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LIGAND-EXCHANGE CHROMATOGRAPHY

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CONTENTS

1. INTRODUCTION

The number of workers employing ligand-exchange chromatography (LEC) for the separation and purification of organic compounds is increasing steadily, as shown by the rapidly expanding volume of information on LEC techniques published in journals, the annual output of such papers having increased about five-fold in the past 3–4 years. However, most of the publications have been contributed by chemists involved in synthetic research and are, therefore, devoted mainly to empirical considerations of the problem, many of the papers involving virtually identical experi-

mental chromatographic work. At the same time one can find descriptions of diverse and fundamental studies in LEC, especially the investigations carried out by H. F. Walton, W. Funasaka, K. Fujimura, K. Shimomura and co-workers. These workers have made significant contributions to the theory of LEC.

About 15 years ago, arising from concomitant developments in the chemistry of complex compounds and high-molecular-weight compounds and in ion exchange, LEC, in its liquid and gas variations, made it possible to resolve many of the outstanding problems in the separation and purification of different substances, which other types of chromatography had failed to do. Moreover, LEC became a powerful and reliable method for the investigation of kinetically labile complexes.

Having acquired comprehensive information on chromatographic processes that involve coordination compounds, we aimed not only to demonstrate the achievements that have been made in chromatography, but also to point out, as far as possible, the future potential of LEC techniques.

There have been two previous reviews on LEC[1,2]. This review covers earlier literature on liquid chromatographic techniques and discusses novel studies that have been published up to 1977. As far as gas and thin-layer LEC is concerned, we have not attempted to cover all of the literature available, but have restricted ourselves to the most important publications. This problem has been elucidated in more detail in some specialized reviews[3–5].

This review consists of two main parts. The first part (section 3) offers some theoretical considerations on LEC while the second part (Section 4) deals with its practical applications.

2. HISTORY AND TERMINOLOGY

Although some LEC techniques had been used previously in both liquid and gas variations[6–11], the term "ligand exchange" as applied to chromatography was first suggested by Helfferich[12] in 1961. It was Helfferich who first interpreted, defined and partially verified the basic principles of this method[13–15], but so far no exact and clear definition of LEC has been suggested.

Let us consider the chromatographic processes that involve ligand exchange. Muzzarelli *et al.*[16] offered the following definition: "ligand-exchange chromatography is based on the principle that a molecule or ion, which is part of a complex fixed on a support, can be released because a different molecule or ion enters to form a more stable complex or because the complex collapses when the medium is altered". They suggest further, "argentation chromatography and related techniques are not ligand-exchange chromatography, because the complexing agent is merely added to silica gel or alumina and is not bound". Such a definition of LEC is not satisfactory and some objections can be made[1,2]. It not only ignores the multiplicity of liquid chromatographic processes in which various ligand exchanges undoubtedly take place, but also rejects gas LEC chromatography which requires a simple addition of complex-forming ion salts to a standard support.

We suggest[2] that "ligand-exchange chromatography" should be defined as a process in which interaction between the stationary phase and the molecules to be separated occurs during the formation of coordination bonds inside the coordination sphere of the complex-forming ion.

LEC differs from ion-exchange, adsorption and other types of chromatography in its basic process of interaction between the sorbate and stationary phase. In this instance the interaction does not take place directly but is accomplished via the coordination sphere of the complex-forming metal ion. It is the exchange of ligands bound to the central ion of the metal that suggests the term "ligand-exchange chromatography".

The marked tendency of Ag^+ ions to bind different complex-forming molecules is the main distinguishing feature of silver-containing sorbents. It is reasonable, therefore, to include thin-layer argentation chromatography in the above definition of LEC.

The formation of some labile organic complexes is closely related to LEC processes. For instance, borate and hydrogen sulphite ions are known to form adducts with glycols and carbonyl compounds, respectively, and therefore it is possible to resolve these compounds on ion-exchange resins in the borate or hydrogen sulphite form[6,7,17]. It is obvious that the deeper our insight into the nature of sorbate–sorbent interactions becomes, the more new or known examples of separations will be attributed to LEC. This involves, in particular, different types of chromatography on inorganic sorbents that contain complex-forming ions or impurities.

Depending on whether the metal ion is fixed on to the stationary phase or whether it is moved along the column by the mobile phase, one can distinguish two types of LEC: the chromatography of ligands and the chromatography of complexes.

In the former instance the metal ion is held by the stationary phase via electrostatic, coordination and/or other bonds to form a stationary complex, $\bar{R}M$. If the coordination sphere of this ion is unsaturated (usually the free coordination sites are temporarily occupied by weakly bound solvent molecules), it can sorb reversively different ligands from the mobile phase. In chromatography the ligands are resolved according to their ability to enter into the coordination ion sphere.

In the latter instance the complex-forming metal ion is bound more strongly to the ligands dissolved in the mobile phase than to those located in the solid phase. Therefore, the mobile phase usually contains several complexes with the same central metal ion. This type of LEC of complexes differs from the ion-exchange chromatography of complexes by the fact that the functional groups of the resin enter into the coordination sphere of the complexes, *i.e.*, they function as stationary ligands.

Sorption in both the LEC of complexes and the LEC of ligands on a chelating phase represents the formation of mixed sorption complexes, $\bar{R}MA$, containing the stationary ligand $\bar{R}$, the complex-forming metal ion M and the mobile ligand A.

Depending on the nature of the mobile phase, LEC can operate in both gas and liquid variations. In the latter instance not only water but also various organic solvents are suitable. Finally, LEC may involve frontal, displacement and elution techniques.

3. PECULIARITIES OF LIGAND-EXCHANGE CHROMATOGRAPHY

A. General features of ligand-exchange chromatography

All chromatographic processes are based on adsorption phenomena. In LEC, as mentioned above, sorption takes place owing to the formation of coordination bonds. The sorption of two compounds, A and B, on a sorbent $\bar{R}$ in the form of a

chelate with a transition metal M takes place by the build-up of a coordination sphere of the stationary complex $\bar{R}M$. This process can be expressed in the form (electrochemical charges being omitted):

$$\bar{R}M + A \overset{K_{RMA}}{\rightleftharpoons} \bar{R}MA$$

$$\bar{R}M + B \overset{K_{\bar{R}MB}}{\rightleftharpoons} \bar{R}MB$$

LEC is possible only in systems where the interaction of the mobile ligand with the stationary phase is a reversible process. The coordination bonds between the ligands and the metal ion must break down readily and re-form, *i.e.*, the sorption complexes must be kinetically labile.

Under equilibrium conditions, ligands A and B are distributed between the mobile and solid phases strictly in accordance with the thermodynamic stabilities of the sorption complexes formed. The greater the difference in the stabilities of these complexes being formed during the separation, the higher is the selectivity of chromatography (Fig. 1). In an ideal case the column selectivity (a) with simultaneous eluation of ligands A and B is determined by the ratio of the formation constants of the sorption complexes[18]:

$$a = \frac{V_B}{V_A} = \frac{K_{\bar{R}MB}}{K_{RMA}}$$

In practice, the selectivity is influenced to a certain extent by the nature and mutual arrangement of the sorbent framework chains, partial coordination to the metal ion of the solvent and the eluent molecules and some other factors.

Complex formation, which is the basis of LEC, is a process that differs considerably in its high selectivity from physical adsorption or ion exchange. Whereas the electrostatic forces of interaction of charged particles are devoid of a definite steric direction and do not place severe requirements upon the distance between the particles, the interaction of the donating ligand groups with the central metal ion in a complex can take place only in a definite direction and at strictly fixed distances. Such a

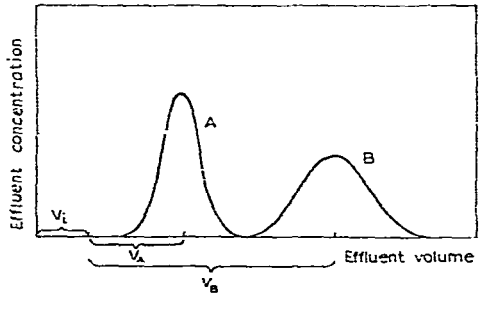


Fig. 1. Elution curves of substances A and B. V_l is the column void volume and V_A and V_B are the retention volumes of compounds A and B, respectively.

feature of complex formation makes it possible, using ligand-exchange techniques to resolve not only ligands that differ considerably from one another in their natures, but also to separate successfully compounds with very similar properties. Geometric and positional isomers, homologues and even isotopes and optical isomers are examples.

The selectivity of complex formation has been used successfully in the ion-exchange separation of rare earth mixtures in the presence of a complex-forming agent[19,20]. LEC employs efficiently the selectivity of the same process to solve an opposite problem, namely the separation of a mixture of complexing agents in the presence of one of the metal ions.

