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Journal of Chromatography (incorporating *Biomedical Applications* and *Chromatographic Reviews*) In the course of 1977, also the cumulative indexes for Vols. 121–130 and 131–140 will appear.

MONTH	J	F	M	A	M	J	J	A	S	O	N	D
Journal of Chromatography	130 131	132/1 132/2 132/3	133/1 133/2	134/1 134/2	135/1 135/2	136/1 136/2 136/3	137/1 137/2	138/1 138/2	139/1 139/2	140/1 140/2 140/3	142 144/1	144/2 144/3
Biomedical Applications	143/1		143/2		143/3		143/4		143/5		143/6	
Chromatographic Reviews				141/1				141/2				141/3

Scope. The *Journal of Chromatography* publishes papers on all aspects of chromatography, electrophoresis and related methods. Contributions consist mainly of research papers dealing with chromatographic theory, instrumental development and their applications. The section *Biomedical Applications*, which is under separate editorship, deals with the following aspects: developments in and applications of chromatographic and electrophoretic techniques related to clinical diagnosis (including the publication of normal values); screening and profiling procedures with special reference to metabolic disorders; results from basic medical research with direct consequences in clinical practice; combinations of chromatographic and electrophoretic methods with other physicochemical techniques such as mass spectrometry. In *Chromatographic Reviews*, reviews on all aspects of chromatography, electrophoresis and related methods are published.

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For further information, see page 3 of cover.

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Microbiological Aspects of Pollution Control

R. K. DART *and* R. J. STRETTON, *Department of Chemistry, University of Technology, Loughborough, Leicestershire, U.K.*

FUNDAMENTAL ASPECTS OF POLLUTION CONTROL AND ENVIRONMENTAL SCIENCE, 2

This book deals with the applied microbiology of pollution and its control in connection with public health and hygiene. Each of the eleven chapters contain a self-contained review of recent developments up until late 1976, including a large number of references to primary sources of information.

The initial chapters discuss the microbial production of pollutants and air pollution with an emphasis on both the bacteria and fungi, their ability to act as pollutants themselves and to act by producing chemical pollutants. The major portion of this work is devoted to the applied microbiology of water, its pollution by sewage and its treatment to render it potable again. The health hazards of polluted water are described, and the measures taken to minimize these risks are considered. In addition, descriptions are given of the problems of eutrophication, thermal pollution, oil pollution and mine drainage. The potential of micro-organisms in mineral recovery is also considered. The biochemistry of degradation is dealt with in the final chapter, and consideration is given to the deliberate manufacture of plasmid containing bacteria which could be seeded into a polluted environment to carry out a specific task.

This book will enable the pure scientist to gain an insight into the applied aspects of microbiology, and will also enable the engineer to have an insight into the pure science basis of plant design and control.

CONTENTS: Introduction. 1. Microbial Production of Pollutants. 2. Air Pollution and Micro-organisms. 3. Health Hazards Arising from Water-Borne Pathogens. 4. Water Testing. 5. Sewage Treatment. 6. Disinfection and Recycling of Water. 7. Eutrophication. 8. Thermal Pollution. 9. The Sulphur Cycle and Waste Recovery. 10. Oil Pollution. 11. Biodegradation.

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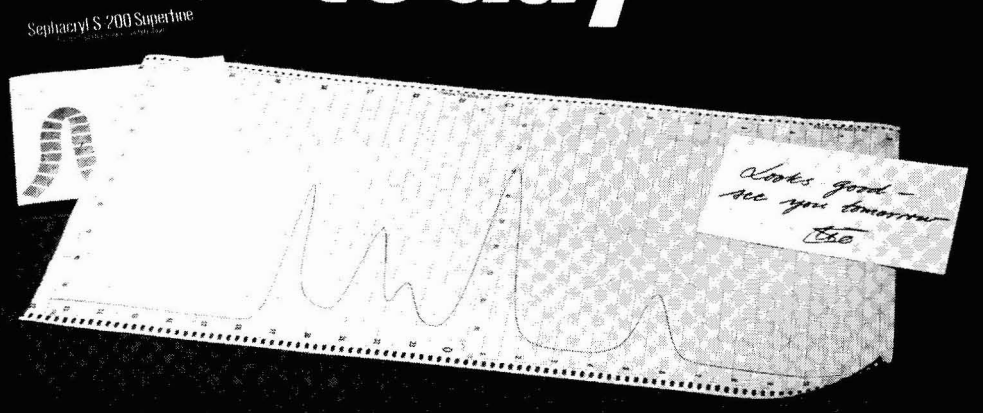

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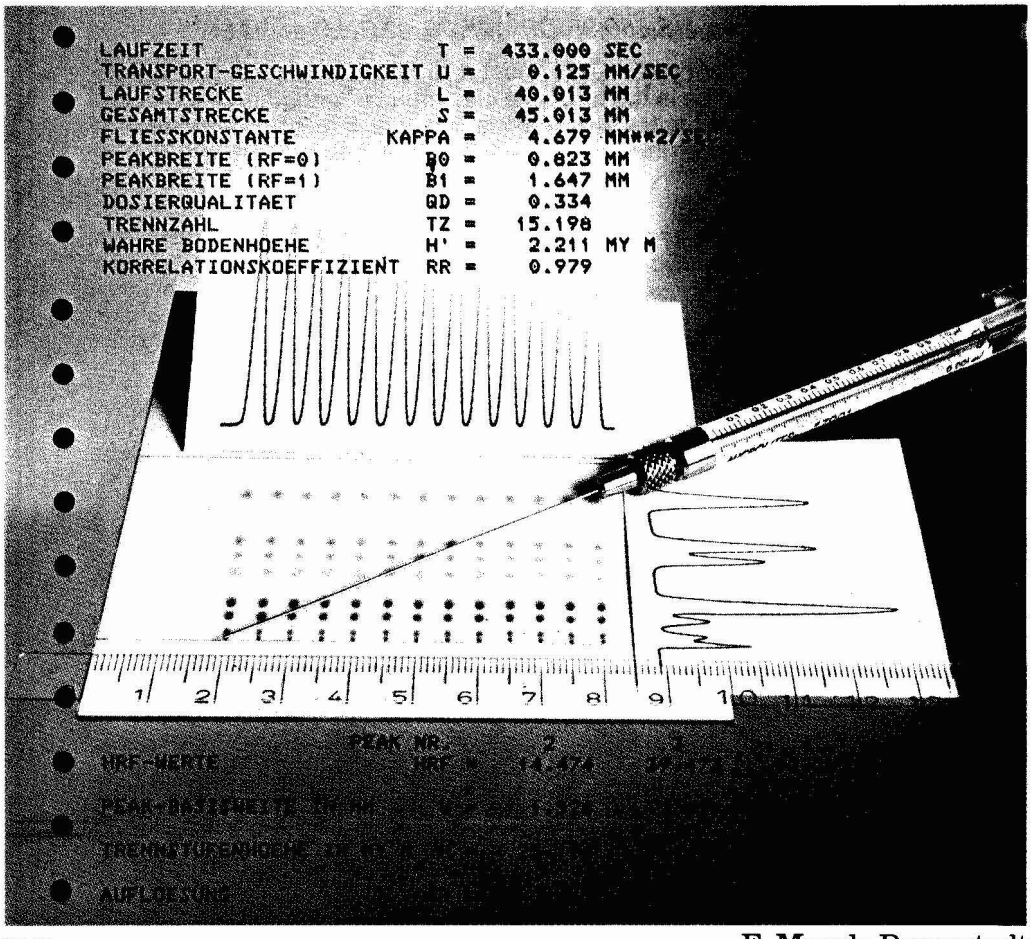

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CHROM. 10,164

INCREMENTATION OF POLAR EFFECT CONSTANTS IN GAS–LIQUID CHROMATOGRAPHY

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(Received March 28th, 1977)

SUMMARY

In chromatographic studies polar effect constants σ_c^*, similar to those of Taft and Ingold, can be obtained from an empirical additivity relationship which takes into account three structural increments.

INTRODUCTION

Each carbon atom of an alkyl group contributes to some extent to the final values of chromatographic retention data. Consequently, in this area σ^* values of alkyl groups beyond those of the classical Taft scale are often needed. This situation differs considerably from that encountered in reactivity studies, where the contribution of the alkyl groups remains constant, once a certain size has been reached.

In previous papers[1,2] we have shown that a scale of polar effect constants σ_c^* can be determined by means of the following relation:

$$\sigma_c^* = \frac{1}{\varrho^*}\left[\log t'_{R_{RZ}} - h\,(n_H - 3)\right] \quad (1)$$

in which $\log t'_{R_{RZ}}$ is the reduced relative retention time of an aliphatic saturated compound RZ (R alkyl group, Z functional group), n_H is the number of hydrogen atoms attached to the carbon atom of R neighbouring the functional group, and ϱ^* and h are the sensitivities to the polar and ramification effects in the chromatographic phenomenon. Using this approach, we determined 42 values of σ_c^* (Table I).

The results reported here indicate the existence of an empirical additivity relationship which can be used for the calculation of any σ_c^* value.

* To whom correspondence should be addressed.

TABLE I

CHROMATOGRAPHIC POLAR EFFECT CONSTANTS σ_c^*

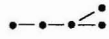
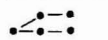
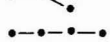
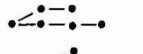
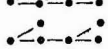
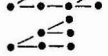
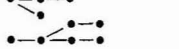
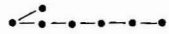
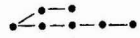
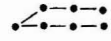
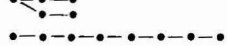
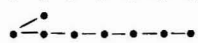
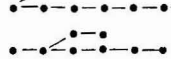
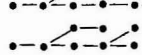
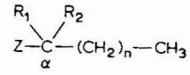
R	*Topological graph*	σ_c^* *set 1 calc. by eqn. 1*	σ_c^* *set 2 calc. by eqn. 2*	*Deviation*
Me		0		
Et		0.101	—	—
nPr		−0.116	−0.115	1
iPr		−0.195	−0.195	—
nBu		−0.131	−0.129	2
iBu		−0.124	−0.123	1
sBu		−0.210	−0.209	1
tBu		−0.288	−0.289	−1
nPent		−0.145	−0.143	2
iPent		−0.140	−0.137	3
sPent		−0.223	−0.223	—
3-Pent		−0.222	−0.223	−1
2-Me-1-Bu		−0.140	−0.137	3
2-Me-2-Bu		−0.305	−0.303	2
3-Me-2-Bu		−0.220	−0.217	3
2,2-DiMe-1-Pr		−0.130	−0.131	−1
nHex		−0.159	−0.157	2
2-Hex		−0.237	−0.237	—
3-Hex		−0.235	−0.237	−2
2-Me-1-Pent		−0.153	−0.151	2
3-Me-1-Pent		−0.156	−0.151	5
4-Me-1-Pent		−0.155	−0.151	4
2-Me-2-Pent		−0.317	−0.317	—
3-Me-2-Pent		−0.235	−0.231	4
4-Me-2-Pent		−0.231	−0.231	—
2-Me-3-Pent		−0.231	−0.231	—
3-Me-3-Pent		−0.319	−0.317	2
2-Et-1-Bu		−0.154	−0.151	3
2,2-DiMe-1-Bu		−0.147	−0.145	2
2,3-DiMe-2-Bu		−0.317	−0.311	6
3,3-DiMe-2-Bu		−0.226	−0.225	1
nHept		−0.173	−0.171	2

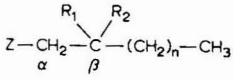
TABLE I *(continued)*

R	*Topological graph*	σ_c^* *set 1 calc. by eqn. 1*	σ_c^* *set 2 calc. by eqn. 2*	*Deviation*
2-Hept		−0.251	−0.251	—
3-Hept		−0.249	−0.251	−2
4-Hept		−0.248	−0.251	−3
2,2-DiMe-1-Pent		−0.159	−0.159	—
2,4-DiMe-3-Pent		−0.238	−0.239	−1
3-Et-3-Pent		−0.333	−0.331	2
nOct		−0.187	−0.185	2
2-Oct		−0.265	−0.265	—
2-Et-1-Hex		0.179	−0.179	—
2-Et-4-Me-1-Pent		−0.173	−0.173	—

CONTRIBUTION OF METHYLENE UNITS

For the determination of this contribution we have defined five structural series:





Series	R_1	R_2
α_0	H	H
α_1	Me	H
α_2	Me	Me
β_1	Me	H
β_2	Me	Me

Basic structures are obtained when $n = 0$; groups belonging to all five series are listed in Fig. 1, in which we have plotted the alkyl group σ_c^* values *vs.* number of methylene units. The slopes of the fairly straight lines obtained are identical; this means that the contribution of a methylene unit is constant over all series (Table II).

For every alkyl group R generated from the basic structures by inserting methylene units, the R σ_c^* values are obtained by adding to the basic σ_c^* value the contributions I_{CH_2} of the corresponding number of methylene groups.

EFFECT OF METHYL SUBSTITUTION

Two successive basic structures of the α or the β series differ by one methyl group. The data analysis (Table II) shows that the contribution of a methyl group is not the same at the α as at the β position.

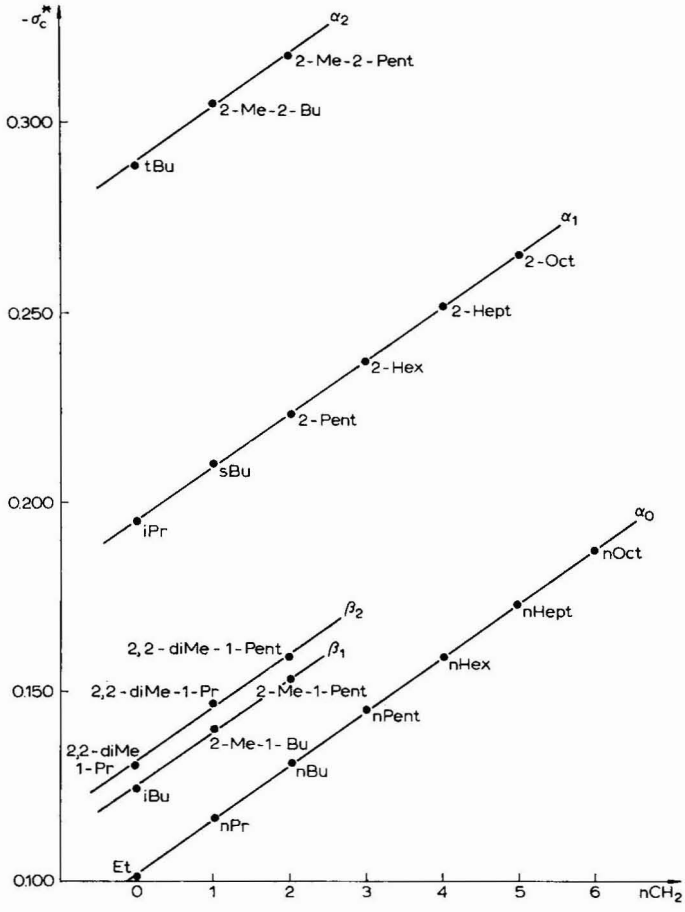


Fig. 1. Plot of the alkyl group σ_c^* values *vs.* number of methylene units.

TABLE II

METHYLENE UNIT INCREMENT AND σ_c^* VALUES OF BASIC STRUCTURES

Series	*Individual terms of each series*	I_{CH_2}*	σ_c^{*}**	r***	ψ***
α_0	Et, nPr, nBu, nPent, nHex, nHept, nOct	−0.014	−0.101	0.999	0.01
α_1	iPr, sBu, 2-Pent, 2-Hex, 2-Hept, 2-Oct	−0.014	−0.195	0.999	0.01
α_2	tBu, 2-Me-2-Bu, 2-Me-2-Pent	−0.014	−0.288	0.995	0.17
β_1	iBu, 2-Me-1-Bu, 2-Me-1-Pent	−0.014	−0.124	0.998	0.10
β_2	2,2-DiMe-1-Pr, 2,2-diMe-1-Bu, 2,2-diMe-1-Pent	−0.014	−0.130	0.995	0.17

* Contribution of a methylene unit.

** σ_c^* of the basic structure, obtained when $n = 0$.

*** r is the correlation coefficient. The Exner test ψ is a measure of the quality of a correlation[3]. It simultaneously takes into account the correlation coefficient r, the number of parameters and the number of experimental points. According to Exner, a perfect correlation is obtained when $\psi < 0.10$, a correct one for $0.10 < \psi < 0.20$.

α-Branching

From examination of σ_c^* values of alkyl groups in the α_0, α_1 and α_2 series we can deduce the mean contribution of each methyl substituent:

$$I_{Me_\alpha} = -0.094$$

When the methyl group is replaced by a longer alkyl chain, the contribution of each methylene is the same as above. For instance, the predictional contribution of an α-ethyl group will be the sum of the contributions of the α-methyl and one methylene

$$I_{Et_\alpha} = (-0.094) + (-0.014) = -0.108$$

β-Branching

Similarly, analysis of the β-branching yields the mean contribution of the β-methyl group

$$I_{Me_\beta} = -0.008$$

It is interesting to note that the increment I_{Me_β} can be considered as a general contribution of a methyl substitution effect in all positions except α.

$$I_{Me} \equiv I_{Me_\beta}, I_{Me_\gamma}, I_{Me_\delta} \cdots$$

ADDITIVITY RELATIONSHIP OF σ_c^* VALUES

Comparison of the basic structures shows that they are generated from the ethyl group by successive methylations. Consequently the ethyl group will be the foundation of our incrementation system.

The above results show that, depending on the branching position along an alkyl chain, the contribution of a methyl group is −0.094 or −0.008. On the other hand, in all cases examined the contribution of a methylene group is fairly constant, wherever it is inserted.

Definitively, the polar effect constant σ_c^* values can be calculated by means of the empirical relation

$$\sigma_{cR}^* = \sigma_{cEt}^* + \Sigma I_{CH_2} + \Sigma I_{Me_\alpha} + \Sigma I_{Me} \qquad (2)$$

The definite values of the increments are obtained by correlation of the values of σ_c^* set 1 (Table I):

$$I_{CH_2} = -0.014$$
$$I_{Me_\alpha} = -0.094$$
$$I_{Me} = -0.008$$

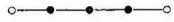
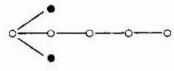
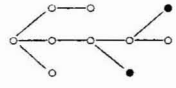
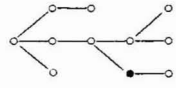
correlation coefficient $r = 0.999$
Exner Test $\psi = 0.04$

As expected, the polar effect constants are strictly additive[4].

