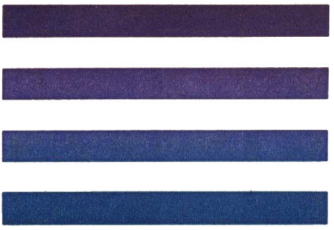
ISSN 0378-4355

VOL. 373 NO. 1 MAY 21, 1986



NAL OF

HROMATOGRAPHY

RNATIONAL JOURNAL ON CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS

CHROMATOGRAPHIC REVIEWS (Vol. 31, No. 1)

edited by

Michael Lederer

ELSEVIER

JOURNAL OF CHROMATOGRAPHY

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.

Submission of Papers. Papers in English, French and German may be submitted, in three copies. Manuscripts should be submitted to: The Editor of *Journal of Chromatography*, P.O. Box 681, 1000 AR Amsterdam, The Netherlands, or to: The Editor of *Journal of Chromatography, Biomedical Applications*, P.O. Box 681, 1000 AR Amsterdam, The Netherlands. Review articles are invited or proposed by letter to the Editors and will appear in *Chromatographic Reviews* or *Biomedical Applications*. An outline of the proposed review should first be forwarded to the Editors for preliminary discussion prior to preparation. Submission of an article is understood to imply that the article is original and unpublished and is not being considered for publication elsewhere. For copyright regulations, see below.

Subscription Orders. Subscription orders should be sent to: Elsevier Science Publishers B.V., P.O. Box 211, 1000 AE Amsterdam, The Netherlands. The *Journal of Chromatography* and the *Biomedical Applications* section can be subscribed to separately.

Publication. The *Journal of Chromatography* (incl. *Biomedical Applications, Chromatographic Reviews* and *Cumulative Author and Subject Indexes, Vols. 326–350*) has 38 volumes in 1986. The subscription prices for 1986 are:

J. Chromatogr. (incl. *Chromatogr. Rev.* and *Cum. Indexes, Vols. 326–350*) + *Biomed. Appl.* (Vols. 346–383): Dfl. 6080.00 plus Dfl. 912.00 (postage) (total *ca.* US$ 2589.75)

J. Chromatogr. (incl. *Chromatogr. Rev.* and *Cum. Indexes, Vols. 326–350*) only (Vols. 346–373): Dfl. 5040.00 plus Dfl. 672.00 (postage) (total *ca.* US$ 2115.50)

Biomed. Appl. only (Vols. 374–383): Dfl. 1850.00 plus Dfl. 240.00 (postage) (total *ca.* US$ 774.00).

Journals are automatically sent by airmail at no extra costs to Argentina, Australia, Brasil, Canada, China, Hong Kong, India, Israel, Japan, Malaysia, Mexico, New Zealand, Pakistan, Singapore, South Africa, South Korea, Taiwan, Thailand and the U.S.A. Back volumes of the *Journal of Chromatography* (Vols. 1 through 345) are available at Dfl. 219.00 (plus postage). Claims for issues not received should be made within three months of publication of the issue. If not, they cannot be honoured free of charge. Customers in the U.S.A. and Canada wishing information on this and other Elsevier journals, please contact Journal Information Center, Elsevier Science Publishing Co. Inc., 52 Vanderbilt Avenue, New York, NY 10017. Tel. (212) 916-1250.

Abstracts/Contents Lists published in Analytical Abstracts, ASCA, Biochemical Abstracts, Biological Abstracts, Chemical Abstracts, Chemical Titles, Current Contents/Physical, Chemical & Earth Sciences, Current Contents/Life Sciences, Deep-Sea Research/Part B: Oceanographic Literature Review, Excerpta Medica, Index Medicus, Mass Spectrometry Bulletin, PASCAL-CNRS, Referativnyi Zhurnal and Science Citation Index.

See page 3 of cover for Publication Schedule, Information for Authors and information on Advertisements.

0378-4355/86/$00.35 per page (minimum $03.50)

Printed in The Netherlands

VOL. 373, NO. 1 JOURNAL OF CHROMATOGRAPHY MAY 21, 1986

(Chromatographic Reviews, Vol. 31, No. 1)

CONTENTS

In articles with more than one author, the name of the author to whom correspondence should be addressed is indicated in the article heading by a 6-pointed asterisk (*)

BALANCE

A Program to Compare the Means of Two Series of Measurements

Authors: D.L. Massart et al.

- *requires no profound* knowledge of statistics
- guides user with playful ease to the correct statistical test
- *incorporates:*
  - paired t-test
  - *Wilcoxon test*
  - Student's t- and Cochran's tests
  - Mann-Whitney's U-test
  - *tests for small and large* numbers of measurements
  - one/two tailed (sided) tests
- full source code listings
- clear, fully descriptive manual with worked examples
- US $ 185.00

No shipping charge if paid in advance

Apple is a registered trademark of Apple Computer, Inc.
IBM-PC is a registered trademark of IBM.

AVAILABLE FROM

Elsevier Scientific Software (JIC)
52 Vanderbilt Avenue
New York, NY 10017 USA
Phone: (212) 916 1250 Telex: 420643
or
Elsevier Scientific Software
P.O. Box 330
1000 AH Amsterdam
THE NETHERLANDS
Phone: (020) 5862 828 Telex: 18582

". . . the BALANCE program and its documentation are so clearly written that it should be quite useful in many areas of academic and industrial chemistry." (R.E. Davis in the Journal of American *Chemical Society*)

"The program and accompanying documentation have been designed to be user-friendly and the authors have succeeded in this regard with this implementation." (J. Marshall in The Analyst)

"Let me say right away that BALANCE is a pleasant surprise. . ."
C.S. Gutteridge in TrAC)

for: IBM-PC and Apple II series

Write to us for further information on our other programs

ELSEVIER SCIENTIFIC SOFTWARE

JOURNAL OF CHROMATOGRAPHY

VOL. 373 (1986)

(Chromatographic Reviews, Vol. 31)


RESPECT COPYRIGHT
BERNE
CONVENTION
100 YEARS
1886-1986
ENCOURAGE CREATIVITY

JOURNAL *of* CHROMATOGRAPHY

INTERNATIONAL JOURNAL ON CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS

CHROMATOGRAPHIC REVIEWS

edited by

Michael Lederer

Vol. 31 1986

ELSEVIER
AMSTERDAM — OXFORD — NEW YORK — TOKYO

0378-4355/86/$00.35 per page (minimum $03.50)

Printed in The Netherlands

Journal of Chromatography, 373 (1986) 1–20
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHREV. 200

APPLICATIONS AND LIMITATIONS OF COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

RICHARD DÄPPEN, HANS ARM and VERONIKA R. MEYER*
Institute of Organic Chemistry, University of Bern, Freiestrasse 3, CH-3012 Bern (Switzerland)
(Received October 11th, 1985)

CONTENTS

1. INTRODUCTION

Interest in chiral separations by chromatographic methods has grown considerably in recent years, presumably for two reasons. In modern gas and liquid chromatography, the number of theoretical plates available for every-day separations has increased enormously; therefore, small chiral effects in a chromatographic system can be utilized to yield a good separation of enantiomers. Moreover, a deeper insight into many biological processes has shown that chiral substances often have different effects on an organism; one isomer may be inactive or even harmful. This fact is of great interest in the design of novel pharmaceuticals and requires efficient methods for the separation of optical isomers.

The chromatographic separation of enantiomers can be performed by various methods, but it is always necessary to use some kind of chiral discriminator or selector. Two different types of selectors can be distinguished: a chiral stationary phase (for a review of the state of the art in liquid chromatography, see ref. 1) or a chiral additive in the mobile phase, *e.g.*, as described in ref. 2. Another possibility is pre-column derivatization of the sample with chiral reagents to produce diastereomeric molecules which can be separated by non-chiral chromatographic methods (for an example, see ref. 3).

0378-4355/86/$07.00

Of the numerous chiral stationary phases described in the literature for the liquid chromatographic separation of enantiomers, a considerable number are commercially available. In this paper we discuss the phases that are suitable for high-performance liquid chromatography (HPLC), *i.e.*, those with a mean particle diameter not larger than 10 μm. The range commercially available in autumn 1985 is surveyed. Although we have tried to achieve completeness, it is difficult as progress is very rapid in this field. Readers interested in the purchase of a chiral column for HPLC should ask the manufacturers for actual details.

The commercially available chiral stationary phases for HPLC use very different separation principles. We propose to classify them as follows:

(a) Chiral ligand-exchange chromatography.
(b) Chiral affinity chromatography.
(c) Chiral chromatography with helical polymers.
(d) Chiral chromatography with cavities.
(e) Chiral chromatography with "brush type" chemically bonded phases.

Using this classification, we discuss the commercially available chiral stationary phases based on data sheets and manuals from the manufacturers and on the scientific literature. The references cited are a selection from the vaste number of papers in this field and an attempt was made to represent the main workers in this area. Further searches in the literature for a certain type of chiral stationary phase can easily be done based on the information given in this paper.

2. LIGAND-EXCHANGE PHASES

Ligand-exchange chromatography for the separation of enantiomers was introduced in liquid chromatography by Davankov *et al.*[4,5]. They prepared various chiral stationary phases from chloromethylated polystyrene and amino acids. After loading the resin with Cu(II), Ni(II), Zn(II) or Cd(II), it was possible to separate racemates of amino acids.

Gübitz and co-workers[6,7] transferred this principle to silica-based stationary phases. They bonded L-proline or L-valine to silica via a 3-glycidoxypropyl spacer. After treatment with aqueous copper sulphate solution, the phases are ready for

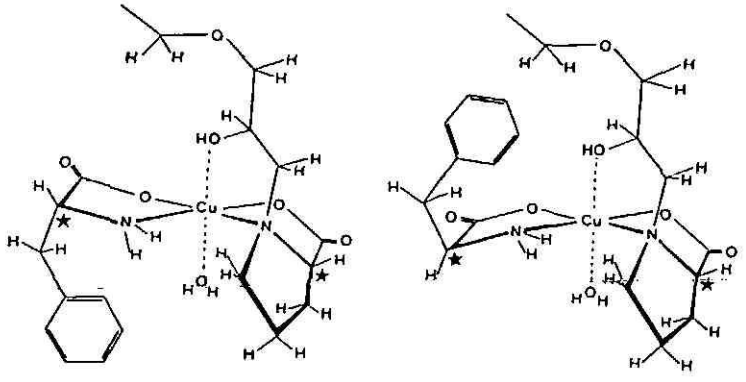
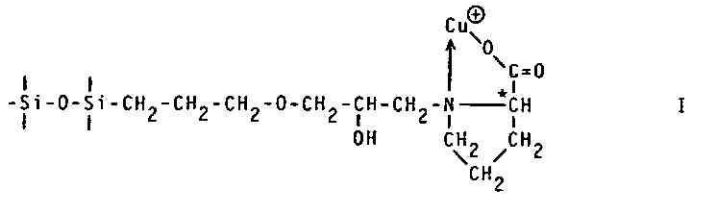
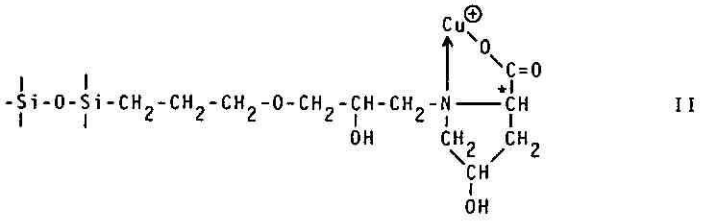


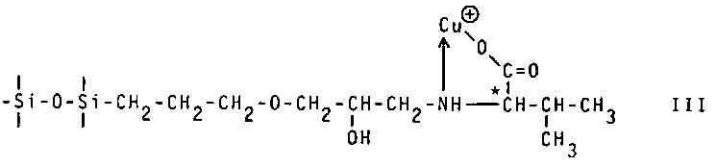
Fig. 1. Complex of D- (left) and L-phenylalanine (right) with copper-loaded L-proline stationary phase. (From ref. 8, with permission.)



I



II



III

Fig. 2. Structures of the commercially available proline–copper (I), hydroxyproline–copper (II) and valine–copper (III) phases.

enantioselective complex formation with amino acids, as shown in Fig. 1[8]. These phases, whose structures are shown in Fig. 2, are commercially available from Serva as Chiral ProCu = Sil00Polyol and Chiral ValCu = Sil00Polyol; a further phase is Chiral HyproCu = Sil00Polyol with hydroxyproline as the amino acid. Similar phases are sold by Daicel as Chiralpak WH and Chiralpak WM and by Macherey, Nagel & Co. as Nucleosil Chiral-1.

A successful separation into enantiomers can be expected if the sample molecule has two polar functional groups with the correct spacing, which can simultaneously act as ligands for the copper ion. Therefore, α-amino acids with their NH_2 and COO^- groups are very suitable for separations with these stationary phases. An example is shown in Fig. 3. Some β-amino alcohols and similar molecules could also be candidates for this method.

The mobile phase is aqueous and should be approximately 0.5 m*M* in Cu(II) in order to prevent a loss of copper from the stationary phase. Increased temperatures (40–50°C) improve both the separation factor and the column efficiency[8]; this indicates that the separation is entropy controlled, a fact that is unique for the phases discussed in this paper. Samples with strong acids or bases should be avoided or derivatized.

Preparative separations are possible with these ligand-exchange phases and preparative-scale columns are available.

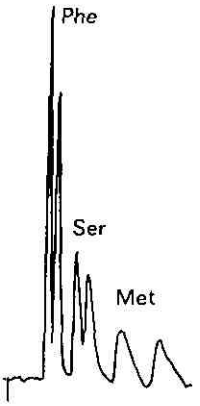


Fig. 3. Separation of racemic amino acids on a chiral ligand-exchange phase (Serva). Column, 25 cm × 4.6 mm I.D.; stationary phase, Chiral ProCu = Sil00Polyol (5 μm); mobile phase, 1 m*M* copper(II) sulphate in water, 1 ml/min, 100 bar; detector, UV (230 nm); sample, 20 μl with 1% of each amino acid.

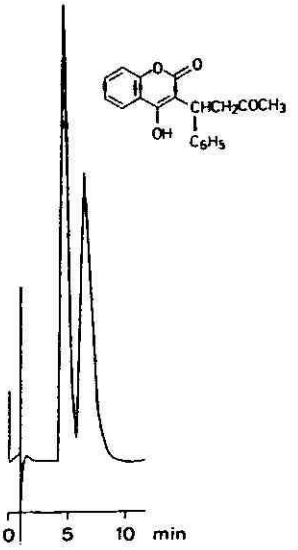
3. AFFINITY PHASES

Several proteins can undergo enantioselective interactions with a large number of pharmacologically active compounds. This effect was first used for chromatographic separations by Stewart and Doherty[9], who succeeded in resolving of D- and L-tryptophan on bovine serum albumin (BSA) bound to agarose. Allenmark *et al.*[10] made the method accessible to HPLC by binding BSA to HPLC-grade silica. Another protein phase was developed by Hermansson[11], who used α_1-acid glycoprotein (α_1-AGP, orosomucoid) coated and cross-linked on silica. The BSA column is available from Macherey, Nagel & Co. as Resolvosil and the α_1-AGP column from LKB as EnantioPac.

The separation mechanism of protein columns is not known, although there is no doubt that it is based on principles of bioaffinity. It includes hydrophobic interactions (similar to a true reversed phase), interactions of polar groups and steric effects[12]. Both phases are excellently suited for the separation of pharmaceuticals into enantiomers and often show high separation factors. Resolvosil is recommended for aromatic amino acids, amino acid derivatives, aromatic sulphoxides, coumarin derivatives, benzoin and benzoin derivatives. The various samples successfully separated on EnantioPac include important drugs such as ephedrine, disopyramide and methadone.

Protein columns are demanding in that the chiral separation depends on the chromatographic conditions, such as pH, ionic strength, organic modifier concentration (charged or uncharged) and temperature[10–14]. Therefore, the optimum combination of these parameters has to be determined for each separation problem. Recommended values are pH 5–9, ionic strength 0–500 m*M*, organic modifiers up to 5% (1- or 2-propanol) or 0–10 m*M* (N,N-dimethyloctylamine, *tert.*-butylammonium bromide, octanoic acid) and temperature up to 35°C.

The selectivity of affinity phases is often excellent (see Figs. 4 and 5). Unfortunately, the column efficiencies are low and, as the load of protein on the silica is



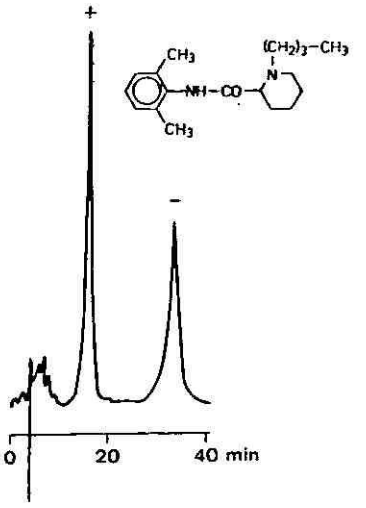


Fig. 4. Separation of racemic warfarin on a bovine serum albumin phase (Macherey, Nagel & Co.). Column, 15 cm × 4 mm I.D.; stationary phase, Resolvosil (10 μm); mobile phase, 0.05 *M* phosphate buffer (pH 6.79)–1-propanol (97:3), 2 ml/min; detector, UV (225 nm); sample, 10 μl, 50 μ*M*.

Fig. 5. Separation of racemic bupivacaine on an α_1-acid glycoprotein phase (LKB). Column, 10 cm × 4 mm I.D.; stationary phase, EnantioPac (10 μm); mobile phase, phosphate buffer, μ = 0.02 + 0.1 *M* sodium chloride (pH 7.2)–2-propanol (96:4), 0.3 ml/min; temperature, 35°C; detector, UV (215 nm).

low, the sample capacity is not more than 1–2 nmol per injection. Preparative separations are impossible. Biological samples need to be analysed with great care, and a reversed-phase pre-column[15] or even a column switching technique may be necessary. For some drugs a pre-column derivatization may be needed in order to obtain a good resolution; this was reported for amino-alcohols (which include some β-blockers) which were derivatized with phosgene to obtain oxazolidones[16].

4. HELICAL POLYMER PHASES

Polymers with a helical structure are able to separate enantiomers by steric effects. Optical antipodes are retained differently if their attachment between the layers of the helix is different and therefore a chiral effect is noticeable.

4.1. Cellulose derivatives

Cellulose may be used for HPLC separations of enantiomers, *e.g.*, tryptophan, as reported by Gübitz *et al.*[17]. However, it seems that cellulose derivatives have a greater resolving capacity. At present, underivatized cellulose of HPLC grade is not commercially available.

Derivatization of the hydroxy groups does not destroy the helical structure of cellulose. Cellulose triacetate was first used by Hesse and Hagel[18] for chiral separations. It was introduced in HPLC by Mannschreck and co-workers[19,20]. This sta-

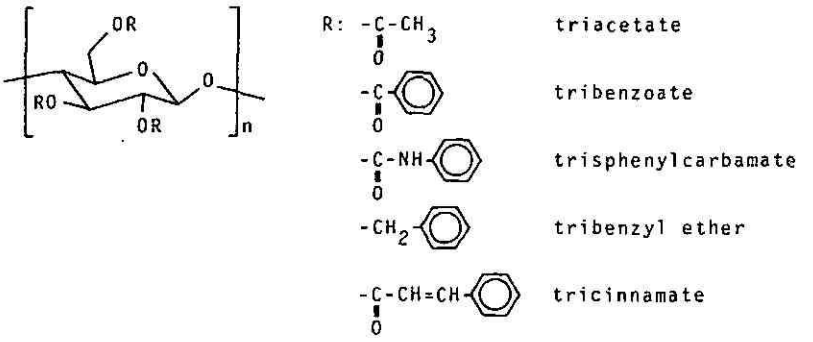


Fig. 6. Structures of derivatized cellulose.

tionary phase (pure cellulose triacetate) is available from Macherey, Nagel & Co. as Cellulose CEL-AC-40 XF. The maximum pressure drop is limited to 80 bar. The standard eluent is 96% ethanol.

Recently some new cellulose phases have been developed by Okamoto's group[21]. These five different cellulose derivatives are all adsorbed on macroporous silica and are available from Daicel: Chiralcel OA is cellulose triacetate, Chiralcel OB is cellulose tribenzoate, Chiralcel OC is cellulose trisphenylcarbamate, Chiralcel OE is cellulose tribenzyl ether and Chiralcel OK is cellulose tricinnamate (Fig. 6). The properties of these phases are described as follows[21]:

Triacetate: for many racemates, especially effective for substrates with a phosphorus atom at an asymmetric centre. In general, low separation factors.

Tribenzoate: for racemates with carbonyl group(s) in the neighbourhood of an asymmetric centre.

Trisphenylcarbamate: for polar racemates, sensitive to the molecular geometry of the substrates (Fig. 7).

Tribenzyl ether: effective with protic solvents as mobile phases.

Tricinnamate: for many aromatic racemates and barbiturates. High retention times.

Samples with strong acids or bases should be avoided. Good results are ob-

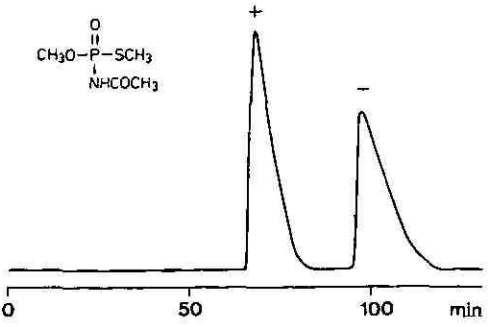


Fig. 7. Separation of racemic oltran on a cellulose trisphenylcarbamate phase (Daicel). Column, 25 cm × 4.6 mm I.D.; stationary phase, Chiralcel OC (10 μm); mobile phase, hexane–2-propanol (9:1), 0.5 ml/min; detector, UV (220 nm).

tained, if carboxylic acids are converted into phenyl esters, amines into benzoic acid amides and alcohols into benzoic acid esters.

The separation factor is strongly influenced by the type of mobile phase[20,21]. Although it is possible to use polar and non-polar eluents, Daicel recommends a limitation to the following solvents: hexane (also used as a storage solvent), hexane–2-propanol, methanol, ethanol, methanol–water and ethanol–water.

As these stationary phases are prone to irreversible adsorption, biological samples should not be injected without a pre-column. Preparative separations are possible[22]. However, because the Daicel phases, which are coated on silica, are very expensive, an alternative is to use the bulk material from Macherey, Nagel & Co. (also available in larger particle diameters) or from Merck (cellulose triacetate, 15–25 or 25–40 μm) for these purposes.

4.2. Poly(triphenylmethyl methacrylate)

The polymerization of triphenylmethyl methacrylate in the presence of a chiral anion catalyst yields an isotactic polymer whose chirality is caused by its helical structure. This phase was first synthesized by Okamoto *et al.*[23] and also used for liquid chromatography[24]. The chemical structure of poly(triphenylmethyl methacrylate) is shown in Fig. 8. Stationary phases for HPLC were polymerized in the presence of a (+)-6-benzylsparteine–butyllithium complex and coated on 10 μm silica[25]. They are available from Daicel as Chiralpak OT(+), which is (+)-poly(triphenylmethyl methacrylate), and Chiralpak OP(+), which is poly(2-pyridyldiphenylmethyl methacrylate).

These phases show an extraordinary selectivity for many enantiomers. Many samples can be successfully separated if they possess a rigid non-planar structure (often with a C_2 symmetry axis), *e.g.*, *trans*-disubstituted cyclic molecules with six-, four- or three-membered rings. An example for the latter class of compounds is *trans*-stilbene oxide, whose separation factor on Chiralpak OT(+) is 5.2[25]. For the separation of *cis*- and *trans*-phenothrin, an insecticidal pyrethroid, into the four possible isomers, see Fig. 9.

The recommended mobile phases for the Chiralpak OT(+) and OP(+) columns are hexane (also as a storage solvent), hexane–2-propanol and methanol. Chiralpak OP(+) may also be used with methanol–water. For Chiralpak OT(+) the temperature should be below 15°C (down to 0°C) because the separation is better at low temperatures. The OP(+) column is used at room temperature.

Samples with strong acids or bases should be avoided. Good results are ob-

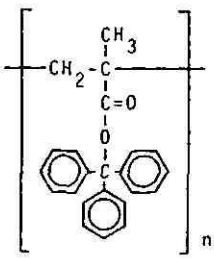


Fig. 8. Structure of poly(triphenylmethyl methacrylate).

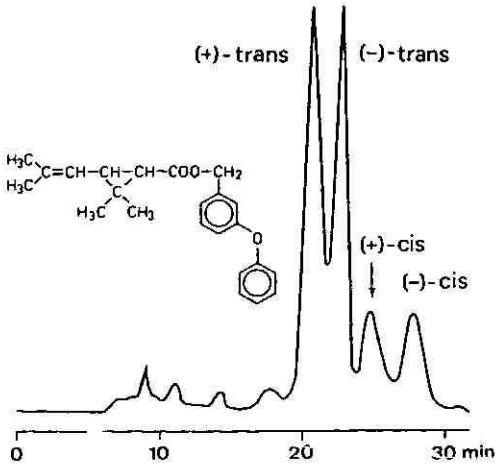


Fig. 9. Separation of racemic *cis*- and *trans*-phenothrin (3-phenoxybenzyl chrysanthemate) on a poly(triphenylmethyl methacrylate) phase (Daicel). Column, 25 cm × 4.6 mm I.D.; stationary phase, Chiralpak OT(+) (10 μm); mobile phase, methanol, 0.5 ml/min; detector, UV (254 nm).

tained if carboxylic acids are converted into phenyl esters, amines into benzoic acid amides and alcohols into benzoic acid esters.

5. CAVITY PHASES

Cyclodextrins are cyclic oligoglucose molecules (Fig. 10). Their structure is

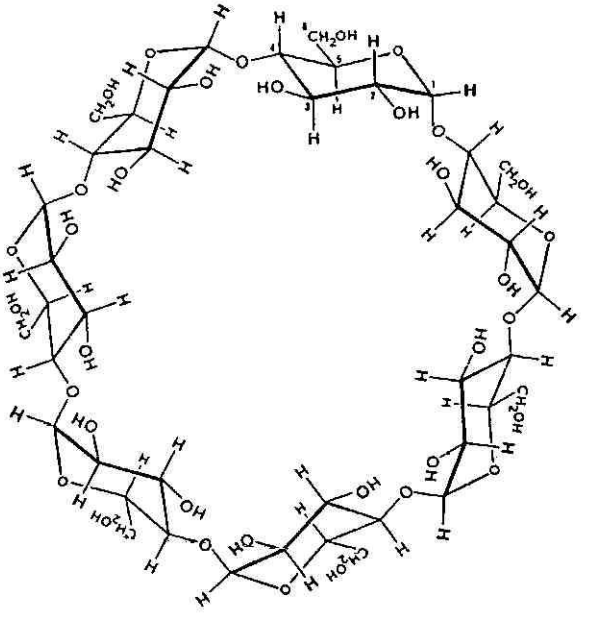


Fig. 10. Structure of β-cyclodextrin.

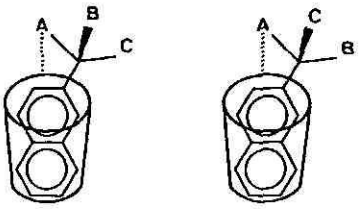


Fig. 11. Model of the chiral recognition by cyclodextrins. Whereas the aromatic part of the molecule dips into the cavity, the three substituents can interact with the clockwise oriented 2-hydroxy groups on the rim.

unique in that it resembles a truncated cone with both ends open. The larger opening of the cone is rimmed with the secondary 2-hydroxy groups of the glucose units, all rotated to the right, and the smaller opening is rimmed with the more polar primary hydroxy groups. The interior of the cyclodextrin cavity contains no hydroxy groups and therefore is relatively hydrophobic. If a chiral molecule fits exactly into the cavity with its less polar (preferably aromatic) side, a separation into the enantiomers can be expected (Fig. 11). Small molecules that are completely enveloped by the cyclodextrin cannot be separated.

This effect was first used for the non-chromatographic separation of racemates (via inclusion complexes) by Cramer and Dietsche[26]. Polymerized cyclodextrin can act as a stationary phase for classical column liquid chromatography[27]. Armstrong and co-workers[28,29] developed a material suitable for HPLC with the cyclodextrin ring bound to silica via a 6- to 10-atom spacer.

Various HPLC-grade cyclodextrins are available from Astec. Cyclobond I is β-cyclodextrin with seven glucose units (cavity I.D. = 8 Å) which can separate a variety of enantiomers: dansyl amino acids, β-naphthylamide and β-naphthyl ester derivatives of amino acids, barbiturates[30] and metallocenes[31]. The separation of racemic hexobarbital is shown in Fig. 12. Cyclobond II is γ-cyclodextrin with eight glucose units (cavity I.D. = 9.5 Å); however, no chiral separations are known with this stationary phase. Cyclobond III is α-cyclodextrin with six glucose units (cavity I.D. = 5.7 Å); little is known about this new phase, which is suitable for small molecules only. α- and β-cyclodextrin for HPLC will also be available from Serva in 1986.

Cyclodextrin phases are used like reversed phases. Suitable eluents are mixtures

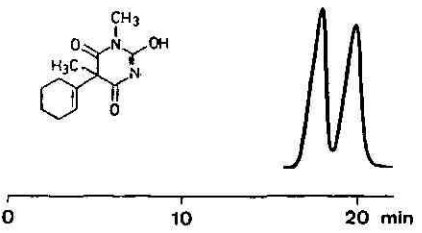


Fig. 12. Separation of racemic hexobarbital on a β-cyclodextrin phase. Column, 10 cm × 4.6 mm I.D.; stationary phase, Cyclobond I (5 μm); mobile phase, water–methanol (8:2), 1 ml/min; temperature, 22°C; detector, UV (254 nm). (From ref. 30, with permission.)

of water with methanol, ethanol or acetonitrile (with less polar mobile phases the chiral separation effect is lost). Gradient elution is possible, and also the regeneration of the column with absolute methanol, ethanol or acetonitrile. Column switching with reversed phases could be very interesting.

Cyclodextrin phases are cheaper than many other chiral phases. Preparative separations are possible and preparative-scale columns are available.

6. "BRUSH TYPE" PHASES

The hydroxy groups of the silica surface are accessible for chemical derivatization. The most widely used phase of this type is $C_{18}H_{37}$-modified silica, the well known RP-18 and ODS phases. As one can imagine that the organic groups are directed away from the silica network, this structure is often called a "brush". Chiral "brush type" phases for HPLC were first used by Mikeš *et al.*[32] for the separation of racemic helicenes. Later, the groups of Pirkle and of Ôi synthesized a large number of "brush type" phases, some of which are commercially available.

6.1. π-acceptor phases

The first commercially available chiral "brush type" phase for HPLC was ionically bound N-(3,5-dinitrobenzoyl)phenylglycine (phase I in Fig. 13). It was synthesized by Pirkle *et al.*[33] by pumping a tetrahydrofuran solution of (*R*)-N-(3,5-dinitro-

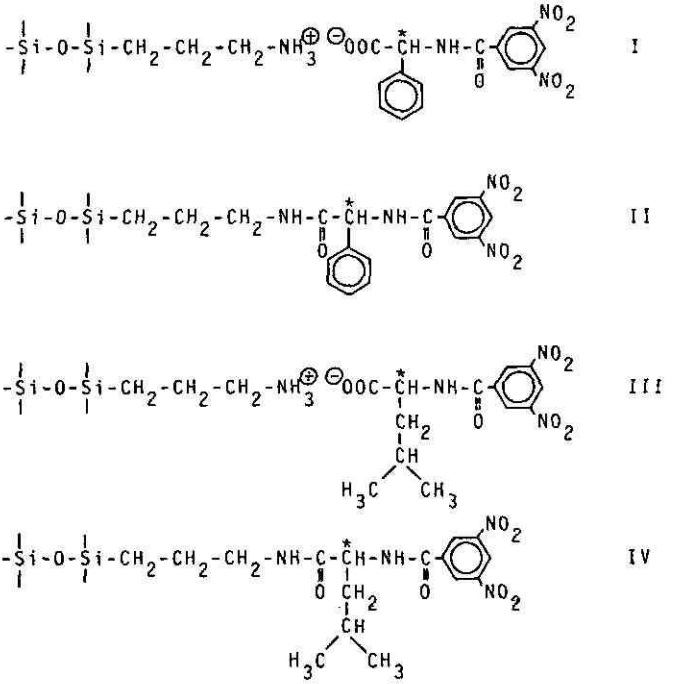


Fig. 13. Structures of commercially available "brush-type" π-acceptor phases. I = Ionic N-(3,5-dinitrobenzoyl)phenylglycine; II = covalent N-(3,5-dinitrobenzoyl)phenylglycine; III = ionic N-(3,5-dinitrobenzoyl)leucine; IV = covalent N-(3,5-dinitrobenzoyl)leucine.

benzoyl)phenylglycine through a pre-packed column of aminopropyl-silica. Soon it was clear that this phase can separate the enantiomers of a wide range of classes of compounds[33,34]. Other dinitrobenzoyl (DNB) phases followed: covalent DNB-phenylglycine (phase II in Fig. 13), ionic DNB-leucine (phase III) and covalent DNB-leucine (phase IV).

All these phases are commercially available. Regis offers Ionic D-Phenyl Glycine, Ionic L-Leucine, Covalent D-Phenyl Glycine, Covalent L-Phenyl Glycine, Covalent D,L-Phenyl Glycine (a racemic column) and Covalent L-Leucine, all from the micro to the preparative scale (D- or D,L-leucine phases on request). Baker offers Bakerbond Chiral Ionic DNBPG, Bakerbond Chiral Covalent DNBPG and Bakerbond Chiral Covalent DNBLeu. Serva offers Chiral DNBPG-C = Sil00Polyol, Chiral DNBDL-C = Sil00Polyol (D-leucine) and Chiral DNBLL-C = Sil00Polyol (L-leucine); all these phases are covalent. Sumitomo offers Sumipax OA-2000 and Sumipax OA-2000A, which are ionic and covalent DNB-phenylglycine, respectively.

Although a lot of work has been done in this field, the separation mechanism is not understood in all instances. However, there is no doubt that charge-transfer interactions, hydrogen-bonding interactions, so-called "dipole stacking" interactions[35] and steric effects are involved. As the dinitrobenzoyl group is a π-acceptor, possible samples for all these phases should possess a π-donor group (*e.g.*, an aromatic ring with an alkyl, OR, NR_2 or SR substituent). Moreover, the sample needs to be able to act as a donor or acceptor for hydrogen bonds or to enter into dipole-stacking processes. Many possible classes of compounds have been indicated[34,36]. Others published later include benzodiazepinones[37] (Fig. 14) and N-acylated heterocyclic amines[38]. If necessary, the sample can be derivatized; this was described for the determination of D- and L-propanolol in human serum as oxazolidones[39].

It often cannot be predicted which of the four phases (ionic and covalent DNB-phenylglycine and DNB-leucine) will give the best results in a particular separation problem. The enantiomeric selectivities in some instances differ markedly[40]. An important restriction is the fact that the ionic phases cannot withstand mobile phases more polar than 20% propanol in hexane. With the covalent phases all

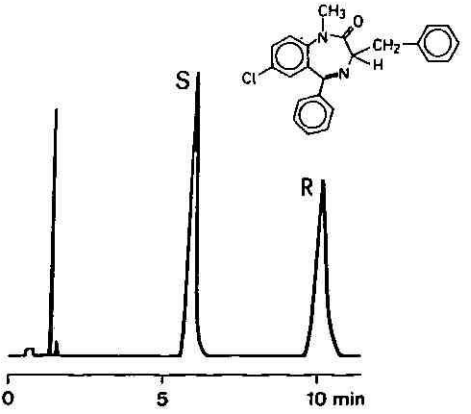


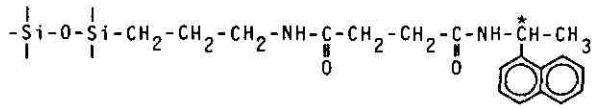
Fig. 14. Separation of racemic 3-benzyldiazepam on a dinitrobenzoylleucine phase. Column, 25 cm × 4.6 mm I.D.; stationary phase, covalent (*S*)-N-(3,5-dinitrobenzoyl)leucine (5 μm); mobile phase, hexane–2-propanol (90:10), 2 ml/min; detector, UV (254 nm). (From ref. 37, with permission.)

eluents, even water, are allowed (this is explicitly declared by all manufacturers with the exception of Baker). However, aqueous mobile phases should not be used as storage liquids and should be replaced with methanol. With a covalent DNB column, the user is free to optimize the separation via the composition of the mobile phase[41].

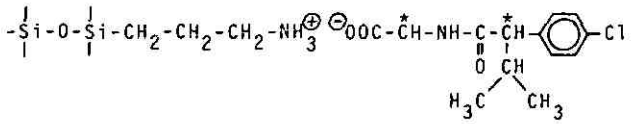
If the active part of the stationary phase of a ionic DNB column has been washed out by a too polar mobile phase, regeneration is possible. Regeneration reagents or ready-to-use solutions are sold by Aldrich, Baker, Regis and Sigma.

The covalent DNB-phenylglycine phase is available from Regis in three types: D-, L- and DL-(racemic). The D- and L-columns give reversed elution orders. This is convenient when a small amount of one enantiomer has to be quantified in the presence of the other. In these instances it is better to use the column which elutes the small peak first. The racemic column gives identical separations to the chiral ones except that the chiral resolution is not present, a valuable aid in the detection of enantiomers in complex mixtures. Chiral and racemic columns can be coupled to influence the extent of chiral separation[42].

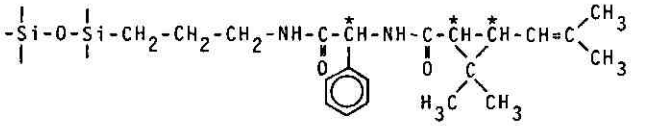
Preparative separations are possible[37,43] and preparative-scale columns (with HPLC-grade phases as well as with larger particle sizes) are available.



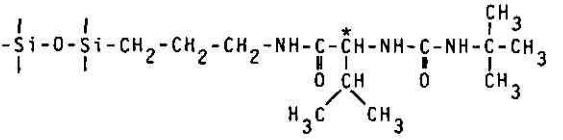
I



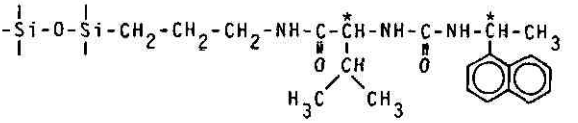
II



III



IV



V

Fig. 15. Structures of commercially available "brush type" phases of Ôi *et al.* I = Sumipax OA-1000 and OA-1000A; II = Sumipax OA-2100; III = Sumipax OA-2200; IV = Sumipax OA-3000; V = Sumipax OA-4000 and OA-4100.

6.2. Other phases

Several "brush type" chiral stationary phases for HPLC were synthesized by Ôi *et al.*[44] and are available from Sumitomo. Their structures are shown in Fig. 15. Sumipax OA-1000 and Sumipax OA-1000A are (*S*)-1-(α-naphthyl)ethylamine covalently bound as amide (I in Fig. 15)[45]. Only the OA-1000A phase is suited for use with aqueous mobile phases because any water-soluble impurities have been removed during the manufacturing process. Sumipax OA-2100 is ionic N-(*S*)-2-(4-chlorophenyl)isovaleroyl-(*R*)-phenylglycine (II)[46], Sumipax OA-2200 is covalent (N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-phenylglycine (III)[47], Sumipax OA-3000 is covalent N-*tert.*-butylaminocarbonyl-(*S*)-valine (IV)[48], Sumipax OA-4000 is covalent N-(*S*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-valine and Sumipax OA-4100 is the optical antipode with both asymmetric centres in the *R* configuration (V)[49].

As these phases were introduced in 1984, little is known about their properties. Their recommended uses are as follows:

OA-1000 and OA-1000A: these are π-donor phases, so the samples need to have a π-acceptor group. The columns are suitable for the separation of amines and amino acids as 3,5-dinitrobenzoyl derivatives and of carboxylic acids as 3,5-dinitroanilide derivatives. The OA-1000A column is for aqueous mobile phases.

OA-2100: this is a phase with two asymmetric carbon atoms and a weak π-donor. It is suitable for amines, amino acid esters or amides as 3,5-dinitrobenzoyl derivatives and for carboxylic acids as 3,5-dinitroanilide derivatives.

OA-2200: this is an extraordinary phase in that it has three asymmetric carbon

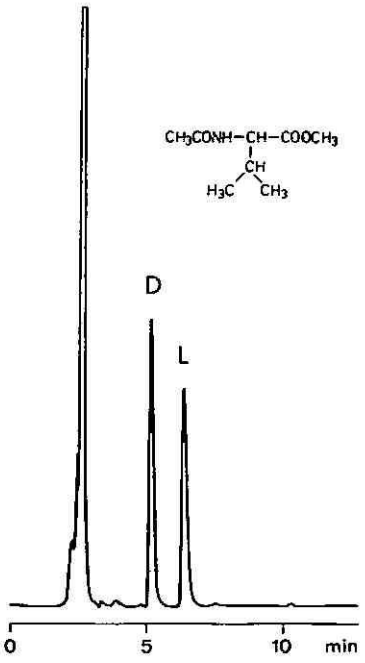


Fig. 16. Separation of racemic N-acetylvaline methyl ester on an N-*tert.*-butylaminocarbonyl-(*S*)-valine phase (Sumitomo). Column, 25 cm × 4 mm I.D.; stationary phase, Sumipax OA-3000 (5 μm); mobile phase, *n*-hexane–1,2-dichloroethane–ethanol (50:15:1). 1 ml/min; detector, UV (230 nm).