The ligand-exchange capacity ($X_{\text{lig.}}$) of a resin is defined as the number of available coordination sites per unit weight or unit volume of the resin. Let us consider a case where the metal ion with a coordination number N and electrochemical valency Z is adsorbed on a cation-exchange resin only by ionic bonds. The relationship between the ion-exchange ($X_{\text{ion.}}$) and ligand-exchange ($X_{\text{lig.}}$) capacities of the resin is

$$X_{\text{lig.}} = \frac{X_{\text{ion.}} \cdot N}{Z}$$

Thus the sorption of ammonia, for instance[13], by sulphonated Dowex-50 (Ni^{2+}) resin ($X_{\text{lig.}} = 15$ mequiv./g) is more advantageous than that by the resin in the H^+ form ($X_{\text{ion.}} = 5$ mequiv./g); $Z = 2$ and $N = 6$ (for Ni^{2+}). However, if the complexes formed are large and the fixed ionic resin groups block part of the coordination sphere of the metal ion, the maximum ligand-exchange capacity will not be reached.

LEC has other advantages[13–15]. Firstly, owing to the high stability of the complexes formed, the sorbents are capable of selectively binding the mobile ligands irrespective of the concentration of foreign salts and non-electrolytes in the external solution. In this manner, ligand sorption can be used successfully both for the separation of complex-forming compounds from all kinds of substances that are devoid of donating groups and for the concentration of dilute ligand solutions.

Secondly, LEC is an exceptionally flexible process as the wide choice of metal ions that possess a great variety of complex-forming features makes it possible to change the properties of the sorbent advantageously by re-loading the resin as appropriate with another metal ion.

Thirdly, in the separation of ligands with different coordinative valencies, the selectivity of their interaction with the sorbent can be adjusted or even reversed simply by varying the total concentration of ligands in the external solution. Let us consider the exchange of a monodentate ligand A for a bidentate ligand B in a nickel complex:

$$NiA_6 + 3B \leftrightarrows NiB_3 + 6A$$

In this instance, at equilibrium:

$$\frac{[NiB_3]\,[A]^6}{[NiA_6]\,[B]^3} = K$$

or

$$\frac{[NiB_3]\,[A]^3}{[NiA_6]} = K\left(\frac{[B]}{[A]}\right)^3$$

For a given constant concentration ratio of B to A, the right-hand side of the last equation remains constant, and the high total ligand concentration favours the formation of an NiA_6 complex while a low total concentration of free ligands favours the formation of NiB_3. Hence, the monodentate ligands must be preferentially sorbed from a concentrated solution and the bidentate ligands from a dilute solution. Therefore, the same resin can sorb preferentially either mono- or bidentate ligands depending on the concentration of the external solution.

To confirm this assumption, Helfferich[14] carried out the following three experiments: a diamine (1,3-diaminopropanol-2) was sorbed from a dilute (0.005 *M*) solution and from a diamine–ammonia mixture (0.1 *M* ammonia + 0.001 *M* 1,3-diaminopropanol-2), and the sorbed diamine was then displaced with concentrated ammonia solution. These experiments were run on small columns containing the carboxylic resin Amberlite IRC-50 (Ni^{2+} form). As far as sorption of the diamine from the dilute solution is concerned, the equilibrium sorption capacity of the resin for diamine is virtually identical with the theoretical value of the ligand-exchange capacity, as the predominant complex in the resin is $[Ni(diamine)_2]^{2+}$. The equilibrium capacity for the sorption of diamine from the diamine–ammonia mixture is slightly lower. However, even from a solution containing a 100-fold excess of competing monodentate ligand, diamine can be removed completely. In this instance about 45% of the ligand-exchange capacity of the resin is utilized for diamine absorption. The absorbed 1,3-diaminopropanol-2 can be recovered from the column {with Amberlite IRC-50 $[Ni(diamine)_2]^{2+}$} by displacement with 15.6 *M* aqueous ammonia. The effluent concentration curves are given in Fig. 2. It can be seen that immediately after one void volume of water, the displaced diamine appears in the effluent in high concentration and in rather small effluent fractions (about 0.9 bed volume).

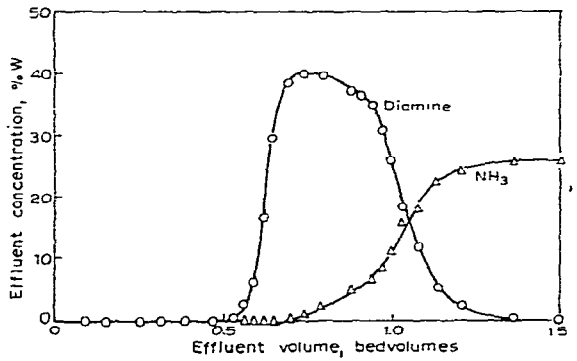


Fig. 2. Variation of effluent concentration in the displacement of 1,3-diaminopropanol-2 by concentrated ammonia (15.6 *M*) from Amberlite IRC-50 (Ni^{2+})[14].

Such sorption–displacement cycles with reversed selectivity make LEC very advantageous for separating ligands with different coordinative valencies. The exchanger prefers ligands of higher valency when the solution is dilute, this ligand (or ligands) thus being selectively removed from mixtures with a ligand (or ligands) of lower dentation. The sorbed polydentate ligands can then be recovered in high concentration by displacement with a concentrated solution of a ligand of lower valency.

No additional regeneration steps, except for washing with solvent, are required in order to regenerate the resin for the next cycle.

B. Compounds being separated

As mentioned above, the conditions necessary for the separation of compounds by LEC lie in their ability to form complexes with metals. A large number of anions and neutral molecules that possess pairs of free electrons are capable of displaying electron-donating properties and of functioning as ligands[21], including representatives of many practically important classes of organic compounds. The most widespread are amines, alcohols, acids, mercaptans and sulphides as well as compounds with several functional groups such as amino acids, hydroxy acids and hydroxyamines.

In addition, when gas chromatography with the ligand-exchange mechanism is used, it is possible to separate compounds that usually do not form complexes in solutions, for instance, ketones, ethers and esters[22,23]. As unsaturated and aromatic compounds are π-donors, they serve as additives in π-complexation[24]. Some gases (such as oxygen and carbon dioxide) can also serve as ligands[25].

C. Complex-forming metal ions

The major requirement for complex-forming ions in LEC is the formation of kinetically labile sorption complexes. This requirement is satisfied by Cu, Ni, Co, Fe Zn, Cd, Mn, Hg, Ag, UO_2^{2+}, VO_2^{2+} and other ions. Ag^+, Cu^+ and Hg^{2+} ions form labile π-complexes and can be used in the LEC of unsaturated compounds[26].

Complex-forming metal ions are capable of binding a definite number of donating groups. Therefore, ligand exchange, like ion exchange, is a stoichiometric process. If a copper-loaded resin is used to sorb molecules of ammonia from aqueous solution, such a "ligand sorption" is, in fact, a stoichiometric process of ligand exchange, as the ammonia molecules coordinating with copper ion displace the equivalent number of water molecules.

The replacement of one metal ion in the resin with another often leads to a substantial alteration in the elution order of the ligands. For instance, 1,6-diamines are eluted before 1,3-diamines from the modified cellulose exchanger Cellex-CM (Cu^{2+}) with carboxylic fixed groups, whereas the situation is reversed, when the metal ion is Zn^{2+} [27].

The effect of the nature of the metal ion on the competitive distribution of two ligands can be illustrated by the example[14] of the sorption of 1,3-diaminopropanol-2 from a solution containing a 50-fold excess of ammonia on the carboxylic resin Amberlite IRC-50 (Fig. 3).

When the total concentration of the two ligands is not too high, there is a marked preference for the sorption of the polydentate ligand by the resin in both the Cu^{2+} and Zn^{2+} forms; however, the resin in the Cu^{2+} form absorbs diamine much more readily than the resin in the Zn^{2+} form, probably because diamine complexes with Cu^{2+} ions are more stable than those with Zn^{2+} ions. When the resin is in the Ag^+ form, both ligands (diamine and ammonia) operate as monodentates, so the concentration dependence of their resolution factor is absent.