The σ_c^* values calculated by eqn. 2 are listed in Table I (set 2). Depending on the precision of chromatographic measurements, the differences between values of the two sets (mean value 1.5/100, upper limit 3/100) enhance the quality of the additivity relationship (eqn. 2).

Contrary to eqn. 1, the latter can be used to determine σ_c^* values without knowledge of any chromatographic data.

The calculation of a σ_c^* value is now fairly simple; it needs six consecutive operating steps. For instance, the polar effect constant of the 3,6-dimethyl-5-ethyl-3-heptyl group is obtained as follows:

Operating steps	*Topological evolution*	*Contributions*	
Foundation			−0.101
Methylene insertions on foundation		3 × − 0.014	−0.042
Me α-branching		2 × − 0.094	− 0.188
Methylene insertions on α branches		1 × − 0.014	− 0.014
Other Me branchings		2 × − 0.008	− 0.016
Other methylene insertions		1 × − 0.014	−0.014
		σ_c^* =	−0.375

Next to its independence of chromatographic data, the main importance of eqn. 2 lies in the fact that only four factors are needed for the determination of the σ_c^* value of any alkyl groups, whatever its structure*. Furthermore, in chromatography this equation shows that there is no levelling effect in σ_c^* values.

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2 R. Fellous, R. Luft and J.-P. Rabine, *J. Chromatogr.*, 136 (1977) 5.
3 O. Exner, *Collect. Czech. Chem. Commun.*, 31 (1966) 3222.
4 J. MacPhee and J. E. Dubois, *Tetrahedron Lett.*, (1976) 2471, and literature cited therein.

* In practice we can determine unknown σ_c^* values of alkyl groups R′, generated from R, by addition of the appropriate methyl and methylene increments to the known σ_c^* of R

$$\sigma^*_{c_{R'}} = \sigma^*_{c_R} + \Sigma(I_{CH_2}, I_{Me\alpha}, I_{Me})$$

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APPLICATION D'UNE ÉQUATION DE TYPE TAFT AUX DONNÉES DE RÉTENTION

ADDITIVITÉ DES EFFETS DE SUBSTITUANTS

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SUMMARY

Application of a Taft-type equation to retention data. Additivity of substituent effects

The previously established linear extrathermodynamic relationship

$$\text{retention data} = \varrho^* \sum_i \sigma^*_{cR_i} + h\left(\sum_i n_{HR_i} - 3i\right)$$

can be applied to a chemical population the common functional group of which is characterized by an axis of symmetry. In all other cases it is possible to differentiate the particular contribution of each alkyl group R_i; consequently their effects must then be considered separately.

INTRODUCTION

Dans le phénomène chromatographique les grandeurs de rétention sont constituées entre autres par un apport dû à la fonction chimique et par celui de chacun des autres atomes de carbone de la molécule, sans que l'on assiste à un nivellement à partir d'un certain nombre d'atomes de carbone.

Ces grandeurs de rétention peuvent être liées aux effets polaires et structuraux des substituants par l'intermédiaire de relations linéaires extrathermodynamiques. Il est donc intéressant de disposer d'une échelle de constantes d'effet polaire des substituants aussi étendue que possible. C'est dans ce contexte que nous avons établi[1] une nouvelle échelle σ_c^*, applicable aux données chromatographiques et qui présente des caractéristiques simples d'additivité[2].

Dans nos précédents mémoires nous avons abordé le comportement de séries de substances RZ où la fonctions chimique Z ne porte qu'un seul groupe alkyle R

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variable. Dans le présent travail nous examinons le comportement de composés possédant deux groupes alkyles R et R′ variables.

Compte tenu de l'hypothèse de l'additivité des effets de substituants[3], le comportement chromatographique est appréhendé grâce à l'expression:

$$\text{grandeur de rétention} = \varrho^* (\sigma^*_{c_R} + \sigma^*_{c_{R'}}) + h(n_{H_R} + n_{H_{R'}} - 6) \qquad (1)$$

où $\sigma^*_{c_R}$, $\sigma^*_{c_{R'}}$ sont les constantes d'effet polaire des groupements alkyles R et R′; n_{H_R}, $n_{H_{R'}}$ le nombre d'atomes d'hydrogène des deux carbones de R et R′ portés par la fonction chimique (carbones α); ϱ^* et h des facteurs traduisant la sensibilité du phénomène chromatographique à l'effet polaire et au nombre de ramifications des substituants au niveau de leur carbone α.

RÉSULTATS

Dans un premier temps nous abordons le comportement de familles R–Z–R′ dont la fonction chimique Z est caractérisée par le groupe de symétrie C_{2v}. Du fait de l'existence d'un axe de symétrie, les substituants R et R′ deviennent indiscernables et il est licite d'envisager l'additivité des effets des substituants d'une manière telle que la reflète l'expression 1, c'est à dire en admettant que la sensibilité à l'effet polaire et à l'effet de ramification soit la même pour les deux substituants.

Les Tableaux I–III regroupent les résultats obtenus à partir du traitement des données de rétention de familles d'éthers, d'acétals et de cétones[4], sur six phases stationnaires recouvrant une gamme de polarité assez large. Nous avons fait appel indifféremment aux logarithmes des temps de rétention réduits relatifs, aux indices de rétention ou aux logarithmes des volumes de rétention. En effet, ces trois grandeurs sont interdépendantes et se déduisent l'une de l'autre à partir de relations linéaires simples. Dans chacun des cas analysés, l'examen des critères statistiques de fiabilité: r coefficient de corrélation et ψ test d'Exner[5], montre que la qualité des relations obtenues pour les phases apolaires est très satisfaisante, elle reste acceptable pour les phases polaires.

Dans un deuxième temps nous avons abordé un cas plus général où, mis à part le plan contenant le groupe fonctionnel, les composés R–Z–R′ ne présentent pas

TABLEAU I

VÉRIFICATION DE L'ADDITIVITÉ DES EFFETS DE SUBSTITUANTS

Familles: éthers R–O–R′. Grandeur de rétention à 120°: logarithme des temps de rétention réduits relatifs.

R(R′): Me (Me, nPr, nBu, iBu, tBu); Et (Et, nBu, tBu); nPr (nPr, iPr); iPr (iPr, tBu); nBu (nBu); nPent (nPent); iPent (iPent); nHex (nHex). p: nombre de points entrant dans la corrélation.

Phase stationnaire	ϱ^*	h	r	ψ	p
Apiezon L	−19.069	1.651	0.996	0.09	14
SE-30	−16.671	1.435	0.995	0.10	14
Ucon LB 1715	−17.066	1.489	0.994	0.11	16
Carbowax 20M	−14.551	1.297	0.991	0.14	16
XF-1150	−12.817	1.145	0.989	0.16	14
DEGS	−11.489	1.031	0.975	0.23	16

TABLEAU II

VÉRIFICATION DE L'ADDITIVITÉ DES EFFETS DES SUBSTITUANTS

Famille: formal $H_2C\begin{cases}OR\\OR'\end{cases}$. Grandeur de rétention à 120°: indices de rétention.

R(R'): Me (Me, Et, iPr); Et (Et, nPr, iPr, sBu); nPr (nPr, sBu); iPr (iPr); nBu (nBu); iBu (iBu); sBu (sBu).

Phase stationnaire	ϱ^*	h	I_0	r	ψ	p
Apiezon L	−6170	531	465	0.997	0.08	13
SE-30	6177	532	502	0.996	0.09	13
Ucon LB 1715	−5972	519	578	0.996	0.09	13
Carbowax 20M	−5571	492	727	0.989	0.15	13
XF-1150	−5752	508	744	0.986	0.17	13
DEGS	−5268	481	961	0.938	0.39	12

TABLEAU III

VÉRIFICATION DE L'ADDITIVITÉ DES EFFETS DE SUBSTITUANTS

Famille: cétones R–CO–R'. Grandeur de rétention à 120°: logarithme des volumes de rétention. R(R'): Me (Me, Et, nPr, iPr, nBu, iBu, sBu, tBu, nPent, néoPent, nHex, nHept); Et (Et, nPr, NBu); nPr (nPr); iPr (iPr); nBu (nBu)

Phase stationnaire	ϱ^*	h	$\log V_{G_0}$	r	ψ	P
Apiezon L	−19.878	1.698	0.714	0.994	0.11	18
SE-30	17.407	1.491	0.787	0.994	0.11	18
Ucon LB 1715	−17.837	1.552	0.937	0.991	0.14	18
Carbowax 20M	15.095	1.348	1.059	0.982	0.20	18
XF-1150	−14.012	1.248	1.138	0.983	0.19	18
DEGS	−12.455	1.138	0.970	0.962	0.29	18

d'élément de symétrie. Nous avons retenu dans ce but les indices de rétention d'une famille de 76 esters[6]. En utilisant l'équation 1 le comportement de cette large population est exprimé par

$$I_{x_{SE-30}} = -6335\,(\sigma^*_{c_R} + \sigma^*_{c_{R'}}) + 556\,(n_{H_R} + n_{H_{R'}} - 6) + 533 \quad (r = 0.996,\ \psi = 0.09)$$

illustrée par la Fig. 1. Dans ce diagramme les données de rétention sont portées en fonction de la somme des effets polaires des substituants. On relève six ensembles de points, correspondant à la somme des degrés de ramification des carbones α de R et R'.

Si notre présentation est satisfaisante, tant par la qualité de la relation obtenue, que par celle du diagramme représentatif, elle ne constitue néanmoins qu'une première approximation. En effet, en admettant que le phénomène chromatographique présente une même sensibilité aux effets polaire et structuraux de R et de R', il n'est pas possible d'expliquer pourquoi on observe des écarts entre les temps de rétention de deux esters RCOOR' et R'COOR; par exemple

nPrCOOMe $I = 715$ et MeCOOnPr $I = 707$,
ou bien sBuCOOMe $I = 767$ et MeCOOsBu $I = 746$, etc.

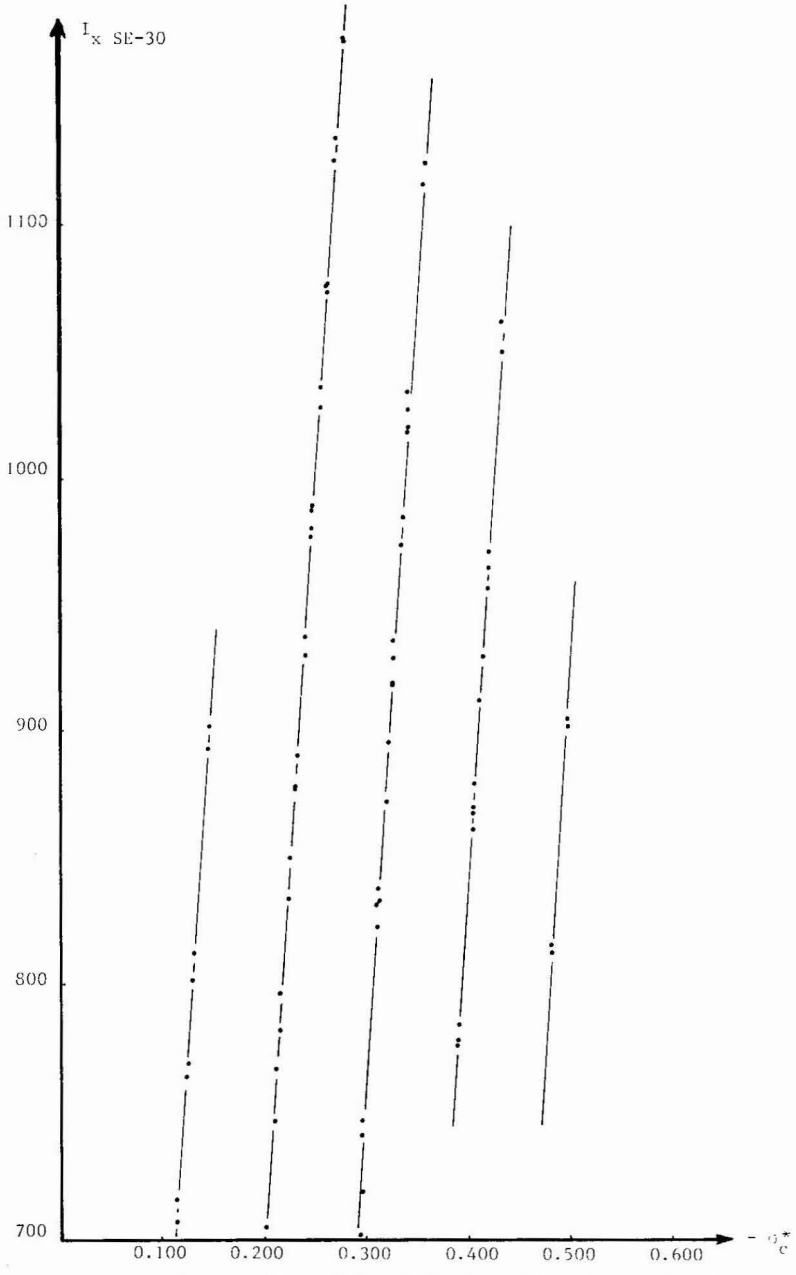

Fig. 1. Représentation graphique de la relation I appliquée à une série d'esters.

C'est pourquoi il nous a paru plus logique d'admettre, pour chacun des substituants R fixés sur des groupes fonctionnels dépourvus d'axe de symétrie, des apports différents aux temps de rétention. La relation 1 est alors modifiée légèrement:

$$\text{grandeur de rétention} = \varrho_1^* \sigma^*_{c_{R_1}} + \varrho_2^* \sigma^*_{c_{R_2}} + h_1(n_{H_{R_1}} - 3) + h_2(n_{H_{R_2}} - 3) \quad (2)$$

Ainsi, avec la famille d'esters dont nous avons examiné les données sur une phase SE-30[6], l'expression 2 devient:

$$I_{x_{RCOOR'}} = -6289\,\sigma^*_{c_R} - 6381\,\sigma^*_{c_{R'}} + 549(n_{H_R} - 3) + 563(n_{H_{R'}} - 3) + 533$$

$(r = 0.997, \quad \psi = 0.08)$

Il est loisible d'envisager l'extension de la relation d'additivité des effets de substituants au cas où le nombre de ceuxci serait supérieur à deux. Si les observations que nous avons faites dans le présent travail se vérifiaient alors, cela permettrait de représenter le lien entre une grandeur de rétention et les facteurs de Taft par la relation très générale

$$\text{grandeur de rétention} = \varrho^* \sum_i \sigma^*_{c_{R_i}} + h\,(\sum_i n_{H_{R_i}} - 3i)$$

où i exprime le nombre de substituants.

RÉSUMÉ

L'équation de type Taft établie précédemment peut être mise sous la forme générale

$$\text{grandeur de rétention} = \varrho^* \sum_i \sigma^*_{c_{R'}} + h(\sum_i n_{H_{R_i}} - 3i)$$

lorsque le groupement fonctionnel d'une famille de composés porte plusieurs groupes alkyle et est simultanément caractérisé par un axe de symétrie. En l'absence de cet élément de symétrie, il convient de traiter séparément les différents effets des substituants car leur participation au phénomène global peut être différenciée.

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ISOELECTRIC FOCUSING AS A METHOD FOR THE CHARACTERIZATION OF AMPHOLYTES

II. pH MEASUREMENTS IN SOLVENT MIXTURES USED IN DENSITY-GRADIENT ISOELECTRIC FOCUSING

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SUMMARY

When pH values are measured in mixed aqueous–organic solvents by means of the usual electrometric procedure, a correction (δ) must be applied in order to obtain physically meaningful pH values (*i.e.*, values that can be interpreted as the negative logarithm of the hydrogen ion activity). δ values have been determined in sucrose–water, glycerol–water and ethylene glycol–water mixtures at 25° and 4°, for use in density-gradient isoelectric focusing.

INTRODUCTION

In a previous paper[1], we drew attention to the shortcomings of isoelectric focusing as a method for the characterization of ampholytes. It was shown that only those variants of the technique in which the solvent composition of the focused zones is standardized (*e.g.*, zone convection, gel and thin-layer isoelectric focusing) are useful as characterization methods. In these variants, the standard deviation of a measured p*I* value is governed by the temperature difference between the final focusing temperature and the temperature of the pH measurement. Its magnitude was estimated to be about 0.005 pH unit per degree of temperature difference if the carrier ampholytes employed are matched to the investigated ampholytes with respect to their protolytic groups.

For density-gradient isoelectric focusing, we showed that, in addition, systematic and random errors are introduced as a result of the influence of the solvent composition on the isoelectric point of an ampholyte and upon the difference between the measured pH and pa_H^{*}*. Definite conclusions about the importance of these errors

* An asterisk is used to denote that the quantity under consideration (here the activity of H^+ ions) is referred to an infinitely dilute solution in the same solvent. When the asterisk is omitted, the quantity under consideration is referred to an infinitely dilute solution in water (or is considered in a general way).

were precluded by the lack of data pertinent to the most utilized solvents in isoelectric focusing, *viz.*, sucrose–water, glycerol–water and ethylene glycol–water mixtures, although it was argued that their importance may be far greater than that due to temperature differences.

In this paper, we present electrometric measurements of $\delta = \mathrm{pH} - \mathrm{p}a_{\mathrm{H}}^{*}$ in these solvents, with the aid of which the measured pH value of a focused zone in the solvents of interest can be converted into the corresponding $\mathrm{p}a_{\mathrm{H}}^{*}$ value, if the solvent composition in the zone is known. This enables one to make a reliable comparison of measured isoelectric points of the same ampholyte in different solvent mixtures.

THEORETICAL

It is well recognized that pH values of solutions in non-aqueous or partly aqueous solvents, obtained by using the operational pH definition and aqueous standard buffer solutions for the calibration of the pH meter, cannot be identified with $\mathrm{p}a_{\mathrm{H}}^{*}$, but differ from it by a quantity δ. This quantity δ accounts for the influence of the solvent on both the liquid junction potential, E_j, at the tip of the calomel electrode and the standard potential, $E^{\circ}_{\mathrm{glass}}$, of the glass electrode:

$$\delta \equiv \mathrm{pH} - \mathrm{p}a_{\mathrm{H}}^{*} = \frac{(E_j^{*} - E_j) - (E^{\circ *}_{\mathrm{glass}} - E^{\circ}_{\mathrm{glass}})}{2.303\ RT/F} \qquad (1)$$

where R represents the molar gas constant, T the absolute temperature and F the Faraday constant.

