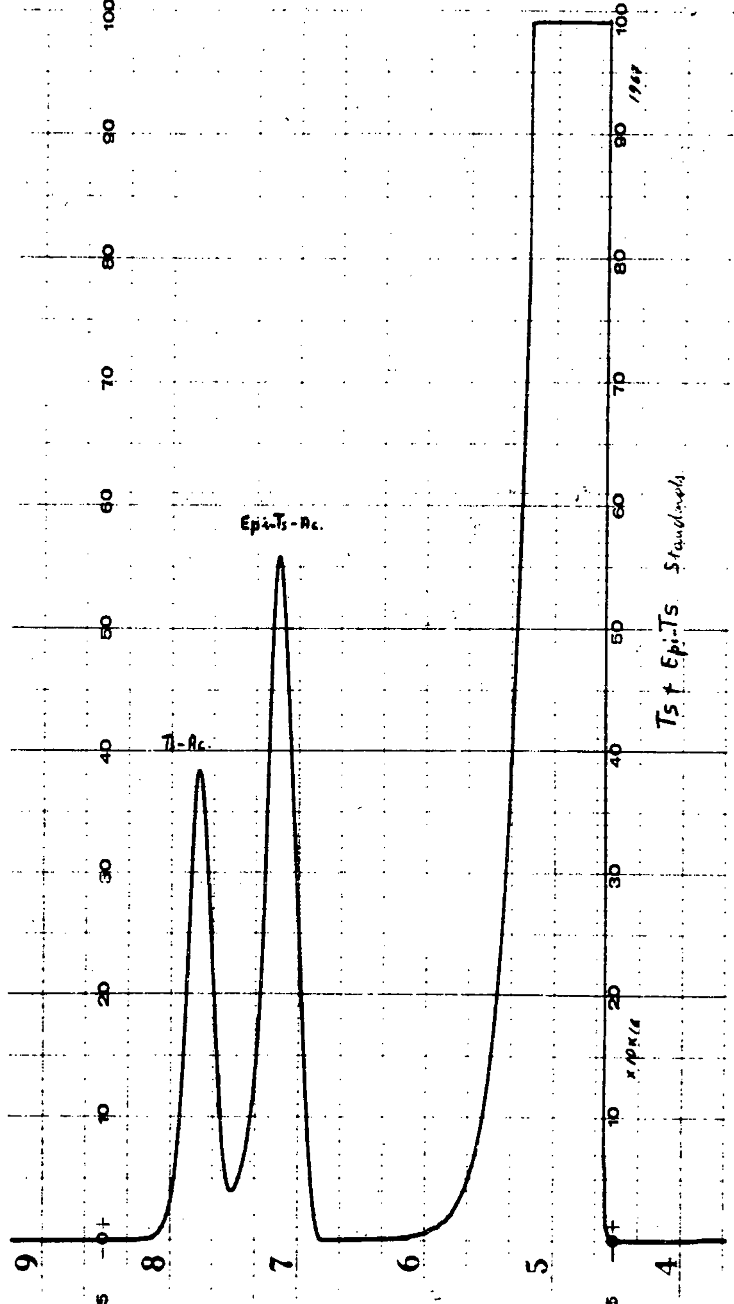

Fig. 9. Gas–liquid chromatographic analysis of authentic testosterone acetate (about 30 ng) and epitestosterone acetate (about 35 ng). Conditions as described in the text.

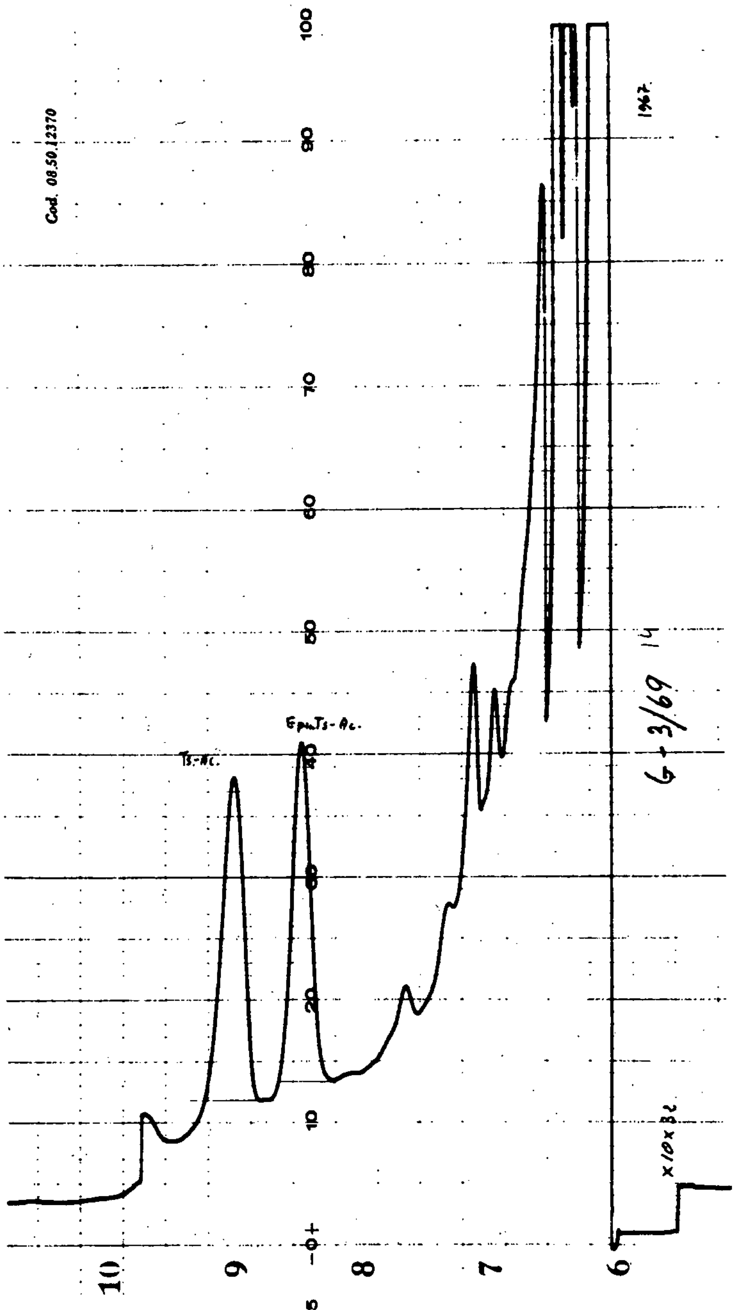

Fig. 10. Gas–liquid chromatographic analysis of urinary testosterone acetate and epitestosterone acetate in normal men after chromatographic partition in solvent system (a). Conditions as described in the text.

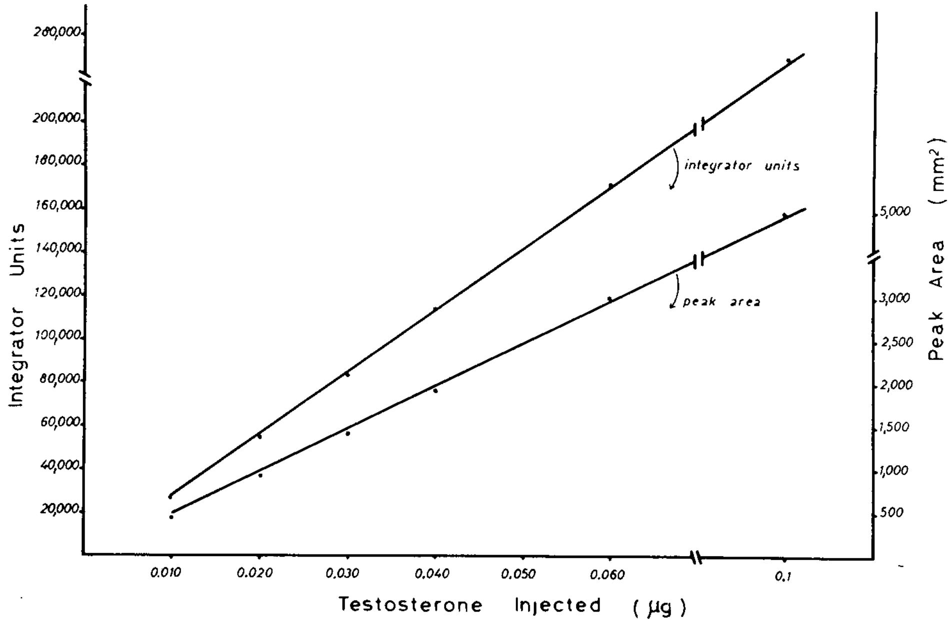

Fig. 11. Calibration curve: linearity and range of detector response to μg quantities of authentic testosterone using integrator units and peak areas, respectively.

Reliability criteria of the method

Accuracy. This was estimated by recovery experiments. A 5.0 μg sample of unlabelled authentic Ts was added to 100 ml of water, and 0.01 μg of labelled Ts were added to 100 ml of female urine. The recoveries obtained by different methods of detection are shown in Table II. Recoveries of 75.1 % and 88.4 % were obtained by using GLC and by LSC. However, the recoveries obtained using SACHS' reaction were only 66.8 %.

Precision. This may be estimated from the difference between duplicate analyses, according to SNEDECOR[20]. The standard deviation from 21 pairs of estimates was ±3.2 μg for a mean of 58.7 μg/24 h (male urine). In 18 pairs of estimates from female

TABLE II

ACCURACY OF THE METHOD USED FOR DETERMINING URINARY TESTOSTERONE

Experimental details are given in the text and the number of observations is given in parentheses.

Method of detection	*Amount of testosterone added after hydrolysis (μg)*	*Mean percentage recovery ± S.D.*
Liquid scintillation counting (radioactivity due to [^{14}C]testosterone)	0.01 (31)	88.4 ± 3.7
Gas–liquid chromatography (as testosterone acetate)	5.0 (18)	75.1 ± 6.8
Spectrophotometric method (SACHS' reaction)	5.0 (23)	66.8 ± 8.2

urine, the standard deviation was ± 1.2 μg for a mean of 8.4 μg/24 h. The coefficients of variation were 4.8 % and 7.3 % for male and female urine, respectively.

Specificity. The identity of the compound determined by the present method was examined at the different stages of analysis. HTLC of the extract containing Ts-acetate showed only one component when the plates were sprayed with the detection reagent. In 75 chromatograms from different samples of male and female urine, only one spot could be detected corresponding in colour and position to Ts. Oxidation of 15 eluates obtained from the second migration produced a compound identical with androstenedione both on HTLC and in GLC on columns coated with SE-30 and XE-60. In all experiments, formation of the acetate of Ts in the final eluate from the second HTL-partition gave a compound of the same R_F and t_R as authentic Ts-acetate. Finally, testosterone could not be detected in 9 urine samples from three women subjected to bilateral ovariectomy and adrenalectomy and accordingly it was concluded that the non-steroidal materials present in urines of these patients did not interfere with the method.

Sensitivity. About 0.5 μg of testosterone could be measured accurately in the final residue by using SACHS' spectrophotometric method, and 0.04 μg as steroid acetate by GLC. The sensitivity, calculated according to the method of BROWN *et al.* [21], was 0.7 μg in a 24 h collection of urine.

Urinary excretion of testosterone in normal subjects

In 100 determinations from 25 normal males aged from 21 to 46 years, the mean excretion of Ts was 61.4 ± 15.7 μg (S.D.) in a 24 h urine (range: 32.4–87.1 μg/24 h). In 24 determinations from 5 young men aged 17–22 years, the mean urinary excretion was 91.7 ± 88 μg (S.D.) per 24 h. When the urinary Ts level was determined in normal women during the follicular phase of the menstrual cycle (111 determinations in 37 women aged 20–41 years), the mean value was 6.0 ± 3.1 μg (S.D.) per 24 h (range: 1.0–92 μg/24 h), while during the luteal phase (69 determinations in 23 women aged 18–40 years) it averaged 12.2 ± 4.3 μg (S.D.) per 24 h (range: 5.1–16.8 μg/24 h). Thus there was a significant difference between the amounts eliminated in male and female urine and between the follicular and luteal phase of the menstrual cycle. Fig. 12 shows the daily Ts excretion pattern during the menstrual cycle of a 21-year-old normally menstruating girl.

CONCLUSIONS

The evidence obtained by GLC shows that testosterone is the major steroid compound present in the final urinary extract. Our experimental results obtained after the more efficient separation and purification of Ts from contaminants and closely related steroids by selective HTLC and GLC, support this finding. However it is necessary to confirm the concept that by using HTLC or GLC it is possible to obtain a satisfactory separation of Ts from its epimer, the latter being the main steroid which interferes with the determination of Ts and is the cause of higher results being obtained with some other methods[4,6,7]. There is considerable evidence to show that the present method is specific for Ts.

The determination of Ts by the colorimetric method is possible only with male urines because of the low sensitivity of the SACHS' reaction. The fact that the re-

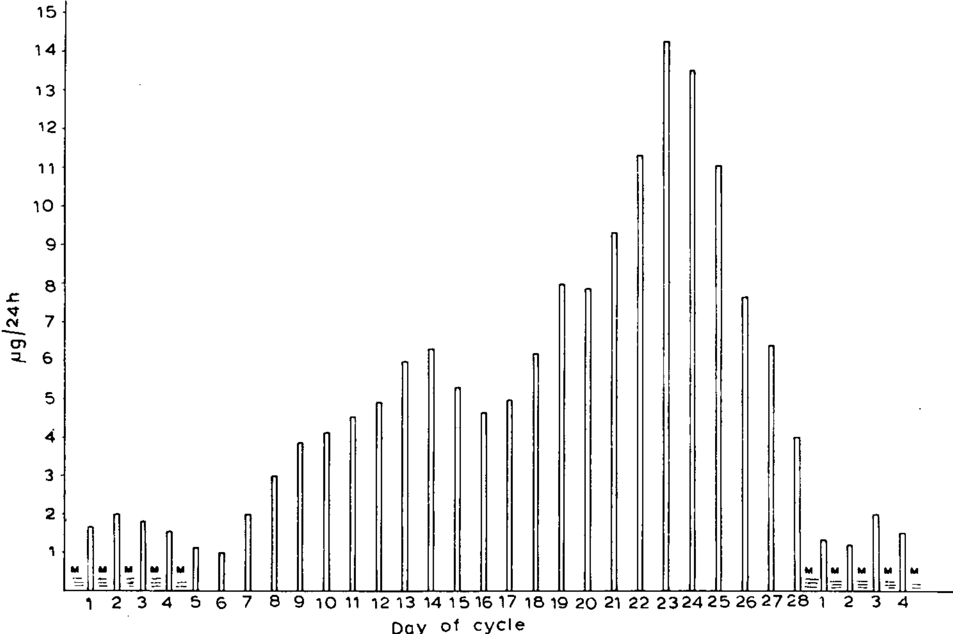

Fig. 12. Daily testosterone excretion pattern during the normal menstrual cycle of a 21-year-old girl.

covery values obtained by GLC on replicates of materials used for determinations by the SACHS' reaction were always higher suggests that impurities from the thin layer may have interfered in the colorimetric determination. GLC represents therefore the procedure of choice.

The values obtained by the method described in this paper are in agreement with the results of some previously reported techniques. However, the upper limits of our results were generally lower than those which have been obtained by some of the previous methods. Finally, the reliability criteria of the method described here permits its use for routine analyses to determine urinary testosterone in normal men, in the follicular and luteal phase of the normal menstrual cycle and in different cases of endocrine diseases.

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CHROMATOGRAPHISCHE TRENNUNG VON ÖSTRON, ÖSTRADIOL-17β UND ÖSTRIOL AN SEPHADEX G-10

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(Eingegangen am 5. März 1971)

SUMMARY

Chromatographic separation of oestrone, 17β-oestradiol and oestriol on Sephadex G-10

A new assay is described to separate urinary oestrogens chromatographically. A 600 × 12 mm Sephadex G-10 column (V_t = 56.6 cm^3) was loaded with samples up to 200 ml. Oestrogens and a few other urinary ingredients were reversibly adsorbed by the gel matrix if the test sample was saturated with Na_2SO_4 and adjusted to pH 4.6 ± 0.2. Almost all non oestrogenic urinary ingredients were eluted prior to the three sexual steroids which left the column in high purified fractions. As eluant a continuous non linear gradient was used produced by mixing a solution of 16% Na_2SO_4 with 0.1 *N* NaOH. An automatic oestrogen determination could be achieved by fluorometry in a flow cell. Additional extractions with organic solvents were unnecessary. 91.3% of the added radioactive oestrogens were discovered in the respective fractions.

EINLEITUNG

Schon nach dem Erscheinen der ersten Veröffentlichungen zur Technik der Gelfiltration[1–3] wurde deutlich, dass sich eine Reihe von Verbindungen während der Passage durch Sephadexsäulen anders verhielt, als es ihrem Molekulargewicht entsprach. In ganz besonderem Masse traf dies für aromatische Verbindungen zu, welche alle eine mehr oder weniger ausgeprägte Affinität zur stationären Phase zeigten.

BELING[4,5] fand bei der Sephadexfiltration von Harnproben eine deutliche Retardation der Östrogenkonjugate. Von besonderem Interesse ist in den Arbeiten dieses Autors, dass eine Retardation nicht zu beobachten war, wenn die Östrogenkonjugate in destilliertem Wasser aufgelöst worden waren. Stärker als die Konjugate wurden die freien Östrogene retardiert[6–8]. Die Elutionsverzögerung war in diesem Falle auch bei den in destilliertem Wasser aufgelösten Östrogenen zu beobachten. Eine quantitative, reversible Adsorption der Östrogene war möglich, wenn die zu untersuchende Probe vor der Beladung der Säule mit NaCl oder Na_2SO_4 gesättigt wurde. Alle Östrogene einer 200 ml-Probe konnten auf diese Weise an eine geringe Menge Sephadex adsorbiert und anschliessend aus der Säule (V_t = 15.7 cm^3) eluiert

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** Leiter: Prof. Dr. E. GRUNERT.

werden[9]. Trotz stark variierender Probenvolumina (1.2–200 ml) blieb das Volumen der östrogenhaltigen Fraktion nahezu konstant. Östron, Östradiol und Östriol lagen zu Beginn der Elution zu einem idealen Startfleck konzentriert an der Säulenspitze vor, so dass ihre Auftrennung durch Gradientenelution denkbar erschien.

MATERIAL UND METHODIK

Reagenzien

Östron (3-Hydroxy-östra-1,3,5(10)-trien-17-on), Östradiol-17β (Östra-1,3,5 (10)-triene-3, 17β-diol), Östradiol-17α (Östra-1,3,5 (10)-triene-3,17α-diol) und Östriol (Östra-1,3,5(10)-triene-3,16α,17β-triol): Schering AG, West-Berlin*. 6,7-Tritium-markiertes Östron, Östradiol-17β und Östriol: Radiochemical Centre Amersham, England. Schwefelsäure (95–97 %), Na_2SO_4, NaOH, Äthanol, Eisessig, *p*-Nitrophenol, Tetrabromäthan, Hydrochinon: E. Merck AG, Darmstadt. Sephadex G-10: Pharmacia, Uppsala, Schweden. Aquasol: NEN Chemicals, Boston, U.S.A.

Geräte

Präzisions-Chromatographierohre (12 × 600 mm), Fraktionensammler Ultrorac 7000. Peristaltische Pumpe, UV-Absorptiometer Uvicord II: LKB-Produkter AB, Stockholm, Schweden. Spektralphotometer (M4 Q III, PMQ II und ZFM 4): Carl Zeiss, Oberkochen. Durchflussküvette (0.5 ml): Hellma GmbH, Müllheim/Baden. 10 Zoll-Schreiber: Beckmann Instruments GmbH, München. Tri-Carb-Scintillations-spektrometer, Modell 3022. Flüssigkeitsscintillationszähler (Mark I): Nuclear Chicago, U.S.A.

Östrogennachweis

(a) Kober-Reaktion nach BAULD[10];

(b) Kober-Reaktion nach Ittrich[11];

(c) Automatisierte Kober-Reaktion durch Mischen von Säuleneluat und Schwefelsäure im Verhältnis 1:2 mit Hilfe einer peristaltischen Pumpe. Erhitzen im Durchfluss (20 Min., 100°) und Messung im Durchflussfluorimeter.

Trennung der Östrogene

Bekannte Aktivitäten von tritiummarkiertem Östron, 17β-Östradiol und Östriol sowie je 1.25 μg der jeweiligen nichtaktiven Verbindung wurden in 50 ml Harn einer nichttragenden Kuh aufgelöst, 8 g festes Na_2SO_4 in die Probe eingewogen und der pH-Wert mit Essigsäure auf 4.6 $\pm$ 0.2 eingestellt. Nach 20 Min. Zentrifugation bei 3000 U/Min. konnte die Säule mit dem Überstand der Probe beladen werden. Um eine vollständige Adsorption zu gewährleisten, war eine langsame Flussrate erforderlich (12.5 ml/cm²/Std.). Die Elution erfolgte mit einem kontinuierlichen exponentiellen Gradienten, der aus 50 ml 16 %iger Na_2SO_4-Lösung im Mischgefäss und etwa 400 ml 0.1 *N* NaOH im Vorratsgefäss erstellt wurde. Dass Mischgefäss war mit der Säule durch einen Kapillarschlauch verbunden und behielt durch den Zustrom von 0.1 *N* NaOH durch einen zweiten Schlauch während der Elution ein konstantes Volumen.

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* Die Hormonpräparate wurden uns in dankenswerter Weise kostenlos für unsere Versuche überlassen.

Versuche zur Östrogenadsorption

Zur Bestimmung der optimalen Bedingungen für die Östrogenadsorption wurden 2 Serien von Versuchen ausgeführt. In einer Serie kamen unterschiedliche Na_2SO_4-Konzentrationen bei einem konstanten pH-Wert von 4.6 zur Anwendung (Fig. 3). In jedem Reagenzglas befanden sich 5 μg eines Östrogengemisches (Östron–Östradiol-17α–Östradiol-17β–Östriol, 1:1:1:1) in 5 ml der betreffenden Natriumsulfatlösung. In jede Probe wurden 100 mg Sephadex G-10 eingewogen und 1 Std. lang geschüttelt. Anschliessend wurde das Sephadex abfiltriert und der Östrogengehalt des Filtrats mit einer Kober-Reaktion[10] bestimmt. Die erhaltenen Werte (Fig. 3 und 4) repräsentieren jeweils 6–12 Probenansätze. 2 Leerwertbestimmungen und 2 Standards ohne Sephadex dienten in jeder Konzentrationsstufe zur Korrektur der erhaltenen Messwerte. Sinngemäss wurde die zweite Versuchsserie ausgeführt. Die Na_2SO_4-Konzentration betrug hierbei in allen Proben 16 %, während der pH-Wert stufenweise von 2.5 bis 12.0 variierte (Fig. 4).

ERGEBNISSE

Bei der chromatographischen Auftrennung der dem Harn zugesetzten 6,7-^{3}H-markierten Östrogene erschien Östriol im Eluat von 175 bis 210 ml, Östron von 225 bis 265 ml und Östradiol-17β von 275 bis 350 ml (Fig. 1). Die meisten Verunreinigungen der 50 ml-Probe verliessen die Säule von 27 ml ($=V_0$) bis 140 ml (Fig. 2). Die Wiederfindungsrate lag bei 91.3 %.

Bei den Adsorptionsversuchen ergab sich ein Anstieg der Bindungskapazität des Sephadex G-10 mit steigender Salzkonzentration. Sie erhöhte sich von 45.5 % in den natriumsulfatfreien Proben auf 99.8 % in Gegenwart von 16 % Na_2SO_4. Der pH-Wert beeinflusste die Adsorptionskapazität nur wenig. Bei Werten zwischen 2.5 und 12.0 resultierte eine Östrogenadsorption von 91 bis 100 %. Von pH 2.5 bis 7.5

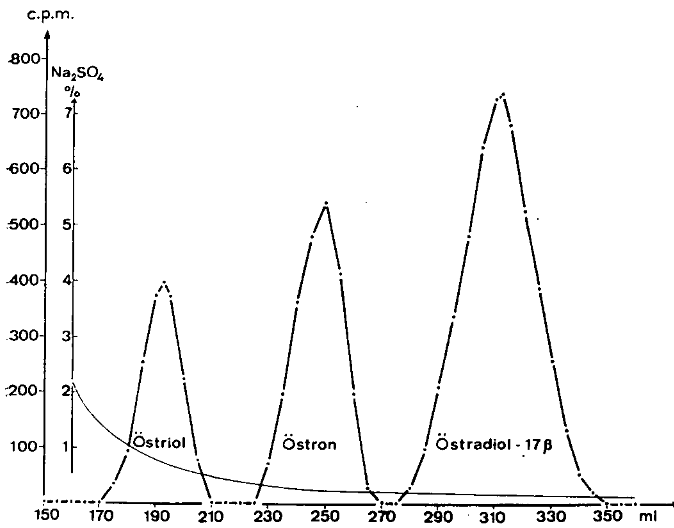

Fig. 1. Elutionskurve von tritiummarkiertem Östriol, Östron und Östradiol-17β (Verhältnis 1:2:4), die in 50 ml Rinderharn aufgelöst wurden. Die Probe enthielt 16 % Na_2SO_4 und war auf pH 4.6 ± 0.2 eingestellt.

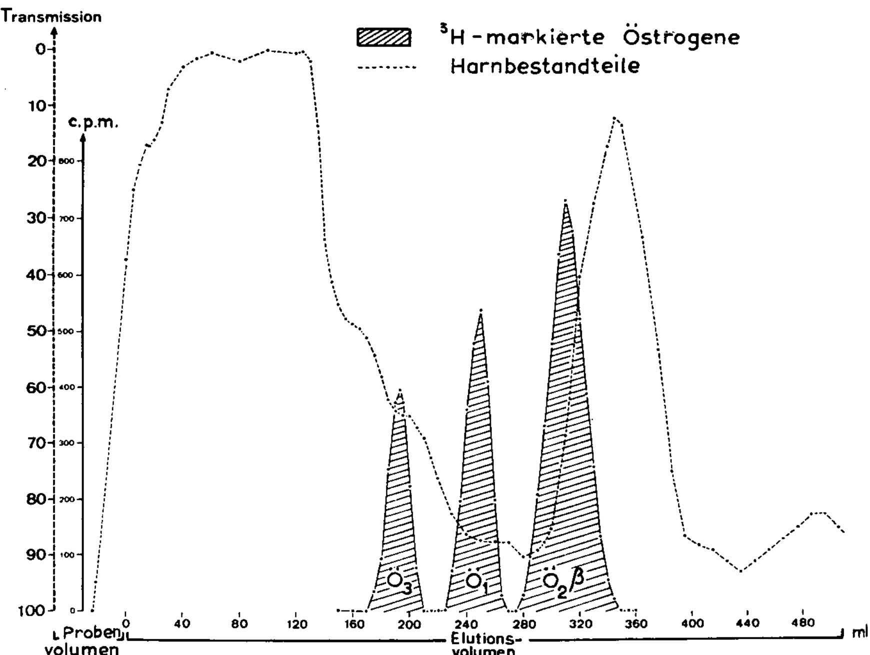

Fig. 2. Trennung der in 50 ml Rinderharn aufgelösten tritiummarkierten Östrogene von Harnbestandteilen, die UV-Licht von 280 nm absorbieren. $Ö_3$ = Östriol, $Ö_1$ = Östron, $Ö_2\beta$ = Östradiol-17β.

waren, mit Ausnahme der Proben von pH 6.5, nur geringe Differenzen in der Adsorptionskapazität (95.6 bis 100 %, S.D. = ± 0.41 bis ± 3.38) zu beobachten. Stärkere Unterschiede (91 bis 99 %) zeigten sich bei den pH-Werten von 8.0 bis 12.0, jedoch wiesen die Werte einer pH-Stufe hier zum Teil grössere Streuungen auf (S.D. = ± 0.78 bis ± 7.51).