The rate of exchange of ligands in kinetically inert complexes is extremely slow. Ligand sorption in inert systems has long been in use in the dyeing of fibres etched with metal ions (Cr^{3+}, Al^{3+}, Fe^{3+}, etc.). Owing to the inertness of the sorption

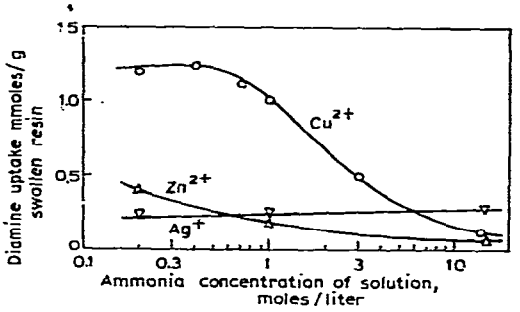


Fig. 3. Influence of nature of metal ion on sorption of 1,3-diaminopropanol-2 by Amberlite IRC-50 in the metal ion form at 30° from solutions containing ammonia and diamine in the molar ratio 50:1[13].

complexes, the dyes formed are very stable[28]. It is clear that the exchange of ligands during chromatography in kinetically inert complexes is impossible. However, we consider the exchange of ligands in the second (external) coordinative sphere of the kinetically inert complexes to be of exceptional interest. As will be shown later, most chromatographic processes with the participation of kinetically inert complexes of Co^{3+} and Cr^{3+} can be interpreted as an external sphere ligand exchange. The properties of the ligand exchange of the external coordination sphere have not yet been studied in detail. We believe that LEC, especially in its gas version, can play a major role in the solution of this problem.

D. Sorbents

Initially, sulphonic resins were widely used in the form of salts with transition metals[29–31], but an important shortcoming of these sorbents was their inability to retain the metal ions[29–32]. The replacement of Ni^{2+} or Cu^{2+} with Ag^{+} in the chromatography of diamines has made it possible to reduce slightly the loss of metal ions; an even greater effect was achieved by using non-aqueous eluents[30].

Cation-exchange resins with carboxylic and phosphonic groups retain the metals sufficiently firmly and are being used advantageously at present as sorbents in LEC.

Chelating resins proved to be even better with regard to their ability to retain metal ions. Unfortunately, among the numerous chelating sorbents synthesized so far, resins with iminodiacetate ligands (Dowex A-1, Chelex-100) and resins with α-amino acid groups are the only ones that have been widely used in LEC.

The application of anion-exchangers in LEC is possible owing to the ability of nitrogen atoms to enter into the coordinative sphere of transition metal ions[8,15]. In addition, LEC is operable with anion exchangers loaded with kinetically labile complex anions, *e.g.*, $[Ni(EDTA)]^{2-}$ (ref. 33).

Sephadexes retain transition metal ions rather weakly[34], but nevertheless they are occasionally used in ligand-exchange techniques[11,35]. Cellulose and to a greater extent its derivatives (diethylaminoethyl-, carboxymethyl-, aminobenzyl-, phosphate, etc.) sorb the metal ions and can be also employed as stationary phases[16,27].

Finally, it is worth mentioning a series of methods involving the use of diverse sorbents impregnated with complex-forming organic substances for chromatographic

separations of metal ions[36,37]. When loaded with metal ions, these impregnated sorbents can probably be used in the LEC of weak ligands that do not strip the metal from the sorbent[38].

In general, the range of stationary phases used in LEC can be substantially increased as almost all of the sorbents that are used for the selective sorption of transition metals are also suitable for LEC. The search for such selective sorbents is going on permanently; also, different new complexing sorbents[39–41], polyurea-based resins[42], natural carbohydrates of the chitosan type[43], soy-bean proteins[44], etc., are now being used for the selective sorption of trace amounts of Cu(II).

In parallel with organic ion exchangers, inorganic sorbents such as zirconium phosphate[29,31] with sorbed metal ions are also being made use of.

Brief mention should also be made of the stationary phases used in gas LEC. Not only in liquid but also in gas chromatography one attempts to increase the sorbent selectivity by employing complex-forming reactions in the column. Ag^+ and Hg^{2+} salts as adducts on the stationary phase make it possible to increase significantly its affinity towards unsaturated and aromatic hydrocarbons[26,45,46]. The introduction of transition metal salts or their coordinatively unsaturated complexes brings about the separation of other classes of compounds that are incapable of acting as ligand. These additives can be used in the column in the solid form on a standard support[47,48], or even without a support[49,50], in the form of melts[51,52], as suspensions in the liquid phase[22,53]. In principle, it is also possible to use many sorbents designed for liquid media in gas LEC. However, thermostable inorganic carriers are, of course, more convenient[54,55].

The nature of the sorbent can have a major impact on the order of elution of complex-forming compounds from the column. In the chromatography of ligands with aromatic rings the sorption complexes become stable on resins with a polystyrene matrix as a result of additional interactions between the aromatic systems. Thus, for example, the retention time of benzylamine on sulphopolystyrene (Ni^{2+}) resin is much higher than that on zirconium phosphate in the same form[56].

The stability constants of nickel complexes with ethanolamine ($K_1 = 950$, $K_2 = 125$) are higher than those with diethanolamine ($K_1 = 620$, $K_2 = 45$). Accordingly, ethanolamine is held longer by the sulphopolystyrene resin in both the Ni^{2+} and Cu^{2+} forms than is diethanolamine[31]. On the contrary, diethanolamine is eluted after ethanolamine from a more "hydrophilic" zirconium phosphate matrix in the Ni^{2+} form. Walton and Navratil[27] noted an increasing binding interaction of aliphatic diamines with the hydrocarbon sorbent matrices with increasing length of the alkyldiamine chain. Evidently, such a type of additional interaction is responsible for the change in the ligand sorption selectivity of different sorbents (Table 1).

The character of the organization of the polymer chains may play an important role in chromatography. From macroreticular sorbents, for instance, metal ions were desorbed faster than from gel cation exchangers of the same chemical nature[31]. Nevertheless, macroporous sorbents are indispensible in work with organic media, *e.g.*, oil[57] and methanol[58].

E. Calculation of ligand-exchange equilibria: properties of stationary and sorption complexes

As with ion exchange, the ligand exchange equilibrium can be described with the help of the law of mass action.

TABLE 1

ORDER OF ELUTION OF AMINES FROM THE CHROMATOGRAPHIC COLUMN[32]

Sorbate	*Type of exchanger*						
	Sulphonated polystyrene resin	*Carboxymethyl cellulose*	*Chelex 100*	*Zirconium phosphate*	*Carboxymethyl cellulose*	*Chelex 100*	*Cellulose phosphate*
	Ni^{2+}				Cu^{2+}		
Diethanolamine	1	1	1	4	1	1	1
Ethanolamine	2	4	2	3	4	2	2
Dimethylamine	3	3	3	2	3	3	4
n-Butylamine	4	2	4	1	2	4	3

If a metal M is bound to the stationary phase $\bar{R}$, the ligand sorption of a mobile ligand A proceeds according to the equation

$$\bar{R}M(H_2O)_n + iA \overset{K_i}{\rightleftarrows} \bar{R}MA_i + nH_2O$$

To describe the ligand sorption isotherm providing the total concentration of ligand A in the resin phase ($\bar{M}_A$) as a function of the concentration of the ligand in the external solution (m_A), the following equation was suggested[14]:

$$\Lambda_A = \frac{\bar{M}_A}{m_A} = \left\{1 + \frac{\bar{M}_M \sum_{i \neq 0} [i K_i (m_A \lambda_A)^{i-1}]}{\sum_i [K_i (m_A \lambda_A)^i]}\right\} \lambda_A$$

where

Λ_A = total distribution coefficient of ligand A (including complexed species);
λ_A = distribution coefficient of the "free" (non-complexed) ligand;
$\bar{M}_A$ = total concentration of ligand A in the resin including the complexed molecules (moles per unit weight of solvent);
m_A = concentration of ligand A in the external solution (molal);
$\bar{M}_M$ = total concentration of metal M in the resin, including the complexed ions (molal);
K_i = stability constant of the sorption complex with i ligands.

To calculate the sorption isotherm, it is necessary to know the values $\bar{M}_M$, λ_A and K_i. The first value is determined from the ion-exchange capacity and the solvent content of the resin, λ_A must be found experimentally, and for K_i Helfferich[14] applied the stability constants of the low-molecular weight complexes MA_i formed in the absence of resin.

When two competing ligands A and B with coordinative valencies a and b are present in the system, a series of mixed sorption complexes can be formed in the resin containing metal M with coordinative valency N_M (with subtraction of the number of coordinative sites blocked by resin groups):

$$\bar{R}M + iA + jB \overset{K_{ij}}{\rightleftarrows} \bar{R}MA_iB_j$$

$$i, j \geqslant 0$$

$$ai + bj \leqslant N_M$$

It is assumed that the formation of polynuclear complexes that contain more than one metal atom does not take place and that ligands A and B do not displace the resin groups from the coordination sphere of the metal ion. Then the following structures can exist in the solid phase:

$$\begin{array}{cccccc} & & \mathrm{A} & & & \\ & \mathrm{M} & \mathrm{MA} & \mathrm{MA_2} & \cdots & \mathrm{MA_p} \\ \mathrm{B} & \mathrm{MB} & \mathrm{MAB} & \mathrm{MA_2B} & \cdots & \\ & \mathrm{MB_2} & \mathrm{MAB_2} & & & \\ & \cdot & & & & \\ & \cdot & & & & \\ & \cdot & & & & \\ & \mathrm{MB_q} & & & & \end{array}$$

where $q = N_M/b$ and $p = N_M/a$ (if the corresponding ratio is not an integer, q or p is the next smallest integer).