δ values can be easily measured, but they can be used universally for the calculation of $\mathrm{p}a_{\mathrm{H}}^{*}$ values from measured pH values only if three conditions are fulfilled:

(1) $E_j^{*} - E_j$ should not depend on the nature of the (buffering) solutes in the standard and sample solutions. In methanol–water and ethanol–water mixtures, this condition has been proved[2–4] to be generally valid for (buffer) solutions with pH (in water) ranging from 2.5 to 10 and for contents of the organic component up to about 70% of methanol and 100% of ethanol.

(2) $E_j^{*} - E_j$ should not depend upon the type of the device forming the liquid junction at the tip of the calomel electrode. This condition has also been proved[5] to be generally valid in methanol–water and ethanol–water mixtures.

(3) $E^{\circ *}_{\mathrm{glass}} - E^{\circ}_{\mathrm{glass}}$ should not depend upon the type of glass electrode. In an investigation[6] of the validity of this condition we found, in methanol–water and ethanol–water mixtures, a slight dependence of $E^{*}_{\mathrm{glass}} - E^{\circ}_{\mathrm{glass}}$ on the method of fabrication and pre-conditioning in the laboratory (rather than on the composition of the glass). This means that the use of average δ values, holding for all glass electrodes, results in a rather large error (about 0.04 pH unit).

Douhéret[7,8] measured δ values in solutions of hydrochloric acid of various concentrations in a series of partly aqueous solvents, using a glass electrode of a manufacture not included in the above-mentioned investigation[6]. In methanol–water, ethanol–water, ethylene glycol–water and 2-ethoxyethanol–water mixtures δ appeared to be a constant, characteristic of the solvent composition and independent of the acid concentration at $\mathrm{p}a_{\mathrm{H}}^{*} > 3$. Moreover, the differences between his δ values in

methanol–water and ethanol–water mixtures and those measured by Gelsema and co-workers[5,6] ranged from 0 to 0.06 pH unit (depending on the solvent composition), which is in reasonable accordance with the variability of $E^{\circ *}_{glass} - E^{\circ}_{glass}$ values mentioned above.

All of this evidence points to the feasibility of obtaining meaningful pH values (*i.e.*, pa^*_H values) by simple correction of measured pH values with appropriate δ values also in the solvents used in isoelectric focusing. Therefore, we measured δ in sucrose–water, glycerol–water and ethylene glycol–water mixtures, for saturated and 3 *M* potassium chloride–calomel electrodes. We also measured the values of $\delta' \equiv (E^*_j - E_j)/2.303\,(RT/F)$ and of $(E^{\circ *}_{glass} - E^{\circ}_{glass})/2.303\,(RT/F)$ separately. These measurements were made at 25°, the standard temperature for pH and p*K* determinations, but, as many investigators perform pH measurements in isoelectric focusing experiments at low temperatures, some δ values were also measured at 4°.

EXPERIMENTAL

E.m.f. measurements were made at 25° with the following cells:

Pt (H_2) | $HClO_4$ (m_H), water | sat. KCl(aq.) | Hg_2Cl_2–Hg (I)

Pt (H_2) | $HClO_4$ (m'_H), water, S(X) | sat. KCl(aq.) | Hg_2Cl_2–Hg (II)

glass | $HClO_4$ (m_H), water | sat. KCl(aq.) | Hg_2Cl_2–Hg (III)

glass | $HClO_4$ (m'_H), water, S(X) | sat. KCl(aq.) | Hg_2Cl_2–Hg (IV)

Hg–Hg_2Cl_2 | 3*M* KCl(aq.) | $HClO_4$ (m_H), water | sat. KCl(aq.) | Hg_2Cl_2–Hg (V)

Hg–Hg_2Cl_2 | 3*M* KCl(aq.) | $HClO_4$ (m'_H), water, S(X) | sat. KCl(aq.) | Hg_2Cl_2–Hg (VI)

where $m_H \approx m'_H \approx 0.001$ mole·kg^{-1}, S represents sucrose, glycerol and ethylene glycol, respectively, and X, representing the weight percentage of component S, has the values 15, 30, 45 and 60% for sucrose and 20, 40, 60 and 80% for glycerol and ethylene glycol. The following chemicals were used: perchloric acid (Merck, Darmstadt, G.F.R., p.a. grade), sucrose (J. T. Baker, Phillipsburgh, N.J., U.S.A., analyzed grade), glycerol (Merck, p.a. grade) and ethylene glycol (Merck, p.a. grade). The design of cells I and III was given by De Ligny and Rehbach[9].

The measurements were performed as follows. A stream of carbon dioxide- and oxygen-free hydrogen was bubbled through the cell. When a stable e.m.f. value (E_I, E_{II}) had been reached (within 1 h), one of the two platinum electrodes was replaced with a glass electrode and the e.m.f. (E_{III}, E_{IV}) was measured. The glass electrode was kept in an aqueous buffer solution between the measurements. Details of the platinization of platinum electrodes and the purification of hydrogen were given elsewhere[10]. A Radiometer 4 pH meter and an Ingold glass electrode*, Type 10271/3005, were used. All e.m.f. measurements were measured in duplicate at least (see Tables I and II).

δ' values for the saturated calomel electrode were calculated by means of the equation

$$\delta'_{sat} \equiv \frac{E^*_j - E_j}{2.303\,RT/F} = \frac{E_{II} - E_I}{2.303\,RT/F} + \log\frac{m'_H}{m_H} + \log\frac{\gamma^*_H}{\gamma_H} \qquad (2)$$

* The glass electrode had been given the pre-treatment prescribed by the manufacturer.

where γ_H and γ_H^* represent the activity coefficients of the hydrogen ion in water and in the partially aqueous solvent, respectively. These data were calculated by the equation

$$-\log \gamma_H (\gamma_H^*) = \frac{1.8144 \cdot 10^6 (DT)^{-3/2} (m_H d_o)^{\frac{1}{2}}}{1 + 201.21 (DT)^{-\frac{1}{2}} (m_H d_o)^{\frac{1}{2}}} \quad (3)$$

where D represents the dielectric constant of the solvent and d_0 its density. Values of D and d_0 were taken from the literature[11–14].

δ values for the saturated calomel electrode were calculated by means of the equation

$$\delta_{sat} \equiv \frac{(E_j^* - E_j) - (E_{glass}^{\circ *} - E_{glass}^{\circ})}{2.303\ RT/F} = \frac{E_{IV} - E_{III}}{2.303\ RT/F} + \log \frac{m'_H}{m_H} + \log \frac{\gamma_H^*}{\gamma_H} \quad (4)$$

Values of $E_{glass}^{\circ *} - E_{glass}^{\circ}$ can then easily be found:

$$E_{glass}^{\circ *} - E_{glass}^{\circ} = 2.303\ RT/F\ (\delta' - \delta) \quad (5)$$

E.m.f. measurements were also performed on the cells III and IV at 4°, at $m_H \approx m'_H\ 0.001 \approx$ mole·kg^{-1} and with X representing 60% of sucrose, 80% of glycerol and 80% of ethylene glycol, respectively. The resulting δ values were calculated by using eqns. 3 and 4.

E.m.f. measurements on cells V and VI (E_V, E_{VI}) were made in quadruplicate. The difference of the δ values, holding for saturated and 3 M calomel electrodes, can be calculated as follows:

$$\delta_{sat} - \delta_{3M} = \frac{E_{VI} - E_V}{2.303\ RT/F} \quad (6)$$

TABLE I

VALUES OF δ', δ AND $(E_{glass}^{\circ *} - E_{glass}^{\circ})/2.303\ (RT/F)$ AT 25° IN SUCROSE–WATER, GLYCEROL–WATER AND ETHYLENE GLYCOL–WATER MIXTURES

Solvent	*X (wt.-%)*	*N*	$-\delta'$ *(pH units)*	$-\delta$ *(pH units)*	$-(E_{glass}^{\circ *} - E_{glass}^{\circ})/2.303\ RT/F$ *(pH units)*
Sucrose–water	15	2	0.114 ± 0.004	0.104 ± 0.003	0.010 ± 0.005
	30	2	0.219 ± 0.001	0.210 ± 0.005	0.009 ± 0.005
	45	2	0.377 ± 0.004	0.333 ± 0.004	0.044 ± 0.006
	60	2	0.611 ± 0.017	0.505 ± 0.004	0.106 ± 0.017
Glycerol–water	20	3	0.134 ± 0.010	0.130 ± 0.010	0.004 ± 0.014
	40	3	0.278 ± 0.012	0.255 ± 0.010	0.023 ± 0.016
	60	3	0.466 ± 0.018	0.414 ± 0.017	0 052 ± 0 025
	80	3	0.780 ± 0.011	0.696 ± 0.007	0.084 ± 0.013
Ethylene glycol–water	20	2	0.113 ± 0.004	0.109 ± 0.003	0.004 ± 0.005
	40	2	0.211 ± 0.001	0.202 ± 0.002	0.009 ± 0.002
	60	2	0.318 ± 0.001	0.297 ± 0.003	0.021 ± 0.003
	80	2	0.667 ± 0.004	0.617 ± 0.016	0.050 ± 0.017

RESULTS

The values of $\delta_{sat}-\delta_{3M}$ did not differ significantly from zero. Values of δ, δ' and $(E^{\circ *}_{glass}-E^{\circ}_{glass})/2.303\ (RT/F)$ at 25° are presented in Table I. The indicated errors are standard deviations calculated from replicate e.m.f. measurements; the number of replicates (N) is given in the table.

δ values are plotted as a function of the solvent composition in Fig. 1; the results obtained by Douhéret[8] in ethylene glycol–water mixtures are included.

In Table II the δ values at 4° are given.

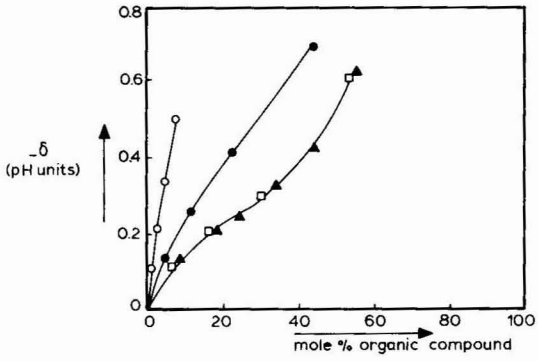

Fig. 1. Values of δ at 25° in sucrose–water (○), glycerol–water (●) and ethylene glycol–water (□) mixtures as a function of the mole fraction of the organic component. ▲, From ref. 8.

TABLE II

VALUES OF δ AT 4° IN 60% SUCROSE, 80% GLYCEROL AND 80% ETHYLENE GLYCOL

Solvent	*X* (*wt.-%*)	*N*	$-\delta$ (*pH units*)
Sucrose–water	60	3	0.475 ± 0.004
Glycerol–water	80	3	0.675 ± 0.006
Ethylene glycol–water	80	3	0.554 ± 0.004

DISCUSSION

It was demonstrated by Douhéret[8] that δ values in mixtures of monovalent alcohols and ethylene glycol with water are related in a qualitative way to the Hammett acidity function[15]: low δ values correspond to a low basicity of the solvent mixture. As has been pointed out by Kalidas and Palit[16], the relatively low basicity of ethylene glycol compared with monofunctional alcohols can be explained by the capacity of the molecules of ethylene glycol to form *inter-* and *intra*-molecular hydrogen bonds. As this tendency for intermolecular hydrogen bonding can be assumed to increase in the order ethylene glycol < glycerol < sucrose, δ values at equal mole fractions of the organic component would be expected to decrease in this order. Fig. 1 shows that the expected order obtains.

The values of $(E^{\circ *}_{glass} - E^{\circ}_{glass})/2.303\ (RT/F)$ and their standard deviations, given in Table I, clearly demonstrate a significant influence of the solvent composition on the standard potential of the glass electrode in the three solvent systems studied. This result corroborates earlier findings in methanol–water and ethanol–water mixtures.

As can be seen in Fig. 1, the differences between the δ values in ethylene glycol–water mixtures given in Table I, and those measured by Douhéret[8] range from 0 to about 0.05 pH unit. These differences are reasonable, in view of the fact that different glass electrodes were used in the two studies and in view of the established variability of $E^{\circ *}_{glass} - E^{\circ}_{glass}$ values between different glass electrodes in methanol–water and ethanol–water mixtures (see Theoretical). The absolute values of $(E^{\circ *}_{glass} - E^{\circ}_{glass})/2.303\ (RT/F)$, given in Table I, are of the same order of magnitude as those found earlier[6] in methanol–water and ethanol–water mixtures (0–0.07 pH unit). Therefore, if the δ values in Table I are used to correct pH values measured with an arbitrary glass electrode, their standard deviation can be estimated to range from 0 to $\pm$ 0.04 pH unit as the solvent composition ranges from water-rich to water-poor, as found before[6]. Evidently, if pH measurements are performed with the same (Ingold) glass electrode as was used in the present investigation (to which the same pre-treatment has been given), the standard deviations of the δ values given in Table I apply.

At 4°, the δ values at the highest concentration of the organic constituent of the solvent mixtures are 0.02–0.06 pH unit less negative than those at 25° (see Table II). At lower concentrations of the organic component, values of δ at 4° can be estimated by assuming that the difference $\delta_{25°} - \delta_{4°}$ is proportional to the weight percentage X.

In Table III, δ values at both temperatures and at various values of X, obtained by graphical interpolation, are given.

TABLE III

VALUES OF δ (IN pH UNITS) AT 25° AND 4° IN SUCROSE–WATER, GLYCEROL–WATER AND ETHYLENE GLYCOL–WATER MIXTURES

X (wt.-%)	*Sucrose–water*		*Glycerol–water*		*Ethylene glycol–water*	
	$-\delta_{25°}$	$-\delta_{4°}$	$-\delta_{25°}$	$-\delta_{4°}$	$-\delta_{25°}$	$-\delta_{4°}$
5	0.03	0.03	0.03	0.03	0.03	0.02_5
10	0.06_5	0.06	0.06_5	0.06	0.05_5	0.04_5
15	0.10_5	0.09_5	0.10	0.09_5	0.08	0.07
20	0.14	0.13	0.13	0.12_5	0.11	0.09_5
25	0.17_5	0.16	0.16	0.15_5	0.14	0.12
30	0.21	0.19_5	0.19	0.18_5	0.16	0.13_5
35	0.24_5	0.23	0.22_5	0.21_5	0.18_5	0.15_5
40	0.29	0.27	0.25_5	0.24_5	0.20	0.17
45	0.33_5	0.31	0.29	0.28	0.22	0.18
50	0.38	0.35_5	0.32_5	0.31_5	0.24	0.20
55	0.43_5	0.41	0.36_5	0.35_5	0.26_5	0.22
60	0.50_5	0.47_5	0.41_5	0.40	0.29_5	0.25
65	—	—	0.47	0.45	0.34	0.28_5
70	—	—	0.53	0.51	0.40	0.34_5
75	—	—	0.60	0.58	0.49	0.43
80	—	—	0.69_5	0.67_5	0.62	0.55_5

CONCLUSIONS

Meaningful pH values (*i.e.*, values which can be interpreted as $-\log a_H^*$) can be obtained in sucrose–water, glycerol–water and ethylene glycol–water mixtures, which are used as solvents in density-gradient isoelectric focusing. This can be done by simply subtracting from the pH meter readings a quantity δ, which is characteristic of the solvent composition.

The resultant interpretation errors, $pH - \delta - pa_H^*$, range from zero in water to $\pm$ 0.04 pH unit in 60% sucrose, 80% glycerol and 80% ethylene glycol, if an arbitrary glass electrode is used. They are less than $\pm$ 0.02 pH unit if a glass electrode of the same manufacture as used in the present investigation is applied.

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DETERMINATION OF 8-METHOXYPSORALEN IN PLASMA BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

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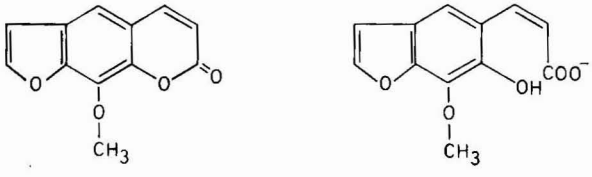
SUMMARY

A method is given for the determination of 8-methoxypsoralen in human plasma at the low ng/ml level using gas chromatography with electron capture detection. 8-Methoxypsoralen was extracted from plasma with methylene chloride at pH 7.0. After addition of the internal standard, 8-butoxypsoralen, the psoralens were hydrolysed in sodium hydroxide and the aqueous phase was purified by extraction with methylene chloride and toluene. The aqueous phase was acidified and the re-lactonized psoralens were extracted with toluene and analysed.

Some determinations of plasma levels of 8-methoxypsoralen after oral administration are presented.

INTRODUCTION

Psoralens have been used in the therapy of vitiligo for more than two decades. Recently increased interest has been shown in their use in the treatment of psoriasis. 8-Methoxypsoralen (8-MOP, Fig. 1) has been used both in local[1] and peroral[2,3]



A B

Fig. 1. A: 8-methoxypsoralen (8-MOP); B: *cis*-3-(6-hydroxy-7-methoxy-5-benzofuranyl)acrylic acid (8-MOP-acid).

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therapy, combined with exposure of the patients to long-wavelength UV light. Little information is available concerning the pharmacokinetics of 8-MOP owing to the lack of analytical techniques with sufficient sensitivity and selectivity. This paper gives a method for the determination of 8-MOP in human plasma at the low ng/ml level.

EXPERIMENTAL

Apparatus

The gas chromatographic (GC) analysis was performed using a Varian 1400 instrument equipped with an electron capture detector (ECD) of ^{63}Ni-type. The following temperatures were used: injector, 250°; column, 195°; detector, 200°.

A silanized glass column (90 cm × 2 mm I.D.) was packed with 3% OV-225 on Gas Chrom Q, 100/120 mesh. Nitrogen was used as carrier gas with a flow-rate of 20 ml/min. The column was conditioned for 24 h at 275°.

Photometry was performed with a Zeiss PMQ III Spectralphotometer equipped with thermostated cells (10 mm).

The pH determinations were performed with an Orion Research Model 701/ digital pH meter equipped with an Ingold combined electrode, Type 401.

The radioactivity was measured with a Packard Tri Carb instrument, Model 3320.

Chemicals

All solvents were of analytical grade. Aqueous and organic phases were equilibrated before use in partition experiments. 8-MOP was supplied by AB Draco, Lund, Sweden.