Weitere Versuche, die unter Optimalbedingungen (pH 4.6 ± 0.2; 16 % Na_2SO_4) ausgeführt wurden, zeigten eine 99 bis 100 %ige Adsorption an 100 mg Sephadex G-10 auch beim Vorliegen grösserer Östrogenmengen (50–100 μg). Die Östrogenkonjugate einer 5 ml Probe von einer hochtragenden Kuh konnten dagegen unter den gleichen Bedingungen nur zu etwa 25 % adsorbiert werden. Die Trennung von Östrogen-3-Methyläthern nach der hier für freie Steroide beschriebenen Methode gelang nicht.

DISKUSSION

Hydrophile Gele werden im allgemeinen zur Trennung von Stoffgemischen auf der Basis von Molekulargewichtsunterschieden verwendet[12,13]. Auf Trennungsvorgänge, die der Molekularsieb- oder Ausschlusstheorie entsprechend verlaufen, üben Adsorptionserscheinungen einen störenden Einfluss aus. Zur Verhinderung der Adsorption wurden verschiedene Techniken entwickelt[1,14]. Die Steroidadsorption liess sich am besten durch menschliches und tierisches Blutserum aufheben[15], wobei eine Steroidbindung an das Serumprotein ausgeschlossen werden konnte.

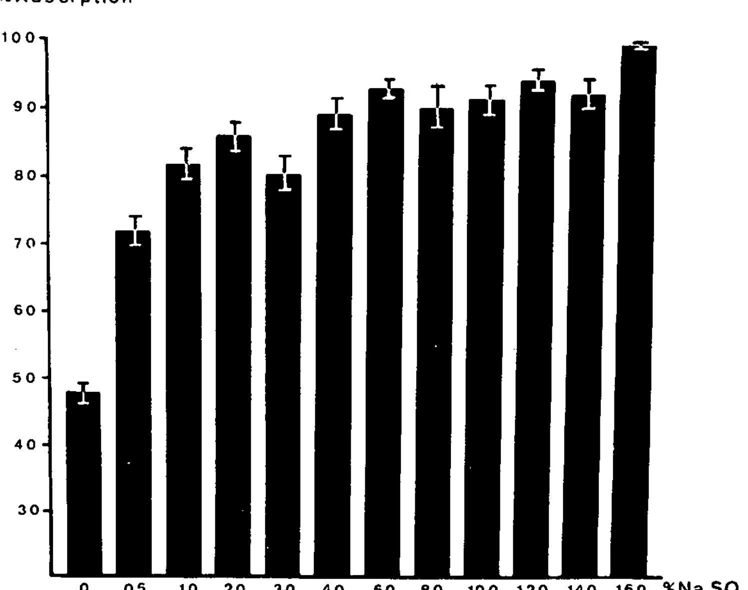

Fig. 3. Änderung der Adsorption von 5 μg eines Östrogengemisches (Östron–Östradiol-17α–Östradiol-17β-Östriol, 1:1:1:1) an 100 mg Sephadex G-10 in Abhängigkeit von der Na_2SO_4-Konzentration. Jede Probe wurde 1 Std. geschüttelt, dann filtriert und der Östrogengehalt des Filtrats bestimmt. Alle Proben wurden auf pH 4.6 $\pm$ 0.2 eingestellt. I = Standardabweichung.

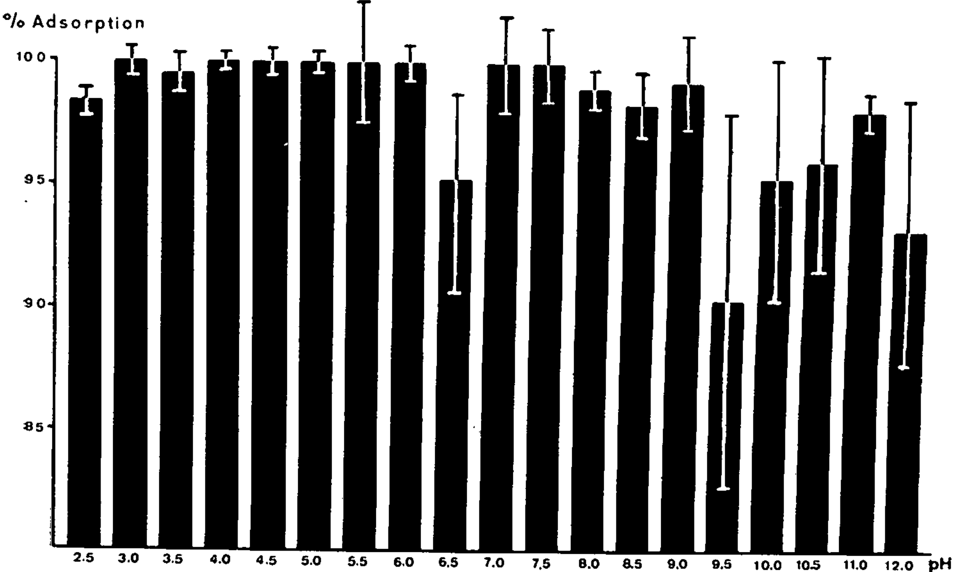

Fig. 4. Änderung der Adsorption von 5 μg eines Östrogengemisches (Östron–Östradiol-17α–Östradiol-17β–Östriol, 1:1:1:1) in Abhängigkeit vom pH-Wert. Alle Proben enthielten 16% Na_2SO_4. I = Standardabweichung.

In den hier vorliegenden Untersuchungen sollte im Gegensatz dazu eine möglichst feste, reversible Adsorption erreicht werden. Dieses gelang durch die Zugabe von entquellend wirkenden Elektrolyten wie NaCl oder Na_2SO_4. Da NaCl bei der automatischen Kober-Reaktion störte (Gasbildung) wurde das Verfahren auf Na_2SO_4 eingestellt. Es zeigte sich, dass sich die Affinität der Gelphase zu den Östrogenen mit steigender Salzkonzentration direkt proportional erhöhen liess. Aus Harnproben wurden ausser Östrogenen auch noch viele andere Verbindungen adsorbiert.

Obwohl in allen Sephadex G Typen Carboxylgruppen vorkommen, kann die Adsorption nicht mit einem Ionenaustausch erklärt werden. Ionenaustauscher wurden jedoch schon mit Erfolg zur Auftrennung einiger Östrogenkonjugate verwendet[16,17].

In früheren Experimenten konnten wir die Beobachtung machen, dass die Adsorption von Östrogenen und der Quellungszustand des Gels in einer Wechselbeziehung stehen. Wurde durch Zufügen von Na_2SO_4 die Ionenstärke der Probe erhöht, so kam es zu einer deutlichen Entquellung des Gels, wenn die Probenlösung in das Gelbett eindrang.

Man kann sich den Adsorptionsvorgang in vereinfachter Form etwa folgendermassen vorstellen. Wurde die mobile Phase einer mit Wasser oder verdünnter Natronlauge bereiteten Sephadexsäule durch die konzentrierte wässrige Lösung eines entquellend wirkenden Elektrolyten verdrängt, so kam es zu einer Dehydratation des Gels. Diese ist in ihrer Stärke von der Art der in die Säule eingeführten Ionen sowie von deren Konzentration abhängig. Die Verkleinerung der Hydrathülle, die die polymerisierten Dextranketten umgab, bewirkte ein stärkeres Hervortreten der lipophilen Eigenschaften der Gelmatrix. Somit wurden gelöste stark lipophile Stoffe wie z.B. Östrogene, die zu ihrem wässrigen Lösungsmittel ohnehin nur eine geringe Affinität aufweisen, leichter an das Sephadex adsorbiert.

Neben Elektrolytlösungen führten auch andere Stoffe, wie z.B. Äthanol zu einer Entquellung des Gels, die ebenfalls eine sehr feste Östrogenadsorption bewirkte. Aus diesem Grunde konnte Äthanol, also ein gutes Östrogenlösungsmittel, nicht zur Elution verwendet werden. Wurde das entquollene Gel langsam wieder zur Quellung gebracht, so traten die Östrogene in der Reihenfolge ihrer pK-Werte wieder in die mobile Phase der Säule über. Hierbei stellte sich je nach dem Quellungsgrad ein Gleichgewicht zwischen Adsorption und Desorption ein.

Bei den stärker quellbaren Sephadextypen kann die Elution der Östrogene mit Wasser erreicht werden. Bei Sephadex G-10 war die Adsorption jedoch so fest, dass erst die weitere Erhöhung der OH-Ionenkonzentration zu praktisch brauchbaren Elutionsvolumina führte. Mit destilliertem Wasser als Eluant wurden zu grosse Flüssigkeitsmengen benötigt.[6] Wie pH-Messungen des östrogenhaltigen Eluats zeigten, wurden die Östrogene auch dann noch retardiert, wenn ihr pK-Wert so weit überschritten war, dass praktisch vollständige Dissoziation herrschen musste. Es kann angenommen werden, dass durch das Sephadex eine starke Verschiebung des Dissoziationsgleichgewichtes zugunsten der undissoziierten Form bewirkt wurde. Sieht man diese als allein adsorbierbar an, so kann die Ursache für die starke Elutionsverzögerung, die auch noch bei pH-Werten über 10.5 zu beobachten war, hierin vermutet werden. Die Abtrennung der übrigen Harnbestandteile von den Östrogenen war zwei verschiedenen Prinzipien unterworfen. Die Masse der höhermolekularen Stoffe sowie die meisten anorganischen Salze passierten die Säule ungehindert nach

dem Prinzip der Molekularfiltration und eluierten spätestens nach dem Durchfluss von $V_i + V_0$.

Alle Stoffe, die vollständig oder teilweise adsorbiert wurden, verliessen die Säule erst dann, wenn ein bestimmter pH-Wert oder ein bestimmter Quellungsgrad des Gels vorlag. Die Fig. 3 und 4 lassen erkennen, dass ein sinkender Salzgehalt und ein erhöhter pH-Wert die Elution in Gang setzten oder beschleunigten.

Da fast alle bei der fluorimetrischen Östrogenbestimmung störenden Harnbestandteile vor den Östrogenen eluierten, traten die Östrogene in einer sehr reinen Fraktion auf. Nach einer einfachen Reinigungstechnik[7,8] wurde mit den trockenen Rückständen der an Sephadex G-10 aufgetrennten Östrogene die Kober-Reaktion nach ITTRICH ausgeführt. In den Tetrabromäthanextrakten von 50 ml-Proben einer Kuh im normalen Zyklus konnten keine gefärbten Verunreinigungen wahrgenommen werden. Bei der automatischen Kober-Reaktion der Eluate von 5 ml-Proben erhöhte sich die Leerwertfluoreszenz von 5 % auf 6 %, wenn beim Vorliegen von 1.25 μg pro Östrogen 80–100 % des Schreiberausschlages erreicht wurden.

Die im Bereich des Östriol und Östradiol auftretende blassrosa Färbung des Eluats hatte keinen wahrnehmbaren Einfluss auf die Östrogenfluoreszenz. Nach unseren bisherigen Erfahrungen scheint es im Harn kaum Verbindungen zu geben, die einerseits gemeinsam mit den Östrogenen eluieren und andererseits bei der Kober-Reaktion stärker in Erscheinung treten.

Die hier beschriebene Methode zur Östrogentrennung an Sephadex G-10 wies gegenüber den bisher gebräuchlichen Verfahren einige wesentliche Vorteile auf. Am stärksten fiel die hohe Kapazität des Gels ins Auge. Es konnten grosse Proben ohne vorherige Reinigung eingesetzt werden, wobei eine Änderung des Elutionsverhaltens der Östrogene nicht wahrnehmbar war. Vom Zeitpunkt des Elutionsbeginns an gerechnet ergaben sich für stark unterschiedliche Probenvolumina identische Elutionskurven. Durch die Anreicherung der Östrogene während der Probenaufgabe scheint die Methode auch bei Probenmaterial mit geringem Östrogengehalt einsetzbar zu sein. Die hohe Wiederfindungsrate von 91.3 % dürfte sich hierbei ebenfalls positiv auswirken.

Da es sich um eine säulenchromatographische Methode handelte, bei der grössere Flüssigkeitsmengen benötigt wurden, konnte die Elution mit den üblichen Geräten automatisiert werden. Steht ein Durchflussfluorimeter zur Verfügung, so ist es möglich, auch die Bestimmung vollautomatisch auszuführen. Als nachteilig muss das recht hohe Elutionsvolumen von 35–75 ml pro Östrogen angesehen werden. Hierdurch wurde bei der automatischen Durchflussbestimmung die Empfindlichkeit gegenüber anderen Methoden herabgesetzt. In urinfreien Standardproben ergaben 0.125 μg eines Östrogens jedoch noch einen Wert von 30 % des Schreiber-Vollausschlages. Die Elution einschliesslich der vollautomatischen Aufzeichnung der Elutionskurven nimmt etwa 24 Std. in Anspruch.

ZUSAMMENFASSUNG

Es wird eine neue Methode zur chromatographischen Auftrennung von Harnöstrogenen beschrieben. Eine 600 × 12 mm Sephadex G-10 Säule ($V_t = 56.6$ cm^3) konnte mit Proben bis zu 200 ml beladen werden. Hierbei wurden die Östrogene sowie einige andere Harnbestandteile reversibel an die Gelmatrix adsorbiert, wenn das

Probengut vorher mit Na_2SO_4 gesättigt und auf pH 4.6 ± 0.2 eingestellt wurde. Fast alle nichtöstrogenen Harnbestandteile wurden vor den drei Sexualsteroiden eluiert, welche die Säule in hochgereinigten Fraktionen verliessen. Als Eluant diente ein kontinuierlicher exponentieller Gradient aus 16 %iger Na_2SO_4-Lösung im Mischgefäss und 0.1 *N* NaOH im Vorratsgefäss. Die Östrogenbestimmung konnte vollautomatisch in einem Durchflussfluorimeter ausgeführt werden. Extraktionen mit organischen Lösungsmitteln waren nicht erforderlich. 91.3 % der zugesetzten radioaktiven Östrogene wurden in den betreffenden Fraktionen wiedergefunden.

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FRACTIONATION OF RAPIDLY-LABELLED NUCLEIC ACIDS FROM *RHODOSPIRILLUM RUBRUM* USING POLYLYSINE KIESELGUHR COLUMN CHROMATOGRAPHY

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SUMMARY

Poly-L-lysine kieselguhr column chromatography has been used to fractionate a rapidly labelled RNA fraction from *Rhodospirillum rubrum*. Labelled nucleic acid mixtures, prepared from *R. rubrum* grown anaerobically in the light, were fractionated on poly-L-lysine kieselguhr columns. Preliminary results of this study are reported in this paper.

INTRODUCTION

The facultative photoheterotroph *Rhodospirillum rubrum* can be cultured either aerobically in the dark or anaerobically in the light, the two types of growth being manifested in the specific differences in cellular structure and metabolic pathways.

The process of transition from heterotrophic growth to photosynthetic growth, is accompanied by the extensive synthesis of protein pigments and other components of the photosynthetic apparatus. The heterotrophic organism possesses very low levels of pigments and structures necessary for photosynthesis, and when transferred to anaerobic light conditions, is able to grow only after a certain lag period. The organism must synthesise pigments and other components of the photosynthetic organelle during this lag phase, and this may require the synthesis of specific messenger RNA's (mRNA).

YAMASHITA AND KAMEN[1,2] extracted pulse-labelled RNA from *R. rubrum* cells labelled with 0.1 mCi of [^{3}H]uracil per ml of culture for 3 min. They examined and compared the properties of pulse-labelled RNA from cells grown in the dark and light, and found no differences in the base composition of the two RNA's. Methylated-albumin coated kieselguhr column chromatography and sucrose gradient centrifugation showed the pulse-labelled RNA to be associated with the ribosomal RNA. They therefore concluded that if a light-specific messenger RNA were required to initiate photopigment synthesis, it was present in an undetectable amount. However, although they could not detect the existence of a light-specific messenger RNA using hybridisation techniques, they observed a marked increase in uracil incorporation into RNA in the presence of light.

In this paper the fractionation of rapidly-labelled RNA extracted from *R. rubrum* using a poly-L-lysine kieselguhr (PLK) column, devised by AYAD AND BLAMIRE[3-5], was achieved.

PROCEDURES

Strain of bacteria

Rhodospirillum rubrum strain S, was used as the source of nucleic acids.

Culture of micro-organisms

R. rubrum were grown in the medium described by ORMEROD *et al.*[6]. Stock liquid cultures were grown in completely filled screw cap bottles in the presence of light. These were maintained by transferring every 24 h into fresh medium. A 24 h-old culture was used as an inoculum. Anaerobic cultures were grown in conical flasks, three-quarter filled with medium, maintained at 28–30° and illuminated on either side of the flask. Sufficient nitrogen gas was bubbled through to disperse the cells in the culture medium and to maintain an atmosphere of nitrogen in the flask. Cells were grown to the middle of logarithmic phase of the growth cycle to give a very bright red colour.

Preparation of DNA and nucleic acid mixtures

Preparation of DNA. R. rubrum DNA was prepared by the method of MARMUR[7].

Preparation of nucleic acid mixtures. Nucleic acid mixtures were prepared by a modification of DNA preparation as described by MARMUR[7]. Ribonuclease treatment was omitted and nucleic acids were precipitated with cold ethanol (95 %) after standing 2 h at −22°. The precipitate was collected by centrifugation for 5 min at 3000 r.p.m. at 4° using MSE Major centrifuge and dissolved in standard saline citrate. Two more deproteinisations were carried out as described by MARMUR[7] and the nucleic acid mixtures were precipitated with cold ethanol (95 %) and dissolved in standard saline citrate and stored in a concentrated form at 4°.

Preparation of rapidly-labelled nucleic acid mixtures. Rapidly-labelled nucleic acid mixtures were prepared by labelling the cells in the log phase of growth with 0.05 to 1 μCi of [^{3}H]uracil per ml of culture incubated under photosynthetic conditions as described previously. After labelling for the appropriate time, the reaction was stopped by the addition of 0.1 volume of 0.1 *M* sodium azide dissolved in saline–EDTA buffer pH 8, and an excess of crushed-ice. Rapidly-labelled nucleic acid mixtures were then prepared as described previously.

Preparation of PLK columns

PLK columns were prepared as described by AYAD AND BLAMIRE[3-5]. Nucleic acid mixtures prepared as described previously were fractionated on 5 g PLK columns in which the fractionating layer consisted of 5 g washed kieselguhr treated with 5 mg of polylysine. PLK column was eluted using a linear gradient of 0.4 *M* NaCl containing 0.02 *M* KH_2PO_4, pH 6.7 to 4 *M* NaCl containing 0.02 *M* KH_2PO_4, pH 6.7 (100 ml of each) according to the method of AYAD *et al.*[8]. The flow rate was about 20 ml/h and the extinction of the effluent was continually measured using LKB Uvicord UV absorptiometer (257 nm) and 2 ml or 4 ml fractions were collected using an LKB (Stockholm) fraction collector linked to the Uvicord system.

Following PLK fractionation of nucleic acids (2 mg), each fraction was estimated at 260 nm using Unicam SP 500 spectrophotometer, and the radioactivity was assayed by precipitating the nucleic acids with 5 % ice-cold TCA and filtering onto a glass fibre disc (grade GF/C). The discs, after washing with 5 % ice-cold TCA and cold ethanol (95 %), were dried using an IR lamp. The discs were placed in 5 ml scintillator, (0.4 % 2,5-diphenyloxazole and 0.04 % 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, dissolved in xylene), and counted in the Packard "Tricarb" liquid scintillation spectrometer using three channels with a gain of 60 % and window settings of 50 to 800 in all the three windows.

DNA was estimated by the diphenylamine test[9], RNA by the orcinal test[10] and protein by the method of LOWRY *et al.*[11]. NaCl gradient was measured by using the conductivity cell.

RESULTS

The results obtained when a sample of nucleic acid mixture prepared as described previously was fractionated on PLK column is shown in Fig. 1. Five distinct peaks are obtained, eluting at the salt molarities of 0.4, 1.0, 1.2, 1.75 and 1.9 *M* NaCl. The peak eluting at 0.4 *M* NaCl consists of nucleotides and oligonucleotides of low molecular weight, and most of this material can be removed by dialysis prior to fractionation. Its immediate elution at 0.4 *M* NaCl indicates that it is not retained by the column. When the fractions from each peak are assayed for DNA[9], RNA[10] and protein[11], the results obtained are shown in Fig. 2. The 0.4 and 1.9 *M* NaCl peaks consist of RNA and DNA with some protein respectively. The other three peaks eluting at 1.0, 1.2 and 1.75 *M* NaCl, consist mainly of RNA with very little DNA. Further confirmation of these findings is provided by the elution profiles shown in Fig. 3. They are: (A) *R. rubrum* DNA prepared by the method of MARMUR[7], (B) transfer RNA extracted from yeast and supplied commercially by Sigma Chemical Co., and (C) highly polymerised RNA supplied by B.D.H. Ltd.

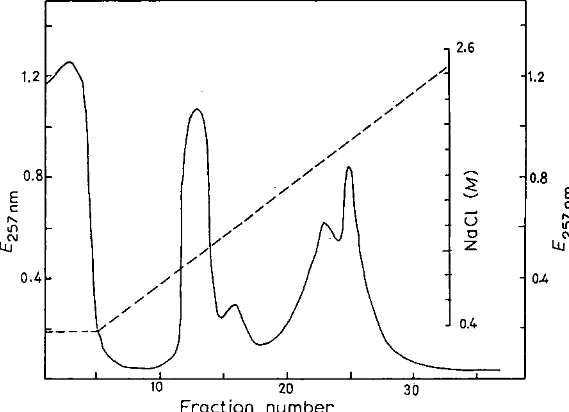

Fig. 1. The continuous elution profile from a 5 g PLK column monitored using an LKB Uvicord UV spectrophotometer of a nucleic acid mixture obtained from *R. rubrum*, using a linear gradient between 0.4 and 4.0 *M* NaCl. 4-ml fractions were collected. $E_{257\,nm}$ (———) and NaCl molarity (- - - -).

The fractionation of a nucleic acid mixture extracted from a culture incubated for 24 h under photosynthetic conditions as described above in the presence of [^{3}H]uracil, 2 μCi/ml of culture, is shown in Fig. 4. It can be seen that the radioactivity is distributed evenly in all the RNA peaks, this is due to the long period of incubation with [^{3}H]uracil.

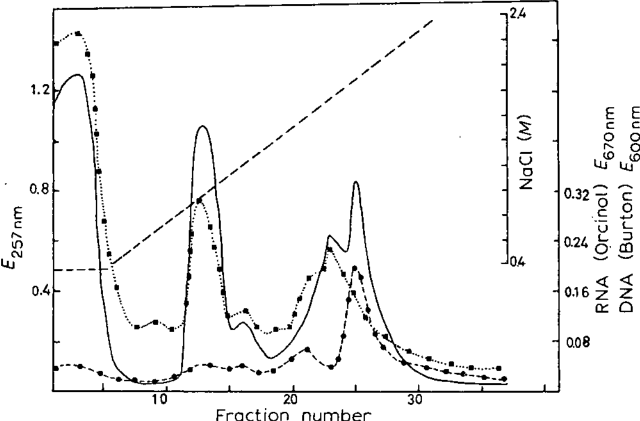

Fig. 2. Samples are taken from a fractionation of a nucleic acid mixture on PLK column (see Fig. 1) and assayed for RNA and DNA. E_{257nm} (———), RNA (■... ■), DNA (●---●) and NaCl molarity (– – – –).

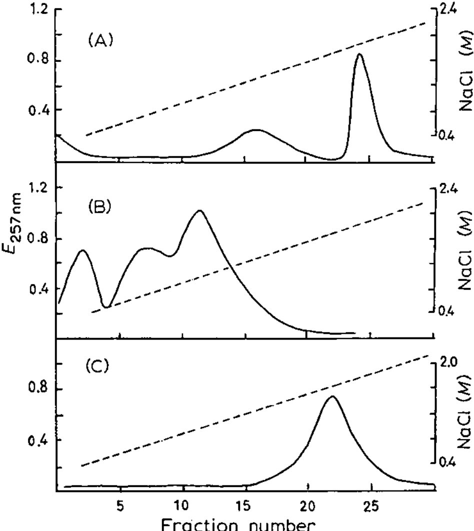

Fig. 3. Continuous elution profile from a 5 g PLK column of (A) DNA isolated from *R. rubrum*, (B) yeast tRNA and (C) highly polymerised RNA. E_{257nm} (———) and NaCl molarity (– – – –).

