

CHARGE-TRANSFER COMPLEXES OF METALS IN THE CHROMATOGRAPHIC SEPARATION OF ORGANIC COMPOUNDS*

O. K. GUHA AND J. JANÁK

*Institute of Instrumental Analytical Chemistry, Czechoslovak Academy of Sciences, Brno
(Czechoslovakia)*

(Received December 12th, 1971)

CONTENTS

1. Introduction	325
2. Metal acceptors	326
3. Chromatographic behaviour and applications	328
A. Argentation chromatography	329
a. Olefins	329
b. Fatty acid esters	333
c. Neutral and polar lipids, sterols and other terpenoid compounds.	334
d. Miscellaneous separations.	335
B. Charge-transfer interactions of other metals in the chromatographic separation of organic compounds	335
4. Conclusions	338
Summary	340
References.	340

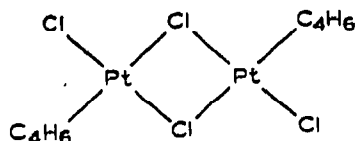
I. INTRODUCTION

Metals form molecular complexes with certain organic compounds such that a variety of types of association product of two or more molecules can be formed. Cationic complexes of organic compounds have been excellently dealt in many text books and reviews^{7,63,77,126}. Many of these complexes are formed by the weak interaction of certain classes of organic compounds, functioning as electron donors, with metals, acting as electron acceptors. These donor-acceptor complexes, the composition of which can be represented by integral mole ratios of the components, are normally very unstable and exist in equilibrium with the free components. The rates of formation of complexes are generally very rapid. The heats of interaction are generally small and there is abundant evidence that the coordination forces are much weaker than those established in the formation of covalent bonds, *i.e.*, the degree to which electron transfer from the donor to the acceptor component takes place¹⁰⁰ is much less than that which ordinarily occurs when new compounds are formed. The above properties are very favourable for use in chromatographic processes.

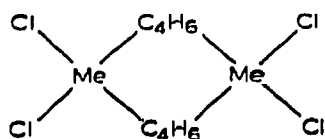
* Part of the thesis of O. K. GUHA.

2. METAL ACCEPTORS

Complexes of a wide variety of metal acceptors have been reported. In both the solid state and aqueous solution, silver salts and salts of copper(I) and mercury(II) interact with alkenes to form complexes⁵⁷. In characterizing new types of unsaturated compounds, a large number of solid silver adducts, which frequently have sharp melting points, have been prepared. With halides of platinum(II), palladium(II), rhodium(II) and related metallic ions, many solid adducts of unsaturated compounds, notably of dienes, have also been prepared, and dienes are known to react with metal carbonyls to form stable compounds³³. In fact, in many instances coordination forces between the components in these solid metal halide complexes with alkenes and dienes approach those established in the formation of strong dative bonds. It is concluded on the basis of available spectroscopic evidence that in most cases in which the geometric situation is favourable, both double bonds of the diene are coordinated with metal in the complex^{34,162,164}. The IR spectra of the butadiene complexes, $K_2PtCl_6 \cdot C_4H_6$, $Cu_2Cl_2 \cdot C_4H_6$, $Cu_2Br_2 \cdot C_4H_6$ and $(PdCl_2 \cdot C_4H_6)_2$, lack absorption bands attributable to $C=C$ stretching in the free diene, while such a bond has been observed¹⁴⁶ in the spectrum of $(PtCl_2 \cdot C_4H_6)_2$. Presumably only one $C=C$ bond per butadiene molecule is coordinated with a platinum atom in the last complex, for which the following structure has been suggested:



while for the palladium complex in which both double bonds are coordinated, the structure

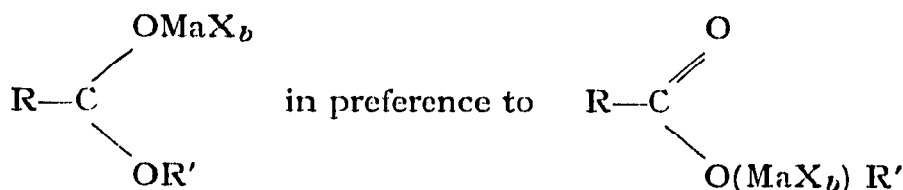


has been proposed. The Raman spectra of the donors also suggests the interaction of a silver ion with either a $C-C$ double or triple bond¹⁵⁹. The $C=C$ stretching frequency of an olefin dissolved in concentrated silver nitrate solution is lowered by about 65 wave-numbers and somewhat greater shifts in the $C=C$ frequency of an acetylene take place when it is dissolved in silver nitrate solution. When benzene is dissolved in concentrated silver perchlorate solution, the carbon-carbon stretching vibrations of the hydrocarbon are also significantly affected.

Lewis acid-type catalysts in Friedel-Crafts and related reactions act as acceptors with respect to π - as well as to n -type donors. For example, aluminium bromide readily dissolves in aromatic hydrocarbons to form coloured complexes (faint yellow in benzene and orange in mesitylene) and by vapour pressure-composition phase studies it has been established that solid complexes, $ArH \cdot Al_2Br_6$, are formed with benzene and toluene²³. Highly coloured aromatic hydrocarbon complexes of the

type $\text{ArH} \cdot \text{HBr} \cdot \text{Al}_2\text{Br}_6$ and $\text{ArH} \cdot \text{HBr} \cdot \text{AlBr}_3$ have been isolated at low temperatures²⁴. Because of the high conductivities and the intense colours of adducts of this type and because of the fact that the aromatic compounds exchange with deuterium under conditions leading to the interaction of $\text{ArH} \cdot \text{DCl}$ and AlCl_3 , it has been concluded that when these complexes are formed a proton becomes tetrahedrally bonded to one of the ring carbon atoms. To distinguish them from the less stable π -complexes, which are formed with relatively little disruption of the π -electron cloud through interaction of the aromatic compounds with hydrogen chloride, the resultant carbonium ion-like products, ArH_2^+X^- (where $\text{X}^- = \text{F}^-, \text{BF}_4^-, \text{AlCl}_4^-$ etc.), are sometimes called σ -complexes.

A decrease in the $\text{C}=\text{O}$ vibration frequency, when a Lewis acid-type Friedel-Crafts catalyst interacts with a ketone, is a direct consequence of the decrease in the stretching force constant of this bond, which accompanies electron transfer from the donor oxygen to the Lewis acid²⁵. Similar changes occur when acetophenone and benzophenone interact with aluminium halides and when acetone forms a complex with titanium tetrachloride^{20,32,151,152}. When ethyl acetate and ethyl formate form complexes with acceptors such as tin(IV) chloride and antimony(III) and antimony(V) chlorides, the carbonyl stretching frequencies of the ester decrease and the $\text{C}-\text{O}$ (ether) stretching frequencies increase¹⁷⁷. The preferred structures for these adducts should be



The results of IR spectroscopic studies^{65,153} support the assumption that in an adduct of an aluminium halide or a structurally related acceptor with an aliphatic or aromatic nitro compound, the oxygen of the nitro group is the centre of coordination. In certain complexes of metallic halides with amides and related compounds, the donor bonding sites have also been established by IR spectroscopy. For example, a marked drop in intensity of the fundamental $\text{C}=\text{S}$ stretching frequency⁹² suggests that methylthiourea, in its complexes with zinc and cadmium salts, coordinates through the sulphur atom. However, with halides of platinum(II), palladium(II) and copper(I), this bond is not significantly different in either position or intensity from that of the free donor, while marked changes in bands corresponding to $\text{N}-\text{H}$ and $\text{C}-\text{N}$ stretching and to NH_2 rocking indicate that nitrogen rather than sulphur is the donor coordination centre. The porphyrin structure of many stable metal phthalocyanines leaves positions available for coordination. Iron(II) phthalocyanine can add two molecules of pyridine or imidazole in chloronaphthalene solution, as shown in spectroscopic studies⁹¹. In an IR study, TERENIN AND SIDOROV¹⁶¹ noticed changes in the spectra of several metal phthalocyanines in the presence of electron donor molecules such as water, hydrogen sulphide, ammonia and amines. A similar study was reported by ELVIDGE AND LEVER⁵⁸.

The changes in IR spectra that accompany the interaction of Friedel-Crafts catalysts (MaX_b) with acyl and aryl halides have been extensively studied and there is evidence that both oxocarbonium ion salts ($\text{RCO}^+\cdot\text{MaX}^-_{b+1}$) and donor-acceptor

complexes, in which the carbonyl oxygen is the donor coordination site, are formed. A liquid form of the 1:1 coordination adduct of acetyl chloride with aluminium chloride prepared by COOK³⁶ appears to be a mixture of an oxocarbonium ion salt and a donor-acceptor complex, in which the latter predominates. The spectrum of the liquid in nitrobenzene solution shows absorption characteristics both of the oxocarbonium ion and the donor-acceptor complex, while in chloroform, a solvent with a relatively low dielectric constant, the adduct appears to exist entirely in the form of the complex. A liquid form of the acetyl chloride-antimony pentachloride adduct is predominantly $\text{CH}_3\text{CO}^+ \cdot \text{SbCl}_6^-$, while the corresponding antimony pentachloride adducts of propionyl and benzoyl chlorides occur largely as the donor-acceptor complexes $\text{R}-\text{CO}^{\delta+} \rightarrow \text{Sb}^{\delta-}-\text{Cl}_5$ (ref. 107). On the basis of the IR spectroscopic



studies, it has been concluded that the aluminium chloride and titanium tetrachloride adducts of 2,6-dimethylbenzoyl chloride are more oxocarbonium ion-like in character than those of *o*-toluyl chloride¹⁵⁴.