8-Butoxypsoralen (8-BOP) was synthesized as follows: 8-hydroxypsoralen (25 mg, prepared from 8-MOP by demethylation[4]) in 25 ml of methylene chloride was mixed with 5 ml of butyl iodide and 10 ml of carbonate buffer (pH 10.5) containing 1 *M* tetrabutylammonium hydrogen sulphate (neutralized with an equivalent amount of sodium hydroxide). The mixture was shaken for 30 min at room temperature. The organic phase was separated and extracted three times with 10 ml of buffer (pH 10.5) and twice with water and dried with Na_2SO_4. The organic solvent was evaporated and the residue recrystallized from methanol–water. The structure was confirmed by mass spectrometry.

Tritium-labelled 8-MOP was synthesized from 8-hydroxypsoralen and [$^3H_3$]-methyl iodide[4] and purified by thin-layer chromatography (TLC) (Silica gel 60, chloroform, $R_F = 0.5$) immediately before use. The radiochemical purity was >99%, as checked by TLC with radiochromatogram scanning.

8-Methoxypsoralen tablets (Neomeladinine, 15 mg) were obtained from the Memphis Chemical Co. (Cairo, Egypt). The doses given were 30 mg to patients weighing 35–55 kg, 45 mg to patients weighing 55–75 kg and 60 mg to patients weighing more than 75 kg.

Determination of partition coefficients

The organic phase containing 8-MOP was equilibrated with phosphate buffer pH 7.0 ($\mu = 0.1$) for 30 min at 25° (equal phase volumes). The concentration of

8-MOP was determined photometrically: in the organic phase by direct measurements and in the aqueous phase after re-extraction into methylene chloride.

Plasma extractions

The extraction from plasma was studied using tritium-labelled 8-MOP (426 mCi/mmole). The radioactivity in aqueous and organic phases was determined by liquid scintillation counting after addition of Instagel®.

The plasma extraction of 8-butoxypsoralen was studied by GC–ECD using 8-MOP as internal standard.

Determination of apparent first-order rate constants for hydrolysis and lactonization

The constants were determined by photometry (*cf.* ref. 5).

The lactonization was studied at 25.0° by mixing 0.200 ml of hydrolysed 8-MOP in 0.01 *M* NaOH with 3.00 ml of the appropriate buffer ($\mu = 0.1$, thermally equilibrated) in the thermostated cells of the spectrophotometer and following the change in absorbance at 300 nm. The absorbance at t_∞ was obtained after the addition of 0.100 ml of 12 *M* HCl.

The hydrolysis was studied as described above by mixing 3.00 ml of an aqueous solution of 8-MOP with 0.300 ml of the appropriate buffer ($\mu = 1$). Hydrolysed 8-MOP in buffer (pH 7.0) and 0.01 *M* NaOH exposed to light for 2 h at 300 nm in the photometer gave, after acidification, the same spectra as a freshly prepared solution of 8-MOP, thus indicating no photochemical degradation.

General procedure for the determination of 8-MOP in plasma

A 2.00 ml plasma sample was mixed with 0.5 ml of phosphate buffer (pH 7.0, $\mu = 1$) and 5.00 ml of methylene chloride and extracted for 15 min. Then 3.00 ml of the organic phase was mixed with 0.200 ml of 8-BOP (internal standard) 125 ng/ml in methanol and evaporated to dryness under nitrogen, after which 0.025 ml of methanol and 0.200 ml of 0.1 *M* NaOH were added and the solution was left for 10 min at room temperature. The aqueous phase was extracted once with 1 ml of methylene chloride (discarded) and twice with 1 ml of toluene (discarded). The aqueous phase was mixed with 0.050 ml of toluene and 0.025 ml of 12 *M* HCl and extracted for 3 min. A 2 μl sample of the organic phase was injected into the chromatograph.

All quantitations were based on peak height measurements.

Blood samples (10 ml) were obtained by venous puncture. After centrifugation the plasma was immediately frozen (−20°). Handling of plasma samples at room temperature was kept to a minimum.

RESULTS AND DISCUSSION

The method consists of extraction of plasma with methylene chloride and separation of 8-MOP from endogenous compounds by extraction, followed by GC determination using an ECD. The high sensitivity of the ECD can be fully utilized only after separation of 8-MOP from co-extracted endogenous material prior to the GC step. A selective extraction was obtained because psoralens hydrolyse in alkaline and re-lactonize in acidic solutions.

TABLE I

PARTITION COEFFICIENTS FOR 8-METHOXYPSORALEN

Aqueous phase: phosphate buffer, pH 7.0, $\mu = 0.1$. Temperature: 25°.

Organic phase	*log k_d*
Methylene chloride	>3
Benzene	2.7
Toluene	2.5
Diethyl ether	1.8
Hexane	0.60

Extraction

The partition coefficients for 8-MOP are given in Table I. Quantitative extraction (>99%) is obtained with methylene chloride, benzene or toluene using equal phase volumes. The internal standard, 8-BOP, should give higher partition coefficients owing to its higher carbon content (*cf.* ref. 6). Methylene chloride was preferred as solvent owing to its good extracting properties and high volatility, facilitating subsequent evaporation.

Extraction studies from plasma revealed, however, a lower recovery compared to aqueous solutions (Table II). This was particularly notable for 8-BOP, which was extracted to *ca.* 70% only (equal phase volumes). The lower extraction of 8-BOP

TABLE II

EXTRACTION OF PSORALENS FROM PLASMA

Organic phase: methylene chloride. Aqueous phase: plasma pH 7.0 (2.00 ml plasma + 0.5 ml phosphate buffer, pH 7.0, $\mu = 1$). Extraction time: 15 min.

Ratio $\frac{org.\ phase}{aq.\ phase}$	*Conc. (ng/ml)*	*Yield (%) (n = 6)*
8-Methoxypsoralen		
1:1	100	91.7 ± 0.2*
2.0:1	100	93.4 ± 0.2*
2.0:1	10	91.4 ± 0.1*
8-Butoxypsoralen		
1:1	100	72 ± 5**

* Determined by liquid scintillation counting.

** Determined by GC–ECD.

TABLE III

APPARENT FIRST-ORDER RATE CONSTANTS FOR HYDROLYSIS OF 8-METHOXYPSORALEN (8-MOP) AND 8-BUTOXYPSORALEN (8-BOP)

Temperature: 25°. Each value is a mean of two determinations.

pH	*− log [k (sec^{-1})]*	
	8-MOP	*8-BOP*
10.25	3.96	—
10.94	3.27	—
12 (0.01 *M* NaOH)	2.34	2.41

TABLE IV

APPARENT FIRST-ORDER RATE CONSTANTS FOR LACTONIZATION OF 8-MOP-ACID AND 8-BOP-ACID

Temperature: 25°. Each value is a mean of two determinations.

pH	$-\log [k\ (\sec^{-1})]$	
	8-MOP-acid	*8-BOP-acid*
1.95	1.93	1.99
2.98	2.74	—
4.34	3.71	—
7.07	4.51	

compared to 8-MOP may be due to a higher degree of plasma protein binding (*cf.* ref. 7). Because of its different plasma extraction properties 8-BOP was added after the initial extraction. A ratio of organic phase to plasma of 2.0 gave an extraction of 8-MOP of 93.4% at 100 ng/ml and 91.4% at 10 ng/ml. The degree of extraction was not increased by varying the extraction time from 15 min to 60 min.

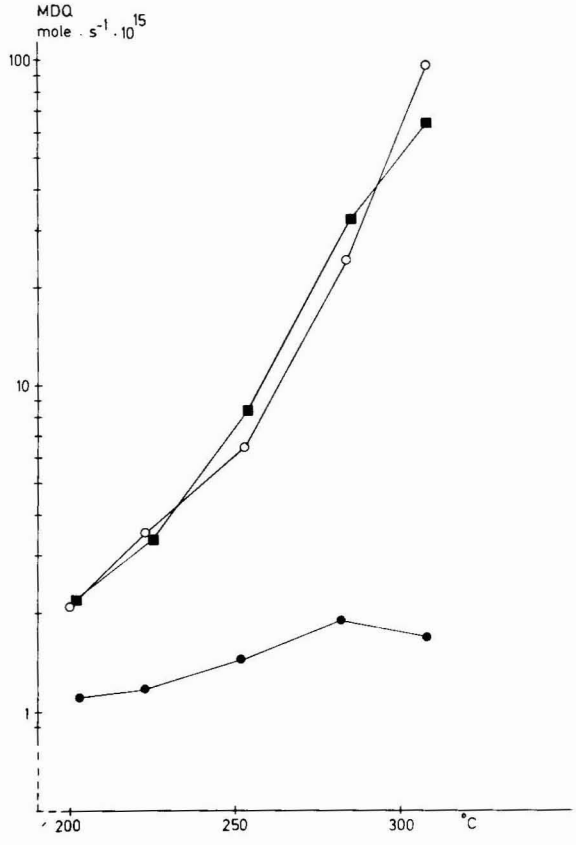

Fig. 2. Electron capture detector response for psoralens as a function of detector temperature. ○: 8-Butoxypsoralen; ■: 8-methoxypsoralen; ●: 5-methoxypsoralen.

Hydrolysis and lactonization

8-MOP is hydrolysed in alkaline solutions to 8-MOP-acid (Fig. 1, *cf.* ref. 8). The apparent first-order rate constants for hydrolysis at three different pH values are given in Table III. At pH 7.0 8-MOP was stable for at least 24 h but in 0.1 *M* NaOH hydrolysis was complete in <3 min.

The rate of lactonization at various pH values is given in Table IV; it was complete within 1 min in 0.1 *M* HCl.

The rates of lactonization and hydrolysis of the internal standard, 8-BOP, differed only slightly from those of 8-MOP (Tables III and IV).

Electron capture detector response

The minimum detectable quantity (MDQ, signal-to-noise ratio 3:1) for 8-MOP, 8-BOP and 5-methoxypsoralen (5-MOP) are given in Fig. 2. The responses for 8-MOP and 8-BOP are strongly affected by the detector temperature: a decrease of the detector temperature from 300° to 200° enhanced the response *ca.* 30-fold. The response of 5-MOP is only slightly temperature-dependent.

The MDQ for 8-MOP at 200° corresponds to an injected amount of 6 pg ($N = 3600$, $t_R = 3$ min).

Precision and selectivity

A chromatogram from a plasma sample obtained from a patient 6 h after an oral dose of 30 mg 8-MOP and containing 45 ng/ml of 8-MOP is given in Fig. 3. No peaks interfering with the analysis of 8-MOP were observed when analysing

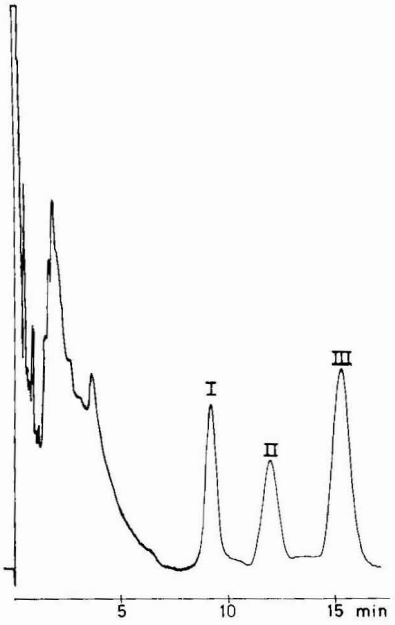

Fig. 3. Chromatogram from patient plasma containing 8-methoxypsoralen at a concentration of 45 ng/ml. For chromatographic conditions see Experimental section. I: 8-Methoxypsoralen; II: unknown compound; III: 8-butoxypsoralen.

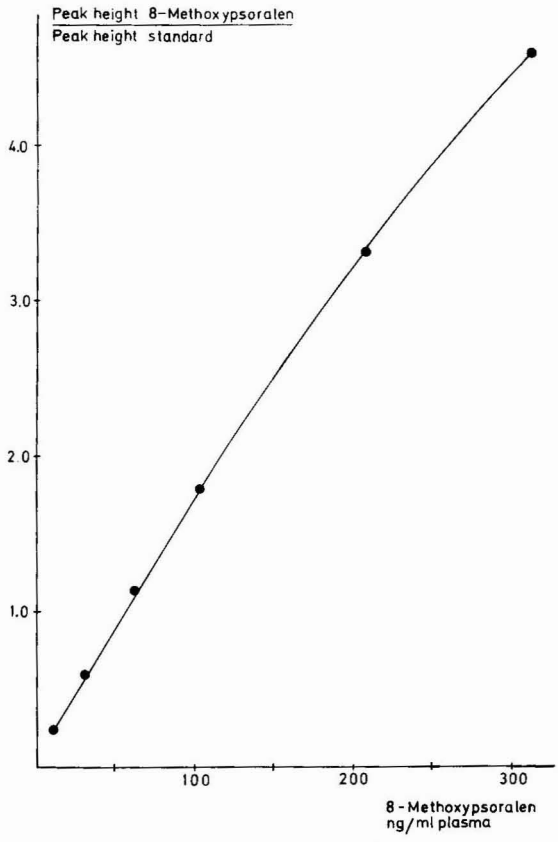

Fig. 4. Standard curve for the determination of 8-methoxypsoralen in plasma.

blank plasma. A standard curve for the determination of 8-MOP in plasma is given in Fig. 4. The relative standard deviation was 2.1% at 100 ng/ml and 4.2% at 10 ng/ml ($n = 5$).

8-Hydroxypsoralen, a likely metabolite of 8-MOP, did not interfere as it has a longer retention time than 8-BOP.

It is reasonable to assume that 8-MOP can be metabolized to some extent by esterases to 8-MOP-acid, which can then spontaneously re-lactonize to 8-MOP. The interference of 8-MOP-acid in the determination of 8-MOP will depend on their relative concentrations and the way the plasma samples are handled (*e.g.* storage time and temperature, pH used for the extraction). Plasma concentrations of 8-MOP-acid were determined in three patients, 1, 3 and 6 h after an oral dose of 30 mg of 8-MOP, using a method based on ion pair extraction and GC–ECD[9], but in all cases were below the detection limit of the technique (<10 ng/ml plasma).

Plasma concentrations of 8-MOP

The plasma levels of 8-MOP in five patients receiving 8-MOP orally are given in Fig. 5 and show considerable variation in the peak plasma levels (20–700 ng/ml). Further pharmacokinetic studies are in progress.

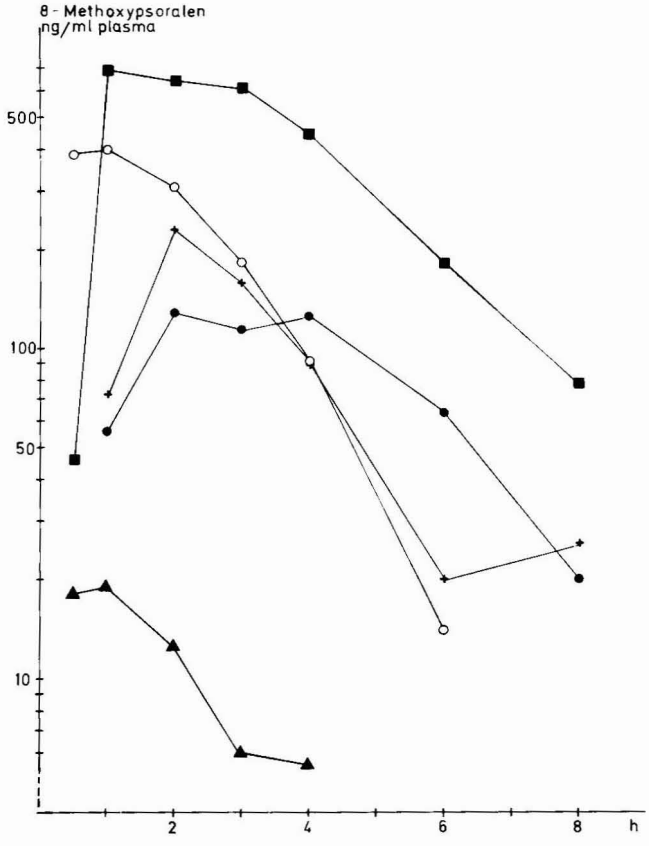

Fig. 5. Plasma levels of 8-methoxypsoralen.

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Note

Gas chromatographic determination of barbiturates by extractive alkylation and support coated open tubular column separation

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The analysis of barbiturates has mostly been based on gas chromatography (GC) or mass spectrometry with packed columns[1–5]. Since the separation of related barbiturates appears to be a problem on packed columns, the use of support coated open tubular (SCOT) columns[6] might be a solution. The chromatography of barbiturates as their 1,3-dimethyl derivatives has been discussed by Brochmann-Hansen and Oke[7]. The disadvantage of using trimethylanilinium hydroxide as a flash methylating agent is the appearance of so-called "early peaks" from phenobarbital and some other barbiturates. These were identified by Osiewicz and collaborators[3] as breakdown products. These peaks interfere with barbiturates eluting at a lower column temperature than phenobarbital. The use of extractive alkylation[9,10] has the advantage that no "early peaks" appear, and different alkyl derivatives can easily be formed. For these reasons a method for the determination of barbiturates in body fluids of overdose patients has been developed.

EXPERIMENTAL

Apparatus

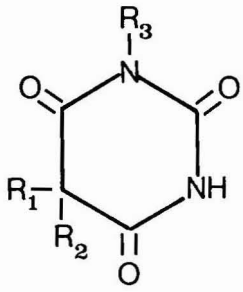
A Hewlett Packard 5720 A gas chromatograph with a temperature programming unit and flame ionization detector (FID) was used. The SCOT column and injection system were from SGE (N. Melbourne, Australia). The gas chromatograph was equipped with an inlet system for the make-up gas.

Chromatographic system

The column was made of glass, 43 m × 0.5 mm, coated with SE-30 (type GSC/SE-30/S). The GC conditions were as follows: injection port 250°, detector 300°, oven temperature isothermal at 170° for 4 min and then programmed from 170° to 260° at 4°/min. The carrier gas was helium at a flow-rate of 2 ml/min. The make-up gas for the FID was nitrogen.