A series of transformations of equations for ligand sorption isotherms led Helfferich[14] to the following final expression for the ligand-exchange separation factor α_A^B, similar to that in the ion-exchange:

$$\alpha_A^B = \frac{\bar{M}_B\, m_A}{\bar{M}_A\, m_B} = \frac{\lambda_B \{1 + \bar{M}_M \sum_{j \neq 0, i} [jK_{ij}(m_A \lambda_A)^i (m_B \lambda_B)^{j-1}] / \sum_{i,j} [K_{ij} (m_A \lambda_A)^i (m_B \lambda_B)^j]\}}{\lambda_A \{1 + \bar{M}_M \sum_{i \neq 0, j} [iK_{ij} (m_A \lambda_A)^{i-1} (m_B \lambda_B)^j] / \sum_{i,j} [K_{ij} (m_A \lambda_A)^i (m_B \lambda_B)^j]\}}$$

From this equation and knowing the distribution factors λ_A and λ_B and the complex formation constants K_{ij}, it is possible to calculate the isotherms of the ligand exchange and the ligand-exchange separation factor α_B^A, the only difficulty being that the mixed complex formation constants K_{ij} $(i, j \neq 0)$ are usually unknown. It is especially difficult to determine them for the complexes $\bar{R}MA_iB_j$ where the stationary ligand $\bar{R}$ participates in the coordination sphere of the metal.

Helfferich[13] checked the proposed equation by calculating the isotherms of the ligand exchange and the separation factors for ammonia and bidentate 1,3-diaminopropanol-2 on the carboxylic resin Amberlite IRC-50 (Ni^{2+}) using the stability constants of low-molecular-weight analogues of the complexes under investigation. As can be seen in Fig. 4, there is some discrepancy between the calculated and experimental results, which is particularly noticeable in the dilute solution (0.1 N) and at small equivalent fractions of diamine $[2\bar{M}_B/(2\bar{M}_B + \bar{M}_A)$ ranging from 0.1 to 0.5]. Helfferich believes this to be due to the fact that the formation constant K_{11} of a mixed complex $[Ni(NH_3)(\text{diamine})]^{2+}$, affording a considerable contribution to the calculation, was not determined independently but only by an approximate extrapolation. Nevertheless, the agreement between the observed and predicted data for this system can be considered to be satisfactory.

The above calculation[13,14] therefore appears to be generally acceptable. In fact, it takes into consideration many of the important factors involved in complex-forming processes in a sterically hindered resin phase, such as: partial blocking of the

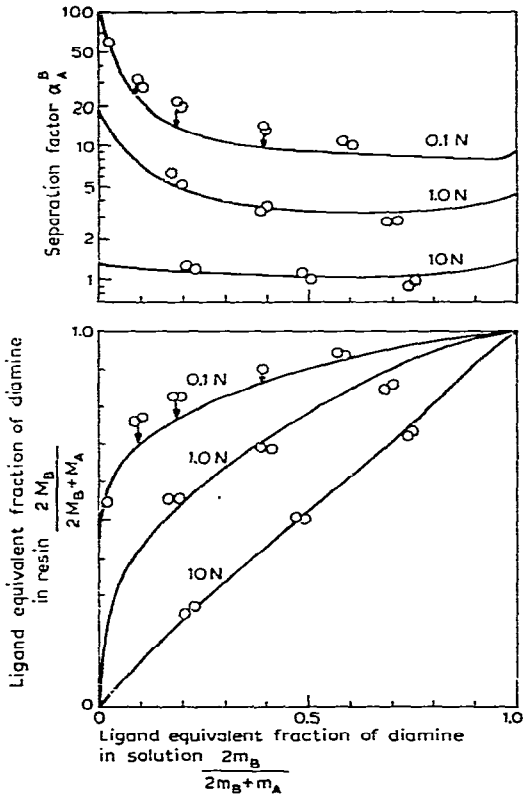


Fig. 4. Calculated (solid lines) and experimental (individual points) ligand-exchange separation factors and isotherms for exchange of ammonia for 1,3-diaminopropanol-2 in Amberlite IRC-50 (Ni^{2+}) in 0.1, 1.0 and 10 *N* aqueous solution[13].

coordinative sphere of the metal ion by ionogenic groups of the resin, formation of coordinative unsaturated and mixed (containing several types of mobile ligands) complexes and sorption of additional uncomplexed ligands due to mechanisms other than ligand exchange. However, the theory does not include the possibilities of the formation of polynuclear complexes and the displacement of earlier bound stationary ligands from the coordinative sites of the metal at high concentrations of mobile ligands. In addition, it seems incorrect to use for thermodynamic calculations the stability constants K_i and K_{ij} for complexes MA_i and MA_iB_j (known from the literature) in cases where the ionogenic groups $\bar{R}$ of the resin participate in the formation of the metal coordination sphere. Here, only low-molecular-weight analogues of species of the type $\bar{R}_xMA_i$ and $\bar{R}_xMA_iB_j$ can simulate adequately the sorption complexes formed in the resin phase.

The experimental investigation of the thermodynamics of ligand exchange should, in general, start with a study of the structures and properties of the stationary complexes $\bar{R}M$ which are the active resin centres that provide mobile ligand sorption and formation of $\bar{R}MA$ sorption complexes.

The structures of stationary complexes of a number of sorbents have been studied in detail and in some instances the thermodynamics of their formation were

also studied. The following techniques were used: study of the distribution of transition metal ions between the resin and the external solution[39,59]; potentiometric titration of resin groups in the presence of metal ions[60]; determination of pH of decomplexation of metals[61]; and spectroscopy[62]. Usually chelating sorbents were used for isolating and purifying metal ions[39].

At this point, it is worthwhile discussing in more detail the impact of the structure of the stationary complex on the sorption of mobile ligands.

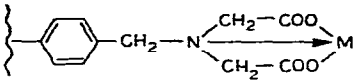
Sulphonic cation exchangers hold the metal ions with purely ionic bonds without blocking the coordinative metal sphere. Therefore, the sorption and exchange of amines with these sorbents take place in a similar manner to the complexing process in aqueous solution:

$$(\bar{R}SO_3)_2M(NH_3)_4 + 2\,NH_2CH_2CH_2NH_2 \rightleftarrows (\bar{R}SO_3)_2M(NH_2CH_2CH_2NH_2)_2 + 4\,NH_3$$

These processes in the phase of sulphonic cation exchangers are characterized by constants that are almost identical with those in solution[10,63,64]. However, the sorption and separation of negatively charged ligands (A^-) on these sorbents are difficult as the uncharged complexes of type MA_2 that are formed are readily eluted from the column.

Carboxylic cation exchangers bind the metal ions more strongly than the sulphonic type as carboxylate anions enter the coordinative metal sphere. This results in a decrease in the affinity of the central ion towards the mobile ligand. Hence the effective stability constants for copper complexes with amines decrease almost 20-fold[10,63,64]. Nevertheless, the partial blocking of the copper ion sphere with the stationary carboxylic groups appears to be reversible. With a sufficient concentration of ammonia the maximum possible ligand-exchange valency of copper ions corresponding to the formation of tetraamino complexes can be achieved[30]. The displacement of stationary ligands from the coordinative sphere of the copper atom and the transformation of its ion-coordination bonds to carboxylic groups into pure ionic bonds lead to a significant decrease in the retention strength of the metal by the stationary phase. Therefore a 1.0 *M* solution of sodium perchlorate initiates desorption of the copper ions from the carboxylic cation exchangers, if the mobile phase also contains ammonia[65] at sufficient concentration (2 *M*). In many respects cation exchangers with phosphonic acid groups[66] behave in a similar manner to the carboxylic type.

Stable stationary complexes are produced when resins with iminodiacetic groups (Dowex A-1, Chelex-100) interact with transition metal ions to give the chelates with the following 1:1 composition[59,62] (one stationary ligand to one metal ion):



The stationary ligand occupies three positions in the metal sphere[32]. Schmuckler[67] calculated that the binding energy of transition metal cations in this instance is 15–25 kcal/mole instead of the 2–3 kcal/mole for the usual cation exchangers. Indeed, the amount of metal ions in the eluate during LEC in ammonia solutions is minimal when resins with iminodiacetic groups are used (Table 2).