3. CHROMATOGRAPHIC BEHAVIOUR AND APPLICATIONS

Spectroscopic evidence of the charge transfer interactions of some metals with organic compounds have been discussed briefly in the preceding section. On several occasions, gas chromatography (GC) has been used in the study of complex formation. An early description of a rather specialised case by DU PLESSIS AND SPONG⁵⁵ deserves recognition as a pioneering attempt in the direct quantitative study of equilibria by GC. Subsequently, stability constants of Ag^+ -unsaturated compound adducts in ethylene glycol were measured by GIL-AV AND HERLING⁶⁸ and by MUHS AND WEISS¹⁰⁵. PHILLIPS AND TIMMS¹¹⁶ suggested the possibility of studying the kinetics of metal-ligand interactions within the column by using the theory advanced by KLINKENBERG⁸⁸. DU PLESSIS⁵⁶ later made further experiments with packings containing silver salts in organic solvents to improve the quantitative treatment used earlier⁵⁵. PURNELL¹²⁵ discussed the various approaches to the study of complexing reactions in solution by GC methods and developed generalised retention theories for each. In the study of complex formation, GC is superior in many instances to other techniques and offers a particularly effective method in the case of hydrolyzable and water-insoluble complexes. The GC method is most valuable when other methods for isomer analysis fail. For example, the visible, UV and IR spectra of the *cis-trans* isomers of chromium(III) trifluoroacetyl acetonate are almost identical⁶², which restricts their analysis by spectroscopic methods. Owing to the paramagnetism of the chromium(III) complex, NMR spectroscopy cannot be used. The easy separation of these two isomers is one of the simplest stereochemical problems for which GC can be used¹⁴⁴. As our main purpose is to discuss the use of charge-transfer interactions of metals with organic donors as a method of chromatographic selectivity, the value of GC in the study of volatile stable metal coordination compounds, where a strong dative bond is formed between the metal and the ligand, is omitted from this discussion. Those interested in this aspect may consult the excellent book by MOSHIER AND SIEVERS¹⁰⁴. The widely recognized value of GC in the study of volatile stable metal coordination compounds mostly lies in the preparation of the sample.

The dependence of the magnitude of the stability constants on the structure of the donor and the metal acceptor and on the solvent system, which is a measure of chromatographic selectivity, has been evaluated in the past mainly by classical methods^{7,78,95,145,174} and by GC^{43,68,105}. This potential selectivity was utilized by a number of workers in chromatographic separations. Silver has been studied more extensively than other metals, both in theory and practice. Thus, for convenience, the following discussion on the use of charge-transfer interactions with metals in the chromatographic separation of organic compounds has been divided into silver ion (argentation) and non-silver metal ion chromatography.

A. *Argentation chromatography*

The potential selectivity of Ag^+ -unsaturated compound complexes, according to the number, type, geometry and the position of unsaturation, provides a relatively recent, but now very important, addition to the range of separation methods available to organic chemists and biochemists and is discussed below according to compound types.

(a) *Olefins*

The first chromatographic application of silver-olefin complexing involved gas-liquid chromatography (GLC). BRADFORD, HARVEY AND CHALKEY²² were the first to report on the high selectivity of the silver nitrate-ethylene glycol stationary phase for the separation of butenes. The striking efficiency of this complex-forming phase for the analysis of olefins with similar boiling points was subsequently confirmed by many investigators^{66,67,70,140-142,147,148}. On the basis of retention data for 83 hydrocarbons (saturated and unsaturated) on a silver nitrate-ethylene glycol column, the analytical potentiality of such columns according to degree, type, geometry and position of unsaturation was evaluated by SMITH AND OHLSON¹⁴⁸. The high selectivity of silver nitrate-containing phases is in general due to the marked effect of relatively small structural changes of olefins on the stability constants of the complexes. The factors responsible for the variation in the stability of the silver ion-olefin complexes with changes in the olefin structure have been investigated in the past mainly by classical methods^{7,78,95,174} and by GC^{68,105}. According to DEWAR⁵¹, the bond between the olefinic ligand and the metal ion is formed by donation of electrons from the ethylenic bond to the vacant *s*-orbital of the silver ion and back donation of *d*-electrons from the metal to the antibonding orbitals of the unsaturated compound. Both polar and steric factors determine the strength of the coordination bond, but the relative importance of the two contributions is not known. In view of this uncertainty, an unambiguous separation of the two effects, electronic and steric, is very difficult and still remains to some extent unsettled. However, the steric effects (and strain relief) have been found to be strong and more readily recognized and in many cases suffice to explain the influence of structure on the stability constants. The following general rules, correlating structure with retention volume and the stability constants of the Ag^+ -olefin complexes, have been established^{60,70,142}:

- (I) Substitution at the double bond decreases the retention volume.
- (II) A 1-alkyl compound has a lower retention volume than those of the 3- and 4-alkyl isomers.

- (III) Olefins having a substituent in the 3-position have higher retention volumes than those of the 4-isomers.
- (IV) Cyclobutenes have less tendency for complex formation than the corresponding five- and six-membered cycloolefins.
- (V) Cyclopentene derivatives have higher retention volumes than those of the corresponding isomeric cyclohexenes.
- (VI) A cumulative double bond system has a lower complex-forming capacity than a single double bond.
- (VII) In contrast to findings in aqueous solutions, conjugated dienes do not show reduced complex stability in ethylene glycol.
- (VIII) Compared with their behaviour in aqueous media, unconjugated dienes coordinate strongly in ethylene glycol solution, probably as a result of chelation.

One of the most interesting features in argentation chromatography is the secondary isotope effect. The pronounced secondary deuterium isotope effects on the stability of Ag^+ -olefin complexes have been studied in detail by GC⁴³. Experiments with specially deuterated propylenes showed that the isotope effect is much larger when deuterium is directly bound to the unsaturated carbon atoms (" α -effect") than when it is in the β -position to the double bond (" β -effect"). Both effects are inverse in the sense that deuteration leads to an increase in complex stability. The isotope effect is logarithmically additive and approximately independent of the degree of alkyl substitution in the olefin. Interpretation of the secondary deuterium isotope effects presents great difficulties⁷⁵. In the empirical approach of HALEVI and co-workers^{75,76}, it was assumed that there is a greater effective inductive electron release from C-D bonds than from the corresponding C-H bonds. The progressive increase in retention time on silver nitrate-ethylene glycol columns by deuterium substitution has, in fact, been used as the basis for the separation and analysis of the various isotopic olefins differing in deuterium atoms^{1,2,9,44,52}. ACHE AND WOLF^{1,2} separated isotopic ethylenes in a much shorter time than by a conventional single column technique by using a highly efficient silver nitrate-ethylene glycol column in combination with a recycling method¹³⁰. With the help of a 250-ft. silver nitrate-ethylene glycol packed column, ATKINSON *et al.*⁹ analysed deuterated olefins with an accuracy similar to that of mass spectral analysis. LEE AND ROWLAND⁹³ separated isotopic positional isomers of tritium-labelled propylenes, *cis*-butenes and 1-butenes (" β -effect"), but could not separate propylene isomers tritiated at the double bond. A similar isotope effect was observed by SGOUTAS AND KUMMEROW¹³⁰ during the chromatography of tritiated unsaturated fatty acid methyl esters on a silver nitrate-impregnated silicic acid column in liquid-solid chromatography. Molecules labelled with tritium at the active centres of unsaturation showed a longer retention time on silica-silver nitrate columns than molecules labelled with carbon-14 at the carboxyl group. The results presented for the experimental conditions used are not sufficiently reliable for the claim of ATKINSON *et al.*⁹ that the deuterated *cis*-isomer is retained longer than the *trans*-isomer to be accepted.