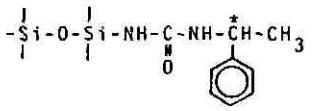


Fig. 17. Structure of commercially available Supelcosil LC-(R)-Urea phase.

atoms. It is suitable for amino acid esters as 3,5-dinitrobenzoyl derivatives. It was possible to separate some fungicidic alcohols into enantiomers[47].

OA-3000: this is a urea phase as it contains the NHCONH group. Therefore, it is suitable for dipole-stacking processes and the samples need not necessarily have a π-acceptor group. Possible samples are amino acids and oxy acids as N-acetyl-O-ester derivatives (Fig. 16) and as 3,5-dinitrophenylurethane-O-ester derivatives.

OA-4000 and OA-4100: these phases are very promising because here a π-donor group is combined with the urea group. Therefore, the possible interactions are charge-transfer and dipole-stacking processes. Moreover, the phases contain two asymmetric carbon atoms, which seems to improve the enantioselectivity[44]. These phases are recommended for many amines, carboxylic acids, amino acids, oxy acids, alcohols, amino alcohols and esters, even without derivatization. All kinds of amides, carbamates and ureas are possible samples, with or even without π-acceptor groups, as these compounds could undergo dipole-stacking with the phases. OA-4000 and OA-4100 are optical antipodes and therefore show a reversed elution order for chiral samples.

Preparative separations are possible and preparative-scale columns are available.

A similar urea-type phase was developed by Supelco[50], an (*R*)-(1-phenyl)ethylurea silica called Supelcosil LC-(R)-Urea (Fig. 17). In comparison with the OA-4000 series from Sumitomo, it seems that the π-donor/dipole-stacking combination is realized in a less effective manner as the π-donor is phenyl instead of naphthyl. Supelcosil LC-(R)-Urea can separate, *e.g.*, racemic PTH-amino acids (see Fig. 18).

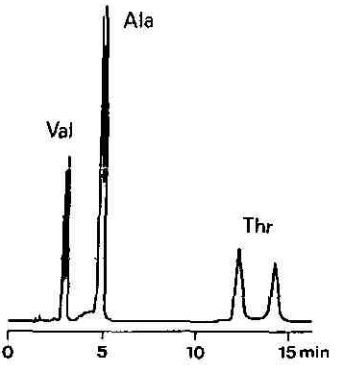


Fig. 18. Separation of racemic PTH-amino acids on a phenylethylurea phase (Supelco). Column, 25 cm × 4.6 mm I.D.; stationary phase, Supelcosil LC-(R)-Urea (5 μm); mobile phase, *n*-hexane–1,2-dichloroethane–ethanol (50:10:1), 2 ml/min; temperature, ambient; detector, UV (254 nm); sample, 5 μl mobile phase solution with 2.5 μg of each isomer.

7. CONCLUSIONS

The range of commercial chiral stationary phases for HPLC is now very broad and it is difficult to decide which phase could solve a given separation problem. Of course, it is not recommended to buy a column with which aqueous mobile phases cannot be used if the sample is soluble only in water. Often the chemical structure of the sample can give a hint of a suitable type of chiral phase. Such structure elements include charge-transfer groups or rigid spatial arrangements. Using the possibility of achiral derivatization, many samples can be adapted to the various chiral stationary phases. This is especially true for amino acids. In other instances the analyst has to search the literature on chiral separations for a similar molecule or has to make an attempt at random. Many companies lend their columns so that the consumer can check the possibilities of a certain phase for his separation problem.

All common detectors can be used with chiral stationary phases. However, as the polarimeter, utilized as a HPLC detector, only gives a signal if a large amount of a chiral sample is injected, its coupling with columns of low loadability seems not to be practicable. This is especially true for affinity phases.

8. LIST OF COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Commercially available chiral stationary phases are listed in Table 1.

9. LIST OF MANUFACTURERS

Aldrich

Aldrich Chemical Co. Inc., P.O. Box 355, Milwaukee, WI 53201, U.S.A.

In Europe: Aldrich-Chemie Gesellschaft mbH & Co. KG, D-7924 Steinheim, F.R.G.

Sells regeneration reagents for ionic DNB phases.

Astec

Advanced Separation Technologies Inc., 37 Leslie Court, P.O. Box 297, Whippany, NJ 07981, U.S.A.

In Europe: Paul Bucher, Analytik und Biotechnologie, Schützengraben 7, CH-4051 Basle, Switzerland.

Baker

J. T. Baker Research Products, 222 Red School Lane, Phillipsburg, NJ 08865, U.S.A.

In Europe: Baker Chemikalien, Postfach 1661, D-6080 Gross Gerau, F.R.G.

Daicel

Daicel Chemical Industries, Ltd., 8-1, Kasumigaseki 3-chome, Chiyoda-ku, Tokyo 100, Japan.

In Europe: Daicel (Europa) GmbH, Königsallee 92a, D-4000 Düsseldorf 1, F.R.G.

TABLE I

COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES

Manufacturer	*Name of phase*	*Class**	*Chiral element***
Astec	Cyclobond I	Cavity	β-Cyclodextrin
Baker	Bakerbond Chiral Ionic DNBPG	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Bakerbond Chiral Covalent DNBPG	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Bakerbond Chiral Covalent DNBLeu	"Brush", π-acceptor	(*S*)-DNB-leucine
Daicel	Chiralcel OA	Helical	Cellulose triacetate
	Chiralcel OB	Helical	Cellulose tribenzoate
	Chiralcel OC	Helical	Cellulose trisphenylcarbamate
	Chiralcel OE	Helical	Cellulose tribenzyl ether
	Chiralcel OK	Helical	Cellulose tricinnamate
	Chiralpak OT(+)	Helical	Poly(triphenylmethyl methacrylate)
	Chiralpak OP(+)	Helical	Poly(2-pyridyldiphenylmethyl methacrylate)
	Chiralpak WH	Ligand exchange	Proline–copper
	Chiralpak WM	Ligand exchange	Amino acid–copper
LKB	EnantioPac	Affinity	α_1-Acid glycoprotein
Macherey, Nagel & Co.	Cellulose CEL-AC-40 XF***	Helical	Cellulose triacetate
	Nucleosil Chiral-1	Ligand exchange	Hydroxyproline–copper
	Resolvosil	Affinity	Bovine serum albumin

Regis	Ionic D-Phenyl Glycine (Pirkle Type 1A)	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Covalent D-Phenyl Glycine	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Covalent L-Phenyl Glycine	"Brush", π-acceptor	(*S*)-DNB-phenylglycine
	Covalent D,L-Phenyl Glycine	"Brush", π-acceptor	(*R,S*)-DNB-phenylglycine
	Ionic L-Leucine	"Brush", π-acceptor	(*S*)-DNB-leucine
	Covalent L-Leucine	"Brush", π-acceptor	(*S*)-DNB-leucine
Serva	Chiral BDex = Sil00Polyol	Cavity	β-Cyclodextrin
	Chiral DNBPG-C = Sil00Polyol	"Brush", π-acceptor	(*R*)-DNB-phenylglycine (covalent)
	Chiral DNBLL-C = Sil00Polyol	"Brush", π-acceptor	(*S*)-DNB-leucine (covalent)
	Chiral DNBDL-C = Sil00Polyol	"Brush", π-acceptor	(*R*)-DNB-leucine (covalent)
	Chiral HyproCu = Sil00Polyol	Ligand exchange	Hydroxyproline–copper
	Chiral ProCu = Sil00Polyol	Ligand exchange	Proline–copper
	Chiral ValCu = Sil00Polyol	Ligand exchange	Valine–copper
Sumitomo	Sumipax OA-1000	"Brush", π-donor	α-Naphthylethylamide
	Sumipax OA-1000A	"Brush", π-donor	α-Naphthylethylamide
	Sumipax OA-2000	"Brush", π-acceptor	(*R*)-DNB-phenylglycine (ionic)
	Sumipax OA-2000A	"Brush", π-acceptor	(*R*)-DNB-phenylglycine (covalent)
	Sumipax OA-2100	"Brush", π-donor	Chlorophenylisovaleroylphenylglycine
	Sumipax OA-2200	"Brush"	Chrysanthemoylphenylglycine
	Sumipax OA-3000	"Brush", urea type	*tert.*-Butylaminocarbonyl valine
	Sumipax OA-4000	"Brush", urea, π-donor	(*S*),(*S*)-α-Naphthylethylaminocarbonylvaline
	Sumipax OA-4100	"Brush", urea, π-donor	(*R*),(*R*)-α-Naphthylethylaminocarbonylvaline
Supelco	Supelcosil LC-(R)-Urea	"Brush", urea type	Phenylethylurea

* "Brush" type phases always include dipole and hydrogen bonding interactions.

** DNB = 3,5-dinitrobenzoyl.

*** This cellulose triacetate from Macherey, Nagel & Co. is the only phase in this table that is not based on silica; this 7-μm material is pure cellulose triacetate.

In U.S.A.: Daicel (USA), Inc., 611 West 6th Street, Room 2152, Los Angeles, CA 90017, U.S.A.

LKB

LKB-Produkter AB, Box 305, S-16126 Bromma, Sweden.

In U.S.A.: LKB Instruments Ltd., 9319 Gaither Road, Gaithersburg, MD 20877, U.S.A.

Macherey, Nagel & Co.

Macherey, Nagel & Co., Neumann-Neander-Strasse 6–8, Postfach 307, D-5160 Düren, F.R.G.

In U.S.A.: Alltech Associates, 2051 Waukegan Road, Deerfield, IL 60015, U.S.A.

Merck

E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt 1, F.R.G.

In U.S.A.: EM Science, 111 Woodcrest Road, P.O. Box 5018, Cherry Hill, NJ 08034, U.S.A.

Sells cellulose triacetate for medium-pressure LC.

Regis

Regis Chemical Company, 8210 Austin Avenue, Morton Grove, IL 60053, U.S.A.

In Europe: Promochem GmbH, Postfach 1246, D-4230 Wesel, F.R.G.

Serva

Serva Feinbiochemica, Carl-Benz-Strasse 7, Postfach 105260, D-6900 Heidelberg 1, F.R.G.

In U.S.A.: Serva Fine Biochemicals Inc., P.O. Box A, Garden City Park, Long Island, NY 11040, U.S.A.

Sigma

Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178, U.S.A.

In Europe: Sigma Chemie GmbH, Am Bahnsteig 7, D-8028 Taufkirchen, F.R.G.

Sells regeneration reagents for ionic DNB columns.

Sumitomo

Sumitomo Chemical, Sumitomo Building, 5-15, Kitahama, Higashi-ku, Osaka 541, Japan.

In Europe: Sumitomo Düsseldorf Representative Office, Nordstern Versicherungs-Gebäude, Georg-Glock-Strasse 14, D-4000 Düsseldorf 30, F.R.G.

In U.S.A.: Sumitomo Chemical America, Inc., 345 Park Avenue, New York, NY 10154, U.S.A.

Supelco

Supelco Inc., Supelco Park, Bellefonte, PA 16823, U.S.A.

In Europe: Supelco SA, Chemin du Lavasson 2, CH-1196 Gland, Switzerland.

10. SUMMARY

This review surveys commercially available chiral stationary phases for HPLC. It is a guide to help the practitioner choose a stationary phase for a particular separation problem. It gives some information about the recommended eluents and also about the compatibility with achiral phases for column switching. Special hints and possibilities for preparative separations are mentioned.

REFERENCES

1 G. Gübitz, *GIT Fachz. Lab., Suppl. Chromatogr.*, 4/85 (1985) 6.
2 C. Pettersson, *J. Chromatogr.*, 316 (1984) 553.
3 R. H. Buck and K. Krummen, *J. Chromatogr.*, 315 (1984) 279.
4 V. A. Davankov, S. V. Rogozhin, A. V. Semechkin and T. P. Sachkova, *J. Chromatogr.*, 82 (1973) 359.
5 V. A. Davankov, *Adv. Chromatogr.*, 18 (1980) 139.
6 G. Gübitz, W. Jellenz, G. Löfler and W. Santi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 145.
7 G. Gübitz, W. Jellenz and W. Santi, *J. Liq. Chromatogr.*, 4 (1981) 701.
8 G. Gübitz, W. Jellenz and W. Santi, *J. Chromatogr.*, 203 (1981) 377.
9 K. K. Stewart and R. F. Doherty, *Proc. Natl. Acad. Sci. USA*, 70 (1973) 2850.
10 S. Allenmark, B. Bomgren and H. Borén, *J. Chromatogr.*, 264 (1983) 63.
11 J. Hermansson, *J. Chromatogr.*, 269 (1983) 71.
12 S. Allenmark, B. Bomgren and H. Borén, *J. Chromatogr.*, 316 (1984) 617.
13 J. Hermansson, *J. Chromatogr.*, 298 (1984) 67.
14 G. Schill, I. W. Wainer and S. A. Barkan, *J. Liq. Chromatogr.*, 9 (1986) 641.
15 J. Hermansson and M. Eriksson, paper presented at the *9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985.*
16 J. Hermansson, *J. Chromatogr.*, 325 (1985) 379.
17 G. Gübitz, W. Jellenz and D. Schönleber, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 31.
18 G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 277.
19 K. R. Lindner and A. Mannschreck, *J. Chromatogr.*, 193 (1980) 308.
20 H. Koller, K.-H. Rimböck and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89.
21 A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, *Chromatographia*, 19 (1984) 280.
22 K.-H. Rimböck, F. Kastner and A. Mannschreck, *J. Chromatogr.*, 329 (1985) 307.
23 Y. Okamoto, K. Suzuki, K. Ohta, K. Hatada and H. Yuki, *J. Am. Chem. Soc.*, 101 (1979) 4763.
24 H. Yuki, Y. Okamoto and I. Okamoto, *J. Am. Chem. Soc.*, 102 (1980) 6356.
25 Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, S. Murata, R. Noyori and H. Takaya, *J. Am. Chem. Soc.*, 103 (1981) 6971.
26 F. Cramer and W. Dietsche, *Chem. Ber.*, 92 (1958) 378.
27 J. L. Hoffman, *Anal. Biochem.*, 33 (1970) 209.
28 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.
29 T. J. Ward and D. W. Armstrong, *J. Liq. Chromatogr.*, 9 (1986) 407.
30 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
31 D. W. Armstrong, W. DeMond and B. P. Czech, *Anal. Chem.*, 57 (1985) 481.
32 F. Mikeš, G. Boshart and E. Gil-Av, *J. Chromatogr.*, 122 (1976) 205.
33 W. H. Pirkle, J. M. Finn, J. L. Schreiner and B. C. Hamper, *J. Am. Chem. Soc.*, 103 (1981) 3964.
34 W. H. Pirkle, J. M. Finn, B. C. Hamper, J. Schreiner and J. R. Pribish, in E. L. Eliel and S. Otsuka (Editors), *Asymmetric Reactions and Processes in Chemistry (ACS Symposium Series*, No. 185), American Chemical Society, Washington, DC, 1982, Ch. 18, p. 245.
35 W. H. Pirkle, C. J. Welch and M. H. Hyun, *J. Org. Chem.*, 48 (1983) 5022.
36 I. W. Wainer and T. D. Doyle, *LC, Liq. Chromatogr. HPLC Mag.*, 2 (1984) 88.
37 W. H. Pirkle and A. Tsipouras, *J. Chromatogr.*, 291 (1984) 291.

38 W. H. Pirkle, C. J. Welch, G. S. Mahler, A. I. Meyers, L. M. Fuentes and M. Boes, *J. Org. Chem.*, 49 (1984) 2504.
39 I. W. Wainer, T. D. Doyle, K. H. Donn and J. R. Powell, *J. Chromatogr.*, 306 (1984) 405.
40 Z.-Y. Yang, S. Barkan, C. Brunner, J. D. Weber, T. D. Doyle and I. W. Wainer, *J. Chromatogr.*, 324 (1985) 444.
41 M. Zief, L. J. Crane and J. Horvath, *J. Liq. Chromatogr.*, 7 (1984) 709.
42 R. Däppen and W. H. Pirkle, paper presented at the *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, 1985*; in press.
43 W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 47 (1982) 4037.
44 N. Ôi, *Bunseki Kagaku*, 33 (1984) E401.
45 N. Ôi, M. Nagase and T. Doi, *J. Chromatogr.*, 257 (1983) 111.
46 N. Ôi, M. Nagase, Y. Inda and T. Doi, *J. Chromatogr.*, 265 (1983) 111.
47 N. Ôi, M. Nagase, Y. Inda and T. Doi, *J. Chromatogr.*, 259 (1983) 487.
48 N. Ôi and H. Kithara, *J. Chromatogr.*, 285 (1984) 198.
49 N. Ôi and H. Kithara, *Abstracts of the 49th Biannual Meeting of the Chemical Society of Japan*, I (1984) 386.
50 D. J. Gisch, paper presented at the *9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985.*

Journal of Chromatography, 373 (1986) 21–44
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHREV. 201

TEMPERATURE GRADIENTS IN GAS CHROMATOGRAPHY

V. G. BEREZKIN*, T. YU. CHERNYSHEVA, V. V. BUZAYEV and M. A. KOSHEVNIK
A. V. Topchiev Institute of Petrochemical Synthesis, U.S.S.R. Academy of Sciences, Leninsky Prospekt 29, Moscow (U.S.S.R.)
(Received October 28th, 1985)

CONTENTS

1. INTRODUCTION

At the very beginning of chromatography[1], most attention was paid to the development of simple types of chromatography in which the parameters (conditions) were maintained constant during the experiment. However, the potential range of chromatography can be substantially extended by varying the conditions in the course of a chromatographic experiment, and it is appropriate to consider the development of new variants of chromatography. In practice, a variety of methods are used, depending on the means employed for moving the components of the sample mixture along the adsorption layer, the physical state of the mobile and stationary phases, the form of the sorption layer, etc. This review is concerned with the development of temperature gradient methods in gas chromatography.

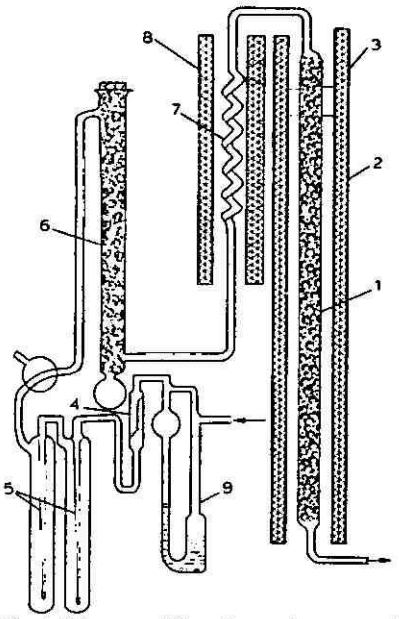
The temperature of separation is one of the major parameters determining the duration of separations, sorbent selectivity and the spreading of the chromatographic zones, and the rational use of the thermal factor considerably extends the potential of gas chromatography. By raising or lowering the temperature of the column (sorbent), it is possible to change dramatically the properties of different substances in the gas–mobile phase system both in time and in space on the sorbent layer. The importance of this factor was demonstrated from the very first days of the development of gas–liquid chromatography by Griffiths *et al.*[2], who suggested a new

method consisting of temperature programming through the column. This method subsequently found wide application in chromatography. The method was discussed in detail by Harris and Habgood[3]. However, temperature programming is not the only method of applying the thermal factor in gas chromatography.

Another application, the use of temperature gradients, was first suggested by Zhukhovitsky and co-workers[4–8]. This variant was called chromathermography, and its discovery was a significant step in the development of the theory and practice of gas chromatographic analysis. Essentially, chromathermography is a variant of gas chromatography in which the separation occurs on the sorbent layer as a result of the effect of the carrier gas flow in the space of the temperature field in time, which is characterized by a defined gradient of temperature in the chromatographic system during the separation.

In chromathermography, the separation is effected by additional moving of the temperature field along the column, *i.e.*, the column temperature varies lengthwise with time, but not simultaneously along the column as in chromatography with temperature programming[9]. Chromathermographic separation, in contrast to separation under isothermal conditions, makes it possible to increase the concentration of substances in the maximum of the chromatographic zone and, consequently, to provide greater sensitivity of determination. The chromathermographic method is exceptionally convenient for the separation of substances that differ greatly in their properties, *e.g.*, boiling point, and it combines the advantages of thermal desorption and elution chromatography and is applicable to the analysis of the mixtures that contain components with both strong and weak sorption abilities, irrespective of their sorption isotherms. The scheme of the first chromathermograph[4] is shown in Fig. 1. A silica gel column (1) is placed in a large electric oven (2) combined with a smaller cylindrical oven (3) moving along the length of the column, thereby heating it gradually, zone by zone. The air entering the column is purged (4, 5), then the air flows through the column filled with calcium chloride (6) where the moisture is absorbed. The air is subsequently delivered to a heat exchanger (7) housed in an electric oven (8), where it is heated to the desired temperature. The flow-rate of the air is measured by means of rotameter (9). The concentration of the substance beneath the silica gel layer is permanently controlled with the help of a gas interferometer. The procedure for analysis is as follows. A certain amount of the mixture of the substances to be separated is applied on to the silica gel. The individual components of the mixture are developed by drawing through the column whilst simultaneously heating it, zone by zone, by lowering the smaller oven along the layer at a given rate. The gas flow passes out from the column to a detecting system, where the concentrations of the components are measured.

Two versions of chromathermography were distinguished: stationary and non-stationary[9]. In non-stationary chromathermography the direction of the moving temperature field coincides with that of the carrier gas flow, and a direct temperature gradient is used (the temperature at the beginning of the oven in the direction of its travel is higher than that at the end). Non-stationary chromathermography was applied by Zhukhovitsky *et al.*[10] in the adsorption retention method. In the separation, weakly sorbed components move ahead over the sorbent layer within the oven and enter the higher temperature field, where their motion is accelerated. Strongly sorbed substances lag behind and enter the lower temperature zone, where their motion is



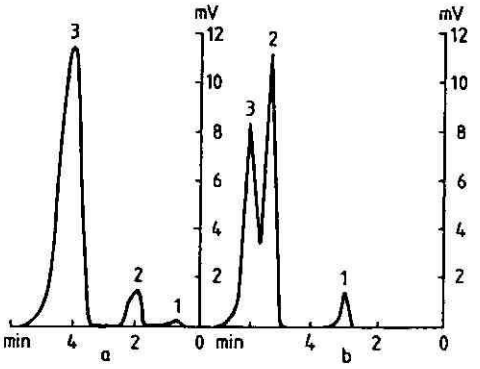


Fig. 1. Diagram of first chromathermograph. 1 = Column containing silica gel; 2 = electric oven (general thermostat); 3 = smaller cylindrical heater; 4, 5 = carrier gas scavenging system; 6 = column containing calcium chloride; 7 = heat exchanger; 8 = electric oven for pre-warming the carrier gas; 9 = rheometer.

Fig. 2. Chromatogram of air–isobutane mixture. Peaks: 1 = air; 2 = isobutane; 3 = *n*-butane. (a) Non-stationary chromathermography; (b) stationary chromathermography.

decelerated. Thus, owing to the temperature gradient of the heater, an additional "stretching" of components takes place, giving rise to an increase in the selectivity of their separation. This is evidenced by the comparison of the chromatograms illustrated in Fig. 2. However, non-stationary chromathermography imposes very stringent requirements on the conditions of chromatography, and is therefore hardly ever used.

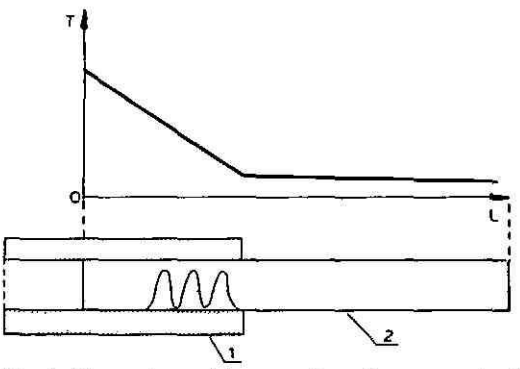
As in the development of any important method after its discovery, many investigators soon turned their attention to chromathermography. With regard to thin-layer liquid chromatography we may mention the method described in 1940 by Laip and Eralp[11]. In their method, a non-fixed adsorbent layer 8 cm long was applied to a glass plate, which was placed on an inclined aluminium support. The latter was cooled from its upper edge and heated from its lower edge. The mixture to be separated was applied to the upper edge of the adsorbent and then gradually eluted down with a solvent.

In 1943, Turner[12] described a method based on creating a moving temperature gradient along a chromatographic column. The column with the sample mixture is placed in a uniform temperature field which heats the adsorption layer, zone by zone, leading to a subsequent displacement of the components. However, instead of elution chromatography with a carrier gas flow, Turner's method makes use of the displacement, the movement of the zone being effected by the displacement of the components of the sample mixture. Therefore, Turner's method can be referred to as a displacement chromathermographic method.

2. STATIONARY CHROMATHERMOGRAPHY

The second variant, stationary chromathermography, is a method in which the temperature field and the flow of the carrier gas move in the same direction, the temperature gradient being a negative value.

In the stationary chromathermographic method (the first moment of separation is shown in Fig. 3), the sample is introduced into the column and a cylindrical heater is moved on to the column, along which there is a temperature gradient; then the oven is moved by a special motor along the column at different speeds prescribed by a pre-set programme. The temperature at the beginning of the heater (in the direction of its travel) is lower than that at the end. If we introduce the sample mixture into the inlet of the column and move the travelling oven on to the beginning of the column, the sample components, depending on their nature, will move along the layer at various velocities. The substances that sorb weakly will move along the layer at a higher velocity, and when they are displaced forwards within the oven they enter a region of lower temperatures where their sorption ability increases while the rate of movement decreases. This retardation will proceed until the rate of movement is equal to that of the oven itself. Substances that sorb more strongly will move along the layer at the beginning more slowly than the oven. As the sorbing substances lag behind, they approach a region of higher temperatures, where their sorption ability decreases with increasing rate of movement. Such an increase in speed will take place until it becomes equal to the rate of movement of the travelling oven. Subsequently, all the components will travel along the layer at the same rate as the oven. In chromathermography, as distinct from traditional isothermal chromatography with temperature programming, there are special conditions that hinder the spreading of the zone and lead to their compression, *i.e.*, concentration, the compression of the zone occurring (within certain limits) automatically.



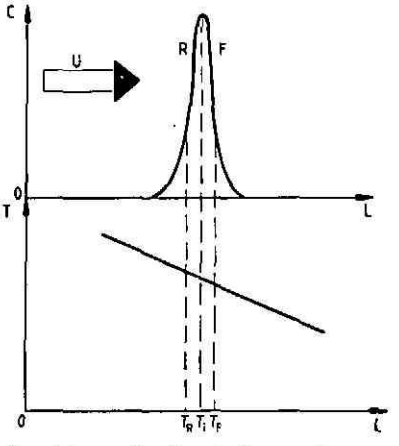


Fig. 3. Chromatographic separation of components of a sample mixture using the stationary chromathermography technique. 1 = Oven with temperature gradient; 2 = chromatographic column. L = column length; T = temperature.

Fig. 4. Compression of chromatographic zone in chromathermography. u = Linear velocity of carrier gas; C = concentration; L = column length; T = absolute temperature; T_i, T_F, T_R = temperature in the centre, front and rear of the zone, respectively; F = front of zone; R = rear of zone.

Fig. 4 shows the mechanism of zone compression under conditions of stationary chromathermography when the rates of movement of the zone and the heater are the same, and illustrates the connection between the zone shape and the temperature distribution. The temperature T_R corresponds to the speed of the analyzed substance i at the rear part of the zone, U_{Ri}, the temperature T_F corresponds to the speed of the substance at the front of the zone, U_{Fi}, and the temperature T_i corresponds to the speed of the substance in the central part of the zone, U_i. u is the linear velocity of the carrier gas, L is the length of the chromatographic column and C is the concentration.

Suppose a substance zone i moves at a speed U_i; considering that the zone moves under conditions when the sorbent temperature in the front part of the zone, T_F, is lower than that in the rear part, T_R ($T_R > T_F$), the speed of movement of the molecules of the substance being chromatographed in the region of the zone front, in general, must be lower than the mean speed of the zone, U_i, whereas the speed of movement of the molecules of the sample substance in the region of the rear part of the zone must be higher than the mean speed of the zones. If we neglect the changes in the gas flow-rate and pressure in the zone, we can write

$$U_i = \frac{u}{K_i} \tag{1}$$

$$U_{Fi} = \frac{u}{K_{Fi}} \tag{2}$$

$$U_{Ri} = \frac{u}{K_{Ri}} \tag{3}$$

where U_i, U_{Fi}, U_{Ri} and u are as defined above and K_i, K_{Fi} and K_{Ri} are the coefficients of distribution of the substances to be chromatographed in the regions of the central, front and rear parts of the zone, respectively.

As the distribution coefficient usually decreases with increasing temperature, *i.e.* (see Fig. 4),

$$K_{Ri} < K_i < K_{Fi} \tag{4}$$

then

$$U_{Ri} > U_i > U_{Fi} \tag{5}$$

Thus, the compression of the chromatographic bands and their movement corresponding to the speed of movement of the temperature field in chromathermography are in the steady state. The characteristic value in this method is not the retention volume, but the characteristic temperature of the chromatographed compound passing through the column. Under real conditions, the chromathermographic zone has limited sizes, which are determined by the effect of two opposing operating factors: zone compression (the effect of this factor was considered above) and factors whose effects lead to broadening of the band (the limiting rate of heat transfer, non-uniform

temperature gradient in the column, the limiting rate of mass exchange, etc.). The temperature gradient also causes the molecules for some reason to be drastically retarded, the motion having "outstripped" the zone, whereas the molecules that lagged behind the zone start to move faster (see Fig. 4). Thus, chromathermography provides the opportunity to achieve very narrow zones and to concentrate the substance.

Zhukhovitsky and Turkel'taub[7] developed a theory of movement of substances in a steady-state chromathermographic regime, on the basis of which a method was suggested for determining the heat of adsorption of substances using chromatographic zone data. In addition, the problems of compression of chromatographic zones being moved by the temperature gradient have also been studied. According to this theory, the concentration maximum in the chromatographic zone will group around one characteristic temperature ($T_{char.}$), which can be determined according to the following equation[9]:

$$T_{char.} = \frac{Q}{R \ln \left(\frac{1}{K \cdot \frac{\omega}{u}} - \kappa \right)} \tag{6}$$

where u is the linear velocity of the carrier gas, ω is the linear velocity of movement of the temperature field, κ is the proportion of the gas phase in the column, Q is the heat of adsorption, R is the gas constant and K is Henry's constant.

It should be noted that the method for performing steady-state chromathermography can differ from that suggested above by Zhukhovitsky and Turkel'taub[9].

Steady-state chromathermography is one of few chromatographic techniques by means of which absolute enrichment of components to be separated can be realized. Chromathermography can also be employed for the preliminary enrichment of a sample mixture. The process of preliminary enrichment was first described by Zhukhovitsky and Turkel'taub in 1957[13]. The process is simple, but requires a carrier gas of high purity. A quantitative comparison of the results of gas chromathermographic analyses with constant temperature and temperature gradients for an *n*-C_5–*n*-C_9 mixture was published[14], and also showed that the duration and efficiency of the latter is considerably higher (Fig. 5). Kaiser[15,16] also described the chromathermographic enrichment of a large sample in a column with a given temperature gradient with a flow of substance inside a special enrichment arrangement (Fig. 6). In Kaiser's variant, the carrier gas (sufficiently pure) is passed through the sample or over it and directed to the enrichment column together with the sample components. The packing used for the enrichment column was Dexsil 300 GC coated on a highly inert support. The main advantage of the enrichment system is the absence of a dosing operation.

Sukhorukov and Vatulya[17] suggested a method for the selection of optimal enrichment parameters and proposed a calculation scheme for a preliminary determination of the enrichment of the impurities in the chromathermographic regime. In our opinion, the multi-step calculation scheme is of interest for establishing the role of the main factors that influence the enrichment process. To calculate the enrichment

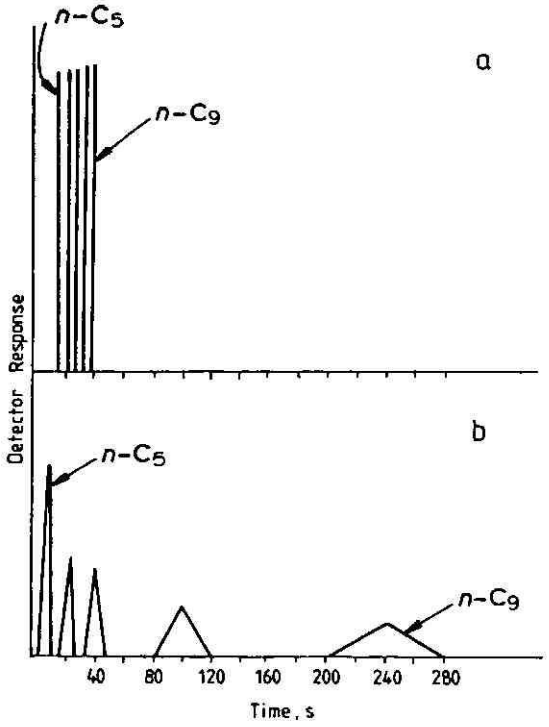


Fig. 5. Chromatogram of n-C_5–C_9 alkane mixture. (a) With temperature gradient; (b) isothermal conditions.

factor (O), Sukhorukov and Vatulya[17] suggested the following equation:

$$O = 2\sqrt{\frac{\mathrm{HETP_{iso.}}}{\mathrm{HETP_{chromatherm.}}} \cdot L\varepsilon\eta K_0} \qquad (7)$$

where K_0 is Henry's constant at the temperature of introduction of the sample into the column, ε is a characteristic value that determines the compression of the band on the layer, η is the relation of the oven velocity to the linear velocity of carrier gas in the outlet of the column, $\mathrm{HETP_{iso.}}$ is the height equivalent to a theoretical plate in isothermal experiments and $\mathrm{HETP_{chromatherm.}}$ is the height equivalent to a theoretical plate in chromathermographic experiments. The authors claimed that the suggested scheme may be varied, depending on the problem to be solved[17]. As indicated by Zhukhovitsky and Turkel'taub[9], the stationary chromathermographic method has a number of advantages over conventional isothermal chromatography: rapid separation of mixtures into their components with different adsorbabilities, the possibility of obtaining symmetrical peaks even with non-linear sorption isotherms and a considerable increase in the concentration of the sample components in the central part of the chromatographic zone.

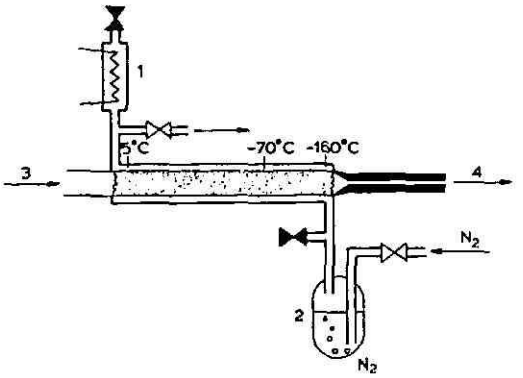


Fig. 6. Diagram of an enriched heat dynamic arrangement using a gaseous coolant. 1 = Electric heater for gas flow (heat carrier); 2 = heat exchanger with liquid coolant (liquid nitrogen) for cooling the gas flow; 3 = sample gas flow; 4 – outlet for the sample gas flow.

3. HEAT DYNAMIC METHOD

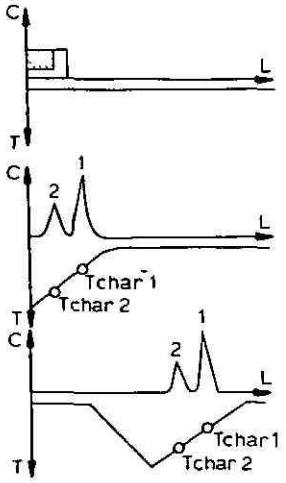
The next step in applying the thermal factor in gas chromatography was the heat dynamic method developed by Zhukhovitsky *et al.*[18]. Of all the variants of chromatography developed so far, the heat dynamic method is closest to a continuous automated separation process. Its application is of interest in both analytical and preparative problems.

The heat dynamic method is a combination of frontal chromatography with moving temperature field. In this method the direction of the temperature gradient is opposite to that of the flow of the mixture to be separated. The separation is achieved in the following manner. The sample mixture is run through the column in a continuous process and travels through the adsorbent layer and, at the same time, the temperature field moves along the layer. The simultaneous effects of the adsorption layer and of the temperature field on the mixture favour its separation into individual components. According to the theory of chromatography, a given component cannot penetrate along the adsorbent layer behind the point of the temperature field where the temperature equals the characteristic temperature for a given compound. It is important to note that at the point responsible for the characteristic temperature, continuous enrichment must take place (an increase in concentration) so as to correspond to the given characteristic temperature of the component. Thus, sharp maxima (peaks) of concentrations of the individual components of the sample mixture will occur in the region of the corresponding characteristic temperatures. By making an appropriate choice of parameters, the mixture can be separated into individual components. Moreover, owing to the high temperature in the upper part of the oven, all the compounds are completely desorbed from the adsorbent, the latter thus being regenerated. After the heater has reached the lowest position, it rises automatically to the top of the column at a high speed and then moves slowly along the layer again, *i.e.*, the separation process re-starts automatically. Essentially, by this means the flow of the gas being analysed is not discontinued. As the length of the oven is smaller than that of the column, the upper part of the column becomes cooled as the oven travels along the column. The "heavy" sample components (char-

acterized by higher sorption abilities) adsorbed in the upper part of the column create a preparatively enriched sample zone for the next separation cycle.

In the analytical variant of the heat dynamic method, a detector is arranged behind the chromatographic column, which automatically records (as chromatograms) any changes in the composition of the gas flow through the column. The heat dynamic method is the most useful procedure for the separation of the least sorbable component of a mixture in the pure state or for the purpose of concentrating (and subsequent analysis) of other components of the sample mixture, and also for the preparative separation of pure substances. Fig. 7 shows schematically the creation of zones of heavy impurities in the heat dynamic method[19]. A circulation apparatus was constructed, supplied with two columns and two moving ovens, which provide a high degree of concentration. The heat dynamic apparatus developed by Zhukhovitsky and Turkel'taub[20] makes it possible to determine automatically the mean concentration of all the sample components in 5–10 min with continuous passage of the gas mixture. With two weakly adsorbable components (*e.g.*, air–methane), their ratio is determined continuously.

The apparatus operates according to the principle of the heat dynamic method, which is intermediate between the chromathermographic method and the moving adsorbent layer method[21]. However, there are three substantial respects in which the chromathermographic method that differs from the latter method: (1) a pre-set optimal temperature gradient moves along the adsorbent layer; (2) the oven (temperature field) moves relative to the adsorbent, which eliminates numerous difficulties connected with wear of the adsorbent layer; and (3) sampling of the separated components is effected at a definite position on the adsorbent layer at the pre-set temperature, which leads to the periodic production of average concentrations during a relatively short time. Unlike the chromathermographic method, the mixture is passed



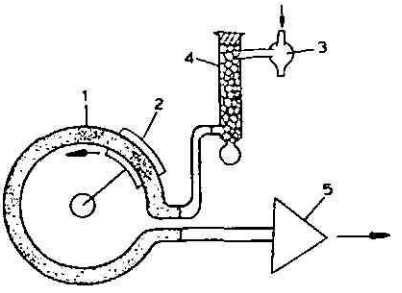


Fig. 7. Diagram of zone formation in the heat dynamic method. C = Concentration of components; T = absolute temperature; L = column length.

Fig. 8. Diagram of first thermodynamic installation (horizontal variant). 1 = Horizontal adsorption tube containing silica gel, having two vertical outlets; 2 = electric heater; 3 = three-way tap; 4 = desiccant (NaOH); 5 = heat-transfer detector.

continuously through the layer. The adsorption column represents an open horizontal circle along which a cylindrical electric oven moves continuously, incorporating a temperature gradient.

Fig. 8 depicts a horizontal heat dynamic installation. As the oven (temperature field) travels, it concentrates all the components accumulated in the adsorbent near the characteristic temperatures and directs them alternately to the detector. The concentrations of the components are generally determined by the heights of the maxima on the basis of a calibration graph. Output curves (chromatograms) representing the separation of a propane–*n*-butane–isopentane–*n*-hexane mixture are illustrated in Fig. 9. The characteristic temperatures, measured with the help of thermocouples inserted into the end part of the layer, are given on the peaks.