Chemicals and materials

The barbiturates and glutethimide were obtained from manufacturers their respective. Tetrabutylammonium hydrogen sulphate (TBA-HSO_4) was purchased from Lab Kemi (Stockholm, Sweden), and prepared as a 1 *M* solution in



	Compound	R_1	R_2	R_3
1	Metharbital	ethyl	ethyl	methyl
2	Barbital	ethyl	ethyl	hydrogen
3	Allobarbital	allyl	allyl	hydrogen
4	Aprobarbital	allyl	isopropyl	hydrogen
5	5,5-dipropyl barbituric acid	propyl	propyl	hydrogen
6	Butethal	ethyl	butyl	hydrogen
7	Amobarbital	ethyl	3-methylbutyl	hydrogen
8	Pentobarbital	ethyl	1-methylbutyl	hydrogen
9	Vinbarbital	ethyl	1-methylbutenyl	hydrogen
10	Secobarbital	allyl	1-methylbutyl	hydrogen
11	Hexobarbital	methyl	1-cyclohexenyl	methyl
12	Glutethimide			
13	Phenobarbital	ethyl	phenyl	hydrogen
14	5-allyl-5-phenyl barbituric acid	allyl	phenyl	hydrogen
15	Heptabarb	ethyl	1-cycloheptenyl	hydrogen

Fig. 1. Structural formulae of the barbiturates.

1 *M* sodium hydroxide. The charcoal used was "Norit A", a neutral, pharmaceutical grade obtained from Amend Drug and Chemical Co., Irwington, N.J., U.S.A. It was prepared as follows: to *ca.* 500 mg of charcoal were added 50 ml of distilled water which was mixed thoroughly with a magnetic stirrer. All other chemicals were of reagent grade.

Analytical method

A 0.5 ml plasma sample was mixed thoroughly with 1 ml of the charcoal suspension and allowed to stand for a few minutes. After centrifugation as much as possible of the supernatant was aspirated off and discarded. Then 0.5 ml of 1 *M* sodium hydroxide, 50 μl of TBA-HSO_4 and 200 μl of ethyl iodide were added to the charcoal suspension and extracted with 1 ml of dichloromethane for 45 min. After centrifugation the aqueous phase was aspirated and the dichloromethane layer was transferred to a new tube and evaporated to dryness in a sandbath at 40° under a gentle stream of nitrogen. The residue was reconstituted in 50 μl of hexane. Standards

were prepared by the addition of known amounts of drugs (see Fig. 1) to human plasma. Drug concentrations were obtained by plotting the peak-height ratio of drug to internal standard.

RESULTS AND DISCUSSION

The adsorption of drugs onto charcoal from plasma and urine is well known and has been used as a clean-up procedure prior to GC[11] and liquid chromatographic analyses[12]. Extractive alkylation can be carried out directly, which simplifies the procedure to a single extraction step. The polar counter-ion, tetrahexylammonium sulphate, which gives a shorter derivatization time[10], could not be used because it contained impurities that would interfere with some barbiturates. To achieve quantitative derivatization with the less polar TBA-HSO_4 it was necessary to extract for 45 min at room temperature. This could, however, be speeded up by performing the extraction in a thermo-block at a higher temperature. The ethyl derivatives of all

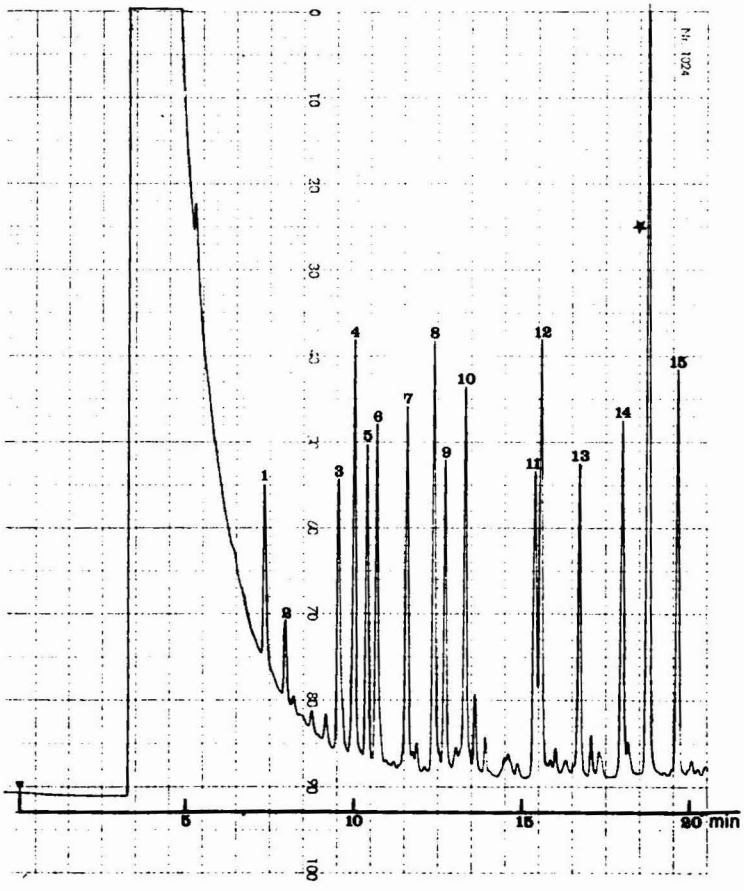


Fig. 2. Chromatogram of a spiked plasma sample: concentration of all drugs 20 μg/ml. Drugs numbered as in Fig. 1. ★ = peak from plasma.

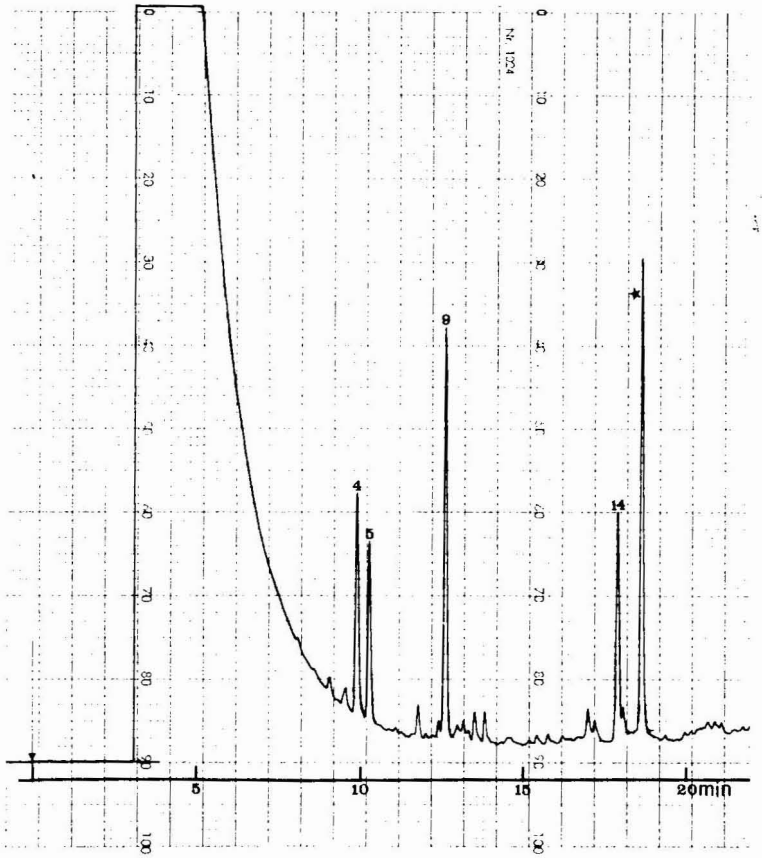


Fig. 3. Chromatogram of a plasma sample containing 19 μg/ml aprobarbital and 46 μg/ml vinbarbital. Internal standards (5,5-dipropyl barbituric acid and 5-allyl-5-phenyl barbituric acid) were added.

TABLE I

MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) FOR 21 SINGLE DETERMINATIONS ON PLASMA SAMPLES

Drug		*y* (μg/ml)*	$\bar{X}$ (μg/ml)	*S.D.*
1	Metharbital	15.0	8.8	3.5
1**	Metharbital	15.0	15.7	2.1
2	Barbital	14.4	10.2	3.4
3	Allobarbital	17.7	14.0	1.6
4	Aprobarbital	20.8	18.5	1.1
6	Buthethal	15.0	14.6	0.6
7	Amobarbital	15.0	15.5	1.5
8	Pentobarbital	15.0	16.5	2.3
9	Vinbarbital	15.0	16.8	2.7
10	Secobarbital	14.4	16.0	2.8
11	Hexobarbital	16.5	17.8	1.5
12	Glutethimide	15.8	15.5	1.3
13	Phenobarbital	15.0	14.5	0.9
15	Heptabarb	15.8	16.1	1.0

* Known plasma concentration.

** Ten determinations of metharbital with barbital as internal standard.

drugs give a better GC separation. Glutethimide and the barbiturates are ethylated in the nitrogen position. The structures of the barbiturates are shown in Fig. 1, and a chromatogram from a spiked plasma sample is shown in Fig. 2.

Standard curves were determined for all barbiturates and glutethimide using two different internal standards, dipropylbarbituric acid for barbiturates 1–10 (Fig. 1) and allylphenylbarbituric acid for the remaining barbiturates and glutethimide. The range of the standard curves was 5–40 μg/ml and they showed a good linear relationship. The correlation coefficient varied from 0.969 (barbital) to 0.999 (butethal). Means and standard deviations (S.D.) for 21 determinations at a concentration of *ca.* 15 μg/ml in plasma (all drugs added to spiked plasma) are in Table I. The quantitation of metharbital and barbital showed a higher S.D. depending on the large differences in retention time to internal standard. This was shown by running 10 determinations of metharbital with barbital as internal standard (Table I). The barbiturates are identified from their retention times. For positive identification the retention time should not differ by more than 0.5% from a standard sample containing all barbiturates. No interference was found by running acetylsalicylic acid, paracetamol, phenytoin, methaqualone or diazepam through the procedure. A chromatogram of a plasma sample from a patient is shown in Fig. 3, and the peaks can be identified as aprobarbital and vinbarbital. These barbiturates are in a multiple drug "Diminal-Duplex", one of the most common hypnotics in Sweden.

SCOT columns are not yet in common use but they appear to be preferable to packed columns when barbiturate separation is a problem. An unsplit inlet system can be used, and up to 1 μl solvent and 10 μg drug/peak could be injected without any detrimental long-term effect or overloading. In the end step of the analyses, 1 μl of hexane contains not more than 1 μg of each drug.

The method has been in use in our laboratory for more than a year, with good results. The sensitivity of the method in 500 μl samples seems sufficient for detection and quantitation in overdose patients.

ACKNOWLEDGEMENT

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CHROM. 10,134

Note

Extractive alkylation of biological samples of clioquinol or chloroquinaldol and determination by electron capture gas chromatography

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The two 8-hydroxyquinolines, clioquinol (5-chloro-7-iodo-8-quinolinol) and and cloroquinaldol (5,7-dichloro-2-methyl-8-quinolinol), which are frequently used as antibacterial and antimycotic agents, are responsible for very severe side-effects after oral administration. Despite the widespread use of these compounds, few methods exist for their determination in biological samples[1–3], and none of these is capable of measuring concentrations below 50 ng/ml.

Recently a method appeared which uses extractive methylation of clioquinol before analysis by gas chromatography with electron capture detection (GC–ECD)[4]. Quantitation of the methyl ether derivative down to 10 ng/ml of biological sample was claimed. However, neither the poor stability of clioquinol in plasma or urine samples, nor the rapid decomposition of the methyl derivative in heptane solution was considered. Furthermore, the reaction conditions would cause pronounced hydrolysis of the methylating reagent owing to a high co-extraction of hydroxide ions into the organic phase.

In the present method tetrabutylammonium is used as counter-ion for the phenolate as ion-pair, which is extracted into methylene chloride where methylation occurs. The influence of pH on the reaction has been studied with respect to time and degradation of formed derivative. The highly selective electron capture response enables determinations directly from urine or plasma samples with a sensitivity equal to that of the previous method[4].

EXPERIMENTAL

Gas chromatography

A Pye GCV gas chromatograph equipped with a flame ionization detector (FID) and a ^{63}Ni electron capture detector (ECD) operated in the constant current mode was used in the evaluation of reaction conditions and in the analysis of biological samples, respectively. The glass column (150 × 0.2 cm) was filled with 3% OV-17 on Gas Chrom Z 80–100 mesh and operated at 215°. Injector and detector temperatures were maintained at 290 and 300°, respectively. The flow-rate of nitrogen carrier gas was 30 ml/min.

Reagents and chemicals

Tetrabutylammonium (0.1 *M*) was prepared by neutralization of tetrabutylammonium hydrogen sulphate (AB Hässle, Mölndal, Sweden) and purified by washing with equal volumes of methylene chloride four times and heptane twice. The tetrabutylammonium solution was then diluted to volume with phosphate buffer (pH 11, $\mu = 1$).

Methyl iodide, methylene chloride and heptane (Uvasol quality) were obtained from E. Merck (Darmstadt, G.F.R.). A saturated solution of silver sulphate in water was used.

Chloroquinaldol was used as internal standard in the determination of clioquinol and *vice versa*. They were dissolved and diluted to 1 μg/ml with buffer. Fresh solutions of clioquinol were prepared each day. β-Glucuronidase was obtained from Sigma (St. Louis, Mo., U.S.A.).

Methods

To evaluate the reaction conditions, clioquinol and chloroquinaldol (1 mg/ml) were dissolved in methylene chloride (1 ml) together with tetracosane (0.5 mg/ml) as internal standard. After addition of methyl iodide, the solution was shaken with 2 ml of 0.1 *M* tetrabutylammonium in phosphate buffer. The reaction was quenched by washing the organic phase with 1 ml of 1 *M* phosphoric acid, and 1 μl of the organic phase was injected into the gas chromatograph equipped with FID. The height ratio of formed derivative to internal standard was then calculated.

To determine the minimum detectable concentration, a solution of the derivatives of clioquinol and chloroquinaldol, prepared in the mg/ml range, was diluted and 10 pg injected into the gas chromatograph with ECD[5].

The plasma or urine sample (0.1 or 0.5 ml) was made alkaline with two drops of 5 *M* sodium hydroxide, and 0.1 ml of internal standard solution was added. To this solution were added 1 ml of tetrabutylammonium solution, 0.1 *M* in buffer, and sufficient water to make up to 2 ml. This was shaken for 30 min with 1 ml of methylene chloride and 100 μl of methyl iodide. After centrifugation for 15 min at 2500 rpm, as much as possible of the organic phase was transferred to another tube, 0.5 ml of heptane was added and methyl iodide and methylene chloride were completely evaporated in a stream of nitrogen. Another 1 ml of heptane was added and the organic phase shaken for 10 min with 1 ml of saturated silver sulphate solution. Some microlitres of the organic phase were injected into the gas chromatograph.

A standard curve was prepared by treating known amounts of the actual 8-hydroxyquinoline in plasma or urine according to the above procedure.

RESULTS AND DISCUSSION

Reaction conditions

Rapid consumption of alkylating reagent in extractive alkylation procedures at pH > 12 with the use of tetrahexyl- or tetrapentylammonium ion has been observed by Gyllenhaal[6]. The reduction in reagent concentration decreases the reaction rate and hence the yield of derivative is low. As the consumption is due to the co-extraction and alkylation of buffer anions, the use of tetrabutylammonium as counter-ion is strongly recommended.

For a short reaction time the acid should be completely ionized. The time course of the methylation of clioquinol and chloroquinaldol is given in Figs. 1 and 2, which show that a pH greater than 10 is required for rapid reaction. The highest reaction rate was observed at pH 12 although the yield decreased, most rapidly for clioquinol, over prolonged reaction times. To avoid this effect a pH value of 11 was used. The methylation was completed in 30 min, and the yield remained constant for more than 3 h. The choice of buffer system was also emphasized by Gyllenhaal[6].

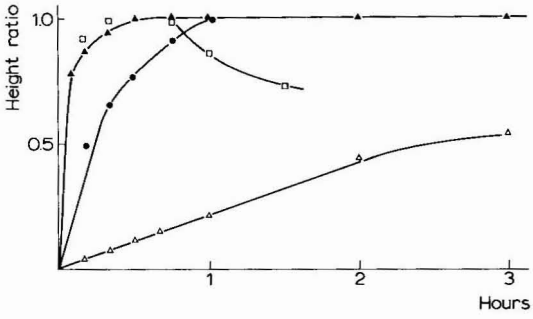

Fig. 1. Time course of the extractive methylation of clioquinol. Organic phase: methylene chloride with 10% of methyl iodide. Aqueous phase: tetrabutylammonium ion, 0.1 *M* in phosphate buffer ($\mu = 1$). □, pH 12; ▲, pH 11, ●, pH 10; △, pH 8.

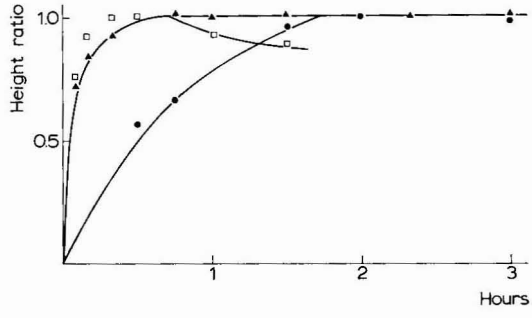

Fig. 2. Time course of the extractive methylation of chloroquinaldol. Organic phase: methylene chloride with 10% of methyl iodide. Aqueous phase: tetrabutylammonium ion, 0.1 *M* in phosphate buffer ($\mu = 1$). □, pH 12; ▲, pH 11; ●, pH 10.

After conversion into the methyl derivatives the 8-hydroxyquinolines are amenable to GC–ECD. The presence of two halogen atoms renders the compounds highly responsive to electron capture. The minimum detectable concentrations of clioquinol and chloroquinaldol as methyl derivatives were 0.5 and 1.6×10^{-16} mole/sec, or corresponding to an injected amount of 0.5 pg of clioquinol on a column with 1500 theoretical plates and with a retention time of 4 min. A reaction time > 2 h was required with 2% of methylating reagent, whereas *ca.* 20 min was necessary with 20%. In practice, 10% of methyl iodide was used.

Injection of the reaction mixture directly into the gas chromatograph was not possible as methyl iodide and methylene chloride seriously disturbed the ECD. After addition of heptane these were easily removed by evaporation. Tetrabutylammonium

iodide, formed as a by-product, was removed using a saturated solution silver sulphate in water[7].

Determination in biological samples

The stability of the two 8-hydroxyquinolines was studied in urine and plasma samples at room temperature. The degradation was measured against an inert standard (Mirex®), and the results are given in Table I. The stability of clioquinol was extremely poor and only 50% remained after 3 days. Samples stored in a refrigerator showed no degradation after 7 days. For minimum degradation the samples were frozen immediately after sampling and were analysed not more than 3 days later. Clioquinol was also unstable in buffer solution at pH 7, even if stored in a dark room. A similar degradation of the methyl derivative of clioquinol in heptane solution was observed. Chloroquinaldol was found to be stable under the above conditions.