A serious drawback of the silver nitrate-ethylene glycol column, which limits its application to low-boiling compounds, in spite of its excellent selectivity, was pointed out by KEULEMANS⁸⁶. According to him, the temperature for argentation GLC should be kept below 40°, because above this temperature the adducts do not form and

the stationary phase is not stable. To improve the stability and selectivity of silver nitrate columns, several solvents were investigated. Comparing the difference in retention times between alkanes and alkenes, including *cis-trans* isomers, BEDNAS AND RUSSEL¹⁰ made a comparative study of ethylene glycol, polyethylene glycol and glycerol as solvents for silver nitrate and found polyethylene glycol to be inferior to the other two. They also modified the temperature limit stated by KEULEMANS⁸⁶ to 65°, above which the silver ion is rapidly reduced. TENNY¹⁰⁰, in connection with studies of various liquid substrates used in GC, also investigated a column of a solution of silver nitrate in triethylene glycol and demonstrated its ability to separate certain types of olefins. HERLING *et al.*⁷⁰ studied the effects of solvents other than ethylene glycol to improve the separation of 3- and 4-ethylcyclohexenes and found that benzyl cyanide and triethylene glycol reduce the selectivity of the stationary phase, while SHABTAI¹⁴³ achieved better separation of methylcycloheptenes and methylenecycloheptane with silver nitrate in triethylene glycol than in ethylene and diethylene glycol solvents. This anomaly possibly lies in the method of column preparation, as no-one, as far as we know, has critically studied reproducibility of silver nitrate columns in which different solvents are used. VAN DE CRAATS¹⁰⁵ and later LUCHSINGER⁹⁶ and ARMITAGE⁸ used benzyl cyanide as a solvent for the silver nitrate. From the retention values determined by ARMITAGE⁸, it appears that the selectivity of this stationary phase for various hydrocarbon types is relatively low. This fact can be attributed to the limited solubility of silver nitrate in benzyl cyanide and the high degree of solute-solvent intermolecular interaction by dispersion forces. On the other hand, benzyl cyanide limits the operating temperature because of its high vapour pressure. The use of silver nitrate in high-boiling liquids, suggested by ZLATKIS *et al.*¹⁷⁸, reduces column bleed at *ca.* 65° but the selectivity is still low and decreases at higher temperatures^{10,70,86}. Later, ZLATKIS¹⁷⁹ combined the high selectivity of the complex-forming stationary phase with the properties of high efficiency, speed and resolution of capillary columns and found the silver nitrate-ethylene glycol bis(cyanoethyl) ether-coated capillary column to be useful for the analysis of single carbon number mixtures. COPE and co-workers³⁷⁻³⁹ and SAUERS¹³⁴ used silver nitrate in di- or tetra-ethylene glycol as the stationary phase for the analysis of alkenes, methylcycloalkenes, methylenecycloalkanes and menthenes. The same type of unsaturated hydrocarbon has been investigated with silver nitrate-ethylene glycol columns^{67,140}. The better separation of mixtures of octenes, hexadienes and hexynes (except 1-hexyne) achieved by BENDEL *et al.*²⁰ using the AgBF₄-β,β'-oxydipropionitrile column cannot be compared with the silver nitrate-ethylene glycol column as the salts are different. One very interesting aspect is that the sequence of elution on the AgBF₄ column differs from that on the silver nitrate-ethylene glycol column¹⁴⁸. For gas-solid systems PHILLIPS¹¹⁷ mentioned the behaviour of different silver salts. While silver nitrate- and silver sulphate-modified supports show very strong retardations for olefins and aromatics, silver chloride and silver iodide are very similar in behaviour to alkali halides as modifiers. Presumably, the ability of the Ag⁺ ion to complex with double bonds is largely quenched by complexing with Cl⁻ and I⁻ ions. POWELL AND WHITING¹¹⁸ separated camphene and tricyclene at 80° by using silver nitrate-diethylene glycol as the stationary phase. On columns of silver nitrate in different organic solvents, COPE *et al.*⁴⁰ analysed *cis-trans* isomers of C₉-C₁₂ cycloalkenes between 88° and 100°. In view of the reported

inactivation of silver nitrate solution above 65° (ref. 19) it is questionable whether the effect of the Ag^+ ion plays any major role in these cases. In the preparation of long-lived silver nitrate columns in GC, VILLALOBOS¹⁰⁷ suggested triethylene glycol as a solvent for silver nitrate because of its vapour pressure of 0.01 mm Hg at the operating temperature of 50° . On the basis of experimental results on silver nitrate-triethylene glycol columns, he also suggested that gradual deterioration of the silver nitrate-benzyl cyanide column was due to gradual inactivation of Ag^+ rather than to loss of the partition liquid. In a number of investigations dealing principally with C_2 - C_4 hydrocarbons, many other investigators^{15,20,35,64,81,97,127,180} used single columns of silver nitrate in different solvents or in series combination with other columns or physical mixtures of packing. BARNARD AND HUGHES¹⁵ achieved the GC separation of an ethane, ethylene, propane and propylene mixture by using a physical mixture of packing of triisobutylene on Celite and silver nitrate-saturated ethylene glycol on Celite, but he failed to separate them on series columns made from the same packing. The reason for the failure on series columns was mentioned by PRIMAVESI¹²¹ as being the loss of triisobutylene during column preparation. None of the above investigators was able to significantly improve the stability and selectivity of silver nitrate-ethylene glycol columns. It is interesting to note in this connection that recently WASIK AND TSANG¹⁷⁰ used aqueous solutions of silver nitrate as GC column liquids. The strongly ionizing properties of water (thus increasing the silver ion activity) make such columns more efficient than the conventional silver nitrate-ethylene glycol columns. These columns have interesting analytical applications, particularly in the field of isotope separation. WASIK AND TSANG¹⁷¹ also demonstrated the possibility of the separation of hydrocarbon groups such as olefins, alkanes and aromatics by GC by using aqueous solutions of silver and mercury(II) salts as the stationary phase. Mercury(II) ions complex very strongly with olefins and act essentially as a barrier for the elution of any olefins, but do not complex with alkanes or aromatics; silver ions complex weakly with aromatics. The result is that the aromatics are eluted with longer times relative to alkanes while the olefins are retained completely. In spite of the extensive investigations, the problem of operating the silver nitrate column at elevated temperatures still remains, which restricts its application to low-boiling compounds.

This is the reason why argentation thin-layer chromatography (TLC) is so popular for the analysis of high-boiling compounds. PETROWITZ¹¹³ used thin layers of silver nitrate-impregnated silica gel for the separation of isomeric 1,5,9-cyclododecatrienes. Recently, a promising technique for the separation of high-boiling olefins from their mixtures with other hydrocarbons of different structure has been described by JANÁK *et al.*⁸⁵. They used a porous polystyrene-divinylbenzene polymer (Porapak Q) as the non-polar stationary phase and silver nitrate solution in a mixture of propanol and water as the liquid mobile phase. Such a variation of reversed-phase chromatography offers new possibilities because of multiplicative effects by dispersion forces in the stationary phase and complexing in the mobile phase. Indeed, they separated C_{14} olefins at room temperature from paraffins and aromatics with great selectivity. A selective shift equivalent to seven carbon atoms has been found. The popularity of argentation TLC will be discussed in the subsequent section in connection with the analysis of lipids, sterols and other high-boiling compounds.

Owing to its obvious disadvantages, argentation chromatography in gas-solid

systems cannot be as popular¹¹⁷ as argentation GLC. At an early date, the selective retention of olefins in gas-solid systems on a silver ion-modified zeolite column was reported by JANÁK⁸⁴. The successful GC separation of *cis-trans* isomers of heptene-3 on a preparative scale¹³⁷ was achieved with silver nitrate-alumina columns at 75° by using a displacement technique. SCOTT AND PHILLIPS¹³⁸ analysed traces of olefins in paraffinic hydrocarbons on silver nitrate-alumina columns with octene-1 vapour as the displacer. The analysis of complex hydrocarbon mixtures by GC is facilitated by using short columns of silver nitrate-alumina or copper(I) chloride-alumina as a subtractor¹¹⁷. In the analysis of hydrocarbon gases, the subtractive strengths of silver and mercury salts towards olefins, acetylenes and aromatics have been compared⁸³. DUFFIELD AND ROGERS⁵³ worked at relatively higher temperatures (in the range 140–220°) with silver nitrate-impregnated Chromosorb W and P columns in order to make unsaturated compounds mobile, and found much higher values for the heats of reaction of 4-methyl-1-pentene than those obtained by using a solution of silver nitrate in polyethylene glycol. At such a high temperature, it is doubtful whether Ag⁺ charge transfer plays any role in the selectivity of such columns.

(b) *Fatty acid esters*

Argentation chromatography was also found to be very suitable for the separation of fatty acid methyl esters according to the degree, type, geometry and the position of unsaturation.

The separation of fatty acid methyl ester mixtures according to the degree of unsaturation has been carried out on a relatively large scale by column chromatography on silver nitrate-impregnated silicic acid^{48,49,122,168}, Florosil^{6,173} and ion-exchange resin^{59,170} columns. On an analytical or smaller preparative scale, TLC on silicic acid impregnated with silver nitrate^{54,99,123,132} or with ammoniacal silver nitrate solution¹⁷⁵ was found to be convenient. The plates prepared from ammoniacal silver nitrate solution¹⁷⁵ were reported to give better separation of fatty acid methyl esters. They retain their resolving power much longer and produce less corrosion of conventional spreaders. The active species in complexing was considered to be the diamminosilver ion, Ag(NH₃)₂⁺.