The elution profiles obtained from fractionation of nucleic acid mixtures on PLK columns, extracted from cultures incubated for 0.5, 1, 5, 10 and 60 min in the presence of [^{3}H]uracil, 0.1 μCi/ml of cultures are shown in Figs. 5, 6, 7, 8 and 9 re-

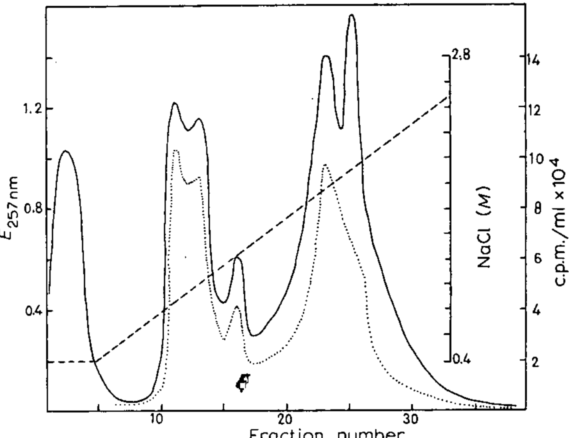

Fig. 4. Fractionation of a nucleic acid mixture on a 5 g PLK column, extracted from a culture of *R. rubrum* incubated for 24 h with [^{3}H]uracil (2 μCi/ml of culture); 4-ml fractions were collected. E_{257nm} (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

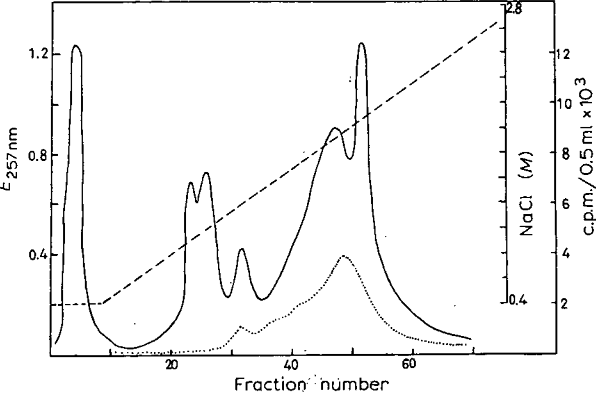

Fig. 5. Fractionation of a nucleic acid mixture extracted from *R. rubrum* pulse-labelled for 30 sec with [^{3}H]uracil (0.1 μCi/ml of culture) on PLK column; 2-ml fractions were collected. E_{257nm} (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

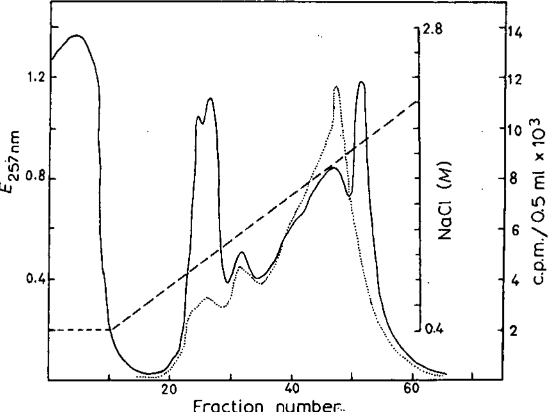

Fig. 6. PLK fractionation of a nucleic acid mixture, extracted from *R. rubrum* pulse labelled for 1 min with [^{3}H]uracil (0.1 μCi/ml of culture); 2 ml fractions were collected. E_{257nm} (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

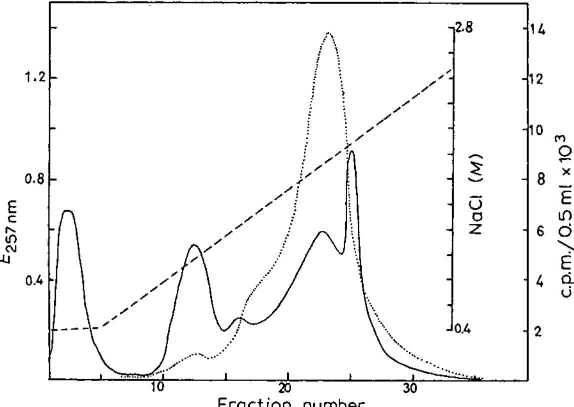

Fig. 7. PLK fractionation of a nucleic acid mixture, extracted from *R. rubrum* labelled for 5 min with [^{3}H]uracil (0.1 μCi/ml of culture); 4 ml fractions were collected. $E_{257\,nm}$ (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

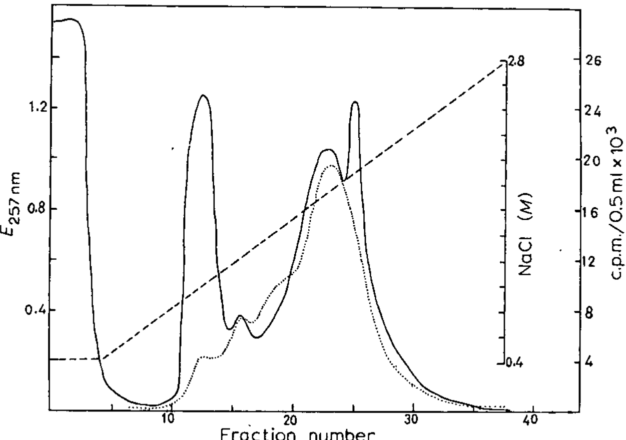

Fig. 8. PLK fractionation of a nucleic acid mixture, isolated from *R. rubrum* labelled for 10 min with [^{3}H]uracil (0.1 μCi/ml of culture); 4 ml fractions were collected. $E_{257\,nm}$ (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

spectively. There is a very marked variation in the distribution of radioactivity in the RNA peaks with the time of incubation.

Proflavine is known to inhibit the synthesis of RNA in *Escherichia coli* in a manner similar to that of actinomycin D in other systems[12,13]. The inhibition of RNA synthesis occurs because actinomycin D complexes with the DNA and subsequently inhibit transcription[14–16]. The synthesis of RNA in viruses is unaffected by the antibiotic[16]. The effect of proflavine on rapidly-labelled RNA in *R. rubrum* was investigated. Total nucleic acid were isolated and fractionated on PLK columns and the results are shown in Figs. 10–12. It can be shown that proflavine is very effective in inhibiting RNA synthesis in *R. rubrum*. During a 1 min exposure to proflavine, at a concentration of 60 μg/ml of culture, in the presence of [^{3}H]uracil (0.05 μCi/ml of culture), the RNA synthesis is inhibited by about 82 %. Moreover, if the cells are

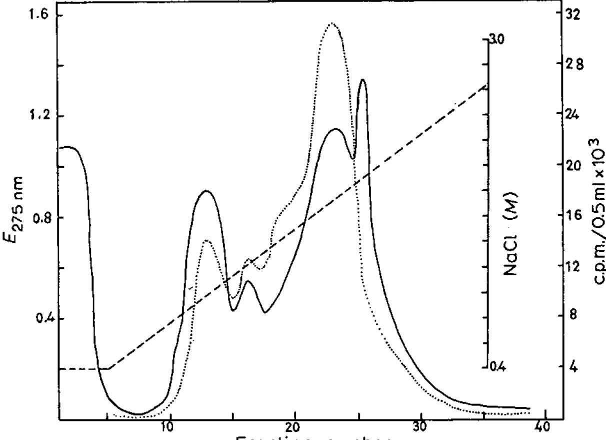

Fig. 9. PLK fractionation of a nucleic acid mixture, extracted from *R. rubrum* labelled for 60 min with [^{3}H]uracil (0.1 μCi/ml of culture); 4 ml fractions were collected. $E_{257\,nm}$ (———), ^{3}H-activity (· · · · · ·) and NaCl molarity (– – – –).

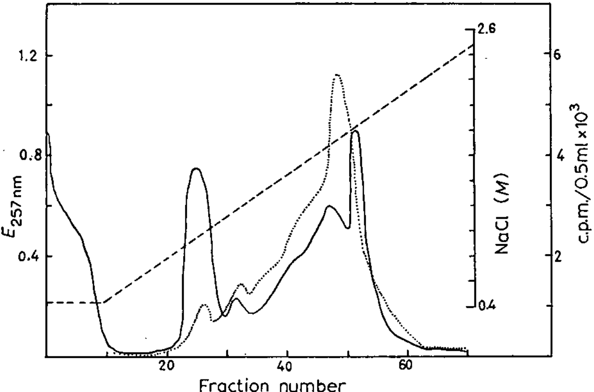

Fig. 10. Fractionation of a nucleic acid mixture, isolated from *R. rubrum* incubated for 1 min with [^{3}H]uracil (0.05 μCi/ml of culture), on PLK column; 2 ml fractions were collected. $E_{257\,nm}$ (———), ^{3}H-activity (· · · · · ·) and NaCl molarity (– – – –).

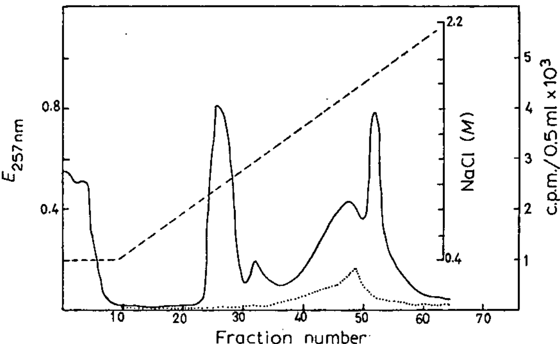

Fig. 11. Fractionation of a nucleic acid mixture, extracted from *R. rubrum*, incubated with [^{3}H]-uracil (0.05 μCi/ml of culture) and proflavine (60 μg/ml), on PLK column; 2 ml fractions were collected. $E_{257\,nm}$ (———), ^{3}H-activity (· · · · · ·) and NaCl molarity (––––).

incubated for 5 min with the same concentration of proflavine and then labelled for 1 min with [^{3}H]uracil (0.05 μCi/ml of culture), the RNA synthesis is totally suppressed. This suggests that the cells pretreated with proflavine for 5 min, have almost entirely lost their ability to synthesise RNA, as the cells are unable to incorporate any radioactivity into RNA species.

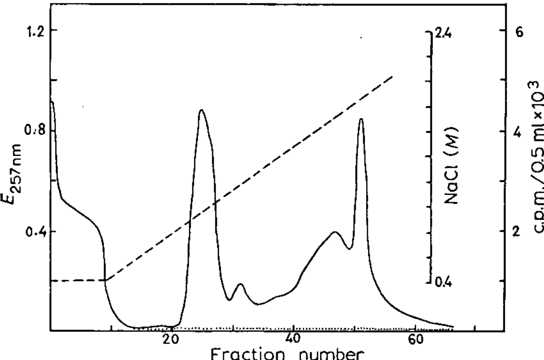

Fig. 12. Fractionation of a nucleic acid mixture, isolated from *R. rubrum* treated for 5 min with proflavine (60 μg/ml) and incubated with [^{3}H]uracil (0.05 μCi/ml) for 1 min, on PLK column; 2 ml fractions were collected. E_{257nm} (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

PLK fractionation of rapidly-labelled RNA of treated and untreated cultures of *R. rubrum* with proflavine (Figs. 10–12), shows that at the end of the treatment the bacteria have lost most of their endogenous supply of "rapidly renewable RNA". The RNA species which is very rapidly labelled in the absence of proflavine must be at least partly equivalent to messenger RNA, the effect of proflavine on *R. rubrum* could be to degrade the rapidly-labelled RNA species and to stop the transcription of RNA by complexing with the DNA molecule.

The effect of actinomycin D (which is known to inhibit RNA synthesis in *Bacillus subtilis*)[21] on rapidly-labelled RNA in *R. rubrum* was also studied, and it was found that this antibiotic (at a concentration of 1 μg/ml of culture) had very little effect on RNA synthesis.

It has been reported that in *B. subtilis* cells during exposure to chloramphenicol, they produce large quantities of RNA, while protein synthesis ceases[22]. The effect of chloramphenicol, at a concentration of 20 μg/ml of culture, on RNA synthesis in *R. rubrum* cells was investigated in the presence of [^{3}H]uracil (0.05 μCi/ml of culture) and the results are shown in Fig. 13. It can be seen that the RNA synthesis was inhibited by about 25%. This finding agrees with the results of YAMASHITA AND KAMEN[2]. However, the effect of proflavine on the rapidly-labelled RNA is very much less in the presence of chloramphenicol as shown in Fig. 14.

DISCUSSION

Fractionation of nucleic acids extracted from *R. rubrum* on PLK columns, shows five distinct peaks, eluting at salt molarities 0.4, 1.0, 1.2, 1.75 and 1.9 *M* NaCl. The peak eluting at 1.9 *M* NaCl is identified as DNA by the diphenylamine test[9],

and also by the fractionation of standard *R. rubrum* DNA prepared by the method of MARMUR[7] (Fig. 3A). Peaks eluting at 1.0, 1.2 and 1.75 *M* are identified as RNA by the orcinol test[10]; these peaks are further identified as transfer RNA and ribosomal RNA by the fractionation of yeast transfer RNA and *E. coli* ribosomal RNA (Figs. 3B and C).

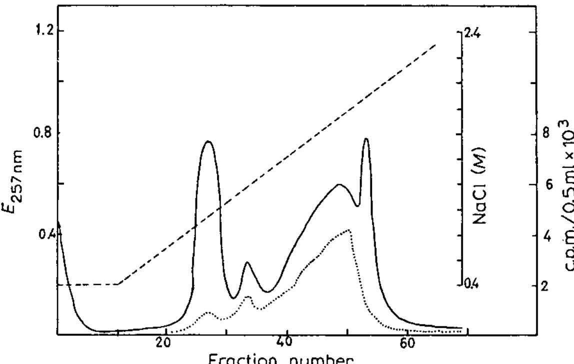

Fig. 13. Fractionation of a nucleic acid mixture extracted from *R. rubrum* treated with chloramphenicol (20 μg/ml) for 5 min and incubated with [^{3}H]uracil (0.05 μCi/ml) for 1 min, on PLK column; 2 ml fractions were collected. E_{257nm} (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

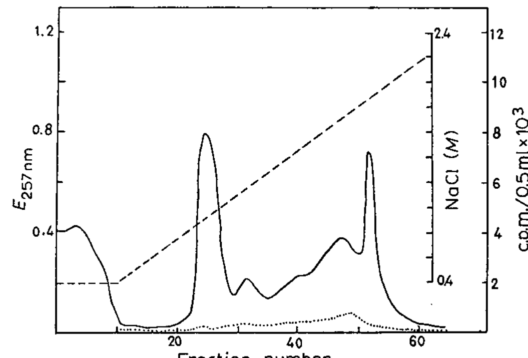

Fig. 14. Fractionation of a nucleic acid mixture, extracted from *R. rubrum* treated with chloramphenicol (20 μg/ml) and proflavine (60 μg/ml) for 5 min and incubated with [^{3}H]uracil (0.05 μCi/ml) for 1 min, on PLK column; 2 ml fractions were collected. E_{257nm} (———), ^{3}H-activity (······) and NaCl molarity (– – – –).

Previous studies on rapidly-labelled RNA in *R. rubrum* have shown that a major portion appears in the region of ribosomal RNA using sucrose gradient centrifugation and methylated albumin kieselguhr columns (MAK)[1,2]. The results reported here show that the rapidly-labelled RNA appears in the region of ribosomal RNA in a PLK fractionation, after a 30 sec exposure to [^{3}H]uracil. Incubation for 1 min however, results in the [^{3}H]activity appearing in the transfer RNA region. Moreover, the lack of coincidence between the radioactivity and the extinction profiles of the peaks indicates that uracil was first incorporated into RNA species other than the main ribosomal and transfer RNA's.

HURWITZ *et al.*[23] reported that proflavine exerts an action similar to that of actinomycin D and inhibits both enzymatic reactions leading to RNA and DNA synthesis. Furthermore, they reported that in the case of proflavine the DNA synthesis is more sensitive than RNA synthesis. SOFFER AND GROS[24] have studied the effect of proflavine and dinitrophenol (DNP) on the synthesis of RNA in *E. coli* and concluded that proflavine is more specific with respect to its inhibition of DNA transcription *in vitro* and effective in degrading the rapidly-labelled RNA *in vivo.* The residual RNA in the proflavine- or DNP-treated cells has lost a significant proportion of its capacity to stimulate amino acid incorporation *in vitro* into protein. However, the protein synthesising machinery remains intact after treatment with DNP, and the inhibition of growth caused by this drug is reversed almost immediately after removal of DNP.

GROS *et al.*[12] observed that if the duration of the radioactive pulse prior to DNP of proflavine treatment in *E. coli* is increased, the percentage of rapidly-labelled RNA fraction decreases and they suggested that the ribosomal RNA and transfer RNA are conserved in DNP- or proflavine-treated cells. In agreement wtth these findings it was observed that when *R. rubrum* cells were treated with proflavine for 5 min, before the addition of [^{3}H]uracil, they lost their ability to incorporate any radioactive uracil into RNA species.The RNA from cells treated with proflavine for 1 min in the presence of [^{3}H]uracil, however, have lost a significant proportion of its rapidly-labelled RNA indicating that RNA synthesis is blocked. Rapidly-labelled RNA synthesised in the absence of proflavine may represent partly a messenger RNA, the only known biologically active RNA *in vitro*[19,20]. The effect of proflavine on *R. rubrum* could be to degrade the rapidly-labelled RNA species and to stop the transcription by complexing with the DNA. There also appears a slow loss of ribosomes from the cells treated with proflavine. COST AND GRAY[18] have observed similar effects of proflavine on DNA synthesis in *Rhodopseudomonas spheroides*. YAMASHITA AND KAMEN[1,2] observed a rapid decrease in the incorporation of [^{3}H]uracil into RNA species after the addition of proflavine to a culture of *R. rubrum* cells.

It is evident from the results presented here that a rapidly-labelled RNA fraction isolated from *R. rubrum* exists and can be fractionated using a PLK column. This RNA species is shown to be degraded by treating the culture with proflavine. Work is in progress to characterise further the rapidly-labelled RNA fraction in the anaerobically grown cells and also to examine and compare its properties with the rapidly-labelled RNA fraction in the aerobically grown cells.

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CHROM. 5338

TEST DE ROUTINE POUR LA DÉTERMINATION DES INSECTICIDES ORGANO-PHOSPHORÉS PAR LA CHROMATOGRAPHIE EN COUCHE MINCE

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SUMMARY

Routine method for the determination of organophosphorus insecticides by thin-layer chromatography

A procedure is described for the identification of organophosphorus insecticides by thin-layer chromatography using two types of adsorbent, *viz.* silica gel and polyamide, six solvent systems, and three main visualisation procedures, *viz.* UV absorption and the use of nitrobenzylpyridine or palladium chloride as chromogenic sprays. This method allows the separation and identification of an organophosphorus insecticide using at most four consecutive chromatograms.

INTRODUCTION

Les insecticides organo-phosphorés sont employés de plus en plus fréquemment non seulement en phytopharmacie (insecticides systémiques ou non) ou dans l'hygiène domestique (bombes insecticides) mais aussi dans la thérapeutique de certaines affections parasitaires animales (hypodermoses, verminoses, etc.). La multiplicité de leurs applications et leur haute toxicité sont à l'origine d'accidents de plus en plus fréquents aussi bien parmi les animaux domestiques que parmi la faune sauvage. Le diagnostic de ces intoxications nécessite la détection et l'identification de ces composés. La chromatographie en couche mince, peu coûteuse, sensible et d'une technique aisée, nous paraît être la méthode la mieux adaptée aux examens de routine. L'utilité de cette technique a été démontrée par divers auteurs[1,2]. Il est toutefois admis qu'aucun système ne permet de séparer valablement et en une seule opération tous les insecticides organo-phosphorés[3]. En combinant divers adsorbants, divers systèmes éluants et divers procédés de révélation, nous nous sommes efforcés d'établir une clef de détermination portant sur 25 organo-phosphorés dont la diversité tient aussi bien à leur structure chimique qu'à leur modalité d'emploi.

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MATÉRIELS ET MÉTHODES

Standards

Les standards de référence sont repris au Tableau I où ils sont groupés en fonction de leur structure chimique. Ils sont mis en solution dans le benzène à la concentration de 1 mg/ml.

TABLEAU I

STANDARDS DE RÉFÉRENCE

Nom chimique	*Formule chimique*	*Nom commercial*
Phosphates		
TEPP	tétraéthyl pyrophosphate	Bladen, Vapotone
Dibrom	O,O-diméthyl-O-1,2-dibromo-2,2-dichloroéthyl phosphate	Naled
Dichlorvos	O,O-diméthyl-O-2,2-dichlorovinyl phosphate	DDVP, Vapona, Atgaard
Phosdrin	O,O-diméthyl-O-2-carbométhoxy-1-méthylvinyl phosphate	Mevinphos
Phosphonates		
Trichlorfon	O,O-diméthyl-2,2,2-trichloro-1-hydroxyéthyl phosphonate	Neguvon, Bayer L13/57, Dipterex, Tugon, Dylox
Phosphoramidates		
Ruélène	O-4-*tert.*-butyl-2-chlorophényl-O-méthyl méthylphosphoramidate	Narlene
Phosphorothionates		
Ronnel	O,O-diméthyl-O-2,4,5-trichlorophényl phosphorothioate	Trolene, Korlane, Dow ET 57, Ectoral, Fenchlorfos, ET 14,
Méthyl parathion	O,O-diméthyl-O-*p*-nitrophényl phosphorothioate	Metacide, Nitrox, Dalf, E 605
Parathion	O,O-diéthyl-O-*p*-nitrophényl phosphorothioate	Thiophos, E 605
Baytex	O,O-diméthyl-O-4-(méthylthio)-3-méthylphényl phosphorothioate	Lebaycid, Fenthion, Tiguvon, Mercaptophos, Figuron
Diazinon	O,O-diéthyl-O-(2-isopropyl-4-méthyl-6-pyrimidinyl) phosphorothioate	Basudine, G 24480
Co-Ral	O,O-diéthyl-O-(3-chloro-4-méthyl-2-oxo-2H-1-benzopyran-7-yl) phosphorothioate	Coumaphos, Resitol Asuntol, Muscatox, Bayer 21/199

TABLEAU 1 (*suite*)

Nom chimique	*Formule chimique*	*Nom commercial*
Phosphorothiolates	$(RO)_2P(=O)SR'$	
Méta-Systox R	O,O-diméthyl-S-[2-(éthylsulfinyl)éthyl] phosphorothioate	Methyldemeton, Metaisosystox
Systox — mélange de		Demeton O et S, Bayer 1059
isomère thiol	O,O-diéthyl-S-[2-(éthylthio)éthyl] phosphorothioate	
isomère thiono	O,O-diéthyl-O-[2-(éthylthio)éthyl] phosphorothioate	
Phosphorodithioates	$(RO)_2P(=S)SR'$	
Méthyl Trithion	O,O-diméthyl-S-(*p*-chlorophénylthiométhyl) phosphorodithioate	
Trithion	O,O-diéthyl-S-(*p*-chlorophénylthiométhyl) phosphorodithioate	Carbophenothion, R 1303
Thiométon	O,O-diméthyl-S-(éthylthioéthyl) phosphorodithioate	Ekatine
Di-Syston	O,O-diéthyl-S-2-(éthylthio)éthyl phosphorodithioate	Disulfoton, Thiodemeton, Dithiosystox, Bayer 19639
Thimet	O,O-diéthyl-S-(éthylthiométhyl) phosphorodithioate	Phorate
Éthion	O,O,O′,O′-tétraéthyl-S,S′-méthylène biphosphorodithioate	Niagara 1240
Malathion	O,O-diméthyl-S-(1,2-dicarbéthoxyéthyl) phosphorodithioate	
Guthion	O,O-diméthyl-S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl-méthyl] phosphorodithioate	Methyl gusathion, Azinphos méthyl
Éthyl Guthion	O,O-diéthyl-S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl-méthyl] phosphorodithioate	Ethyl gusathion, Azinphos ethyl
Diméthoate	O,O-diméthyl-S-(N-méthylcarbamoylméthyl) phosphorodithioate	Rogor, Cygon
Delnav	2,3-*p*-dioxanedithiol-bis(O,O-diéthyl) phosphorodithioate	Dioxathon, Kavadel, Hercules, AC-528

Tous proviennent de la firme Polyscience Corporation, excepté les suivants: Baytex, Systox et Éthyl Guthion, produits techniques fournis par la firme Bayer; Diazinon, produit technique fourni par Geigy; Dichlorvos, produit commercial vendu sous le nom de Atgaard par Shell; Thiométon, produit commercial vendu sous le nom de Ekatine par Defoer.

Adsorbants

Dans toutes nos expériences, nous avons utilisé les supports suivants: (a) plaques de gel de silice avec agent fluorescent de Macherey, Nagel & Cie. "MN Polygram Sil NHR UV_{254}" et (b) plaques de polyamide avec agent fluorescent de Macherey, Nagel & Cie "MN Polygram Polyamid-6 UV_{254}".

Systèmes d'élution

Parmi la multitude de systèmes éluants proposés par la littérature, nous avons retenus les suivants:

(a) sur gel de silice: (I) xylène[1]; (II) hexane–acétone (80:20)[2]; (III) acétate d'éthyle–dichlorométhane (50:50)[4].

(b) sur polyamide: (IV) hexane–acétone (80:20); (V) hexane–acide acétique (95:5)[5]; (VI) méthanol–eau (50:50)[5]; (VII) éthanol–eau–ammoniaque (50:40:20)[6].

Les plaques sont utilisées sans activation préalable. 10 μl de l'échantillon (ce qui correspond à 10 μg du standard de référence) sont déposés à 2 cm du bord inférieur. La chromatographie ascendante est effectuée dans des cuves préalablement saturées. Lorsque la distance de migration atteint 15 cm, le chromatogramme est retiré, sèché et ensuite révélé.

TABLEAU II

VALEURS R_F DES 25 STANDARDS ÉTUDIÉS

Dans la détermination de ces valeurs R_F, la nitrobenzylpyridine a été employée comme révélateur général sauf pour les composés marqués d'une astérisque qui ont été révélés par le chlorure de palladium sur les plaques de polyamide.

	Systèmes éluants (temps de migration)						
	I (*30 min*)	*II* (*20 min*)	*III* (*30 min*)	*IV* (*30 min*)	*V* (*40 min*)	*VI* (*2 h*)	*VII* (*3 h*)
TEPP	0	11	18	7/71	13/30	94	85
Dibrom	2	20	55	66	41	49	84
Dichlorvos	0	18	50	87	48	77	77
Phosdrin	0	10/6	39/30	57/65	17/25	90	80
Trichlorfon	0	3	19	11	9	80	78
Ruélène	4	16/20	33/52	56	50	35	61
Ronnel	64	52	74	83	80	4	15
Méthyl parathion	33	26	72	65	39	17	29
Parathion	39	37	76	77	61	9	21
Baytex	44	39	73	78	64	6	23/18
Diazinon*	3	41	65	87	81	35	54
Co-Ral	8	25	73	66	30	6	80
Méta-Systox R	0	14	44	15	2	95	81
Systox*	0/24	18/48	54/74	78/88	58/82	72/25	75/42
Méthyl Trithion	57	40	75	73	65	4	12
Trithion	64	52	75	80	78	3	6
Thiométon	39	45	72	82	72	19	36
Di-Syston*	38	54	74	88	80	12	26
Thimet*	49	54	76	88	80	12	28
Éthion	44	44	78	86	82	4	13
Malathion	4	27	70	76	59	28	55
Guthion	4	16	65	54	19	26	40
Éthyl Guthion	5	24	66	66	33	16	33
Diméthoate	0	4	25	26	2	74	78
Delnav	10	27	74	79	64	3	15

Techniques de révélation

(1) Absorption dans l'UV à 254 mμ: Les plaques sont examinées après développement au moyen d'une lampe Camag type TL 900 ou Desaga UVIS. Les spots apparaissent en violet foncé sur fond vert.

(2) Réactif à la 4-*p*-nitrobenzylpyridine: La technique décrite par RAGAB[7] nous a donné entière satisfaction. On obtient des spots bleu-violet sur fond blanc. Le Diazinon apparaît en rouge.

(3) Réactif au chlorure de palladium: Il s'agit d'un révélateur classique en chromatographie sur couche mince décrit par BÄUMLER ET RIPPSTEIN[8]. Les thiophosphorés se colorent en jaune ou brun.

(4) Réactif à l'iodoplatinate: Ce réactif a été appliqué à la révélation des organophosphorés par GUTH[1]. Les spots apparaissent en jaune sur fond brunâtre. Le Diazinon se colore en bleu.

(5) Réactif à la potasse alcoolique: Après développement, une solution d'hydroxyde de potassium dans l'alcool est pulvérisée sur les chromatogrammes. Ceux-ci sont ensuite placés à l'étuve à 100° pendant 10 min. Ce réactif qui met en évidence les groupements *p*-nitrophénols colore en jaune sur fond blanc, le Parathion et le Méthyl Parathion.

(6) Test d'inhibition des cholinestérases: Nous avons adopté la méthode de SCHUTZMANN ET BARTHEL[9]. Les organo-phosphorés apparaissent en blanc sur fond mauve.

(7) Test au nitrate d'argent: Cette technique est employée couramment pour la révélation des insecticides chlorés et a été décrite par KOVACS[10]. Les organo-phosphorés qui contiennent des groupes chlorés se manifestent par des spots brun-noir sur fond blanc.