TABLE 2

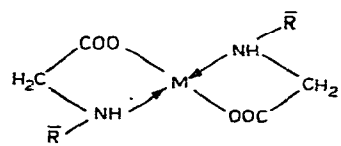
METAL SORPTION CAPACITY OF DIFFERENT SORBENTS AND EQUILIBRIUM METAL ION CONCENTRATIONS DURING ELUTION WITH AMMONIA SOLUTION[32]

Ion exchanger	*Dowex 50*		*Amberlite IRC-50*	*Chelex 100*	*Cellulose*	
	X8	*X12*			*Phosphate*	*Carboxymethyl*
Metal sorption capacity (mmole/ml column volume)	0.95	1.0	0.65	0.5	0.05	0.06
NH_3 concentration in eluent (*M*)	1.0	1.5	—	1.4	1.5	1.5
Cu concentration in eluate (*M*)	High	High	$5 \cdot 10^{-4}$	$7 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-3}$
Ni concentration in eluate (*M*)	$2 \cdot 10^{-4}$	$5 \cdot 10^{-5}$	—	$4 \cdot 10^{-5}$	$3 \cdot 10^{-4}$	$4 \cdot 10^{-3}$

The absolute stability constants determined by different workers for stationary complexes formed by Cu^{2+} ions with the iminodiacetic groups of Dowex A-1 vary widely, and the following log *K* values have been reported: 16.90 [estimated from the distribution of copper ions between the resin and a solution of N-(β-hydroxyethyl)ethylenediaminetriacetic acid[59]]; 9.01 (calculated from the decomplexation pH value[39,61]); and 10.54 (determined by potentiometric titration[60]). The last value seems to be the most correct and lies close to the value of 10.61 for copper N-benzyliminodiacetate[60], which can be considered as a model compound simulating the stationary complexes of Dowex A-1.

When sorbed on iminodiacetic resin, Cu^{2+} and Zn^{2+} ions leave only one coordination site for ammonia molecules, while the Ni^{2+} ion leaves three sites. However, the sorption of ligands probably proceeds with a partial rearrangement of the stationary complex (for instance, with the displacement of one of the carboxylic groups).

The extensive rearrangement of stationary complexes during the sorption of mobile ligands is obvious with chelating sorbents on an α-amino acid base[68,69]. Such resins together with metal ions give stable 2:1 stationary complexes[39,70]:



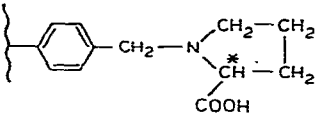
Weak complex-forming agents such as acylated amino acids are hardly retained by such sorbents in the M form under chromatographic conditions[70]. The sorption of stronger ligands such as α-amino acids or diamines proceeds readily with a reversible breakdown of the stationary complexes:

$$\bar{R}\text{–M–}\bar{R} + A \rightleftarrows \bar{R}\text{–M–A} + \bar{R} \qquad (1)$$

The thermodynamics of ligand exchange on chelate-forming resins remained almost unstudied until recently. The only earlier serious work was carried out by

Loewenschuss and Schmuckler[59]. They calculated the formation constants of stationary and sorption complexes on a resin with iminodiacetic groups by studying the distribution of Cu(II) ions and mobile ligands A (A = glycine, glutamic and iminodiacetic acid) between two phases. The value of log $K = 16.90$ was obtained for the stationary complex $\bar{R}Cu$, while values as high as 17.10, 15.61 and 16.72, respectively, were found for the sorption complexes with the above amino acids. However, having reported these values, the authors did not note that the absolute values of the formation constants of the sorption complexes with glutamic and iminodiacetic acid are lower that those for the formation of the initial stationary complex. Such a relationship between constants is evidence that the sorption of these two amino acids as distinct from glycine is thermodynamically disadvantageous. Indeed, later studies showed the absence of sorption of acidic α-amino acids on this sorbent during chromatography in alkaline solutions[71–73].

The investigation of the thermodynamics of ligand sorption on chelating resins[39] that form 2:1 stationary complexes is complicated. In this instance the relationship between the stabilities of the stationary and sorption complexes can change according to the experimental conditions used, particularly when the amount of metal ions present in the system is changed[69,70]. The main feature of resins that form 2:1 complexes is that the stationary complexes acquire a new form of additional crosslinks between the polymer chains of the resin framework. Moreover, it is important to bear in mind that the average stability of these complexes depends on the degree of saturation of the resin with metal ions. Davankov and co-workers[18,68–70] made a detailed study of the sorbent with L-proline stationary ligands:



The determination of the formation constants of the stationary complexes with copper(II) ions showed that the value log $K_{\bar{R}-Cu-\bar{R}}$ decreases from 16.3 to 15.1 with an increase in the degree of loading of the resin with copper ions from 14.4 to 92.0%[70]. This is probably due to the fact that the metal ions in the resin phase form stationary complexes, first of all, with those pairs of stationary fixed ligands which are favourably distributed in space. As the degree of saturation of the sorbent by metal ions increases, the pairs of fixed ligands that are less fortunately distributed become involved in the complex-forming reaction with metal ions. Thus, in order to form the bis-chelate, it is necessary to overcome the resistance of the polymeric chains that are bound to the stationary ligands by displacing them to a distance demanded by the geometry of the bis-complex (Fig. 5).

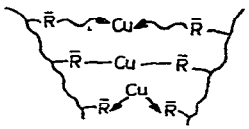


Fig. 5. Scheme of formation of 2:1 stationary complexes[74].

According to Hering and co-workers[74,75] the formation of 2:1 stationary complexes apparently takes place provided that the energy (Q_C) liberated in the course of complexation is sufficient to overcome the framework deformation energy (Q_D), *i.e.*, $Q_C - Q_D \geqslant 0$. Beyond this limit, for a favourable composition of the external solution (sufficient concentration of free Cu^{2+} ions), considerably less stable 1:1 complexes can be formed in the resin.

Unlike the ligand sorption on resins with 1:1 stationary complexes, the processes that occur on resins of the amino acids fixed ligand type are much more complicated[70]. Here, the equilibrium position of the mobile ligand sorption process (see eqn. 1) is determined by the relationship between the stabilities of the final sorption and the initial stationary complexes. The difference between these two values increases with increasing degree of loading of the resin with copper ions, because the stability of the starting stationary complex in this instance declines. This implies that the average affinity of the mobile ligand for the sorbent is higher the more metal ions are present in the resin. For a system containing L-proline, copper(II) ions and the sorbent with L-proline ionogenic groups, the sorption affinity ($\log K_{\bar{R}-Cu-A} - \log K_{\bar{R}-Cu-\bar{R}}$) changes from 0.40 to 1.05 on changing the degree of saturation of the resin with copper ions from 13.7 to 94.0%[70]. This regularity, naturally, also manifests itself in the sorption of other mobile ligands. For instance, amino alcohols, possessing a reduced tendency for complex formation compared with amino acids, are sorbed by proline resins only with high degrees of loading with copper ions, which is not an essential condition for sorbents of the iminodiacetic type.

The thermodynamics of the formation of stationary complexes with copper(II) ions and of sorption complexes with L-proline as mobile ligands in a resin containing L-proline stationary groups was recently studied in more detail. The potentiometric titration method provides a value of $\log K_{\bar{R}-Cu-\bar{R}} = 12.4$ for the stability constant of the stationary complex[76], which lies close to the stability of bis-(N-benzyl-L-prolinato)copper ($\log K = 12.39 \pm 0.10$). Studying the distribution of Cu^{2+} and L-proline between the two phases[77] allows one to observe clearly the decrease in the effective stability of stationary complexes in the resin phase upon increasing the degree of its saturation with metal ions (Fig. 6).

However, stability constants obtained in this way depend strongly on the experimental conditions, such as pH range and ionic strength of the external solution. Nevertheless, no other methods have yet been developed for the determination of the stability constants of sorption complexes. On saturating the resin with Cu^{2+} ions, the difference between these constants and the stability constants of starting stationary complexes rises continuously (Fig. 6), this difference being a measure of the affinity of mobile ligands and the resin phase.