The separation of compounds with non-conjugated unsaturation according to the geometry of the double bond is one of the most useful attributes of argentation methods. *Cis-* and *trans*-monoenoic esters are readily separated by argentation adsorption chromatography in columns^{48,49} or on thin layers^{99,100} and by partition methods on thin layers^{21,110}. Various geometric isomers of polyenoic esters are also readily resolved by TLC^{50,94}.

The ability to fractionate ester mixtures according to the geometry and the degree of unsaturation has been particularly useful in the elucidation of the structure of polyunsaturated fatty acids. In combination with other techniques, argentation TLC of polyunsaturated esters partially reduced with hydrazine, which causes no positional or geometric isomerization of unreduced double bonds, has become a general procedure in the elucidation of the structures of polyunsaturated fatty acids present in various seed oils^{11,101,110,124}.

Besides effecting separation according to the degree of unsaturation and to the geometry of the double bonds, the third major attribute of argentation chromatography is its ability to separate suitable positional isomers of unsaturated fatty acid

esters. DE VRIES AND JURRIENS⁵⁰ showed that with dienoic esters the effect of silver complexing increased with increasing separation of the two double bonds and thus TLC readily separated 9,11-, 9,12- and 9,15-octadecadienoates. On this basis, a number of positional isomers of linoleic acid were detected in butter fat by argentation TLC⁴⁶. The separation of 6-, 9- and 12-octadecenoate isomers by argentation TLC was also demonstrated by DE VRIES AND JURRIENS⁵⁰.

MORRIS⁹⁰ and MORRIS AND WHARRY¹⁰² separated a considerable range of epoxy, halohydroxy, hydroxy and dihydroxy esters by argentation TLC according to the degree, type and position of unsaturation. By double impregnation with silver nitrate and boric acid⁹⁰, dihydroxy esters were separated not only on the basis of unsaturation but also according to the *threo* or *erythro* configuration of the glycol group.

Argentation chromatography, as with all forms of chromatography, is most effectively used in conjunction with other types of chromatography. The fact that it effects separation basically according to the degree and the type of unsaturation, with little if any separation of different chain lengths, makes it particularly suitable for combination with other partition methods (*e.g.*, GLC and liquid chromatography). For complete separation according to both chain length and the degree of unsaturation, BERGELSON *et al.*²¹ described the two-dimensional TLC of esters, with reversed-phase TLC in the first and argentation TLC in the second dimension. An elegant method of a combined chromatographic technique has been described¹³². The first dimension of chromatography was GLC, the components emerging from the column to be eluted on the edge of a logarithmically-travelling thin-layer plate impregnated with silver nitrate, which was developed to provide the second dimension. A similar sequence, preparative GLC followed by silver ion modified-resin column chromatography, was used by EMKEN *et al.*⁶⁰ for the separation of conjugated methyl octadecadienoate and trienoate geometric isomers. The reverse sequence, *i.e.*, preliminary fractionation by preparative argentation TLC followed by GLC of the individual unsaturation classes, has been used by many workers^{74,90,108} for the analysis of complex mixtures. A combination of silver nitrate-silicic acid column chromatography followed by GLC facilitated the elucidation of the structures of parinaric and punicic acids¹⁶⁸.

(c) *Neutral and polar lipids, sterols and other terpenoid compounds*

GOERING, CLOSSON AND OLSON⁷¹ appear to be the first workers to apply argentation partition chromatography, which is similar in effect to the more popular adsorption system in which silver nitrate is used, in lipid separation. They used a column of silica gel impregnated with silver nitrate solution as the stationary phase for the separation of *cis*- and *trans*-5-cyclodecenols. At about the same time, reversed-phase partition chromatographic methods based on silver complexing and developed by WICKBERG¹⁷² were being used for the separation of various natural terpenoid materials^{16,46,61,131}. In these methods silver fluoroborate was used instead of silver nitrate in aqueous methanol as the mobile phase in conjunction either with columns of polyvinyl chloride powder or with glass papers impregnated with hexadecane as the stationary phase. VERESHCHAGIN¹⁶⁶ and PAULOSE¹¹⁰ have described similar reversed-phase partition methods in the separation of lipids by paper chromatography and TLC, respectively, with a non-polar stationary phase and silver nitrate in the

mobile phase. These procedures effect separation according to the chain length and the degree of unsaturation simultaneously and avoid the "critical" groups that frequently occur in conventional reversed-phase chromatography. As it was the Ag^+ ions in the mobile phase that effected the separation, the re-impregnation of the silicone oil-impregnated layer with silver nitrate by PAULOSE¹¹⁰ was unnecessary. Simultaneously, MORRIS⁹⁹, who used TLC, and DE VRIES⁴⁸, who used columns, first described true argentation adsorption chromatography particularly applied to lipids. At the same time, BARRET, DALLAS AND PADLEY¹⁷ separated glyceride mixtures on silver nitrate-impregnated thin layers. These first three communications^{17,48,99} indicated the potentialities of argentation chromatography in virtually all important classes of lipid separations. The largest range of sterols so far examined, mostly as their acetates, was described by COPIUS PEEREBOOM and co-worker^{41,42} and by KLEIN *et al.*⁸⁷. The great influence of the molecular environment around the centres of unsaturation on the degree to which they participate in complexing was clearly shown in these three excellent papers. For analytical and structural studies of sterols, they also incidentally demonstrated the considerable potentiality of argentation TLC, particularly in conjunction with reversed-phase partition^{41,42} or adsorption⁸⁷ chromatography. Separation of lipid materials by argentation chromatography has been excellently reviewed by MORRIS¹⁰³. The bulk of this review described the application of separation methods involving silver ions to the separation of the various categories of lipids. Separations of fatty acid esters, sterols and other lipophilic materials were also considered.

(d) Miscellaneous separations

The ability of Ag^+ ions to complex nitriles, amines, pyridine and other nitrogen heterocyclics extended the method to the separation of a variety of organic compounds. Some excellent separations of aldehydes and ketones as their dinitrophenylhydrazone derivatives have been carried out by TLC on silver nitrate-impregnated alumina^{47,103}, kieselguhr¹⁰ and silica gel⁹⁸. BADINGS AND WASSINK¹⁰ concluded that besides complexing with $\text{C}=\text{C}$ bonds, the silver ions also complexed with $\text{C}=\text{N}$ bonds of the dinitrophenylhydrazones. Pyridine and other nitrogen heterocyclics are known to complex with silver ions, giving crystalline complexes^{111,155}, with both silver nitrate and silver perchlorate. Silver oxide has recently been shown to complex with pyridine and its homologues¹⁵⁶. The main disadvantage of using silver nitrate with more polar solvents has been overcome by TABAK and co-workers using argentation TLC with silver oxide for the separation of pyridine homologues¹⁵⁵ and of amines and unsaturated and aromatic carboxylic acids¹⁵⁷.

B. Charge-transfer interaction of other metals in the chromatographic separation of organic compounds

Relative to silver, the potential selectivity of other metals¹⁴⁵ in complexing with organic molecules has limited their application in chromatographic separation. In spite of the excellent selectivity of silver nitrate columns, silver has the serious drawback of decomposition at elevated temperatures¹⁰, which restricts its application to high-boiling compounds. To improve the instability of silver nitrate columns at higher temperatures, BANTHORPE *et al.*¹³ replaced the silver nitrate by thallium

nitrate in diethylene or polyethylene glycol as the stationary phase in GC without loss of selectivity. However, the separation of *cis*- and *trans*-but-2-enes was poorer on the thallium nitrate column than on the silver nitrate-ethylene glycol column. Recently, thallium nitrate has also been used as a substitute for silver nitrate in the TLC analysis of monoterpene hydrocarbons¹². Thallium nitrate-silica layers were found to be more stable and to have a longer usable life than the silver nitrate-impregnated layers and gave more reproducible R_F values.

It is well known that mercury salts readily form complexes with organic sulphides, preferentially with cyclic sulphides. Formation of coordination complexes with mercury(II) acetate was used by ORR¹⁰⁸ for the liquid chromatographic separation of alkyl sulphides containing 12 to 18 carbon atoms from complex mixtures on stationary phases containing mercuric acetate in aqueous acetic acid. ORR¹⁰⁹ later suggested the separation of sulphides containing 2 to 6 carbon atoms using relatively dilute aqueous zinc chloride as the stationary phase. Preferential formation of zinc chloride complexes with cyclic sulphides of low molecular weight was reflected in the values of the distribution constants for non-cyclic and cyclic sulphides. The latter values differ by about an order of magnitude for molecules containing the same number of carbon atoms. Various metal salts have been added to silica and alumina as complexing agents to improve separation. Ferric oxide hydrate and chromium oxide have been used in TLC to complex hydroxy and amino derivatives^{80,135} or other polar samples¹⁶⁰. Silica modified with cobalt oxide has been used in column chromatography for selective sulphur-type separation¹²⁰. Salts of cobalt and copper have been suggested as useful adsorbent modifiers¹³⁵. As with silver nitrate, improvements in separation by using plates impregnated with mercury salts have been proposed¹⁶⁰. Ferric chloride-impregnated silica³¹ has been found to be useful in the TLC separation of oxine derivatives.