RÉSULTATS

Les R_F des 25 standards étudiés dans les sept couples adsorbants–éluants retenus sont donnés au Tableau II. Ce sont des chiffres moyens obtenus à partir d'au moins trois déterminations. Ce tableau reprend également les temps de migration pour chacun des systèmes.

Le Tableau III regroupe toutes les données concernant les diverses techniques de révélation.

DISCUSSION

En tenant compte des résultats groupés dans les Tableaux II et III et en les combinant d'une manière rationnelle, nous avons pu établir un schéma dichotomique qui permet de déterminer aisément un organo-phosphoré inconnu pourvu qu'il fasse partie des 25 composés étudiés (voir Tableau IV).

Le premier chromatogramme (1) est effectué sur gel de silice avec comme éluant le xylène. Ce couple adsorbant–éluant a été choisi parce qu'il sépare les organo-phosphorés étudiés en trois groupes nettement distincts:

(a) Un premier groupe à migration nulle ou très faible (R_F entre 0 et 10), comprend tous les phosphates, les phosphonates, les phosphoroamidates, les phosphorothiolates, auxquels s'ajoutent quelques phosphorothionates et phosphorodithioates dont le radical R a une structure chimique complexe.

TABLEAU III

RESULTATS DES DIVERSES TECHNIQUES DE RÉVÉLATION

Les symboles suivants ont été employés dans ce tableau: + = réaction positive, — = réaction negative, B = spot bleu, R = spot rouge, J = spot jaune et Br = spot brun.

	Révélateurs						
	1	*2*	*3*	*4*	*5*	*6*	*7*
TEPP	—	B	—	—	—	+	—
Dibrom	—	B	—	—	—	+	+
Dichlorvos	—	B	—	—	—	+	+
Phosdrin	—	B	—	—	—	+	—
Trichlorfon	—	B	—	—	—	—	+
Ruélène	+	B	—	—	—	+	+
Ronnel	—	B	Br[a]	—	—	+	+
Méthyl parathion	+	B	Br[a]	—	+	+	—
Parathion	+	B	Br[a]	—	+	+	—
Baytex	+	B	J-Br	J	—	+	—
Diazinon	+	R[b]	J	B	—	+	—
Co-Ral	+	B	Br	—	—	+	±
Méta-Systox R	—	B	J	J	—	+	—
Systox	—	B[b]	J	J	—	+	—
Méthyl trithion	+	B	J	J	—	+	+
Trithion	+	B	J	J	—	+	+
Thiométon	±	B	J	J	—	+	+
Di-Syston	—	B[b]	J	J	—	+	—
Thimet	±	B[b]	J	J	—	+	—
Éthion	—	B	J	J	—	+	—
Malathion	—	B	J	J ±	—	+	—
Guthion	+	B	J	J ±	—	+	—
Éthyl guthion	+	B	J	J ±	—	+	—
Diméthoate	—	B	J	J	—	+	—
Delnav	—	B	Br	J	—	+	—

[a] Ces spots apparaissent 20 min après pulvérisation sur les plaques de gel de silice, un jour après, sur les plaques de polyamide.
[b] Le réactif à la nitrobenzylpyridine ne révèle pas ces composés sur les plaques de polyamide.

(b) Un second groupe à migration moyenne (R_F entre 25 et 50) comprend la majorité des phosphorothionates et phosphorodithioates.

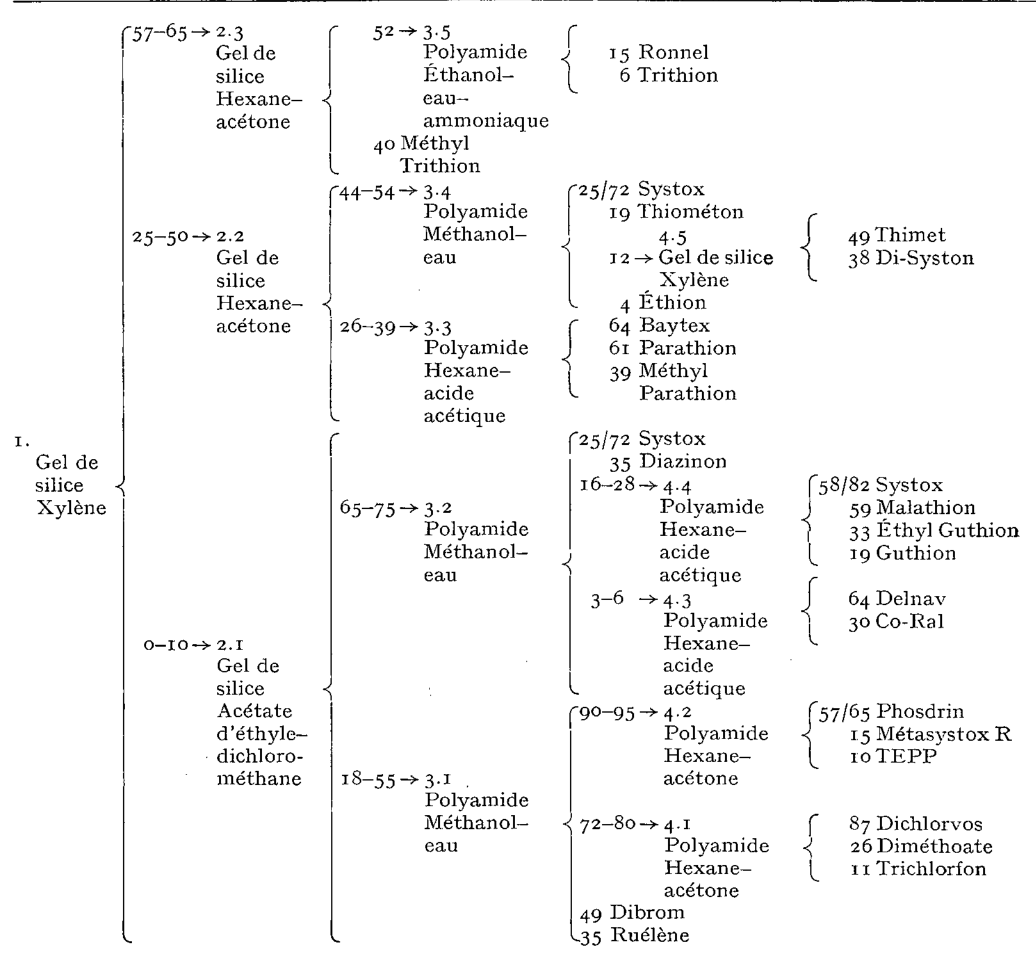
(c) Un petit groupe à forte migration (R_F entre 57 et 65) comprend le reste des phosphorothionates et -dithioates. Ces composés ont tous un cycle chlorophényl dans leur radical R.

Les standards de référence appliqués en même temps que les échantillons à déterminer sont ceux dont les R_F délimitent ces trois groupes: à savoir le Delnav (R_F 10), le Méthyl Parathion (R_F 33) que nous substituons au Systox dont le spot supérieur (R_F 24) n'est pas toujours bien visible, le Thimet (R_F 49) et le Méthyl Trithion (R_F 57). Nous y adjoignons le Parathion (R_F 39) en raison de la fréquence des intoxications par ce produit.

Pour ce premier chromatogramme nous préconisons comme révélateur la nitrobenzylpyridine qui colore tous les organo-phosphorés. En cas de doute, la présence

TABLEAU IV

SCHÉMA DICHOTOMIQUE



d'un organo-phosphoré dans un échantillon inconnu sera mise en évidence par le test d'inhibition des cholinestérases. Il est à noter que les phosphorés du troisième groupe se distinguent de ceux du deuxième par le fait qu'ils réagissent avec le nitrate d'argent.

Dès ce premier chromatogramme, la détermination de l'échantillon inconnu est orientée clairement: l'organo-phosphoré est classé dans le groupe a, b ou c.

La plaque 2.1 reprend les organo-phosphorés à migration faible (groupe a). Le système éluant acétate d'éthyle-dichlorométhane (50:50), beaucoup plus polaire que le xylène, donne un étalement des spots de R_F 18 à R_F 74 avec un hiatus entre les R_F 55 et 65, ce qui permet la scission de l'ensemble en deux groupes. Les standards de référence utilisés sont le Trichlorfon (R_F 11) et le Dibrom (R_F 55) qui délimitent le premier groupe, et le Diazinon (R_F 65) qui marque la limite inférieure du second groupe.

Le révélateur employé est la nitrobenzylpyridine. Notons dès à présent la coloration rouge que ce réactif donne avec le Diazinon.

La plaque 2.2 reprend tous les organo-phosphorés qui migrent moyennement dans le xylène (groupe b). Le support est le gel de silice et le système éluant est l'hexane–acétone (80:20). Les standards appliqués sont le Méthyl Parathion (R_F 26) et le Baytex (R_F 39) qui délimitent un premier groupe et l'Ethion (R_F 44) et le Thimet (R_F 54) qui en délimitent un second.

Cette plaque est révélée au chlorure de palladium qui colore les organo-phosphorés du premier groupe en brun et ceux du second en jaune. En outre, les organo-phosphorés du premier groupe absorbent fortement l'UV, ceux du second peu ou pas du tout.

La plaque 2.3 reprend les organo-phosphorés qui migrent fortement dans le xylène (groupe c). La séparation est effectuée sur gel de silice; le système éluant est l'hexane–acétone (80:20). Les standards appliqués sont le Méthyl Trithion, le Trithion et le Ronnel. Les révélateurs sont l'UV, le chlorure de palladium et l'iodoplatinate. Cette plaque permet de caractériser le Méthyl Trithion (R_F 40). Le Trithion et le Ronnel (R_F 52) ne sont pas séparés mais se distinguent par leur colorations: le Trithion absorbe dans l'UV, il est coloré en jaune par l'iodoplatinate et par le chlorure de palladium; le Ronnel n'absorbe pas dans l'UV, il n'est pas coloré par l'iodoplatinate et il apparaît en brun avec le chlorure de palladium. L'identité de l'échantillon peut être confirmée sur plaque de polyamide dévéloppée dans un mélange éthanol–eau–ammoniaque (50:40:20) et révélée par la nitrobenzylpyridine: le Ronnel (R_F 15) est nettement séparé du Trithion (R_F 6).

La plaque 3.1 reprend sur polyamide les organo-phosphorés à faible migration de 2.1. Le système éluant est le mélange méthanol–eau (50:50). Les standards sont le Ruélène (R_F 35, le Dibrom (R_F 49), le Trichlorfon (R_F 80) et le Phosdrin (R_F 90). Les révélateurs utilisés sont l'UV et la nitrobenzylpyridine. Le Ruélène est nettement séparé du Dibrom par son R_F, il s'en distingue en outre par son absorption dans l'UV. Les autres organo-phosphorés se séparent en deux groupes: le premier à R_F compris entre 72 et 80 et le second à R_F compris entre 90 et 95.

Si l'échantillon inconnu a un R_F compris entre 72 et 80, il est repris sur la plaque 4.1 en même temps que les standards, à savoir le Trichlorfon, le Diméthoate et le Dichlorvos. L'adsorbant est le polyamide, l'éluant le mélange hexane–acétone (80:20). Les révélateurs sont le chlorure de palladium et la nitrobenzylpyridine. Ces trois organo-phosphorés sont nettement séparés: le Trichlorfon a un R_F de 11 et n'est pas coloré par le chlorure de palladium, le Diméthoate a un R_F de 26 et est coloré en jaune par le chlorure de palladium, le Dichlorvos a un R_F de 87, ce qui permet d'identifier l'échantillon inconnu à un de ces trois composés.

Si le R_F de l'échantillon est compris entre 90 et 95, il est repris sur la plaque 4.2 en même temps que le Phosdrin, le Métasystox R et le TEPP qui constituent ce groupe. L'adsorbant, l'éluant et les révélateurs sont les mêmes que pour la plaque 4.1. Ce chromatogramme permet de séparer nettement les trois organo-phosphorés cités: le TEPP a un R_F de 10 et n'est pas coloré par le chlorure de palladium, le Métasystox R a un R_F de 15 et est coloré en jaune par le chlorure de palladium, le Phosdrin présente deux spots (R_F 57 et 65).

La plaque 3.2 reprend les organo-phosphorés qui avaient un R_F élevé en 2.1. (R_F 65–75). Le support et l'éluant sont les mêmes qu'en 3.1. Les standards appliqués

sont le Co-Ral (R_F 6), l'Éthyl Guthion (R_F 16), le Malathion (R_F 28), le Diazinon (R_F 35) et le Systox (R_F 25 et 72). Les spots sont révélés par l'UV et le chlorure de palladium.

Ce chromatogramme permet d'isoler le Diazinon (R_F 35, UV +) et le Systox (R_F 25 et 72, UV —). En outre il sépare les organo-phosphorés restants en deux groupes l'un à R_F compris entre 3 et 6 et l'autre à R_F compris entre 16 et 28.

Si l'organo-phosphoré a un R_F compris entre 3 et 6, il est repris sur la plaque 4.3 en même temps que le Co-Ral et le Delnav. Le support est le polyamide, l'éluant l'hexane–acide acétique (95:5). Les spots sont révélés par l'UV et la nitrobenzylpyridine. Le Co-Ral (R_F 30) à fluorescence bleue dans l'UV se distingue nettement du Delnav (R_F 64).

Si l'organo-phosphoré inconnu a un R_F compris entre 16 et 28, il est repris en 4.4. Le support et le système éluant sont les mêmes qu'en 4.3. Les standards de rérérence sont le Guthion, l'Éthyl Guthion, le Malathion et le Systox (dont un des constituants avait un R_F de 25). Les spots sont révélés par l'UV et le chlorure de palladium. Ces quatre composés ont des R_F nettement distincts, ce qui permet de les caractériser aisément.

La plaque 3.3 reprend les organo-phosphorés à faible migration en 2.2 (R_F 26–39). La séparation est effectuée sur polyamide avec le mélange hexane–acide acétique (95:5) comme éluant. La plaque est révélée par la nitrobenzylpyridine et la potasse alcoolique. Ce chromatogramme permet de caractériser le Méthyl Parathion (R_F 39), le Parathion (R_F 61), tous deux colorés par la potasse alcoolique, et le Baytex (R_F 64), non coloré par ce réactif.

La plaque 3.4 reprend les organo-phosphorés dont les R_F étaient compris entre 44 et 54 en 2.2. La séparation s'effectue sur polyamide avec le mélange méthanol–eau (50:50) comme éluant et le chlorure de palladium comme révélateur. Le chromatogramme permet de caractériser l'Éthion (R_F 4), le Thiométon (R_F 19) et le Systox (R_F 25/72). Le Thimet et le Di-Syston (R_F 12) doivent être repris sur 4.5 qui seul permet de les séparer.

CONCLUSION

Ce schéma dichotomique permet avec un maximum de quatre chromatogrammes successifs d'identifier un insecticide organo-phosphoré pourvu qu'il appartienne à la liste des 25 composés étudiés.

Nous pensons que ce schéma peut être appliqué à la séparation et à l'identification d'autres organo-phosphorés par l'adjonction d'autres standards qui, à notre avis, devraient s'y intégrer aisément.

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RÉSUMÉ

Les auteurs ont mis au point un procédé méthodique pour l'identification des insecticides organo-phosphorés par la chromatographie en couche mince. L'étude

porte sur un ensemble de 25 composés. La méthode basée sur l'utilisation de deux types d'adsorbant (gel de silice et polyamide), de six systèmes éluants et de trois procédés principaux de révélation (nitrobenzylpyridine, chlorure de palladium et absorption dans l'UV à 254 mμ) permet la séparation et l'identification d'un organophosphoré avec un maximum de quatre chromatogrammes.

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DÜNNSCHICHTCHROMATOGRAPHISCH-ENZYMATISCHER NACHWEIS VON CARBAMATEN

II. NACHWEIS HERBIZIDER CARBAMATE MIT RINDERLEBER-ESTERASE

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SUMMARY

Thin-layer chromatographic–enzymatic identification of carbamates. II. Identification of carbamate herbicides with bovine liver esterase

Carbamate-type herbicides can be detected by means of a thin-layer chromatographic–enzymatic inhibition technique with bovine liver esterase. The minimum quantities detected were in the range of 3–100 μg for the N-phenylcarbamates and 5–30 μg for the thiolcarbamates, depending on pretreatment. Under normal assay conditions and after bromine treatment N-phenylcarbamates show a more intense colour. No activation of the esterase takes place, however, and it is supposed that this phenomenon is due to a reaction between enzymatic degradation products and Fast Blue Salt B. After UV irradiation these substances are real inhibitors. The thiolcarbamates studied show inhibitory activity against bovine liver esterase. After UV irradiation only Diallate and Triallate are stronger inhibitors, while Eptam, Vernolate, and Pebulate remain unaffected. Bromine treatment has only a slight effect. Kinetic studies with Eptam and Vernolate show that thiolcarbamates are degraded to other antiesterase substances and it is supposed that the degradation products are the sulphoxides and/or sulphones.

EINLEITUNG

Über die Wirkung der herbiziden Carbamate ist noch immer relativ wenig bekannt. Die ersten Arbeiten liessen vermuten, dass es sich bei dieser Wirkstoffklasse um Mitosegifte handelt, da es nach Propham-Behandlung zu mitotischen Aberrationen in bestimmten Wurzel- und Schösslingszellen von Avena und Allium kommt[1]. Als Ursache dieser Veränderungen wird eine Beeinträchtigung der Wirkung der Spindelfasern und damit das Ausbleiben einer Trennung der Chromosomen in der Metaphase angesehen[1]. Dieser Eingriff in den Spindelmechanismus dürfte nach IVENS UND BLACKMAN[2] auf einer Wechselwirkung der Carbamate mit Spindelproteinen und einer sich daraus ergebenden intramolekularen Präzipitation und Faltung der Proteinketten unter Spindeldesintegration beruhen. Da andererseits Äthyl-phenylcarbamat

das Wachstum von Gerste schon in Konzentrationen hemmt, die noch keinen Einfluss auf die Spindel haben, müssen daneben noch andere Hemmmechanismen vorliegen[2]. Ein erhöhter Gehalt an reduzierenden Zuckern und Sucrose deutet auf eine Blockierung des Kohlenhydratabbaus hin[3]. Ausserdem findet man eine deutliche Reduzierung der Atmung[3]. Zu ähnlichen Ergebnissen kamen auch SWANSON *et al.*[4], die feststellten, dass Chlorpropham bei Baumwollwurzeln die Atmung um 50 % hemmt, ein Effekt, der auf einer Hemmung der Glycolyse durch Eingreifen in die Phosphorylierung von Hexosen beruhen könnte[4]. Auch eine Verstärkung der Phosphatase-Aktivität mit einem gleichzeitigen Anstieg von Phosphat und freien Zuckern konnte bei Reis-Sämlingen nach Propham-Behandlung festgestellt werden[5].

Den bisher geschilderten Beobachtungen über die Wirkung herbizider Carbamate auf Stoffwechselvorgänge bei Pflanzen dürfte zweifelsohne eine gewisse Bedeutung zukommen, doch scheint ihr Eingriff in den Photosyntheseprozess für ihre Wirkung als Herbizide viel tiefgreifender zu sein. Von den Alkyl-N-chlorphenylcarbamaten ist seit langem bekannt, dass sie die Hill-Reaktion hemmen[6,7]. Allerdings weisen auch diese Autoren[6,7] darauf hin, dass der Wirkungsmechanismus der Carbamat-Herbizide nicht so einfach ist und nicht ausschliesslich auf einer Hemmung der Photosynthese beruht, sondern dass die Substanzen in zahlreiche Stoffwechselprozesse der Zelle eingreifen.

Während über die Wirkung der herbiziden Carbamate in Pflanzen wenigstens schon einige Hinweise vorliegen, ist über ihre Beeinflussung tierischer Enzyme bisher noch nichts bekannt. Vorliegende Arbeit berichtet erste Ergebnisse über die Wirkung von herbiziden Carbamaten auf Rinderleber-Esterase auf dünnschichtchromatographischer Basis.

MATERIAL UND METHODEN

Reagenzien

Alle verwendeten Lösungsmittel und Chemikalien waren analysenrein und stammten von der Firma Merck, Darmstadt. Zur Plattenbeschichtung wurde Kieselgel G nach Stahl mit *ca.* 13 % $CaSO_4$ und einer mittleren Korngrösse von 10–40 μ von der gleichen Firma genommen.

Enzym- und Substratlösung

Die Herstellung der Enzympräparation und Substratlösung erfolgte in Anlehnung an ACKERMANN[8], doch wurde, wie schon früher beschrieben[9], das Homogenisieren der Leber und die Verdünnung der Enzympräparation mit Phosphatpuffer pH 7.0 durchgeführt. Für das Besprühen der Platten wird eine etwa 1:60 verdünnte Enzymlösung genommen.

Wirkstofflösungen und Dünnschichtchromatographie

Die untersuchten Wirkstoffe (Tabelle I) wurden in Konzentrationen von 10 mg/ml in Aceton gelöst, auf handgegossene Kieselgel G-Platten[9] aufgetragen und in verschiedenen Laufmittelsystemen (Tabelle II) chromatographiert.

Durchführung des enzymatischen Hemmtestes

Die Platten wurden nach dem Entwickeln sofort oder nach halbstündiger Bestrahlung mit UV-Licht der Wellenlängen 254/366 nm einer Desaga Uvis-Lampe bei

TABELLE I

NAME UND STRUKTUR DER UNTERSUCHTEN HERBIZIDEN CARBAMATE

Trivial-Name	*Chemische Bezeichnung*	*Strukturformel*
CEPC	N-(3-Chlorphenyl)-2-chloräthylcarbamat	(Cl)-Phenyl-NH–C(=O)–O–CH_2–CH_2Cl
Propham (IPC)	N-Phenyl-isopropylcarbamat	Phenyl-NH–C(=O)–O–C(H)(CH_3)CH_3
Chlorpropham (CIPC)	N-(3-Chlorphenyl)-isopropylcarbamat	(Cl)-Phenyl-NH–C(=O)–O–C(H)(CH_3)CH_3
CPPC	N-(3-Chlorphenyl)-1-methyl-2-chloräthylcarbamat	(Cl)-Phenyl-NH–C(=O)–O–CH(CH_3)–CH_2Cl
Chlorbufam	N-(3-Chlorphenyl)-1-methylpropin-(2)-ylcarbamat	(Cl)-Phenyl-NH–C(=O)–O–C(H)(C≡C–H)CH_3
Barban	N-(3-Chlorphenyl)-4-chlorbutin-(2)-ylcarbamat	(Cl)-Phenyl-NH–C(=O)–O–CH_2–C≡C–CH_2Cl
Phenmedipham	N-(3′-Methylphenyl)-3-methoxy-carbamylaminophenylcarbamat	CH_3O–C(=O)–NH-Phenyl-O–C(=O)–N(H)-Phenyl-CH_3
Eptam (EPTC)	S-Äthyl-N,N-dipropylthiolcarbamat	C_2H_5–S–C(=O)–N(CH_2–CH_2–CH_3)$_2$
Vernolate	S-Propyl-N,N-dipropylthiolcarbamat	C_3H_7–S–C(=O)–N(CH_2–CH_2–CH_3)$_2$
Pebulate (PEBC, Tillam)	S-Propyl-N,N-butyläthylthiolcarbamat	C_3H_7–S–C(=O)–N(CH_2–CH_3)(CH_2–CH_2–CH_2–CH_3)
Diallat	S-2,3-Dichlorallyl-N,N-diisopropylthiolcarbamat	((CH_3)$_2$CH)$_2$N–C(=O)–S–CH_2–C(Cl)=CHCl
Triallat	S-2,3,3-Trichlorallyl-N,N-diisopropylthiolcarbamat	((CH_3)$_2$CH)$_2$N–C(=O)–S–CH_2–C(Cl)=C(Cl)Cl

TABELLE II

VERWENDETE LAUFMITTELSYSTEME (v/v) ZUR TRENNUNG DER UNTERSUCHTEN HERBIZIDEN CARBAMATE

Nr.	*Laufmittelsysteme*
1	Cyclohexan
2	Äthylmethylketon
3	Benzol–Aceton (95:5)
4	Benzol–Aceton (66:34)
5	Benzol–Dichlormethan (30:120)
6	Benzol–Chloroform (30:120)
7	Chloroform–Acetonitril (20:10)
8	Chloroform–Wasser (untere Phase) (90:50)
9	Cyclohexan–Dioxan (70:30)
10	Cyclohexan–Äthyläther (10:90)
11	Cyclohexan–Aceton (80:20)
12	Cyclohexan–Aceton–Toluol (50:10:10)
13	Cyclohexan–Aceton–Toluol–Äthylacetat (20:20:10:10)

einem Abstand Strahler–Platte von 15 cm zunächst leicht mit Puffer, anschliessend mit Enzymlösung besprüht und nach halbstündiger Inkubation bei 37° in der von ACKERMANN[8] beschriebenen Weise weiterbehandelt. Die Brombehandlung der Platten erfolgt in der an anderer Stelle[10] beschriebenen Weise.

ERGEBNISSE UND DISKUSSION

Unter den hier angewandten Bedingungen reagieren grundsätzlich alle untersuchten herbiziden Carbamate mit der Rinderleber-Esterase. Die Nachweisempfindlichkeit liegt für alle Wirkstoffe im Mikrogrammbereich.

Die N-Phenylcarbamate zeigen in ihrer Wirkung auf die Rinderleber-Esterase weitgehende Übereinstimmung (Tabelle III). Für alle Verbindungen liegt die Nachweisgrenze bei 10 μg — lediglich Propham mit 40 μg und Phenmedipham mit 100 μg machen in diesem Zusammenhang eine Ausnahme. Die Empfindlichkeit ist damit nur wenig schlechter als bei den Chlorkohlenwasserstoffen[9]. Betrachtet man jedoch zum Vergleich die Nachweisgrenzen für die insektiziden Carbamate, so liegt diese mit Rinderleber-Esterase um etwa drei Zehnerpotenzen günstiger[10]. Diese erheblichen Unterschiede in der Nachweisempfindlichkeit zwischen herbiziden und insektiziden Carbamaten sind jedoch angesichts der Tatsache, dass letztere über eine Hemmung der Acetylcholinesterase wirken sollen, mehr als verständlich.

Während die insektiziden Carbamate nach UV-Bestrahlung stark an Antiesterase-Aktivität einbüssen[10], kommt es bei den N-Phenylcarbamaten zu einer leichten Aktivierung (Tabelle III). Damit verhalten sie sich ähnlich wie die Chlorkohlenwasserstoffe[9]. Brombehandlung dagegen führt mit einer Ausnahme zu einer leichten Verschlechterung der Nachweisempfindlichkeit (Tabelle III), eine Eigenschaft, die sich auch bei den insektiziden Carbamaten findet[10].

Die N-Phenylcarbamate nehmen beim Nachweis mit Rinderleber-Esterase eine gewisse Sonderstellung ein. Diese zeigt sich besonders nach Brombehandlung, aber in gewissem Umfange auch bei den unbehandelten Wirkstoffen. Nach Brombehandlung zeigen sich nicht die beim Nachweis durch Esterasehemmung üblichen weissen

TABELLE III

UNTERE NACHWEISGRENZEN VON SIEBEN N-PHENYLCARBAMATEN NACH VERSCHIEDENEN VORBEHANDLUNGEN INFOLGE BEEINFLUSSUNG DER RINDERLEBER-ESTERASE

Nachweisgrenze in μg; Laufmittelsystem Benzol–Aceton (95:5).
I = Farbintensivierung nach Ausfärbung der Platte; (I) = Farbintensivierung, die manchmal an die Stelle von Hemmflecken tritt, bzw. "Hofbildung".