Finally, it is worth considering here a specific case of ligand exchange. Bernauer and co-workers[33,78–80] suggested using, for the separation of optical isomers of N-acetylated α-amino acids, the optically active complex of Fe^{3+} ion with N-(β-hydroxyethyl)-D-propylenediaminetriacetic acid. The complex being held by electrostatic forces on the anion-exchange resin Dowex-1, the coordination sphere of Fe^{3+} ion in this instance is completely saturated. However, the sorption of amino acids probably takes place at the expense of re-arrangement of the coordination sphere of iron. It is interesting that chromatography on such a resin is successful only in an alcoholic medium, the water contributing to rapid dissociation of the sorption complexes being

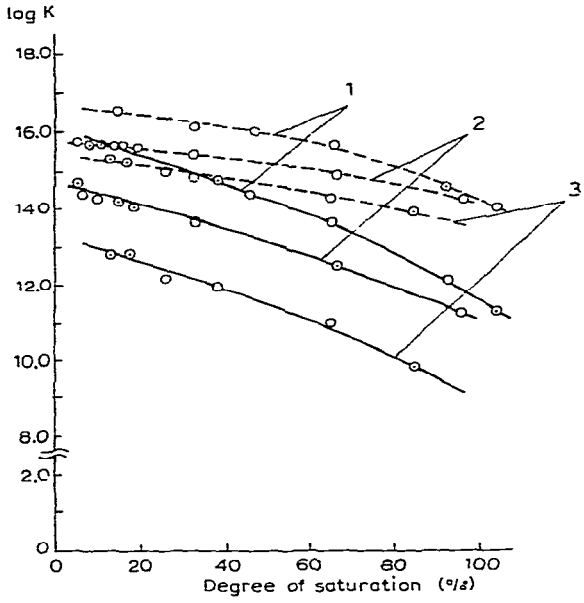


Fig. 6. Stability constants of stationary $\bar{R}$–Cu–$\bar{R}$ (———) and sorption $\bar{R}$–Cu–Pro (– – –) complexes over a wide range of saturation of asymmetric resin with Cu^{2+} ions. 1, pH > 11; 2, pH > 11, 1.0 *N* KCl; 3, pH ≈ 8, 1.0 *N* KCl.

used as a displacing eluent. For this system it is also unusual that the sorption complex has a larger negative charge than the stationary one, and that it is bound by the anion exchanger more strongly than is the starting complex.

In conclusion, it should be pointed out that the study of the structures and properties of the complexes in sorbents and of the thermodynamics of the ligand exchange is of paramount importance in order to understand the processes that occur in the resin phase under LEC conditions.

F. Ligand-exchange kinetics and efficiency of ligand-exchange chromatography

The establishment of ligand-exchange equilibrium between the resin and external solution depends on two basic processes: firstly, diffusion of mobile ligands, metal ions and their complexes in solution, in the resin granules and through the phase boundary, and secondly, the exchange of ligands in the coordination sphere of the sorbed metal. No publications devoted to the study of the diffusion processes and the kinetics of ligand exchange in stationary and sorption complexes have yet appeared in the literature.

Of the variety of metal cations that are capable of forming complexes, Fujimura *et al.*[81] suggested the use of metal ions with full d orbitals [Zn(II), Cd(II), etc.] rather than ions with vacant d orbitals [Ti(III), Y(IV), etc.] for LEC. In general, the rate of complex formation in solution for these ions, producing labile complexes, is sufficiently high. The rate of coordination of amines by Ni^{2+} ions (*i.e.*, the rate of replacement of water molecules with amino groups in its coordination sphere) reaches 10^3–10^5 $mole^{-1} \cdot sec^{-1}$ (ref. 82), whereas for Cu^{2+} ions it is much higher.

Hence, the rate of metal sorption and ligand exchange in chelating resins must

also be high. Indeed, kinetic investigations of the sorption of different metal ions[83,84] and of their self-exchange[85] in resins of the Dowex A-1 type led to the conclusion that the rate-determining step, as a rule, is the particle-diffusion of ions in the gel phase. However, the mechanism of sorption in this instance is different from that in sulphonic cation exchangers. Owing to the high energy of complex formation, chelated metal ions must be almost deprived of their freedom to change their sorption sites in the resin phase. In order to reach vacant stationary ligands, the complex-forming metal ion must therefore pass a dense metal-saturated layer of the resin bead. Even in carboxylic resins partially loaded with Cu^{2+} ions, the compensatory re-distribution of metal ions is an extremely slow process[86]. The presence of mobile ligands in the resin phase can obviously accelerate this re-distribution.

The kinetics of ligand exchange in the resin phase have not yet been studied. In general, the chromatographic peaks in LEC are broader than the peaks in ion-exchange chromatography. However, this fact alone does not enable one to solve the problem of correlation between the diffusion rate of the mobile ligands and the rate of ligand exchange in the resin phase.

The efficiency of LEC, which is a function of the overall kinetics, has been studied in more detail. For example, for dimethylamine it was found[31] that the HETP on a carboxylic cation exchanger in the Ni^{2+} form was 0.10 cm, on a sulphonic resin 0.28 cm and on zirconium phosphate 0.55 cm. We consider that such considerable broadening and asymmetry of the dimethylamine peak during chromatography on the inorganic ion exchanger are due to the low diffusion rate. It should be stressed, however, that the comparison of the kinetic characteristics of different sorbents can be considered to be correct only if the sorbents possess identical swelling capacities and granule sizes. Also, in studying the influence of the nature of the metal on the efficiency of LEC on the same sorbent, it is necessary to consider how the swelling of the sorbent changes on changing the metal ions. In the chromatography of phenylethylamine on a carboxylic cation exchanger of the Bio-Rex 70 type, Hernandez and Walton[87] obtained the following HETP values, depending on the nature of the metal ion: Cu^{2+}, 0.10–0.15 cm; Cd^{2+}, 0.39 cm and Ni^{2+}, 0.59 cm.

In general, the nature of the metal ion has a significant effect both on the selectivity of the chromatography, characterized by the ratio of the retention times of the components, and on its efficiency, characterized by the sharpness of the peaks. Some workers have noted that Cu^{2+} ions result in a high resolution power of the columns, but give poorer peak sharpness than other ions. Therefore, for optimal solution of a specific chromatographic problem, it is advisable to devise a system that would ensure complete separation of all of the desired components of the mixture in the shortest time. Arikawa and Toshida[88] recommended the use of Cd^{2+} instead of Cu^{2+} ions in a sulphonated resin in order to shorten the time of analysis of amino acids and to give narrower peaks. For the same reasons, Zn^{2+} was found to be more advantageous than Cu^{2+} or Ni^{2+} ions[27] in the analysis of polyamine mixtures on different resins.

In general, the efficiency of LEC on sulphonic cation exchangers, carboxylic resin and chelating resins of the iminodiacetic type can be regarded as satisfactory although this question requires more thorough investigation. For resins that form 2:1 stationary complexes the efficiency is apparently lower. Semechkin *et al.*[70] considered the following two aspects of ligand exchange on resins with 2:1 stationary complexes

to be the cause of broadening of the chromatographic peaks. The first cause is of a thermodynamic nature: the stability of 2:1 stationary complexes varies over a wide range, which considerably changes their ability to interact with mobile ligands. This is equivalent to the sharply defined non-linearity of the isotherm of mobile ligand sorption. The second cause is of a kinetic nature: in accordance with the scheme (see eqn. 1) of transformation of $\bar{R}$–Cu–$\bar{R}$ stationary bis-complexes in $\bar{R}$–Cu–A sorption complexes the displaced stationary ligand $\bar{R}$ has a polymeric nature. Its transfer in space results in the conformational rearrangement of the adherent polymeric chain segment and, consequently, in the rearrangement of the whole system of neighbouring stationary and sorption complexes. This process should proceed relatively slowly. In fact, we found[18] that during the chromatography of proline on one of the sorbents with L-proline groups in the Cu^{2+} form the HETP value can be as high as 0.7–1.7 cm.

Further, the additional broadening and tailing of the peaks in LEC are favoured by the interference of some non-specific interactions between the sorbent and sorbate, *i.e.*, of a hydrophobic or aromatic nature.

4. APPLICATIONS OF LIGAND-EXCHANGE CHROMATOGRAPHY

A. Experimental peculiarities of liquid chromatography

From the practical point of view, experiments in LEC differ little from those of other types of column chromatography. However, LEC using liquid eluents has a number of peculiarities which are worth discussing.

Let us start with the preparation of the sorbent. Nowadays chelating resins or sorbents with carboxylic ionogenic groups are most frequently applied, the latter also being a complex-forming material[30]. Before loading the column, the resin has to be converted into a chelate with metal ions. It is kept for 1.0–1.5 h in contact with an excess of a salt solution of a corresponding metal in the presence of a base (usually ammonia) to bind the liberated protons:

$$\bar{R}H + M^{+} \rightleftarrows \bar{R}M + H^{+}$$

The sorbent obtained (often intensively coloured) is washed with water in order to remove excess ions, introduced into the column and washed with a buffer solution or water to equilibrium. If the chromatography is performed in an organic solvent*, it is useful to wash the sorbent first with water and subsequently with acetone.

Most experiments in LEC are performed under simultaneous or step elution conditions. It should be noted that there are numerous elution methods in this type of chromatography[70]. In aqueous solutions ammonia is usually used as the eluting agent. The displacing ligand (B) breaks down the sorption complex as a result of re-complexation:

$$\bar{R}\text{–M–A} + B \rightleftarrows \bar{R}\text{–M–B} + A$$

* The sorption of weak complex-forming agents occurs from media of organic solvents (alcohols, ketones, hydrocarbons) that are capable of coordinating to a lesser degree than water.

The equilibrium position of this process and, consequently, the efficiency of elution of ligand A depend both on the nature of ligand B and on its concentration. To elute the derivatives of α-amino acids in non-aqueous solutions, we have often used ethanolamine. The elution of amines in hydrocarbon media can be performed with methanol[57], while for alkyl sulphides diethyl ether can be used[89].