Genkin and Sazonov[22] developed methods for determining argon–oxygen impurities in helium, where the advantages of the heat dynamic method and the method ensuring complete component separation are combined. The experiments were carried out on a special low-temperature heat dynamic installation[23]. The installation accommodates a chromatographic column fixed rigidly to a rotary shaft of a drive, so that at the same time one end of the column is inserted in a coolant bath and the other end in the heater, and as the shaft rotates along the chromatographic column the temperature field with a constant temperature gradient also travels. In this manner it is possible to determine neon, hydrogen, argon, oxygen and nitrogen with sensitivities of $5 \cdot 10^{-3}$, $3 \cdot 10^{-3}$, $2 \cdot 10^{-5}$, $2 \cdot 10^{-5}$% (by volume), respectively, in 9–12 min. The sensitivity increases with increasing time. The method may find application in the analysis of different mixtures that are difficult to separate.

A chromathermographic system using frontal adsorption accumulation at cryogenic temperatures (liquid nitrogen) has been described[24]. The heat dynamic

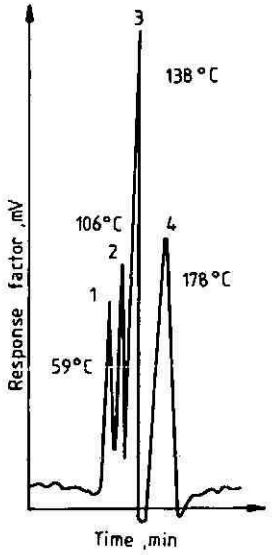


Fig. 9. Analysis of mixture separated on a chromathermographic installation. 1 = Propane; 2 = *n*-butane; 3 = isopentane; 4 = *n*-hexane; ω = 8.7 cm/min; u = 78 cm/min. The indicated temperatures correspond to the temperatures of separation of the corresponding peaks.

method of enrichment employed in the installation makes it feasible to determine O_2, N_2, CH_4, C_2H_6, C_3H_8, C_4H_{10}, NO, NO_2, CO and CO_2 impurities in hydrogen, helium and air at concentrations of 10^{-5}–10^{-7}% (by volume).

4. COMBINED CHROMATHERMOGRAPHIC METHODS

A variety of combined methods have been suggested for the development of chromathermographic and heat dynamic methods. A variant of chromathermography in which separation is performed simultaneously along the length of the column under a negative temperature gradient was described[25]. The method consists in the use of a constant gradient of temperatures along the column together with temperature programming. According to Zhukhovitsky and Turkel'taub[9], the employment of a non-stationary gradient does not provide any advantages over separation under isothermal conditions. However, variation of the temperature with time with a negative gradient along the column is equivalent, to a first approximation, to the thermal field under chromathermographic conditions.

Consider the simplest case where the gradient is constant over the entire layer and unchanged with time. The relationship between temperature, length and time is expressed by the following equations:

$$T = T_0 - \gamma L + mt \tag{8}$$

$$T = T_0 - \gamma\left(L - \frac{m}{\gamma} \cdot t\right) \tag{9}$$

where T_0 is the temperature at the beginning of the column at the starting moment, m is the temperature gradient with time, γ is the temperature gradient along the length and t is time.

The movement of a certain temperature field at a constant velocity is described by the following equation:

$$T = (L - \omega t) \tag{10}$$

From eqns. 9 and 10, it follows that

$$\omega = m/\gamma \tag{11}$$

In a general case, $T = f(L, t)$ for any distribution of temperature across the layer ($\gamma \neq$ const) and with time, which does not comply with a certain field moving at a constant velocity.

With constant gradients the characteristic temperatures are expressed by the same equation as in chromathermography:

$$T_{char.} = \frac{Q}{R \ln K\omega/u} = \frac{Q}{R \ln Km/\gamma u} \tag{12}$$

where Q is the heat of adsorption, K is Henry's constant, R is the gas constant and u is the flow velocity. The width of the peak (μ) is given by an equation similar to

that in chromathermography:

$$\mu = 2\sqrt{2}\, u/\omega \sqrt{\frac{\overline{\mathrm{HETP}}}{\sigma}} = 2\sqrt{2}\, u\gamma/\omega \sqrt{\frac{\overline{\mathrm{HETP}}}{\sigma}} \qquad (13)$$

where $\sigma = Q\gamma/RT_0^2$.

The selection of different gradients, including those varying with time, allows one to use the intermediate variants between chromathermography and temperature programming. Both methods have advantages; a decision should be made for each particular case as to which is preferable: a decrease in the peak width or an increase in the difference $\Delta V_g = V_1 - V_2$ (where V_1 and V_2 are retention volumes).

With a negative temperature gradient, the change in temperature with time along the column is equivalent to the moving heat field under chromathermographic conditions. In this instance, as follows from eqn. 11, the change in the rate of temperature leads to a corresponding change in the velocity of gradient field movement. In chromathermography the separation occurs only in the zone of the moving heater with a gradient, *i.e.*, on the restricted section of the chromatographic column. In the combined chromathermographic method, the separation occurs along the whole length of the column. Existence of such field is apparently an important advantage, especially when analysing complex mixtures with a wide range of boiling temperatures. In fact, when performing analyses of such mixtures, the characteristic temperatures of the first and last component may differ markedly, which necessitates the use of an oven that provides a greater difference in temperature at the cold and hot ends. If the oven is of limited length, this may necessitate the use of very sharp temperature gradients, which will have an adverse effect on the separation achieved. Apparently, the combined method allows one to achieve a large difference between the initial and final temperatures with a small gradient, as the "length" of the heater is not restricted. The analysis may be performed on a standard chromatograph with temperature programming.

The method was checked by determining, as an example, mixtures such as nonane, mesitylene and decane in toluene. A negative linear temperature gradient of 1.8°C/cm was produced with the help of a heating coil with variable winding. Spherochrom-1 solid carrier, containing 10% of apiezon L was used as the sorbent. Fig. 10 shows the chromatogram of the analysis of the mixture using a 35-cm column. The carrier gas flow-rate was 15 ml/min and the temperature programming rate was 10°C/min, which corresponds to the heat field movement of 6 cm/min. A chromatogram of the separation of the mixture with ordinary temperature programming is also presented for comparison. The combined chromathermographic variant provides a sharp compression and concentration of the samples components, giving an improved separation of nonane mesitylene, the concentration at the peak maxima being increased 10–15-fold compared with the isothermal method. The application of this method is simpler in the gas adsorption variant, where thermostable materials are used as the sorbents. By adopting the automatic heating–cooling procedure, the method may be used in the heat dynamic variant of chromathermography.

According to Nerheim[26], the temperature gradient along the column was produced several times in succession with a view to improving the chromatographic separation. At the beginning of the procedure the temperature gradient was less than

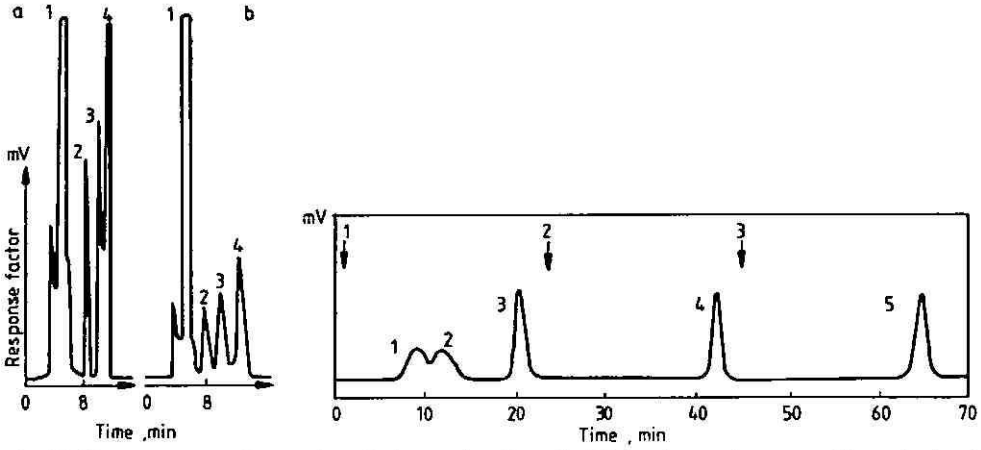


Fig. 10. Chromatogram of separation of mixtures in toluene by (a) the chromathermographic method and (b) the temperature programming method. Peaks: 1 = toluene; 2 = nonane; 3 = mesitylene; 4 = decane. γ = 1.8°C/cm; m = 10°C/min; u = 15 ml/min; L = 35 cm.

Fig. 11. Chromathermography with successive passages of the heater. 1, 2, 3 = First, second and third passages of heater. Peaks: 1 = isopentane; 2 = *n*-pentane; 3 = *n*-hexane; 4 = *n*-heptane; 5 = *n*-octane.

the characteristic temperature of high-boiling components, and only low-boiling components were liable to elution. Subsequently a higher gradient with higher temperatures was created that enabled the higher-boiling components to be eluted as separated peaks.

The method was exemplified by the separation of isopentane, *n*-pentane, *n*-hexane, *n*-heptane and *n*-octane. The improvement in separation resulting from multiple effects of the temperature gradient is shown in Fig. 11. The initial movement of the oven along the column has no effect on the separation of pentanes, as the rates of movement of the corresponding zones are higher, whereas the *n*-hexane zone, travelling at the speed of the oven, is compressed and becomes narrower than the zones of *n*- and isopentane. When a gradient with still high temperatures is produced for the second and third times, *n*-heptane and *n*-octane are also separated in the form of narrow zones; the *n*-octane zone, with a retention time of 65 min, is not wider than the *n*-hexane zone, with a retention time of 20 min.

Zizin and Makov[27] suggested a new moving gradient variant of chromathermography; they designed an installation that produces the chromathermographic effect by using a special attachment operating in an isothermal regime (Fig. 12). A chromatographic column (1) enclosed in a casing (2) (tube into tube) is accommodated in a chromatograph thermostat operating in an isothermal regime. The casing of the column is connected at the inlet and outlet with a coil (4) via a piston pump (5) in such a way that a closed contour is formed, through which the heat-carrier can circulate continuously. The coil is incorporated in a heat exchanger (6) with thermal insulation (7) and filled with a coolant. If the temperatures in the chromatograph thermostat, T_0, and in the heater exchanger, t_0, are constant, then if $T_0 \gg t_0$ a steady-state gradient of temperatures is fixed in the column casing, its magnitude being dependent on $T_0 - t_0$ and on the circulation velocity of the heat carrier in the closed contour.

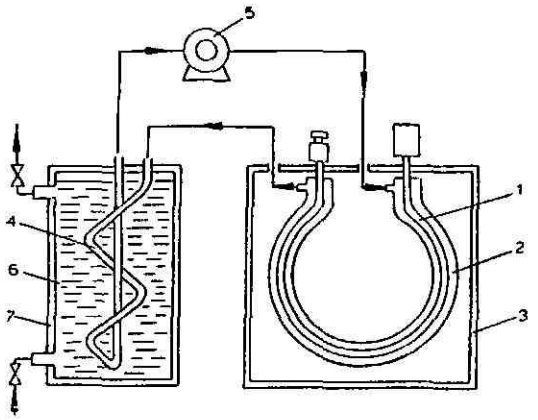


Fig. 12. Diagram of chromathermographic installation with liquid coolant. 1 = Chromatographic column; 2 = column casing; 3 = thermostat; 4 = heat exchanger; 5 = pump; 6 = heat-exchanger casing; 7 = thermal insulation.

Zizin and Makov[27] presented an equation expressing the temperature distribution during the movement of the gradient along the column. The process is equivalent to the movement along a heater column of infinite length with a temperature gradient varying exponentially. The suggested installation[27] combines the advantages of chromathermography and chromatography with temperature programming.

An original impulse–thermal method for gas analysis was developed by Dantsig[28]. This method ensures high enrichment of admixtures during the component separation in addition to compression of the chromatographic bands in different column sections. This method involves three separation stages: (1) preliminary separation; (2) compression of the chromatographic bands on the column during movement of a narrow thermal field in the direction opposite to the flow of the carrier gas; and (3) elution of the separated components with temperature programming. This method was shown to be effective for determining ethane and ethylene in air at a 10^{-4}% concentration using a device supplied with a catharometer.

5. ELUTION–THERMAL DISPLACEMENT METHOD

Berezkin and Rastyannikov[29] suggested an elution–thermal displacement method in which separation takes place as a result of the joint action of a moving thermal field and a low flow of carrier gas. The column filled with sorbent and purged with an inert carrier gas is supplied with an initial mixture until a part of the layer at the beginning of the column is saturated. A narrow oven then moves on to the column and a carrier gas flow is started at the same time. The moving oven causes a sharp decrease in the sorbability of the component in the heated zone, and therefore even a small gas carrier flow is sufficient to remove the component from this zone and direct it to the cold section of the sorbent. The sorbability of the component increases sharply on the cold section of the sorbent and its rate of movement slows accordingly, so that it becomes much lower than that of the heater. Thus, the heater will "overtake" the heavy component and the process of "pushing out" the com-

ponent from the heated zone by means of the carrier gas will be repeated. As the heavy component zone moves ahead it will force out the heavier component, obliging it to move at the speed of the zone. This elution–displacement method makes it possible to decrease considerably the temperature of the thermal field and reduce the contact time of the separating components with the heated zone. This allows the separation of substances that decompose or react at higher temperatures.

Further development of the thermal displacement method was carried out by Harris and co-workers[30,31]. They showed[31] that this method, depending on the ratio of the heater movement speed, U_H, to the carrier gas flow-rate, U_r, makes it possible to operate under different column regimes. If $U_H \gg U_r$, adsorbent condensation takes place; if $U_r \gg U_H$, the column works as under elution chromatographic conditions. Operation under thermal displacement conditions requires a particular optimal U_H/U_r ratio. Equations were suggested[31] for the maximal dose of the initial mixture and the concentration profile in the thermal zone. The latter equation was derived from the assumption that the stationary front moving at the speed of the heater is fixed in the column. These equations were verified on binary mixtures of *n*-hexane with 2,2- and 2,3-dimethylbutane and 2- and 3-methylpentane as examples. Alumina and Porasil B were used as packings. It was shown that when the flow velocity changes and the ratio U_H/U_r is constant, the efficiency of separation can be described by a Van Deemter type equation; at low velocities the spreading of the zone is determined by the longitudinal diffusion, whereas at high velocities it is determined by the mass-transfer resistance. The shape of the bands in the thermal displacement method, as calculated from the suggested equation, is close to that derived from experiment. It has been shown[32], using as an example the analysis of *o*-xylene, *n*-nonane, *n*-decane, butylbenzene and other impurities in light volatile solvents, that the chromathermographic method has several advantages over ordinary chromatography under isothermal and linear temperature programmed conditions. Under isothermal conditions, it was not possible to separate the above mixture, and the resolution obtained with linear temperature programming was significantly worse than that obtained under chromathermographic conditions.

Gel'man[33] demonstrated that methods such as chromathermographic, heat dynamic and elution–thermal preparative displacement are convenient when they are used at high concentrations. On the basis of a general chromathermography theory, he discussed the transition from chromathermography with an increase in the amount of sample to chromathermography without a carrrier and, subsequently, the transition from the latter to the so-called method of concentration fixing.

6. OVERLOADED CHROMATHERMOGRAPHY

In their extensive investigations of the potential of chromathermography, Zhukhovitsky and co-workers[34–36] developed a method without employing a carrier gas. This method was termed "overloaded chromathermography" (OCTG). It was found[35] that even in the presence of diffusion spreading under OCTG conditions, the amount of the substance in the zone is linearly dependent on the square of the band width of the component. As a rule, the band width is ascribed as time from the moment of the beginning of chromatographic zone output to the moment when the point corresponding to the characteristic temperature reaches the column output.

This method proved to be convenient for measuring both high and low concentrations of different substances. It was stated that the time corresponding to the characteristic temperature is constant, and it can be determined for each component.

The applicability of this method to the analysis of five-component mixtures, *e.g.*, C_2–C_6 *n*-alkanes, was demonstrated[37]. The theoretical aspects of OCTG have been discussed[38], and in particular an equation was derived for calculating the profile of the chromathermographic curve of carbon dioxide when determining it in air on silica gel MSM. The experimental and calculated (theoretical) data were in good agreement.

The search for effective separation methods that allow a decrease in the spreading, an increase in productivity and the determination of the physico-chemical properties stimulated the elaboration of a method lying at the borderline between chromatography and distillation[39–41], referred to as "chromadistillation". According to this method, the mixture to be separated is contained on a column with a solid filler (glass or metal spherules) and when the carrier gas is passed through the mixture a steady-state temperature field with a negative gradient is established. Separation of the mixture on the contacting zone of individual components takes place as a result of multiple condensation and evaporation.

The application of the chromadistillation method makes it possible to perform separations with a liquid stationary phase, which is important when analysing high-boiling compounds (including solids under ordinary conditions). The theory of chromadistillation and a variety of applications were discussed by Zhukhovitsky and co-workers[42,43].

Chromathermography, as has been shown above, is exceptionally efficient for the analysis of admixtures, as it makes it possible to increase the concentrations of the components at the peak maxima. Chromadistillation also allows one to enrich the components of the mixture[44]. Yanovsky *et al.*[45] combined these two methods and suggested a new method which they termed "chromathermodistillation". This method works in a similar manner to that described earlier[25] by combining the temperature gradient which exists in chromadistillation with temperature programming and that is equivalent to the moving of temperature field. This allows one to enhance the effects of enrichment that take place both under chromathermographic and chromadistillation conditions. As distinct from chromadistillation with a stationary temperature gradient, chromathermodistillation makes it feasible to solve a variety of problems concerning the analysis of multi-component mixtures containing components with a wide range of boiling temperatures.

7. THERMOCHROMATOGRAPHY

In connection with the development of investigations of short-lived isotopes, methods for the rapid separation and detection of elements have acquired great importance. A very promising method for the separation of elements is gas thermochromatography. This method is based on the entrainment of compounds by the carrier gas, which in some instances is also a reagent gas, from the high-temperature heating zone where such compounds are formed into a column having an pre-set inverse temperature gradient. As reported in a number of publications[46–55], the gas thermochromatographic method makes it possible to separate elements when their concentration in the initial sample is as low as 10^{-14}–10^{-11} mole.

Thermochromatography is usually regarded as a chromatographic method in which the separation of a sample mixture (with a sufficiently large volume) is carried out with a moving carrier gas (which in some instances also performs the function of a reagent) on a column having a constant negative temperature gradient, and the detection of the zones being separated is usually effected by scanning the column after completion of separation. As a rule, thermochromatography is employed for separating radioactive compounds, which substantially simplifies subsequent identification of the composition of the sample mixture by the scanning method.

Substances that can be separated by gas thermochromatography include a group of comparatively highly volatile oxides formed as a result of various thermochemical reactions (decomposition, combustion in an oxygen atmosphere, etc.). For example, the method was used for the rapid separation of preparations of rhenium, osmium, iridium and mercury[46]. The target was metallic gold of high purity, which was placed in a special quartz ampoule and irradiated with a beam of protons for a definite period of time. After irradiation, the target was allowed to stand for about 40 h for the short-lived isotopes to decay. Volatilization of the reactive products of rhenium, osmium, iridium and mercury from the gold melt and their distribution over the length of a thermochromatographic column having a negative temperature gradient were studied under the following conditions: sublimation temperature, 1160 ± 20°C; sublimation time, 3–60 min; carrier gas velocity, 10–40 ml/min; and carrier gas, oxygen, air and helium.Gaseous products entrained from the gold melt by the carrier gas were fed to the thermochromatographic column, which was a 480 mm × 2 mm I.D. quartz tube. A constant negative temperature gradient was maintained with the aid of special heaters. The end of the column was connected to a special trap and detector. This method makes it possible to effect selective deposition on the walls of the column of volatile oxides of rhenium, osmium, iridium and mercury in the following sequence of decreasing temperature: Re (500–350°C), Ir (180–80°C), Hg (80–25°C), Os.

Thermochromatography has also been used for the separation and identification of nuclear reaction products[56], and results for the thermochromatography of Na, K, Cs, Ba, Eu, Yb, Tm, Tl, Pb, Bi, Po, Am and Cf in a titanium column were presented. The carrier gas was helium containing small amounts of sodium or potassium. The atoms of the initial elements were entrained by evaporation from lanthanum. The column, made of titanium foil, was placed in a heater having a negative temperature gradient.

The formation and thermochromatographic behaviour of carrier-free traces of volatile oxides and hydroxide of tungsten were studied[47]. The samples were metallic foils of gold and tantalum irradiated with high-energy protons in which tungsten isotopes were formed by the corresponding nuclear reactions. First, high-temperature sublimation was carried out. Oxygen saturated with water vapour was used as the reagent gas. The temperature in the reaction section of the installation was 1060–1160°C, the carrier gas flow-rate was 15–45 ml/min and the sublimation time was 5–60 min. The distribution of ^{185}W along the thermochromatographic column (550 × 5 mm I.D.) was determined on completion of sublimation by drawing the tube in front of a scintillation detector connected to an automatic recorder. This modified gas-thermochromatographic method for the separation of radiochemically pure tungsten, under conditions without a carrier gas and without gold and tantalum targets irradiated with high-energy protons, is sufficiently effective and rapid.

A study using gas thermochromatography was carried out on the rapid production of short-lived neutron-deficient isotopes of zirconium and niobium from volatile chlorides for the purpose of further nuclear-spectroscopic investigations[48]. An ampoule containing silver chloride was irradiated with an ejected beam of protons on a synchrocyclotron at the Joint Institute of Nuclear Research (Dubna, Moscow Region, U.S.S.R.). After the ampoule had been irradiated, it was introduced into a reaction vessel into which 3–5 min earlier a thoroughly washed thermochromatographic column had been inserted. After completing the experiment, the column was removed quickly from the vessel, cooled and cut into separate zones, then each zone was measured for radioactivity using a Ge(Li) detector to determine the total yield and distribution of the separated radioactive elements along the thermochromatographic column.

Based on the thermochemical properties of osmium and rhenium oxides, a rapid gas thermochromatographic method was elaborated for separating rhenium from a complex mixture of products that are formed as a result of irradiation of targets with high-energy protons[49]. This method allows the production of radioactive preparations of rhenium for studying the nuclear spectroscopic properties of short-lived isotopes of this element, the half-life of which is several minutes.

Eichler[50] separated thermochromatographically the products of carrier-free nuclear reactions without a carrier gas. A column having a temperature gradient in the axial direction was used to separate the mixture thermochromatographically, a carrier gas being passed through the column in the direction of the temperature drop. It was shown that when the gas was passed through the column continuously, no equilibrium distribution was achieved. In an interrupted experiment, the distribution of the components along the column is "frozen" and an "internal chromatogram" is obtained in the column. An equation was derived[50] expressing the relationships between the total retention volume and the cross-section of the stationary phase, initial temperature, temperature gradient, heat of adsorption and the temperature of precipitation on the column walls. Equations that determined the precipitation temperature and described the processes of separation of the nuclear reaction products were obtained[51]. The method was successfully used in radiochemistry to identify elements 105[49] and 107[57] and to separate the products of uranium fission[52].

Radioactive iridium and platinum compounds in a carrier-free state, which were formed at 725 ± 25°C, were separated on a quartz thermochromatographic column. The influence of the method for producing the initial preparation and the experimental conditions was studied, particularly the effect of moisture in the carrier gas on the yield of oxygen-containing volatile compounds of iridium and platinum. Both elements formed two adsorption zones; under certain conditions the iridium zone centres were at 265 ± 20 and 175 ± 20°C and those of platinum at 280 ± 20 and 55 ± 15°C[58].

Travnikov and co-workers[59,60] studied the behaviour of actinides and fission products in the process of transfer of their chlorides in a column with a temperature gradient, with variable starting temperature, gradient value, carrier gas velocity and other factors. It was shown that the separation of micro-amounts of elements having similar properties could be achieved, provided that there is a difference in the characteristic temperatures of adsorption of not less than 50°C. New effective methods for the chromathermographic separation of trans-plutonium elements and chloride

were suggested. The thermochromatographic behaviour of trace amounts of rare-earth metal trichlorides was examined[61]. Distinct adsorption zones of the trichlorides of individual rare-earth elements were obtained in an open quartz gas thermochromatographic column of I.D. 1.25 mm. In was shown that the temperatures of the centres of gravity of the adsorption zones of the trichlorides of all the rare-earth elements were almost identical and, consequently, the gas thermochromatographic separation under these conditions was impossible.

Novgorodov[62] studied the separation and purification of radionuclides by thermochromatography. Theoretical calculations were made of the distribution of a substance during its movement through a thermochromatographic column having a constant temperature gradient. The thermochromatographic behaviour of nuclear reaction products during the combustion of uranium and molybdenum irradiated with high-energy protons in oxygen was studied. The behaviour of trace amounts of certain rare earth elements in a tantalum column under vacuum was also investigated. A description was given[62] of selective and rapid methods for the thermochromatographic separation of radioactive isotopes under vacuum and in a gas phase containing chlorine, hydrogen chloride, oxygen and their mixtures. Thermochromatography of ^{106}Ru and ^{233}Pu was used to study the products of the reaction of fluorine containing 1% of oxygen with ruthenium and plutonium oxides and fluorides[63]. Methods for the separation of radiochemically pure fission products developed on the basis of thermochromatography can be used in the production of radioactive isotopes valuable for applied investigations.

8. APPLICATION OF CHROMATOGRAPHIC METHODS WITH A TEMPERATURE GRADIENT TO THE DETERMINATION OF PHYSICO-CHEMICAL CHARACTERISTICS

The application of chromatography is not restricted only to the solution of problems concerning separation and analysis of complex mixtures, *i.e.*, problems of a pure analytical nature. In our opinion, the potential of chromathermography for investigating the physico-chemical properties of substances has been insufficiently studied, although its application to physico-chemical measurements was considered briefly in one of the first papers on this method[6]. The application of this method made it possible to determine the heats of adsorption of ethane, propane and butane on silica gel; the results were in good agreement with those obtained by other methods. Eichler and Zvara[64] substantiated the validity of calculations of heats of adsorption from thermochromatographic data. They also considered[65] the possibilities of determining the enthalpy (ΔH) and entropy (ΔS) of adsorption from chromatographic data obtained in a column having a constant negative temperature gradient in the direction of flow. A time retention equation was derived on the basis of the theory of linear chromatography. Equations for the entropy of adsorption were given for two models of steady-state and unsteady-state adsorption layers. Approximate methods were developed for determining ΔS and ΔH on the basis of experimental data.

Fan and Gaeggeler[66] investigated the adsorption of lead on quartz and calculated the enthalpy and entropy of adsorption from thermochromatographic results. Eichler *et al.*[67] determined the heats of adsorption of metal chlorides using thermochromatography. These methods made it possible to determine the activation

energy and the reaction order of dehydration of complexes of certain cations[68] and the kinetics of the separation of gaseous products of perchlorate decomposition[69].

A simple arrangement was suggested[70] for conducting thermochromatographic experiments on the basis of the Tsvet-100 chromatograph and testing it by comparing the data from the thermochromatographic and thermogravimetric decomposition of carbonyltriphenylphosphine complexes of rhodium, *viz.*, $Rh(CO)_3(PPh)_3ClO_4$ and $Rh(CO)(PPh)_2Cl$. Weighed amounts of ground sample were placed in a Pyrex glass reaction tube, which was incorporated in one of the chambers of the chromatograph evaporator and coupled directly to one of the heat conductivity cells. Linear heating of the evaporator in the temperature range 20–320°C at an increasing rate from 0.5 to 10°C/min was effected by means of a laboratory motor transport supplied with a small electric motor and pressure regulator.

The suggested thermochromatographic technique is more universal in application than the thermogravimetric method and can be used for studying solid-phase conversions of compounds whose heat resistance does not exceed 400°C and whose decomposition is accompanied by the evolution of volatile products.

The thermochromatographic technique was used to study the physico-chemical properties of mendelevium and a variety of other actinoids in comparison with some known metals[71]. The thermochromatographic method was also successfully employed for studying the chemical products of uranium fission[72].

Another interesting physico-chemical application is associated with the study of heterogeneous catalytic reactions. In 1961, the possibility of coupling in the reactor heterogeneous catalytic reactions and the chromatographic separation of the components of the reaction mixture[73]. In 1964, Roginsky and Yanovsky[74] indicated that in a number of instances, carrying out the reaction in a chromatographic regime under isothermal conditions is not effective. This takes place, for example, in reactions of the $A \rightleftarrows B$ type because, irrespective of the difference in the adsorption coefficients K_A and K_B, the separation of A and B on the column does not give advantageous yields of either A or B. The same situation is observed with reactions of the $A \rightleftarrows B + C$ type, with $K_B = K_C$. In view of this, a suggestion was made as to the prospects of employing stationary chromathermography for such reactions.

Let us introduce into the flow pulses of substance AB (which in the presence of the catalyst filling the column reacts at a limited rate in accordance with the scheme $AB \rightleftarrows A + B$), and starting with a rate ω we displace the temperature field. The adsorption coefficient a_{AB} is high at the beginning on the cold catalyst and the rate V_{AB} of the displacement of AB along the layer of the catalytic packing is low, *i.e.*, the temperature field passes the substance AB. In view of the presence of a negative temperature gradient, the band AB moves to the region of higher temperature together with the travelling oven, and accelerates its movement. Finally, at some characteristic temperature, $T_{char.}$, AB starts to move at a velocity equal to that of the oven, *i.e.*, $V_{AB} = \omega$. The rate of movement of the oven, ω, the linear flow velocity, u, and the heat of adsorption, Q, are related as follows:

$$T_{char.} = \frac{Q}{RT \ln \omega/u} \tag{13}$$

If $T_{char.} < T_{react.}$, where $T_{react.}$ is the temperature at which the reaction rate becomes appreciable, AB will pass through the reactor without any change as one peak. A

gradual decrease in ω/u leads to $T_{char.} = T_{react.}$ and then significant formation of substances A and B begins, each of them tending to enter the zone with its own characteristic temperature, $T_{char.A}$ and $T_{char.B}$. These attributes make chromathermography very useful for conducting reactions under chromatographic conditions. The selection of u and ω may alter the temperature of the zone where AB is located. It should be emphasized that a reverse reaction $A + B \rightleftharpoons AB$ is suppressed not only by the process of separation, as in the isothermal pulse variant, but also by localization of at least one of the products in the low-temperature zone. By measuring the temperature $T_{char.AB}$ at the outlet of the moment the maximum of AB with concentration C_{AB} appears, we are able to determine the reaction temperature ($T_{char.AB} = T_{react.AB}$). The application of the chromatographic method for effecting reactions under chromatographic conditions is also useful because in this instance a continuous enrichment or compression of the band takes place because the separated chromatographic band is affected by the temperature gradient and therefore the front part of the band moves slowly and the rear part faster, the concentration must be constant and determined by the external pressure. Therefore, reactions higher than first order under chromatographic conditions must proceed at elevated constant velocities in comparison with the isothermal chromatographic regime. In particular, it is advantageous under such conditions to carry out a reaction in which the sorption coefficients of the products are smaller than those of the initial substance (for instance, in high-temperature cracking), when $T_{char.AB} > T_{char.B} > T_{char.C}$, as the reacting substance is in the zone with elevated temperatures while the products are in the zone with low temperatures, thus preventing secondary reactions.

The possibility of isomerization reactions of isohexanes, *viz.*, 3-methylpentene-2 $\rightleftharpoons$ 3-methylpentene-1 and 4-methylpentene-1 $\rightleftharpoons$ 4-methylpentene-2, occurring under chromathermographic conditions was studied experimentally[75]. The results of a study of the catalytic reactions of double bond migration in isohexane molecules on a zinc–iron–copper catalyst under conditions of isothermal elution chromatography and under conditions of stationary chromatography were also described. Conducting the reaction under chromathermographic conditions allows one to obtain higher yields of α-olefins than are obtained under isothermal conditions. This result may be accounted for by the coupling of the catalytic reactions and the chromatographic separation in the reactor.

9. CONCLUSION

Methods involving temperature gradients in gas chromatography may be applied successfully in various fields of analytical and physical chemistry. Unfortunately, the advantages of these methods are not fully realized and their field of application is relatively narrow. One of the main reasons for such a situation is the absence of standard equipment and insufficient development of the technical basis of the method.

Further study and modifications of chromatographic methods with a temperature gradient should lead to new effective methods for the separation and analysis of substances and to the establishment of the areas of optimal application of existing methods. We hope that this review will arouse interest among investigators to develop further chromathermographic methods.

10. ABBREVIATIONS

u	linear velocity of carrier gas;
u_0	linear velocity of carrier gas at column outlet;
U_i	linear velocity of substance i in the centre of the zone;
U_{Fi}	linear velocity of substance i in the front region of the zone;
U_{Ri}	linear velocity of substance i in the rear region of the zone;
U_{AB}	linear velocity of substance AB;
ω	linear velocity of the temperature field and heater;
K	Henry's constant;
K_0	Henry's constant at the temperature of introduction of the sample into the column;
K_i	Henry's constant in the centre of the zone;
K_{Fi}	Henry's constant in the front region of the zone;
K_{Ri}	Henry's constant in the rear region of the zone;
K_A, K_B, K_{AB}	Henry's constant for substances A, B and AB, respectively;
T	absolute temperature;
T_0	temperature at the beginning of the column at the initial moment of time;
T_i, T_F, T_R	temperatures at the centre, front and rear of the zone, respectively;
T_B, T_C	temperature of substances B and C, respectively;
Q	heat of adsorption;
R	gas constant;
L	column length;
C	substance concentration;
κ	proportion of the gas phase in the column;
γ	dT/dL temperature gradient along the column length;
m	$dT/d\tau$ temperature gradient with time;
HETP	height equivalent to a theoretical plate;
$HETP_{iso.}$	height equivalent to a theoretical plate under isothermal conditions;
$HETP_{chromatherm.}$	height equivalent to a theoretical plate under chromathermographic conditions;
ε	characteristic value for determining the compression of the band on the layer;
O	enrichment factor;
μ	peak width at half-height;
V	retention volume of sample substance;
ΔV	difference in retention volumes of two sample substances.

11. ACKNOWLEDGEMENT

The authors thank Dr. L. S. Ettre for his help and valuable discussion.

12. SUMMARY

The main applications of temperature gradients in gas chromatography are reviewed, including stationary chromatothermography, combined chromathermographic methods, elution–thermal displacement, overloaded chromathermography and thermochromatography. The theoretical basis of the methods, the possibility of practical applications for analytical and physico-chemical measurements and prospects for development of the methods are considered.

REFERENCES

1 V. G. Berezkin, in V. G. Berezkin, V. S. Gavrichev, L. N. Kolomiets, A. A. Korolev, V. N. Lipavsky, N. S. Nikitina and V. S. Tatarinsky (Editors), *Gazovaya Khromatografiya v Neftekhimii (Gas Chromatography in Petrochemistry)*, Nauka, Moscow, 1975, p. 17.
2 J. H. Griffiths, D. H. James and O. S. Phillips, *Analyst (London)*, 77 (1952) 897.
3 W. E. Harris and H. W. Habgood, *Programmed Temperature Gas Chromatography*, Wiley, New York, London, Sydney, 1966.
4 A. A. Zhukhovitsky, N. M. Turkel'taub, O. V. Solotareva and V. A. Sokolov, *Dokl. Akad. Nauk SSSR*, 77 (1951) 435.
5 A. A. Zhukhovitsky, V. M. Turkel'taub and V. A. Sokolov, *Dokl. Akad. Nauk SSSR*, 88 (1953) 859.
6 N. M. Turkel'taub, V. P. Shvartsman and T. V. Georievskaya, *Zh. Fiz. Khim.*, 27 (1953) 1827.
7 A. A. Zhukhovitsky and N. M. Turkel'taub, *Dokl. Akad. Nauk SSSR*, 94 (1954) 77.
8 A. A. Zhukhovitsky, N. M. Turkel'taub and V. P. Shvartsman, *Zh. Fiz. Khim.*, 28 (1954) 1901.
9 A. A. Zhukhovitsky and N. M. Turkel'taub, *Gazovaiya Khromatografiya (Gas Chromatography)*, Gostoptekhizdat, Moscow, 1962.
10 A. A. Zhukhovitsky, E. V. Vagin and S. S. Petukhov, *Dokl. Akad. Nauk SSSR*, 102 (1955) 771.
11 C. Lapp and K. Erali, *Bull. Sci. Pharmacol.*, 47 (1940) 49.
12 N. G. Turner, *Nat. Pet. News*, 35 (1943) 234.
13 A. A. Zhukhovitsky and N. M. Turkel'taub, *Dokl. Akad. Nauk SSSR*, 116 (1957) 986.
14 R. W. Ohline and D. D. De Ford, *Anal. Chem.*, 35 (1963) 227.
15 R. E. Kaiser, in K. V. Chmutov and K. I. Sakodynskii (Editors), *Uspekhi Khromatografii (Advances in Chromatography)*, Nauka, Moscow, 1972, p. 193.
16 R. E. Kaiser, *Fresenius Z. Anal. Chem.*, 236 (1968) 168.
17 O. A. Sukhorukov and N. M. Vatulya, in K. I. Sakodynskii (Editor), *Transactions Gazovaiya Khromatografiya (Gas Chromatography). NIFKhI im. L. Ya. Karpov*, NIITEKhim, Moscow, 1970.
18 A. A. Zhukhovitsky, N. M. Turkel'taub and T. V. Georgievskaya, *Dokl. Akad. Nauk SSSR*, 92 (1953) 987.
19 V. G. Berezkin and V. S. Tatarinsky, *Gas-Chromatographic Analysis of Impurities*, Consultants Bureau, New York, London, 1973, p. 127.
20 A. A. Zhukhovitsky and N. M. Turkel'taub, *Zavod. Lab.*, 10 (1956) 1252.
21 N. Berg, *Trans. Am. Inst. Chem.*, 42 (1946) 665.
22 Yu. M. Genkin and M. L. Sazonov, *Gazov. Delo*, 1 (1971) 19.
23 V. I. Samuilov, Yu. M. Genkin and M. D. Suvorova, *Auth. Cert.*, No. 257848; *Byull. Izobr.*, No. 36 (1969).
24 D. E. Bodrina, L. V. Kovalev, T. V. Adamiya and G. A. Krasnova, *Zavod. Lab.*, 46 (1980) 216.
25 V. G. Berezkin, V. S. Tatarinsky and A. A. Zhukhovitsky, *Zavod. Lab.*, 11 (1970) 1299.
26 A. G. Nerheim, *Anal. Chem.*, 32 (1960) 436.
27 V. G. Zizin and N. I. Makov, *Zavod. Lab.*, 45 (1979) 1082.
28 G. A. Dantsig, *Zavod. Lab.*, 30 (1964) 1313.
29 V. G. Berezkin and E. R. Rastyannikov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2 (1968) 58.
30 C. M. A. Badger, J. A. Harris, K. F. Scott, M. J. Walker and C. S. G. Phillips, *J. Chromatogr.*, 126 (1976) 11.
31 J. P. Horrocks, J. A. Harris, C. S. C. Phillips and K. F. Scott, *J. Chromatogr.*, 197 (1980) 109.
32 V. G. Berezkin and N. G. Starostina, *Chromatographia*, 8 (1957) 395.
33 L. G. Gel'man, *Thesis Essay*, VNIGNI, Moscow, 1972.