Wirkstoff	*Ohne Vorbehandlung*	*Nach Brombehandlung*	*Nach UV-Bestrahlung*
CEPC	10 (I)	15 I	6
Propham	40	50 (I)	30
Chlorpropham	10 (I)	15 I	6
CPPC	10 (I)	15 (I)	6
Chlorbufam	10 (I)	15 I	6
Barban	10 (I)	10 I	3
Phenmedipham	100 (I)	(3)	6

Hemmflecke, sondern es ist in der Regel eine intensivere Ausfärbung der Flecke zu beobachten, die sich deutlich vom Untergrund abhebt. Von einer Aktivierung des Enzyms kann jedoch nicht die Rede sein, da die Herbizidflecke nicht von Anfang an durch eine schnellere Ausfärbung auffallen, sondern erst nach Ausfärbung der Platte permanente blaue Flecke auf rotviolettem Grund auftreten — ähnlich der Ausfärbung in der Lösungsmittelfront. Beim Nachweis ohne Vorbehandlung kommt es bei allen N-Phenylcarbamaten gelegentlich zu solchen Farbintensivierungen, die dann an die Stelle der Hemmflecke treten. Neben der gelegentlichen Farbintensivierung im Gesamtfleck findet man häufig eine Hofbildung; d.h. um die Hemmflecke bildet sich ein intensiv blaugefärbter Ring. Die Ursache für diese Farbintensivierung ist unbekannt. Da sie jedoch nur bei den N-Phenylcarbamaten auftritt, ist anzunehmen, dass diese Erscheinung für die Gruppe charakteristisch ist. Es ist zu vermuten, dass es zwischen Esterase und dem N-Phenylcarbamat zu einer Reaktion kommt, ähnlich jener zwischen Esterase und den insektiziden Carbamaten[11] und Phosphorsäureestern[12]. Während es jedoch bei den Insektiziden zu einer mehr oder weniger stabilen Acylierung des Enzyms kommt, dürfte diese bei den Herbiziden aufgrund des Phenylrestes nur sehr instabil sein und unter Bildung des entsprechenden Isocyanats und schliesslich des Anilins hydrolysiert werden. Letzteres könnte mit Echtblausalz B, einem Diazo-Reagenz für Phenole und kupplungsfähige Amine, unter Farbstoffbildung reagieren. Um die Richtigkeit dieser Hypothese zu prüfen, wurden die N-Phenylcarbamate in Konzentrationen von 10–100 μg auf Dünnschichtplatten aufgetragen, mit Bromwasser behandelt und anschliessend eine Stunde mit 1:30 verdünntem Enzym inkubiert. Nach dem Besprühen mit Echtblausalz B zeigen Barban ab 50 μg und CEPC ab 70 μg aufwärts schwache blaue Flecke, Phenmedipham hingegen ab 50 μg aufwärts bräunliche Flecke auf weissem Grund. Ohne Esterase sind dagegen keinerlei Flecke festzustellen. Entsprechendes gilt für eine Inkubation der Platten mit normalem Substrat ohne Enzymgabe. Ob das Substrat eine Rolle bei dieser Carbamat-Spaltung spielt und wenn ja welche, bleibt abzuwarten. Durch die Einwirkung von Brom könnte die enzymatische Bildung von Anilin-Derivaten in bisher unbekannter Weise erleichtert werden. Da jedoch bei den unbehandelten N-Phenylcarbamaten in vielen Fällen zumindest eine Hofbildung zu beobachten ist, darf an-

genommen werden, dass die Carbamate bei geringen Konzentrationen, wie sie am Rande der Flecke herrschen dürften, in geringem Umfange auch ohne vorherige Veränderung des Wirkstoffes durch Bromeinwirkung zu Anilin-Derivaten gespalten werden. Diese Derivate würden dann durch die Kupplung mit Echtblausalz zu einer Blaufärbung führen und dadurch die Hofbildung hervorrufen. Bei den höheren Konzentrationen im Zentrum der Flecke scheint es hingegen zu einer echten Hemmung des Enzyms zu kommen, da hier keine Farbausbildung auftritt. Eine Klärung der Frage, ob unter Einwirkung der Rinderleber-Esterase Anilin-Derivate entstehen, ist zwar von grosser Bedeutung, muss jedoch späteren Arbeiten vorbehalten bleiben, da es hier primär um einen Nachweis der Carbamat-Herbizide geht und erst in zweiter Linie toxikologische und Stoffwechsel-Fragen zur Debatte stehen.

Die untersuchten Thiolcarbamate reagieren wesentlich einheitlicher als die N-Phenylcarbamate. Vor allem handelt es sich bei ihnen um echte Esterasehemmer, deren Nachweisgrenze allerdings um den Faktor 3 höher liegt als bei den meisten N-Phenylcarbamaten (Tabelle IV). Nach UV-Bestrahlung gehen nur Diallat und Triallat in stärkere Antiesterase-Substanzen über, während alle übrigen Wirkstoffe keine Veränderung zeigen. Durch Brombehandlung scheinen die Thiolcarbamate eher eine leichte Verbesserung der Nachweisempfindlichkeit zu erfahren. Damit unterscheiden sie sich deutlich von den N-Phenylcarbamaten.

TABELLE IV

UNTERE NACHWEISGRENZEN VON FÜNF THIOLCARBAMATEN NACH VERSCHIEDENEN VORBEHANDLUNGEN INFOLGE HEMMUNG DER RINDERLEBER-ESTERASE

Nachweisgrenze in μg; Laufmittelsystem, Benzol–Aceton (95:5).

Wirkstoff	*Ohne Vorbehandlung*	*Nach Brombehandlung*	*Nach UV-Bestrahlung*
Eptam	30	20	30
Vernolate	30	20	30
Pebulate	20	20	20
Diallat	30	30	6
Triallat	30	20	5

Während sich die N-Phenylcarbamate durch die Farbintensivierung nach Brombehandlung und die Hofbildung bei den unbehandelten Wirkstoffen auszeichnen, zeigen die Thiolcarbamate eine andere interessante Eigenschaft. Schon bei der Ermittlung der unteren Nachweisgrenze in Benzol–Aceton (95:5) fiel auf, dass die Wirkstoffe nach einiger Zeit neben dem ursprünglichen einen weiteren Hemmfleck zeigten. Diese Erscheinung trat besonders auffällig bei der Bestimmung der hR_F-Werte in den verschiedenen Laufmitteln hervor. Schon nach wenigen Tagen mussten die Lösungen neu angesetzt werden, da zwei Flecke auftraten. Oft traten sogar schon am Tage der Neueinwaagen zwei Substanzflecke auf. Daraufhin wurden Eptam und Vernolate etwas eingehender untersucht, wobei die Beurteilung visuell nach der Stärke der Hemmflecke erfolgte. Die in Tabelle V wiedergegebene Kinetik des Auftretens eines zweiten Hemmfleckes unter verschiedenen Bedingungen zeigt, dass der Hemmfleck mit dem hohen hR_F-Wert, der dem Originalwirkstoff zugeschrieben wurde, oft erst nach einigen Tagen seine volle Hemmwirkung erreicht. Weiter ergibt sich,

TABELLE V

AUFTRETEN EINES UMWANDLUNGSPRODUKTES MIT ANTIESTERASE-WIRKUNG BEI ZWEI THIOLCARBAMATEN IN ABHÄNGIGKEIT VON DER ZEIT BEI UNTERSCHIEDLICHER MÖGLICHKEIT DER SAUERSTOFFEINWIRKUNG

Auftragmenge, 0.1 mg; Laufmittelsystem, Benzol–Dichlormethan (30:120).
+++ starke, ++ mittlere, + schwache, (+) gerade noch sichtbare Hemmflecke; kT nicht getestet.

Wirkstoff	*Ansatz in*	hR_F	*Tage*								
			0	*2*	*5*	*9*	*12*	*16*	*19*	*22*	*26*
Eptam	10-ml-Kolben	62	+	+	++	++	++	++	++	++	++
	Enghals	15	—	—	—	—	(+)	(+)	(+)	(+)	(+)
	1-ml-Zylinder	62	+	+	+	++	++	++	++	kT	kT
	Weithals	15	+	++	++	+++	++	+++	+++	kT	kT
Vernolate	10-ml-Kolben	65	+	++	++	++	+++	+++	+++	+++	+++
	Enghals	15	(+)	+	+	+	+	++	++	++	+
	1-ml-Zylinder	65	++	+++	+++	+++	+++	+++	+++	kT	kT
	Weithals	15	+	+	+	++	++	++	+++	kT	kT

TABELLE VI

hR_F-WERTE DER UNTERSUCHTEN HERBIZIDEN CARBAMATE IN VERSCHIEDENEN LAUFMITTELSYSTEMEN

Auftragmenge, 0.1 mg.

Wirkstoff	*Laufmittel*												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
CEPC	0	100	65	84 94	81	64	100	67	45 62	93	55	52	88
Propham	0	100	66	95	78	69	100	68	45 70	94	62	58	90
Chlorpropham	0	100	72	95	83	74	100	73	43 71	95	60	64	92
CPPC	0	100	71	86 95	62 85	72	100	34 71	45 65	94	39 51	33 59	79 92
Chlorbufam	0	100	71	94	84	73	100	72	45 63	95	58	55	90
Barban	0	100	73	84 95	47 86	78	100	39 73	46 62	61 95	30 47	27 49	76 90
Phenmedipham	0	100	28	84	35	24	100	15	37 44	81	25	20	77
Eptam	0	100	26 76	83 97	15 62	75	100	22 71	47 89	100	30	27 82	76 96
Vernolate	0	100	25 81	83 97	15 65	82	100	83	48 93	100	85	85	97
Pebulate	0	100	27 82	83 97	28 77	79	100	75	48 91	100	84	85	96
Diallat	0	100	89	83 96	27 90	85	100	16 86	46 87	85 100	30 85	85	78 97
Triallat	0	100	92	97	92	91	100	28 90	93	100	88	88	76 97

dass der zweite Hemmfleck beim Ansatz in einem 1-ml-Weithalsmesszylinder wesentlich schneller und intensiver auftritt als beim Ansatz in einem 10-ml-Enghalsmesskölbchen. Aufgrund der Tatsache, dass die Proben im Kühlschrank im Dunkeln standen und nur bei Bedarf ins Licht kamen, dürften weder Licht noch Temperatur eine grössere Rolle bei dieser Umwandlung spielen. Vielmehr ist anzunehmen, dass der Luftsauerstoff der entscheidende Faktor ist und eine Oxidation der Thiolcarbamate zum Sulfoxid und/oder Sulfon für das Auftreten des zweiten Hemmfleckes verantwortlich ist. Theoretisch sollte man drei Flecke erwarten. Dieser Fall trat jedoch während der Bestimmung der hR_F-Werte in den verschiedenen Laufmitteln nur ein einziges Mal ein. Die Tatsache, dass man in den ersten Tagen einen deutlichen Anstieg in der Antiesterase-Aktivität feststellen kann (Tabelle V), lässt vermuten, dass das Thiolcarbamat selbst gar nicht die Esterase hemmt, sondern erst die Oxidationsprodukte die Inhibitoren darstellen. Da nach UV-Bestrahlung mit Ausnahme von Diallat und Triallat bei keinem Thiolcarbamat eine Verstärkung der Hemmintensität zu beobachten ist (Tabelle IV), gewinnt die Interpretation an Wahrscheinlichkeit, dass bereits Oxidationsprodukte vorlagen. Vom chemischen Standpunkt dürfte der Mercaptoschwefel sehr schnell ins Sulfoxid übergehen, das seinerseits sehr schnell weiter zum Sulfon oxidiert würde.

Versuche zur dünnschichtchromatographischen Trennung der untersuchten Wirkstoffe in einer Reihe von Laufmittelsystemen brachten keine befriedigenden Ergebnisse, wie aus Tabelle VI hervorgeht. Aus der Tabelle ist zu ersehen, dass unter diesen Bedingungen nur einige Substanzen von den übrigen getrennt werden können. Die Ergebnisse zeigen allerdings auch, dass sich speziell das Laufmittel 9 (Cyclohexan–Dioxan, 70:30) zu Untersuchungen über das Auftreten von Abbauprodukten und Verunreinigungen bei den einzelnen Wirkstoffen eignet und sich für Stoffwechseluntersuchungen bei den Thiolcarbamaten auch das Laufmittel 5 (Benzol–Dichlormethan, 30:120) sehr gut verwenden lässt.

Zusammenfassend lässt sich sagen, dass hiermit erstmals die Wirkung von herbiziden Carbamaten auf Rinderleber-Esterase demonstriert wurde. Die Erfassungsgrenzen liegen dabei in einem vertretbaren Rahmen, obwohl chemische Nachweise zum Teil erheblich empfindlicher sind.

DANK

Mein besonderer Dank gilt Fräulein I. BLEICH für die sorgfältige Mitarbeit bei der Durchführung der Versuche.

ZUSAMMENFASSUNG

Die untersuchten herbiziden Carbamate lassen sich dünnschichtchromatographisch-enzymatisch mit Rinderleber-Esterase nachweisen, wobei die Nachweisgrenzen je nach Vorbehandlung zwischen 3 und 100 μg für die N-Phenylcarbamate und 5 und 30 μg für die Thiolcarbamate liegen. N-Phenylcarbamate zeigen ohne Vorbehandlung und nach Brombehandlung eine Farbintensivierung, die allerdings nicht auf einer Enzymaktivierung beruht. Vielmehr ist anzunehmen, dass dieses Phänomen auf einer Reaktion zwischen enzymatischen Abbauprodukten und Echtblausalz B beruht. Durch UV-Bestrahlung gehen diese Substanzen in echte Esterase-Hemmer über. Die

untersuchten Thiolcarbamate zeigen deutliche Antiesterase-Wirkung, und nur Diallat und Triallat gehen nach UV-Bestrahlung in stärkere Hemmer über, während Eptam, Vernolate und Pebulate unbeeinflusst bleiben. Brombehandlung hat nur einen geringen Einfluss. Kinetikuntersuchungen mit Eptam und Vernolate zeigen, dass die Thiolcarbamate zu anderen Antiesterase-Substanzen abgebaut werden, wobei es sich bei diesen Abbauprodukten möglicherweise um das Sulfoxid und/oder Sulfon handelt.

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THE EFFECT OF ANIONS ON THE GEL CHROMATOGRAPHIC BEHAVIOR OF MAGNESIUM IONS

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SUMMARY

Magnesium ions were chromatographed on a Sephadex G-15 column using sodium chloride, sodium sulfate, sodium nitrate, sodium perchlorate and sodium triphosphate as eluting agents. In order to evaluate the solute–gel interaction and then to estimate the contribution of the anions, the variation in elution curves of magnesium ions with sample concentrations was observed in various eluent systems. It was concluded that the elution volume of the magnesium ions is dependent on the sizes, the complexing ability and the adsorptive property of the anions in the elution system.

INTRODUCTION

Gel chromatography is a technique for separating solute molecules according to their sizes in solution. Larger solute molecules are eluted earlier than smaller solute molecules. In most cases the gel chromatographic behavior of inorganic compounds on a Sephadex column has been explained in terms of steric exclusion or a sieving effect in the gel phase[1–11]. However, it has also been reported that side effects arising from solute–gel interactions, such as ion exclusion (Donnan effect), ion exchange and adsorption, play an important role in some cases[11–16].

In addition to the solute–gel interaction the solute–solute interaction between solute molecules and the eluting agent may affect the chromatographic behavior of the solute in question. Some investigators[2,13,16] have demonstrated that the elution behavior of metal ions was greatly dependent on the chemical form of the samples and the kind of eluting agent. However, the interpretation of the mechanism of such a solute–solute interaction is still inadequate. This work was undertaken to investigate the effect of the anion on the gel chromatographic behavior of magnesium ions on a Sephadex G-15 column using sodium chloride, sodium sulfate, sodium nitrate, sodium perchlorate and sodium triphosphate as eluting agents. In order to evaluate the solute–gel interaction and then to estimate the contribution of the anion, the variation in elution curves of the magnesium ions with sample concentration was observed in various eluent systems. It was concluded that the elution volume of magnesium ions is dependent on the size, the complexing ability and the adsorptive property of the anions in the elution system.

EXPERIMENTAL

Unless otherwise stated, all experiments were carried out according to our previous paper[15]. It should be noted that the total bed volume of a Sephadex G-15 column was adjusted so that it was 100 ml and the volume of each fraction was 0.98 ml.

Determination of triphosphate

Triphosphate was determined colorimetrically with an Mo(V)–Mo(VI) reagent[6].

Determination of magnesium

Magnesium ions were determined by the method of MANN AND YOE[17] with some modification. To each fraction 2.0 ml of borate buffer (0.08 *M*) and 6.0 ml of deionized water were added. Then 5.0 ml of Dotite XB-1 reagent (150 mg in 1 l of 95 % ethyl alcohol) was added and the total volume was adjusted to 25 ml with 95 % ethyl alcohol. The absorbance at 510 mμ was measured.

In case of the presence of triphosphate the effluent in each fraction was adjusted to pH 4.2 with 0.01 *M* hydrochloric acid and the total volume was adjusted to 10 ml with deionized water. 1 g of dry ion-exchange resin (Dowex 1 X 8, Cl^--form, 100–200 mesh)was added to remove triphosphate ions by ion-exchange adsorption. The magnesium was then determined according to the procedure mentioned above.

RESULTS

Preliminary experiment

Fig. 1 shows the elution pattern of magnesium chloride (0.01–2.0 *M*) eluted on a Sephadex G-15 column with a 0.1 *M* sodium sulfate solution. At lower sample concentrations symmetrical elution curves appear and their elution volumes are smaller than those of magnesium ions eluted with 0.1 *M* sodium chloride solution[15]. With increasing sample concentration, however, the peak skewness becomes remarkable and the elution band spreads irregularly to give a complicated elution curve with

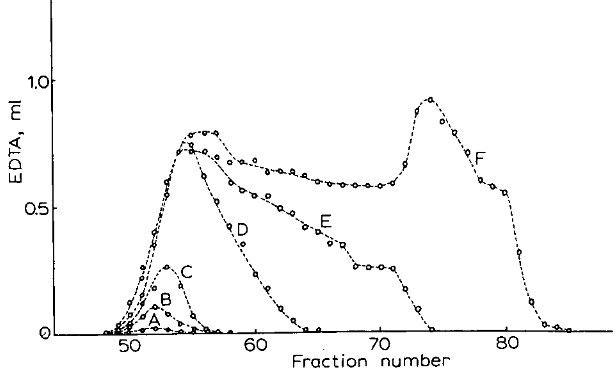

Fig. 1. Concentration dependence of the elution curves in the $MgCl_2$–Na_2SO_4 system. Eluent: 0.1 *M* Na_2SO_4. Concentrations of sample ($MgCl_2$): A = 0.01 *M*; B = 0.05 *M*; C = 0.1 *M*; D = 0.5 *M*; E = 1.0 *M*; F = 2.0 *M*.

a plateau. At extremely high concentration, 2.0 M, a secondary peak appears in a position corresponding to the elution volume of magnesium ions eluted with a 0.1 M sodium chloride solution.

The above fact suggests that not only the chloride ions in the sample solution but also the sulfate ions in the eluent play an important role in determining the gel chromatographic behavior of magnesium ions. In order to characterize this effect due to the anions four "homogeneous" sample–eluent systems were investigated, *viz.*, $MgCl_2$–NaCl, $MgSO_4$–Na_2SO_4, $Mg(ClO_4)_2$–$NaClO_4$ and $Mg(NO_3)_2$–$NaNO_3$ systems. In such homogeneous systems the anions of both the sample and the eluent are common. In addition, two "heterogeneous" systems, $MgCl_2$-(NaCl + Na_2SO_4) and $MgCl_2$–(NaCl + $NaClO_4$), were examined. The heterogeneous systems contain more than one kind of anion.

The homogeneous $MgCl_2$–NaCl system

This system has already been discussed in our previous paper[15].

The homogeneous $MgSO_4$–Na_2SO_4 system

Fig. 2 shows the elution behavior of magnesium sulfate eluted with a 0.1 M sodium sulfate solution. The elution volumes of the magnesium ions in this system are smaller than those in the $MgCl_2$–NaCl system and the general features of the variation in the elution curves with sample concentration is identical to that at lower sample concentrations in Fig. 1.

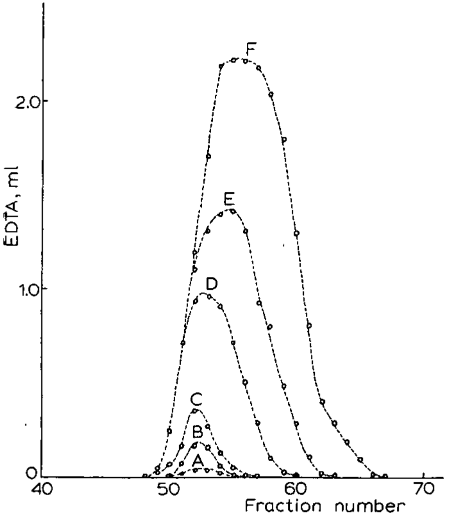

Fig. 2. Concentration dependence of the elution curves in the $MgSO_4$–Na_2SO_4 system. Eluent: 0.1 M Na_2SO_4. Concentrations of sample ($MgSO_4$): A = 0.01 M; B = 0.05 M; C = 0.1 M; D = 0.5 M; E = 1.0 M; F = 2.0 M.

The homogeneous $Mg(ClO_4)_2$–$NaClO_4$ system

Figs. 3(a) and 3(b) show the concentration dependence of the elution curves of magnesium perchlorate eluted with 0.1 M sodium perchlorate solution. In contrast to the elution behavior in the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 systems the K_d

values are larger than unity and decrease with the increase in the sample concentrations. The elution curves are unsymmetrical and of a T-shape (tailing)[15] over a wide range of sample concentrations, which is identical to the elution pattern of barium ions in the $BaCl_2$–NaCl system.

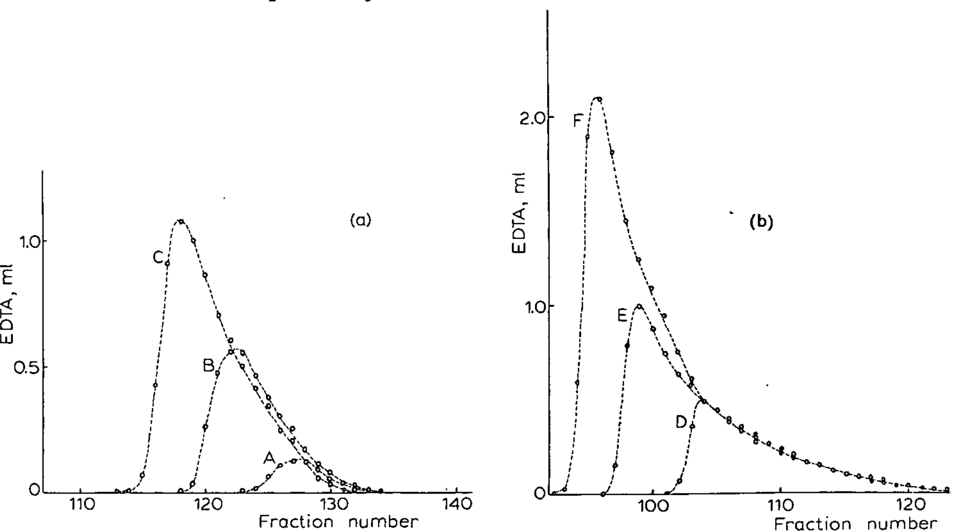

Fig. 3. Concentration dependence of the elution curves in the $Mg(ClO_4)_2$–$NaClO_4$ system. Eluent: 0.1 *M* $NaClO_4$. Concentrations of sample ($Mg(ClO_4)_2$): (a) A = 0.01 *M*; B = 0.05 *M*; C = 0.1 *M*. (b) D = 0.5 *M*; E = 1.0 *M*; F = 2.0 *M*.

The homogeneous $Mg(NO_3)_2$–$NaNO_3$ system

Figs. 4(a) and 4(b) show the elution behavior of magnesium nitrate eluted with a 0.1 *M* sodium nitrate solution. The elution volumes are somewhat larger than those

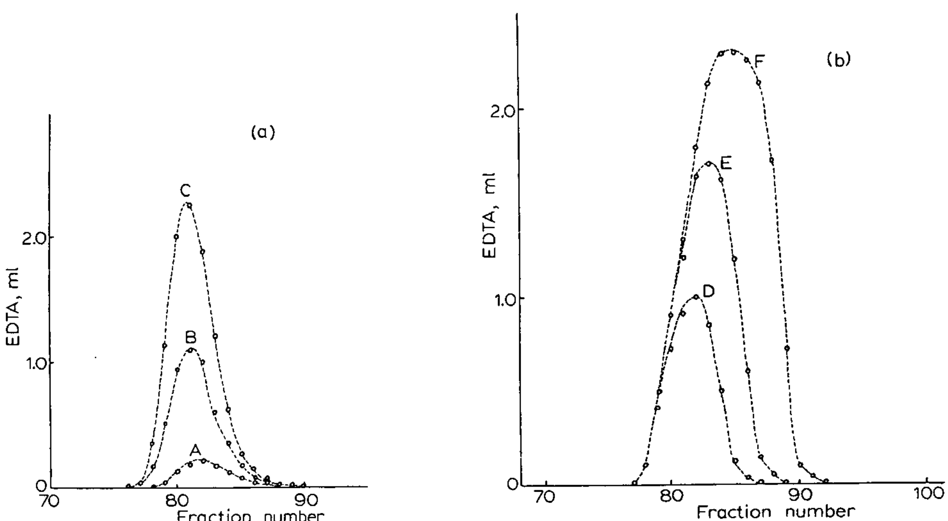

Fig. 4. Concentration dependence of the elution curves in the $Mg(NO_3)_2$–$NaNO_3$ system. Eluent: 0.1 *M* $NaNO_3$. Concentrations of sample ($Mg(NO_3)_2$): (a) A = 0.01 *M*; B = 0.05 *M*; C = 0.1 *M*. (b) D = 0.5 *M*; E = 1.0 *M*; F = 2.0 *M*.

in the $MgCl_2$–NaCl system and increase with the increase in sample concentration. Some tailing is observed at the lower portion of the descending sides of the elution curves in Fig. 4(a).

The overall feature for the elution volumes (the elution-peak positions) of magnesium ions in the four homogeneous systems can be seen in Fig. 5, in which the elution volumes were plotted against the concentrations of magnesium ions. It shows that magnesium ions in the $Mg(ClO_4)_2$–$NaClO_4$ system are eluted at positions far behind the total liquid volume of the column ($V_i + V_0 = 84$ ml).