TABLE I

STABILITY OF CLIQUINOL AND CHLOROQUINALDOL

Determination by GC–ECD by comparison to inert standard (Mirex®). Concentration of 8-hydroxyquinoline: $5 \cdot 10^{-6}$ *M* (1 μg/ml). Values are the height ratio to the internal standard.

Day	*Plasma*		*Urine*		*Phosphate buffer (pH7)*		*Derivative in heptane*	
	Clio-quinol	*Chloro-quinaldol*	*Clio-quinol*	*Chloro-quinaldol*	*Clio-quinol*	*Chloro-quinaldol*	*Clio-quinol*	*Chloro-quinaldol*
0	1.00	1.02	1.00	0.99	1.00	1.00	0.99	1.01
1	0.88	1.05	0.68	1.00	0.68	1.05	0.82	1.02
2	0.66	1.08	0.62		0.56	1.00	0.53	0.95
3	0.48	1.00	0.50	0.96	0.36	0.96	0.40	0.96
6	0.36	0.98	0.49	1.02	0.24	0.93	0.10	0.98

The method has been used to determine clioquinol and chloroquinaldol in plasma and in urine. The main fraction of the 8-hydroxyquinolines is excreted in the urine as conjugated metabolites. The selectivity of the extractive alkylation procedure to the conjugated metabolites was previously verified[4]. The total amount of the compounds in urine was determined following enzymatic hydrolysis with β-glucuronidase. Interference from other metabolites is not likely.

Rectilinear standard curves through the origin were obtained for the two compounds in the range 25–150 ng added to plasma or urine samples. The method was quantitated down to 10 ng of clioquinol in a 0.5 ml plasma sample. The relative standard deviation was 6.6% for 25 ng of clioquinol in 0.5 ml of plasma ($n = 8$).

The clinical applications of this study are reported elsewhere[8].

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Note

High-performance liquid chromatographic analysis of gramicidin, a polypeptide antibiotic

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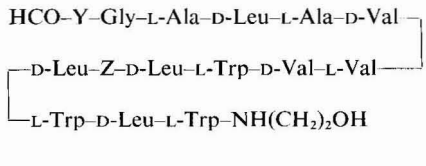
Gramidicin (Gdin), a polypeptide antibiotic isolated from tyrothricin, has previously been fractionated into three major components by countercurrent distribution (CCD)[1–3] and by droplet countercurrent chromatography (DCCC)[4]. There were strong indications that each of the major components, designated Gdin A, Gdin B and Gdin C, actually consists of two closely related components, designated valine-gramicidin ([Val]-Gdin) and isoleucine-gramicidin ([Ile]-Gdin)[5].

The gramicidins are linear N-acylated pentadecapeptide-ethanolamides with their terminal amino groups blocked by a formyl group. The structures of the individual components which have been elucidated are shown in Fig. 1[6–13].



	Y	Z	Mol.wt.
[Val]-Gdin A:	L-Val	L-Trp	1882
[Ile]-Gdin A:	L-Ile	L-Trp	1896
[Val]-Gdin B:	L-Val	L-Phe	1843
[Ile]-Gdin B:	L-Ile	L-Phe	1857
[Val]-Gdin C:	L-Val	L-Tyr	1859
[Ile]-Gdin C:	L-Ile	L-Tyr	1873

Fig. 1. Structure of the gramicidin components.

This paper describes a high-performance liquid chromatographic (HPLC) method that provides an efficient analytical separation of the gramicidin components. The method is used to determine the ratio of components in the World Health Organization (WHO) international reference preparation of gramicidin[14] and to study the effect of recrystallizations on ratio of components.

EXPERIMENTAL

Materials

The WHO international reference preparation of gramicidin (and the U.S. Food and Drugs Administration internal working standard) is identical with crystalline gramicidin lot No. 27 produced by H. Lundbeck (Copenhagen, Denmark)[14].

After an initial determination of the ratio of components this was used as the HPLC working standard.

For the identification and quantification of components in the working standard, relatively pure components were available from a CCD separation performed by Dr. Lyman C. Craig of the Rockefeller Institute, New York[14].

A non-purified gramicidin was used as starting material for the recrystallization experiment.

Recrystallizations

A saturated solution was prepared in 96% ethanol by gentle heating, and was left in a refrigerator for 48 h. The crystals were filtered off and dried in a vacuum oven at 60° for 5 h. Eight consecutive crystallizations were performed.

High-performance liquid chromatography

A DuPont 830 liquid chromatograph was used, with a DuPont 837 spectrophotometer detector operated at a wavelength of 220 nm. The column was a 25 cm × 2.1 mm I.D. stainless steel column prepacked with Zorbax ODS (DuPont, Hitchin, Great Britain), which is a microspheroidal silica packing with a chemically bonded octadecyl stationary phase (particle size 5 μm). The column was operated at 60° and at a pressure of 2000 p.s.i.

The mobile phase was a mixture of analytical grade methanol and a 0.005 *M* aqueous solution of ammonium sulphate in the ratio 74:26. Samples were dissolved in the mobile phase and introduced into the column by means of a six-port valve with a 10 μl loop corresponding to an injected amount of 1 μg gramicidin.

RESULTS AND DISCUSSION

The reference preparation

A typical chromatogram of lot No. 27 is shown in Fig. 2, and as expected from CCD and DCCC data, the elution sequence turned out to be:

[Val]-Gdin C
[Ile]-Gdin C
[Val]-Gdin A
[Ile]-Gdin A
[Val]-Gdin B
[Ile]-Gdin B

↓ increasing lipophilicity

The quantitative determination of components showed that lot No. 27 contains 78% w/w Gdin A, 14% w/w Gdin C, and 8% w/w Gdin B. The amount of [Ile]-Gdin in each of these is from 10 to 15% w/w. These values are listed in Table I and compared with values calculated on the basis of an amino-acid analysis[14] and the basic structures shown in Fig. 1. A very good agreement is observed.

The recrystallization experiment

It has been observed that the gramicidin components show quantitative differences in their antibacterial activity, and it is doubtful whether reliable micro-

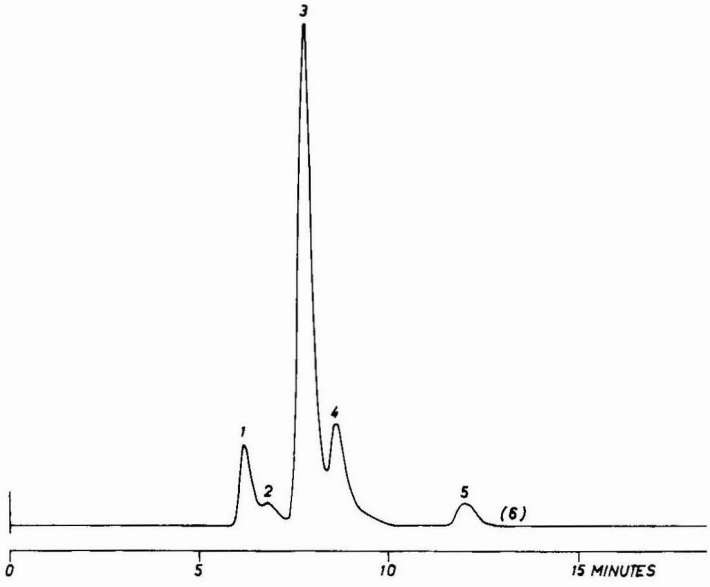


Fig. 2. Crystalline gramicidin lot No. 27. Chromatogram showing the separation of: [Val]-Gdin C (1), [Ile]-Gdin C (2), [Val]-Gdin A (3), [Ile]-Gdin A (4), [Val]-Gdin B (5), [Ile]-Gdin B (6).

biological assays can be performed if the sample and the standard preparation have different component ratios. Consequently it is of great importance that the influence of manufacturing procedures, such as recrystallizations, is known.

The ratios of components found in the amorphous starting material and in the crystalline fractions obtained are shown in Fig. 3, from which it appears that the ratios are influenced considerably by the first three or four crystallizations. Additional recrystallizations have little effect. It also appears that pure Gdin A cannot be obtained in this way. The influence of recrystallizations on melting points and solubilities of gramicidin has previously been reported[15].

Other applications

The HPLC method described is not restricted to gramicidin analysis. It is also

TABLE I

CRYSTALLINE GRAMICIDIN LOT No. 27 HPLC RESULTS COMPARED WITH CALCULATED VALUES BASED ON AMINO-ACID ANALYSIS AND THE BASIC STRUCTURE OF THE COMPONENTS (Fig. 1)

Components	*Quantitative determination by HPLC (% w/w)*	*Calculated on the basis of amino-acid analysis* (% w/w)*
[Val] + [Ile]-Gdin A	78	79**
[Val] + [Ile]-Gdin B	8	7
[Val] + [Ile]-Gdin C	14	14
[Ile]-Gdin A + B + C	10–15	14

* 0.98% Ile, 1.31% Tyr, 0.58% Phe (% w/w as free amino acids).

** Calculated as difference.

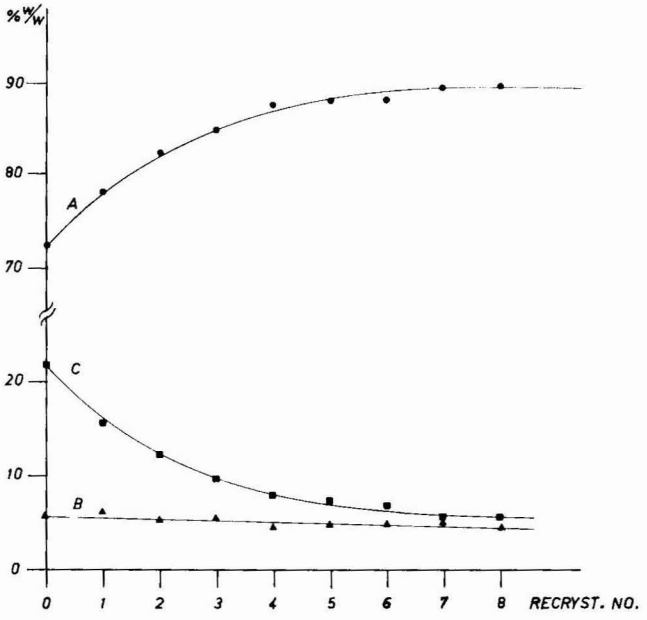


Fig. 3. Relationship between the ratio of components (Gdin A, Gdin B, and Gdin C) and the number of recrystallizations performed.

applicable to tyrothricin (gramicidin and tyrocidin), and to any intermediate between fermentation broth and crystalline gramicidin.

Fig. 4 shows a chromatogram of an extract of a fermentation broth. Several peaks are seen in the tyrocidin area, and it appears that a fermentation broth contains considerably more Gdin C than a crystalline gramicidin.

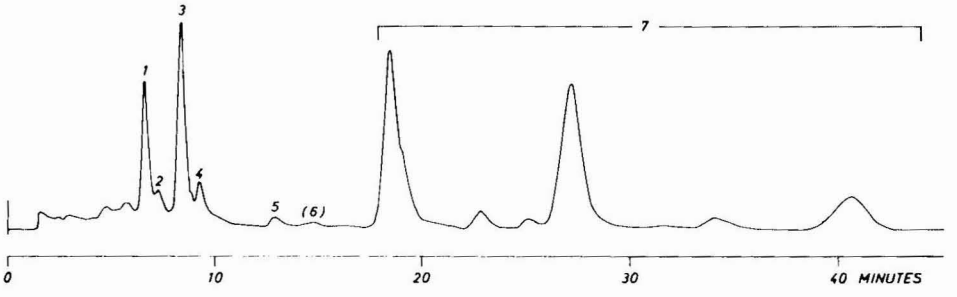


Fig. 4. Chromatogram of a fermentation broth showing gramicidins (1–6) (Fig. 2) and tyrocidins (7).

CONCLUSION

HPLC has provided a rapid and powerful tool for the component analysis of gramicidin and its intermediates. From an analytical point of view HPLC is considerably more efficient than earlier applied CCD- and DCCC-separations. The separation of gram-size samples would, however, require a very large and costly

increase in scale, and from a preparative point of view CCD- and DCCC-separations are still to be preferred.

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The authors express their thanks to Dr. L. Szabo, who has provided a major part of the chemical background knowledge necessary to carry out this study.

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CHROM. 10,225

Note

Improved thin-layer chromatographic method for the separation of major phospholipids and glycolipids from plant lipid extracts and phosphatidyl glycerol and bis(monoacylglyceryl) phosphate from animal lipid extracts

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A large number of solvents and stationary phases exist for the separation of both plant and animal glycerolipids using both one- and two-dimensional thin-layer chromatography (TLC)[1–4]. Two-dimensional TLC, while providing the best separation, is time consuming and often results in oxidation of some of the poly-unsaturated fatty acids of the lipids. A further disadvantage is that the amount of lipid that can be applied to a plate is very limited. On the whole, the one-dimensional TLC systems that exist do not completely separate all plant lipids. Similarly no one-dimensional TLC system has been reported that satisfactorily and consistently separates phosphatidyl glycerol and bis(monoacylglyceryl) phosphate from animal tissues.

In our work with phospho- and glycolipids from plant tissue we needed a rapid and complete separation of individual lipids for quantitative analysis by gas–liquid chromatography. Many of the TLC systems used to separate plant lipids did not separate phospholipids from the galactolipids, particularly digalactosyl diglyceride (DGDG), and often resulted in considerable overlap of other phospholipids and glycolipids. The solvent system of Pohl *et al.*[1] provided good separation of monogalactosyl diglyceride (MGDG) and DGDG but did not separate phosphatidyl glycerol and phosphatidylethanolamine consistently. We report here a TLC system which not only allows a highly reproducible separation of plant lipids but also separates phosphatidyl glycerol (PG) and bis(monoacylglyceryl) phosphate (bis-MP) from the crude lipid extracts of various mammalian tissues.

MATERIALS AND METHODS

Plant lipids

Lipids were extracted from leaves of *Vicia faba* by homogenization in a high-speed blender (VirTis Model 45, Fisher Scientific) with chloroform–methanol (2:1, v/v). Lipid extracts were filtered through PTFE-coated Millipore filters and washed thoroughly with chloroform–methanol (2:1) and chloroform. Water soluble non-lipid contaminants were removed by the method of Williams and Merrilees[5]. The chloroform solution of lipids was dried in a flash evaporator, redissolved in a suitable volume of chloroform and stored under nitrogen in a freezer until use. Labelled

lipids (^{14}C) were extracted from leaf discs infiltrated with [^{14}C]acetate solution or fed $^{14}CO_2$ in light (1100 ft-c.)[6,7].

Animal lipids

Male rats of the Wistar strain, weighing 200–250 g, received chlorphentermine (50 mg/kg) intraperitoneally for two weeks[8]. Animals were killed after fasting for 16 h, livers and spleens were removed and the lipids were extracted with 19 volumes of chloroform–methanol (2:1). The crude lipids were purified according to the method of Folch *et al.*[9].

Purification of bis(monoacylglyceryl) phosphate

Male Wistar rats weighing 200–250 g were fasted for 16 h before sacrifice. Livers from 2 rats were removed and the lipids were extracted as described previously. Lipids were dissolved in 100 ml of methanol–chloroform–water (60:30:8) and put on to a DEAE-Sephadex column, 30 × 2.5 cm (ref. 10). The column was eluted sequentially with 1 l of methanol–chloroform–water (60:30:8) and 1 l of methanol–chloroform–0.2 *M* sodium acetate (60:30:8). The acidic phospholipids, containing bis-MP, were eluted with the second eluting medium and were concentrated. The lipids were redissolved in 25 ml of chloroform–methanol (2:1) and sodium acetate was removed by washing with water. Lipids were concentrated and chromatographed to separate bis-MP[11]. After elution from the gel the lipid was rechromatographed to check the purity[12]. A single spot of bis-MP was detected after spraying sulphuric acid and charring at 150°. The purified bis-MP was used as a reference compound. Phosphatidyl glycerol standard was purchased from Supelco.

Preparation of TLC plates

Normal silica gel G plates were prepared by applying a slurry of 40 g of gel in 100 ml of water to glass plates (20 × 20 cm) at a thickness of about 0.37 mm using a Desaga spreader. Ammonium sulphate impregnated plates were prepared by substituting 0.15 *M* ammonium sulphate solution for water. Both types of plates were left at room temperature for 5 min and then dried in an oven at 110° for 4 h. The plates were cooled to room temperature just prior to use. If the plates are stored at room temperature they must be activated for 1 h at 110° before use.

Application of samples and developing solvents

The samples and reference compounds were dissolved in chloroform or chloroform–methanol (2:1) and applied to the plate in a band with a glass applicator. The TLC chambers were lined on both sides with filter paper saturated with the chromatographic solvents. The ammonium sulphate impregnated plates were developed in acetone–benzene–water (91:30:8). For comparison normal plates were developed in the same solvent and also in chloroform–methanol–water (65:25:4)[2]. The plates were developed by the ascending technique until the solvent front had reached a distance of 2 cm from the top whereupon they were removed and dried under nitrogen.

Identification of lipids

Lipid bands were identified using reference compounds and appropriate spray

reagents. The following spray reagents were used: phospholipid spray reagent of Vaskovsky and Kostetsky[13], ninhydrin (2% in ethanol) for phosphatidylethanolamine and phosphatidyl serine, cresyl-violet acetate for sulphoquinovosyl diglyceride[14]. Lipids were also visualized by charring the plates after spraying with sulphuric acid. When the lipids were used for subsequent GLC analysis, chromatoplates were sprayed with 2′,7′-dichlorofluorescein and visualized under UV light.

Gas–liquid chromatography

Fatty acid methyl esters of individual lipids were prepared as described previously[6] and analysed on a dual-column gas chromatograph (Packard Model 7401) equipped with flame ionization detector and a digital integrator (Infotronics Model CRS 208). Samples were injected onto a glass column (4 mm × 180 cm) packed with 10% EGSS-X on Chromosorb P and run isothermally at 180°.