Complexes between a metal and an organic ligand have interesting properties when used as the stationary phase or adsorbent in GC. Use of complexes having low solubilities in water and high thermal stabilities as the stationary phase or adsorbent in the GC separation of isomeric hydrocarbons and other compounds has been reviewed by ILIE⁸². The dependence of the retention volumes of various organic ligands on the environment surrounding a transition metal ion was shown by PFLAUM AND COOK¹¹⁴ using columns packed with several nickel complexes coated on Gas-Chrom P. Owing to the weak interaction that occurs at the unoccupied coordinate positions, the complexes of the *N*-dodecylsalicylaldehydes of nickel, palladium, platinum and copper and the methyl *n*-octylglyoximes of nickel, palladium and platinum, when used as column liquids, specifically retarded those molecules which act as ligands, especially amines, ketones, alcohols and molecules containing double bonds²⁷. Selective differences were observed in the behaviours of different metals. The strong interaction of nickel with amines was expected, as a large number of square planar diamagnetic complexes of nickel are known to react with two molecules of amine to give octahedral paramagnetic complexes¹³³. No such favourable rearrangement of electrons is possible in copper complexes. A similar pronounced interaction between ligands and other nickel salts has also been observed^{14,18,28,115}. The selective retardation for specific organic donors on divalent metal caproates was reported by BAYER¹⁸ and that on metal stearates by PHILLIPS¹¹⁵, and the latter has also been excellently described by BARBER *et al.*¹⁴. With stearates of divalent man-

ganese, cobalt, nickel, copper and zinc as column liquids¹⁴, retardation factors for the series plotted against the atomic number of the metal in the stearate showed a maximum for nickel followed by a sharp fall to copper for alcohols and ketones. Such a pattern is similar to that obtained for the complex stabilities for substitution at the fifth and sixth positions on the metal, as was found in studies of the metal ions in aqueous solution where the effect has been interpreted in terms of ligand-field theory⁷². A very strong interaction was observed when amines were passed through the columns of metal salts. For secondary and tertiary amines on manganese, cobalt and zinc stearates, the factors were proportional to the basic dissociation constants of amines. The special interaction with metal stearates suggested their use for effecting difficult separations. The retention of organic compounds on columns packed with complexes of *n*-nonyl- β -diketones with copper, nickel, aluminium, beryllium and zinc salts as column liquids²⁸ showed alcohols to be effective ligands for complex formation with metal diketones. The complexing action increases from the tertiary to the primary alcohols and is quite effective in the case of zinc and beryllium and more pronounced for nickel. Recently, CASTELLS AND CATTOGGIO³⁰ found a very anomalous sequence in the interactions between aliphatic amines and metallic ions by GC using different metallic stearate solutions in quadrol [N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine] as stationary phases. Greater interaction with cadmium and zinc, which have completed atomic sub-levels, than with nickel, which has incompleting sub-levels, cannot be explained exclusively on the basis of amine-cation interaction. Possibly, the presence of quadrol in the stationary phase complicated the system. The porphyrin structure of metal phthalocyanines leaves a position available for additional ligands. In GC studies using dispersed metal phthalocyanines as substrates, PECSOK AND VARY¹¹² found that phthalocyanine complexes of those metals with fewer filled *d*-orbitals were the most active towards extra electron-rich ligands. Hexane-cyclohexane-pentanone and pentane-water-methanol mixtures were well separated on columns of ferrous phthalocyanine dispersed in silicone oil. GIL-AV AND SCHURIG⁷⁰ compared complexation for 39 aliphatic and cyclic mono-olefins with Rh(CO)₂ (3-trifluoroacetylcamphorate) in squalane at 50° and silver nitrate in ethylene glycol at 40°. The K_{Rh} values were generally much higher, although the measuring temperature was 10° higher. A higher complexation constant has been measured for C₂D₄ than for C₂H₄. Compared with silver nitrate solution, a difference in retention sequences has been found for the pairs isobutene-*trans*-2-butene and 1-pentene-*cis*-2-pentene. SCHURIG AND GIL-AV¹³⁶ proposed that the olefin interaction with the rhodium(I) complex occurs via a five-coordination intermediate and also found a marked selectivity for dicarbonyl-Rh(I)- β -diketonates in their interactions with olefins. CARTONI *et al.*²⁷ earlier mentioned a marked olefin selectivity on Pd₂[(C₂H₅O)₃P]₂Cl₄ when used as column liquid. Recently, a complex-forming stationary phase of palladium dichloride (10-25%) in N-methylacetamide solution has been studied by KRAITR⁸⁰ for the selective GC separation of olefins. These types of complexes have two metal ion units on one olefin molecule so that steric situations are different from those of silver nitrate-N-methylacetamide and thereby influence the retention sequences. The separation of hexenes has been achieved at 20-40°.

The great potential selectivity of metal salts together with their high thermal stability makes gas-solid chromatography especially suitable for the separation of

isomers and high molecular weight compounds. The selectivity of columns obtained by the modification of activated alumina, silica or silica-alumina with metal salts and complexes has been discussed in general by PHILLIPS¹¹⁷ and SCOTT AND PHILLIPS¹³⁷. On the basis of spectroscopic evidence, an increase in the retention of benzene on salt-modified adsorbents compared with the unmodified ones was suggested by ROGERS¹²⁸ as a consequence of charge-transfer or electrostatic effects. It is questionable whether the charge-transfer interaction, as suggested by spectroscopic evidence at ambient temperatures, plays any role in the retention of benzene at such a high temperature (100°) with alkali halide-modified alumina. In fact, the effect of the metal ion (charge-transfer) on the selectivity of a salt-modified, highly specific adsorbent (such as alumina or silica) may not be a major contribution.

Selective adsorbents in GC were made by careful elimination of volatile compounds from crystals of metal salts. GC studies of organic compounds with such adsorbents prepared from selected heavy metal salts^{3,4,120} showed a selective retardation for many specific donors. The observed selectivity may be largely a consequence of charge-transfer interactions in which π - or non-bonded electrons of the adsorbate are involved. For columns of $\text{Cu(Py)}_2(\text{NO}_3)_2$, Cu(Py)SO_4 and $\text{Cu(Py)}_4\text{SO}_4$, the number of groups coordinated around the Cu(II) ion had a major effect on the retention characteristics of the adsorbent³. Columns of $\text{Cu(Ph)}_2(\text{NO}_3)_2$ (ref. 4) and $\text{Cu(Py)}_2(\text{NO}_3)_2$ (refs. 3 and 129) gave good separations of numerous aliphatic and aromatic hydrocarbons, alcohols, esters, ethers and ketones with minimum tailing of peaks. The latter column also exhibited some outstanding complete separations of isomeric ketones (2- and 3-pentanone and 2-, 3- and 4-heptanone), of *n*-pentyl alcohol and 2-hexanol, and of ethyl butyrate and *n*-butyl acetate. A problem due to fine particles clogging the column with unsupported copper-amine-filled columns^{3,4,120} was eliminated by using a support⁵.

4. CONCLUSIONS

The potential selectivity of the charge-transfer interaction with metals in chromatographic separations has been emphasized throughout the discussion. In connection with argentation GLC, KEULEMANS⁸⁰ reported that no adducts are formed above 40°. In fact, this is a general consequence. Charge-transfer interaction is reduced considerably at elevated temperatures and in many instances these donor-acceptor complexes are so unstable that they cannot be isolated in the pure state at ordinary temperatures, but exist only in solution in equilibrium with their components. This is the reason why argentation TLC became so popular for the analysis of high boiling compounds. Owing to practical disadvantages, classical liquid chromatography could not be popular compared with argentation TLC. Modern high-pressure liquid chromatography has removed the practical disadvantages of the classical method, both in speed and efficiency. The latter compares with GC. In future, liquid chromatography should prove to be a promising technique in this area.

The distribution coefficient in liquid-solid systems with polar adsorbents (*e.g.*, silica or alumina) is expressed by SNYDER¹⁴⁰:

$$\log K^0 = \log V_a + \alpha(S^0 - A_s \epsilon^0) + \Delta e a s$$

where the first term reflects the contribution of adsorbent surface area. V_a is the so-called adsorbent surface volume and is the volume of the adsorbed solvent monolayer per gram of the adsorbent. The second term expresses the basic adsorbent-solute-solvent interaction. Both S^0 , the free energy of adsorption of the solute molecule on the adsorbent surface of standard activity, *i.e.*, $\alpha = 1$ and A_s , the area which is effectively covered by one solute molecule on the adsorbent surface, are related to the solute molecule. The surface activity α is related to the adsorbent. The solvent strength parameter ε^0 is the free energy of adsorption of the solvent molecule per unit area of the adsorbent surface of standard activity, *i.e.*, $\alpha = 1$. The third term collects all secondary contributions of adsorbent-solute-solvent interactions.