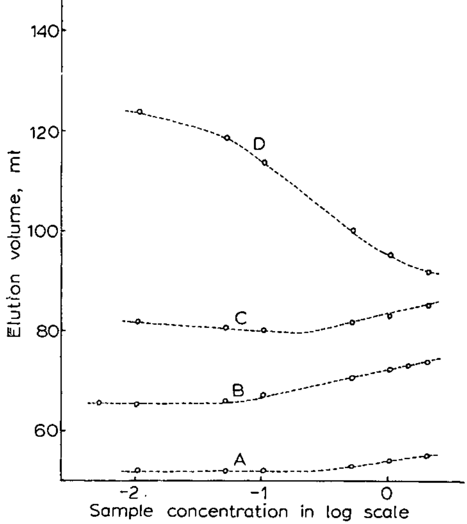

Fig. 5. Concentration dependence of the elution volumes in the four homogeneous systems. A = $MgSO_4$–Na_2SO_4 system; B = $MgCl_2$–NaCl system; C = $Mg(NO_3)_2$–$NaNO_3$ system; D = $Mg(ClO_4)_2$–$NaClO_4$ system.

The heterogeneous $MgCl_2$–(NaCl + Na_2SO_4) and $MgCl_2$–(NaCl + $NaClO_4$) systems

Figs. 6 and 7 show the variation in the elution volumes of magnesium ions with the variation in the compositions of the eluents. In these systems the total molar concentrations of the eluents were kept constant, 0.1 *M*. The elution volumes increase

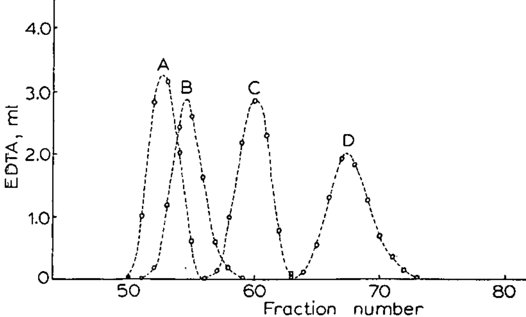

Fig. 6. The effect of the composition of the eluent on the elution volumes in the $MgCl_2$–(NaCl + Na_2SO_4) system. Sample: 0.01 *M* $MgCl_2$. Composition of the eluents: A = 0.1 *M* Na_2SO_4; B = 0.075 *M* Na_2SO_4 + 0.025 *M* NaCl; C = 0.05 *M* Na_2SO_4 + 0.05 *M* NaCl; D = 0.1 *M* NaCl.

with the increase of the molar ratio, $NaCl/Na_2SO_4$, in the $MgCl_2$–($NaCl + Na_2SO_4$) system but decrease with the increase of the ratio, $NaCl/NaClO_4$, in the $MgCl_2$–($NaCl + NaClO_4$) system. All elution peaks appear in the intermediate regions between the elution positions of magnesium ions in the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 or in the $MgCl_2$–NaCl and the $Mg(ClO_4)_2$–$NaClO_4$ systems mentioned above.

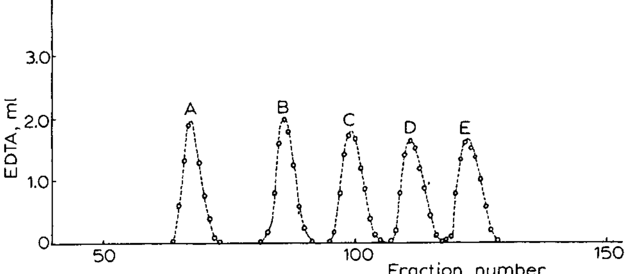

Fig. 7. The effect of the composition of the eluent on the elution volumes in the $MgCl_2$–($NaCl + NaClO_4$) system. Sample: 0.01 *M* $MgCl_2$. Composition of the eluents: A = 0.1 *M* NaCl; B = 0.075 *M* NaCl + 0.025 *M* $NaClO_4$; C = 0.05 *M* NaCl + 0.05 *M* $NaClO_4$; D = 0.025 *M* NaCl + 0.075 *M* $NaClO_4$; E = 0.1 *M* $NaClO_4$.

DISCUSSION

It is evident from Figs. 2–5 that the elution volume of magnesium ions is greatly dependent on the type of counter anion. As has been described in a previous paper[16], this can be discussed with respect to the elution behavior of counter anions. It was found, by using an automatic chromatograph with a thermal detector[18], JLC-2A (Japan Electron Optics Laboratory), that, when 0.1 *M* solutions of sodium salts of the four counter anions were eluted on a Sephadex G-15 column (bed volume = 110 ml) with a 0.1 *M* sodium chloride solution as eluent, the elution volumes increased in the order of $Na_2SO_4 < NaCl < NaNO_3 < NaClO_4$. Sodium nitrate appeared at a position somewhat larger than the total liquid volume of the column. Sodium perchlorate was eluted more slowly, about 50 ml behind the elution position of sodium nitrate. The shapes of the elution curves for both nitrate and perchlorate were unsymmetrical and of a T-shape, which suggests the interaction of the solute anions with the gel matrix.

Steric exclusion

Sulfate ions and chloride ions seem to have no direct interaction with the gel matrix. Therefore, the behavior of magnesium ions in both the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 systems can be expected to be based on steric exclusion considerations. In order to explain the difference between the elution patterns of magnesium ions in the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 systems it seems appropriate to introduce the idea of the polyfunctional character of the gel phase[11]. For convenience of interpretation the features of polyfunctional character are shown schematically in Fig. 8. If a background electrolyte, *e.g.*, sodium chloride, can only penetrate to a limited extent into the internal volume because of steric exclusion the gel phase is considered to be divided into two phases, phases I and II in Fig. 8A, resulting from the presence

or absence of the background electrolyte. The degree of the penetration of the background electrolyte, *i.e.*, the magnitude of phase II, will be primarily determined by the larger one of the two constituent ions because of the requirement of the electrical neutrality. Since the effective sizes[16] of chloride, sodium and sulfate ions have been estimated to increase in this order, the magnitude of phase II in the sulfate system (Fig. 8B) may be smaller than that in the chloride system.

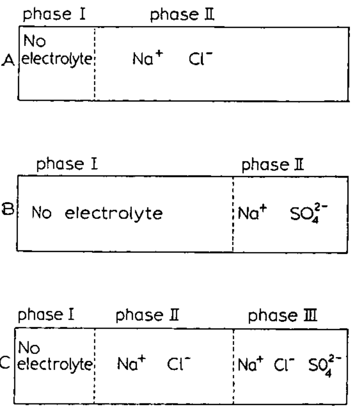

Fig. 8. Schematic representation of polyfunctional character in the gel phase.

In a homogeneous sample–eluent system the elution volume of magnesium ions is dependent on the penetrability of counter anions in phase II, because magnesium ions must be present near their counter anions in order to satisfy the principle of electrical neutrality. In the $MgCl_2$–NaCl system magnesium ions can penetrate freely according to their ionic size because counter anions, chloride ions, are smaller than the magnesium ions. On the other hand the penetration of magnesium ions in the $MgSO_4$–Na_2SO_4 system is restricted by the sulfate ions whose effective size is larger than that of the magnesium ions. Thus the available volumes or K_d values of the magnesium ions in the $MgSO_4$–Na_2SO_4 system become smaller than those in the $MgCl_2$–NaCl system.

When a mixed solution of sodium chloride and sodium sulfate is used as an eluent the internal liquid phase is considered to be divided into three regions (Fig.8C). An additional, new phase III is composed of sodium cations and chloride and sulfate anions. If magnesium ions do not form any complex with the sulfate ions these ions can distribute both in phases II and III according to their ionic size. The elution volume of magnesium ions in the $MgCl_2$–(NaCl + Na_2SO_4) system is expected to be comparable to that in the $MgCl_2$–NaCl system. Since the formation of a sulfato-complex of magnesium is well known, however, it would rather be expected that magnesium ions would tend to be retained preferentially in phase III. Therefore, the elution volume decreases with the increase in the ratio of Na_2SO_4/NaCl, which is consistent with the experimental results in Fig. 6.

The preliminary observation in Fig. 1 can be explained in the same way. When the sample concentration is low, *i.e.*, the molar ratio of chloride ions to sulfate ions is small, magnesium ions in the $MgCl_2$–Na_2SO_4 system behave as in the homogeneous

$MgSO_4$–Na_2SO_4 system. With an increase of sample concentration the elution system in the sample zone is considered to become heterogeneous as represented in Fig. 8C. When the concentration of magnesium ions is larger than that of sulfate ions or, in other words, the sample is loaded over the capacity of phase III (the sulfate anion phase is regarded as a sort of liquid cation-exchanger) a portion of magnesium ions will be distributed over not only phase III but also phase II, which results in the slower migration of the magnesium ions. Magnesium ions thereby remove into an adjacent plate (behind the sample zone) successively to be re-equilibrated. The sample band spreads through such a successive re-equilibration of magnesium ions in the elution process. At extremely high concentrations, the large excess of magnesium ions behave as in the $MgCl_2$–NaCl system to give a secondary peak.

Complex formation

In order to confirm the effect of complex formation on the gel chromatographic behavior of magnesium ions, an additional experiment was done using sodium triphosphate instead of sodium sulfate in the heterogeneous $MgCl_2$–(NaCl + Na_2SO_4) system. The gel chromatographic behavior[7] and the complex formation[19] of triphosphate have been well characterized. Fig. 9(A) shows the elution behavior of magnesium obtained by eluting a 0.004 *M* magnesium chloride solution on a Sephadex G-15 column. The eluent is composed of a mixed solution of 0.1 *M* NaCl + 0.01 *M* $Na_5P_3O_{10}$, adjusted to pH 7.2. Fig. 9(B) shows a negative peak for triphosphate anions obtained by eluting pure water as a sample with the same eluent and determining the triphosphate colorimetrically. Magnesium ions in the $MgCl_2$–(NaCl + $Na_5P_3O_{16}$) system appear at the same position as the triphosphate ions that is about 22 ml before the elution volume of magnesium ions in the $MgCl_2$–NaCl system. The fact that the elution volume of magnesium ions is greatly influenced by the presence of smaller amounts of triphosphate ions than sulfate ions in the $MgCl_2$–(NaCl + Na_2SO_4) system is attributable to the larger formation constant of the magnesium triphosphate

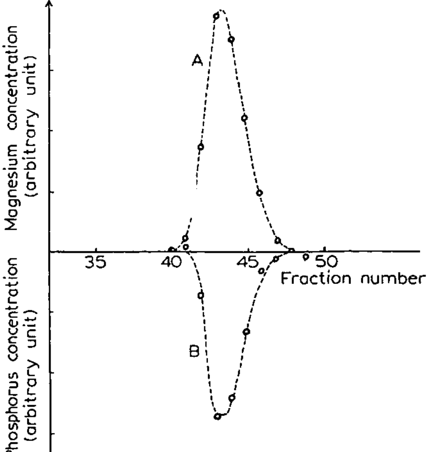

Fig. 9. Elution curves of magnesium and triphosphate ions in the $MgCl_2$–(NaCl + $Na_5P_3O_{10}$) and the H_2O–(NaCl + $Na_5P_3O_{10}$) systems. Samples: A = 0.004 *M* $MgCl_2$; B = H_2O. Eluent: 0.1 *M* NaCl + 0.01 *M* $Na_5P_3O_{10}$ (pH 7.2).

complex. Such complex formation may increase the probability of the retention of magnesium ions in phase III, which results in a decrease of the apparent elution volume of the magnesium ions. It can be assumed from Fig. 9 that the effective size of magnesium triphosphate may be comparable to that of free triphosphate ions.

Adsorption

In a previous paper[11] it was observed that when a sodium chloride solution was used as eluent the shape of the elution curves for magnesium ions at lower sample concentrations was symmetrical and its elution volume did not vary with sample concentration, while strontium and barium ions gave elution curves of the T-shape and their elution volumes varied inversely with sample concentrations. The elution behavior of strontium and barium ions was interpreted in terms of the interaction of the solutes with the gel matrix. On the other hand, it was concluded that magnesium ions are not influenced by such an adsorption effect.

In the $Mg(ClO_4)_2$–$NaClO_4$ system, however, magnesium ions give elution patterns which are similar to the behavior of barium ions mentioned above. As has already been discussed in a previous paper[16] it is suggested that a side effect such as solute–gel interaction may play an important role in this system. Since direct adsorption of magnesium ions on the gel matrix was concluded to be unlikely, another possible mechanism is that magnesium ions are adsorbed electrostatically by perchlorate anions which were preliminarily adsorbed on the gel matrix. This assumption can be expressed by eqns. 1 and 2.

$$R + ClO_4^- \rightarrow R\text{–}ClO_4^- \quad (1)$$

$$R\text{–}ClO_4^- + Mg^{2+} \rightarrow R\text{–}ClO_4\text{–}Mg^+ \text{ or } (R\text{–}ClO_4)_2Mg \quad (2)$$

where R represents the gel matrix.

The mechanism of direct adsorption of perchlorate anions is not yet clear. However, the fact that the elution curve of sodium perchlorate is of a T-shape which is typical in adsorption chromatography[15] and the heat of interaction of sodium perchlorate with a Sephadex gel[18] is extraordinarily large compared with those of sodium chloride and sodium sulfate indicates the predominant adsorption of perchlorate anions. PECSOK AND SAUNDERS[13] suggested the formation of hydrogen bonds between perchlorate anions and the amide hydrogens of Bio-Gel P-2. It is not unlikely that the hydroxy groups in the Sephadex gel skeleton and the polarized water molecules in the hydration sphere of the Sephadex gel may interact with perchlorate anions to form hydrogen bonds.

With increasing concentration of perchlorate anions in the eluent the concentration of $R\text{–}ClO_4^-$ in the gel phase increases according to eqn. 1 and the apparent distribution coefficient of magnesium ions would be expected to increase correspondingly. According to such an expectation the elution volume of magnesium ions increases with the increase in the concentration of perchlorate anions at constant ionic strength.

Nitrate anions are considered to interact with the Sephadex gel to a much smaller extent so that these ions do not affect the chromatographic behavior of magnesium ions so markedly as perchlorate anions.

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Notes

CHROM. 5326

A new type of micro-analysis of steroid hormones in biological materials by gas chromatography with flame ionisation and electron capture detection

I. The urinary profile

For the gas phase analysis of a mixture of steroids in biological materials, steroid derivatives stable under the temperature conditions of gas chromatography (GC) are necessary. O-Methyloxime-trimethylsilyl derivatives (MO-TMSi) introduced by GARDINER AND HORNING[1] in 1966 allowed them to perform the so-called "steroid biological profile" with flame ionisation detection (FID). Electron capture detection (ECD) brings GC sensitivity to the nanogram and even the subnanogram level using heptafluorobutyrate derivatives, but these derivatives, depending on the type of steroid, are not always stable in GC. With 17-desoxycorticosteroid, and more extensively with 17-hydroxycorticosteroid esters, multiple peaks appear which probably involve side-chain cleavage[2], whilst with a ketosteroid, like dehydroepiandrosterone (DHEA), we found that the 17-keto group is partially enolised leading to two derivatives. If methyloxime formation is carried out before the perfluoroacylation, stable derivatives are obtained and enolisation is prevented. In 1969, HORNING AND MAUME[3] studied O-methyloxime-heptafluorobutyrate derivatives (MO-HFB) for aldosterone. The formation of these derivatives applied to other steroids and their metabolites allows good thermal stability and sensitivity at the nanogram level.

Besides being used for biological fluids containing a small amount of steroid, this method appears very interesting for steroid analysis on small tissue samples in which steroid metabolism exists. Because it has been shown that adrenal tumor cells grown in culture[4] and adrenal cell suspension[5] synthesize steroids[5,6], our method should be valid for a more specific and complete study of steroidogenesis in the course of cell differentiation or development. Following the work of HARARY AND FARLEY[7] showing that single normal heart cells can be kept and grown in Petri dishes, recent progress in maintaining normal non-embryonic cells from organs related to steroid metabolism, like the liver[8,9] and adrenal glands[10], prompted us to ascertain the validity of our analytical method. Therefore for comparison with other methods, this preliminary work has been done on human urine, the most investigated mixture of steroids.

Methods

For the standards, the formation of MO-HFB derivatives is described elsewhere[11]. O-Methyloxime (MO) derivatives were made by the usual method[12]. Excess reagent was eliminated by washing with a sodium chloride solution. Heptafluorobutyrylimidazole was used as the reagent for HFB derivative formation. These derivatives were then extracted by hexane at $-18°$. The final amount injected was 1 μl containing 1 μg for FID and 1 ng for ECD.

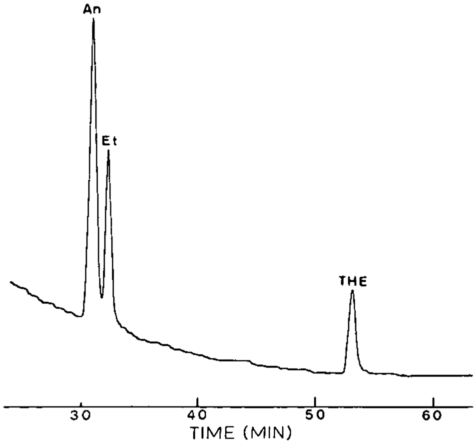

Fig. 1. Separation of a synthetic mixture of MO-HFB steroids on a 12-ft. 1 % OV-1 column made according to HORNING *et al.*[16]. Gas chromatograph is a model 7400 Packard with FID. Compounds are androsterone (An), etiocholanolone (Et) and tetrahydrocortisone (THE). Temperature settings: programming at 1°/min from 170 to 230°, 240° for the injection, 260° for the oven detector.

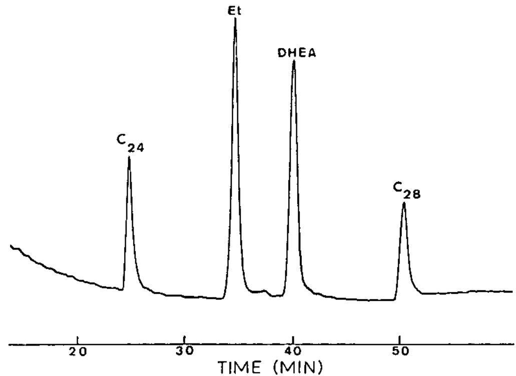

Fig. 2. Same as Fig. 1, but the separation was carried out on a 1 % OV-17 column. Two alkanes (C_{24} = *n*-tetracosane, C_{28} = *n*-octacosane) were added as internal standards for methylene unit (MU) calculation.

TABLE I

METHYLENE UNIT VALUES (MU) OF HFB AND MO-HFB DERIVATIVES OF SOME HUMAN URINARY STEROIDS

Steroids	*OV-1*[a]	*OV-17*[a]
Androsterone (3α-hydroxy-5α-androstan-3-one)	24.07	25.47
Etiocholanolone (3α-hydroxy-5β-androstan-3-one)	24.25	25.55
Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one)	24.85	26.41
Pregnanediol[b] (3α,20α-dihydroxy-5β-pregnane)	25.69	25.15
Pregnanolone (3α-hydroxy-5β-pregnan-20-one)	26.04	27.36
Tetrahydrocortisone (3α,17α,21-trihydroxy-5β-pregnane-11,20-dione)	27.52	28.08

[a] 12-ft. column; 1 % liquid phase; temperature, 1°/min from 170°.
[b] HFB ester, other steroids are as MO-HFB derivatives.

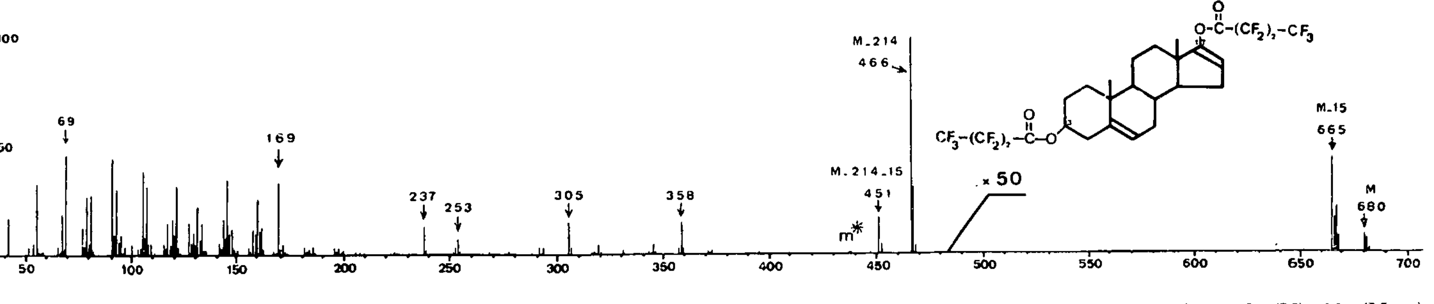

Fig. 3. Mass spectrum of 3β,17-(enol)-diHFB-dehydroepiandrosterone. Fragments providing structural information are m/e = 680 (M), 665 (M-15), 466 (M-214) corresponding to the loss of heptafluorobutyric acid from the molecular ion, 451 (M-214-15), 253 (M-214-213), 252 (M-2 × 214), 237 (M-2 × 214-15). Peaks at 69, 119 and 169 a.m.u. correspond to CF_3^+, $C_2F_5^+$, $C_3F_7^+$, respectively. Metastable ion found at m/e = 436.7 corresponds to the transition 466 $\xrightarrow{-15}$ 451 (calculated m/e = 436.48). This spectrum was obtained with an LKB 9000 gas chromatograph–mass spectrometer; ionising energy: 70 eV; ionisation source temperature 200°.

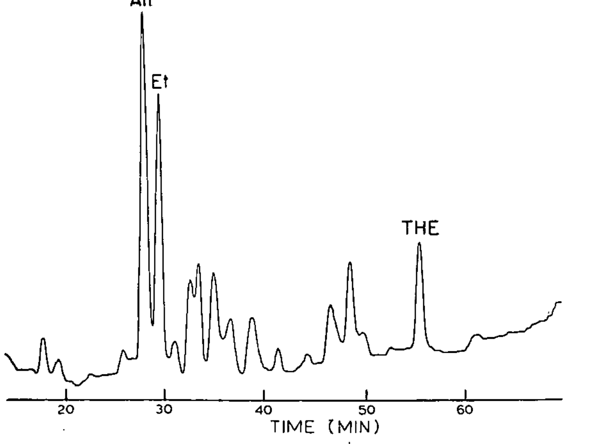

Fig. 4. Same conditions as Fig. 1. Separation of adult male urinary steroids.

25-ml urinary samples were each hydrolysed enzymatically, and the free steroids were transformed to MO-HFB derivatives by the previous method. Estrogens were separated by partition between aqueous ethanol (2 %) and benzene–hexane (1:1). The GC apparatus equipped with FID and ECD are described in the legend to Fig. 1.

Results and discussion

Figs. 1 and 2 show that MO-HFB derivatives are thermostable and each give a single peak. The side chain of C_{21} steroid-like tetrahydrocortisone is protected from cleavage by MO-HFB formation. If no MO formation occurs before perfluoroacylation, we have found that, in the case of 17-ketosteroid, HFB anhydride induces a partial enolisation of the keto group under the usual reaction conditions. For example, DHEA heated 1 h at 60° with HFB anhydride in acetonitrile (3:10) gives, besides the 3β-HFB derivative, a minor peak which has been identified by mass spectrometry as the 3β,17-diHFB (enol) derivative (Fig. 3). The second enol peak increases if the reaction time is longer (65 % of HFB enol after 15 h) (ref. 13). Although it may be satisfactory to obtain 100 % enol formation by reacting for 36 h at 60° instead of avoiding it, it is preferable, especially if acylation is carried out over a shorter time, to perform the MO reaction first.

The methylene unit values[14] of MO-HFB derivatives shown in Table I are smaller than those given by corresponding MO-TMSi[15].

Therefore faster elution is another interesting feature of these MO-HFB derivatives. Moreover the separation between isomeric steroids such as MO-HFB or HFB compounds is generally improved in comparison with other types of derivatives Examples are given for the separation between MO-HFB androsterone and MO-HFB etiocholanolone (Figs. 1 and 4) and more strikingly between isomeric HFB estriols[11] (Fig. 7).

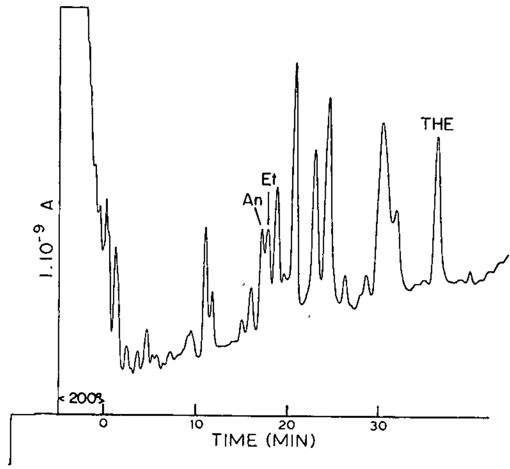

Fig. 5. Same urinary sample as in Fig. 4, but diluted to 1/1000. Column was a 6-ft. 1 % OV-1 under isothermal conditions at 200° for 5 min and then temperature programming at 1°/min. Detection was done by ^{63}Ni-ECD with 2 V DC, the detector oven being set at 255°. Comparison with Fig. 4 shows a smaller relative response for "An" and "Et", bearing one HFB, than for THE bearing two HFB.

Fig. 4 shows a characteristic separation of male adult urinary steroids as MO-HFB derivatives with the use of FID. When the gas chromatograph is operated with ECD, relative heights of peaks are modified because the response of this detector is dependent on the number of fluorine atoms and consequently on the number of OH groups which have been esterified in the molecule (Fig. 5).

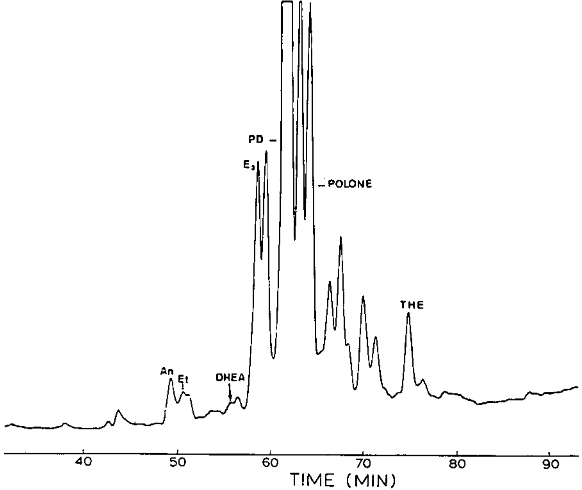

Fig. 6. Separation of MO-HFB steroids of a urinary sample from pregnant woman. Temperature programmed from 150° at 1°/min; other conditions as in Fig. 1. Other compounds identified are estriol (E3), pregnanediol (Pd), pregnanolone (Polone).

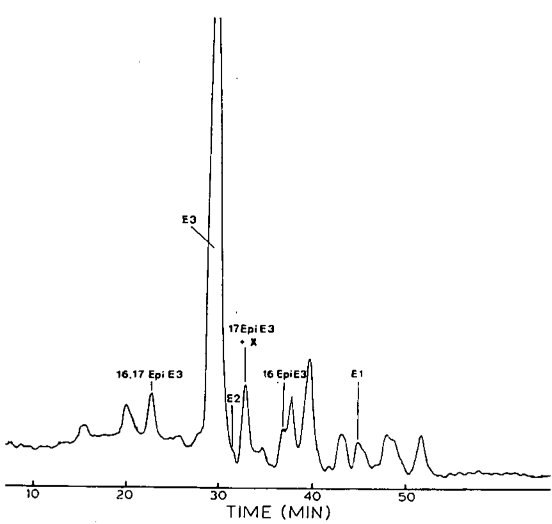

Fig. 7. Separation of HFB and MO-HFB steroids of a urinary sample from a pregnant woman in the last month of pregnancy. 6-ft. 1% OV-17 column; temperature programmed from 170° at 1°/min; ECD. See conditions in Fig. 5 for detection by electron capture. The amount injected was equivalent to 3 μl of the original urine sample. Compounds identified are E1 = estrone; E2 = 17β-estradiol; E3 = 3,16α,17β-estriol; 17epiE3 = 3,16α,17α-estriol; 16,17-epiE3 = 3,16β,17α-estriol; 16-epiE3 = 3,16β,17β-estriol.