34 A. A. Zhukhovitsky, M. L. Sazonov, L. G. Gel'man and V. P. Schvarzman, *Chromatographia*, 4 (1971) 547.
35 S. M. Yanovsky, I. A. Silayeva, L. G. Gel'man, V. P. Shvartsman, M. L. Sazonov and A. A. Zhukhovitsky, *Zavod. Lab.*, 38 (1972) 526.
36 I. A. Silayeva, A. A. Zhukhovitsky, M. L. Sazonov and V. P. Shvartsman, *Zavod. Lab.*, 38 (1972) 1297.
37 I. A. Silayeva and S. M. Yanovsky, *Zh. Anal. Khim.*, 30 (1978) 387.
38 S. M. Yanovsky, I. A. Silayeva and G. L. Kogan, *Inz.-Fiz. Zh.*, 27 (1974) 466.
39 A. A. Zhukhovitsky, *Auth. Cert.*, No. 536429; *Byull. Izobr.*, No. 43 (1976).
40 A. A. Zhukhovitsky, S. M. Yanovsky and V. P. Shvartsman, *Zh. Fiz. Khim.*, 49 (1975) 2954.
41 A. A. Zhukhovitskii, S. M. Yanovskii and V. P. Shvarzman, *J. Chromatogr.*, 119 (1976) 591.
42 A. A. Zhukhovitsky, S. M. Yanovsky and V. P. Shvartsman, in A. A. Zhukhovitsky (Editor), *Khromatographiya, Itogi Nauki i Tekhniki*, VINITI, Moscow, 2 (1978) 49.
43 A. A. Zhukhovitsky and S. M. Yanovsky, *Zavod. Lab.*, 47 (1981) 7.
44 A. A. Zhukhovitsky, B. P. Okhotnikov, S. M. Yanovsky and L. G. Novikov, *Zh. Anal. Khim.*, 34 (1979) 545.
45 S. M. Yanovsky, G. S. Birun and A. A. Zhukhovitsky, *Zh. Fiz. Khim.*, *55 (1981) 1813.*
46 B. Boyar, I. Votsilka, N. G. Zaitseva and A. F. Novgorodov, *Radiokhimiya*, 16 (1974) 329.
47 B. Boyar, I. Votsilka, N. G. Zaitseva and A. F. Novgorodov, *Radiokhimiya*, 16 (1974) 385.
48 B. Boyar, I. Votsilka, N. G. Zaitseva and A. F. Novgorodov, *Radiokhimiya*, 16 (1974) 343.
49 B. Boyar, N. G. Zaitseva and A. F. Novgorodov, *Radiokhimiya*, 15 (1973) 553.
50 B. Eichler, *Seiffen/Erzgebirge*, (1974) 1.
51 B. Eichler and V. P. Domanov, *J. Radioanal. Chem.*, 28 (1975) 143.
52 A. W. Castelman and I. N. Tang, *Nucl. Sci. Eng.*, 29 (1957) 2159.
53 I. Zvara and V. V. Belov, *Preprint Ob'edineny Institut Yadernih Issledovaniy*, Dubna, 1970, P12-5120.
54 L. Westgard, G. Rudstam and O. C. Jonsson, *J. Inorg. Nucl. Chem.*, 31 (1969) 3747.
55 B. Boyar, N. G. Zaitseva and A. F. Novgorodov, *Preprint Ob'edineny Institut Yadernih Issledovaniy*, Dubna, 1971, P6-5955.
56 S. Hübener and I. Zvara, *Radiochim. Acta*, 27 (1980) 157.
57 I. Zvara, V. P. Domanov, S. Hübener, M. R. Shalayevsky, S. N. Timokhin, B. L. Zhuikov, B. Eichler and G. V. Buklanov, *Radiokhimiya*, 26 (1984) 76.
58 V. P. Domanov, B. Eichler and I. Zvara, *Radiokhimiya*, 26 (1984) 66.
59 A. V. Davydov, S. S. Travnikov and B. F. Myasoyedov, *Radiokhimiya*, 17 (1975) 684.
60 S. S. Travnikov, *Thesis Essay*, Institut Geokhimii i Analiticheskoy Khimii AN SSSR, Moscow, 1976.
61 Nguen Kong Chang, A. F. Novgorodov, M. Kaskevich, A. Kolachkovsky and V. A. Khalkin, *Radiokhimiya*, 26 (1984) 60.
62 A. F. Novgorodov, *Thesis Synopsis*, Institut Atomnoy Energii imeni I. V. Kurchatova, Moscow, 1982.
63 B. Jouniaux, J. Ledoux and J. Merinis, *Radiochem. Radioanal. Lett.*, 39 (1979) 129.
64 B. Eichler and I. Zvara, *Preprint Ob'edineny Institut Yadernih Issledovaniy*, Dubna, 1975, P12-8443.
65 B. Eichler and I. Zvara, *Radiochim. Acta*, 30 (1982) 233.
66 W. Fan and H. Gaeggeler, *Radiochim. Acta*, 31 (1982) 94.
67 B. Eichler, V. P. Domanov and I. Zvara, *Preprint Ob'edineny Institut Yadernih Issledovaniy*, Dubna, 1978, P12-9454.
68 V. A. Logvinenko, V. M. Gorbatchov, G. F. Nikitchenko, L. I. Myachina and A. V. Nikolaev, *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk*, N12 (5) (1970) 11.
69 L. Berezkina, S. Borisova and N. Tamm, *J. Chromatogr.*, 69 (1972) 31.
70 N. P. Fedyanin, *Zavod. Lab.*, 46 (1980) 1032.
71 S. Hübener and I. Zvara, *Radiochim. Acta*, 31 (1982) 89.
72 A. W. Castelman and I. N. Tang, *J. Inorg. Nucl. Chem.*, 32 (1970) 1057.
73 S. Z. Roginsky, M. I. Yanovsky and P. A. Gaziyev, *Dokl. Akad. Nauk SSSR*, 40 (1961) 1125.
74 S. Z. Roginsky and M. I. Yanovsky, in *Gazovaya Khromatografiya, Proceedings, III All-Union Conference on Gas Chromatography, Moscow, May 27–30, 1964*, Dzerzhinsk, 1966, p. 54.
75 I. A. Degtarev and M. I. Yanovsky, *Kinet. Katal.*, 17 (1976) 973.

Journal of Chromatography, 373 (1986) 45–60
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHREV. 204

THEORY OF MULTICOMPONENT CHROMATOGRAPHY

A STATE-OF-THE-ART REPORT*

FRIEDRICH G. HELFFERICH
Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802 (U.S.A.)
(Received January 28th, 1986)

CONTENTS

1. INTRODUCTION

No three inventions have had greater impact on the working day of the physical scientist of our time than the computer, the photocopier and the chromatograph. Of these, at least one, the chromatograph, can rightly be called a Russian invention, attributable to Tswett[1], who, in 1906, described his success in separating chlorophyll components on a calcium carbonate column. As has often been the case with a fundamentally new idea, an incubation period was needed before the full potential of chromatography was recognized and realized, a development that owes much to the genius of Nobel laureate A. J. P. Martin[2]. Today, the chemist, biochemist, chemical engineer, geologist, metallurgist, nuclear engineer, clinical laboratory technician, almost anyone in practical physical science would find it hard to imagine how he could conduct his work without the almost instantaneous information on composition of matter that chromatography can supply.

The principal impact of chromatography has been as a fast, convenient and reliable tool of chemical analysis. The vast majority of publications and applications to date relate to analytical separations, to the extent that thinking about chromatographic problems has been conditioned to follow along the trails the pioneers of analysis have blazed. This has not always been so. Indeed, Tswett seems to have had a preparative rather than analytical separation in mind, and much of the early prac-

* Plenary lecture presented at the *5th Danube Symposium on Chromatography, Yalta, U.S.S.R., 11–16 November 1985.*

0378-4355/86/$05.60

tical and theoretical work on chromatography, predating the gas chromatography revolution of the 1950s, had this orientation. And in the present decade, preparative chromatography seems to be moving again out of the shadows cast by the immense success of analytical chromatography.

It will serve us well not to lose sight of the fundamental difference in objectives between analytical and preparative separations. Chemical analysis calls for a separation as complete as reasonably possible, in a single operation; small sample size and low concentration are advantages in that they reduce the amount of material required (and expended), and have been made possible by the marvels of detector technology of today. Much of the work on chromatography —and some most spectacular progress— has been in reduction of analysis time, sample size and equipment size, without sacrifice in sharpness of separation. In contrast, a preparative separation calls primarily for processing of large quantities of material and recovery at concentrations as high as possible; sharpness of separation can be compromised to some extent as cuts with overlaps of components can easily be recycled. The difference in goals and outlook cuts deeper than is apparent at first glance, as an examination of the theoretical basis of chromatography can show.

From this vantage point, analytical chromatography presents itself as a science and art based on the fundamental equation for the average rate of travel, in direction of mobile phase flow, of a species i with partition coefficient K_i between the stationary and the mobile phase:

$$v_i = v^0/(K_i + 1) \tag{1}$$

(for symbols see list at end of paper). In various guises, usually expressed in terms of retention time or adjusted retention time, this equation is the starting point for almost all developments in analytical chromatography. Stationary phases are chosen so that different substances have different partition coefficients, and thus travel through the column at different speeds, affording separation. The work of the analytical chromatographer now is to fight the effects that lead to spreading and distortion of the "peaks" and so to obtain a sharp separation in as small as possible a column in as short as possible a time. This has been the mainspring of technical advance in analytical chromatography, to mention capillary columns, thin-layer chromatography and high-performance liquid chromatography as only some of the more remarkable examples.

The chemist or engineer attempting to design a preparative separation faces different problems. The large amounts of matter to be separated force him to work with bands occupying even initially a substantial length of column, making separation harder than with minuscule analytical samples. The desire for effective recovery forces him to work with high concentrations; here, the partition coefficients are no longer constant, and this leads to "self-sharpening" or "non-sharpening" behavior of the traveling concentration variations in his column (formation of "shocks" and "rarefaction waves", in the physicist's language), complicating separations. Worst of all, at high concentrations the various species in his column will interfere with one another: the curvature of a sorption isotherm at moderate to high concentration reflects the fact that the behavior of a solute molecule is affected by the presence of others of its kind, and it would then be naive to assume it to remain unaffected by

the presence of molecules of other species. What is needed here is theory of "multicomponent" chromatography, accounting for such interference. It is not possible to use ordinary chromatographic theory as the starting point and introduce interference as a refinement. Rather, multicomponent theory is fundamentally different in that it must deal with simultaneous rather than single differential equations and that interference can produce entirely new effects. On the other hand, this higher complexity and the lesser need for absolute sharpness of separation in preparative applications have let theory retain to this day quite simple assumptions regarding perturbations (deviations from local phase equilibrium, non-ideal flow pattern, etc.), a stage of development which theory of analytical chromatography has long outgrown.

As one might expect in view of the preeminence of analytical chromatography, there has been no lack of attempts to develop preparative separations by scale-up of successful analytical techniques. This approach amounts to fighting the facts inherent in analytical chromatography that high concentration is detrimental to ease of separation and that separation necessarily entails a dilution of the components with the eluent. From the theoretical point of view, the true challenge in preparative chromatography is not to fight the complexities of high concentrations and interference, but to make them work for better efficiency. Indeed, they make it possible to achieve concentration as well as separation in the same operation, though at the expense of having to reconstitute the column for renewed use.

2. ELUTION *VERSUS* DISPLACEMENT

In conventional, analytical operation with injection of a sample into a continuous stream of carrier gas or solvent, the components of the original mixture travel at different speeds in accordance with eqn. 1, increasing the distances from one another while their peaks flatten owing to perturbations (see Fig. 1, left). The distances between adjacent peaks increase in proportion with the distance traveled while the peaks themselves spread in proportion with the square root of that distance. Accordingly, one can attain any desired sharpness of separation by making the column long enough. In the language of preparative chromatography this mode of operation is termed "elution development".

In contrast, for preparative separation, the use of an agent with greater affinity

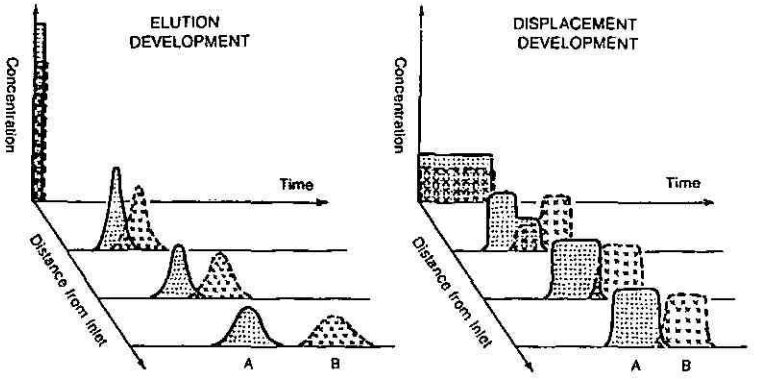


Fig. 1. Comparison of elution and displacement development.

for the sorbent than possessed by any component of the original mixture offers an advantage. Such an agent effectively displaces the mixture from the sorbent in a piston-type fashion. As the mixture is pushed through the column, its components arrange themselves in the order of increasing affinity for the sorbent. A state is attained in which bands of individual components follow one another close-up, with some overlap, all traveling at the same speed, and with a concentration essentially dictated by that of the displacing agent (see Fig. 1, right). Large sample size is an advantage rather than disadvantage in that the individual bands will be wider, so that overlaps constitute a lesser fraction of total material. If the agent is used at a sufficiently high concentration, the constitutents of the mixture can be recovered in concentrations much higher than charged. The benefits for preparative separations are obvious. The price to be paid is that the column after separation is saturated with the strongly held displacing agent, which must be removed before another separation can be conducted. This oldest and most common mode of preparative chromatography is termed "displacement development".

Displacement development is by no means a recent innovation. The idea, first suggested by Tiselius[3] in 1943, predates the wide-spread adoption of gas chromatography. It was used little later with spectacular success in the preparative separation of rare-earth elements in kilogram quantities and spectroscopic purity by Spedding and Powell[4]. Indeed, one might speculate that preparative methods based on this principle would have become standard long ago and the philosophy of solving preparative problems by scale-up of analytical techniques might never have become prevalent, had not the phenomenal success of analytical gas chromatography focused attention on the elution development mode.

3. EARLY THEORY IN RETROSPECTIVE

Chromatography in any form relies on differences in equilibrium sorption behavior and, conversely, much of the interest in sorption phenomena has been stimulated by the attempt to devise chromatographic and other separations. In the early days, before the gas chromatography revolution, preparative problems commanded much attention, and so theoretical developments included high-concentration and multicomponent behavior almost as a matter of course. Langmuir[5] derived his adsorption isotherm from the outset for multicomponent systems with interference, and the single-component form we have become used to associate with his name is but a later simplification. Likewise, much of the early theoretical work on chromatography, most notably by DeVault[6] and Glueckauf[7–9], included non-linear isotherms and interference, making it applicable to high concentrations and multicomponent systems.

Specifically for displacement development, Claesson[10] in 1946 presented procedures and formulas to predict quantitatively the pattern of individual bands that would eventually arise and remain unchanged on further travel through the column. This left unanswered the practical question how much time and column length would be required to attain that final pattern. The way to solve this problem in principle by calculation of transient behavior was shown by Glueckauf[7], although it took much longer to develop mathematics capable of handling mixtures of more than three or four components without prohibitive calculation effort[11–14].

Apart from its great practical importance, displacement development is of special interest in that it sheds an interesting sidelight on the evolution of multicomponent theory. As will be discussed later, the conventional theoretical approach to chromatography runs into trouble when applied to the general case of arbitrary, gradual or repeated variations in feed composition. Displacement development involves introduction of two different feeds in succession: the mixture, followed by the displacing agent. Nevertheless, Glueckauf[7], with genius and intuition, correctly evaluated this situation, if with an assumption that at the time lacked a rigorous proof of validity and that is not applicable in the general case. This remarkable achievement may have helped to obscure the fundamental difficulty which a rigorous multicomponent theory for arbitary and repeated composition variations was facing.

4. THE CRUX OF MULTICOMPONENT THEORY: WAVE EQUATION AND PARTIAL DERIVATIVES

Eqn. 1, although a starting point for analytical chromatography, is of little use in multicomponent systems. It is still valid (with composition-dependent K_i) but describes the rate of travel of molecules of a species rather than, what is much more convenient to evaluate, that of a given concentration or concentration variation. Its place is taken by the "wave equation"

$$v_{C_i} \equiv (\partial z/\partial t)_{C_i} \equiv v^0/[1 + (\partial \bar{C}_i/\partial C_i)_z] \tag{2}$$

easily derived from a differential material balance[15] and giving the velocity of a concentration C_i, that is, the velocity at which a given (constant) value of C_i travels through the column in the direction of flow. The trouble is with the partial derivative $\partial \bar{C}_i/\partial C_i$. If species i is the only solute present, this quantity (then a total derivative) is well defined: as the variation in sorbed with fluid-phase concentration, it simply is the slope of the isotherm. In a multicomponent system with interference, however, the sorbed concentration $\bar{C}_i$ depends not only on the fluid-phase concentration C_i of the same species, but on those of all other solutes as well. This alone necessitates solving simultaneous equations. What is worse, however, is that even for a given composition $C_1, C_2, \ldots, C_n$ in an n-component system the derivative remains indefinite, being dependent on the local gradients of all concentrations. This becomes apparent without an excursion into calculus if one considers that the isotherm of

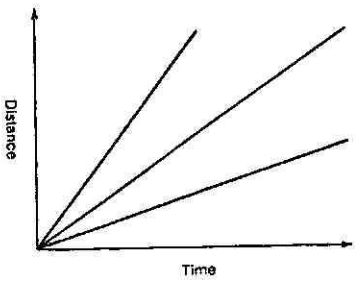


Fig. 2. Trajectories of given concentrations C_i in distance-time plane (C_i constant along lines shown), traceable to single perturbation at column inlet and start of operation (schematic).

species i in the multicomponent system is a multidimensional hypersurface rather than a curve on a plane, and so allows a tangent to be placed at any specified point in an infinite number of directions in space, with different partial slopes $\partial \bar{C}_i/\partial C_i$.

For the simple case of constant feed and uniform initial column compositions it is plausible that one should be able to trace all concentration variations back to the same point in distance and time: the column inlet and time of first entrry of feed (see Fig. 2). The concentrations of all species coexisting at any point in space and time in the column should then have traveled jointly, from that same origin, and with the same speed for all. In other words, the wave equation should give the same velocity for all concentrations C_1, C_2, etc., that coexist at a distance–time point in the column, necessitating the partial derivatives to be equal for all species. This eminently plausible conclusion was intuitively accepted without proof by Glueckauf and his successors (with the notable exception of Baylé and Klinkenberg[16]), for this situation as well as for more complex cases with successive feed changes, such as displacement development. Granted the equality of the partial derivatives, wave velocities can now be calculated.

Unfortunately, matters in reality are more complex. In the general case of arbitrary, variable starting conditions, the wave velocities of the species coexisting at a distance-time point in the column may well be unequal. What happens then is best understood in terms of a new concept, that of "coherence".

5. "COHERENCE"

Inherently, propagation of a perturbation in a system otherwise at equilibrium or steady state is a dynamic, transient phenomenon. It is over when the system has returned to its "stable" equilibrium or steady state. Yet, in a multicomponent system (more generally, in a system with several dependent variables), an arbitrary initial perturbation does not traval as such, but shakes itself down to a state it is more comfortable with. Such a state is called "coherent" (a word different from "stable" being needed)[12]. Coherence thus is a state which a transient, non-equilibrium, non-steady state perturbation seeks to assume, in much the same way as a closed system

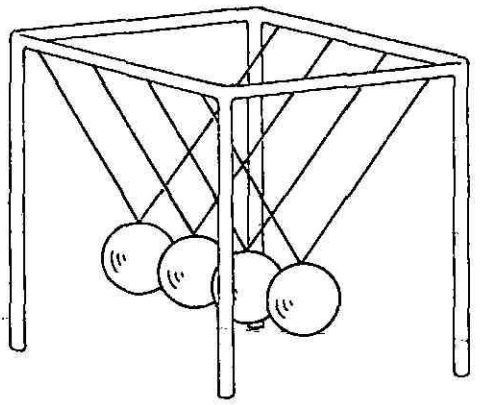


Fig. 3. Array of suspended steel balls, for demonstration of attainment of a well defined dynamic state from arbitrary starting conditions.

seeks to attain equilibrium and an open system with fixed boundaries and constant boundary values seeks to attain a steady state. This idea is unfamiliar to the physical chemist and chemical engineer, who have been preoccupied with equilibrium and steady state as conditions a system settles down to; it is not to the physicist and mechanical engineer, well acquainted with damped harmonic oscillations —say, the flutter of an aircraft wing.

A manifestation of such attainment of a well defined transient state from arbitrary starting conditions can be given with a linear array of steel balls suspended by strings and just touching one another (see Fig. 3). An arbitrary initial variation soon settles down to a regular, fully synchronized motion in which the balls have stopped bouncing one another. (One might say they are wiser than mankind in that they understand how to achieve peaceful coexistence.)

The concept of coherence can be illustrated with a simple thought experiment[17]. Imagine a mixture of oil and water being sparged into a vertical tube initially filled with air. Granted that our oil is lighter than water, a layer of oil will form on top of the growing column of liquid in the tube and will increase in size as does that column (see Fig. 4). This experiment demonstrates that a single perturbation at the feed port (the switch from air to water–oil injection) is propagated through the tube not as a single wave, but as a set of waves traveling at different speeds. Here, there are two waves: the air–oil interface and the boundary between the oil layer and the oil–water mixture. In principle, any number of waves can arise, depending on the number of components of the system.

Actually, the system in Fig. 4 can be said to involve three waves rather than only two, the third being the boundary between air and the oil–water mixture. This wave, however, existed only for a fleeting instant, at the sparger at the start of oil–water injection, for buoyancy immediately saw to it that the oil layer would begin to form. In other words, that third wave was incapable of surviving and, instead, broke up to form the two waves that travel with integrity until they exit the tube at its upper end. In the language of coherence theory, the initial, non-surviving wave is

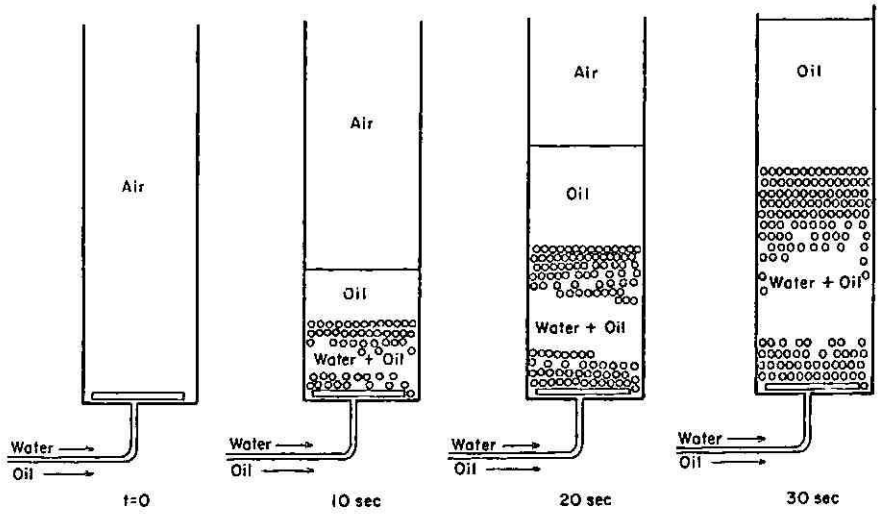


Fig. 4. Water–oil injection into air-filled tube, shown at regular time intervals.

called "non-coherent"; the two others, traveling with integrity, are called "coherent". In those words, the original non-coherent wave has been resolved into two coherent waves, which travel without further change in state.

From this simple model, a sufficient and necessary condition for a wave to be coherent is easily deduced. Any wave in a multicomponent system involves concentration variations of all species present at that point and can thus be viewed as a superposition of individual, single-component waves. if the wave is to be coherent, it must be definition travel with integrity rather than break up into several separate waves. Obviously, it can do so only if the wave velocities with respect to all component represented are equal. This is the fundamental coherence condition[18]. (The presentation here, aiming only at conceptual understanding, glosses over the complications arising from the composition dependence of the coherent wave velocity as calculated from eqn. 2 and the coherence condition, which leads to formation of shock or rarefaction waves; for details, see ref. 12.)

The coherence condition, in combination with the wave equation, can now be seen to demand the equality of partial derivatives, the equality that was taken for granted by the early chromatography theoreticians. This equality leads to an eigenvalue problem and allows only certain discrete composition variations rather than any arbitrary ones. These variations can be mapped in a coordinate system with the species concentrations as axes (hodograph space). Such as grid of so-called composition paths[12] constitutes the groves into which the system will tend to settle. An example of a calculated grid is shown in Fig. 5. Once the grid has been constructed, the response of the system to almost any perturbation is readily deduced without much further recourse to mathematics[12,17], relying on the principle that, given enough time, the system will settle down to entirely coherent behavior and thus follow a route exclusively along the paths of the grid. Observing a few simple selection rules one can trace that route much as one would project a vacation trip by car on a road map.

Of particular interest in this context is interference of waves as will occur when faster waves generated later will catch up with slower ones generated earlier (see Fig.

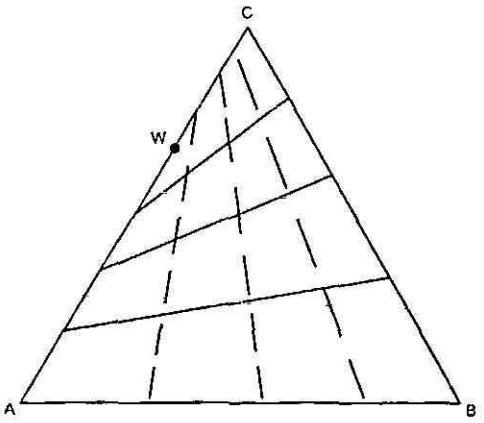


Fig. 5. Composition path grid, calculated for three-component ion-exchange system A–B–C with constant separation factors $\alpha_{AB} = 2$ and $\alpha_{AC} = 5$.

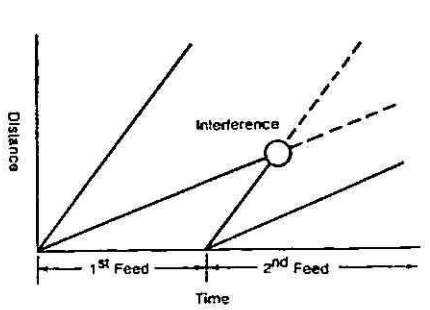


Fig. 6. Interference of waves: wave trajectories in distance–time plane for two stepwise feed composition variations (schematic).

6). In principle, such collision of two waves gives rise to local non-coherence. This non-coherence does not know or care whether it was introduced at the feed port at start or arose from wave interference later and somewhere in the column; it is unable to survive and is resolved into new coherent waves under the same rules as apply to any non-coherent feed variation. With use of the composition path grid the resulting waves are easily predicted, at least qualitatively[12,17]. Overall, the evolving picture much resembles wave interference phenomena in optics and mechanics. The simplest case is interference of two shock waves (see Fig. 7, left). Here, non-coherence remains confined to a single point in the distance–time plane. This has made displacement development an exceptionally simple case to calculate, for all its waves are shocks. The most difficult situation arises when two diffuse waves interfere; here, non-coherence persists within a finite distance–time region (see Fig. 7, right) as, incidentally, it does in the case of a gradual non-coherent initial or feed composition variation.

The possibility of presence of finite regions of non-coherence brings home the fact that not all problems of multicomponent chromatography can be solved taking the coherence condition for granted. Rather, theory of coherence has served to elucidate what this condition signifies and when it does and does not apply, and so, paradoxically, has been of greatest value in situations involving non-coherence.

For coherence to be relied upon as a key concept, proof is needed that an arbitrary initial perturbation will indeed evolve into a coherent pattern. For the idealized case of so-called equilibrium theory —that is, for local equilibrium between

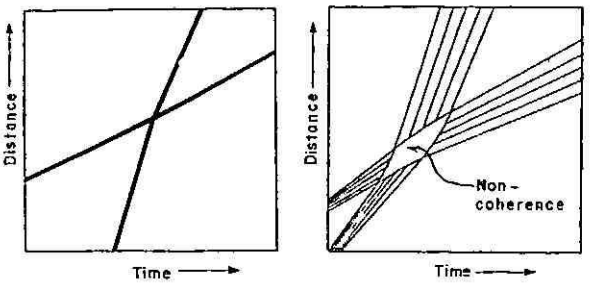


Fig. 7. Types of wave interfererence: interference of two shocks (left), with single point of non-coherence (at cross-over); of two diffuse waves (right), with finite distance–time region of non-coherence (schematic).

stationary and mobile phases, ideal plug flow, and absence of dispersion effects such as diffusion in axial direction— a rigorous proof based on the method of characteristics has been given[19]. A brief outline of this proof is attached in the Appendix. In the language of the method of characteristics (see, for example, refs. 20 and 21) the coherence principle can be formulated as follows:

An arbitrary starting variation, if embedded between sufficiently large regions of constant state, is resolved into simple (that is, coherent) waves between which new regions of different constant states arise.

It is indeed surprising that this relatively straightforward corollary of the method of characteristics seems not to have been explicitly formulated much earlier. The reason may be that the mathematician regards the statement as self-evident, but has in the past not realized its great predictive power.

It is interesting to note that the proof, based on the method of characteristics, is restricted to hyperbolic systems, as is that method, whereas the coherence principle appears to be more generally valid. Thus, if dispersion effects (axial diffusion, deviation from local phase equilibrium because of finite sorption-desorption rate, etc.) are included and contribute second-order terms to the material balance and wave equations, the method of characteristics is no longer applicable, but coherence is nevertheless approached asymptotically as the second-order terms fade out or dispersive and self-sharpening effects come into balance[22]. It should, therefore, be possible to find a more fundamental basis of the coherence principle, perhaps in statistics or statistical thermodynamics. No serious attempts in this direction have so far been made. Here is a challenge which, I hope, will eventually be picked up by experts in those fields.

6. LANGMUIR-TYPE SYSTEMS, VACANCY GAS CHROMATOGRAPHY AND *h* TRANSFORMATION

The key to easy prediction of multicomponent column performance under almost any operating conditions is a knowledge of the composition path grid —that is, of the mapping of composition variations compatible with the coherence condition. Such grids can be constructed mathematically or graphically by solution of the eigenvalue problem that results from equating the wave velocities of the components. However, this procedure is cumbersome and time-consuming if the equilibrium equations are complex. The art in working with the technique of composition path grids is to find short cuts how to construct at least approximate grids by calculating prominent features (such as loci of singularities, envelopes, etc.) and then filling in the rest of the grid by interpolation. In some instances of practical importance, however, even this can be dispensed with and simple algebraic relations can be obtained. Cases in point are sorption with multicomponent Langmuir isotherms and the mathematically equivalent situation of (stoichiometric) ion exchange with constant separation factors.

The short cut applicable in these cases is provided by the so-called *h* transformation (see, for example, ref. 23). For ion-exchange chromatography as an example, the fluid-phase concentration variables (counterion equivalent fractions x_i) are transformed into new variables h_i obtained as the roots of the polynomial[23]

$$\sum_{i=i}^{n} x_i/(1 - \alpha_{1i}) = 0 \tag{3}$$

For Langmuir sorption systems a slightly different transformation[13,14] achieving the same purpose is algebraically more convenient. Velocities of coherent waves and compositions of zones between the waves can then be calculated from simple algebraic expressions involving the separation factors α_{ij} or coefficients of the Langmuir isotherm equations and the roots h_i of the initial and feed compositions. Even where non-coherence calls for numerical integration, the transformation offers substantial savings in effort in that the partial differential equations assume a much simpler form if written in terms of the h_i. Specifically, the velocity $(\partial z/\partial t)_{h_k}$ of a given root value h_k is a point property, that is, uniquely given by the local composition $h_1, h_2, \ldots, h_n$ rather than being dependent on gradients, as is the concentration velocity $(\partial z/\partial t)_{C_i}$.

In terms of the composition path grid —which now is superfluous— the transformation amounts to an orthogonalization[17,23]: in the coordinate space of the transformed variables h_i, each set of paths is parallel to one axis and normal to all others. As a corollary, only one h_i varies across a coherent wave, all others remain constant.

In chromatography, the use of the h transformation (not under that name) was first suggested by Davidson[24] in 1949 for ion exchange in a discussion contribution that went largely unnoticed. The transformation has also been applied independently by Zhukhovitskii and co-workers[25,26] to a physically rather different situation in chromatography. These authors considered interference between components in gas chromatography at high concentrations through occupancy of space in the gas phase, while assuming constant partition coefficients. The most notable of his countless contributions in this context propose "vacancy chromatography"[25], where a pulse of inert gas is injected into a continuous gas stream of sorbable components, and "chromatography without carrier gas"[26], both with considerable interest for process control. Interestingly, even though composition-independent partition coefficients are assumed, the interaction of the components in the gas phase —loosely speaking, by taking away room from one another— again leads to mathematics for which Zhukhovitskii showed the h transformation to be applicable.

The h transformation, indeed, seems to be broadly applicable to systems with what one might call indiscriminate interference, that is, if species compete at constant relative strengths in sharing some function. This function may be occupancy of sorption sites in chromatography, occupancy of vapor space in vacancy chromatography or distillation, transport of electric current in electrophoresis, etc., and "indiscriminate" competition would be reflected in constant binary interaction coefficients: separation factors in chromatography, relative volatilities in distillation, relative electrochemical mobilities in electrophoresis.

The polynomial of eqn. 3 appears in differential geometry in the description of homofocal surfaces[27], and Binet is credited with having derived, in 1811, a theorem essentially equivalent to a reverse h transformation (see ref. 28). Over the years and in addition to the work previously quoted here, the transformation has been reinvented for multicomponent electrodiffusion by Pleijel[29] and Schlögl[30], multicomponent electrophoresis by Dole[31], and multicomponent distillation by Underwood[32] in his well-known stripping factor method, each author apparently being unaware of

previous uses. The broad applicability of the transformation to systems with several interacting dependent variables certainly deserves more attention, and it would be interestinging to determine the exact prerequisites for its use.

7. SELECTED RECENT APPLICATIONS

The most intriguing theory is of no more than academic interest unless it leads to progress in practical applications. Therefore, some such applications are mentioned here in passing. The selection is small, quite subjective, and confined to work inspired by or drawing heavily on theory; in particular, the selection also is unlikely to do justice to the many recent developments in this symposium's host country with which I am not sufficiently familiar.

Perhaps the most promising use that multicomponent chromatographic theory recently has found is in ongoing work on HPLC separations by displacement development[14] under Hováth, originator of HPLC (high-performance liquid chromatography). Molecules too large for efficient separation on conventional chromatographic sorbents can be analytically separated by HPLC. The combination of HPLC with displacement development extends the scope to preparative separations. For effective design, this work is drawing on multicomponent theory[12,13] and, incidentally, has provided experimental confirmation for a number of theoretical predictions.

Another interesting development illustrating how multicomponent theory, suitably extended to more complex systems, can help to solve a difficult practical problem is the work by Wang and Huang[33] on removal of ammonium carbonate from physiological dialysis fluids. Here, it is essential that the pH and the concentrations of K^+, Ca^{2+}, and Mg^{2+} be maintained so that delicate subsequent readjustment becomes unnecessary. Aided by multicomponent theory, Wang and Huang achieved this with a mixed bed containing a zeolite and a weak-acid cation-exchange resin.

Other recent theory-inspired studies aim at improving the efficiency of preparative chromatographic operations by a program of injections alternatingly from one and the other end of the column (Bailly's and Tondeur's "two-way chromatography"[34]), by using temperature- or pressure-swing techniques (Camero and Sweed[35], Tondeur and Grevillot[36], and Wankat and Tondeur[37]), or by operating the column with moving feed ports (Wankat[38]).

An example of a fairly straightforward application of multicomponent theory (with h transformation) to an important practical problem in anion exchange is provided by the work by Clifford[39] on removal of nitrate from drinking water supplies also containing chloride, sulfate, and carbonate.

Work involving extensions of multicomponent theory has been carried out on systems with precipitation and dissolution of precipitates, namely, by Klein[40] with regard to scale formation in water treatment and by Walsh *et al.*[41] in connection with problems or uranium leach mining. Other work by Klein *et al.*[42] is concerned with carbonate regeneration in water treatment systems and incorporates ionic dissociation equilibria into multicomponent theory. In a similar application, work in our laboratory[43] predicted and experimentally confirmed pH excursions induced by association–dissociation reactions of weak-acid anions of buffers in multicomponent anion-exchange columns, demonstrating that in ion-exchange chromatography the pH is not necessarily what the analyst may think it to be.

Ongoing work by Schweich *et al.*[44] entails extensions of multicomponent theory in a different direction by inclusion of catalytic reactions taking place on the chromatographic support.

In our laboratory, work on an extension of multicomponent theory to the general problem of propagation of perturbations in continuous counter-current mass-transfer operations such as fractionation, liquid–liquid extraction, gas absorption, and continuous counter-current ion exchange has been started by Hwang[45].

Still farther afield but addressing a clientele larger than the community of chromatographers are applications and extensions of multicomponent chromatographic theory to problems of enhanced oil recovery. These range from straightforward application to the cation balance in reservoir waters ion exchanging with clays in water or surfactant floods[46] to chromatographic behavior of surfactant mixtures[47,48] used in the latter and to an extension of multicomponent chromatographic theory to the presence of several fluid phases with arbitrary equilibrium partitioning of components and arbitrary flow properties[49,50], as may occur in surfactant, carbon dioxide, caustic, steam and other floods.

This brief catalogue, though far from comprehensive and biased by my own research interests, may serve to illustrate that multicomponent chromatographic theory is progressing, is finding many uses, and is stimulating advance in other fields. Undoubtedly, this symposium will add many other entries that would have deserved to be included.

8. SYMBOLS

C_i concentration of species i in fluid phase (mol m^{-3}, column*)
$\bar{C}_i$ concentration of species i in stationary phase (mol m^{-3} column*)
h variable in H function (eqn. 3)
h_i ith root of H function (eqn. 3) (transformed concentration variable)
K_i $= \bar{C}_i/C_i$ partition coefficient of species i
t time (s)
v_i velocity of molecules of species i
v_{C_i} velocity of concentration C_i
v^0 velocity of bulk fluid phase
x independent variable for coherence proof (Appendix)
x_i $= z_iC_i/\Sigma z_iC_i$ equivalent counterion fraction (z_i = electrochemical valence)
y independent variable for coherence proof (Appendix)
z distance from column inlet (m)
α_{ij} $= \bar{C}_iC_j/\bar{C}_jC_i$ separation factor of species i and j

9. APPENDIX: COHERENCE PROOF[19]

A proof of attainment of coherence from arbitrary starting conditions can be briefly summarized as follows. The proof uses the method of characteristics[20,21] and is phrased in the language of that method.

Stock-in-trade of the method of characteristics is that[20,21]:

* Note concentrations are per unit volume of column, not of respective phase.

(a) In the plane of physical variables (distance and time in chromatography), regions traversed by characteristics originating exclusively from a region or regions of constant state are themselves regions of constant state.

(b) Regions traversed by characteristics of which one set originates from a starting variation, the other or others from a region or regions of constant state, are simple (*i.e.*, coherent) waves.

(c) Regions traversed by characteristics of which more than one set originates from a starting variation are non-simple (i.e., non-coherent) waves.

In Fig. 8, for a system with two independent and two dependent variables, a starting condition is given along the curve AD in the physical plane, containing a variation along BC and constant states along AB and CD. The domain of dependence, BCF, of BC is a non-simple (non-coherent) wave as within it all characteristics have originated from the variation BC. Since the characteristics of the two sets have different slopes, all those originating from BC will cross one another inside BCF. The range of influence, FIH and beyond, of F thus is a region of constant state, with characteristics originating exclusively from the constant-rate starting regions AB and CD. Also, the regions BFHE and CGIF are simple (coherent) waves as each is traversed by one set of characteristics originating from the starting variation BC and another set originating from the constant-state starting region AB or CD.

This argument shows that the non-coherent starting variation BC is resolved into two coherent waves (BFHE and CGIF), between which a new region of constant state (FIH) arises.

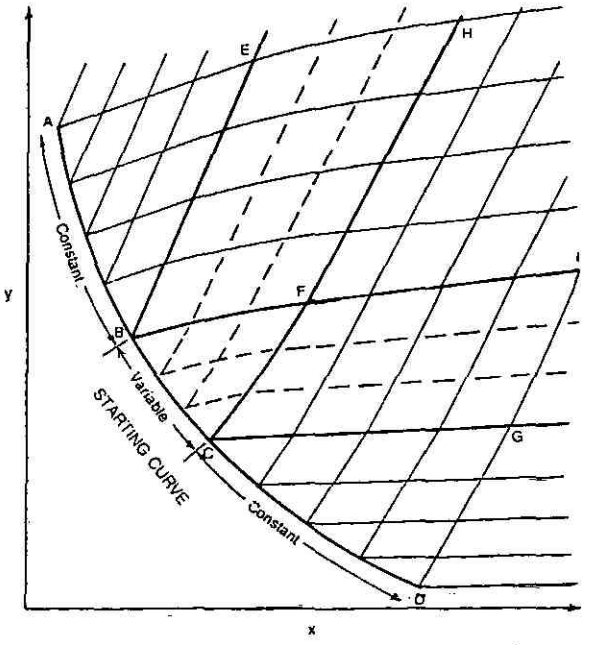


Fig. 8. Physical plane with characteristics emanating from starting curve with regions of constant state AB and CD and variation BC, for system with two independent variables (x and y) and two dependent variables (two sets of characteristics). Characteristics originating from starting variation are shown as broken lines; those from regions of constant state as solid lines.

The extension of the proof to more than two dependent variables is straightforward. The extension to more than two independent variables is much more difficult and has so far not been worked out in detail.

10. SUMMARY

Chromatography at high concentration and with large sample size, as desired for preparative separations, involves non-linear isotherms and interference of sorbable components with one another. Theoretical developments of the last fifteen years and their implications for separation methods are reviewed in the light of the concepts of wave propagation and coherence.