Further, S^0 is expressed in detail as follows:

$$S^0 = \sum^i Q_i^0 - f(Q_k^0) \sum^{i \neq k} Q_i^0 + \sum^i \sum^j q_{ij}^0$$

where Q_i = adsorption energy of the group i on the adsorbent surface of standard activity, *i.e.*, $\alpha = 1$.

$f(Q_k^0) \sum^{i \neq k} Q_i^0$ = net losses in the adsorption energies of all the groups i except k due to delocalisation.

$\sum^i \sum^j q_{ij}^0$ = net changes in the adsorption energies of all the groups i due to the presence of the groups j .

In this relation, the first term is related to the basic interaction given by the nature of the solute molecule since the two other terms reflect the induced interaction during sorption of solute from solvent on the adsorbent surface.

Porapak Q, a non-polar variety of Porapak (Water Associates, Framingham, Mass., U.S.A.), is believed to behave as a non-polar stationary phase-adsorbent and retains compounds, mainly by dispersion forces, according to their boiling points or carbon numbers. With Porapak Q as adsorbent, the non-dispersive functions

$(Q_k^0) \sum^{i \neq k} Q_i^0$ and $\sum^i \sum^j q_{ij}^0$ can be neglected to a first approximation and the expression

for the distribution coefficient simplifies to:

$$\log K^0 = \log V_a + \alpha (\sum^i Q_i^0 - A_s \varepsilon^0) + \Delta eas$$

In a chromatographic system with Porapak Q as the column packing and metal salt solutions as the mobile phase, which can form charge-transfer complexes with solute molecules, the magnitude of Δeas will have negative values. Thus, depending on the magnitude of Δeas , which is a measure of the stability constant, such a chromatographic system, unlike polar adsorbents (*e.g.*, silica or alumina), can provide group- as well as compound-type separation.

Polymers such as Porapak Q or Synachrom (Research Institute of Lacquers and Resins, Pardubice, Czechoslovakia) give, owing to their polyhydrocarbon nature, an excellent possibility of realizing reversed-phase chromatographic systems in which the sorbent-solute interactions are determined largely by dispersion forces alone⁸⁶. Extreme equilibria may be expected with the use of a complex-forming mobile phase. Contrary to common opinion, liquid chromatographic columns of high

efficiency have been found by one of us²³ with narrow-mesh Porapak Q (3–15 μ). Thus, the use of porous organic polymers now available with specially designed porosities and chemical natures as stationary phases and complexing agents as mobile phases, seems to indicate new prospective systems in TLC and in high-efficiency liquid chromatography.

New separations can be expected particularly in hydrocarbon chemistry, but not only in this field.

SUMMARY

Charge-transfer complexes of metals with organic compounds are discussed. For convenience, the application of charge-transfer interactions with metals in the chromatographic separation of organic compounds is divided into silver ion-argentation and non-silver metal ion chromatography. The use of argentation chromatography in the analysis of fatty acid esters and natural products is discussed only briefly as it has been excellently reviewed elsewhere. The use of charge-transfer complexes of non-silver metals in the chromatographic separation of organic compounds is reviewed, including most of the important applications in this field. Chromatographic systems of organic porous polymers with systematically modified structures as column materials and metal salt solutions as mobile phases are suggested for the new potential separations of complex organic mixtures.

REFERENCES

- 1 H. J. ACHE AND A. P. WOLF, *J. Amer. Chem. Soc.*, **88** (1966) 888.
- 2 H. J. ACHE AND A. P. WOLF, *Z. Anal. Chem.*, **230** (1967) 19.
- 3 A. G. ALTENAU AND L. B. ROGERS, *Anal. Chem.*, **36** (1964) 1726.
- 4 A. G. ALTENAU AND L. B. ROGERS, *Anal. Chem.*, **37** (1965) 1432.
- 5 A. G. ALTENAU AND C. MERRITT, JR., *J. Gas Chromatogr.*, **5** (1967) 30.
- 6 R. L. ANDERSON AND E. J. HOLLENBACH, *J. Lipid Res.*, **6** (1965) 577.
- 7 L. J. ANDREWS AND R. M. KEEFER, *Molecular Complexes in Organic Chemistry*, Holden-Day, London, 1964.
- 8 F. ARMITAGE, *J. Chromatogr.*, **2** (1959) 655.
- 9 J. G. ATKINSON, A. A. RUSSELL AND R. S. STUART, *Can. J. Chem.*, **45** (1967) 1963.
- 10 H. T. BADINGS AND J. G. WASSINK, *Neth. Milk Dairy J.*, **17** (1963) 132.
- 11 M. O. BAGBY, C. R. SMITH, JR. AND I. A. WOLFF, *Lipids*, **1** (1966) 263.
- 12 D. A. BAINES AND R. A. JONES, *J. Chromatogr.*, **47** (1970) 130.
- 13 D. V. BANTHORPE, C. GATFORD AND B. R. HOLLEBONE, *J. Gas Chromatogr.*, **6** (1968) 61.
- 14 D. W. BARBER, C. S. G. PHILLIPS, G. F. TUSA AND A. VERDIN, *J. Chem. Soc.*, (1959) 18.
- 15 J. A. BARNARD AND H. W. D. HUGHES, *Nature*, **183** (1959) 250.
- 16 H. S. BARRETO AND C. ENZELL, *Acta Chem. Scand.*, **15** (1961) 1313.
- 17 C. B. BARRETT, M. S. J. DALLAS AND F. B. PADLEY, *Chem. Ind. (London)*, (1962) 1050.
- 18 E. BAYER in D. H. DESTY (Editor), *Gas Chromatography 1958*, Butterworths, London, 1958, p. 333.
- 19 M. E. BEDNAS AND D. S. RUSSEL, *Can. J. Chem.*, **36** (1958) 1272.
- 20 E. BENDEL, B. FELL, W. GARTZEN AND G. KRUSE, *J. Chromatogr.*, **31** (1967) 531.
- 21 L. D. BERGELSON, E. V. DYATLOVITSKAYA AND V. V. VORONKOVA, *J. Chromatogr.*, **15** (1964) 191.
- 22 B. W. BRADFORD, D. HARVEY AND D. E. CHALKEY, *J. Inst. Petrol.*, **41** (1955) 80.
- 23 H. C. BROWN AND W. J. WALLACE, *J. Amer. Chem. Soc.*, **75** (1953) 6265.
- 24 H. C. BROWN AND W. J. WALLACE, *J. Amer. Chem. Soc.*, **75** (1953) 6268.
- 25 T. L. BROWN AND M. KUBOTA, *J. Amer. Chem. Soc.*, **83** (1961) 4175.
- 26 J. N. BUTLER AND G. B. KISTIAKOWSKY, *J. Amer. Chem. Soc.*, **82** (1960) 759.
- 27 G. P. CARTONI, R. S. LOWRIE, C. S. G. PHILLIPS AND L. M. VENANZI, in R. P. W. SCOTT (Editor), *Gas Chromatography*, Butterworths, London, 1960, p. 273.