In some elutions we introduced into the eluent a sufficient amount of transition metal ions[68]. In this instance the mobile ligand leaves the column not in a free but in a complexed form:

$$2\bar{R}\text{–M–A} + M \rightleftarrows 2\bar{R}\text{–M} + MA_2$$

With this method, the elution curves become narrower and symmetrical, although the resolution power of the column is usually reduced.

Breakdown of the sorption complex can also result from a change in the pH of the eluent; competition in this instance takes place between the metal ion and proton, tending to protonate the stationary ligand:

$$\bar{R}\text{–M–A} + 2H^+ \rightleftarrows \bar{R}H + M^{2+} + AH$$

It is interesting that in some instances the sorption of ligands takes place so strongly that this method of elution appears to be the only one possible.

In addition, an increase in the column temperature brings about an increase in the degree of dissociation of the sorption complexes and the elution of mobile ligands from the column. This method of elution is promising in that it enables one to prevent contamination of the separated ligands by foreign materials.

Often it is possible to observe visually how the composition of the coordination sphere of metal ions changes during the eluation process. For example, an increase in the intensity of the colour of the sorbent with the moving ammonia front can be seen distinctly in the column. Helfferich[15] pointed to the possibility of the direct observation of the chromatographic zones of some amines.

The problem of automatic detection of eluates is a weakness of LEC which, in many respects, prevents further development of this method. The presence of a large excess of ammonia makes it impossible to use many of the methods for detecting amines that are based on obtaining coloured compounds. We were therefore obliged to use aqueous solutions of pyridine instead of ammonia in the LEC of α-amino acids on an amino acid analyzer.

Walton[1] devoted much attention to the unsolved problem of the automatic detection of amines. Detection with the aid of a flow refractometer and by measuring the heat of sorption failed to give acceptable results. Flame emission detection seems to be more advantageous, but this method is not automatic and is rather laborious. The most universal solution to the problem of detection is the application of radioactively labelled compounds, and polarimetric detection is just as precise[70]. The latter method is considerably simpler, but requires the use of optically active compounds, which, like labelled compounds, are not always available.

Substances that contain aromatic rings are readily detected with the aid of a spectrophotometer. However, it is necessary to take into account the possibility of

absorption, in the same spectral region, of complexed metal ions, which are partially desorbed from the resin.

With the development of the Uvicord-III instrument (LKB, Stockholm, Sweden), which records the absorption of carbonyl groups at 206 nm, the problem of detecting carbonylic compounds (carbonic acids, ketones, etc.) became much easier.

Special attention should be paid to the partial elution of metal ions from the column. Firstly, this process leads to a change in the sorption parameters of the system[1,2] and, in addition, contaminates the separated compounds. Because of the significant loss of metal ions, sulphonated exchangers are now rarely employed in LEC. Complex-forming sorbents, carboxylic and phosphonic resins retain the metal ions much more strongly, but it is impossible to prevent completely their elution from the column. In order to keep constant the exchange capacity of the sorbent and its affinity for the sorbate, it is convenient to add to the eluent an amount of metal salt that corresponds to its equilibrium concentration in the eluate.

In order to remove trace amounts of metals from the eluate, Hering and Heilmann[75] suggested that after operation of the principal column, an additional smaller column that contains the same, but metal-ion-free, resin should be used. This method, however, makes the real sizes of the operating sorbent layer and its degree of metal-saturation unknown.

B. Ligand-exchange chromatography in organic chemistry

(a) Amines.

Amines are typical ligands and, although they are difficult to detect, they have been studied in the most detail[90].

A mixture of triethylamine, ethylamine and diethylamine was separated on a sulphonic cation exchanger (Ni^{2+})[32]. In this instance the order of elution of amines followed that of their basicities. However, this regularity does not always apply, and methylamine is eluted after trimethylamine and dimethylamine on a resin with iminodiacetatic groups (Ni^{2+}). A more detailed study of the behaviour of amines established that primary amines are usually retained more strongly than the corresponding secondary, tertiary and heterocyclic amines[87]. Also, the more substituted is the α-carbon atom of the coordinating amine group, the more weakly is the ligand retained on the sorbent. This regularity, reflecting steric complications in the complex-forming process, is probably also valid for other classes of compounds, as has been observed, for instance, with some alkyl sulphides[89].

A mixture of diethylamine, butylamine, diethanolamine and ethanolamine[31] and also a mixture of benzylamine, pyridine and aniline[56] were separated by elution with aqueous ammonia on columns filled with Dowex 50-X8 (Ni^{2+}). Several isomeric picolines and bipyridines were separated on the sulphonated polystyrene resin KY-2 (Cu^{2+} and Zn^{2+})[91].

Bernauer[33] separated a mixture of α and γ-picolines and pyridine by LEC on the anion-exchange resin Dowex 1-X2 [$Ni(EDTA)^{2-}$]. A mixture of aniline, trimethylamine and dimethylamine was separated on DEAE-cellulose (Co^{2+} and Sb^{3+})[16].

By shaking the disodium salt of *p*-phenylenedi(methoxyphenyldithiophosphonic acid) in the presence of carbon tetrachloride with an aqueous solution of a transition metal (Co^{2+}, Ni^{2+}) salt, Kuchen *et al.*[92] obtained a series of coordination

polymers. By chromatographic techniques diethylamine and tributylamine were separated in pentane; pyridine was isolated from the aliphatic amines mixture. These sorbents were also employed as carriers in gas LEC[54].

Both on sulphonated polystyrene and on chelating resins (Ni^{2+}), 1,2-diaminoethane and 1,2-diaminopropane are bound so strongly that they can be readily isolated from concentrated monoamine solutions[29,30], whereas 1,3-diaminopropane, 1,6-diaminohexane and 1,4-diaminobutane are easily eluted from the column with 1.22 *M* aqueous ammonia with satisfactory separation[30]. For the chelating resin (Cu^{2+}), it was established that 1,4-diaminobutane binds with copper ions as a chelate-forming agent occupying two positions in the coordination sphere, although 1,6-diaminohexane, in such an instance, takes the role of a monodentate ligand occupying only one position[1].

Recently, Walton and Navratil[27] carried out a systematic study of a series of diamines and polyamines. The carboxylic resin Bio-Rex 70, the sulphopolystyrene resin Aminex Q-150s and the sorbent Cellex-CM with carboxylic groups on cellulose (Cu^{2+}, Ni^{2+}, Zn^{2+} and other forms) served as the sorbents. The retention parameters of the amines with these resins are given in Table 3.

TABLE 3

RETENTION VOLUMES (IN FREE VOLUME UNITS)[27]

Amine	*Cellex CM*			*Aminex Q-150 S*		*Bio-Rex 70*	
	Cu (1.1 M NH_3)	*Zn (1.7 M NH_3)*	*Ni (1.1 M NH_3)*	*Cu (7.1 M NH_3)*	*Zn (7.6 M NH_3)*	*Cu (3.8 M NH_3)*	*Zn (4.9 M NH_3)*
1,3-Diaminopropane	14.3	2.0	5.4	12.2	2.1	5.6	1.5
1,4-Diaminobutane	2.9	2.9	1.7	—	3.6	2.6	2.3
1,5-Diaminopentane	3.4	4.4	2.3	—	7.4	3.3	2.5
1,6-Diaminohexane	4.4	5.6	2.4	8.4	14.4	3.5	2.8
Spermidine	4.2	3.6	1.9	3.9	4.6	2.3	1.7
Spermine	8.9	4.2	2.0	6.4	5.1	1.9	1.3
Histamine	4.4	1.4	—	18.6	—	4.9	2.0
Lysine	1.1	0.8	1.6	1.0	—	1.0	—
Histidine	2.0	2.1	1.5	1.0	—	1.5	—
Arginine	3.1	1.3	4.0	5.8	1.3	6.0	1.2
n-Butylamine	1.9	2.0	1.4	—	4.3	1.2	1.0

Walton and Navratil[27] arrived at the following general conclusions: of all the diamines investigated, 1,3-diamines were retained most strongly on sorbents loaded with Cu^{2+} ions while Zn^{2+} displayed a marked affinity for 1,6-diamines; with respect to histamine, Cu^{2+} has a greater affinity than Zn^{2+}; the columns with Cu^{2+} ions have a greater resolution capacity with respect to spermine and spermidine mixtures than the same columns loaded with other metal ions. The best results were obtained on the microspherical sulphonated polystyrene resin Aminex A-7 (Zn^{2+}) and on the polyacrylic resin Bio-Rex 70 (Cu^{2+}), on the basis of which a method was developed for the analysis of diamine and polyamine mixtures.