REFERENCES

1 M. Tswett, *Ber. Dtsch. Botan. Ges.*, 24 (1906) 316, 384.
2 A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358.
3 A. Tiselius, *Ark. Kemi Mineral. Geol., A*, 16 (1943) No. 18.
4 F. H. Spedding and J. E. Powell, in F. C. Nachod and J. Schubert (Editors), *Ion Exchange Technology*, Academic Press, New York, 1956, Chap. 15.
5 I. Langmuir, *J. Am. Chem. Soc.*, 38 (1916) 2221; 40 (1918) 1361.
6 D. DeVault, *J. Am. Chem. Soc.*, 65 (1943) 532.
7 E. Glueckauf, *Proc. Roy. Soc. (London), Ser. A*, 186 (1946) 35.
8 E. Glueckauf, *Trans. Faraday Soc.*, 7 (1949) 12.
9 E. Glueckauf, *Ion Exchange and its Applications*, Society of Chemical Industry, London, 1955, p. 49.
10 S. Claesson, *Ark. Kemi Mineral. Geol., A*, 23 (1946), No. 1.
11 F. Helfferich and D. B. James, *J. Chromatogr.*, 46 (1970) 1.
12 F. Helfferich and G. Klein, *Multicomponent Chromatography*, Marcel Dekker, New York, 1970, Sec. 4.IV.B; reprinted by University Microfilms International, Ann Arbor, MI.
13 H.-K. Rhee and N. R. Amundson, *AIChE J.*, 28 (1982) 423.
14 J. Frenz and C. Horváth, *AIChE J.*, 31 (1985) 400.
15 F. Helfferich and G. Klein, *Multicomponent Chromatography*, Marcel Dekker, New York, 1970, p. 41, eqn. 3–13; reprinted by University Microfilms International, Ann Arbor, MI.
16 G. G. Baylé and A. Klinkenberg, *Rec. Trav. Chim.*, 73 (1954) 1037.
17 F. G. Helfferich, *AIChE Symp. Ser.*, 80 No. 233 (1984) 1.
18 F. Helfferich and G. Klein, *Multicomponent Chromatography*, Marcel Dekker, New York, 1970, Sec. 3IV.A; reprinted by University Microfilms International, Ann Arbor, MI.
19 F. G. Helfferich, presented at *AIChE Diamond Jubilee Meeting, Washington, DC, November 1983; Chem. Eng. Commun.*, in preparation.
20 A. Jeffrey, *Quasilinear Hyperbolic Systems and Waves*, Pittman, London, 1976.
21 R. Aris and N. R. Amundson, *Mathematical Methods in Chemical Engineering*, Vol. II Prentice-Hall, Englewood Cliffs, NJ, 1973, Ch. 8.
22 F. Helfferich and G. Klein, *Multicomponent Chromatography*, Marcel Dekker, New York, 1970, Ch. 5; reprinted by University Microfilms International, Ann Arbor, MI.
23 F. Helfferich and G. Klein, *Multicomponent Chromatography*, Marcel Dekker, New York, 1970, Sec. 3.IV.D and Appendix 1; reprinted by University Microfilms International, Ann, Arbor, MI.
24 B. Davidson, *Trans Faraday Soc.*, 7 (1949) 45.25.
25 A. A. Zhukhovitskii and N. M. Turkeltaub, *Dokl. Acad. Nauk SSSR*, 143 (1962) 646.
26 A. A. Zhukhovitskii, M. L. Sazonov, A. F. Shlyakhov and A. I. Karymova, *Zavod. Lab.*, 31 (1965) 1048.
27 L. P. Eisenhart, *A Treatise on Differential Geometry of Curves and Surfaces*, Constable, London, 1909, Ch. VII; reprinted by Dover, New York, 1960.
28 T. Muir, *The Theory of Determinants in the Historical Order of Development*, Vol. 2, Macmillan, London, 1911, p. 156.
29 H. Pleijel, *Z. Phys. Chem.*, 72 (1910) 1.
30 R. Schlögl, *Z. Phys. Chem. (Frankfurt)*, 1 (1954) 305.

31 V. P. Dole, *J. Am. Chem. Soc.*, 67 (1945) 1119.
32 A. J. V. Underwood, *J. Inst. Petrol.*, 32 (1946) 614; *Chem. Eng. Progr.*, 44 (1948) 603.
33 N.-H. L. Wang and S. Huang, *Sep. Purif. Methods*, 13 (1984) 43; *AIChE Symp. Ser.*, 79, No. 230 (1984) 26.
34 M. Bailly and D. Tondeur, *Chem. Eng. Sci.*, 36 (1981) 455.
35 A. A. Camero and N. H. Sweed, *AIChE J.*, 22 (1976) 369.
36 D. Tondeur and G. Grevillot, in A. Rodrigues (Editor), *Ion Exchange, Science and Technology, Troia, Portugal, August 1985 (NATO Advanced Study Institute)*, Martinus Nijhoff, Alphen aan de Rijn, and NATO Scientific Affairs Division, in press.
37 P. C. Wankat and D. Tondeur, in A. Rodrigues (Editor), *Ion Exchange, Science and Technology, Troia, Portugal, August 1985 (NATO Advanced Study Institute)*, Martinus Nijhoff, Alphen aan de Rijn, and NATO Scientific Affairs Division, in press.
38 P. C. Wankat, *I&EC Fundam.*, 23 (1984) 256.
39 D. A. Clifford, *I&EC Fundam.*, 21 (1982) 141.
40 G. Klein, in A. Rodrigues (Editor), *Ion Exchange, Science and Technology, Troia, Portugal, August 1985 (NATO Advanced Study Institute)*, Martinus Nijhoff, Alphen aan de Rijn, and NATO Scientific Affairs Division, in press.
41 M. P. Walsh, S. L. Bryant, R. S. Schechter and L. W. Lake, *AIChE J.*, 30 (1984) 317.
42 G. Klein, J. Sinkovic and T. Vermeulen, *OWRT Report RU-82-7*, U.S. Department of the Interior, University of California, Berkeley, CA, 1982.
43 F. G. Helfferich and B. J. Bennett, *React. Polym.*, 3 (1984) 51; Solvent Extract. Ion Exchange, 2 (1984) 1151.
44 D. Schweich, J. Villermaux and M. Sardin, *AIChE J.*, 26 (1980) 477.
45 Y.-L. Hwang, *Chem. Eng. Sci.*, submitted for publication.
46 G. A. Pope, L. W. Lake and F. Helfferich, *SPE J.*, 18 (1978) 418.
47 J. H. Harwell, F. G. Helfferich and R. S. Schechter, *AIChE J.*, 28 (1982) 448.
48 J. H. Harwell, R. S. Schechter and W. H. Wade, *AIChE J.*, 31 (1985) 415.
49 F. G. Helfferich, *SPE J.*, 21 (1981) 51.
50 G. J. Hirasaki, *SPE J.*, 21 (1981) 191.

Journal of Chromatography, 373 (1986) 61–80
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHREV. 202

LIQUID CHROMATOGRAPHY ON CHEMICALLY BONDED ELECTRON DONORS AND ACCEPTORS

L. NONDEK*
Institute of Chemical Process Fundamentals, Czechoslovak Academy of Sciences, 165 02 Prague 6 (Czechoslovakia)
(Received November 19th, 1985)

CONTENTS

1. INTRODUCTION

In addition to physisorption, hydrogen bonding, solvophobic effects and other retention mechanisms, the formation of electron donor–acceptor (EDA) complexes also plays a role in liquid chromatography (LC)[1]. Complexes (substances with a defined stoichiometry and geometry) are formed by the interaction of two or more component molecules or ions. The formation is an equilibrium process and the complex formed dissociates reversibly into its components.

EDA complexes result from a weak interaction of electron donors (D) with electron acceptors (A)[2,3]:

$$A + D \overset{K_{eq}}{\rightleftarrows} AD \qquad (1)$$

The enthalpy of EDA complexation is usually of the order of a few kcal/mol and the rates of formation and dissociation of AD are very high. As the stability of EDA complexes depends not only on the structure of components but also on the polarity of the solvent, their formation has been utilized in LC for several decades[1–3].

Electron donors are defined as molecules capable of giving up an electron and their ionization potential, I_D, is a measure of the donation ability. Electron acceptors

* Present address: Water Research Institute, Analytical Department, Podbabská 30, 16000 Prague, Czechoslovakia.

0378-4355/86/$07.00

are able to accept an electron and this is related to their electron affinity, EA, or reduction potential. In some instances, the same molecule can act as an electron donor or acceptor, depending on the circumstances. In large molecules such as pharmaceuticals, biologically active compounds and synthetic dyestuffs, independent electron-accepting and -donating parts of the molecule can exist[2–5].

Small unsaturated or aromatic hydrocarbons are usually weak donors or very weak acceptors. Their donating or accepting capability increases with increase in the number of C=C double bonds or aromatic rings. Polynuclear aromatic hydrocarbons (PAHs) and azaarenes are therefore efficient donors of π-electrons. The replacement of a hydrogen atom in the parent molecule of PAHs with an electron-releasing substituent such as an alkyl, alkoxy or amino group increases the capability of molecule to donate π-electrons.

On the other hand, aromatic or unsaturated compounds containing several electron-withdrawing substituents such as NO_2, Cl or CN are efficient acceptors. Picryl chloride, tetracyanoethylene, *p*-phenylendiamine and other compounds are examples of strong π-donors or π-acceptors. The EDA complexation not only involves an interaction between π-electrons, but σ- or n-electrons and vacant or antibonding σ-orbitals can also play a role in the interaction.

The formation of EDA complexes is assumed to be the main interaction mechanism governing the chromatographic behaviour of solutes in so-called "charge-transfer" or EDA liquid chromatography (EDA-LC). The EDA complexation can take place in either the mobile phase or the stationary phase[1]; the latter alternative is possible using stationary phases with electron-accepting or-donating ability. Thus, the separation of PAHs on silica gel impregnated with 2,4,6-trinitrobenzene or another acceptor was reported several decades ago[6]. Nevertheless, the adsorbed stationary phases are easily washed out and this not only causes a decrease in retention but also interferes with UV detection often used in high-performance liquid chromatography (HPLC).

Various metal oxides can also have adsorption sites with acceptor or donor capability[7,8]. Pairs of these sites have been found on alumina surfaces in addition to Brönsted acid sites. As a consequence, this so-called "Lewis acidity" of alumina enhances the retention of PAHs or other electron donors compared with nearly neutral silica gel. However, the acid–base properties of many metal oxides, including alumina, are strongly dependent on the concentration of surface hydroxy groups or adsorbed water[9]. The chromatographic behaviour of thermally activated alumina is therefore easily changed by rehydration.

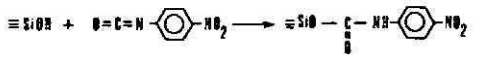
Organic electron acceptors bound to the surface of polymers have been studied in LC for over 20 years since the first such sorbent based on polystyrene gel bearing aromatic groups was reported by Ayres and Mann[10] in 1964. Hydrophilic gels such as polydextrans modified with various donors or acceptors have been prepared and tested by Porath[11]. In this instance, the separation of donors and acceptors of biological importance was achieved in aqueous mobile phases.

The advent of chemically bonded stationary phases for HPLC made it possible to prepare more selective and more efficient electron-accepting or -donating sorbents. In practice, the organic donors or acceptors are immobilized on the silica surface via a suitable silanization reaction. This review considers silica and organic polymers modified with covalently bound donors and acceptors. An attempt has been made

to review all the relevant contributions to this class of HPLC sorbents characterizing their preparation, chromatographic behaviour and practical applications.

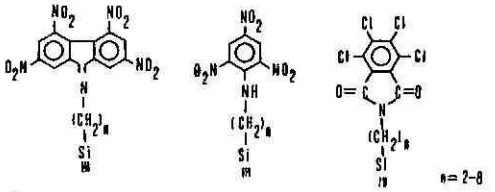
2. PREPARATION OF CHEMICALLY BONDED DONORS AND ACCEPTORS

Stationary phases for EDA-LC are mostly bonded to porous silica. The first silica modified with chemically bonded acceptor ligands was prepared via the reaction of surface silanols with *p*-nitrophenyl isocyanate by Ray and Frei[12] (Scheme 1). The resulting sorbent is unstable, being easily decomposed by moisture and light.



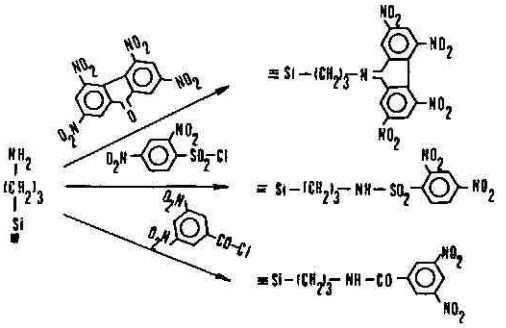
Scheme 1

Since then, several acceptor-modified silicas have been prepared and tested. Most of them are based on nitroaromatic ligands such as 2,4,5,7-tetranitrofluorenonimino, 2,4-dinitroanilino or 2,4,6-trinitroanilino groups bonded to the silica surface by means of a short aliphatic chain (see, *e.g.*, refs. 13–16) (Scheme 2). In addition to nitroaromatic ligands, tetrachlorophthalimidopropylsilica[17] has also been prepared (Scheme 2).



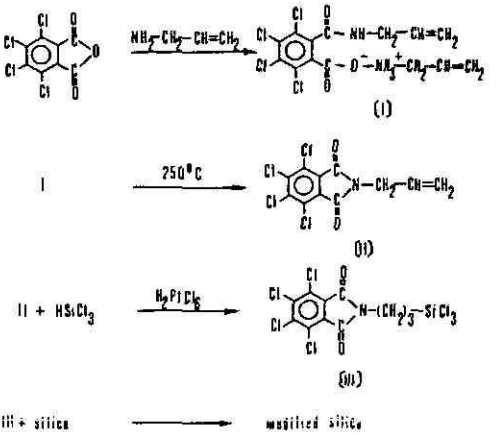
Scheme 2.

In principle there are two ways to prepare sorbents with chemically bonded electron donors or acceptors. First, silica is modified with a silane having a sufficiently reactive group that is utilized in the next step of the synthesis. Amino groups are often used for this purpose (see, *e.g.*, refs. 13–16 and 18–21) (Scheme 3). In the second method, the corresponding silanes are first prepared and then allowed to react with the silica surface, yielding the chemically bonded stationary phase[17,22–25]. The synthesis of the above-mentioned tetrachlorophthalimidopropylsilica reported by



Scheme 3.

Holstein[17] can serve as an example (Scheme 4).



Scheme 4.

With polynitroaromatic ligands, it is usually impossible to isolate the resulting silane from the reaction mixture and the crude product is therefore used in the silanization step[25].

The first method is simpler and does not require special synthetic skills, but the unreacted amino groups, being efficient n-electron donors, can interact with the bonded acceptor ligands. Hence, the surface reaction is not only to be selective but also quantitative. For example, unreacted propylamine groups have been found to form a red complex with picramidopropyl ligands. The conversion of amino groups into picramido groups can be checked by means of photoacoustic spectroscopy[26].

The low selectivity of the surface reaction, resulting in low yields of the desired ligands, is a plausible explanation for why many sorbents prepared via the surface reaction have shown poor chromatographic performance[14,19] (Fig. 1). However, the reaction of 2,4-dinitrofluorobenzene and 2,4,6-trinitrobenzenesulphonic acid with bonded primary amines can be recommended for the preparation of bonded nitroan-

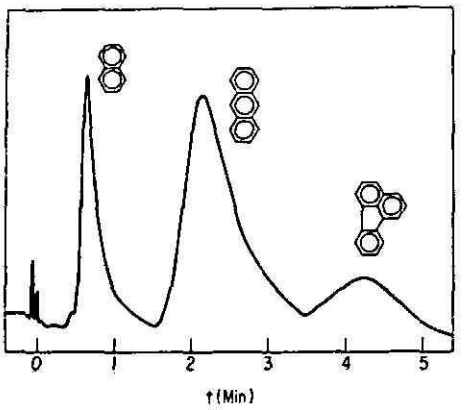
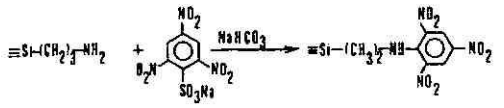


Fig. 1. Separation of naphthalene, anthracene and fluoranthene on silica modified with 3-(2,4,5,7-tetranitrofluorenimino)propyl ligands (taken from ref. 19).

ilines[27,28]. The reproducible preparation of 2,4-dinitroanilinopropylsilica has been checked by several workers[14–16,20,29,30] and the identity of the bonded ligands demonstrated by photoacoustic spectroscopy[31].

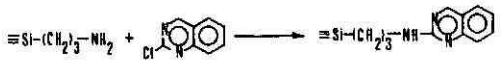
Both of the above reactions are carried out under mild conditions in aqueous media owing to the hydrolytic stability of 2,4-dinitrofluorobenzene and 2,4,6-trinitrobenzenesulphonic acid[27,28]. It has been noted that the free amino groups on γ-aminopropylsilica are largely adsorbed on silanol groups in non-polar solvents[32]. On the other hand, the use of polar aqueous media ensures better accessibility of amino groups for the surface reaction owing to their solvation. The quantitative conversion of amino groups into bonded picramide illustrates the advantageous use of polar reaction media[33] (Scheme 5).



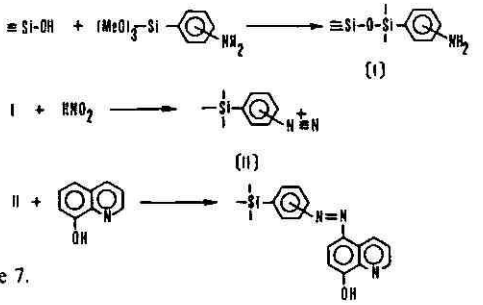
Scheme 5.

If trinitrochlorobenzene is used under the above reaction conditions, free picric acid, which forms stable picrates with bonded amines, is formed. For this reason, an excess of picryl chloride was used by Eppert and Schinke[34]. The poor reproducibility of picramide-modified silica prepared from picryl chloride has been also reported by Nondek and Ponec[15].

Chemically bonded PAHs, azaaromatics and alkoxybenzenes are expected to be efficient electron donors. Phenoxy[23] or pyrene[24] ligands have been bonded to the silica surface via corresponding silanes. The reactivity of aminosilica has been utilized by Lochmüller *et al.*[31] for the preparation of a 2-quinazoline stationary phase (Scheme 6). Marshall and Mottola[35] described the preparation of silica with covalently bonded 8-quinolinol. A diazo coupling procedure was used in the last step of the synthesis (Scheme 7).



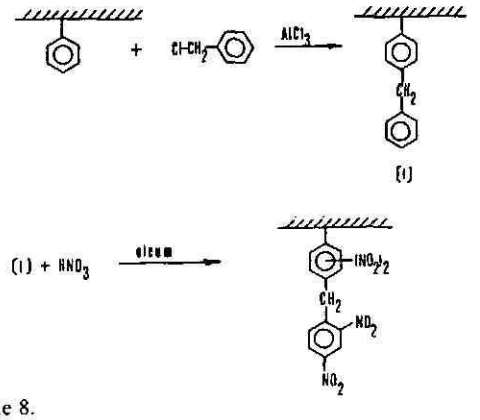
Scheme 6.



Scheme 7.

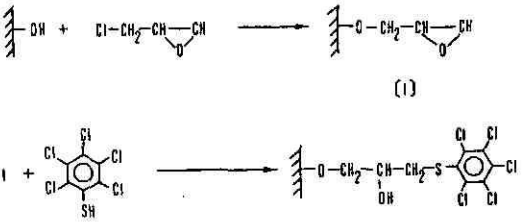
Organic gels modified with donors or acceptors are the second group of sorbents for EDA-LC. Divinylbenzene–styrene copolymer was modified by a conventional Friedel–Crafts benzylation with benzyl chloride (Scheme 8). The reaction prod-

uct was nitrated with a mixture of oleum and fuming nitric acid. The resulting polynitrobenzylpolystyrene resin permitted the complete separation of anthracene and pyrene despite the very low efficiency of the column used. In the early work of Ayres and Mann[10], charge-transfer spectral bands of complexes formed by the resin and PAHs were observed in the region of 350–500 nm. Polymers modified by electron acceptors were also reported by Smets *et al.*[36].



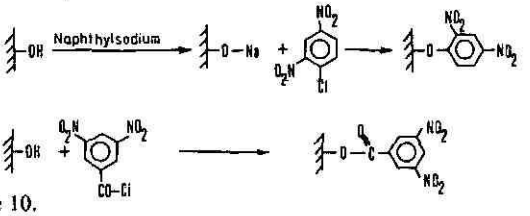
Scheme 8.

Porath and co-workers[11,37,38] modified Sephadex G-25 polydextran gel with several organic electron donors and acceptors. In the first step, the gel was treated with 1-chloro-2,3-epoxypropane and the reactive epoxide groups bonded to the polymer surface permitted the immobilization of various ligands, *e.g.*, the pentachlorothiophenyl group[11] (Scheme 9).



Scheme 9.

Šmidl and Pecka[39,40] prepared methacrylate copolymers modified with 2,4-dinitrophenoxy and 3,5-dinitrobenzoyl ligands. They utilized the reactivity of hydroxy groups present on the surface of the methacrylate gel used. The copolymer was arylated with dinitrochlorobenzene or esterified with dinitrobenzoyl chloride (Scheme 10).



Scheme 10.

Natural polymers have also been chemically modified with electron donors and acceptors. Glucose esterified with 3-(pentamethylphenyl)propionic or 3-(9-phenanthryl)propionic acid has been used for the thin-layer chromatography (TLC) of nitrotoluenes and quinones[41]. Riboflavin has been covalently bonded to cellulose for the separation of biologically active electron donors[42].

3. RETENTION MECHANISM IN EDA-LC

In this type of LC, the chromatographic retention depends on the stability of EDA complexes formed between the stationary phase and the solute. The formation of these complexes, which is assumed to be the governing retention mechanism, is influenced by factors identical with those in a homogeneous phase.

The ideas about the formation of EDA complexes in the gas phase or dilute solutions are based on Mulliken's theory of charge-transfer complexes[43]. In this theory, an electron donor (D) and acceptor (A) usually form a weak complex stabilized by charge transfer. Two electronic states are assumed: non-bonding (DA) and dative (D^+A^-). The resulting wave function is

$$\psi_N(DA) = a\psi_0(DA) + b\psi_1(D^+A^-) \tag{2}$$

The formation of the dative state contributes to the stabilization of the ground state ($a \gg b$). Hence, the main factor influencing the stability of an EDA complex is the electronic structure of D and A.

However, the main difference between the complexation in homogeneous dilute solutions and on a sorbent surface, which may play a decisive role in EDA-LC, is given by two additional factors: (1) a large surface concentration of bonded ligands permits the formation of non-stechiometric complexes; and (2) the limited motion of immobilized ligands causes steric hindrance of EDA complexation. For these reasons, the equilibrium constants, K_{eq}, measured in dilute solutions may not correlate well with log k'.

As stated above, the stability of EDA complexes is influenced by the structure of both participants, the solute and the bonded ligands. To separate the contribution of other experimental factors such as temperature and solvent effects or varying concentration of "active" ligands, the structure–retention relationships will be discussed in terms of chromatographic selectivity. This permits the comparison of different experiments, the evaluation of various sorbents and a better discussion of the retention mechanism.

3.1. Chromatographic selectivity in EDA-LC

The chromatographic selectivity, $\alpha_{i,1}$ is defined as the ratio of capacity factors, k'_i/k'_1, for a given pair of solutes. As the individual capacity factors are proportional to an equilibrium constant K_{eq}:

$$\log k' \approx \log K_{eq} = -\Delta G^\circ/RT \tag{3}$$

$\log \alpha_{i,1}$ can be approximated as

$$\log \alpha_{i,1} \doteq (\Delta H^\circ)_i - (\Delta H^\circ)_1 \tag{4}$$

$$(i = 1, 2, 3, \ldots, n)$$

in a series of n structurally related solutes (ΔS° = constant). In EDA-LC, it is possible to substitute the enthalpic terms in eqn. 4 with an interaction energy, ΔE, which is a measure of the stability of the complex.

Nondek and Ponec[15] attempted to predict log $\alpha_{i,1}$ using a simple quantum-chemical model, in which only π-electrons are transferred between frontier orbitals of A and D. Thus, ΔE may be approximated by the equation of Klopman and Salem[44–46]:

$$-\Delta E = \frac{2\Sigma c^2_{\mathrm{HOMO}}\, c^2_{\mathrm{LUMO}}\, \beta^2}{E_{\mathrm{HOMO}} - E_{\mathrm{LUMO}}} \tag{5}$$

where c_{HOMO} and c_{LUMO} are frontier orbital coefficients, E_{HOMO} and E_{LUMO} are energies of these orbitals and β is the corresponding resonance integral. Assuming that the numerator in eqn. 5 is a constant in a series of structurally related solutes, the following relationship between the energies of frontier orbitals and the chromatographic selectivity or relative stability of EDA complexes has been found[15]:

$$\log \alpha_{i,1} = \text{Constant} \cdot \frac{(\Delta E_{\mathrm{HOMO}})_i}{(E_{\mathrm{HOMO}} - E_{\mathrm{LUMO}})_1}$$
$$(\Delta E_{\mathrm{HOMO}})_i = (E_{\mathrm{HOMO}})_i - (E_{\mathrm{HOMO}})_1 \tag{6}$$

Nondek and Ponec[15] tried to verify this relationship by studying the retention of several PAHs on four electron acceptor-modified silicas. They observed a qualitative agreement despite the fact that the selectivity is influenced by many experimental factors such as the surface concentration of the acceptor ligands[47].

Despite its crudeness, the above model can serve as a rational basis for the synthesis of more selective electron acceptor-modified sorbents bearing aromatic ligands[15]. The selectivity depends not only on the number of electron-accepting substituents (NO_2, Cl, CN, etc.) attached to the aromatic skeleton, but also on the nature of the spacer connecting the skeleton with the silica surface. The dependence of $\alpha_{i,1}$ on the structure of bonded ligands is also shown in the separation of methylcholanthrenes on nitrofluorenone ligands studied by Lochmüller *et al.*[13]. The selectivity increases with increase in the number of nitro groups attached to the fluorenonimino-propyl ligands (Fig. 2).

The experimental results of Šmídl[39] are also in qualitative agreement with the quantum-chemical model, as 3,5-dinitrobenzoyl ligands attached to a gel matrix show greater selectivity than 2,4-dinitrophenoxy ligands. In this instance, the selectivity $\alpha_{i,1}$ does not depend on the preparation procedure or the surface concentration of EA ligands and clearly reflects the influence of substituent position and the nature of the spacer (Table 1).

As mentioned above, the quantum-chemical model is of only limited value for numerical calculations of $\alpha_{i,1}$. However, it shows that log $\alpha_{i,1}$ is a combination of two independent factors: the structural difference of the solutes and the ability of bonded ligands to form EDA complexes. Thus, the selectivity can be expressed as[47]

$$\log \alpha_{i,1} = \kappa\delta_i \tag{7}$$

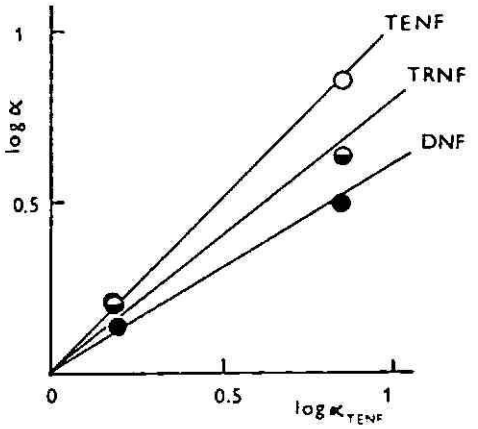


Fig. 2. Replotted selectivities of 2,4,5,7-tetranitro- (TENF), 2,4,7-trinitro-(TRNF) and 2,6-dinitrofluorenimino (DNF) ligands in the separation of 1-, 2- and 3-methylcholanthene. Data taken from ref. 13; TENF-silica used as a reference sorbent (taken from ref. 15).

This linear free energy relationship (LFER) is more general than eqn. 6 because it can be used for the quantification of several independent interaction mechanisms.

As PAHs are frequently used in testing acceptor-modified silicas, the set of δ constants has been calculated (Table 2). They can serve for the calculation of the κ parameter characterizing the stationary phase. In Table 3, various acceptor-modified silicas are surveyed; it is evident that the most efficient sorbent is 2,4,7,9-tetranitrofluorenoneoximesilica prepared by Hemetsberger *et al.*[22] and the weakest is Nucleosil-NO_2, which is assumed to be *p*-nitropropylbenzenesilica[21,48].

Nevertheless, it is evident from Table 3 that the chromatographic selectivity of the sorbent expressed by κ is influenced by the density of bonded acceptor ligands, the temperature and the polarity of the mobile phase. Sorbents with low coverages

TABLE I

CHROMATOGRAPHIC SELECTIVITY IN EDA-LC OF PAHs OVER MODIFIED HYDROXYETHYLMETHACRYLATE GELS*

PAH	*Chromatographic selectivity, $\alpha_{i,1}$*			
	*Acylation (%)***		*Arylation (%)****	
	18	*63*	*20*	*60*
Naphthalene§	0.00	0.00	0.00	0.00
Anthracene	0.52	0.61	0.49	0.45
Phenanthrene	0.59	0.67	0.47	0.51
Fluoranthene	0.87	1.01	0.72	0.64
Chrysene	1.11	1.18	0.91	0.92

* Recalculated from ref. 39; *n*-hexane as mobile phase.
** Surface OH groups acylated with 3,5-dinitrobenzoyl chloride (% conversion).
*** Surface OH groups arylated with 2,4-dinitrochlorobenzene (% conversion).
§ Taken as a reference solute.

TABLE 2

RETENTION CONSTANTS (δ) OF PAHs*

PAH	δ	*PAH*	δ
Naphthalene**	0.00	Chrysene	1.32
Phenanthrene	0.66	Benzo[*a*]pyrene	1.69
Anthracene	0.64	Perylene	1.79
Fluoranthene	1.01	Picene	1.95
Pyrene	1.04		

* Taken from ref. 47.
** Taken as a reference solute.

show poor chromatographic performance, probably owing to non-homogeneity of the surface layer. This non-homogeneity is increased by unreacted amino groups, provided that aminosilica is used as a starting material.

Thomson and Reynolds[16] prepared four chemically bonded nitroaniline stationary phases: 2,4-dinitroanilinopropyl-, 2,4,6-trinitroanilinopropyl-, 2,4-dinitroanilinooctyl- and 2,4,6-trinitroanilinooctylsilica. The results of elemental analysis showed excellent correlation between the carbon and nitrogen percentages for surface coverage of the 2,4-dinitroanilinopropylsilica prepared according to Nondek and Málek[20] in aqueous solution. The other three sorbents show higher carbon contents, which indicates incomplete conversion of bonded amine to nitroaniline ligands, probably owing to unsuitable experimental conditions being used. The trinitroanilinopro-

TABLE 3

COMPARISON OF VARIOUS ACCEPTORS BOUND ON SILICA IN EDA-LC OF PAHs

Acceptor ligand A	κ	*[A] (μmol/m²)*	*Mobile phase composition*	$r_{correl.}$	$n_{exp.}$*	*Ref.*
2,4-Dinitroaniline	1.03	2.5	*n*-Hexane (25°C)	0.9953	12	14
	0.94	2.5	*n*-Hexane (40°C)	0.9969	12	14
	0.54	0.7	*n*-Hexane	0.9585	9	47
2,4,6-Trinitroaniline	0.75	–	Cyclohexane–10% EtOAc	0.9928	5	25
3,5-Dinitrobenzamide	0.71	–	*n*-Hexane–5% CH_2Cl_2	0.9907	7	21
	1.03	–	*n*-Hexane–5% CH_2Cl_2	0.9876	5	75
2,4,6-Trinitrophenyl propyl ether	0.88	2.5	*n*-Hexane–25% CH_2Cl_2	0.9975	5	75
2,4,7,9-Tetranitro-fluorenoneoxime	1.75	3.0	*n*-Hexane–20% CH_2Cl_2	0.9741	6	22
	1.44	3.0	*n*-Hexane–40% CH_2Cl_2	0.9573	7	22
	1.15	3.0	CH_2Cl_2	0.9760	7	22
Tetrachlorophthalimide	0.82	3.3	*n*-Hexane–20% CH_2Cl_2	0.9813	11	17
Pentafluorobenzamide	0.52	4.1	*n*-Hexane	0.9482	7	64
Caffeine	0.98	2.5	*n*-Hexane–25% CH_2Cl_2	0.9974	5	62
	0.88	2.3	*n*-Hexane–25% CH_2Cl_2	0.9958	5	63
Nucleosil-NO_2	0.57	–	*n*-Hexane–10% $CHCl_3$	0.9924	10	48
	0.45	–	Isooctane 10% CH_2Cl_2	0.9937	5	60
	0.68	–	*n*-Hexane–5% CH_2Cl_2	0.9990	5	75

* Number of experimental points used in correlation to give $r_{correl.}$.

pyl- and octyl-substituted sorbents show lower selectivity than 2,4-dinitroanilinopropylsilica[16].

Šmídl[39] studied the retention of several PAHs on Separon H-1000 arylated or acylated with dinitrochlorobenzene or dinitrobenzoyl chloride. Recalculating the reported k' values to selectivities relative to naphthalene, one can see the independence of selectivity from the surface concentration of acceptor ligands (Table 1). As the surface concentration of bonded ligands is unknown in the above gels, it is impossible to compare Šmídl's results with the data given in Table 3.

3.2. Temperature and solvent effects in EDA-LC

Obviously the retention of PAHs on bonded acceptors decreases with increasing temperature and solvent polarity[14,20,22,29]. The same holds for the chromatographic selectivity, $\alpha_{i,1}$, as is evident from κ values given in Table 3. As the enthalpy of complexation, $-\Delta H$, decreases with increasing complex stability, the retention of stronger donors will be decreased more with increase in temperature[20,22]. Consequently, a lower selectivity is observed at higher temperatures[48].

As for the composition of mobile phase, both the retention and the selectivity decrease with increasing polarity. It is interesting that similar trends in the relative stability of EDA complexes have been observed in solutions, *e.g.*, the relative K_{eq} are decreased in a series of several donors with fluoranil or 1,4-dicyano-2,3,5,6-tetrafluorobenzene as acceptors if tetrachloromethane is replaced with the more polar chloroform[49].

It must be pointed out that the solvent effects on EDA complexation can be characterized as either non-specific or specific solvation of A or D. The latter can be described as competing equilibria[2]:

$$D + A \overset{K_{eq}}{\rightleftarrows} DA \tag{8}$$

$$D + S \overset{K_{solv}}{\rightleftarrows} DS \tag{9}$$

Assuming that [S] $\gg$ [DS] and [A°] $\gg$ [DA], which is perfectly fulfilled under LC conditions, one can derive

$$k'_n = k'_s (1 + K^D_{solv} [S]) \tag{10}$$

where

$$k'_n = K_{corr} [A^\circ] \tag{11}$$

$$k'_{solv} = K_{eq} [A^\circ] \tag{12}$$

The concentration of bound acceptor ligands is [A°], K_{eq} is the equilibrium constant given by eqns. 8 and 9 and K_{corr} is a corrected equilibrium constant derived by Drago *et al.*[50]. K_{corr} does not involve the specific solvation competing with the solute–stationary phase complexation. Eqn. 10 enables K_{solv} and k'_n to be determined. A similar equation can be derived for the specific solvation of A.

If the solvent S competes for the both A and D, an analogous equation has been derived by Bishop and Sutton[51]:

$$K_{eq} = K_{eq}^{solv} (1 + K_{solv}^{D} [S]) (1 + K_{solv}^{A} [S]) \quad (13)$$

The specific solvation effects characterized by the equilibrium constants K_A^{solv} and K_D^{solv} proceed via EDA interaction and/or hydrogen bonding. Such competition is assumed to be the major solvent effect of dioxane and ethers, which are known to be effective n-donors; chloroform and dichloromethane have been shown to hydrogen bond to aromatic π-donors[2].

Recalculating the retention data taken from previous papers[22,29], one can plot reciprocal capacity factors, $1/k'_s$, against the concentration [S] of chloroform or dichloromethane in *n*-hexane and *n*-heptane (Fig. 3). The slope of the lines is K_{solv}^{D} according to eqn. 10; for the retention of phenanthrene and naphthalene on 2,4-dinitroanilino- and 2,4,5,7-tetranitrofluorenoneoximesilica, it is evident that k'_n agrees well with the capacity factors measured in pure *n*-alkanes ([S] = 0). The estimated value of K_{solv}^{D} is 4.6 l/mol for phenanthrene–chloroform, 1.7 l/mol for naphthalene–chloroform and 0.6 l/mol for naphthalene–dichloromethane complexes.

In this way, the decrease in the κ factor with increasing concentration of the polar component S complexing with solutes can be explained. If only the bonded acceptor ligands were solvated specifically ($K_{solv}^{D} = 0$), all the experimental points obtained for different solutes should fit only one correlation line having a slope of K_{solv}^{A}. Non-linear correlations are expected, provided A and D form complexes with S (K_{solv}^{A}, $K_{solv}^{B} \neq 0$). Using a similar approach, Hemetsberger *et al.*[22] found that the bonded tetranitrofluorenoneoxime ligands form A_2S complexes with several polar solvents. They assumed that D competes with *n* molecules of S for A. This way, a "sandwich" structure of swollen ligand layers is formed. The complexing strengths of the solvents increase in the order isopropyl chloride < dichloromethane < tet-

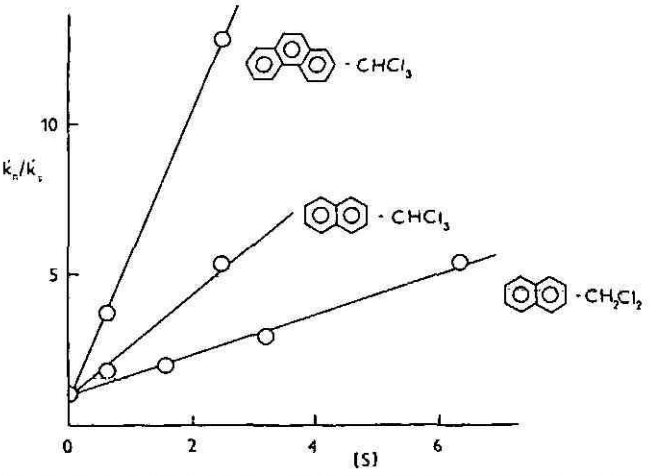


Fig. 3. Plot of reciprocal capacity factors, k'_s, of naphthalene and phenanthrene against the concentration, [S], of polar solvent ($CHCl_3$, CH_2Cl_2) in a non-polar mobile phase (*n*-hexane, *n*-heptane) according to eqn. 13. Data taken from refs. 22 and 29.

rahydrofuran < acetone < ethyl acetate. The specific solvation of bonded ligands with n-donors, *e.g.*, alcohols, esters or ethers, can be assumed. However, chlorinated hydrocarbons such as dichloromethane or chloroform are not known to interact specifically with organic electron acceptors.

The effect of mobile phase polarity on the retention of PAHs over tetranitrofluorenoneoximesilica has been thoroughly discussed by Hemetsberger *et al.*[22]. They used a model derived by Filakov and Borovikov[52] under the assumption that the stability of EDA complexes is determined by dipole–dipole electrostatic actions. Assuming that the dipole moments of D and A are unchanged by the complexation, a linear relationship (eqn. 14) has been derived[52]:

$$\log k' = a + b/\varepsilon \tag{14}$$

The plots of log k' versus $1/\varepsilon$ are not exactly linear, however, for the experimental data of Hemetsberger *et al.*[22].

As has been mentioned elsewhere[2], there is no simple correlation between K_{eq} and bulk polarity parameters of solvents such as dielectric constant, ε, although a general trend to lower K_{eq} with increasing ε is observed. For strong complexes, the reverse trend occurs, however[2]. These complexes with significantly larger dipoles gain extra stabilization from the inductive interactions with polar solvents.

In very polar solvents, the ionic states of both components are stabilized. The stabilization energy will depend on the dielectric constant of the solvent according to Born's equation[3,11]:

$$-\Delta H_{solv} \approx \left(1 - \frac{1}{\varepsilon}\right)\left(\frac{1}{R_{A^-}} + \frac{1}{R_{D^+}}\right) \tag{15}$$

where R_{A^-} and R_{D^+} are the ionic radii of ions A^- and D^+, respectively. Thus, the solvation energy calculated with eqn. 14 may amount to about 100 kcal/mol in water[3]. EDA-LC in reversed-phase systems therefore seems to be an interesting alternative to the normal-phase separations discussed above.

The first attempt in this direction was made by Hunt *et al.*[53], who used bonded phthalimide for the separation of PAHs. The retention order of these solutes is very

TABLE 4

RETENTION OF NITROBENZENES IN REVERSED-PHASE LC ON BONDED ARYL ETHER AND C-18 STATIONARY PHASES

Solute	*Capacity factor**	
	Aryl ether	*C-18*
Benzene	2.98	8.18
Nitrobenzene	3.76	6.95
1,2-Dinitrobenzene	4.40	5.54
1,3-Dinitrobenzene	4.88	4.58
1,3,5-Trinitrobenzene	6.94	3.13

* Taken from ref. 23.