RESULTS

Plant lipids

The autoradiographs of ^{14}C-labelled lipids separated by three different chromatographic systems are shown in Fig. 1. Although the solvent system chloroform–methanol–water (65:25:4) allowed a distinct separation of MGDG from the rest of the lipids, the nearly equal R_F values of PG and PC, PE and DGDG and the complete overlapping of PE with an unidentified lipid made the whole system difficult to use for qualitative as well as quantitative studies (see legend of Fig. 1 for abbreviations). Besides, the lipid bands were often contaminated with pigments. The TLC system recommended by Pohl *et al.*[1] was used to separate galactosyl diglycerides but the separation of phospholipids was found to be very inconsistent and often resulted in the overlapping of PG with PE, and SL with the unidentified lipid. The method described here offered advantages over the above TLC techniques because of the complete separation of PC, PE, PG and SL as well as the galactosyl diglycerides. With the exception of PG and the unidentified lipid, the mobilities of the glycolipids and the phospholipids were not significantly different from the system used by Pohl *et al.* In order to check the purity of each of these lipids, individual lipids were separated by preparative TLC, eluted from the gel and rechromatographed separately by the above three systems; each lipid gave a single band.

Fatty acid methyl esters of individual lipids separated on ammonium sulphate plates were prepared[6] and analysed by GLC; each lipid showed its characteristic fatty acid profile (Table I). *Trans*-Δ^3-hexadecenoic acid is only found in PG and thus used as a marker for PG in green photosynthetic tissues. Galactosyl diglycerides were obtained free of contaminants. GLC analyses of deacylated products of MGDG and DGDG, as reported previously[7], was used to confirm their identity.

Animal lipids

PG and bis-MP are present in mammalian tissues in such small quantities that it becomes impossible to separate and detect them by two-dimensional TLC unless the lipids are previously labelled with radioactive precursors. Attempts were made to increase the level of bis-MP in liver and spleen by drug treatment and the total lipids were chromatographed by using our chromatographic system. The details of

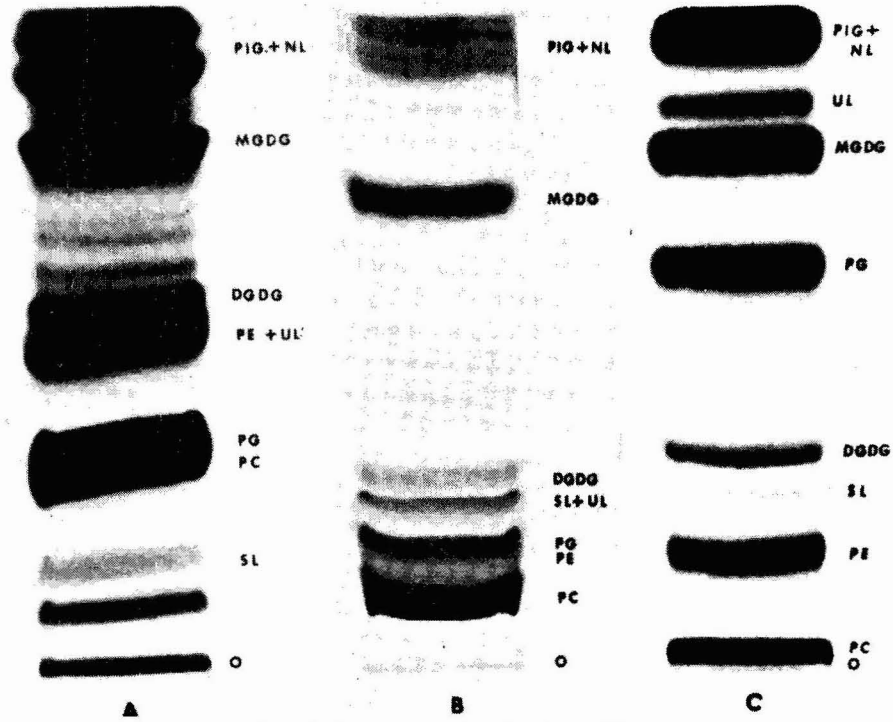

Fig. 1. Autoradiographs of TLC plates comparing the three different systems employed during the separation of ^{14}C-labelled lipids from *Vicia faba* leaves. A, silica gel G plates in chloroform–methanol–water (65:25:4); B, silica gel G plates in acetone–benzene–water (91:30:8): C, ammonium sulphate impregnated silica gel G plates in acetone–benzene–water (91:30:8). O = origin; PC = phosphatidyl choline; PE = phosphatidylethanolamine; PG = phosphatidyl glycerol; MGDG = monogalactosyl diglyceride; DGDG = digalactosyl diglyceride; SL = sulphoquinovosyl diglyceride; UL = unidentified lipid; PIG + NL = pigments and neutral lipids.

TABLE I

FATTY ACID COMPOSITIONS (mole %) OF PHOSPHOLIPIDS AND GLYCOLIPIDS FROM GREEN LEAVES OF *VICIA FABA*

For abbreviations of lipids, see legend to Fig. 1.

Lipids	*No. of C at. in fatty acid: no. of unsaturated bonds*						*Quantity (μmole/g fresh wt.)*
	16:0	*16:1*	*18:0*	*18:1*	*18:2*	*18:3*	
PC	19	—	3	14	49	15	1.90
PE	28	—	3	6	45	18	0.64
PG	40	11	2	6	16	25	0.83
MGDG	3	—	trace	2	9	85	2.96
DGDG	10	—	3	1	4	82	1.72
SL	28	—	4	5	17	46	0.29
UL	23	—	4	6	17	50	0.16

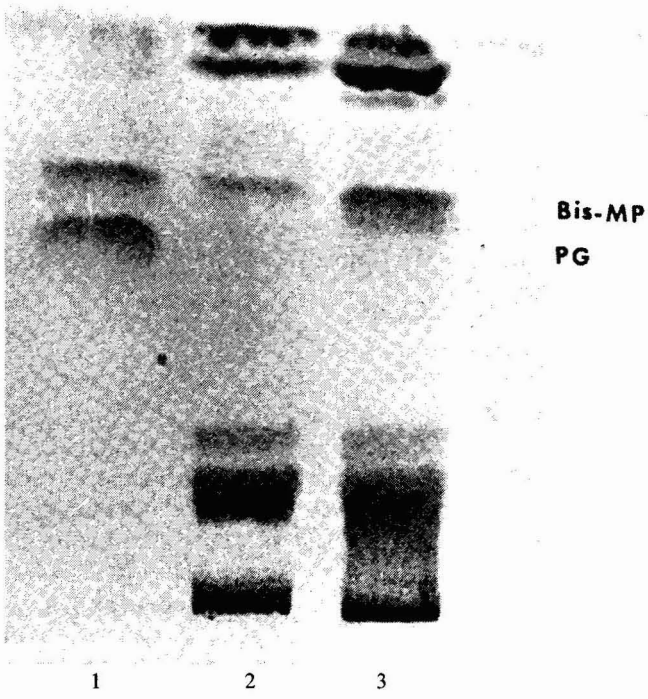

Fig. 2. TLC of total lipids from rat liver and spleen. Samples in lanes 1–3 are reference compounds of PG + bis-MP, spleen and liver lipids, respectively. Samples were applied to silica gel G plates impregnated with ammonium sulphate and developed with acetone–benzene–water (91:30:8).

TABLE II

FATTY ACID COMPOSITION OF BIS(MONOACYLGLYCERYL) PHOSPHATE DERIVED FROM RAT LIVER AND SPLEEN TISSUES AFTER TREATMENT WITH CHLORPHENTERMINE (50 mg/kg)

Fatty acid (no. of C at.: no. of unsaturated bonds)	*% by weight*	
	Liver	*Spleen*
14:0	tr*	2
16:0	3	9
16:1	1	3
18:0	3	2
18:1	8	37
18:2	7	19
20:0	1	tr*
20:1	1	2
Unidentified	tr*	2
22:0	3	4
20:5	2	2
24:0	1	4
24:1	2	2
Unidentified	3	2
22:6	64	6
Unidentified	1	3

* Trace amount, $< 0.5\%$.

the drug effect on the metabolism of phospho- and glycolipids will be published elsewhere[15].

Fig. 2 shows the separation of bis-MP from total lipids extracted from liver and spleen tissues of rats previously treated with chlorphentermine. Most non-acidic phospholipids remained near the origin whereas bis-MP moved further up the plate and also separated distinctly from its closely related lipid, PG. Thus the two lipids can be scraped out of the plate with the silica gel and recovered quantitatively. Fatty acid methyl esters were prepared from bis-MP and analysed by GLC. The percent composition of the fatty acids of rat liver bis-MP (Table II) were very similar to the results obtained from liver "tritosomes"[16].

DISCUSSION

The use of ammonium sulphate to minimize absorption effects has previously been recommended by Chalvardjian *et al.*[17]. Mangold and Kammereck[18] have used silica gel G TLC plates impregnated with 10% ammonium sulphate for the separation of phospholipids and strong acidic fatty acid derivatives. Horrocks[19] has employed ammonium sulphate to suppress the streaking problem in some phospholipids. Recently, Walker[20] has reported a technique to detect the lipids separated on silica gel G TLC plates impregnated with ammonium sulphate, simply by charring.

The present method utilizes the solvent system of Pohl *et al.* that was used to separate plant phospho- and glycolipids on silica gel G plates, and the purpose of using ammonium sulphate impregnated silica gel G plates is to modify the mobility of some acidic phospholipids. In contrast to many methods for the separation of lipids this system has proved to be a rapid technique which gives reproducible results and allows a complete separation of the major phospholipids and glycolipids. The chromatoplates can be loaded with the plant lipid extract equivalent to 1.5–2 mg of chlorophyll (8–10 μmoles of lipids) and separation is complete in 40–45 min.

Since PG and bis-MP are present in most mammalian tissues in extremely small quantities[21] a large amount of total lipid extract should be loaded onto a TLC plate in order to detect these lipids. The advantage of the TLC system reported here is that it can be loaded with approximately 10–12 mg of total lipids and still give excellent separation of PG and bis-MP. We believe this method should be useful for scanning and quantitative recovery of phosphatidyl glycerol and its derivatives synthesized during incubations of rat liver mitochondrial preparations with cytidine diphosphate diglyceride and radioactive *sn*-glycerol-3-phosphate.

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Book Review

Modern methods of chemical analysis, by R. L. Pecsok, L. D. Shields, T. Cairns and I. G. McWilliam, Wiley, New York, Santa Barbara, London, Sydney, Toronto, 2nd ed., 1976, XVIII + 573 pp., price £ 13.50, US$ 21.50, ISBN 0-471-67662-4.

This is the second edition of a student textbook on instrumental analytical techniques first written about ten years ago and it takes recent developments into account. Although the word "chemical" appears in the title, gravimetric and volumetric analyses are not included in this publication, since these are usually dealt with in chemistry courses. The authors' aim to reach a compromise between an elementary survey and a sophisticated treatment in depth has been successfully achieved, so that the book will appeal to chemists and non-chemists alike.

The book, divided into eight sections, opens with 114 pages covering phase changes and separations. After introducing and developing the concepts of distribution coefficients and flow-through columns, chromatography is dealt with in four chapters (70 pages). The first of these describes the various forms of chromatography and basic theory. The second chapter discusses the whole range of liquid chromatography in columns. However, the modern version of high-performance liquid chromatography only occupies a page and a half. Judging by its content, it was written in 1972, *i.e.* a long time ago at its present rapid rate of development.

A third, short chapter describes layer techniques, whilst the final and strongest chapter in this section gives a satisfactory introduction to the student of gas chromatography. Two further sections of direct interest to the chromatographer, which were not in the original edition, discuss statistics and data processing, automatic and process analysers.

Other parts of this book include a very strong section on electromagnetic radiation (particularly infrared, atomic absorption, X-ray and nuclear magnetic resonance spectroscopy), mass spectrometry, electro-analytical and radiochemistry, acid–base and complex ion equilibria.

The book is well organised into self-contained sections, with a well-presented text and clear diagrams, all of which contribute to enjoyable reading. Whilst the book is clearly aimed at the student population, it will also enable the specialist chromatographer to revise his understanding of the principles of other analytical instrumental techniques, which he may have neglected in recent years.

Abingdon (Great Britain) R. AMOS

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2. FUNDAMENTALS, THEORY AND GENERAL

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11. ORGANIC ACIDS AND LIPIDS

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See also: 3444.

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See also: 3313, 3320, 3462.

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See also: 3473.

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See also 4054.

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See 3769.

Thin-Layer Chromatography

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See also 3739.

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See also 3740, 3954, 3971.

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See also 3743.

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See also 3745.

9. OXO COMPOUNDS

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See also 3950.

10. CARBOHYDRATES

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11. ORGANIC ACIDS AND LIPIDS

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See also 3756, 3758, 4115.

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21. PURINES, PYRIMIDINES, NUCLEIC ACIDS AND THEIR CONSTITUENTS

21a. Purines, pyrimidines, nucleosides, nucleotides

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21b. Nucleic acids: RNA

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32a. Synthetic drugs and systematic analysis

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See also 4196, 4201.

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4479 Kovach, A.: Analytical questions of radiochemical purity control of protein aggregates labeled with radioiodine. *Izotoptechnika*, 18 (1975) 228-235; *C.A.*, 84 (1976) 111618m - Whatman 3MM paper.

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35. SOME TECHNICAL PRODUCTS AND COMPLEX MIXTURES

35a. Surfactants

4482 Akhtar, S. and Lai, E.L.: Use of electrophoresis in determining the chemisorption of surfactants on mineral surfaces. *AIChE Symp. Ser.*, 71 (1975) 110-117; *C.A.*, 84 (1976) 141076a.

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Vol. 140, No. 2

journal of
chromatography news section

APPARATUS

N-1044

RECORDER CHECKER

The portable, battery-operated recorder checker from Fisher Scientific Co. verifies linearity and full-scale accuracy of any 1, 5 or 10 mV recorder with input impedance of 100K ohms or greater.

N-1045

"RETENTION TIMES"

The current issue of Retention Times from Tracor features the Tracor Hall electrolytic conductivity detector and its application for analysis of halomethanes in drinking water: capillary columns for Tracor gas chromatographs; Mini-Lab integrator for use with the Tracor 560 gas chromatograph; 980A solvent programmer for liquid chromatography; and the Auto Scan option for the 970 LC detector.

N-1047

PERKIN-ELMER LIQUID CHROMATOGRAPHS

A new range of modular liquid chromatographs is announced by Perkin-Elmer, the Series 2. Each chromatograph is compatible with all commonly available detectors. The new instruments complement the Model 601 research-grade liquid chromatograph. The Series 2/1 is a single module providing solvent delivery and sample injection. The instrument features high-pressure capability and has an adjustable flow-range between 0.1 and 29.9 ml/min. The model is intended for routine quality control, instructional situations, and dedicated analysis.

The Series 2/2 combines the operating features of two Series 2/1 pumps and a sample injection system. The instrument provides linear solvent and flow programming capability from 0.1 to 30 ml/min, and also offers direct mixing of selected solvent compositions and allows independent solvent changeover. The Series 2/2 is intended for analytical methods development, organic synthesis and natural products research, and quality control problem solving.

N-1035

MULTI-RANGE CHART RECORDER

A two-page bulletin describes the new flat-bed multi-range (from 1 mV to 100 V) chart recorder from McKee-Pedersen (MP-1027-MR). Complete specifications of the instrument are given, and features such as the remotely controllable chart drive and pen lift are described in detail.

N-1058

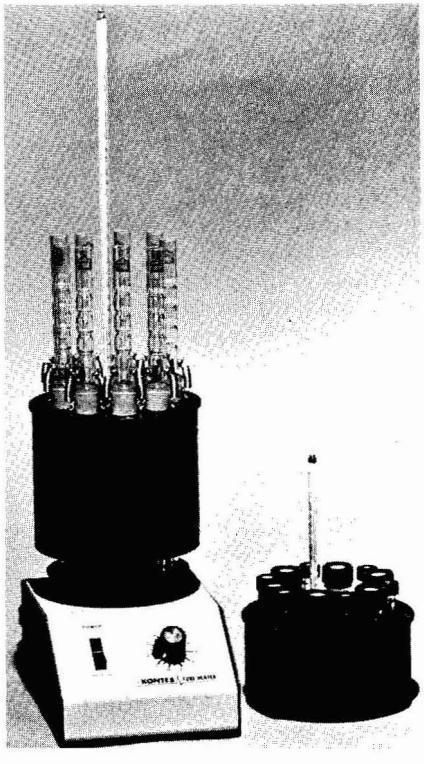
SGE SYRINGES

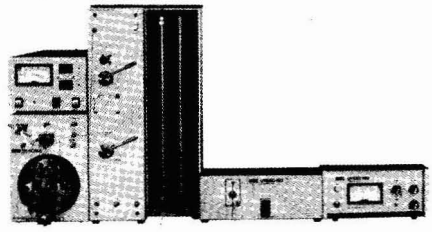
Scientific Glass Engineering (UK) announce that they can now supply their A-type and gas-tight syringes in 50-μl versions. This size is of particular use with syringe loading HPLC valves.

N-1050

TUBE HEATER/CONCENTRATOR

Kontes announces the availability of a new multiple tube heater/concentrator. Eight interchangeable heater blocks allow the accommodation of a wide range of tubes. Temperature control is $\pm 0.5^{\circ}$ in the range 30–160°. The movable base plate allows tubes to protrude below the heating zone to prevent evaporation of samples. The unit is particularly suitable for hydrolysis, enzyme reactions, concentrating solutions and derivatization reactions.





N-1052

HPLC PUMP

The Tri Rotar from Japan Spectroscopic (JASCO) is a new three-head HPLC pump. The suction and discharge rates are balanced, allowing continuous liquid delivery, with essentially pulseless flow. Gradients obtained by low-pressure mixing can be arranged with the one pump over a wide dynamic range. Recycle is possible simultaneously with fractionation. A variety of columns, detectors and accessories are available to form an appropriate HPLC system for the required purpose.

N-1062

HIGH-TEMPERATURE SIZE-EXCLUSION CHROMATOGRAPHIC SYSTEM

DuPont Instruments announce the development of a new high-temperature size-exclusion chromatographic (SEC) system which characterizes polymers by molecular weight at temperatures up to 140°. Separation times are reduced over conventional methods: polyethylene can be seperated in 10 minutes. Molecular weight is pin-pointed to within $\pm$ 3%. The new SEC system features a modified infrared detector which provides identification of specific functional groups in polymer systems. Other components include a solvent pre-heater, a high-temperature injection valve and the flow-controlled DuPont 830 liquid chromatograph. Incorporated in the system are DuPont's new porous-silica-based SEC columns which are inherently insensitive to temperature cycling.

For further information concerning any of the news items, apply to the publisher, using the reply cards provided, quoting the reference number printed at the beginning of the item.