Total urinary steroids at the end of the pregnancy of a woman are shown in Fig. 6 while the analysis of the estrogenic fraction of the same sample is shown in Fig. 7, ECD being used. Thanks to this method, six of the many possible estrogens have been identified. This technique improves the separation and the sensitivity of detection of minute amounts of isomeric estriols in a simple analysis of a total estrogenic fraction avoiding preliminary subfractionation. The complete structure of these derivatives and the homogeneity of each peak in biological profile are undertaken with the use of combined gas chromatography–mass spectrometry. The application of this method to the study of small amounts of steroids in plasma, for instance, or in a small amount of tissue sample, as in normal single cell adrenal[10] or liver[9] culture, is under development.

We are grateful to Dr. E. C. HORNING for mass spectrometry of our 3β-HFB and 3β,17-diHFB(enol)-DHEA samples and for the fruitful discussions during the course of this work.

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CHROM. 5345

Device for automatic preparative gas chromatographic collections on an equal time basis

In our work on the identification of volatile components of natural products, it was necessary to separate the complex material into a number of arbitrary crude fractions by preparative scale gas chromatography (GC).

We had available for this purpose the widely-used Aerograph Model A705 Autoprep gas chromatograph (Varian Aerograph Inc., Walnut Creek, Calif.). This is an automated preparative scale gas chromatograph which controls fraction collection by changing trap positions at a pre-set, but always identical, signal level of the recorder.

In complex natural products the relative concentrations of the components vary by a factor of a thousand or more and the recorder-actuated trap-changing switch is not applicable. One solution to this problem is to collect at a variably adjusted response level (*i.e.* use a peak height discriminator); however, this is not possible with the Autoprep. Another solution that has been proposed is to utilize a collection programmer[1] which incorporates a complex sensing and actuating mechanism to effect trap changing. Although such a system would achieve the required results, a simpler device was desired. Still another method for actuating the trap changing mechanism is to collect on an equal time basis, using a timer system. Although GC collection on a time basis has been suggested before[2,3] such a modification to a commercial instrument has not been previously described. Such a system is effective only if retention times are reasonably reproducible, and the Autoprep demonstrates acceptable reproducibility. The present communication describes a simple, inexpensive device to change trapping positions on an equal time basis.

The present modification, designed to allow collection on an equal time basis,

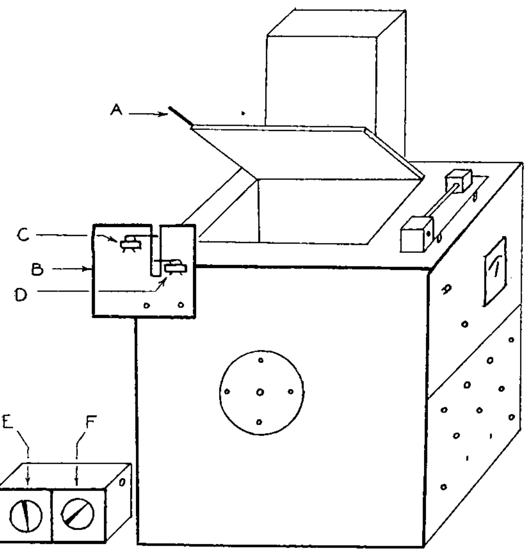

Fig. 1. Modifications to preparative chromatograph.

is shown in Fig. 1. The $\frac{1}{4}$ in. steel rod A is attached to the left side of the oven door and allowed to extend about $1\frac{1}{2}$ in. The 6×6 in. steel plate B which has a $\frac{1}{2} \times 3$ in. slot is attached to the rear left side of the instrument and adjusted so rod A will pass through the slot when the door closes. Micro-switch C is modified by cutting off the standard arm and replacing it with a piece of spring steel and is positioned as shown. This is wired in the normally open position in parallel with the Autoprep manual turntable advance pushbutton switch. It is positioned so that the contact is closed briefly by rod A as the oven door is closed. Micro-switch D is positioned such that the contacts are closed by rod A when the oven door is closed, and open when the oven door is open. Micro-switch D is wired such that when closed by rod A it actuates a Dual-Trol timer (Industrial Timer Corporation, Parsippany, N.J.). The Dual-Trol is a recycling timer consisting of two individual timing mechanisms sequentially pulsing a latch relay. When micro-switch D is closed, timer E, continuously variable between 0 and 5 min, will start its cycle. When the pointer reaches 0, the load contacts are closed and timer F, variable between 0 and 6 sec, is actuated. When timer F completes its cycle it restarts timer E and opens the load contact. This sequence continues until microswitch D is opened. The load contacts of timer F are connected to the leads of the recorder peak sensor switch which normally actuates the collector turntable. As long as the oven door is closed the collector turntable will rotate at equal time intervals, as determined by the time setting on timer E. In operation, timer E is set for the approximate collection interval (*i.e. ca.* 2 min); timer F is set for about 0.5 sec.

In the completely automated temperature programming mode, the following sequence of events occurs: as sample injection occurs and the heater is turned on, the oven door closes striking switch C and closing switch D. Switch C positions trap 1 in the collection position; switch D actuates timer E. At the end of the first collection cycle (*i.e.* 2 min) timer F is actuated momentarily and this actuates the turntable thus placing trap 2 in position. This sequence continues until the selected number of fractions has been collected. At this time the door opens, switch D opens, collection is terminated and the timers reset.

One of the advantages of this system is that up to sixteen fractions can be collected in a reproducible manner. In addition, by utilizing switch C the first trap can be positioned immediately after injection, thus allowing components with short retention times to be collected. The primary disadvantage of this system is that certain components will overlap two fractions if turntable rotation occurs while a peak is emerging.

Although this device was designed with the Aerograph Autoprep in mind, a similar modification should be possible for other automatic preparative GC units which collect at a constant signal response level.

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CHROM. 5346

The degradation of Sephadex G-150 by 50% formic and acetic acids

We are currently engaged in the preparation of synthetic polypeptide collagen models for immunological studies. In the course of this work we have encountered the need to fractionate these polydisperse materials, which are insoluble in nearly all solvents except carboxylic acids and aqueous mixtures containing high proportions of carboxylic acids. Sephadex G media have often been used for preparative gel filtration of peptides using solvent mixtures containing large amounts of acetic or formic acid. To cite but a few examples, G-50 has been used with 45 %[1], 70 %[2] or 88 %[3] formic acid, G-25 with 50 % acetic acid[4], G-50 with 30 %[5] or 50 %[6-8] acetic acid, G-75 with 50 % acetic acid[9,10], and G-25 with phenol–acetic acid–water (1:1:1:1)[11,12]. Although the lability of dextran gels to aqueous mineral acids is well known,[13] it seemed implicit from the large number of examples in the literature that serious detriment was not caused by exposure to aqueous mixtures containing in the region of 50 % acetic or formic acid. We were therefore encouraged to use such a system for our purposes.

Although we are not acquainted with any published application of Sephadex

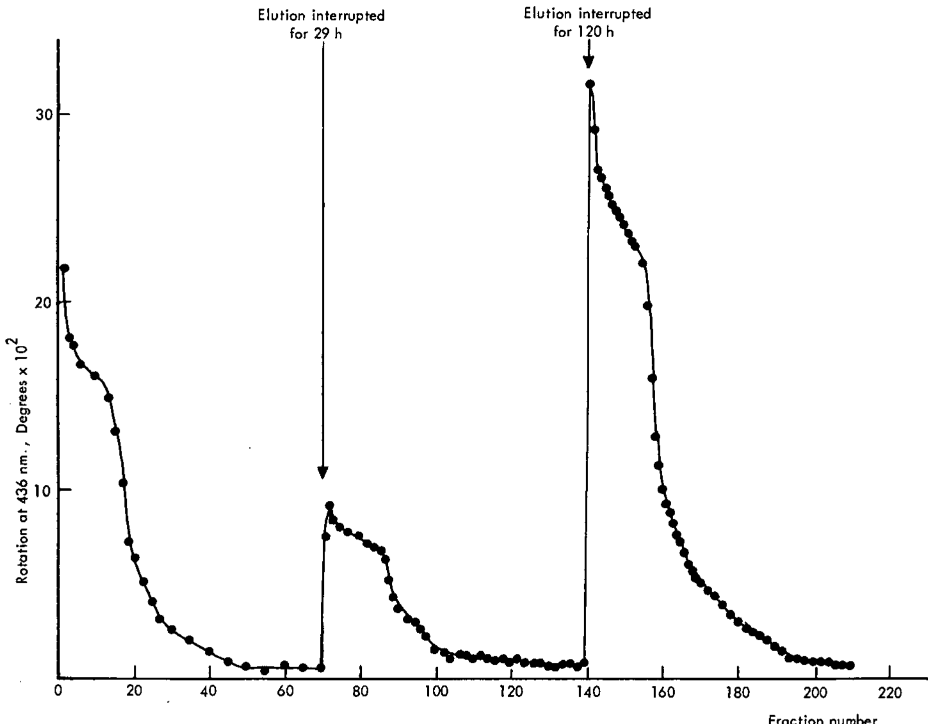

Fig. 1. Sephadex G-150 (lot No. 860) in 50% (v/v) aqueous AnalaR formic acid. Bed dimensions, 1.2 × 76 cm; bed volume, 85 ml; void volume, 28 ml; fraction size, 1.4 ml; flow rate, 7 ml/h. The optical rotations of the fractions were determined in a Perkin-Elmer 141 polarimeter using a 1 dm cell at 25°.

G-150 in conjunction with *ca.* 50 % acetic or *ca.* 50 % formic acids we chose these combinations for our work because preliminary small scale experiments on the fractionation of poly-(L-alanylglycyl-L-proline) showed them to be the most suitable for the molecular weight range of our material. Since this polymer has a high optical rotation and lacks convenient chromophores, eluants were monitored polarimetrically. It was observed that with both 50 % acetic acid and 50 % formic acid the eluant was significantly optically active before the void volume had emerged and remained so even after one bed volume had passed. Because it seemed likely that this was evidence of degradation of the gel we investigated this by running the solvent mixtures alone through fresh columns. A column of G-150, made up in 50 % formic acid and stabilised by passage of several bed volumes of solvent, was allowed to stand at room temperature for 75 h before examination. The first fractions of eluant collected were highly optically active: the rotation fell as elution progressed, attaining a constant small positive value after one bed volume had passed (Fig. 1). The fractions corresponding to the first bed volume were pooled and lyophilised to give 105 mg of a pale yellow solid which was shown to be mainly carbohydrate by the anthrone reaction. When the flow of eluant was interrupted and resumed after 29 h the rotations of the first fractions then obtained were again highly optically active, falling off with continued elution. Repetition of the same operations caused recurrence of the same effects (Fig. 1). Use of 50 % acetic acid gave qualitatively similar evidence for degradation

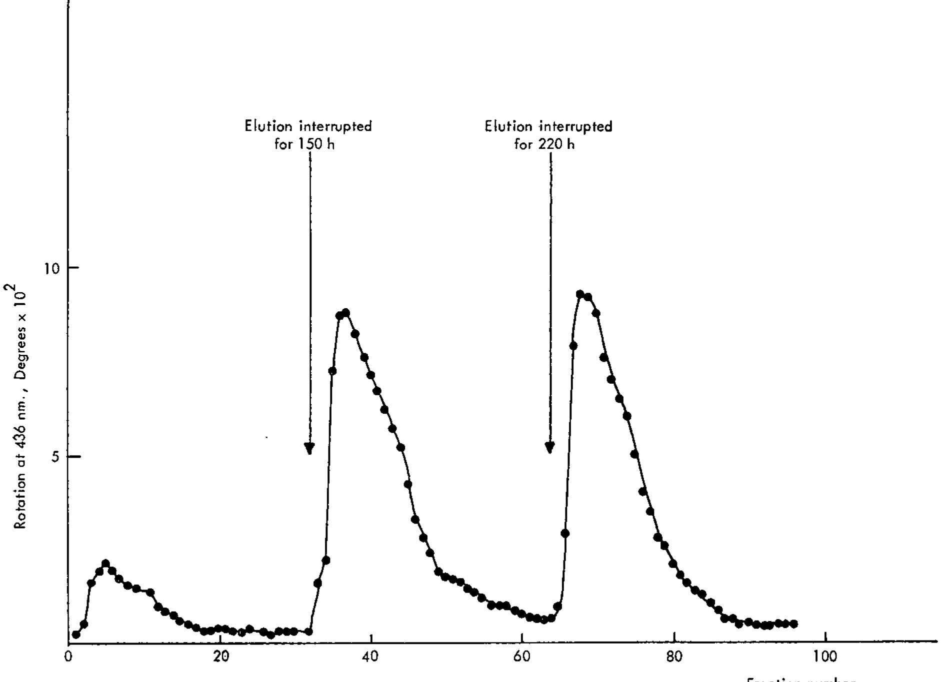

Fig. 2. Sephadex G-150 (lot No. 860) in 50 % (v/v) aqueous AnalaR acetic acid. Bed dimensions, 1.9 × 11 cm; bed volume, 30 ml; void volume, 10.2 ml; fraction size, 1.25 ml; flow rate, 15 ml/h. The optical rotations of the fractions were determined in a Perkin-Elmer 141 polarimeter using a 1 dm cell at 25°.

of the gel (Fig. 2). Distilled water alone did not elute significant amounts of optically active material from a column of G-150.

We conclude that if contamination of the applied material is to be minimised when aqueous mixtures of acetic or formic acid are used as eluants with Sephadex G-150, then the column should be washed through with at least one bed volume of solvent immediately prior to use, since carbohydrate artefacts accumulate in the column on standing. Even if this procedure is followed, some contamination which would not be detected by standard monitoring techniques seems inevitable. For many purposes the introduction of carbohydrate traces is not disastrous, but our polypeptides are intended for immunological work in which the presence of small amounts of carbohydrate antigens is highly undesirable. We have therefore abandoned the use of dextran gels for fractionation of our polypeptides, and have not examined the stability to carboxylic acid mixtures of gels with lower exclusion limits than G-150. However, it seems probable that solubilisation of the more highly cross-linked gels by strong formic and acetic acids also occurs but has escaped notice because the most commonly applied procedures for examining peptide-containing eluants would not have detected the presence of carbohydrate.

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Purification of ganglioside fractions by column chromatography on Sephadex G-100*

Gangliosides are generally extracted from brain tissue with chloroform–methanol (2:1) according to FOLCH-PI *et al.*[1]. After partitioning with water, the aqueous upper phase contains the gangliosides together with free amino acids, peptides, carbohydrates and a small quantity of lipids from the lower phase. The contamination of ganglioside fractions has long been recognized as a problem in radioactive and metabolic studies and various methods have been described for their purification[2,3].

This paper presents a simple and rapid technique for the purification of gangliosides by gel filtration on Sephadex G-100.

Experimental

Chemicals. Analytical grade reagents and redistilled organic solvents were used throughout.

Preparation of the lipid extracts. The experiments were carried out on Wistar rats, 30–40 days old. They were killed by decapitation and the whole brain quickly removed. The tissue was homogenized with chloroform-methanol (2:1) according to FOLCH-PI *et al.*[1]. The total lipid extract was washed once with water and twice with the "theoretical" upper phase (chloroform–methanol–water, 3:48:47), and the resulting upper phase plus the washing were taken to dryness, dissolved in 10 ml of chloroform–methanol (2:1) and "re-partitioned" with 2 ml of water according to SUZUKI AND CHEN[2].

The "crude ganglioside fraction" (CGF) obtained was kept for further experiments.

Purification of the CGF. The CGF was purified using two different procedures: (A) Sephadex G-100 chromatography or (B) dialysis.

(A) Sephadex G-100 was suspended in distilled water and poured into a 2 cm I.D. and 35 cm long column fitted with a Teflon stopcock and a porous glass plate. The final column height was adjusted to 25 cm by aspiration of the excess gel. Samples of the CGF taken to dryness and dissolved in 0.6 ml water containing between 300–1800 μg NANA were applied to the top of the column and eluted with distilled water at a flow rate of 8 ml/h. 2-ml fractions were collected in each tube and aliquots of these were used for further analysis. The effluent fractions were also monitored at 260 and 280 nm.

(B) In parallel experiments, a sample of CGF was exhaustively dialyzed against frequent changes of water, for four days, and used to check the results obtained by the Sephadex procedure.

Chemical procedures. The following analyses were carried out on the effluent from the column and on the dialyzed CGF: Ganglioside NANA was determined by the method of SVENNERHOLM[4] as modified by SUZUKI[5]. Protein determination was done according to LOWRY *et al.*[6]. The modified technique of ROSEN[7] was used for the estimation of amino acids, and lipid phosphorus was determined by the procedure of CHEN *et al.*[8].

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Analysis of the purified ganglioside fraction. The fractions eluted from the Sephadex G-100 column which contained gangliosides were pooled. Aliquots were withdrawn from the pool for: (a) total hydrolysis, followed by quantitative analysis of amino acids; (b) separation of the amino acids by descending paper chromatography; (c) separation of the gangliosides by thin-layer chromatography (TLC).

Hydrolysis was carried out in 6 N HCl at 105° *in vacuo* and qualitative analysis of amino acids was done by descending chromatography on 3 MM Whatman paper using butanol–acetic acid–water (60:15:25) for development. The solvent front reached the bottom of the paper after a 20 h period, at 37°. The amino acids were detected by spraying with 0.2 % ninhydrin in 95 % ethanol, drying and heating at 70° for 5 min.

TLC of gangliosides was done on 20 × 20 cm glass plates coated with Silica Gel G (0.5 mm thickness). The plates were developed in a horizontal Desaga BN-chamber with propanol–butanol–water (65:10:25) at 20–25°. The solvent reached the front of the plate in about 3 h, and the chromatography was continued for 2 more h. Detection of the spots and quantitation of the individual gangliosides were carried out according to MacMillan and Wherret[9].

Radioactive assay. In some cases, rats were injected subcutaneously with 1 μCi/g body weight of U-[^{14}C]glucose. 1 h after injection, the animals were killed by decapitation and their brains processed as described above. The radioactive samples from each fraction collected from the column were placed in vials containing 12 ml of Bray's solution[10] and counted in a Packard TriCarb Spectrometer (model 3003). Correction for quenching was done by the channel's ratio method[11,12].

Results

The pattern obtained with the CGF eluted through a column of Sephadex G-100 is shown in Fig. 1. Two peaks of absorbing material were resolved on this column. Peak I was eluted with the exclusion volume and contained the gangliosides and a small amount of contaminants. The bulk of amino acids and peptides, as well as other contaminating material was eluted with the total volume of the column (Peak II). Both peaks were clearly separated without overlapping.

A small amount of material absorbing at 260 nm was detected in Peak I. However most of the contaminants absorbing at this wavelength, possible nucleotide-sugars, were eluted together with Peak II.

Chromatography of labelled CGF showed that 99 % of the radioactivity was eluted with Peak II and only 1 % with Peak I.

In agreement with observations made by other investigators[13,14] the CGF still contained peptides and amino acids which were detectable by both the ninhydrin and Lowry procedures. Fig. 1 shows that even after purification on Sephadex G-100, our ganglioside fraction (Peak I) contained small amounts of peptides. Table I shows the results obtained by hydrolysis of this peak; there was fourteen-fold increase in free amino acids after hydrolysis. Paper chromatography did not show positive ninhydrin material, but after hydrolysis five well defined spots appeared (Fig. 2). No attempt was made to characterize these spots. A few TLC plates of gangliosides were sprayed with ninhydrin reagent, and purple spots appeared at the individual ganglioside positions.

The ganglioside NANA recovered in Peak I was 88 %, and TLC of this fraction

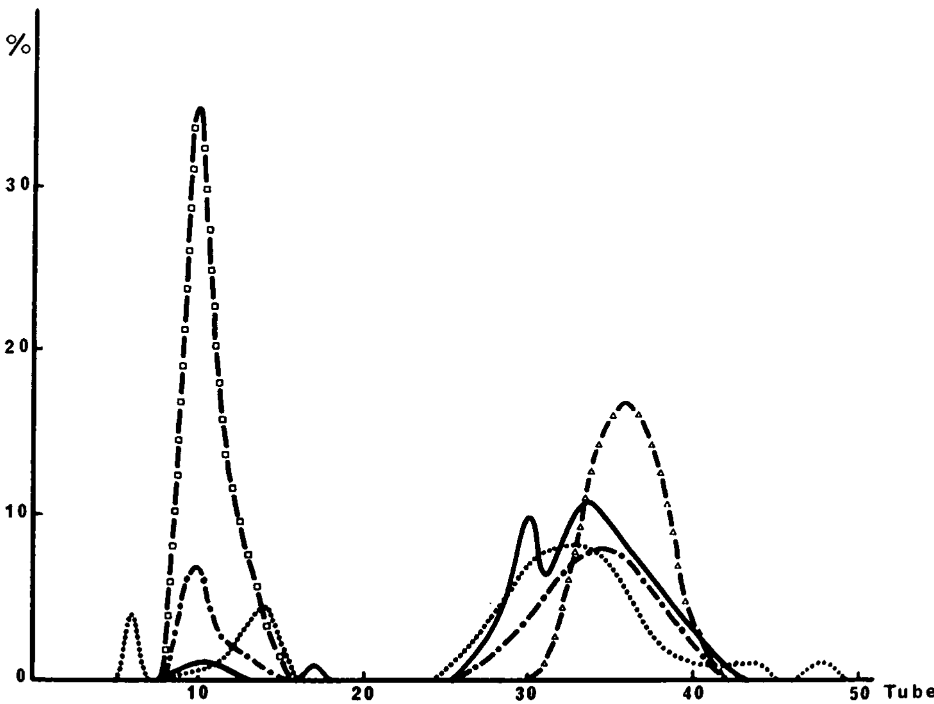

Fig. 1. Chromatographic pattern of the crude ganglioside fraction after elution from the Sephadex G-100 column. 2-ml fractions were collected in each tube as described under *Experimental*. Results are expressed as a percentage of the total amount eluted from the column. (□—□) gangliosides; (———) amino acids; (······) protein; (●—●) absorbance at 260 nm; (△—△) radioactivity.

TABLE I

ACID HYDROLYSIS OF PEAK I

Each figure represents the mean value of two separate experiments. For technical details see text.

	μmoles amino acid/g fresh tissue
Before hydrolysis	0.97
After hydrolysis	14.50

showed that the percentage distribution of the individual gangliosides was similar to that found in dialyzed CGF (Table II). It is clear (Table III) that our purified ganglioside fraction contained a much smaller amount of all the contaminants.

Discussion

Various methods have been described for the purification of the ganglioside fraction. Gangliosides are soluble in water and form high molecular weight micelles (mol. wt. 250000–450000) which do not dialyze through cellulose membranes[17]. This

property has been used for the purification of ganglioside fractions, but the long time required for dialysis (four days) is, however, its major limitation.

To ensure the complete elimination of contaminant lipids, SUZUKI[2] developed the "re-partition" technique. A rather exhaustive dialysis was necessary, anyway,

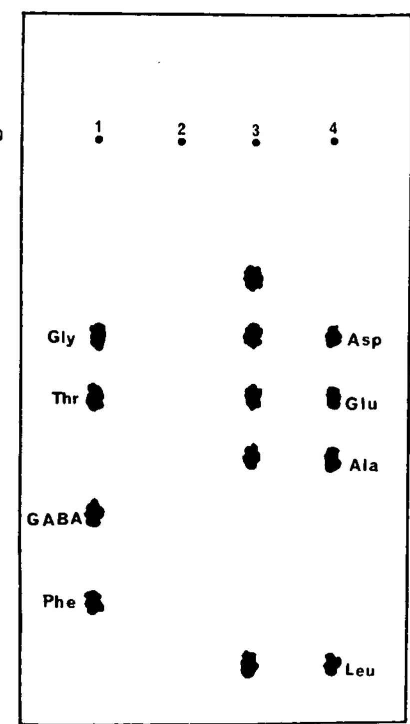

Fig. 2. Tracing of a one-way descending paper chromatogram of amino acids. Chromatographic conditions: 3 MM Whatman paper; solvent, butanol–acetic acid–water (60:15:25); development time, 20 h; development temperature, 37°; spray reagent, 0.2% ninhydrin in 95% ethanol. Samples: 1 and 4 = reference mixtures of amino acids; 2 = Peak I; 3 = Peak I after hydrolysis; 0 = origin.

TABLE II

COMPARISON OF THE INDIVIDUAL GANGLIOSIDE DISTRIBUTION ON TLC

Nomenclature of gangliosides is that of KOREY AND GONATAS[15] with the corresponding SVENNERHOLM[16] nomenclature in parentheses. Results are expressed as a percentage of the total gangliosides recovered from the plate. A = CGF purified on the Sephadex G-100 column; B = CGF purified by dialysis. For technical details see text.

Ganglioside	*A*	*B*
G_0	11.0	7.1
G_1 (G_{T1})	16.1	19.5
G_2 (G_{D1b})	13.8	16.9
G_3 (G_{D1a})	32.3	30.1
G_4 (G_{M1})	23.2	21.4
G_5 (G_{M2})	3.5	4.9

TABLE III

COMPARISON OF THE TWO PURIFICATION METHODS

A = CGF purified on the Sephadex G-100 column; B = CGF purified by dialysis; % = percentage of the total amount found in CGF. Experimental details, see text.

	CGF	*A*	*B*
Gangliosides			
μg NANA/g fresh tissue	374	329	380
%	100	88	103
Amino acids			
μmoles leucine/g fresh tissue	34.10	0.51	5.06
%	100	1.5	14.9
Protein			
μmoles albumin/g fresh tissue	320	120	230
%	100	37	72
Phospholipids			
μg P/g fresh tissue	69	16	33
%	100	23	48

to eliminate the trace amounts of free amino acids and other low molecular weight substances.

WELLS AND DITTMER[18] used Sephadex G-25 columns to exclude water soluble precursors and metabolites from the total lipid extract, but these authors did not remove amino acids from extracts which had been previously partitioned with water or salt solutions, and this procedure did not give quantitative separation of brain lipids. SIAKOTOS AND ROUSER[19] used a Sephadex G-25 column for the complete separation of the major lipid classes from water soluble non-lipids; they eluted with four different solvent systems and obtained gangliosides as a separate fraction. ROUKEMA AND HEIJLMAN[3] combined both purification methods: 24 h dialysis and Sephadex G-25 columns.

Our chromatographic procedure on Sephadex G-100 columns, as outlined, requires a minimum of working time and yields a consistently good recovery. The purified ganglioside fraction does not show any modification with regard to the pattern of migration on TLC, and it is available for further analytical and metabolic studies. The contamination of our purified fraction by phospholipids and specially by peptides, is considerably lower than the contamination obtained by dialysis.