TABLE 5

SELECTIVITY IN EDA REVERSED-PHASE LC OF ALKYLAROMATICS ON 2,4,5,7-TETRANITROFLUORENONEOXIME (TNF) IN COMPARISON WITH C-18 STATIONARY PHASE*

Solute	*Chromatographic selectivity,* $\alpha_{i,1}$	
	TNF	*C-18*
1-Methylnaphthalene	1.00	1.00
2-Methylnaphthalene	1.22	1.00
Acenaphthene	1.58	1.25
Acenaphthylene	2.41	1.12

* Taken from ref. 54: mobile phase, methanol–water (95:5).

close to that for the C-18 phase used for the comparison. Porath[11] also pointed out that EDA complexation might be enhanced owing to solvent effects in water-mediated EDA-LC over modified Sephadex gels. It was clearly shown by Mourey and Siggia[23] that EDA complexation can operate along with the solvatophobic effect obvious in reversed-phase LC. They studied the retention of nitrobenzenes on bonded phenoxy groups acting as an acceptor. The elution order of nitrobenzenes is completely changed by EDA complexation compared with a conventional C-18 phase (Table 4).

Hemetsberger and Ricken[54] studied systematically the chromatographic behaviour of bonded tetranitrofluorenoneoxime ligands in the reversed-phase LC of PAHs. They found that the EDA complexation acted together with the solvophobic effect. The heats of adsorption, $-\Delta H_{ads}$, are much higher for the nitro than for C-18 phase as a result of EDA complexation; the $-\Delta H_{ads}$ values vary to a greater extent with the structure of the solutes, indicating that more specific solute–ligand interactions are involved. In Table 5, the chromatographic selectivities of four structurally related solutes with the same number of carbon atoms are given. Under conventional reversed-phase LC conditions, the selectivity on a C-18 phase is lower than that on the nitro phase; *e.g.*, acenaphthylene, forming relatively stronger EDA complexes with acceptors than naphthalene or acenaphthene, possesses a considerably enhanced retention in EDA reversed-phase LC.

3.3. Surface EDA complexes

It must be pointed out that the existence of well defined EDA complexes between solutes and surface-bound ligands is deduced mainly by analogy with the complexation taking place in homogeneous solutions. Visual or spectral observations of these complexes have not been reported, the only exception being the early work of Ayres and Mann[10]. Various correlations between the retention of solutes and their stability constants for EDA complexation observed in dilute solutions, ionization potentials, I_D, electron affinities, EA, etc., have been used as indirect evidence of the existence of surface EDA complexes[15,22,29].

PAHs have mainly been used as model solutes in these studies and their retention is increased with increasing molecular size, as in other known LC systems such as reversed-phase or adsorption LC. Nondek and Minárik[47] discussed various cor-

relations between the retention of PAHs on 2,4-dinitranilino bonded ligands and various structural parameters. The correlation analysis reveals that the retention depends on molecular size more than on I_D or E_{HOMO}. The same holds for all the systems included in Table 3 as they correlate well with the δ constants.

The dependence of the retention of PAHs retention on their molecular size may be explained by the formation of surface complexes between one molecule of a PAH and several adjacent ligands[29,47,55]. This non-stoichiometric complexation could be enhanced by a non-uniform distribution of bound ligands even at very low concentrations of ligands forming "clusters" or "islands" on silica surface. Nondek and Minárik[47] observed that the retention of PAHs expressed as log $\alpha_{i,1}$ correlates better with K_{eq} at low concentrations of 2,4-dinitroanilino ligands. On the other hand, the results of Šmídl[39] obtained for organic gels bearing nitroaromatic ligands show that the selectivity is independent of the surface concentration of ligands.

Aromatics with polar substituents such as OH, NO_2 and $N(CH_3)_2$ seem to interact with bonded nitroaromatic ligands not only via simple π–π complexation. Their chromatographic behaviour therefore does not correspond with the expected retention order and the nitroaromatic ligands probably act as a strongly polar stationary phase[56].

In connection with the discussion of surface EDA complexes, the structure and properties of the ligand layer are important. Individual polar ligands interact either mutually or with the silica surface. The aggregation depends on the temperature and solvation, as shown by Hammers *et al.*[32]. The self-association of ligands occurs to some extent in the presence of weakly polar solvents. If the association equilibrium is perturbed by a temperature change, a new state of the ligand layer is established after a few hours. In non-polar solvents, adsorption of solute molecules on top of the ligand layer prevails, whereas in more polar solvents the solute molecules are assumed to penetrate into the swollen layer. The "swelling" and "shrinking" of the layer following a change in solvent composition is a relatively slow process. As has been shown by Nondek and Chvalovský[57], the sorptive properties of 2,4-dinitranilino- and 2,4-dinitrobenzenesulphonamidosilica depend on the extent of swelling.

The structure of the surface layer of 2,4-dinitranilino ligands has also been studied by Lochmüller *et al.*[31] by means of photoacoustic spectroscopy. It was found that the bonded ligands behave as a solid in non-polar solvents. This is a consequence of the inability of these solvents to overcome the attraction of adjacent ligands for each other and for the silica surface. The attraction mimics the precipitation of ligands and could yield solid-like spectra. A decreased motional freedom of bound dinitranilino ligands has been proposed by Van Miltenburg and Hammers[58] on the basis of specific heat measurements.

4. PRACTICAL APPLICATIONS

Chemically bonded electron acceptors have been used by various workers for the analysis of complex mixtures of PAHs such as crude oil, petrochemical fractions and products and coal hydrogenates. Holstein[17] demonstrated the practical utility of EDA-LC in the analysis of the above samples over tetrachlorophthalimidopropylsilica. Eppert and Schinke[34] achieved a class separation of higher boiling PAH

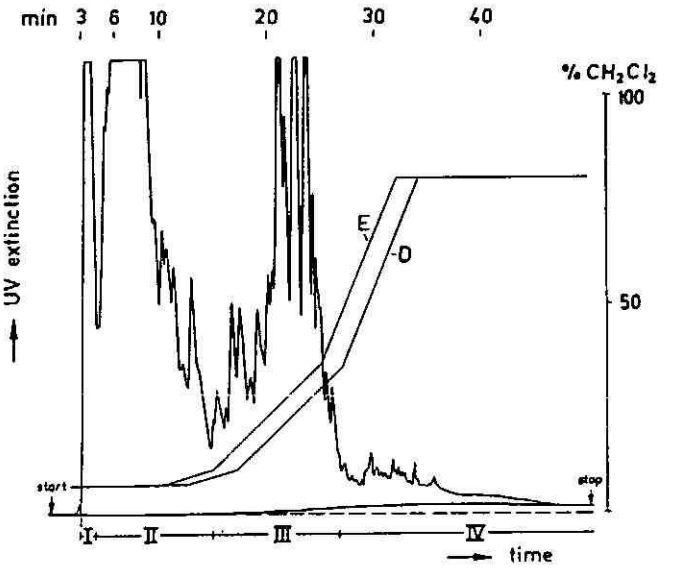


Fig. 4. Gradient separation of PAHs present in diesel fuel on chemically bonded 2,4,6-trinitroanilinopropylsilica. I–IV, Number of benzene rings; *n*-hexane–dichloromethane used as mobile phase (taken from ref. 34).

mixtures over 2,4,6-trinitroanilinopropylsilica (Fig. 4). Thomson and co-workers[16,30] used 2,4-dinitroanilino- and 2,4,6-trinitroanilinopropylsilica for the analysis of liquid fossil fuels. From their comparative study, it is evident that this type of chemically modified silica is superior to alumina and aminopropylsilica, which are often used for this purpose.

Matsunaga[59] found Nucleosil-NO_2 to have greater selectivity than -NH_2, -CN or bare Nucleosil in separations of PAHs. The same was observed earlier by Lankmayr and Müller[60]. Holstein and Severin[61] separated a recycle oil into 23 fractions by means of tetrachlorophthalimidopropylsilica. Felix and co-workers[62,63] tested chemically bonded caffeine in the separation of PAHs and petroleum asphaltenes. The performance of the caffeine-modified silica is comparable to that of nitroaromatic stationary phases (Table 3). Another novel sorbent for HPLC[64], pentafluorobenzimidopropylsilica, seems to be a weak electron-accepting phase with a similar selectivity to that of Nucleosil-NO_2 (Table 3).

All the above studies show that chemically bonded acceptors permit the group analysis of PAHs according to the number of aromatic rings as the retention of alkylaromatics is insensitive to the degree of alkylation to a great extent[16,17,30,34].

As azaarenes and other nitrogen bases are efficient donors of π- and n-electrons, the use of chemically bonded acceptors for their HPLC analysis seems to be advantageous. The first separation of azaarenes by EDA-LC was attempted by Ray and Frei[12]. Tetrachlorophthalimidopropylsilica[17] and the commercial sorbent Nucleosil-NO_2[48] have been also used for this purpose. Nondek and Chvalovský[57] measured the retention of 22 azaarenes, anilines and alkylaromatic amines on 2,4-dinitrobenzensulphonamidosilica and 2,4-dinitroanilinosilica. As PAHs with three or four rings and weak nitrogen bases such as pyrroles and anilines are less retained

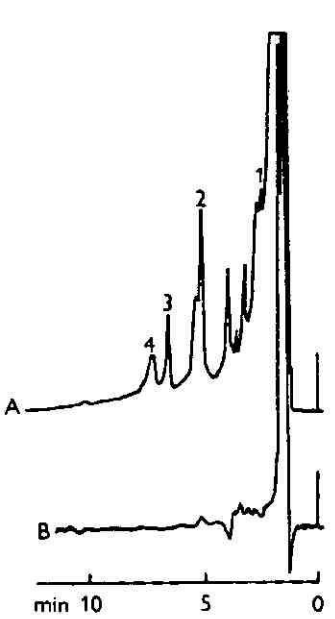


Fig. 5. On-line pre-concentration of various impurities present in kerosene: peak 1 is identical with aniline, 2 with quinoline, 3 with pyridine and 4 with isoquinoline. All the impurities are present in parts per million amounts. The retention of PAHs was checked by means of fluorimetric detection (B); the upper chromatogram was obtained with a UV detector (A) (taken from ref. 57).

than pyridines and other azaaromatics, the above sorbents have been tested for the on-line pre-concentration and LC analysis of azaarenes in gasoline, kerosene and diesel fuel (Fig. 5).

Many workers have used gradient elution for the analysis of complex mixtures of PAHs. Hammers *et al.*[32] pointed out that a layer of nitroaromatic ligands is in a

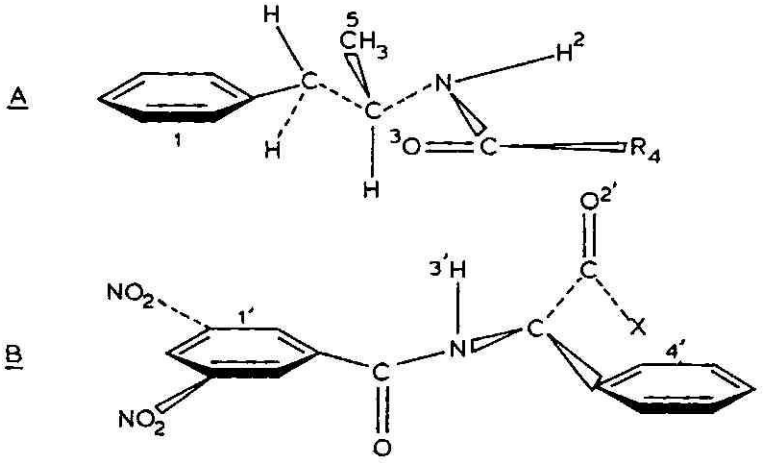


Fig. 6. Bonding interaction between solute [(*S*)-1-phenyl-2-aminopropanamide] and chiral nitroaromatic stationary phase [(*R*)-N-(3,5-dinitrobenzyl)phenylglycine] (taken from ref. 74).

swollen state depending on the polarity of the mobile phase. In dichloromethane, the layer is fully swollen and the subsequent shrinking in pure *n*-heptane is a relatively slow process. Nondek and Chvalovský[57] showed that the adsorption capacity of nitroaromatic phases depends on the extent of swelling. Hence these sorbents act under non-equilibrium conditions if a gradient regime is used.

The practical use of pyrene ligands bonded to silica has been reported by Lochmüller *et al.*[24]. The sorbent shows a good selectivity in the separation of nitroaromatics present in the particulate matter emitted by diesel engines. The formation of EDA complexes may play an important role in the separation of enantiomers on optically active nitroaromatic stationary phases[65–68]. This type of chiral stationary phase was developed by Pirkle and co-workers[69–72] using the concept of three-centre interaction between the solute and chiral ligands[73]. EDA interaction takes place simultaneously with other types of interaction mechanisms, ensuring better complexation of one enantiomer[74,75] (Fig. 6).

5 CONCLUSIONS

The rational utilization of EDA complexation in LC offers not only a better physico-chemical understanding of the separation process, but also improved chromatographic separations in many instances. Both normal-phase separations and the use of reversed-phase LC systems are possible with the broad range of chemically bonded electron acceptors and donors available. EDA complexation used in combination with other specific interactions such as hydrogen bonding leads to efficient separations of racemic mixtures.

EDA-LC has been used frequently in separations of PAH mixtures. However, other classes of organic compounds such as azaaromatics, amines and chlorinated and nitrated aromatics could be separated efficiently by means of EDA-LC. The specific sorption properties of sorbents modified with electron donors or acceptors make it possible to use on-line pre-concentration techniques in connection with EDA-LC.

Finally, the study of complexation processes taking place in solutions may be supported by the use of HPLC, as has been demonstrated here. Relative stability constants, specific solvation effects and enthalpies of complexation found by means of HPLC agree well with the results of spectroscopic measurements.

The main aim of this review has been to draw the attention of users of LC to this promising field. The potential of the application of EDA-LC, however, must be demonstrated by its ability to solve more real-life problems.

6. SUMMARY

The preparation and chromatographic properties of chemically bonded electron donors and acceptors are reviewed. The retention mechanism based upon the formation of donor–acceptor complexes is critically examined. Chromatographic selectivity expressed as a linear free energy relationship is used to compare various electron acceptors chemically bonded on silica in the separation of polynuclear aromatic hydrocarbons. The practical applications of donor–acceptor interactions in liquid chromatography are discussed.

REFERENCES

1 W. Holstein and H. Hemetsberger, *Chromatographia*, 15 (1982) 178.
2 R. Foster, *Organic Change-Transfer Complexes*, Academic Press, London, 1969.
3 K. Tamaru and M. Ichikawa, *Catalysis by Electron Donor–Acceptor Complexes*, Kodansha, Tokyo, 1975.
4 M. A. Slifkin, *Charge-Transfer Interactions of Biomolecules*, Academic Press, London, 1971.
5 H. A. H. Craenen, J. W. Verhoeven and J. de Boer, *Recl. Trav. Chim. Pays-Bas*, 91 (1972) 405.
6 M. Godlewicz, *Nature (London)*, 164 (1949) 1132.
7 K. Tanabe, *Catal. Sci. Technol.*, 2 (1981) 232.
8 H. Noller and J. M. Parera, *J. Res. Inst. Catal., Hokkaido Univ.*, 29 (1981) 95.
9 Z. Vít, J. Vala and J. Málek, *Appl. Catal.*, 7 (1983) 159.
10 J. T. Ayres and C. K. Mann, *Anal. Chem.*, 36 (1964) 2185.
11 J. Porath, *J. Chromatogr.*, 159 (1978) 13.
12 S. Ray and R. W. Frei, *J. Chromatogr.*, 71 (1972) 451.
13 C. H. Lochmüller, R. R. Ryall and C. W. Amoss, *J. Chromatogr.*, 178 (1979) 298.
14 W. E. Hammers, A. G. M. Theeuwes, W. K. Brederode and C. L. de Ligny, *J. Chromatogr.*, 243 (1982) 321.
15 L. Nondek and R. Ponec, *J. Chromatogr.*, 294 (1984) 175.
16 J. S. Thomson and J. W. Reynolds, *Anal. Chem.*, 56 (1984) 2434.
17 W. Holstein, *Chromatographia*, 14 (1981) 469.
18 M. Verzele and N. Van de Velde, *Chromatographia*, 20 (1985) 239.
19 C. H. Lochmüller and C. W. Amoss, *J. Chromatogr.*, 108 (1975) 85.
20 L. Nondek and J. Málek, *J. Chromatogr.*, 155 (1978) 187.
21 G. Felix and C. Bertrand, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 160.
22 H. Hemetsberger, H. Klaar and H. Ricken, *Chromatographia*, 13 (1980) 277.
23 T. H. Mourey and S. Siggia, *Anal. Chem.*, 51 (1979) 763.
24 C. H. Lochmüller, A. S. Colborn, L. M. Hunnicutt and J. M. Harris, *Anal. Chem.*, 55 (1983) 1344.
25 S. A. Matlin, W. J. Lough and D. G. Bryan, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 33.
26 L. Nondek, unpublished results.
27 F. Sanger, *Biochem. J.*, 39 (1945) 507.
28 A. F. S. A. Habbeb, *Anal. Biochem.*, 14 (1966) 328.
29 L. Nondek, M. Minárik and J. Málek, *J. Chromatogr.*, 178 (1979) 427.
30 P. G. Grizzle and J. S. Thomson, *Anal. Chem.*, 54 (1982) 1073.
31 C. H. Lochmüller, S. F. Marshall and D. R. Wilder, *Anal. Chem.*, 52 (1980) 19.
32 W. E. Hammers, C. H. Kos, W. K. Brederode and C. L. de Ligny, *J. Chromatogr.*, 168 (1978) 9.
33 L. Nondek, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 302.
34 G. Eppert and I. Schinke, *J. Chromatogr.*, 260 (1983) 305.
35 M. A. Marshall and H. A. Mottola, *Anal. Chem.*, 55 (1983) 2089.
36 G. Smets, V. Balogh and Y. Castrill, *J. Polym. Sci.*, 4 (1964) 1467.
37 J. Porath and B. Larsson, *J. Chromatogr.*, 155 (1978) 47.
38 J.-M. Egly and J. Porath, *J. Chromatogr.*, 168 (1979) 35.
39 P. Šmídl, *PhD Thesis*, VŠCHT, Prague, 1982.
40 P. Šmídl and K. Pecka, *Chem. Listy*, 77 (1983) 468.
41 H. Setter and I. Schroeder, *Angew. Chem., Int. Ed. Engl.*, 7 (1968) 948.
42 C. Arsenis and D. B. McCormick, *J. Biol. Chem.*, 241 (1966) 330.
43 R. S. Mulliken and W. B. Person, *Molecular Complexes, A Lecture and Reprint Volume*, Wiley, New York, 1969.
44 G. Klopman, *J. Am. Chem. Soc.*, 90 (1968) 223.
45 L. Salem, *J. Am. Chem. Soc.*, 90 (1968) 543.
46 I. Fleming, *Frontier Orbitals and Organic Chemical Reactions*, Wiley, New York, 1979.
47 L. Nondek and M. Minárik, *J. Chromatogr.*, 324 (1985) 261.
48 G. P. Blümer and M. Zander, *Fresenius Z. Anal. Chem.*, 288 (1977) 277.
49 N. M. D. Brown, R. Foster and C. A. Fyfe, *J. Chem. Soc. B*, (1967) 406.
50 R. S. Drago, T. F. Bolles and R. J. Niedzielski, *J. Am. Chem. Soc.*, 88 (1966) 2717.
51 R. J. Bishop and L. E. Sutton, *J. Chem. Soc.*, (1964) 6100.

52 Y. Y. Filakov and A. Y. Borovikov, *Zh. Obsch. Khim.*, 48 (1978) 248.
53 D. C. Hunt, P. J. Wild and N. T. Crosby, *J. Chromatogr.*, 130 (1977) 320.
54 H. Hemetsberger and H. Ricken, *Chromatographia*, 15 (1982) 236.
55 G. H. Schenk, G. L. Sullivan and P. A. Fryer, *J. Chromatogr.*, 89 (1974) 49.
56 L. Nondek and M. Minárik, *4th Danube Symposium on Chromatography, Bratislava, 1983*, Abstract E27.
57 L. Nondek and V. Chvalovský, *J. Chromatogr.*, 312 (1984) 303.
58 J. C. van Miltenburg and W. E. Hammers, *J. Chromatogr.*, 268 (1983) 147.
59 A. Matsunaga, *Anal. Chem.*, 55 (1983) 1375.
60 E. P. Lankmayr and K. Müller, *J. Chromatogr.*, 170 (1979) 139.
61 W. Holstein and D. Severin, *Chromatographia*, 15 (1982) 231.
62 G. Felix and C. Bertrand, *J. Chromatogr.*, 319 (1985) 432.
63 G. Felix, C. Bertrand and F. Van Gastel, *Chromatographia*, 20 (1985) 155.
64 G. Felix and C. Bertrand, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 363.
65 F. Mikeš, G. Boshart and E. Gil-Av, *J. Chromatogr.*, 122 (1976) 205.
66 C. H. Lochmüller and R. R. Ryall, *J. Chromatogr.*, 150 (1978) 511.
67 C. H. Lochmüller, in D. E. Leyden and W. T. Collins (Editors), *Silylated Surfaces, Midland Macromolecular Monographs*, Vol. 7, Gordon and Breach, New York, 1980, p. 231.
68 S. A. Matlin, L. A. Tito-Loret, W. J. Lough, D. G. Bryan, T. Browne and S. Mehani, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 81.
69 W. H. Pirkle and D. W. House, *J. Org. Chem.*, 44 (1979) 1957.
70 W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143.
71 W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 46 (1981) 2935.
72 W. H. Pirkle and J. L. Schreiner, *J. Am. Chem. Soc.*, 46 (1981) 4988.
73 C. E. Dalgliesh, *J. Chem. Soc.*, (1952) 3940.
74 I. W. Wainer and T. D. Doyle, *J. Chromatogr.*, 259 (1983) 465.
75 T. D. Doyle and I. W. Wainer, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 38.

Journal of Chromatography, 373 (1986) 81–110
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHREV. 203

CHROMATOGRAPHY OF MONOSACCHARIDES AND DISACCHARIDES

K. ROBARDS* and M. WHITELAW
School of Applied Science, Riverina–Murray Institute of Higher Education, P.O. Box 588, Wagga Wagga 2650 (Australia)
(Received January 24th, 1986)

CONTENTS

1. INTRODUCTION

The determination of mono- and disaccharides has been achieved by indirect physical (refractometry, polarimetry, hydrometry) or by semi-empirical chemical methods (volumetric analysis, gravimetry, colorimetry). These chemical methods, which are non-specific in that they detect a class of sugar such as pentoses or reducing sugars rather than individual sugars, are based on either colour reactions effected by the condensation of degradation products of sugars in strong mineral acids with various organic compounds (Bial and Molisch test)[1]; the reducing properties of the carbonyl group (Fehling's test)[1]; or on oxidative cleavage of neighbouring hydroxyl groups[1,2]. However, the determination of individual carbohydrates is of considerable importance and the specificity of such tests has often been enhanced by using fractions separated by paper (PC), thin-layer (TLC) or column chromatography. Today, enzymatic methods are available[3–6] but these are limited to a few sugars. Moreover, because of their high specificity, such methods do not allow the simultaneous determination of several sugars. On specificity requirements and the need for multiple determinations, chromatographic methods offer distinct advantages.

Several reviews[7–15,359–361] attest to the importance of chromatography in carbohydrate analyses. With this consideration in mind, the analytical applications of chromatography to monosaccharides and disaccharides are reviewed. Because of their relevance in gas chromatographic methods, selected derivatives are included. Emphasis is placed on food and clinical applications in the period from 1980 to June 1985. However, earlier work of particular significance is also covered.

0378-4355/86/$10.50

2. PAPER AND THIN-LAYER CHROMATOGRAPHY

PC and TLC have been used extensively in the analysis for sugars. The reasons for this include low cost, simplicity and the ability to simultaneously isolate and identify the sugars. PC was first applied to carbohydrates[16,17] in 1947 and, although partition is the predominant separation mechanism, adsorption phenomena[18] also occur. On paper-partition chromatograms, where compounds partition between aqueous phases held stationary on the paper and mobile organic phases, free sugars migrate as single substances[19–21] indicating that continuous equilibration occurs to prevent the resolution of the anomers present in solution. The relations between mobility of the sugars and their structures were studied primarily by Isherwood and Jermyn[22] and by Levy[23]. Thus, the influence of furan–pyran ring formation and the nature of the ring substituent at C-4 or C-5 (ref. 20), *cis–trans* isomerisation[22] at C-2 or C-3, and the type of linkage (-1,4- or -1,6-) in disaccharides[24] have been examined.

For reproducible results in PC the solution to be chromatographed must, as much as possible, be free of non-carbohydrate materials. This means that animal or vegetable extracts should be subjected to preliminary purification[25] to remove impurities such as salts and proteins. Moreover, excessive alkalinity, which may cause epimerization, must be avoided during the preliminary manipulations. Originally, the organic phase of biphasic solvent mixtures[16] such as water-saturated *n*-butanol was proposed for use as mobile phase. However, the temperature dependence of the composition of such systems may result in phase separation during use and, as a result, monophasic solvent systems[26–28] came into use. The latter consist of water, a water-miscible and a water-immiscible organic solvent. In such systems the mobility of the sugars, which increases[22] with the water content, is generally the same[29]; namely, pentoses, hexoses, disaccharides and trisaccharides nearest the origin.

Although the PC of carbohydrates is actively pursued[30–34], it has been superceded by TLC for carbohydrate analysis. This view is substantiated by an examination of *Chemical Abstracts* (covering January 1980 to June 1985) where the number of TLC-based methods for carbohydrates outweighs the PC-based methods in the ratio of 7:1. However, such an analysis makes no allowance for the total number of routine analyses performed by PC. This decline in use of PC can be traced to the long development times[35] frequently involved. Indeed, development times of up to 16 h are not uncommon.

Although partition TLC of simple sugars on microcrystalline cellulose has had limited use[36–39], it offers the resolution of PC with the advantage that the solvent systems and spray reagents most suitable for PC are directly applicable[36] to cellulose TLC. Carbohydrates, being strongly hydrophilic, require very polar solvents which have relatively slow migration rates[40]. Despite this the separations are considerably faster than those attained by PC[41] although slower than can be achieved on inorganic adsorbents. Moreover, TLC is more sensitive[41] than PC for the determination of sugars. Frequently, TLC separations depend upon preferential adsorption phenomena, and occasionally resolutions must incorporate both partition and adsorption factors as, for example, in separations on silica gel G using butanone–acetic acid–water as eluting solvent[20]. Several inorganic adsorbents have been used for the TLC of sugars including magnesium silicate[42], alumina[43], Kieselguhr[44,45], silica gel[46–55], or mixtures of the last two[56,57], and aminopropyl-bonded silica[58,59]. Caution in the

interpretation of chromatograms is required. For example, aminated sugars are formed[60] when sugars are chromatographed on silica gel G with ammoniacal solvents resulting in hexoses, pentoses and disaccharides being split into at least two different compounds. The silica gel apparently exerts a catalytic effect as the formation of amino sugars is not observed on other adsorbents. The mobility of the sugars on silica gel depends primarily on the molecular weight and the number of hydroxyl groups[49,61] and consequently the diastereoisomers are poorly resolved. Resolution is improved by impregnating silica gel and Kieselguhr with salts of weak acids[44,46,62,63,359].

The effects on chromatographic behaviour of the type and concentration of impregnating salt, which react with the carbohydrates by reasonably well known mechanisms, have been systematically investigated[362] using several solvents. Phosphates were identified as suitable impregnants. Although borates were not examined, excellent separations on thin layers of silica gel impregnated with borate have been reported subsequently[363]. Bisulphite, known for its characteristic addition reactions with aldoses and ketoses, also gave[66] excellent separations of certain sugars. Several other salts including molybdate and tungstate have been investigated[364].

A significant feature of inorganic layers is that more corrosive spray reagents can be employed[64,65] for detection. Thus, sulphuric acid alone or admixed with nitric acid[64] or permanganate[65] has proved suitable for detecting sugars at the microgram level. Various other reagents which have been proposed include naphthol–resorcinol–sulphuric acid[47], aniline–diphenylamine[63] and admixtures of sulphuric acid with anisaldehyde[44], naphthol[62], thymol[66], carbazole[66] and phenol[66]. Reagents suitable for PC are also applicable to TLC and of these iodine vapour warrants mention for despite being less sensitive than sulphuric acid it is non-destructive in the short exposure time required. The adsorbed iodine evaporates when the plate is exposed to the air.

Although PC and TLC are now generally regarded as inferior methods for carbohydrates, it is interesting to note that high-performance TLC has recently been applied[45,58,67] to the separation of sugars.

3. GAS CHROMATOGRAPHY

The application of gas chromatography (GC) to carbohydrates was slower than with other classes of compounds. The major problem was the lack of volatility of the polar compounds and the fact that volatile derivatives could not readily be prepared in quantitative yields. As an alternative to derivative formation, Greenwood *et al.*[68] in 1961 investigated pyrolysis–GC of several sugars. However, this approach does not appear to have been followed by other workers, possibly because of the simultaneous development of suitable derivatising reagents in the early 1960's.

3.1. Derivatives

The first derivatives to be chromatographed[69] were those already used for the chemical analysis of sugars. Thus, the fully methylated methyl glycopyranosides of simple pentoses and hexoses were separated[70] on a column of methylated hydroxyethylcellulose. The anomeric forms of a single monosaccharide were also resolved[70] using this technique and methylated sucrose was successfully chromatographed.

Bishop[71] demonstrated in 1962 that mixtures of methylated disaccharides could be resolved and an extensive literature[9] now covers the methyl derivatives. The extent of methylation and the application of the method to different carbohydrates have been examined by several workers[72–75].

The separation of carbohydrates as acetyl derivatives was first described by Gunner *et al.*[76] who found that anomeric glycose acetates gave separate peaks, and derivatives of epimers had different retention times. The technique was further developed by Sawardeker *et al.*[77] and extended to the separation of disaccharides by using lower loadings of more thermostable silicone liquid phases[78,79]. In this procedure the carbonyl group of monosaccharides is reduced with sodium borohydride to the corresponding sugar alcohol which is then acetylated, eliminating the formation of multiple derivatives[77] from a single sugar. Although the preliminary reduction and acetylation steps proceed quantitatively potential difficulties include errors in quantification due to naturally occurring polyols, the possibility of forming a single sugar alcohol from different monosaccharides (*e.g.*, glucose and sorbose), interference by the borate (formed in the reduction step) in the acetylation and tailing peaks caused by the pyridine solvent. Despite this the method has been used widely[80–82].

The occurrence of more than one peak per sugar presents a significant complication in the analysis of carbohydrates. Solutions of carbohydrates undergo mutarotation and an initially pure sugar may result in an equilibrium mixture of the linear form and the α- and β-anomer of both the pyranose and furanose ring forms. Thus, derivatisation of solid α-D-glucose gave[83] a single major peak in the chromatogram. Similarly, β-D-glucose showed a single peak. On the other hand, derivatisation of the residue obtained from evaporation of an aqueous equilibrium solution of glucose, showed two peaks on being chromatographed, corresponding to the peaks obtained with the two separate α- and β-anomers. These observations have been extended[83] to many sugars other than glucose. Derivatisation is generally a faster reaction than mutarotation[84] and, hence, compositional changes during derivatisation are assumed minimal. To reduce further any mutarotation of sugars, dimethyl sulphoxide (DMSO) may be used as derivatising solvent whence, for example, the α- to β-D-glucose anomerization rate[85] is essentially zero and remains low in aqueous DMSO. With respect to water, the α-D-glucose anomerization in 50% aqueous pyridine is about 48 times faster than in either 50% aqueous DMSO or 50% aqueous dioxane. Catalysts (for example, 0.2% lithium perchlorate) have been used[84] to effect mutarotation equilibrium prior to derivatisation. In the case of glucose, an aqueous equilibrium mixture contains[83] about 36% α-D-glucopyranose, 64% β-D-glucopyranose and less than 1% of the linear aldose or of either possible furanose form. In contrast, Acree *et al.*[86] have determined the composition of an aqueous equilibrium solution of galactose to be 1.0% α-galactofuranose, 3.1% β-galactofuranose, 32.0% α-galactopyranose and 63.9% β-galactopyranose. The importance of solvent is seen by comparing the corresponding compositional data[87], namely, 13.7%, 23.4%, 31.7% and 31.2%, respectively, for pyridine solutions of galactose. Additional factors affecting mutarotation equilibria in aqueous systems include[88] pH and metal ion concentrations.

An alternative approach[89] to acylation is the preliminary conversion of aldoses to the corresponding methyloxime, followed by acetylation of free hydroxyl groups. Since the oximation involves reaction at the C-1 position, the incidence of multiple

derivatives from a single sugar is reduced. Although single peaks are obtained for glucose, fructose, mannose and xylose, some carbohydrates give two peaks[89], presumably due to formation of *syn*- and *anti*-isomers. A similar procedure has been used[91] for disaccharide estimation, one peak being obtained for maltose, but two resulting from lactose.

Bourne *et al.*[90] originally described the derivatisation of sugars with trifluoroacetic anhydride to form the corresponding esters. Several different trifluoroacetylation procedures have been described[91–95]. The advantages of trifluoroacetylation are enhanced volatility[91] and sensitivity. Thus, Tamura and Imanari[93] prepared the trifluoroacetate derivatives of glucose, galactose and mannose and exploited the sensitivity obtained with electron-capture detection for their quantification. Derivatisation with N-methyl-bis(trifluoroacetamide) is claimed[96] to be more reproducible.

By far the most popular of the volatile compounds of sugars used for GC purposes are the trimethylsilyl (TMS) derivatives. Sweeley *et al.*[97] were the first to produce a viable method for carbohydrates based on the formation of these derivatives. Their study included pentoses and hexoses as well as monosaccharides through to tetrasaccharides. A mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and anhydrous pyridine was added to dried sugars, derivatisation occurring rapidly at room temperature with virtually quantitative yields. Since the extract was injected directly, ammonium chloride (formed as a by-product in the reaction) contamination of the column occurred. Various modifications of the procedure have been described[98,99] including hexane extraction[100] of the derivatised sugars prior to injection. Although ammonium chloride may be removed by centrifugation, solvent extraction[100,101] has the advantage of simultaneously eliminating pyridine which produces a severely tailing peak on some stationary phases.

It was originally believed that the procedure of Sweeley *et al.*[97] required rigorous drying for silylation. This is not the case for, although drying prevents anomerisation, water does not prevent silylation as it immediately silylates to hexamethyldisiloxane. Thus, its presence merely requires more silylating reagent and this fact has been used[102] to perform the reaction directly in aqueous solution. Brobst and Lott[103] substituted trifluoroacetic acid for TMCS and used a sequential addition of reagents to the sugar. However, the silylation of glucose was claimed[104] to be incomplete by this method.

More powerful silyl donors than HMDS have been proposed. For example, N-trimethylsilylimidazole[105–108] and N,O-bis(trimethylsilyl)acetamide (BSA)[109–111], with TMCS as catalyst, have been used to derivatise sugars in a range of biological fluids. However, BSA in pyridine causes the anomerisation of hexoses, and the chromatogram of a single sugar may contain[112] four or five peaks. Other reagents in this category include N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-N-trimethylsilyltrifluoroacetamide[113,114]. Although BSTFA should be a self-catalyzing silyl donor, it has been found necessary[84,111,113] to add TMCS as an additional catalyst for rapid quantitative derivatisation.

Pyridine has been used as solvent in the majority of silylation studies although other solvents, such as DMSO and dimethylformamide[115–118] and including a large excess of silylating reagent itself[15], have also been used. The advantages claimed for these solvents compared to pyridine are lower anomerization rates[85], improved chromatographic behaviour[112] and less noxious odours.

TABLE I

STATIONARY PHASES AND PACKED COLUMNS USED FOR GC OF SUGARS

Derivative	*Column**	*Column packing*	*Ref.*
TMS	12 ft.	5% SE-30 on Gas Chrom P	114
	2–6 ft. × 6 mm O.D.	2–3% SE-30 on silanised Chromosorb W	103
	1.5 m × 3 mm	3% OV-1 on Gas Chrom Q	111
	3 m × 3 mm	13% OV-1 on Gas Chrom Q	137
	6 ft. × 3 mm	2% OV-1 on silanized Gas Chrom S AW	116
	2.7 m × 3 mm	10% OV-17 on Gas Chrom Q	109–111
	2–6 ft. × 6 mm O.D.	2–3% SE-52 on silanised Chromosorb W	103
	6 ft. × 6 mm O.D.	3% SE-52 on silanised Chromosorb W AW	97
	2 ft. × 6 mm O.D.	0.25% SE-52 on glass beads	138
	2.7 m × 3 mm	3% QF-1 on Gas Chrom Q	111
	6 ft. × 3 mm	3% SP-2250 on Supelcoport	132
	2.7 m × 3 mm	3% XE-60 (cyanoethyl methyl silicone) on Gas Chrom Q	111
	8 ft. × 6 mm O.D.	15% EGS on Chromosorb W AW	97
	6 ft. × 6 mm	15% EGS on Chromosorb W	98
	6 ft. × 2 mm	3% Dexsil 300GC on Chromosorb W AW DMCS	136
Oxime, TMS	15 ft. × 3 mm O.D.	1% SE-30 on Gas Chrom Q	124
	0.5 and 2.0 m × 3 mm	3% SE-30 on Chromosorb W AW DMCS	134
	7 ft. × 6 mm O.D.	3% SE-30 on Chromosorb W	139
	6 ft. × 6 mm O.D.	3% OV-17 on Chromosorb W AW DMCS	125
	0.5 and 2.0 m × 3 mm	3% SP-2250 on Supelcoport	134
TMS, acetyl	No details	3% SE-52	140
Alditol, TMS	2 ft. × 6 mm O.D.	3% SE-52 on Diataport S	133
Alditol acetate	8 ft. × 6 mm O.D.	5% XE-60 on Chromosorb W	77
	4 ft. × 6 mm O.D.	10% Carbowax 20M on Chromosorb W	77
	10 ft. × 6 mm O.D.	3% ECNSS-M (ethylene succinate silicone copolymer) on Chromosorb W	77
	2 m × 3 mm	5% OV-275 on Chromosorb W	122
	2 m × 2 mm	2% Silar-7CP on Chromosorb W AW	141
Alditol trifluoroacetate	1.5 m × 4 mm	2% XF-1105 (cyanoethyl methyl silicone) on Gas Chrom P	142
Trifluoroacetate	6 ft. × 4 mm	20% SE-30 on Chromosorb W AW	95
Methyl	1.8 m × 3 mm	1% SE-30 on Gas Chrom Q	75
	1.8 and 3.6 m × 3 mm	1% OV-17 on Gas Chrom Q	75
Oxime, acetyl	6 ft. × 2 mm	5% QF-1 on Supelcoport	143
Methoxime, acetyl	9 ft. × 4 mm	3% ECNSS-M on Celite	89
Underivatised glucose	2 m × 3 mm	Chromosorb 101 (porous polymer)	135

* Length × internal diameter, except when indicated otherwise.

Additional derivatisation procedures have been combined with trimethylsilylation to decrease the incidence of multiple derivatives. Thus, reduction of monosaccharides to their corresponding alditols followed by the formation of (acetate or) TMS ether derivatives avoids the problem of peak multiplicity[119–123] by removing the carbonyl group normally involved in ring formation. However, the method which involves significant chemical manipulation of sugars, may lead to information loss[124] because certain sugars yield the same alcohol. Moreover, ketosugars yield two epimeric alcohols. For example, fructose and glucose yield sorbitol as their reduction product and fructose, in addition, yields mannitol. The alditol-TMS derivatives are claimed[9] to be less satisfactorily resolved than the corresponding acetyl esters and this evidently accounts for the low incidence of their use. Combined oximation–acetylation[89] and oximation–trimethylsilylation[97,124] also reduces the problem of peak multiplicity (two possible derivatives per sugar, with only one found for most sugars[125]) but some problems have been reported[124] due to poor sample stability of the sugar oximes.

Dimethylsilyl (DMS) and halomethyldimethylsilyl derivatives are prepared using similar conditions as those for TMS ethers. As expected the retention times of the halogen compounds are considerably longer than those of TMS ethers and the application of the halogen derivatives is limited[126]. DMS derivatives can be employed to advantage with high-molecular-weight sugars since the DMS ethers have retention times that are half of those of TMS derivatives. Various other derivatives have been studied[127–130] but offer little by way of improved ease of derivatisation, resolution and/or reduction in the number of derivatives.

In summary, the major difficulty in the GC of carbohydrate derivatives relates

TABLE 2

OPEN TUBULAR COLUMNS USED FOR SUGAR ANALYSIS

SCOT = Support-coated open tubular column; WCOT = wall-coated open tubular column.