CHEMICALS

N-1030

PIERCE HANDBOOK AND GENERAL CATALOGUE

Just published, Pierce Chemical Company's new 1977–1978 handbook and general catalogue features chemicals and reagents used in amino acid analysis, sequence determination, structure and function analysis, peptide synthesis, and gas and liquid chromatography. Laboratory accessories include vials and closure systems, syringes, gas and liquid chromatography accessories and Corning® controlled pore glass. Also included, reference books available through the Company, and description of the Pierce line of clinical diagnostic kits, reagents and related products.

N-1031

GC PERFORMANCE STANDARDS

Pierce Chemical Co. has made available performance standards individually designed for flame-ionization (FID), electron-capture (ECD), and thermoconductivity (TCD) detection systems, allowing routine monitoring of column efficiency, resolution, detector response and other performance characteristics. They will enable the user to troubleshoot problems as they occur, compare and standardize new columns with columns currently in use, and establish system optimization. Each standard is available in a package of ten 1-ml ampoules. The FID test standard contains 0.03% w/v each of normal C_{14}, C_{15} and C_{16} in isooctane; the ECD standard 33 pg/μl each of lindane and aldrin in isooctane; and the TCD test 0.3% w/v each of normal C_{14}, C_{15} and C_{16} in isooctane.

N- 1063

APPLIED SCIENCE LABS' CATALOGUE

Now available is Applied Science Labs' 1977 Catalogue No. 20, comprising 150 pages of products for GC, LC and TLC as well as sections for biochemicals and standards, isotope-labelled chemicals, reagents and pilot plant services (custom synthesis).

N-1060

ANALTECH CATALOGUE

The new Analtech catalogue of TLC and laboratory equipment is now available. Featured are amongst others the new Analtech hard-layer TLC plates.

N-1061

ALLTECH CATALOGUE

Now available is the 1977–78 Alltech catalogue listing their supplies in GC, HPLC and TLC. A 13-page section on capillary columns and accessories is also included.

N-1070

CHROMPACK CATALOGUE

Now available is the Chrompack 1977 catalogue No. 9 listing in 238 pages their supplies for gas chromatography, column liquid chromatography and thin-layer chromatography.

PROCEDURES

N-1049

CHROMATOGRAPHY TEACHING MEDIA

Among the multimedia science programmes of Communication Skills Corporation (CSC) listed in the Prentice Hall Media 1977 catalogue are three lecture support programmes on basic and advanced gas chromatography and basic liquid chromatography. Each programme consists of a set of slides or filmstrip, an audio cassette, and a frame-by-frame teacher's guide.

N-1057

GC APPLICATIONS

Scientific Glass Engineering have produced the first two of a series of GC Application Data Sheets featuring the use of their glass SCOT columns for the analysis of (i) lavandin oil, and (ii) a paraffin/naphthene mixture isolated from a crude oil.

NEW BOOKS

Treatise on analytical chemistry, Part 3, Analytical chemistry in industry, Vol. 3, edited by I.M. Kolthoff, P. Elving and F.H. Stross, Wiley-Interscience, New York, London, 1976, xxi + 598 pp., price US$ 46.95, £27.70, ISBN 0-471-50012-7.

Fluorescence and phosphorescence spectroscopy: physicochemical principles and practice, by S.G. Schulman, Pergamon, Oxford, New York, 1977, x + 288 pp., price £ 11.00, US$ 20.00, ISBN 0-08-020499-6.

Electrochemistry – the past thirty and the next thirty years, (A volume in honor of J. O'M. Bockris), edited by H. Bloom and F. Gutmann, Plenum, New York, London, 1977, xiii + 450 pp., price US$ 49.50, ISBN 0-306-30921-1.

X-Ray fluorescence analysis of environmental samples, by T.G. Dzabay, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1977, 312 pp., price US$ 30.25, ISBN 0-250-40134-7.

Colorimetric and fluorimetric analysis of steroids, by J. Bartos and M. Pesez, Academic Press, New York, London, 1976, xii + 274 pp., price US $ 21.50, £ 9.80, ISBN 0-12-080150-7.

Trends in electrochemistry, edited by J.O'M. Bockris, D.A.J. Rand and B.J. Welch, Plenum, New York, London, 1976, 388 pp., price US $ 35.00, ISBN 0-306-30990-4.

Fundamentals of integrated GC–MS, Part III, The integrated GC–MS analytical system (Chromatographic Science Series, Vol. 7, Part III), by B.J. Gudzinowicz, M.J. Gudzinowicz and H.F. Martin, Marcel Dekker, New York, Basel, 1977, ix + 603 pp., price SFr. 190.00, ISBN 0-8247-6431-5.

Progress in drug metabolism, Vol. II, edited by J.W. Bridges and L.F. Chasseaud, Wiley, Chichester, New York, 1977, *ca.* 368 pp., price *ca.* £ 14.00, US $ 30.00, ISBN 0-471-99442-1.

Modern methods of trace element analysis, by M. Pinta, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1977, price US$ 29.50, ISBN 0-250-40152-5.

MEETING

TWELFTH INTERNATIONAL SYMPOSIUM ON CHROMATOGRAPHY

The 12th International Symposium on Chromatography will be held in Baden-Baden, G.F.R., from September 25–29, 1978.

To be included in the programme are discussion papers in all fields of chromatography and related techniques as well as a number of invited plenary lectures and reviews. Lectures may be held in English, French or German; in order to facilitate communication however, it is recommended that English be used where possible.

The symposium is jointly organised by the "Chromatography Discussion Group", the "Groupement pour l'Avancement des Méthodes Spectroscopique et Physicochimique d'Analyse" (G.A.M.S.) and the "Arbeitskreis Chromatographie der Fachgruppe 'Analytische Chemie' der Gesellschaft Deutscher Chemiker".

Persons interested in participation in this symposium are requested to contact the address given below. Notification of proposed discussion papers, together with an abstract of approximately 300–600 words should be sent before November 15, 1977 to: Geschäftsstelle der Gesellschaft Deutscher Chemiker, Abteilung Fachgruppen, Postfach 900440, 6000 Frankfurt/Main 90, G.F.R., before November 15, 1977.

In conjunction with the symposium, an exhibition of instruments and accessories is planned. This exhibition is to be of a new and concentrated form. Firms interested in participation in this exhibition should direct their enquiries to: Dr. K.-P. Hupe, Ohmstrasse, 7500 Karlsruhe 41, G.F.R.

JOURNAL OF CHROMATOGRAPHY LIBRARY

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A series of books devoted to chromatographic techniques and their applications.

Although complementary to the Journal of Chromatography, each volume in the library series is an important and independent contribution in the field of chromatography. It should be stressed that the library contains no material reprinted from the journal itself.

Volume 11

LIQUID CHROMATOGRAPHY DETECTORS

by R. P. W. Scott

1977. x + 248 pages
Price: US $34.50/Dfl. 84.00
ISBN 0-444-41580-7

The rapid development of liquid chromatography over the past decade has been due to the introduction of highly sensitive linear liquid chromatography detectors. This book provides a comprehensive treatment of the function and optimal working conditions of liquid chromatography detectors. It is divided into four parts.

Part 1 includes a detailed discussion of properties of the detecting system that can impair column performance and how these effects can be minimized. In Parts 2 and 3, the various types of detectors that have been developed are described and a detailed treatment given of commercially available detectors. Part 4 discusses the practical operation of liquid chromatography, including methods for quantitative analysis as well as practical hints on detector operation and special detector techniques. The final chapter deals with spectroscopic detectors and provides a detailed description of LC/UV and LC/MS systems.

This work will be particularly useful because of the presentation of the necessary detector specifications which will enable readers to make a rational comparison of the performance of one detector with that of another.

CONTENTS: Introduction. General characteristics of liquid chromatography detectors. Bulk property detectors. Solute property detectors. The use of detectors in liquid chromatography. Subject index.

Volume 10

GAS CHROMATOGRAPHY OF POLYMERS

by V.G. Berezkin, V.R. Alishoyev and I.B. Nemirovskaya

1977. xiv+226 pages.
Price: US $41.95/Dfl. 103.00.
ISBN 0-444-41514-9.

At present, gas chromatography is the most widespread method for the analysis of organic compounds.

This book is devoted to the strategy of application of gas chromatography in polymer chemistry and discusses, in detail, the use of gas chromatography in research work and the polymeric compounds industry. It is the second, revised and enlarged edition of the original version published in the USSR in 1972.

The following principal applications are covered: analysis of monomers and solvents, determination of the contents of volatile substances in polymers, study of polymer formation processes, investigation into types of disintegration of high-molecular-weight compounds, polymer analysis by reaction and pyrolytic chromatography, and study of polymers and their reactivity with the aid of inverse chromatography.

This work will be of value to research institutions, industrial enterprises and senior students engaged in the fields of polymer or analytical chemistry and gas chromatography.

CONTENTS: Introduction. Basic principles of GC. GC methods for the analysis of monomers and solvents. The study of polymer formation reactions. Determination

of volatile compounds in polymer systems. Study of the kinetics and mechanisms of chemical transformations of polymers at elevated temperatures. Reaction GC of polymers. Pyrolysis GC. Inverse GC. Conclusion.

Volume 9

HPTLC - HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

edited by A. Zlatkis and R.E. Kaiser.

1977. 240 pages.
Price: US $44.95/Dfl. 110.00.
ISBN 0-444-41525-4.

HPTLC is the advanced technology of thin-layer chromatography and is defined as the combined action of several variables which include: an optimized coating material with a separation power superior to the best high performance liquid chromatographic separation material; a new method of feeding the mobile phase; a novel procedure for layer conditioning; a considerably improved dosage method and a competent data acquisition and processing system. Thus a complete system and procedure is discussed here. This should be understood as a stepwise improvement of an analytical method, which has been a powerful tool since the pioneering work of E. Stahl.

The results achieved, as well as the promising aspects of the new method are encouraging enough to refer to the technique as the second generation of thin-layer chromatography. The final judgement however, will be left to those who use this new methodology.

CONTENTS: Simplified theory of TLC *(R.E. Kaiser).* The separation number in linear and circular TLC *(J. Blome).* Advantages, limits and disadvantages of the ring developing technique *(J. Blome).* The U-chamber *(R.E. Kaiser).* Dosage techniques in HPTLC *(R.E. Kaiser).* High performance thin-layer chromatography: development, data and results *(H. Halpaap, J. Ripphahn).* Consideration on the reproducibility of TLC separations *(D. Jaenchen).* Potential and experience in quantitative HPTLC *(U.B. Hezel).* Application of a new high-performance layer in quantitative TLC *(J. Ripphahn, H. Halpaap).* Appendix. Index.

Volume 8

CHROMATOGRAPHY OF STEROIDS

by E. Heftmann.

1976. xiv+204 pages.
Price: US $36.75/Dfl. 90.00.
ISBN 0-444-41441-x

The qualitative and quantitative analysis of individual steroids is of great interest to pharmacologists, physicians, biochemists, plant and animal physiologists and microbiologists.

The principal chromatographic methods of analysis applicable to steroids are: liquid column chromatography (including its recent modification, high-pressure liquid chromatography), thin-layer chromatography and gas chromatography (including the recently introduced coated capillary chromatography).
Since Neher's book "Steroid Chromatography" published by Elsevier in 1964, these applications have not been surveyed in a single volume. Here, the author takes up where Neher left off and presents a detailed description of the currently used techniques. Although some theory is included, this is mainly a laboratory handbook, arranged according to the steroids analyzed as well as according to the methods used.

CONTENTS: Introduction. Liquid column chromatography. Paper and thin-layer chromatography. Gas chromatography. Relations between structure and chromatographic mobility. Sterols. Bile acids and alcohols. Estrogens. Androstane derivatives. Pregnane derivatives. Corticosteroids. Miscellaneous steroid hormones. Vitamins D. Molting hormones. Steroid sapogenins and alkaloids. Cardenolides and bufadienolides. List of Abbreviations. References. Subject Index.

Volume 7

CHEMICAL DERIVATIZATION IN LIQUID CHROMATOGRAPHY

by J.F. Lawrence and R.W. Frei

1976. viii+214 pages.
Price: US $36.75/Dfl. 90.00.
ISBN 0-444-41429-0

This book is intended for all investigators concerned with the use of physical separation techniques for solving complex

analytical problems. It is the first publication to provide a comprehensive account of modern derivatization in liquid chromatography with special emphasis on the practical aspects.

An introductory chapter familiarizes the reader with the basic philosophy of using chemical reactions and labelling procedures to enhance sensitivity, specificity and separation properties in liquid chromatographic techniques. The second chapter enables the practical worker to refresh his memory on some fundamental principles necessary to this work. The third deals with equipment and gives the analyst an idea of the choice of tools available to suit his needs. The final chapter helps the investigator to solve some concrete problems, to extend the concept of compounds and types of problems of immediate interest to him and to become familiar with the literature.

CONTENTS: Introduction. Background. Instrumentation. Applications. Subject Index.

Volume 6

ISOTACHOPHORESIS

Theory, Instrumentation and Applications

by F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen.

1976. xiv+418 pages.
Price: US $65.50/Dfl. 160.00.
ISBN 0-444-41430-4

This book is the only text currently available providing full information on the new separation technique known as Isotachophoresis. There is rapidly growing interest in this technique which will compete with other microanalytical techniques such as liquid and gas chromatography. All kinds of ionic materials can be separated using isotachophoretic equipment. Moreover, several classes of components can be analysed in quick succession as a proper rinsing of the equipment is all that is needed between separations. Each part is detailed and comprehensive.

The various chapters can be referred to more or less independently by scientists interested in fundamental aspects, by research groups intending to construct an instrument and by workers who are mainly concerned with the analytical aspects.

CONTENTS: Historical review. **Theory.** Principles of electrophoretic techniques. Concept of mobility. Mathematical model for isotachophoresis. Choice of electrolyte systems. **Instrumentation.** Detection systems. Instrumentation. **Applications.** Introduction. Practical aspects. Quantitative aspects. Separation of cationic species in aqueous solutions. Separation of anionic species in aqueous solutions. Amino acids, peptides and proteins. Separation of nucleotides in aqueous systems. Enzymatic reactions. Separations in non-aqueous systems. Counter flow of electrolyte. Appendices. Subject Index.

Volume 5

INSTRUMENTAL LIQUID CHROMATOGRAPHY

A Practical Manual on High-Performance Liquid Chromatographic Methods

by N.A. Parris.

1976. x+330 pages.
Price: US $40.95/Dfl. 100.00.
ISBN 0-444-41427-4

Available texts on liquid chromatography have tended to emphasize the developments in the theoretical understanding of the technique and methodology or to list numerous applications, complete with experimental details.

This work intends to bridge the gap between these two treatments by providing, with the minimum of theory, a practical guide to the use of technique for the development of separations. The material is based largely on practical experience and high-lights details which may have important operational value for laboratory workers. Information regarding the usefulness of available equipment and column packings is given, together with chapters devoted to the methodology of each separation method. Applications of liquid chromatography are described with reference to the potential of the technique for qualitative, quantitative and trace analysis as well as for separative applications. Numerous applications from the literature are tabulated and cross-referenced to sections concerned with the optimisation procedures of the particular methods. In addition, many of the figures have been drawn from hitherto unpublished works.

CONTENTS: Introduction and historical background. Basic principles and terminology. Chromatographic support and column.

Liquid chromatographic instrumentation. Liquid chromatographic detection systems. Nature of the mobile phase. Liquid-solid (adsorption) chromatography. Liquid-liquid (partition) chromatography, Ion-exchange chromatography. Steric exclusion chromatography. Qualitative analysis. Quantitative analysis. Practical aspects of trace analysis. Practical aspects of preparative liquid chromatography. Published LC applications information. The latest trends and a glimpse into the future. Subject Index.

Volume 4

DETECTORS IN GAS CHROMATOGRAPHY

by J. Ševčík.

1976. 192 pages.
Price: US $24.50/Dfl. 60.00.
ISBN 0-444-99857-8

This publication is devoted to the function and optimal working conditions of gas chromatographic detectors.

The first systematic treatment of gas chromatographic detection techniques, it devotes special attention to so-called specific detectors and working conditions which strongly influence results (e.g. gas flow, effect of additives in gases, working temperature, detector form and dimensions). Anomalous detector responses are explained and the form and size of response for various working conditions are indicated. The problems presented are illustrated by experimental data which are summarized in numerous tables and figures.

The book should be of interest to all who use gas chromatography in research and who would like to explore the possibilities and working conditions of different detector systems.

Volume 3

LIQUID COLUMN CHROMATOGRAPHY

A survey of modern techniques and applications.

edited by Z. Deyl, K. Macek and J. Janák.

1975. xxii+1176 pages.
Price: US $118.50/Dfl. 290.00.
ISBN 0-444-41156-9

This book provides an up-to-date account of liquid column chromatography for the specialist and non-specialist. The main attention is focussed on techniques developed or widely used during the past 10 years. Both classical and modern techniques of chromatographic separation are treated in detail, thus providing a clear reflection of the present situation in the field.

The wide selection of applications in various fields of chemistry and biochemistry, written by specialists in the area, makes this volume a necessary reference work for those involved in chromatographic investigations.

CONTENTS: Theoretical Aspects of Liquid Chromatography. Techniques of Liquid Chromatography. Practice of Liquid Chromatography. Applications. Subject index. List of compounds chromatographed.

Volume 2

EXTRACTION CHROMATOGRAPHY

edited by T. Braun and G. Ghersini.

1975. xviii+566 pages.
Price: US $52.95/Dfl. 130.00.
ISBN 0-444-99878-0

This volume is the result of the collective work of many specialists, each responsible for a chapter in which a definite aspect of column extraction chromatography is thoroughly presented and discussed.

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3 H. C. S. Wood and R. Wrigglesworth, in S. Coffey (Editor), *Rodd's Chemistry of Carbon Compounds, Vol. IV, Heterocyclic Compounds*, Part B, Elsevier, Amsterdam, Oxford, New York, 2nd ed., 1977, Ch. 11, p. 201.
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Trace-Element Contamination of the Environment

DAVID PURVES, *Spectrochemistry Department, Edinburgh School of Agriculture, Edinburgh, Great Britain.*

FUNDAMENTAL ASPECTS OF POLLUTION CONTROL AND ENVIRONMENTAL SCIENCE, 1

The purpose of this book is to evaluate the global consequences of dispersal of trace elements, originally mined from localised limited deposits in the environment. Until now, this kind of environmental pollution has received less attention than the problem deserves for it could have profound ecological consequences in the long term.

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