- 28 G. P. CARTONI, A. LIBERTI AND R. PALOMBARI, *J. Chromatogr.*, 20 (1965) 278.
29 D. CASSIMATIS AND B. P. SUSZ, *Helv. Chim. Acta*, 43 (1960) 852.
30 R. C. CASTELLS AND J. A. CATOGGIO, *Anal. Chem.*, 42 (1970) 1268.
31 M. A. CAWTHORNE, *J. Chromatogr.*, 25 (1966) 164.
32 P. CHALANDON AND B. P. SUSZ, *Helv. Chim. Acta*, 41 (1958) 697.
33 J. CHATT AND L. M. VENANZI, *J. Chem. Soc.*, (1957) 4735.
34 J. CHATT, L. M. VALLARINO AND L. M. VENANZI, *J. Chem. Soc.*, (1957) 2496.
35 J. P. CHESICK, *J. Amer. Chem. Soc.*, 82 (1960) 3277.
36 D. COOK, *Can. J. Chem.*, 37 (1959) 48.
37 A. C. COPE, N. A. LEVEL, H. H. LEE AND W. R. MOORE, *J. Amer. Chem. Soc.*, 79 (1957) 4720.
38 A. C. COPE, C. L. BUMGARDNER AND E. E. SCHWEIZER, *J. Amer. Chem. Soc.*, 79 (1957) 4729.
39 A. C. COPE AND E. M. ACTON, *J. Amer. Chem. Soc.*, 80 (1958) 355.
40 A. C. COPE, P. T. MOORE AND W. R. MOORE, *J. Amer. Chem. Soc.*, 82 (1960) 1744.
41 J. W. COPIUS PEEREBOOM, *Z. Anal. Chem.*, 205 (1964) 325.
42 J. W. COPIUS PEEREBOOM AND H. W. BEEKES, *J. Chromatogr.*, 17 (1965) 99.
43 H. J. CVETANOVIĆ, F. J. DUNCAN, W. E. FALCONER AND R. S. IRWIN, *J. Amer. Chem. Soc.*, 87 (1965) 1827.
44 R. J. CVETANOVIĆ, F. J. DUNCAN AND W. E. FALCONER, *Can. J. Chem.*, 41 (1963) 2095.
45 P. DANIELS AND C. ENZELL, *Acta Chem. Scand.*, 16 (1962) 1530.
46 K. DE JONG AND H. VAN DER WEL, *Nature*, 202 (1964) 553.
47 K. DE JONG, K. MOSTERT AND D. SLOOT, *Rec. Trav. Chim. Pays-Bas Belge*, 82 (1963) 837.
48 B. DE VRIES, *Paper Presented at the Vth Congress, International Society for Fat Research, London, April, 1962; Chem. Ind. (London)*, (1962) 1049.
49 B. DE VRIES, *J. Amer. Oil Chem. Soc.*, 40 (1963) 184.
50 B. DE VRIES AND G. JURRIENS, *Fette Seifen Anstrichm.*, 65 (1963) 725.
51 M. J. S. DEWAR, *Bull. Soc. Chim. Fr.*, 18 (1951) C71.
52 J. DUBRIN, C. MACKAY AND R. WOLFGANG, *J. Amer. Chem. Soc.*, 86 (1964) 959.
53 J. J. DUFFIELD AND L. B. ROGERS, *Anal. Chem.*, 34 (1962) 1193.
54 E. DUNN AND P. ROBSON, *J. Chromatogr.*, 17 (1965) 501.
55 L. A. DU PLESSIS AND A. H. SPONG, *J. Chem. Soc.*, (1959) 2027.
56 L. A. DU PLESSIS, *J. Gas Chromatogr.*, 1, No. 11, (1963) p. 6.
57 W. F. EBERZ, *The Chemistry of Petroleum Derivatives*, Chemical Catalog Co., New York, pp. 142 and 582.
58 J. A. ELVIDGE AND A. B. P. LEVER, *J. Chem. Soc.*, (1961) 1257.
59 E. A. EMKEN, C. R. SCHOLFIELD AND H. J. DUTTON, *J. Amer. Oil Chem. Soc.*, 41 (1964) 388.
60 E. A. EMKEN, C. R. SCHOLFIELD, V. L. DAVISON AND E. N. FRANKEL, *J. Amer. Oil Chem. Soc.*, 44 (1967) 373.
61 C. ENZELL, *Acta Chem. Scand.*, 15 (1961) 1303.
62 R. C. FAY AND T. S. PIPER, *J. Amer. Chem. Soc.*, 85 (1963) 500.
63 E. O. FISHER AND M. WERNER, *Metal π -Complexes*, Vol. I, Elsevier, Amsterdam, 1966.
64 H. M. FREY, *Nature*, 183 (1959) 743.
65 P. GAGNAUX, D. JANJIC AND B. P. SUSZ, *Helv. Chim. Acta*, 41 (1958) 1023.
66 E. GIL-AV, J. HERLING AND J. SHABTAI, *Chem. Ind. (London)*, (1957) 1483.
67 E. GIL-AV, J. HERLING AND J. SHABTAI, *J. Chromatogr.*, 1 (1958) 508.
68 E. GIL-AV AND J. HERLING, *J. Phys. Chem.*, 66 (1962) 1208.
69 E. GIL-AV AND J. HERLING, *Proc. Journ. Hell. d'Étude des Méthodes de Séparation Immédiate et de Chromatographie, Athens, September, 1965*, Vol. 1, Union des Chimistes Hellènes, Athens, 1966, p. 167.
70 E. GIL-AV AND V. SCHURIG, in A. ZLATKIS (Editor), *Advances in Chromatography 1971*, University of Houston, 1971, p. 111.
71 H. L. GOERING, W. D. CLOSSON AND A. C. OLSON, *J. Amer. Chem. Soc.*, 83 (1961) 3507.
72 M. GRIFFITH AND M. ORGEL, *Quart. Rev.*, 11 (1957) 387.
73 O. K. GUHA, *Some Preliminary Tests on the Evaluation of Porapak Q Columns for Liquid Chromatography*, Unpublished Data.
74 F. D. GUNSTONE AND A. J. SEALY, *J. Chem. Soc.*, (1964) 4407.
75 E. A. HALEVI, *Prog. Phys. Org. Chem.*, 1 (1963) 109.
76 E. A. HALEVI, M. NUSSIM AND A. RON, *J. Chem. Soc.*, (1963) 866.
77 F. R. HARTLEY, *Chem. Rev.*, 69 (1969) 799.
78 F. R. HEPNER, K. N. TRUEBLOOD AND H. J. LUCAS, *J. Amer. Chem. Soc.*, 74 (1952) 333.
79 J. HERLING, J. SHABTAI AND E. GIL-AV, *J. Chromatogr.*, 8 (1962) 349.
80 G. HESSE AND M. ALEXANDER, *Vortragsref., Journ. Int. d'Étude des Méthodes de Séparation Immédiate et de Chromatographie, 13-15th June, 1961, Paris*.
81 R. A. HIVELEY, *J. Chem. Eng. Data*, 5 (1960) 237.
82 V. ILIE, *Rev. Chim. (Bucharest)*, 20 (1969) 43.

- 83 W. B. INNES, W. E. BAMBRICK AND A. J. ANDREATCH, *Anal. Chem.*, 35 (1963) 1198.
- 84 J. JANÁK, in D. H. DESTY (Editor), *Vapour Phase Chromatography*, Butterworths, London, 1957, p. 247.
- 85 J. JANÁK, Z. JAGARIČ AND M. DRESSLER, *J. Chromatogr.*, 53 (1970) 525.
- 86 A. I. M. KEULEMANS, *Gas Chromatography*, Reinhold, New York, 1957, p. 205.
- 87 P. D. KLEIN, J. C. KNIGHT AND P. A. SZCZEPANIK, *J. Amer. Oil Chem. Soc.*, 43 (1966) 275.
- 88 A. KLINKENBERG, *Chem. Eng. Sci.*, 15 (1961) 255.
- 89 M. KRAITR, *Thesis*, Technical University, Prague, 1972.
- 90 D. F. KUEMMEL, *J. Amer. Oil Chem. Soc.*, 41 (1964) 667.
- 91 W. LAGENBACK, H. SCHUBERT AND H. GIESEMANN, *Ann. Chem.*, 585 (1954) 68.
- 92 T. J. LANE, A. YAMAGUCHI, J. V. QUAGLIANO, J. A. RYAN AND S. MIZUSHIMA, *J. Amer. Chem. Soc.*, 81 (1960) 3824.
- 93 E. K. C. LEE AND F. S. ROWLAND, *Anal. Chem.*, 36 (1964) 2181.
- 94 A. M. LEES AND E. D. KORN, *Biochim. Biophys. Acta*, 116 (1966) 403.
- 95 H. J. LUCAS, R. S. MOORE AND D. PRESSMAN, *J. Amer. Chem. Soc.*, 65 (1943) 227.
- 96 W. LUCHSINGER, in H. G. STRUPE (Editor), *Gas Chromatographie 1958*, Akademie-Verlag, Berlin, 1959, p. 118.
- 97 R. H. LUEBBE, JR. AND J. E. WILLARD, *J. Amer. Chem. Soc.*, 81 (1959) 761.
- 98 P. W. MEIJBOOM AND G. JURRIENS, *J. Chromatogr.*, 18 (1965) 424.
- 99 L. J. MORRIS, *Paper Presented at the Vith Congress, International Society for Fat Research, London, April, 1962; Chem. Ind. (London)*, (1962) 1238.
- 100 L. J. MORRIS, D. M. WHARRY AND E. W. HAMMOND, *J. Chromatogr.*, 31 (1967) 69.
- 101 L. J. MORRIS AND M. O. MARSHALL, *Chem. Ind. (London)*, (1966) 1493.
- 102 L. J. MORRIS AND D. M. WHARRY, *J. Chromatogr.*, 20 (1965) 27.
- 103 L. J. MORRIS, *J. Lipid Res.*, 7 (1966) 717.
- 104 R. W. MOSHIER AND H. E. SIEVERS, *Gas Chromatography of Metal Chelates*, Pergamon Press, New York, 1965.
- 105 M. A. MUHS AND F. T. WEISS, *J. Amer. Chem. Soc.*, 84 (1962) 4697.
- 106 R. S. MULLIKEN, *J. Phys. Chem.*, 56 (1952) 801.
- 107 G. A. OLAH, S. J. KUHN, W. S. TOLGYESI AND E. B. BAKER, *J. Amer. Chem. Soc.*, 84 (1962) 2733.
- 108 W. L. ORR, *Anal. Chem.*, 38 (1966) 1558.
- 109 W. L. ORR, *Anal. Chem.*, 39 (1967) 1163.
- 110 M. M. PAULOSE, *J. Chromatogr.*, 21 (1966) 141.
- 111 W. J. PEARD AND R. T. PFLAUM, *J. Amer. Chem. Soc.*, 80 (1958) 1593.
- 112 R. L. PECSOK AND E. M. VARY, *Anal. Chem.*, 39 (1967) 289.
- 113 H. J. PETROWITZ, *Z. Anal. Chem.*, 213 (1965) 194.
- 114 R. T. PFLAUM AND L. E. COOK, *J. Chromatogr.*, 50 (1970) 120.
- 115 C. PHILLIPS, in V. J. COATES, H. J. NOBELS AND I. S. FAGERSON (Editors), *Gas Chromatography*, Academic Press, New York, 1958, p. 58.
- 116 C. S. G. PHILLIPS AND P. L. TIMMS, *Anal. Chem.*, 35 (1963) 505.
- 117 C. S. G. PHILLIPS, in J. H. PURNELL (Editor), *Progress in Gas Chromatography*, Interscience Publishers, New York, 1967, p. 121.
- 118 J. W. POWELL AND M. C. WHITING, *Tetrahedron*, 7 (1959) 305.
- 119 R. G. POWELL, C. R. SMITH, JR., AND I. A. WOLFF, *J. Amer. Oil Chem. Soc.*, 42 (1965) 450A, Abstr. No. 35.
- 120 H. PREZLER AND R. OEHLER, *Chem. Tech., Leipzig*, 5 (1963) 31.
- 121 G. R. PRIMAVESI, *Nature*, 184 (1959) 2010.
- 122 O. S. PRIVETT AND E. C. NICKELL, *J. Amer. Oil Chem. Soc.*, 40 (1963) 189.
- 123 O. S. PRIVETT, M. L. BLANK AND O. ROMANUS, *J. Lipid Res.*, 4 (1963) 260.
- 124 O. S. PRIVETT AND E. C. NICKELL, *Lipids*, 1 (1966) 98.
- 125 H. J. PURNELL, in A. B. LITTLEWOOD (Editor), *Gas Chromatography 1966*, Institute of Petroleum, London, 1967, p. 3.
- 126 H. W. QUIN AND J. H. TSAI, *Adv. Inorg. Chem. Radiochem.*, 12 (1969) 217.
- 127 B. S. RABINOVITCH AND K. W. MICHEL, *J. Amer. Chem. Soc.*, 81 (1959) 5065.
- 128 A. R. ROGERS, in A. GOLDUP (Editor), *Gas Chromatography 1964*, Institute of Petroleum, London, 1965, p. 284 (discussion).
- 129 L. B. ROGERS AND A. G. ALTENAU, *Anal. Chem.*, 35 (1963) 915.
- 130 J. W. ROOT, E. K. C. LEE AND F. S. ROWLAND, *Science*, 143 (1964) 676.
- 131 J. RUNEBURG, *Acta Chem. Scand.*, 14 (1960) 1985.
- 132 N. RUSEVA-ATANASOVA AND J. JANÁK, *J. Chromatogr.*, 21 (1966) 207.
- 133 L. SACCONI, G. LOMBARDO AND P. PAOLETTI, *J. Chem. Soc.*, (1958) 853.
- 134 R. R. SAUERS, *J. Amer. Chem. Soc.*, 81 (1959) 4873.
- 135 P. J. SCHORN, *Z. Anal. Chem.*, 205 (1964) 298.