Derivative	*Column*	*Stationary phase*	*Ref.*
TMS	30 m × 0.28 mm glass WCOT	SE-52	108
	25 m glass WCOT	SE-54	146
	Short × 0.32 mm glass WCOT	OV-1, SE-30	147
	30 m × 0.25 mm fused-silica WCOT	SE-30	84
Oxime, TMS	15 m × 0.25 mm glass WCOT	SP-2250	145
Alditol, TMS	25 and 50 m × 0.23 mm fused-silica WCOT	OV-101	148
Alditol acetate	25 m × 0.25 mm glass WCOT	OV-275	149
	28.5 m × 0.5 mm glass SCOT	Silar 10C	150
	28 m × 0.5 mm glass SCOT	Silar 10C	151
	6 m × 0.2 mm fused-silica WCOT	BP-75	151
	25 m × 0.2 mm fused-silica WCOT	SE-52	152
	20–25 m × 0.3 mm glass WCOT	Chiral phase	153
Methoxime, TMS	25 m × 0.28 mm glass WCOT	SE-30	129
Oxime, acetyl	60 m × 0.3 mm glass WCOT	SE-30	154–156
N-Ethoxycarbonyl-O-TMS	25 m × 0.28 mm WCOT	OV-101; Chirasil-Val	157

to the production of several products from a single glycose owing to either faulty derivatisation or the formation of anomeric derivatives of possible furanose and pyranose ring forms[131]. With respect to faulty derivatisation, incomplete silylation[112], for instance, may produce several peaks per component[104]. Complications may also arise from the order of addition of reagents and the heating (often necessary for dissolution of the sugars) of the derivatisation mixture[83,97]. Thus, heating and the sequential addition of reagents apparently increase the number of derivatives.

3.2. Columns and packing materials

Columns, constructed of copper[103] and stainless steel[103,124,132–134] have been used successfully for the GC of sugar derivatives. However, as in other applications of GC, the trend has been to the increasing use of more inert glass columns[122,125,135,136]. Several stationary phases are suitable for chromatographing sugar derivatives and, in general, the non-polar TMS derivatives have been chromatographed[9] on non-polar phases while the more polar acetyl esters have been better resolved on more polar phases (Table 1). However, many cases are observed in which incomplete resolution results on non-polar, non-selective phases such as SE-30 and SE-52, while analyses on polar columns, such as polyethylene glycol, polyester and nitrile silicone, are often more selective. On the other hand, polar columns are less useful for chromatographing mixtures containing substances with a wide range of boiling points.

The derivatisation procedure can restrict the choice of a stationary phase. For example, in the typical silylation procedure[84,136] where the reaction mixture is injected directly, the presence of excess unreacted reagent precludes the use of stationary phases with reactive hydrogens. Although loading of the stationary phase on the inert support has varied[9] from 0.25 to 25% (Table 1) low percentages have been more common. Of the various materials used as the inert support, Chromosorb W and Gas Chrom Q have found widest success (Table 1).

Capillary GC is a powerful technique for the analysis of complex samples such as carbohydrate derivatives. Stainless-steel capillaries coated with OV-17 have been utilized[144] for an extensive study of carbohydrate changes during sugar boiling. However, the reduced activity of glass[145] and, more recently, fused-silica capillaries has resulted in the increased use of such columns (Table 2). An added advantage of capillary systems is the ability to employ cold on-column injection[147] thereby reducing the incidence of sample decomposition in the heated injection port. The possibility of catalytic and adsorptive phenomena occurring in the injection port and column has not been studied in relation to carbohydrates.

4. COLUMN CHROMATOGRAPHY

Column chromatography of carbohydrates dates back to 1939, when Reich[158] described the separation of azoyl derivatives of sugars. Alumina has found little application in the carbohydrate field since the sugars are too polar and too strongly adsorbed. Moreover, their hydrophilic character makes it impossible to use non-polar solvents, and this limits the choice of suitable solvents. Finally, the basic character of alumina involves the danger of epimerization. Cellulose partition columns[159,160] and ion-exchange columns[161,162] have found limited use. In contrast, low-resolution

charcoal columns dominated[163–165] the column separations of carbohydrates until 1970. Although the development of strong cation exchange columns[166] was a considerable advance in column technology, it is undoubtedly the development of polar bonded-phase materials prepared from silica (5 and 10 μm) which has led to the full advantages of high-performance liquid chromatography (HPLC) becoming applicable to carbohydrate analyses. Indeed, HPLC is now claimed[167] to be superior to GC as a technique for carbohydrate analysis. Thus HPLC often offers direct injection of sample with little or no pretreatment and sugars are not subjected to high temperatures. The interpretation of many HPLC chromatograms is simple because anomeric forms of sugars are normally not resolved[15]. On the other hand, GC detectors are generally the more sensitive and in those cases where only a limited amount of sample is available, GC can be readily employed.

Columns and conditions used for the HPLC of carbohydrates are summarised in Table 3. Anion exchange of carbohydrate-borate complexes was first applied[168] in 1952 but was a tedious, time-consuming procedure. By contrast, a current technique[169] can determine all nine naturally occurring aldoses in 65 min. Various other modes of separation have been applied to HPLC of simple sugars and, of these, reversed-phase partition is the most important. Although both alkylated, cyano- and amino-bonded phases have been utilized for carbohydrates, the latter have been most extensively studied. The first evaluation of the preparation and properties of an amino-bonded stationary phase was reported[195] in 1976. Such columns had limited lifetimes because of the hydrolysis of the bonded phase and the reactivity of the amino function. The resulting deterioration required a mobile phase of gradually decreasing water content[196] so that retention times and resolution could be approximately maintained. Moreover, chemically bonded particles are readily aggregated by contact with hydrophilic substances, and the column packing is altered. Therefore, guard columns are usually used to extend column life. Despite these limitations commercial packings exhibit reasonable stability and provide excellent separations of sugars. *In situ* preparation of amino columns[197] ensures stability of column conditions by constantly regenerating the surface of the stationary phase.

Carbohydrates have been successfully chromatographed[198] on Partisil-10 PAC, a bonded phase containing both cyano and amino groups. The effect of the addition of acids and salts to the aqueous acetonitrile mobile phase has also been examined.

Separations on amino-bonded phase columns have been considered normal-phase partition by others[199] because increasing water content in the mobile phase speeds up the elution. However, it is not clear whether the retention is caused by a competitive interaction of the water and carbohydrate or by adsorption via hydrogen bonding between hydroxyl groups of the carbohydrate molecule and the amino groups of the stationary phase[179]. Indeed, some prefer to call this use reversed-phase adsorption while others feel that there are at least three mechanisms occurring simultaneously: adsorption, partition and surface tension[200].

Ion-moderated partitioning is increasingly being used for the separation of carbohydrates[187]. Porous-polymer-based ion exchangers are used as the stationary phase together with aqueous mobile phase. Both anion and cation exchangers are used, but the latter more frequently. The elution profile is determined by the counterion with separation proceeding in the partition mode[201,202]. However, the change

TABLE 3

HPLC SYSTEMS USED FOR SUGAR ANALYSIS

Mode	*Column*	*Mobile phase*	*Detector*	*Ref.*
Anion exchange: borate complex	15 cm × 4.0 mm, Hitachi 2633	0.50 *M* Borate buffer	Fluorimeter, post-column derivatisation to 2-cyanoacetamide	169
	25 cm × 4.0 mm, Aminex A-25	Borate buffer	UV, 199 nm	170
	12 cm × 8.0 mm, Jeolco LC-R-3	Borate buffer	Visible, 425 nm; post-column derivatisation with orcinol–sulphuric acid	171
Reversed-phase partition	30 cm × 4.0 mm, Bondapak Carbohydrate	Acetonitrile–water (83:17) and (85:15)	Refractometer	172,173
	μBondapak NH_2	Acetonitrile–water (80:20)	Visible, post-column derivatisation with tetrazolium blue	174
	25 cm × 4.6 mm, Amino	70–80% Acetonitrile in water; ethyl acetate–ethanol–water (40:50:10)	Refractometer; UV, 188 nm	175
	25 cm × 4.6 mm, Resolution NH_2 5	Acetonitrile–0.01 *M* KH_2PO_4, pH 7	Refractometer	176
	15 cm × 4.6 mm, Micropak NH_2 (phosphate form)	Acetonitrile–water (70:30)	Refractometer; fluorimeter, post-column derivatisation to 2-cyanoacetamide	177
	15 cm × 4.6 mm, Lichrosorb NH_2 and Nucleosil 5 NH_2	Acetone–water–acetic acid (100:15:1)	Refractometer	178
	25 cm × 4.6 mm, Zorbax NH_2 and Supelcosil LC-NH_2	72–80% Acetonitrile in water	Refractometer	179
	15 cm × 4.6 mm, Resolution ODS 5	Water	Refractometer	176
	25 cm × 4.9 mm, ODS Hypersil	Acetonitrile–water (22:78)	Fluorimeter, pre-column derivatisation to dansyl derivative	180

Ion-moderated partition	100 cm × 4 mm, Aminex A6 (Li^+)	70–85% Ethanol in water	Moving wire	181
	15 and 25 cm × 6.4 mm, Resolution Carbohydrate (Na^+ and Ca^{2+})	Water	Refractometer	176
	30 cm × 7.0 mm, Aminex HPX-85C	Water	Refractometer; fluorimeter	177
	30 cm × 7.8 mm, Aminex HPX-87H	0.01 *N* Sulphuric acid	Refractometer	182
	25 cm × 6.0 mm polystyrene and silica-based cation exchangers (Ca^{2+})	Water	UV, 276 nm; post-column derivatisation to 2-cyanoacetamide	183
	Sugar-Pak 1 (Ca^{2+})	Water	UV, 190 nm	184
	15 cm × 6.0 mm, Shodex RS Pak DC-613 (Na^+ and Ca^{2+})	70–90% Acetonitrile in water	UV, 280 nm; post-column derivatisation to 2-cyanoacetamide	185
	30 cm × 7.8 mm, Aminex HPX-87	Water containing 0.02 g/l calcium propionate	Refractometer	174
	30 cm × 7.8 mm, Aminex HPX-87H and HPX-42A	Water; 0.01 *N* sulphuric acid	Refractometer	186
	20 cm × 9.0 mm, Aminex A7	0.06 *M* Trifluoroacetic acid	Refractometer	187
	25 cm × 4.0 mm, Shodex RS Pak DC-613	Acetonitrile–water (90:10)	Electrochemical	188
Adsorption–normal-phase partition	25 cm × 4.6 mm, Partisil 5	Ethyl acetate–hexane (1:49) to (1:199)	UV-260 nm; pre-column derivatisation to dimethylphenylsilyl	189,190
	25 cm × 4.6 mm, Zorbax SIL	Hexane–chloroform–acetonitrile (10:3:1.9) with 0.1% water	UV, 260 nm; pre-column derivatisation to nitrobenzoates	191
	60 cm × 9.0 mm, Porasil A	Methyl ethyl ketone–water–acetone (85:10:5)	Refractometer	192
	25 cm × 2.1 mm, LiChrosorb Si 60	Water–acetonitrile (0.1:99.9)	Refractometer	193
	100 cm × 4.0 mm, Pellosil HC	Dichloromethane	UV, 254 nm; benzoates	194

TABLE 4

TECHNIQUES USED FOR THE DETERMINATION OF MONO- AND DISACCHARIDES

Analyte	*Sample*	*Method*	*Ref.*
Sucrose and other sugars	Syrups	TLC on silica gel	220
Glucose, rhamnose, xylose, galactofuranose	Microorganism	GC–MS of TMS ethers	221
Simple sugars	Cereals	HPLC	222
Mono- and disaccharides	Urine	PC after desalting	30
Pentoses, hexoses	Not applied	GC on packed columns	223
Aldoses	Biological	GC of TMS diethyldithioacetals on capillary columns	224
Sugars	Wheat bran hydrolysate	GC of alditol acetates on packed columns	225
Xylose, fructose, glucose, sucrose, maltose, lactose etc.	Amylose and inulin hydrolysate	HPLC on amine-bonded phase	226
Mono- and oligosaccharides	Body fluids	Anion-exchange HPLC of borate complexes	227
Mono- and oligosaccharides	Not applied	TLC on phosphate-impregnated silica gel	228
Sorbitol and other sugar alcohols	Bulk sorbitol	HPLC on cation exchanger	229
Mono- and disaccharides	Serum, faeces, urine	TLC on silica gel	230
Mono-, di- and oligosaccharides	–	Reversed-phase HPLC	231
Dissolved carbohydrate hydrolysate	Natural water	GC of alditol acetates	122
Maltose, ribose, xylose, fructose, galactose, glucose	Serum; amylose	HPLC on various columns	232
Mono- and disaccharides	Foods	HPLC on cation exchangers	233
Monosaccharides	[Comparison of UV and refractive index detection]	HPLC on amine-bonded phase	175
Mono-, di- and trisaccharides	Foods	TLC on silica gel	52
Mono- and disaccharides	Body fluids	TLC on silica gel	53
Fucose, mannose, galactose	Serum	GC of alditol acetates on packed columns	141

Mono-, di- and trisaccharides	[Thermal degradation]	GC of underivatised sugars on porous polymer	135
Monosaccharides	Corn bran	GC of TMS alditols on fused-silica WCOT columns	148
Mono- and disaccharides	Not applied	TLC on cellulose	234
Ketoses, aldoses	Not applied	GC–MS of acetyl-oximes	235
Monosaccharides	Plant gums	GC on chiral phase	121
Amino acids, sugars	–	HPTLC on cellulose and silica	236
Alditols	Not applied	HPLC on cation exchanger with several metal ions	237
C_2–C_7 carbohydrates	Reaction products	GC of TMS and TMS-oxime derivatives on packed and capillary columns	238
Glucose, fructose, sucrose, galactose, mannitol, sorbitol	Not applied	HPLC on boronic acid substituted silica	239
Free sugars	Plant materials	GC	240
Sugars	Corn syrup	HPLC on ion exchanger	241
Fructose and other sugars	Urine	HPLC on cation exchanger	242
Sugars	Foods	HPLC on cation exchanger	243
Mono- di- and trisaccharides	[Use of radially compressed columns]	HPLC on modified silica	197
Glucose, mannose, xylose, galactose	Wood pulp	HPLC on various columns	244
Rhamnose, fucose, xylose, arabinose, glucose, galactose, mannose	Rice endosperm, cell wall hydrolysate	GC of methylated alditol acetates on glass SCOT column	245
Glucose, maltose	Foods	HPLC on amine-bonded phase	246
Mono- and disaccharides	Papermill effluent	GC on capillary column	247
Monosaccharides, sugar acids	Microorganisms	GC of alditol acetates and aldonitrile acetates on packed columns	248
Arabinose, xylose, fructose, sorbose glucose	–	GC of TMS ethers	249
Galactose, glucose, mannose	Galactomannans from *Aspergillus* spp.	GC of methylated alditol acetates on WCOT columns	250
Alditols	Diatomaceous ooze sediment	GC of alditol acetates on glass WCOT columns	149

(Continued on p. 94)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref.*
Neutral sugars	Hydrolysate from Daemonorops	GC of alditol acetates on glass WCOT columns	153
Monosaccharides	[Comparison of aldose/ketose derivatisation]	GC of N-ethoxycarbonyl-O-TMS ethers on WCOT columns	157
Fructose, glucose, sucrose	Cane molasses	GC of oxime-TMS ethers on glass WCOT columns	145
Arabinose, fructose, glucose, sucrose, trehalose, sugar alcohols	Wine	HPLC	251
Acids, sugars, polyols	Wine	GC of TMS ethers on glass WCOT columns	252
Sugar	Bakery products	Colorimetry, ebulliometry, iodometry	253
Carbohydrates	Meats	Infrared transmission spectrophotometry	254
Aldoses	Glycoconjugates	Anion-exchange HPLC of borate complexes	255
Glucose, fructose, ribose	Plasma lipids	TLC on silica gel 60, alumina, cellulose and polyamide	256
Mono- and oligosaccharides	Cotton	TLC on silica gel	257
Carbohydrates	Wine	GC of TMS-oximes	258
Mono- and disaccharides	–	HPLC on cation exchanger (Li^+)	259
Reducing sugars, uronic acids	Pneumococcal polysaccharide hydrolysate	PC on DEAE-cellulose	31
Reducing sugars	Sugar refinery products	Potentiometry following copper reduction	260
Glucose	[Mutarotation rate and retention mechanism]	HPLC on amine-bonded silica	261
Sorbose, lactose, lycose, sucrose, glucose, fructose, maltose	Candies	TLC on silica gel	262
Twelve sugars	[Several mobile phases examined]	TLC on silica gel, cellulose and Kieselguhr	263

Glucose, mannose, ribose, xylose, arabinose, fucose, galactose, lyxose	[Separation of enantiomers]	GC on chiral stationary phases	264
Carbohydrate	Beer	HPLC on amine-bonded phase	265
Free sugars	Green tea	HPLC	266
Invert sugar	Sugar beet	Colorimetry using autoanalyser	267
Tetroses, aldopentoses	Not applied	GC of methoxime and butoxime pertrifluoroacetates on packed and capillary columns	268
Rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose	Sheep rumen fluid and milled barley straw	GC of methylated alditol acetates on glass WCOT columns	269
Sugars	[Retention mechanism]	HPLC on amine-bonded phase	270
Free sugars	Oilseeds	HPLC on amine-bonded phase	271
Glucose, sucrose, fructose	Cantaloupe melon juice	HPLC on silica	272
Glucose	[Flow injection and electrochemical detection]	HPLC on amine bonded phase	273
Dextrose	Foods	HPLC on cation exchanger (Ca^{2+})	274
Sugars	[Method for determining pore size in exclusion chromatography]	HPLC on cation exchangers	275
Fructose, sucrose, glucose, raffinose	–	GC and HPLC	276
Fructose, sucrose glucose, sorbitol mannoheptulose	Apple and pear juices	HPLC on ODS and amine-bonded phases	277
Fructose, glucose, sucrose, maltose, lactose	Chocolate and cacao	HPLC on amine-bonded phase	278
Monosaccharides	–	GC of alditol acetates on packed columns	279
Fructose, glucose, sucrose, maltose, lactose	Fruit juices	HPLC on amine-bonded phase	280
Mannose, ribose, xylose, glucose, fucose, rhamnose, arabinose, etc.	Glycoproteins	GC of TMS ethers on fused-silica WCOT columns	281

(Continued on p. 96)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref.*
Carbohydrate	Cocoa	Near-infrared reflectance	282
Arabinose, lyxose, fucose, rhamnose, galactose, glucose, mannose	Flax-seed mucilage hydrolysate	Enantiomer separation by GC of dithioacetals	283
Mannose, fructose, fucose, galactose, glucose, rhamnose	Bovine glycoproteins	HPLC on cation exchanger	284
Sugars	Culture media	PC following ion exchange	32
Glucose, fructose	Spruce and pine needles	TLC on silica gel	285
Sugars	Foods	HPLC and GC	286
Reducing sugars	Candy	Fehling's solution	287
Aldoses, ketoses	Serum	GC–MS of methoxylated TMS ethers on glass WCOT columns	288
Ribose, xylose, lyxose, fucose glucose, mannose, sorbose, etc.	[Enantiomer separation]	GC of menthyloxime pertrifluoroacetates on WCOT columns	289
Rhamnose, fucose, ribose, xylose, mannose, galactose, glucose, etc.	[Column activity tests]	GC of alditol acetates on glass WCOT columns	290
Fructose, glucose, lactose, sucrose	Yogurts	HPLC on amine-bonded phase	291
Glucose, sucrose, fructose, maltose	Pizza extract	HPLC using ion-pairing reagent	292
Fructose, glucose sucrose	Grape musts	GC of TMS ethers (oximes)	293
Glucose, fructose, mannitol, xylose, rhamnose and saccharose	Green olives	PC	294
Fructose, glucose, sucrose, lactose, maltose and sorbitol	Food	GC of TMS-oximes	295
Fructose, glucose, lactose, sucrose, mannitol	Narcotics	GC of TMS ethers on glass WCOT columns	146
Monosaccharides	Plant cell wall hydrolysates	GC of alditol acetates on glass SCOT columns	150

Fructose, glucose, sucrose, maltose, maltotriose	Wort, beer	HPLC using an *in situ*-modified amine column	296
Sugars	[On-column reactions and their influence on elution]	HPLC	297
Glucose, fructose, sucrose	Honey	HPLC	298
Maltose, maltulose, isomaltose	High-fructose syrups	PC	299
Glucitol, galactitol, mannitol, xylitol, arabinitol	[Relation between structure and retention time]	GC of methylated alditol acetates on packed columns	300
Arabinose, mannose, allose, fucose, ribose, xylose, glucose, galactose, etc.	Not applied	GC of alditol acetates on fused-silica WCOT columns	301
Sugars	Infant foods	TLC	302
Glucose, galactose	–	HPLC on anion exchanger and amine-bonded phase, TLC	303
Xylose, arabinose	[On-column interaction of aldose and NH_2 groups]	HPLC on *in situ*-modified amine phase	304
Xylose	–	HPLC on anion exchanger	305
Mono-, di- and oligosaccharides	Physiological fluids	HPLC on silica	306
Reducing sugars	Wood hydrolysate, beverages	HPLC of dansyl derivatives on an ODS column	212
Fructose, maltose, glucose, sucrose, raffinose	–	TLC on silica gel	307
Glucose, mannose, galactose, fucose, arabinose, xylose, glucosamine, 2,5-anhydromannose	Chitin, ovalbumin, horseradish peroxidase	GC of alditol acetates on glass WCOT columns	308
Rhamnose, fucose, ribose, xylose, mannose, glucose, galactose, etc.	Bacterial cell walls	GC of alditol acetates on packed and WCOT columns	309
Sugar alcohols	Not applied	GC of alditol acetates on fused-silica WCOT columns	310

(Continued on p. 98)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref.*
Sugars	Foods	Comparison of GC and HPLC	311
Fructose, glucose, sucrose	[Thermodynamic parameters]	HPLC on amine and cobalt(III) complex-bonded phases	199
Aldoses, alditols	–	GC of TMS ethers and alditol acetates on packed columns	312
Mono- and disaccharides	Beverages	HPLC on an amine-bonded phase	313
Fructose, glucose, sucrose	Fruit juices	HPLC on an amine column	314
Sugars	[NaCl interference]	HPLC on aminopropyl columns	315
Galactose, saccharose, maltose, fructose, raffinose, glucose, lactose	–	TLC on phosphate-impregnated Kieselguhr and silica gel	316
Arabinose, lyxose, mannose, ribose, glucose, fucose, galactose, xylose	Gum arabic, gum tragacanth	GC of trifluoroacetylated derivatives	317
Sucrose, glucose, fructose	Molasses	HPLC on several columns	176
Reducing sugars	Beer	HPLC on amine-bonded phase	177
Monosaccharides	[Pre-column derivatisation]	HPLC on amine-bonded phases	189,190
Monosaccharides	[Plasticizers]	GC of alditol acetates on SCOT and WCOT columns	151
Fructose, galactose, glucose, sucrose, lactose, maltose	Yogurt	GC of TMS ethers on packed columns	139
Glucose, fructose, xylitol	Infusion solution	Colorimetry	318
Sugars	Confectionery	Improved HPLC method	319
Fructose, sucrose, glucose, glycerol, ethanol	Wines	HPLC	320
Fructose, glucose, sucrose, maltose, glycerol	Food, beverages	HPLC on amine-modified silica columns	321

Dextrose, fructose, glycerol, ethanol	Wines	HPLC on cation exchanger	322
Sucrose, glucose fructose	Plant tissue	HPLC on cation exchanger	323
Galactose, glucose, lactose, fructose, maltose, sucrose	Infant foods	TLC on silica gel	324
Glucose, sucrose, lactose, opiates	Illicit heroin	HPLC on silica	325
Mono- and disaccharides	[Electrochemical detection]	HPLC	326
Glucose, fructose, sorbitol, lactic acid, citric acid	Infusion solutions	HPLC	327
Glucose, galactose, saccharose, maltose, fructose, raffinose, lactose	–	TLC on Kieselguhr	328
Maltose, glucose	Dough	HPLC	329
Glucose, galactose, rhamnose	–	TLC of dansylated sugars on polyamide	330
Monosaccharides	Plankton, wood sediments	GC of TMS ethers on fused-silica WCOT columns	84
Mono-, di- and trisaccharides	–	TLC on aminopropyl-bonded-phase silica	58,59
Reducing sugars	[Flow injection analysis]	HPLC on amine-bonded phase	331
Glucose	[Electrochemical detection]	HPLC	332
Glucose, fructose, inositol, sorbitol, mannitol	Lens, erythrocytes, plasma	HPLC on silica	191
Sucrose, lactose, glucose, galactose, fructose	Yogurt	HPLC of cation exchanger (Ca^{2+})	333
Lactose, glucose, galactose	Whey, whey permeate	HPLC on exclusion resin	334
Aldoses	[Anomer separation]	HPLC on cation exchanger	185
Glucose, fructose, sugar alcohols	Lens	GC of trifluoroacetates on WCOT columns	335
Sugars	Fruit juices	HPLC	336
Fructose, glucose, sucrose, maltose	Licorice	HPLC on amine-bonded phase	172
Sucrose, raffinose, invert sugar	–	Reversed-phase HPLC	337
Mono- to tetrasaccharides	Foods	Normal- and reversed-phase HPLC	338

(Continued pn p. 100)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref.*
Reducing sugars	Infusions	TLC on cellulose and HPLC	339
Reducing sugars, sucrose, raffinose, sorbitol	Almonds	GC of TMS ethers on packed columns	340
Fructose, glucose, sucrose maltose, maltotriose	Beer, wort	GC of TMS ethers on packed columns	341
Aldoses	Glycoproteins	HPLC on cation exchanger	342
Sucrose, glucose, fructose	Plant material	TLC on silica gel	54
Glucose, fructose, sucrose, raffinose	Wheat tissue	GC of TMS ethers on packed columns	343
Sucrose	[Adsorption]	GC of TMS ethers on glass WCOT columns	147
Fructose, glucose, sucrose	Nectars	GC of TMS oximes on packed columns	125
Neutral and amino sugars	Microorganisms	GC of alditol acetates on fused-silica WCOT columns	152
Sugar alcohols	Anhydride formation	GC	344
Sucrose, glucose, fructose, raffinose	Sugar beet leaves	HPLC on cation exchanger (Ca^{2+})	345
Reducing sugars	[Pressurized reactor outlet]	HPLC	346
Erythrose, ribose, arabinose, fructose, glucose	Formosation reaction mixtures	HPLC on C_{18} column	347
Sucrose, glucose, galactose	Plants	TLC	348
Sucrose, maltose, raffinose	Beer, malt	HPLC on cation exchanger (Ca^{2+})	349
Sugars	[Retention mechanisms on copper(II)-modified silica gel]	LC	350
Mono- and disaccharides, sugar alcohols	[Details of several systems]	HPLC	351

Sucrose, fructose, glucose	Juices, wines, molasses	HPLC on cation exchanger (Cu^{2+})	352
Raffinose, oligosaccharides	Soy beans	GC of TMS oximes on packed columns	134,353
Neutral and amino sugars	Glycoconjugates	Reversed-phase HPLC	354
Glucose, (xylose)	Body fluids	HPLC on ODS column	180
Disaccharides	[Relation between structure and elution time]	HPLC on amine-bonded phase	179
Glucose-1-phosphate, sucrose, fructose, phosphate	–	HPLC on cation exchanger	187
Glucose, fructose, ribose, mannose, galactose, sugar alcohols	Plasma, amniotic fluid	HPLC on cation exchanger	184
Sugars	Diet composite	GC and HPLC	355
Fructose, glucose, mannose, sugar alcohols	Fructose–mannitol conversion mixture	HPLC on cation exchanger	356
Mono- and disaccharides	[Anomer separation]	HPLC on amine-bonded phase	178
Fructose, glucose, raffinose, sucrose, stachyose, cellobiose	[Detection systems]	HPLC on cation exchangers	183
Monosaccharides	Biomass	HPLC on several columns	186
Reducing sugars	[Optimisation of electrochemical detection]	HPLC on several columns	188
Dextrose, levulose, sucrose	Invert sugar	HPLC on cation exchanger	174
Alcohols, aldehydes, ketones, acids, sugars	[Retention behaviour]	HPLC on cation exchanger	182
Aldoses	Sap of lac trees	GC of alditol acetates on fused-silica WCOT columns	205,357
Glucose, xylose, galactose, arabinose, mannose, erythritol	Wood and pulp hydrolysate	HPLC and PC	358

in elution profile effected by alteration of the metal counterion of cation exchangers suggests that an additional mechanism is involved. Goulding[203] postulated that this was a ligand-exchange interaction between the water molecules in the aquated metal ion and the hydroxyl groups of the aldose molecules.

During the course of liquid chromatography mutarotation occurs[19] thereby restricting the separation of sugar anomers. However, successful separations have been achieved[204] on an amino-bonded phase and a phosphoric acid-modified amino packing using low column temperatures to suppress anomerization. In contrast, several anomers have been separated[205] at ambient temperatures using anion-exchange chromatography.

Normal-phase partition and adsorption chromatography have been applied to carbohydrates (Table 3) generally following pre-column derivatisation to perbenzoates[206,207], per-*p*-nitrobenzoates[208], per(dimethylphenylsilyl)ethers[189,190], peracetates[209] and pernaphthoates[210]. However, the need for derivatisation has usually been related to the requirements of detection[180,189–191] and not to altering the chromatographic behaviour of the sugars by endowing them with hydrophobic properties. Dansyl hydrazone derivatives[211,212] have been chromatographed on reversed-phase[213] and normal-phase[214] systems whereas adsorption chromatography of the N-acetyl-α-methoxybenzyl derivatives of glycamines has enabled separation of D- and L-enantiomers of aldoses[215].

Glycamine derivatives[216] can be separated by cation exchange and finally, underivatised sugars have been chromatographed on the bonded-phase anion-exchange resin, Bondapak AX/Corasil, using mixtures of acetone, ethanol and water[217] and ethyl acetate, propan-2-ol and water[218] as mobile phases.

4.1. Detectors

Detection systems for carbohydrates have recently been reviewed by Honda[10] and for this reason only a brief overview is presented here. However, choice of detection system is an important consideration in HPLC of sugars because, unlike GC, there is no universal LC detector and carbohydrates lack chromophoric and fluorophoric groups necessary for UV and fluorescence detection. Indeed, problems with detection have limited[219] the application of HPLC to carbohydrate analysis. Refractivity measurement is suitable but is highly susceptible to changes of column temperature and solvent composition. Moreover, the low sensitivity of refractive index detectors restricts application to relatively concentrated samples[15], such as soft drinks, confectionery and syrups.

Detection based on absorbance in the near-ultraviolet (180–210 nm)[170,184] is non-selective and involves high capital and running costs due to the requirement for expensive instrumentation and mobile phases. Pre-column[180] and post-column[177] derivatisation to provide colorimetric[174], UV-absorbing[191] or fluorescent[169] species provides the best means of detection with currently available instrumentation.

5. APPLICATIONS

Table 4 summarises the chromatographic analyses of mono- and disaccharides cited by *Chemical Abstracts* during the period January 1980 to June 1985. An attempt has been made also to indicate the range of non-chromatographic techniques which

have been applied during this same period. The distribution of citations between the various chromatographic techniques for the period was 52% HPLC, 31% GC, 14% TLC and 3% PC. The greater use of HPLC relative to GC is even more pronounced when considering foods and clinical–biochemical applications. For foodstuffs, practical considerations strongly favour the use of HPLC over GC since component sugars of foods are generally present in adequate concentrations to be detected by the less sensitive detectors of HPLC. On the other hand, biochemical applications are more likely to require the greater sensitivity of GC detectors. The increasing use of pre- and post-column derivatisation techniques in HPLC coupled with UV and fluorescence detectors has had an obvious impact. However, the main advantage of HPLC, namely, the ability to inject directly with little or no sample preparation, is partially eliminated by pre- and post-column derivatisation.

6. SUMMARY

Chromatographic procedures for measuring monosaccharides and disaccharides are reviewed. Although gas and high-performance liquid chromatographic methods predominate, interest continues in the older techniques using paper and thin layers. However, the most significant developments of the last decade are the increasing use of open tubular columns and bonded-packings based on silica.

REFERENCES

1 D. T. Plummer, *An Introduction to Biochemistry*, McGraw-Hill, Maidenhead, 1978, pp. 173–192.
2 L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis*, Wiley, New York, 1967, p. 815.
3 H. U. Bergmeyer and E. Bernt, *Methods of Enzymatic Analysis*, Academic Press, New York, 1963, p. 127.
4 J. M. Sempere, C. Gancedo and C. A. Senio, *Anal. Biochem.*, 12 (1965) 509.
5 W. Postel, F. Drawert and W. Hagen, *Dtsch. Lebensm.-Rundsch.*, 67 (1971) 107.
6 K. Zurcher and A. Hadorn, *Mitt. Geb. Lebensmittelunters. Hyg.*, 68 (1977) 200.
7 E. Heftmann, *Chromatography*, Van Nostrand-Reinhold, New York, 1967, Ch. 20.
8 G. J. Dickes and P. V. Nicholas, *Gas Chromatography in Food Analysis*, Butterworth, London, 1976, Ch. 15.
9 M. F. Laker, *J. Chromatogr.*, 184 (1980) 457.
10 S. Honda, *Anal. Biochem.*, 140 (1984) 1.
11 E. V. Evtushenko and Yu S. Ovodov, *Khim. Prir. Soedin.*, (1984) 411.
12 E. H. Reimerdes and K. D. Rothkitt, *Fresenius' Z. Anal. Chem.*, 318 (1984) 220.
13 D. Patoraux-Prome and J. C. Prome, *Gas Chromatography–Mass Spectroscopy: Applications in Microbiology*, Plenum, New York, 1984, pp. 105–156.
14 A. Soerensen and M. Guldborg, *Publ. — Statens Levnedsmiddelinst. (Den.)*, 104 (1984) 54 pp.
15 K. J. Schaffler and P. G. Morel du Boil, *Sugar Technol. Rev.*, 11 (1984) 95.
16 S. M. Partridge, *Nature (London)*, 158 (1947) 270.
17 S. M. Partridge, *Biochem. J.*, 42 (1948) 238.
18 C. K. Hordis and G. N. Kowkabany, *Anal. Chem.*, 30 (1958) 1210.
19 H. Jaeger, A. Ramel and O. Schindler, *Helv. Chim. Acta*, 40 (1957) 1310.
20 R. J. Ferrier and P. M. Collins, *Monosaccharide Chemistry*, Penguin, Harmondsworth, London, 1972, pp. 293–297.
21 A. E. Flood, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, (1948) 1679.
22 F. A. Isherwood and M. A. Jermyn, *Biochem. J.*, 48 (1951) 515.
23 M. F. Levy, *Anal. Chem.*, 26 (1954) 1849.
24 A. Jeanes, C. S. Wise and R. J. Dimler, *Anal. Chem.*, 23 (1951) 415.
25 J. E. Courtois, *Mises au Point de Chimie Analytique*, Vol. IV, Masson, Paris, 1956, p. 1.
26 M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, 44 (1949) 402.

27 L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, (1950) 1702.
28 J. E. Courtois and U. Ariyoshi, *C.R. Hebd. Seances Acad. Sci.*, 250 (1960) 1369.
29 I. Smith, *Chromatographic and Electrophoretic Techniques, Vol. 1, Chromatography*, William Heinemann, Bath, 1969, p. 311.
30 M. Piccardo, L. Russo and M. Rosa, *Boll.-Soc. Ital. Biol. Sper.*, 55 (1979) 1039.
31 G. Caldes, B. Prescott and P. J. Baker, *J. Chromatogr.*, 234 (1982) 264.
32 L. I. Trusova and L. Ya Telishevskaya, *Tr., Vses. Gos. Nauchno-Kontrol'n. Inst. Vet. Prep.*, (1980) 29, 114.
33 D. M. Alperin, V. P. Idoyaga-Vargas and H. Carminatti, *J. Chromatogr.*, 242 (1982) 299.
34 P. W. Simon and R. E. Freeman, *Hort. Science*, 20 (1985) 133.
35 J. C. Touchstone, *Quantitative Thin Layer Chromatography*, Wiley, New York, 1973, p. 81.
36 A. Schweiger, *J. Chromatogr.*, 9 (1962) 374.
37 M. L. Wolfrom, D. L. Patin and R. M. de Lederkremer, *J. Chromatogr.*, 17 (1965) 488.
38 J. W. Walkley and J. Tillman, *J. Chromatogr.*, 132 (1977) 172.
39 M. Hoton-Dorge, *J. Chromatogr.*, 116 (1976) 417.
40 B. A. Lewis and F. Smith, in E. Stahl (Editor), *Thin Layer Chromatography*, George Allen and Unwin, London, 1969, p. 807.
41 I. Smith, *Chromatographic and Electrophoretic Techniques, Vol. 1, Chromatography*, William Heinemann, Bath, 1969, p. 318.
42 H. Grasshof, *Dtsch. Apotheker-Z.*, 103 (1963) 1396.
43 P. Barbetti and S. C. Leopizzi, *Ann. Chim. (Rome)*, 65 (1975) 403.
44 E. Stahl and U. Kaltenbach, *J. Chromatogr.*, 5 (1961) 351.
45 K. C. Guven, *Acta Pharm. Turc.*, 27 (1985) 1.
46 V. Prey, H. Berbalk and M. Kausz, *Mikrochim. Acta*, (1961) 968.
47 G. Pastuska, *Fresenius' Z. Anal. Chem.*, 179 (1961) 427.
48 F. Micheel and O. Berendes, *Mikrochim. Acta*, (1963) 519.
49 G. W. Hay, B. A. Lewis and F. Smith, *J. Chromatogr.*, 11 (1963) 479.
50 S. A. Hansen, *J. Chromatogr.*, 107 (1975) 224.
51 B. B. Pruden, G. Pineault and H. Loutfi, *J. Chromatogr.*, 115 (1975) 477.
52 R. Gauch, U. Leuenberger and E. Baumgartner, *J. Chromatogr.*, 174 (1979) 195.
53 Ž. Žilić, N. Blau and M. Knob, *J. Chromatogr.*, 164 (1979) 91.
54 P. Macuha and A. Chudziak, *Pol'nohospodarstvo*, 30 (1984) 774.
55 A. I. Makarov, *Fiziol. Biokhim. Kul't. Rast.*, 16 (1984) 292.
56 L. Wassermann and H. Hanus, *Naturwissenschaften*, 50 (1963) 351.
57 V. Prey, H. Scherz and E. Bancher, *Mikrochim. Acta*, (1963) 567.
58 L. W. Doner and L. M. Biller, *J. Chromatogr.*, 287 (1984) 391.
59 L. W. Doner, C. L. Fogel and L. M. Biller, *Carbohydr. Res.*, 125 (1984) 1.
60 H. Weicker and R. Brossmer, *Klin. Wochenschr.*, 39 (1961) 1265.
61 E. Guilloux and S. Beaugiraud, *Bull. Soc. Chim. Fr.*, (1965) 261.
62 H. Jacin and A. R. Mishkin, *J. Chromatogr.*, 18 (1965) 170.
63 P. G. Pifferi, *Anal. Chem.*, 37 (1965) 925.
64 G. G. Dutton, K. B. Gibney, P. E. Reid and K. N. Slessor, *J. Chromatogr.*, 20 (1965) 163.
65 H. Ertel and L. Horner, *J. Chromatogr.*, 7 (1962) 268.
66 S. Adachi, *J. Chromatogr.*, 17 (1965) 295.
67 K. Y. Lee, D. Nurok and A. Zlatkis, *J. Chromatogr.*, 174 (1979) 187.
68 C. T. Greenwood, J. H. Knox and E. Milne, *Chem. Ind. (London)*, (1961) 1878.
69 A. G. McInnes, D. H. Ball, F. P. Cooper and C. T. Bishop, *J. Chromatogr.*, 1 (1958) 556.
70 H. W. Kircher, *Anal. Chem.*, 32 (1960) 1103.
71 C. T. Bishop, *Methods Biochem. Anal.*, 10 (1962) 1.
72 D. Anderle, P. Kováč and H. Anderlová, *J. Chromatogr.*, 64 (1972) 368.
73 B. Fournet and J. Montreuil, *J. Chromatogr.*, 75 (1973) 29.
74 W. J. Lewicki and J. R. Edwards, *Anal. Lett.*, 3 (1970) 151.
75 R. M. Thompson, R. D. Coffin, M. R. Glick and J. F. Fitzgerald, *Clin. Chim. Acta*, 84 (1978) 185.
76 S. W. Gunner, J. K. W. Jones and M. B. Perry, *Can. J. Chem.*, 39 (1961) 1892.
77 J. S. Sawardeker, J. H. Sloneker and A. Jeanes, *Anal. Chem.*, 37 (1965) 1602.
78 W. J. A. Vanden Heuvel and E. C. Horning, *Biochem. Biophys. Res. Commun.*, 4 (1961) 399.
79 H. G. Jones and M. B. Perry, *Can. J. Chem.*, 40 (1962) 1339.
80 E. Pitkanen, *Clin. Chim. Acta*, 38 (1972) 221.