An association of gangliosides and peptides has been demonstrated by ROSENBERG AND CHARGAFF[13] and other authors[14, 20]. TRAMS AND LAUTER[21] obtained a ganglioside fraction which was peptide free, after a tedious and time consuming procedure. The presence of small amounts of peptides in our purified ganglioside fraction is consistent with BOOTH's hypothesis[22] of some association between gangliosides and peptides in aqueous systems, by ionic interaction.

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Very long Z-shaped thin-layer chromatography and its application to the separation of dyes

Thin-layer chromatographic (TLC) separation of substances with R_F values about 0.1 calls for special procedures, such as the continuous flow method, which differs only from the normal technique in that the development time can be made as long as required. However, if low polarity components are present in the initial mixture, they will migrate to the end of the plate and as a result of this they will appear mixed together with the solvent and plate impurities in the case of the ascending procedure, or they will be washed off the plate in the case of descending chromatography.

Although these difficulties can be overcome by employing very long plates[1], special equipment is required for their handling. However, it is also possible to increase the actual length of the development zone considerably by the suitable arrangement of the absorbing material on commercial size plates.

To this end we have used TLC plates from which we have partially removed the adsorbing layer in order to form a zigzag development path with the remaining adsorber; development zones up to 2 m long can be made this way from 20 × 20 cm plates, the length depending on the width of the development zone.

Experimental and results

The shape we have adopted can be seen in Fig. 1. Particular attention has been

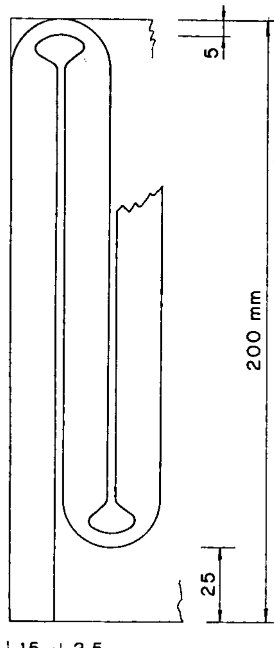

Fig. 1. Arrangement of the adsorbent on the plate.

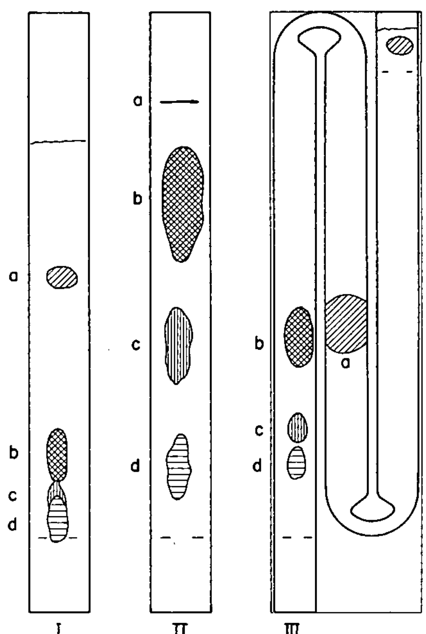

Fig. 2. Comparison of the present technique with other TLC methods. I normal; II continuous flow; III Z-shaped. (a) dimethyl yellow; (b) rhodamine B; (c) methyl red; (d) 2',7'-dichlorofluorescein.

paid to the bent portions in order to ensure that the distance traveled at both edges of them is almost the same, otherwise the spot length would increase at each turn. It has been found experimentally that 5 mm is a suitable width at corners for straight development zones 15 mm wide, although other widths can be used, according to GRIESEMER[2], because the influence of this dimension on R_F values is slight.

The scraped portions of the plate shall be cleaned very carefully in order to avoid solvent transfer from one strip to the next through residual particles of adsorbent. A few minutes only are needed to prepare a plate if a template is used for drawing the path and as a guide for removing the excess of adsorbent.

As a demonstration of the advantages of this procedure as compared with other methods, the separation of four dyes (dimethyl yellow, methyl red, 2',7'-dichlorofluorescein and rhodamine B) has been carried out with acetone as solvent, on precoated silica gel plates 20 × 20 × 0.025 cm (Merck, ref. 5715), in cylindrical tanks 5.5 cm diam. and 21 cm high with saturated atmosphere. The results achieved with three different separation procedures can be seen in Fig. 2; these procedures are as follows:

(a) Normal ascending chromatography, development time 30 min: only dimethyl yellow is separated from the other dyes.

(b) Ascending chromatography with continuous flow: the procedure described by BOBBITT[3] has been employed. A continuous flow of the solvent is maintained by passing the top of the plate through a slot in the tank lid. After a development time of 4 h 30 min, the four dyes were separated but dimethyl yellow is found at the front along with the solvent and plate impurities.

(c) Very long, Z-shaped chromatography: a plate 50 mm wide with an effective adsorbent width of 14 mm is used; the actual length of the development zone is 56 cm. Contact of the adsorbent with the saturating paper wick is avoided by scraping a zone 1 mm wide at each side of the plate. After 5 h 30 min of development time, a good separation of the four dyes is achieved with the advantage that dimethyl yellow can be recovered free of impurities. It becomes increasingly difficult to visualize the position of the solvent front along the path; therefore, in order to know when the solvent front arrives at the end of the path or at any other place, it is advisable to apply a small amount of a low-polarity dye, such as dimethyl yellow, at this point. Its development points out the end of the experiments.

The separated substances can sometimes be identified by the normal methods of TLC to find out their relative positions; at other times, an independent Z-shaped chromatogram will be needed in order to determine the R_F value of a standard. We have used the first method to distinguish between dimethyl yellow and methyl red; the other two dyes are fluorescent and they have been identified with UV light.

R_F values obtained by this procedure are lower than those from normal TLC because the dry adsorbent is excessively exposed to the vapors of the solvent; this is equivalent to saturating the adsorbent previously with solvent vapor[4].

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Die dünnschichtchromatographische Trennung einiger Isoflavone neben Cumöstrol

Die östrogene Aktivität einiger Flavonoide gab den Anlass zu ihrer chromatographischen Trennung (Dünnschichtchromatographie siehe z.B. Lit. 1–8). Für den gleichzeitigen Nachweis von synthetisiertem Daidzein, Genistein, Pratensein, Formononetin, Biochanin A und Cumöstrol wurde eine schnelle und einfache Methode gesucht.

Eine Trennung dieser Aglycone erwies sich sowohl auf Kieselgel G Merck- als auch auf Cellulose MN 300-Schichten mit den verschiedensten Laufmitteln als unbefriedigend. Kritische Paare waren vor allem Formononetin und Biochanin A sowie Daidzein und Genistein, die sich auf diesen Sorptionsmitteln überlagerten. Erst mit Polyamid Woelm DC wurden wesentliche Unterschiede zwischen den hR_F-Werten der einzelnen Substanzen erzielt.

Für eine Platte 10 × 20 cm ist 1 g Polyamid mit einem Zusatz von 0.2 g Cellulose erforderlich. Die Platten wurden im Giessverfahren mit 6 ml 96 %igem Äthanol hergestellt und luftgetrocknet. Das geeignetste Fliessmittel wurde in der Mischung von Petroläther (Kp. 30–60°)–Benzol–Methyläthylketon–Methanol–Essigsäure (30:30:20:20:2) gefunden. Die Laufstrecke betrug 17 cm nach 30 min bei 23° und Kammersättigung. Auftragsmenge: Formononetin, Daidzein und Pratensein, 2 μg; Genistein und Biochanin A, 6 μg; Cumöstrol, 0.2 μg.

TABELLE I

hR_F-WERTE EINIGER ISOFLAVONE UND CUMÖSTROL

	hR_F	
	Nach 30 min	*Nach 90 min*
Cumöstrol	6	6.5
Daidzein	28	30
Genistein	32	39
Pratensein	41	49
Formononetin	62	67
Biochanin A	66	79

Die hR_F-Werte sind in Tabelle I zusammengefasst. Die Trennung der Substanzen lässt sich noch verbessern, wenn die Platte weitere 60 min bei gleichen Bedingungen ohne vorheriges Trocknen in der Kammer bleibt, nachdem das Fliessmittel die zuvor markierte Linie im Abstand von 17 cm zum Start erreicht hat (siehe Tabelle I). Wird die Polarität des Laufmittels erhöht, so erzielt man zwar grössere hR_F-Werte, aber keine schärfere Trennung. Die Reproduzierbarkeit der Zahlen war nur mit einem frisch bereiteten Fliessmittel gewährleistet. Mit dem wiederholtem Gebrauch des Lösungsmittelgemisches nehmen sowohl die hR_F-Werte als auch ihre Differenzen zu, woraus sich eine Verkürzung der erforderlichen Trennzeit ergibt.

Tabelle II zeigt einige Nachweismethoden der untersuchten Substanzen mit

TABELLE II

NACHWEISMÖGLICHKEITEN EINIGER ISOFLAVONE UND DES CUMÖSTROLS AUF DER POLYAMID-DÜNNSCHICHT-PLATTE

Abkürzungen: TG = Tageslicht; Wärme = 5 min auf etwa 110° erhitzen. Sprühreagentien: (1) —; (5) 2 % Zirkonium (IV)-oxidchlorid in Methanol; (6) 1 % Diphenylborsäure-2-aminoäthylester in Methanol; Natrium in Äthanol; (9) Benedict-Reagens: 1.73 g Kupfersulfat crist., 17.3 g Natriumcitrat, 10.0 g 2 % Eisen (III)-chlorid in Wasser, Lösungen 1:1 kurz vor Gebrauch mischen; (11) 0.5 % Echtblausalz B Sulfanilsäure[9]; (13) Diazotiertes Benzidin[10]; (14) 5 % Phosphormolybdänsäure in Äthanol.

	1	*2*		*3*	*4*	
	UV	*UV*	*Wärme + UV*	*UV*	*Wärme + UV*	*Ammoniak + UV*
Cumöstrol	violett	rosaviolett	blau, später gelb	violett	violett	violett
Daidzein	blassblau	weissblau	weissblau	weissblau	—	blau
Genistein	dunkel	dunkel	dunkel	—	gelbgrün	gelb
Pratensein	dunkel	dunkel	dunkel	—	gelbgrün	gelb
Formononetin	blassblau	weissblau	weissblau	weissblau	—	blau
Biochanin A	dunkel	—	dunkel	—	gelbgrün	gelb
Bemerkungen						

TABELLE II (*Fortsetzung*)

	8	*9*	*10*			*11*
	UV	*UV*	*TG*	*Wärme*		*TG*
				TG	*UV*	
Cumöstrol	hellviolett	violett	—	—	violett	—
Daidzein	dunkel-violett	weiss-blau	—	blaugrün	—	—
Genistein	dunkel violett	dunkel	—	blau	—	violett
Pratensein	dunkel-violett	dunkel	blaugrün[a]	kräftig blau	—	intensiv violett
Formononetin	dunkel-violett	weiss-blau	—	—	—	—
Biochanin A	dunkel-violett	dunkel	—	blaugrün[b]	—	violett
Bemerkungen	Untergrund grün		[a] Sofort sichtbar Nach Stunden alle Flecken ausser Formononetin intensiv blau	[b] Am schwächsten		Untergrund gelb, Färbung der Flecken sehr langsam

(2) 5 %ige, wässrige Natronlauge; (3) 25 %ige Ammoniaklösung; (4) 1 % Aluminiumchlorid in Äthanol; (7) 2 % Fluoreszenzindikator Woelm 254 nm wurden dem Polyamid beigemischt; (8) 0.04 % Fluorescein-Natriumcarbonat wasserfrei zu 100 ml in Wasser lösen; (10) 1 % Kaliumhexacyanoferrat (III) in Wasser, in Wasser, frisch herstellen, 0.1 *N* Natronlauge, Lösungen nacheinander aufsprühen. (12) Diazotierte

5		*6*					*7*
Wärme + UV	*Ammoniak + UV*	*UV*	*Wärme*		*Ammoniak*		*UV*
			TG	*UV*	*TG*	*UV*	
violett	violett	violett	—	violett	—	violett	hellviolett
—	weissblau	—	—	—	—	weissblau	schwach dunkelblau
gelbgrün	olivgrün	gelb	schwach gelb	leuchtend gelb	grüngelb	leuchtend gelb	dunkelblau
gelbgrün	olivgrün	gelb	schwach gelb	leuchtend gelb	grüngelb	leuchtend gelb	dunkelblau
—	weissblau	—	—	—	—	weissblau	schwach dunkelblau
gelbgrün	olivgrün	gelb	schwach gelb	leuchtend gelb	grüngelb	leuchtend gelb	dunkelblau
							Untergrund hellgrün

12				*13*		*14*	
TG	*UV*	*Ammoniak*		*TG*	*UV*	*Wärme*	
		TG	*UV*			*TG*	*UV*
—	violett	—	violett	—	violett	—	violett
—	—	—	weissblau	—	blassblau	—	blau
blassgelb	dunkel	gelb	dunkel	orangebraun[c]	dunkel	—	dunkel
kräftig gelb	dunkel	gelb	dunkel	stark[d] orangebraun	dunkel	graugrün	schwarz
—	—	—	weissblau	—	blassblau	—	blau
blassgelb	dunkel	gelb	dunkel	orangebraun[e]	dunkel	—	grau

[c] Farbe sehr blass, spät sichtbar
[d] sofort sichtbar
[e] Färbung gering, wird allmählich sichtbar
Farben nicht beständig, Platte dunkelt nach

verschiedenen Sprühmitteln. Ohne Besprühen geben sich im UV-Licht Formononetin und Daidzein durch eine schwache, helle-, Cumöstrol durch eine stark violette Fluoreszenz und Genistein, Pratensein sowie Biochanin A als dunkle Flecken zu erkennen. Eine Identifizierung ist somit nicht nur durch die hR_F-Werte, sondern auch durch die verschiedenen Farbreaktionen der Isoflavone und des Cumöstrols bei Tages- und UV-Licht möglich (siehe Fig 1).

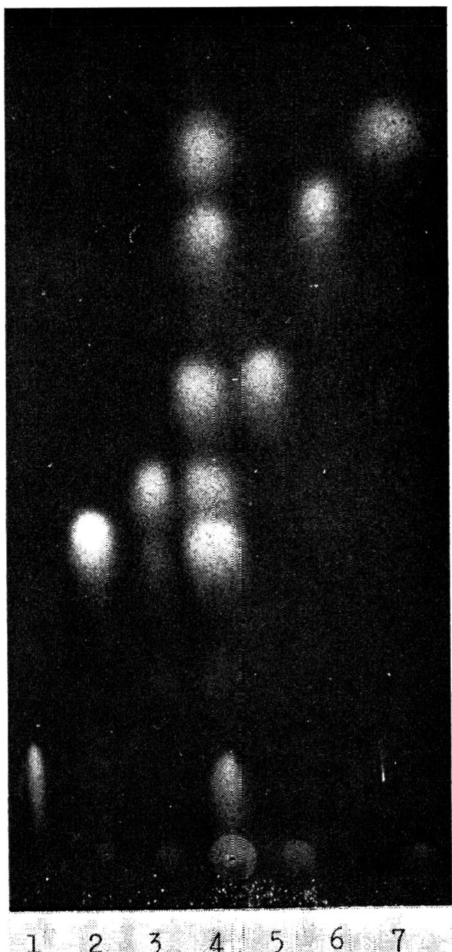

Fig. 1. Dünnschichtchromatogramm einiger Isoflavone und Cumöstrol im UV-Licht aufgenommen. 1 = Cumöstrol; 2 = Daidzein; 3 = Genistein; 4 = 1–3 und 5–7; 5 = Pratensein; 6 = Formononetin; 7 = Biochanin A. Laufzeit, 90 min; Sprühmittel, Reagens 6 und 9, siehe Tabelle II. Weitere Einzelheiten im Text.

Wie aus den Angaben zu ersehen ist, können mit dieser Methode 6 chemisch engverwandte Stoffe im eindimensionalen Dünnschichtverfahren leicht und rasch getrennt werden.

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CHROM. 5312

A new chromatographic spray for the detection of catechins

The colour tests hitherto described in the literature for the detection of catechins on paper chromatograms are rather non-specific and apply to phenolic compounds in general[1] (*e.g.* vanillin–sulphuric acid, sulphanilic acid, *p*-nitroaniline and ferric chloride–potassium ferricyanide reagents). A colour reaction is now described which appears to be specific for the catechins on paper chromatograms. It possesses a high degree of sensitivity and very stable red spots are formed.

The dry chromatogram was sprayed with a 1 % solution of 2,4,6-trinitrophenol in 95 % ethanol, and when the papers were visibly dry, the chromatograms were sprayed again with a 5 % solution of potassium hydroxide in 80 % ethanol (Jaffé-reaction). After 30 sec, stable red spots were given by the catechins. (This colour could be matched with Chinese Red of the Permoglaze colour chart or Geranium Lake of the Derwent colour chart).

It has been recorded rather ambiguously in the literature that the formation of orange or red colours with the above reagents was a characteristic of the guanidines[1] (for a detailed discussion of this reaction see *e.g.* ref. 2). Tests were carried out in our laboratories in order to verify the validity of this statement. The common guanidines such as creatinine, glycocyamine and guanidine gave orange colours which were quite distinct from the characteristic red given by the catechins. Tests were also carried out to determine whether other polyphenols would give a similar colour reaction with these reagents. The polyphenols tested included quercitin, myricetin, caffeic acid, naringenin, coumarin, catechol, phloroglucinol and leucocyanidin (polymer). None of these compounds responded to the test.

The four main catechins found in tea leaves, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate, all gave positive results. The attachment of the galloyl residue in position 2 did not seem to affect the colour reaction (*e.g.* epigallocatechin), but the formation of galloyl esters in position 3 (*e.g.* epigallocatechin gallate) retarded the colour formation to some extent. Similarly the theaflavins formed during tea fermentation answered this test as the basic catechin residue remained unaltered. It was also observed that the closely related leucoanthocyanins (flavane-3,4-diols) present in tea leaves did not give this test. A similar observation was made with the flavones such as naringenin and quercitin which possess double bonds between positions 2 and 3. It was also interesting to note that the use of *o*-nitrophenol, *p*-nitrophenol or even 2,4-dinitrophenol in place of the trinitrophenol, did not give the characteristic red colour with the catechins.

On paper, this colour was very stable and the chromatogram could be preserved for a week or even more unlike the pink colour given by the vanillin–sulphuric acid reagent which faded after 20 min. This method could detect concentrations of catechins of the order of 10 p.p.m. by weight (0.01 mg) and was more sensitive than most of the other reagents, except the vanillin–sulphuric acid reagent which could detect even smaller concentrations (1 p.p.m.).

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CHROM. 5296

Tritium exchange in Sephadex® G-10

Deuterium of deuterated water exchanges freely with the hydroxyl hydrogens of glucose[1], amylose and starch[2] even if the latter contains crystalline regions. In cellulose, however, only the hydroxyl groups in the amorphous regions are apparently accessible to deuterium[3,4]. Since an X-ray powder diagram of Sephadex G-10 did not indicate any crystallinity[5] it is to be expected that tritium of tritiated water will exchange completely or almost completely with the gel hydroxyls, and some exchange has been reported briefly in G-25[6]. Since, however, complete information regarding the cross-linking structure of the gel is not available it is not possible to predict with any precision the magnitude of the expected exchange. A very rough calculation of this value was made from an approximate value of the cross-linking density (kindly provided by Mr. B. SÖDERQVIST) by assuming, what is almost certainly, an over-simplified version of the cross-linking structure. The cross-links were assumed to be all single without any polymerisation into chains or branching at their non-terminal hydroxyl groups; half of the units were assumed to be linked to two dextran chains and thus to form complete cross links, while the other half were assumed to be fixed to the dextran chains at one end only.

Assuming, further, that the equilibrium constant of the exchange reaction is unity[2], the ratio between the number of exchange sites (hydroxyl groups) in the gel matrix (dextran chains and cross-linking structures) and the number of sites in the imbibed water of the gel (two exchange sites per water molecule) should be about 0.1. This means that the K_d value for tritium loaded as tritiated water (HTO) would be about 1.1 and provided, as seems to be the case at least under certain conditions, that column experiments are quasi-equilibrial, the column K_d value for tritium should also be the same. The exchange reaction will thus retard the movement of tritium introduced as HTO along the column. If water labelled with an oxygen isotope is eluted, the isotopic oxygen should move through the column at the same rate as water, since ^{18}O of $H_2{}^{18}O$ does not exchange with hydroxyl or carboxyl groups[7], of which there are also a few of the latter in the G-type Sephadex gels. Thus, the difference between the elution volumes of $H_2{}^{18}O$ and tritium of HTO should be an estimate of the extent of the tritium exchange.

Since it can be shown that the ratio between the number of hydroxyls in the gel matrix and the number of water molecules in the imbibed water of the gel increases with decreasing water regain, the G-10 gel should exhibit the greatest exchange among the members of the G-type series at present available.

Experimental

Tritiated water (Radiochemical Centre, Amersham, Great Britain) and $H_2{}^{18}O$ (loading concentration 0.4 atoms excess per cent, obtained from Nuclear Equipment Chemical Corp., New York, U.S.A. as 97 % $H_2{}^{18}O$) were eluted together with Dextran® 500 ($\overline{M}_W$ = 450000, Pharmacia Ltd., Uppsala, Sweden) as void volume indicator through a column (60 cm × 0.8 cm²) of Sephadex G-10 (Batch No. 2154). The volume of the loading solution was 0.5 ml.

Dextran was determined by an anthrone method[8], tritium by liquid scintillation

(Beckman C PM 200) and ^{18}O of $H_2{}^{18}O$ was exchanged with CO_2 (refs. 9, 10) and determined as $C^{18}O^{16}O$ in a mass spectrometer (Consolidated Electronics Corp., Model 21/201).

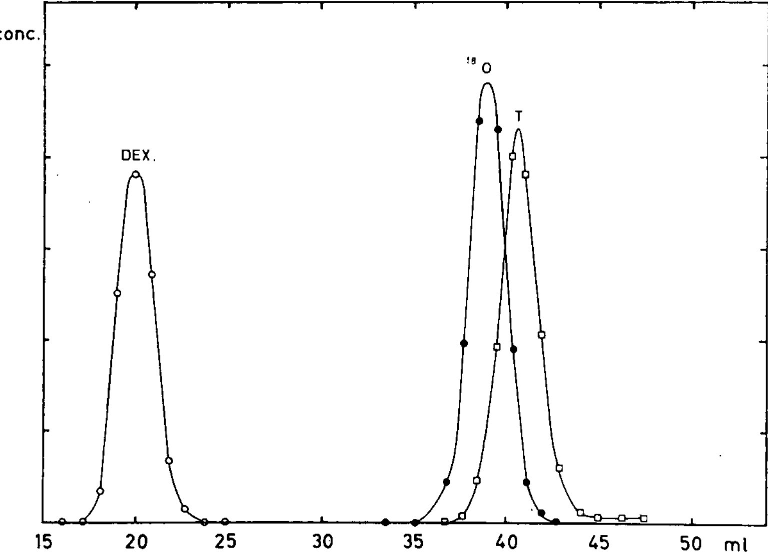

Fig. 1. Elution diagram for Dextran 500, tritium (T) and ^{18}O; the latter two components were introduced on to the column as labelled water.

The results of an experiment are shown in Fig. 1. The ^{18}O peak precedes that of tritium and if the distribution coefficient (K_d) is defined as

$$K_d = \frac{V_T - V_0}{V_w - V_0} \qquad (1)$$

where V_T, V_w and V_0 are the elution volumes of tritium, ^{18}O and dextran, respectively, the K_d value of tritium equals 1.091, which is quite near the value of 1.1 predicted above. This value means that if tritiated water is used as a reference solute a correction must be made, in calculating K_d values since tritiated water gives an overestimate of the water space in the column and hence an underestimate of the K_d value.

Thus, if[6]

$$K_{T_i} = \frac{V_i - V_0}{V_T - V_0} \qquad (2)$$

and

$$K_{d_i} = \frac{V_i - V_0}{V_w - V_0} \qquad (3)$$

where i refers to any solute, then

$$K_{d_i} = \phi\, K_{T_i}, \qquad (4)$$

where

$$\phi = \frac{V_T - V_0}{V_w - V_0}$$

For G-10, ϕ is thus 1.09, while for G-25 it is about 1.06 (ref. 6). ϕ will become increasingly smaller as the water regain value increases and should be less than about 1.02 in G-200.

I should like to thank Miss Å. HAGLUND and Mr. O. FEX for their help and Mr. MAX WAHLBERG for the mass spectrometric measurements.

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Book Reviews

CHROM. 5388

Interacting Macromolecules, by JOHN R. CANN, with a contribution by WALTER P. GOAD, Academic Press, New York and London, 1970, 249 pages, prices $ 12.50.

This very useful book was designed to cover the theory and practice of electrophoresis, ultracentrifugation and chromatography of interacting macromolecular systems. It provides a concise treatment of the mass transport of noninteracting systems and weak electrolyte moving boundary theory. Rather extensive coverage is given to the problem of interpreting, as well as predicting, the detailed shapes of moving boundaries as well as zones in such systems, with the aid of numerical methods and digital computers. The possible consequences of the shifting of chemical equilibria by pressure during sedimentation are also covered in some detail.

Although much of the material presented in this book has already been published, by the author of the book and by many others, there has been no single up-to-date publication to which one might refer for both a methodical treatment of the whole field and a rather thorough bibliography of pertinent literature. This book carries a message, which cannot be emphasized too strongly, to the investigator handling materials of natural origin: most macromolecular systems are likely, while being analyzed, to be reacting internally in a real chemical sense, showing either macromolecule–macromolecule interactions or macromolecule–buffer component interactions. Such possible interactions during analytical separations must be investigated and understood. If this is not done, the investigator is likely to provide a purely classical, but perhaps faulty foundation for a huge seemingly beautiful superstructure which will all come tumbling down, once it has been built.

This reviewer has used this book as a textbook in a graduate Biophysics course called *Separation Methods*, with considerable success, and intends to do so again. It is a book which should be strongly recommended as a reference text for investigators using separation techniques as analytical tools.

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CHROM. 5406

Allgemeine und anorganische Chemie, by VIKTOR GUTMANN AND EDWIN HENGGE, Verlag Chemie, Weinheim/Bergstr., 1971, 362 pages, price DM 39.

Inorganic chemistry has made such advances in the last twenty-five years that in some fields new books have little in common with the older ones. This short textbook is one of the best of the books that have appeared recently. It contains a wealth of material and is equally suitable as textbook and as reference work. To those organic chemists and biochemists who want to consult an inorganic text for quick information on topics such as polyphosphates or metal complexes, this book can be recommended, as well as for the teacher looking for a first-year text.

There are very few typing errors (p. 269 last paragraph is repeated) and the subject index is adequate.

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Errata

J. Chromatogr., 56 (1971) 177

Page 177, line 2, "price £ 7" should read "price £ 5.00".

J. Chromatogr., 56 (1971) 168–171

Page 168, paragraph 5, *Staining for enzyme activity*, first sentence should be changed to two sentences: "The gel was removed from the glass tubes and placed in 4 % starch in phosphate buffer, pH 7, for 2 h. The gels were then washed briefly in running water, placed in a 0.4 % solution of H_2O_2 in phosphate buffer pH 7 for 30 sec, washed with running water and placed in a 1 % solution of potassium iodide acidified with acetic acid."