- 136 V. SCHURIG AND E. GIL-AV, *Chem. Commun.*, (1971) 650.
137 C. G. SCOTT AND C. S. G. PHILLIPS, in A. GOLDUP (Editor), *Gas Chromatography 1964*, Institute of Petroleum, London, 1965, p. 266.
138 C. G. SCOTT AND C. S. G. PHILLIPS, *Nature*, 199 (1963) 66.
139 D. S. SGOUTAS AND F. A. KUMMEROW, *J. Chromatogr.*, 16 (1964) 448.
140 J. SHABTAI, J. HERLING AND E. GIL-AV, *J. Chromatogr.*, 2 (1959) 406.
141 J. SHABTAI, *Isr. J. Chem.*, 1 (1963) 300.
142 J. SHABTAI, J. HARTING AND E. GIL-AV, *J. Chromatogr.*, 11 (1963) 32.
143 J. SHABTAI, *J. Chromatogr.*, 18 (1965) 302.
144 R. E. SIEVERS, B. W. PONDER, M. L. MORRIS AND R. W. MOSHIER, *Inorg. Chem.*, 2 (1963) 693.
145 L. G. SILLEN AND A. E. MARTELL, *Stability Constants*, Metcalfe and Cooper Limited, London, 1964.
146 P. E. SLADE, JR. AND H. B. JONASSEN, *J. Amer. Chem. Soc.*, 79 (1957) 1277.
147 B. SMITH AND R. OHLSON, *Acta Chem. Scand.*, 13 (1959) 1253.
148 B. SMITH AND R. OHLSON, *Acta Chem. Scand.*, 16 (1962) 351.
149 L. R. SNYDER, *Principles of Adsorption Chromatography: The Separation of Non-ionic Organic Compounds*, Marcel Dekker, New York, 1968, pp. 218, 296 and 385.
150 E. STAHL, in E. STAHL (Editor), *Thin-Layer Chromatography: A Laboratory Handbook*, Academic Press, New York, 1965, p. 34.
151 B. P. SUSZ AND I. COOKE, *Helv. Chim. Acta*, 37 (1954) 1273.
152 B. P. SUSZ AND A. LACHAVANNE, *Helv. Chim. Acta*, 41 (1958) 634.
153 B. P. SUSZ AND P. CHALANDON, *Helv. Chim. Acta*, 41 (1958) 1332.
154 B. P. SUSZ AND D. CASSIMATIS, *Helv. Chim. Acta*, 44 (1961) 395.
155 S. TABAK AND M. R. M. VERZOLA, *J. Chromatogr.*, 51 (1970) 334.
156 S. TABAK AND M. MOLINA, *Tetrahedron Lett.*, 11 (1970) 813.
157 S. TABAK, A. E. MAURO AND A. DEL'ACQUA, *J. Chromatogr.*, 52 (1970) 500.
158 T. TAKAGI, *J. Amer. Oil Chem. Soc.*, 43 (1966) 249.
159 H. J. TAUFEN, M. J. MURRAY AND F. F. CLEVELAND, *J. Amer. Chem. Soc.*, 63 (1941) 3500.
160 H. M. TENNY, *Anal. Chem.*, 30 (1958) 2.
161 A. N. TERENIN AND A. N. SIDOROV, *Spectrochim. Acta*, (1957) 573.
162 J. G. TRAYNHAM, *J. Org. Chem.*, 26 (1961) 4694.
163 G. URBACH, *J. Chromatogr.*, 12 (1963) 196.
164 J. H. VAN DEN HENDE AND W. C. BAIRD, JR., *J. Amer. Chem. Soc.*, 85 (1963) 1009.
165 F. VAN DE CRAATS, *Anal. Chim. Acta*, 14 (1956) 136.
166 A. G. VERESHCHAGIN, *J. Chromatogr.*, 17 (1965) 382.
167 R. VILLALOBOS, in L. FOWLER, R. G. HARMON AND D. K. ROE (Editors), *Analysis Instrumentation*, Plenum Press, New York, 1963, p. 13.
168 H. WAGNER, J. D. GOETSCHEL AND P. LESCH, *Helv. Chim. Acta*, 46 (1963) 2986.
169 D. WALDI, in E. STAHL (Editor), *Thin-Layer Chromatography: A Laboratory Handbook*, Academic Press, New York, 1965, p. 3.
170 S. P. WASIK AND W. TSANG, *J. Phys. Chem.*, 74 (1970) 2970.
171 S. P. WASIK AND W. TSANG, *Anal. Chem.*, 42 (1970) 1648.
172 B. WICKBERG, *J. Org. Chem.*, 27 (1962) 4652.
173 D. WILLER, *Chem. Ind. (London)*, (1965) 1839.
174 S. WINSTEIN AND H. J. LUCAS, *J. Amer. Chem. Soc.*, 60 (1938) 836.
175 R. WOOD AND F. SNYDER, *J. Amer. Oil Chem. Soc.*, 43 (1966) 53.
176 C. F. WURSTER, JR., J. H. COPENHAVER, JR. AND P. R. SCHAFFER, *J. Amer. Oil Chem. Soc.*, 40 (1963) 513.
177 M. ZACKRISSON AND I. LINDQVIST, *J. Inorg. Nucl. Chem.*, 17 (1961) 69.
178 A. ZLATKIS, G. S. CHAO AND H. R. KAUFMAN, *Anal. Chem.*, 36 (1964) 2354.
179 A. ZLATKIS, *Chromatographia*, 2 (1969) 298.
180 A. D. ZORIN, A. E. EZHELEVA AND G. G. DEVYATYKH, *Tr. Khim. Khim. Tekhnol.*, 1 (1958) 605.