81 H. Schwind, F. Scharbert, R. Schmidt and R. Katterman, *J. Clin. Chem. Clin. Biochem.*, 16 (1978) 145.
82 L. J. Griggs, A. Post, E. R. White, J. A. Finkelstein, W. E. Moeckel, K. G. Holden, J. E. Zarembo and J. A. Weisbach, *Anal. Biochem.*, 43 (1971) 369.
83 I. M. Campbell and R. Bentley, in R. F. Gould (Editor), *Advances in Chemistry Series, Carbohydrates in Solution, ACS Symposium*, American Chemical Society, Washington, DC, 1971, pp. 1–19.
84 G. L. Cowie and J. I. Hedges, *Anal. Chem.*, 56 (1984) 497.
85 H. Jacin, J. M. Slanski and R. J. Moshy, *J. Chromatogr.*, 37 (1968) 103.
86 T. E. Acree, R. S. Shallenberger, C. Y. Lee and J. W. Einset, *Carbohydr. Res.*, 10 (1969) 355.
87 T. E. Acree, R. S. Shallenberger and L. R. Mattick, *Carbohydr. Res.*, 6 (1968) 498.
88 G. G. S. Dutton, *Adv. Carbohydr. Chem. Biochem.*, 30 (1973) 38.
89 D. Murphy and C. A. Pennock, *Clin. Chim. Acta*, 42 (1972) 67.
90 E. J. Bourne, C. E. M. Tatlow and J. C. Tatlow, *J. Chem. Soc.*, (1950) 1367.
91 M. Vilkas, H.-I. Jan, G. Boussac and M.-C. Bonnard, *Tetrahedron Lett.*, (1966) 1441.
92 J. Shapira, T. Putkey, A. Furst and G. A. McCasland, *Carbohydr. Res.*, 25 (1972) 535.
93 Z. Tamura and T. Imanari, *Chem. Pharm. Bull.*, 15 (1967) 246.
94 J. P. Zanetta, W. C. Breckenridge and G. Vincendon, *J. Chromatogr.*, 69 (1972) 291.
95 M. A. Luke, *J. Assoc. Off. Anal. Chem.*, 54 (1971) 937.
96 J. E. Sullivan and L. R. Schewe, *J. Chromatogr. Sci.*, 15 (1977) 196.
97 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497.
98 W. W. Wells, T. Chin and B. Weber, *Clin. Chim. Acta*, 10 (1964) 352.
99 T. Bhatti and J. R. Clamp, *Clin. Chim. Acta*, 22 (1968) 563.
100 J. de Neef, *Clin. Chim. Acta*, 26 (1969) 485.
101 J. Lehrfeld, *J. Chromatogr. Sci.*, 9 (1971) 757.
102 A. H. Weiss and H. Tambawala, *J. Chromatogr. Sci.*, 10 (1972) 120.
103 K. M. Brobst and C. E. Lott, *Cereal Chem.*, 43 (1966) 35.
104 R. Bentley and N. Botlock, *Anal. Biochem.*, 20 (1967) 312.
105 W. C. Butts and R. L. Jolley, *Clin. Chem.*, 16 (1970) 722.
106 T. Vander Meulen and R. A. Hudson, *Anal. Biochem.*, 53 (1973) 639.
107 K. Osaka, M. Kuboyama, I. Kiyosawa, T. Suzaki and M. Itoh, *J. Nutr. Sci. Vitaminol.*, 21 (1975) 129.
108 S. L. Smith, M. Novotny and E. L. Weber, *Clin. Chem.*, 24 (1978) 545.
109 M. F. Laker and J. N. Mount, *Clin. Chem.*, 26 (1980) 441.
110 M. F. Laker and W. G. Gunn, *Clin. Chim. Acta*, 96 (1979) 265.
111 M. F. Laker, *J. Chromatogr.*, 163 (1979) 9.
112 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, IL, 1968.
113 C. Petersson, *Carbohydr. Res.*, 33 (1974) 47.
114 E. C. Horning and M. G. Horning, *Methods Med. Res.*, 12 (1970) 396.
115 P. E. Reid, B. Donaldson, D. W. Secret and B. Bradford, *J. Chromatogr.*, 47 (1970) 199.
116 W. W. Wells, *Methods Med. Res.*, 12 (1970) 115.
117 D. V. Phillips and A. E. Smith, *Anal. Biochem.*, 54 (1973) 95.
118 A. Ericsson, J. Hansen and L. Dalgaard, *Anal. Biochem.*, 86 (1978) 552.
119 E. P. Crowell and B. B. Burnett, *Anal. Chem.*, 39 (1967) 121.
120 L. G. Borchardt and C. V. Piper, *Tappi*, 53 (1970) 257.
121 G. Holzer, J. Oró, S. J. Smith and V. M. Doctor, *J. Chromatogr.*, 194 (1980) 410.
122 M. Ochiai, *J. Chromatogr.*, 194 (1980) 224.
123 M. S. Sweet and E. M. Perdue, *Environ. Sci. Technol.*, 16 (1982) 692.
124 B. S. Mason and H. T. Slover, *J. Agric. Food. Chem.*, 19 (1971) 551.
125 B. D. Tanowitz and D. M. Smith, *Ann. Bot.*, 53 (1984) 453.
126 W. R. Supina, R. F. Kruppa and R. Henley, *J. Am. Oil Chem. Soc.*, 44 (1967) 965.
127 J. R. Clamp, *Biochem. Soc. Trans.*, 5 (1977) 1693.
128 J. R. Clamp, *Biochem. Soc. Symp.*, 40 (1974) 3.
129 P. Størset, O. Stokke and E. Jellum, *J. Chromatogr.*, 145 (1978) 351.
130 F. Eisenberg, *Carbohydr. Res.*, 19 (1971) 135.
131 I. M. Morrison and M. B. Perry, *Can. J. Biochem.*, 44 (1966) 1115.
132 B. W. Li, P. J. Schuhmann and J. M. Holden, *J. Agric. Food Chem.*, 31 (1983) 985.
133 T. Cayle, F. Viebrock and J. Schiaffino, *Cereal Chem.*, 45 (1968) 154.
134 I. Molnar-Perl, M. Pinter-Szakacs, A. Kövágó and J. Petróczy, *J. Chromatogr.*, 295 (1984) 433.

135 K. Uobe, K. Nishida, H. Inoue and M. Tsutsui, *J. Chromatogr.*, 193 (1980) 83.
136 G. A. Janauer and P. Englmaier, *J. Chromatogr.*, 153 (1978) 539.
137 B. Mueller and G. Goeke, *Dtsch. Lebensmitt.-Rundsch.*, 68 (1972) 222.
138 K. M. Brobst and C. E. Lott, *Proc. Am. Soc. Brew. Chem.*, (1966) 71.
139 D. J. Heaf and D. J. Galton, *Clin. Chim. Acta*, 63 (1975) 41.
140 J. F. Aloia, *J. Lab. Clin. Med.*, 82 (1973) 809.
141 C. W. Gehrke, T. P. Waalkes, E. Borek, W. F. Swartz, T. F. Cole, K. C. Kuo, M. Abeloff, D. S. Ettinger, N. Rosenshein and R. C. Young, *J. Chromatogr.*, 162 (1979) 507.
142 H. Nakamura and Z. Tamura, *Clin. Chim. Acta*, 39 (1972) 367.
143 G. J. Manius, T. M. Y. Lui and L.-F. L. Wen, *Anal. Biochem.*, 99 (1979) 365.
144 P. G. Morel du Boil and K. J. Schäffler, *Proc. S. African Sug. Technol. Assoc.*, (1978) 96.
145 K. J. Schäffler and P. G. Morel du Boil, *J. Chromatogr.*, 207 (1981) 221.
146 I. Barni Comparini, F. Centini and A. Pariali, *J. Chromatogr.*, 279 (1983) 609.
147 H. Traitler, S. Del Vedovo and T. F. Schweizer, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 558.
148 A. G. Bradbury, D. J. Halliday and D. G. Medcalf, *J. Chromatogr.*, 213 (1981) 146.
149 J. Klok, E. H. Nieberg-van Velzen, J. W. de Leeuw and P. A. Schenck, *J. Chromatogr.*, 207 (1981) 273.
150 A. B. Blakeney, P. J. Harris, R. J. Henry and B. A. Stone, *Carbohydr. Res.*, 113 (1983) 291.
151 R. J. Henry, P. J. Harris, A. B. Blakeney and B. A. Stone, *J. Chromatogr.*, 262 (1983) 249.
152 M. D. Walla, P. Y. Lau, S. L. Morgan, A. Fox and A. Brown, *J. Chromatogr.*, 288 (1984) 399.
153 C. Green, V. M. Doctor, G. Holzer and J. Oró, *J. Chromatogr.*, 207 (1981) 268.
154 J. Szafranek, C. D. Pfaffenberger and E. C. Horning, *Anal. Lett.*, 6 (1973) 479.
155 C. D. Pfaffenberger, J. Szafranek, M. G. Horning and E. C. Horning, *Anal. Biochem.*, 63 (1975) 501.
156 C. D. Pfaffenberger, J. Szafranek and E. C. Horning, *J. Chromatogr.*, 126 (1976) 535.
157 H. Frank, H. J. Chaves das Neves and E. Bayer, *J. Chromatogr.*, 207 (1981) 213.
158 W. S. Reich, *C.R. Hebd. Seances Acad. Sci.*, 208 (1939) 589.
159 L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, (1949) 2511.
160 R. Dedonder, *Bull. Soc. Chim. Biol.*, 34 (1952) 144.
161 J. X. Khym and L. P. Zill, *J. Am. Chem. Soc.*, 73 (1951) 2399.
162 J. K. N. Jones and R. A. Wall, *Can. J. Chem.*, 38 (1960) 2290.
163 A. Tiselius, *Ark. Kemi. Mineral. Geol.*, 14B (1940) 4.
164 R. L. Whistler and D. F. Durso, *J. Am. Chem. Soc.*, 72 (1950) 677.
165 R. S. Alm, *Acta Chem. Scand.*, 6 (1952) 1186.
166 J. S. Hobbs and J. G. Lawrence, *J. Sci. Food Agric.*, 23 (1972) 45.
167 D. J. Folkes and P. W. Taylor, in R. Macrae (Editor), *HPLC in Food Analysis*, Academic Press, London, 1982, p. 150.
168 J. X. Khym and L. P. Zill, *J. Am. Chem. Soc.*, 74 (1952) 2090.
169 S. Honda, M. Takahashi, K. Kakehi and S. Ganno, *Anal. Biochem.*, 113 (1981) 130.
170 L. A. T. Verhaar and J. M. H. Kirkx, *Carbohydr. Res.*, 62 (1978) 197.
171 J. F. Kennedy and J. E. Fox, *Carbohydr. Res.*, 54 (1977) 13.
172 P. S. Vora and R. M. Tuorto, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 764 and 529.
173 R. B. Meagher and A. Furst, *J. Chromatogr.*, 117 (1976) 211.
174 M. Wnukowski, *Int. Sugar J.*, 86 (1984) 170.
175 H. Binder, *J. Chromatogr.*, 189 (1980) 414.
176 E. Rajakylä and M. Paloposki, *J. Chromatogr.*, 282 (1983) 595.
177 T. D. Schlabach and J. Robinson, *J. Chromatogr.*, 282 (1983) 169.
178 M. Moriyasu, A. Kato, M. Okada and Y. Hashimoto, *Anal. Lett.*, 17 (1984) 689.
179 Z. L. Nikolov, M. M. Meagher and P. J. Reilly, *J. Chromatogr.*, 319 (1985) 51.
180 D. A. Finden, R. R. Fysh and P. C. White, *J. Chromatogr.*, 342 (1985) 179.
181 T. G. Lawrence, *Chimia*, 29 (1975) 367.
182 R. Pecina, G. Bonn, E. Burtscher and O. Bobleter, *J. Chromatogr.*, 287 (1984) 245.
183 P. Vrátný, R. W. Frei, U. A. Th. Brinkman and M. W. Nielen, *J. Chromatogr.*, 295 (1984) 355.
184 J. A. Owens and J. S. Robinson, *J. Chromatogr.*, 338 (1985) 303.
185 S. Honda, S. Suzuki and K. Kakehi, *J. Chromatogr.*, 291 (1984) 317.
186 G. Bonn, R. Pecina, E. Burtscher and O. Bobleter, *J. Chromatogr.*, 287 (1984) 215.
187 R. M. Stikkelman, T. T. Tjioe, J. P. van der Wiel and F. van Rantwijk, *J. Chromatogr.*, 322 (1985) 220.

188 S. Honda, T. Konishi and S. Suzuki, *J. Chromatogr.*, 299 (1984) 245.
189 C. A. White, S. W. Vass, J. F. Kennedy and D. G. Large, *J. Chromatogr.*, 264 (1983) 99.
190 C. A. White, S. W. Vass, J. F. Kennedy and D. G. Large, *Carbohydr. Res.*, 119 (1983) 241.
191 M. Petchey and M. J. Crabbe, *J. Chromatogr.*, 307 (1984) 180.
192 G. P. Belue, *J. Chromatogr.*, 100 (1974) 233.
193 H. van Olst and G. E. Joosten, *J. Liq. Chromatogr.*, 2 (1979) 111.
194 R. S. Ward and A. Pelter, *J. Chromatogr. Sci.*, 12 (1974) 570.
195 R. Schwarzenbach, *J. Chromatogr.*, 117 (1976) 206.
196 J. C. Linden and C. L. Lawhead, *J. Chromatogr.*, 105 (1975) 125.
197 D. L. Hendrix, R. E. Lee, Jr., J. G. Baust and H. James, *J. Chromatogr.*, 210 (1981) 45.
198 F. M. Rabel, A. G. Caputo and E. T. Butts, *J. Chromatogr.*, 126 (1976) 731.
199 C. A. Chang, *Anal. Chem.*, 55 (1983) 971.
200 R. W. Yost, L. S. Ettre and R. D. Conlon, *Practical Liquid Chromatography — An Introduction*, Perkin-Elmer, Norwalk, CT, 1980, p. 79.
201 P. Jonsson and O. Samuelson, *Anal. Chem.*, 39 (1967) 1156.
202 E. Martinsson and O. Samuelson, *J. Chromatogr.*, 50 (1970) 429.
203 R. W. Goulding, *J. Chromatogr.*, 103 (1975) 229.
204 V. Kahle and K. Tesařik, *J. Chromatogr.*, 191 (1980) 121.
205 R. Oshima, N. Takai and J. Kumanotani, *J. Chromatogr.*, 192 (1980) 452.
206 C. A. White, J. F. Kennedy and B. T. Golding, *Carbohydr. Res.*, 76 (1979) 1.
207 R. Oshima and J. Kumanotani, *J. Chromatogr.*, 265 (1983) 335.
208 F. Nachtmann and K. W. Budna, *J. Chromatogr.*, 136 (1977) 279.
209 J. Thiem, J. Schwentner, H. Karl, A. Sievers and J. Reimer, *J. Chromatogr.*, 155 (1978) 107.
210 J. Golik, H. W. Liu, M. Dinovi, J. Furukawa and K. Nakanishi, *Carbohydr. Res.*, 118 (1983) 135.
211 H. Iwase, S.-C. Li and Y.-T. Li, *J. Chromatogr.*, 267 (1983) 238.
212 K. Mopper and L. Johnson, *J. Chromatogr.*, 256 (1983) 27.
213 W. F. Alpenfels, *Anal. Biochem.*, 144 (1981) 153.
214 M. Takeda, M. Maeda and A. Tsuji, *J. Chromatogr.*, 244 (1982) 347.
215 R. Oshima, Y. Yamauchi and J. Kumanotani, *Carbohydr. Res.*, 107 (1982) 169.
216 F. Perini and B. P. Peters, *Anal. Biochem.*, 123 (1982) 357.
217 H. Hyakutake and T. Hanai, *J. Chromatogr.*, 108 (1975) 385.
218 J. N. Little, D. F. Horgan, R. L. Cotter and R. V. Vivilecchia, in E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, MI, 1974, p. 54.
219 A. Pryde and M. T. Gilbert, *Applications of High Performance Liquid Chromatography*, Cambridge University Press, Cambridge, 1979, p. 136.
220 S. Farag, *J. Am. Soc. Sugar Beet Technol.*, 20 (1979) 251.
221 H. A. Aluyi and D. B. Drucker, *J. Chromatogr.*, 178 (1979) 209.
222 J. W. De Vries, J. C. Heroff and D. C. Egberg, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 1292.
223 Y. N. Elkin, *J. Chromatogr.*, 180 (1979) 163.
224 S. Honda, N. Yamauchi, S. Suzuki and K. Kakehi, *Koen Yoshishu-Seitai Seibun no Bunseki Kagaku Shimpojumu*, 4th. (1979) 65; *C.A.*, 92 (1980) 176702u.
225 N. E. Anderson and F. M. Clydesdale, *J. Food Sci.*, 45 (1980) 336.
226 M. D'Amboise, D. Noel and T. Hanai, *Carbohydr. Res.*, 79 (1980) 1.
227 S. Honda, Y. Matsuda, M. Takahashi, K. Kakehi and S. Ganno, *Anal. Chem.*, 52 (1980) 1079.
228 N. Iizima, M. Fujihara and T. Nagumo, *J. Chromatogr.*, 193 (1980) 464.
229 J. Dokladalova, A. Y. Barton and E. A. Mackenzie, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 664.
230 Y. Vaysse, P. Pilardeau and M. Garnier, *Pathol. Biol.*, 28 (1980) 206.
231 A. Heyraud and M. Rinaudo, *J. Liq. Chromatogr.*, 3 (1980) 721.
232 T. Kato and T. Kinoshita, *Anal. Biochem.*, 106 (1980) 238.
233 J. L. Di Cesare, *Chromatogr. Newsl.*, 8 (1980) 52.
234 N. I. Kovalevskaya, *Lab. Delo*, (1980) 565.
235 F. R. Seymour, E. C. Chen and J. E. Stouffer, *Carbohydr. Res.*, 83 (1980) 201.
236 H. E. Hauck and H. Halpaap, *Chromatographia*, 13 (1980) 538.
237 L. Petrus, V. Bilik, L. Kuniak and L. Stankovic, *Chem. Zvesti*, 34 (1980) 530.
238 D. E. Willis, *J. Chromatogr. Sci.*, 21 (1983) 132.
239 M. Glad, S. Ohlson, L. Hansson, M.-O. Månsson and K. Mosbach, *J. Chromatogr.*, 200 (1980) 254.
240 H. Englyst, *Basic Clin. Nutr.*, 3 (1981) 71.

241 D. L. Kiser, R. L. Hagy and R. L. Olson, in G. Charalambous (Editor), *Anal. Control Less Desirable Flavors Foods Beverages. [Proc. Symp.] 1980, 71*, Academic Press, New York; *C.A.*, 94 (1981) 137883a.
242 J. C. Kuo and E. S. Yeung, *J. Chromatogr.*, 223 (1981) 321.
243 P. Hou, B. G. Archer and R. Won, *Altex Chromatogram*, 4 No. 2 (1981) 7.
244 F. E. Wentz, A. Dale Marcy and M. J. Gray, *J. Chromatogr. Sci.*, 20 (1982) 349.
245 N. Shibuya, *J. Chromatogr.*, 208 (1981) 96.
246 R. Macrae and J. Dick, *J. Chromatogr.*, 210 (1981) 138.
247 G. A. Reid, A. W. Harper, P. F. Sanders and K. B. Pugh, *Chem. Ind. (London)*, No. 2, (1981) 64.
248 J. Lehrfeld, *Anal. Biochem.*, 115 (1981) 410.
249 T. Li, C. Wu, T. Jiang, Y. Zhang and X. Fan, *Fen Hsi Hua Hsueh*, 9 (1981) 295; *C.A.*, 95 (1981) 187542e.
250 E. Barreto-Bergter, L. Hogge and P. A. Gorin, *Carbohydr. Res.*, 97 (1981) 147.
251 W. Flak, *Mitt. Klosterneuburg*, 31 (1981) 204.
252 P. De Smedt, P. A. Liddle, B. Cresto and A. Bossard, *J. Inst. Brew.*, 87 (1981) 349.
253 S. Sidorenko and A. Vangelov, *C.A.*, 96 (1982) 50111w.
254 O. C. Bjarnoe, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 1392.
255 S. Honda, M. Takahashi, Y. Nishimura, K. Kakehi and S. Ganno, *Anal. Biochem.*, 118 (1981) 162.
256 R. Segura and X. Navarro, *J. Chromatogr.*, 217 (1981) 329.
257 J. Bourely, *Coton Fibres Trop. (Fr. Ed.)*, 35 (1980) 189.
258 K. Iwano, S. Sato, A. Mizuno, Y. Takahara, E. Sano, Y. Kizaki, K. Tsuji, N. Umeda, A. Totsuka and H. Kawashima, *Nippon Jozo Kyokai Zasshi*, 76 (1981) 778; *C.A.*, 96 (1982) 67195t.
259 R. A. Boykins and T. Y. Liu, *Chromatogr. Sci.*, 18 (1981) 347.
260 J. Dobrzycki and M. Ludwicki, *Gaz. Cukrow.*, 89, No. 3 (1981) 65.
261 L. A. T. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 234 (1982) 57.
262 Y. Ou, *Shipin Kexue (Beijing)*, 13 (1981) 35.
263 J. I. Vucetic, M. M. Vrvic and B. M. Vujovic, *Med. Podmladak*, 32 (1980) 151; *C.A.*, 96 (1982) 123113d.
264 W. A. Koenig, I. Benecke and S. Sievers, *J. Chromatogr.*, 217 (1981) 71.
265 M. Toth, E. Laszlo and J. Morvai, *Soripar*, 28 (1981) 127; *C.A.*, 96 (1982) 141082x.
266 T. Anan, H. Takayanagi, K. Ikegaya and M. Nakagawa, *Nippon Shokuhin Kogyo Gakkaishi*, 28 (1981) 632; *C.A.*, 96 (1982) 141283p.
267 B. Ticha, Z. Folbergerova and M. Friml, *LC, Listy Cukrov.*, 97 (1981) 271.
268 P. Decker and H. Schweer, *J. Chromatogr.*, 236 (1982) 369.
269 J. A. Lomax and J. Conchie, *J. Chromatogr.*, 236 (1982) 385.
270 P. Orth and H. Engelhardt, *Chromatographia*, 15 (1982) 91.
271 R. Yamauchi, K. Kato and Y. Ueno, *Nippon Nogei Kagaku Kaishi*, 56 (1982) 123; *C.A.*, 96 (1982) 216127b.
272 N. L. Wade and S. C. Morris, *J. Chromatogr.*, 240 (1982) 257.
273 W. Buchberger, K. Winsauer and C. Breitwieser, *Fresenius' Z. Anal. Chem.*, 311 (1982) 517.
274 S. Hughes and D. C. Johnson, *J. Agric. Food Chem.*, 30 (1982) 712.
275 T. Crispin and I. Halász, *J. Chromatogr.*, 239 (1982) 351.
276 H. M. Rodriguez and A. M. Escudey, *Bol. Soc. Chil. Quim.*, 27 (1982) 319.
277 P. E. Shaw and C. W. Wilson, *J. Chromatogr. Sci.*, 20 (1982) 209.
278 C. Corradi, D. Carreri and G. Micheli, *Boll. Chim. Unione Ital. Lab. Prov., Parte Sci.*, 33 (1982) 33.
279 A. Okahira, H. Kobatake and H. Kushida, *Bunseki Kagaku*, 31 (1982) 163.
280 T. T. Fang and K. J. Chang, *Kuo Li T'ai-wan Ta Hsueh Nung Hsueh Yuan Yen Chiu Pao Kao*, 21 (1981) 62; *C.A.*, 97 (1982) 108606u.
281 M. F. Chaplin, *Anal. Biochem.*, 123 (1982) 336.
282 K. J. Kaffka, K. H. Norris, F. Kulcsar and I. Draskovits, *Acta Aliment.*, 11 (1982) 271.
283 M. R. Little, *Carbohydr. Res.*, 105 (1982) 1.
284 A. K. Shukla, N. Scholz, E. H. Reimerdes and R. Schauer, *Anal. Biochem.*, 123 (1982) 78.
285 I. Aguinagalde and R. Hueser, *An. Edafol. Agrobiol.*, 40 (1981) 1485.
286 C. Date, H. Tanaka, K. Yoshikawa, T. Baba, M. Hayashi, K. Owada, K. Okazaki, Y. Nakayama and R. Ishii, *Osaka City Med. J.*, 28 (2) (1982) 67.
287 K. Ma, *Shipin Kexue (Beijing)*, 27 (1982) 57; *C.A.*, 97 (1982) 143191b.
288 O. Pelletier and S. Cadieux, *J. Chromatogr.*, 231 (1982) 225.
289 H. Schweer, *J. Chromatogr.*, 243 (1982) 149.

290 J. R. Hudson, S. L. Morgan and A. Fox, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 285.
291 Y. K. Lee, M. H. Song and S. B. Chun, *Hanguk Ch'uksan Hakhoe Chi*, 24 (1982) 326.
292 R. B. Wills, R. A. Francke and B. P. Walker, *J. Agric. Food Chem.*, 30 (1982) 1242.
293 J. E. Marcy and D. E. Carroll, *Am. J. Enol. Vitic.*, 33 (1982) 176.
294 J. Fernandez-Bolanos, M. J. Fernandez Diez, M. Rivas Moreno and A. Gil Serrano, *Grasas Aceites (Seville)*, 33 (1982) 208.
295 C. Date, H. Tanaka, K. Yoshikawa, Y. Ueda, T. Baba, M. Hayashi, Y. Tanaka, T. Shimada and H. Yamamoto, *Nippon Eiseigaku Zasshi*, 37 (1982) 516; *C.A.*, 97 (1982) 214356x.
296 G. K. Buckee and D. E. Long, *J. Am. Soc. Brew. Chem.*, 40 (1982) 137.
297 L. A. Verhaar, *Starch/Staerke*, 34 (1982) 351.
298 J. Jary, M. Marek and J. Bacilek, in W. Baltes, P. B. Czedik-Eysenberg and W. Pfannhauser (Editors), *Recent Developments in Food Analysis, Proceedings of the 1st European Conference on Food Chemistry, 1981*, Verlag Chemie, Weinheim, 1982, p. 453.
299 S. Osaki and Z. Yoshino, *Denpun Kagaku*, 29 (1982) 210.
300 A. Okahira and H. Kobatake, *Bunseki Kagaku*, 31 (1982) 304.
301 A. B. Blakeney, P. J. Harris, R. J. Henry, B. A. Stone and T. Norris, *J. Chromatogr.*, 249 (1982) 180.
302 M. Fayez, M. El-Tarras, A. S. Ahmed and F. A. Emam, *Egypt. J. Pharm. Sci.*, 21 (1982) 215.
303 T. Kato, F. Iinuma and T. Kinoshita, *Nippon Kagaku Kaishi*, (1982) 1603.
304 B. Porsch, *J. Chromatogr.*, 253 (1982) 49.
305 T. Kato and T. Kinoshita, *Bunseki Kagaku*, 31 (1982) 615.
306 G. K. Grimble, H. M. Barker and R. H. Taylor, *Anal. Biochem.*, 128 (1983) 422.
307 E. Elimer and J. Kwasnik, *Pr. Nauk. Akad. Ekon. im. Oskara Langego Wroclawiu*, 199 (1982) 49; *C.A.*, *98 (1983) 122225w.*
308 R. J. Henry, A. B. Blakeney, P. J. Harris and B. A. Stone, *J. Chromatogr.*, 256 (1983) 419.
309 A. Fox, S. L. Morgan, J. R. Hudson, Z. T. Zhu and P. Y. Lau, *J. Chromatogr.*, 256 (1983) 429.
310 R. Oshima, J. Kumanotani and C. Watanabe, *J. Chromatogr.*, 250 (1982) 90.
311 J. L. Iverson and M. P. Bueno, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 139.
312 V. G. Kostenko and G. G. Senchenko, *Zh. Anal. Khim.*, 38 (1983) 300.
313 R. Gaub, *Monatsschr. Brau.*, 36 (1983) 125.
314 P. E. Shaw and C. W. Wilson, *J. Sci. Food Agric.*, 34 (1983) 109.
315 J. W. De Vries, H. L. Chang, J. C. Heroff and K. D. Johnson, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 197.
316 T. Hodisan, E. Moraru and G. Murgu, *Stud. Univ. Babes-Bolyai, [Ser.] Chem.*, 27 (1982) 34.
317 H. Schweer, *J. Chromatogr.*, 259 (1983) 164.
318 A. Baer and H. Kraemer, *Krankenhauspharmazie*, 4 (1983) 115; *C.A.*, 99 (1983) 10918p.
319 S. Johncock and P. Wagstaffe, *Analyst (London)*, 105 (1980) 581.
320 C. Otoguro, S. Ogino and M. Watanabe, *Nippon Jozo Kyokai Zasshi*, 78 (1983) 220.
321 R. E. Lee, D. Friday, R. Rojas, H. James and J. G. Baust, *J. Liq. Chromatogr.*, 6 (1983) 1139.
322 S. Hughes and D. C. Johnson, *Anal. Chim. Acta*, 149 (1983) 1.
323 G. G. McBee and N. O. Maness, *J. Chromatogr.*, 264 (1983) 474.
324 M. T. Valdehita, A. Carballido and M. D. Tenorio, *An. Bromatol.*, 33 (1983) 283.
325 P. C. White, I. Jane, A. Scott and B. E. Connett, *J. Chromatogr.*, 265 (1983) 293.
326 W. Buchberger, K. Winsauer and C. Breitwieser, *Fresenius' Z. Anal. Chem.*, 315 (1983) 518.
327 M. Ishikawa, M. Yamamoto, T. Masui and M. Nara, *Shizuoka-ken Eisei Kankyo Senta Hokoku*, 25 (1982) 131; *C.A.*, *99 (1983) 146179f.*
328 E. Moraru, T. Hodisan and C. Sarbu, *Rev. Chim. (Bucharest)*, 34 (1983) 144.
329 L. Bianco and M. Marucchi, *Boll. Chim. Unione Ital. Lab. Prov., Parte Sci.*, 34 (1983) 209.
330 A. Murakami, E. Otani, F. Yamamura, K. Hirota, K. Maeda and H. Nishizawa, *Kyoritsu Yakka Daigaku Kenkyu Nenpo*, (27) (1982) 27; *C.A.*, 99 (1983) 186768d.
331 D. Betteridge, N. G. Courtney, T. J. Sly and D. G. Porter, *Analyst (London)*, 109 (1984) 91.
332 W. Buchberger, K. Winsauer and C. Breitwieser, in E. Pungor (Editor), *Modern Trends in Analytical Chemistry, Part A, Electrochemical Detection in Flow Analysis, Proceedings of Scientific Symposium, Matrafüred, October 17–20, 1982 (Analytical Chemistry Symposia Series*, Vol. 18, Part A), Elsevier, Amsterdam, 1984, p. 267.
333 C. Vidal-Valverde, C. Martin-Villa and J. Herranz, *J. Dairy Sci.*, 67 (1984) 759.
334 I. J. Jeon, S. J. Galitzer and K. J. Hennessy, *J. Dairy Sci.*, 67 (1984) 884.
335 M. Tomana, J. T. Prchal, L. C. Garner, H. W. Skalka and S. A. Barker, *J. Lab. Clin. Med.*, 103 (1984) 137.

336 E. D. Coppola, *Food Technol. (Chicago)*, 38 (4) (1984) 88.
337 G. Palla, *Anal. Chem.*, 53 (1981) 1966.
338 R. Galensa, *Z. Lebensm.-Unters.-Forsch.*, 178 (1984) 199.
339 J. Guardiola and K. W. Schultze, *Fresenius' Z. Anal. Chem.*, 318 (1984) 237.
340 F. Saura-Calixto, J. Canellas and A. Garcia-Raso, *J. Agric. Food Chem.*, 32 (1984) 1018.
341 I. Kabzev, S. Manchev and N. Genov, *Nauchni Tr.-Vissh Inst. Khranit. Vkusova Prom-st., Plovdiv*, 30 (1983) 27.
342 S. Honda and S. Suzuki, *Anal. Biochem.*, 142 (1984) 167.
343 A. V. Maidanyuk, Yu P. Shalin, Z. A. Dobrovolskaya and A. S. Shcherbatenko, *Fiziol. Biokhim. Kul't. Rast.*, 16 (1984) 500.
344 G. J. Gerwig, J. P. Kamerling and J. F. Vliegenthart, *Carbohydr. Res.*, 129 (1984) 149.
345 P. Vratny, U. A. Brinkman and R. W. Frei, *Anal. Chem.*, 57 (1985) 224.
346 J. H. Van den Berg, H. W. Horsels and R. S. Deelder, *J. Liq. Chromatogr.*, 7 (1984) 2351.
347 M. Matisova, E. Gasparovicova, L. Koudelka and M. Singliar, *Chem. Prum.*, 34 (1984) 543.
348 I. I. Gavrilyuk, *Prikl. Khromatogr.*, (1984) 294; *C.A.*, 102 (1985) 109171g.
349 S. Ondrousek, G. Basarova and H. Loukova, *Kvasny Prum.*, 30 (1984) 98.
350 F. Guyon, M. Caude and R. Rosset, *Analusis*, 12 (1984) 321.
351 C. Vidal-Valverde, C. Martin-Villa, B. Olmedilla and I. Blanco, *J. Liq. Chromatogr.*, 8 (1985) 75.
352 A. C. Duarte-Coelho, E. D. Dumoulin and J. T. Guerain, *J. Liq. Chromatogr.*, 8 (1985) 59.
353 I. Molnar-Perl, M. Szakacs-Pinter, A. Kovago and J. Petroczy, *Carbohydr. Res.*, 138 (1985) 83.
354 H. Takemoto, S. Hase and T. Ikenaka, *Anal. Biochem.*, 145 (1985) 245.
355 B. W. Li, P. J. Schuhmann and W. R. Wolf, *J. Agric. Food Chem.*, 33 (1985) 531.
356 M. Makkee, A. P. Kieboom and H. Van Bekkum, *Int. Sugar J.*, 87 (1985) 55.
357 R. Oshima, A. Yoshikawa and J. Kumanotani, *J. Chromatogr.*, 213 (1981) 142.
358 R. C. Pettersen, V. H. Schwandt and M. J. Effland, *J. Chromatogr. Sci.*, 22 (1984) 478.
359 M. Ghebregzabher, S. Rufini, B. Monaldi and M. Lato, *J. Chromatogr.*, 127 (1976) 133.
360 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 220 (1981) 313.
361 H. Scherz, G. Stehlik, E. Bancher and K. Kaindl, *J. Chromatogr.*, 10 (1968) 1.
362 Yu. S. Ovodov, E. V. Evtushenko, V. E. Vaskovsky, R. G. Ovodova and T. F. Solo'eva, *J. Chromatogr.*, 26 (1967) 111.
363 M. Ghebregzabher, S. Rufini, G. Ciuffini and M. Lato, *J. Chromatogr.*, 95 (1974) 51.
364 T. Mezzetti, M. Lato, S. Rufini and G. Ciuffini, *J. Chromatogr.*, 63 (1971) 329.

PUBLICATION SCHEDULE FOR 1986

Journal of Chromatography (incorporating *Chromatographic Reviews*) and *Journal of Chromatography, Biomedical Applications*

MONTH	O 1985	N 1985	D 1985	J	F	M	A	M	J	
Journal of Chromatography	346 347/1	347/2 347/3 348/1	348/2 349/1 349/2 350/1 350/2	351/1 351/2 351/3	352 353 354	355/1 355/2 356/1	356/2 356/3 357/1	357/2 357/3 358/1 358/2 359		The publication schedule for further issues will be published later
Chromatographic Reviews								373/1		
Bibliography Section					372/1		372/2		372/3	
Biomedical Applications				374/1 374/2	375/1	375/2	376 377	378/1	378/2 379	

INFORMATION FOR AUTHORS

(Detailed *Instructions to Authors* were published in Vol. 329, No. 3, pp. 449–452. A free reprint can be obtained by application to the publisher.)

Types of Contributions. The following types of papers are published in the *Journal of Chromatography* and the section on *Biomedical Applications:* Regular research papers (Full-length papers), Short communications and Notes. Short communications are preliminary announcements of important new developments and will, whenever possible, be published with maximum speed. Notes are usually descriptions of short investigations and reflect the same quality of research as Full-length papers, but should preferably not exceed four printed pages. For review articles, see page 2 of cover under Submission of Papers.

Submission. Every paper must be accompanied by a letter from the senior author, stating that he is submitting the paper for publication in the *Journal of Chromatography*. Please do not send a letter signed by the director of the institute or the professor unless he is one of the authors.

Manuscripts. Manuscripts should be typed in double spacing on consecutively numbered pages of uniform size. The manuscript should be preceded by a sheet of manuscript paper carrying the title of the paper and the name and full postal address of the person to whom the proofs are to be sent. Authors of papers in French or German are requested to supply an English translation of the title of the paper. As a rule, papers should be divided into sections, headed by a caption (*e.g.*, Summary, Introduction, Experimental, Results, Discussion, etc.). All illustrations, photographs, tables, etc., should be on separate sheets.

Introduction. Every paper must have a concise introduction mentioning what has been done before on the topic described, and stating clearly what is new in the paper now submitted.

Summary. Full-length papers and Review articles should have a summary of 50–100 words which clearly and briefly indicates what is new, different and significant. In the case of French or German articles an additional summary in English, headed by an English translation of the title, should also be provided. (Short communications and Notes are published without a summary.)

Illustrations. The figures should be submitted in a form suitable for reproduction, drawn in Indian ink on drawing or tracing paper. Each illustration should have a legend, all the *legends* being typed (with double spacing) together on a *separate sheet*. If structures are given in the text, the original drawings should be supplied. Coloured illustrations are reproduced at the author's expense, the cost being determined by the number of pages and by the number of colours needed. The written permission of the author and publisher must be obtained for the use of any figure already published. Its source must be indicated in the legend.

References. References should be numbered in the order in which they are cited in the text, and listed in numerical sequence on a separate sheet at the end of the article. Please check a recent issue for the layout of the reference list. Abbreviations for the titles of journals should follow the system used by *Chemical Abstracts*. Articles not yet published should be given as "in press", "submitted for publication", "in preparation" or "personal communication".

Dispatch. Before sending the manuscript to the Editor please check that the envelope contains *three copies of the paper complete with references, legends and figures.* One of the sets of figures must be the originals suitable for direct reproduction. Please also ensure that permission to publish has been obtained from your institute.

Proofs. One set of proofs will be sent to the author to be carefully checked for printer's errors. Corrections must be restricted to instances in which the proof is at variance with the manuscript. "Extra corrections" will be inserted at the author's expense.

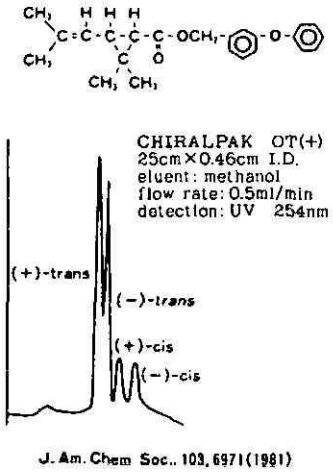
Reprints. Fifty reprints of Full-length papers, Short communications and Notes will be supplied free of charge. Additional reprints can be ordered by the authors. An order form containing price quotations will be sent to the authors together with the proofs of their article.

Advertisements. Advertisement rates are available from the publisher on request. The Editors of the journal accept no responsibility for the contents of the advertisements.

HPLC columns for optical resolution

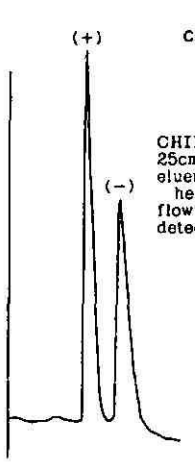
CHIRALPAK
CHIRALCEL

Daicel Chemical Industries, Ltd. has developed excellent chiral columns for the separation of optical isomers. "CHIRALPAK" or "CHIRALCEL" in combination with High Performance Liquid Chromatography has proven to be a highly successful technique for separation of optical isomers and for the determination of enantiomeric purity of compounds. Now a wide variety of optically pure compounds are readily available without laborious resolution techniques!

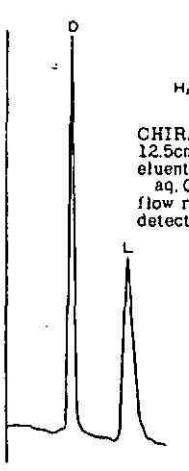


CHIRALPAK OT(+)
25cm×0.46cm I.D.
eluent: methanol
flow rate: 0.5ml/min
detection: UV 254nm

J. Am. Chem Soc., 103, 6971 (1981)



CHIRALCEL OC
25cm×0.46cm I.D.
eluent:
hexane-2-propanol
flow rate: 1.5ml/min
detection: UV 220nm



CHIRALPAK WH
12.5cm×0.46cm I.D.
eluent:
aq. 0.25mM $CuSO_4$
flow rate: 1.5ml/min
detection: UV 254nm

For more details about HPLC columns, please contact:

DAICEL CHEMICAL INDUSTRIES, LTD.

Tokyo
8-1, Kasumigaseki 3-chome,
Chiyoda-ku, Tokyo 100, Japan
Phone: 03 (507) 3173, 3178
Telex: 222-4632 DAICEL J

Düsseldorf
Königsallee 92a,
4000 Düsseldorf 1, F R Germany
Phone: (0211) 134158
Telex: (41) 8588042 DCEL D

New York
Pan-Am Bldg., 200 Park Avenue,
New York, N.Y. 10166-0130, U.S.A.
Phone: (212) 878-6765, 6766
Telex: (23) 236154 DCC UR

DAICEL (U.S.A.), INC.
611 west 6th Street, Room 2152
Los Angeles California 90017, U.S.A.
Phone: (213) 629-3656
Telex: 215515 DCIL UR