Aliphatic diamines and benztriazole were sorbed from naphtha oil on the

macroreticular sulphonic resin Amberlyst 15 (Cu^{2+}, Ni^{2+}, Fe^{2+})[57], and the diamines were then desorbed readily with methanol. Different phenylenediamine isomers were separated on columns with the sulphonic resin Amberlite CG-120 (Fe^{2+})[93]. Schiermaul *et al.*[94] achieved a partial separation of nitrogen isotopes in ammonia under displacement chromatographic conditions. Using a resin (Cu^{2+}) saturated with ammonia, they passed a solution of ethylenediamine through the column. The first fractions of displaced ammonia were enriched with ^{14}N and the latter portions with ^{15}N.

(b) Aziridines and hydrazines

On both sulphonated and carboxylic resins (Ni^{2+}) it is possible to separate several derivatives of hydrazines[32]. On the other hand, Cu^{2+} ions, which catalyse the oxidation of hydrazines, are not suitable for this purpose. Unsymmetric dimethylhydrazine, monomethylhydrazine and hydrazine were completely separated in a step-elution process with aqueous ammonia. The retention time decreased considerably with an increase in the number of methyl groups in the hydrazine molecule, which agrees well with the stability constants of the corresponding complexes in water[1]. The hydrazine itself is retained by the sulphonic resin (Ni^{2+}) as strongly as if it were a bidentate diamine ligand.

Aziridines are very unstable in neutral and especially in acidic solutions. However, their ability to form complexes in alkaline media enables one to achieve the separation of aziridines by LEC. For example, a mixture of N-(2-hydroxyethyl)aziridine, propyleneimine and ethyleneimine was separated on a sulphonic polystyrene resin (Ni^{2+}) by elution with 1.0 *M* ammonia solution[95]. However, ethyleneimine and the product of its hydrolysis (ethanolamine) can be separated successfully only on Chelex 100 (Cu^{2+}) whereas on a sulphonic resin (Ni^{2+}) no separation was observed[1].

(c) Carboxylic acids and phenols

The number of papers describing applications of LEC to the separation of carboxylic acids mixtures is considerably less than that describing separations of nitrogen-containing ligands. This difference can probably be ascribed to the convenience of traditional ion-exchange methods for the separation and purification of these substances. However, for separating the isomers and homologues of acids, LEC is the most suitable technique.

For example, the isomers of aminobenzoic acid were separated in aqueous ammonia on Amberlite CG-120 (Cu^{2+}) [93]. Benzoic, 4-methoxy- and 4-chlorobenzoic acids were partially separated on the anion exchanger Dowex 1-X2 saturated with a complex of Fe^{3+} with N-(β-hydroxyethyl)ethylenediaminetriacetate in a 0.5 *M* solution of acetic acid in ethanol[33].

The complete separation of α-hydroxy-β-naphthoic and β-hydroxy-α-naphthoic acids and the separation of *o*- or *m*- from *p*-hydroxybenzoic acid were achieved on sulphonic Amberlite CG-120 (Fe^{3+} and Ti^{4+}). The separation of *o*- and *m*-isomers under such conditions failed[81]. In studies of the electronic and NMR spectra of hydroxybenzoic acid isomers in the presence of iron(III) and mercury(II), Fujimura *et al.*[81] found that in an ethanol solution iron(III) ions are chelated only by the *o*-isomer, while the *m*- and *p*-isomers form intermolecular hydrogen bonds. In a chloroform solution the situation is reversed for the mercury(II) ions: the intramolecular hydrogen bonds block the *o*-isomer. This effect makes it possible to achieve the separation of

the isomers on the carboxylic resin Amberlite CG-50 (Fe^{3+} or Hg^{2+}) by elution with ethanol or chloroform, respectively[96].

Alkylphenols and naphthenic and salicylic acids were successfully sorbed from mineral oil on the macroreticular resin Amberlyst-15 (Cu^{2+}, Ni^{2+} and Fe^{3+})[57].

Bedetti *et al.*[97] carried out an interesting separation of oxalic, malonic, succinic and hydroxyacetic acids. The complete separation of this mixture was effected on Chelex-100 (Ni^{2+}) by elution with 10^{-5} *M* aqueous ammonia (Fig. 7). Fig. 7 also demonstrates another general regularity of LEC: bifunctional molecules producing five-membered chelate rings have, as a rule, the greatest affinity towards resins in the Cu^{2+} or Ni^{2+} form.

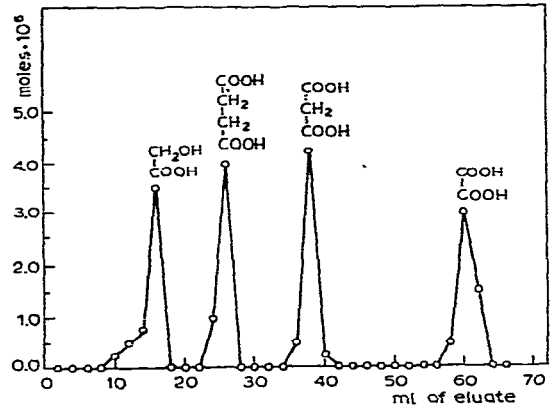


Fig. 7. Elution curve of a mixture of glycolic, oxalic, malonic and succinic acids from Chelex 100 (Ni^{2+}) in 10^{-5} *M* ammonia solution[97].

An interesting study to determine the nature of the chelation of nitrosonaphthols was carried out by Fujimura *et al.*[98]. The retention times of α-hydroxy-β-naphthoic and β-hydroxy-α-naphthoic acids as well as α-amino-β-naphthol and β-amino-α-naphthol on Amberlite CG-120 (Fe^{3+}) were determined and compared with those of analogous nitrosonaphthols. The results indicated that five-membered chelate complexes of Fe^{3+} with nitrosonaphthols are formed with the hydroxylic oxygen atom and the nitrogen atom of the nitroso group participating.

(d) Alkanolamines

A mixture of ethanolamine, diethanolamine, butylamine and dimethylamine was separated on Dowex 50-X8 (Ni^{2+}) by elution with aqueous ammonia. A mixture of triethanolamine, diethanolamine and monoethanolamine can also be separated under similar conditions[1]. The order of elution of alkanolamines corresponds to the order of increasing basicity of the nitrogen atom in their molecules. In contrast, the order of elution of the alkanolamines is reversed during their ion-exchange separation on the same sulphonic resins in the absence of Ni^{2+} [99].

(e) Sulphur-containing compounds

Owing to its marked nucleophilic character, the sulphur atom has a greater

tendency than nitrogen to form coordination compounds. So far, however, LEC has not been used extensively for analysing sulphur-containing compounds. For mercaptans the reasons are probably their sensitivity to oxygen and their tendency to bind heavy metal ions too strongly. Dialkyl sulphides are, in this respect, easier to handle.

Ligand sorption is used to remove sulphur-containing components from effluent water. An inorganic sorbent consisting of silicon, aluminium and tin oxides, after impregnation with cadmium, zinc, copper and mercury salts, readily extracts most sulphur-containing ligands and sulphide anions from factory effluent water[100]. A similar investigation devoted to the sorption of dialkylsulphides was made by Ishibashi *et al.*[101]. They also observed a strong adsorption of dialkyl sulphides from a hexane solution on Cu^{2+} and Ag^{+} forms of sulphonated polystyrene resins.

The use of the mercury(II) form of strongly acidic macroreticular cation exchangers makes it possible to separate sulphides from other oil components[102]. This ion, however, is inconvenient in practice because it catalyses the breakdown of the sulphides and because sorption complexes containing mercury are much too stable. Against this, the application of Bio-Rex 70 (Cu^{2+} and Ag^{+}) has made it possible to attain a reversible sorption of sulphur-containing compounds from oil[89], desorption being performed with the help of diethyl ether. Dihexyl sulphide, cyclohexylthioethane, methylthiocyclohexanes, methylthiocyclopropanes and 2-propylthionaphthalene were sorbed more strongly than other compounds. This method is used to purify oil fractions to remove sulphur-containing impurities.

(f) Hydroxy and keto compounds

The sorption of glucose, galactose, phenols and other oxygen-containing organic substances from sea water was performed on Chelex 100 (Fe^{3+})[103].

Ethylene glycol, glycerine and other polyhydroxy compounds were sorbed on the macroporous resin Amberlyst 15 (Fe^{3+}). Separate elution of the components is possible using water-free methanol[1,58], the elution volumes of ethylene glycol, glycerine and sorbitol being 1.4, 1.9 and 2.4 free column volumes, respectively. It is interesting that the resin in the Ni^{2+} and Cu^{2+} forms failed to sorb these components. A study of the sorption of sugars and polyhydroxy alcohols on different foms of the sulphonic cation exchanger Aminex A-5 was made by Goulding[104].

Acetylacetone was adsorbed on Amberlyst 15 (Fe^{3+}, Ni^{2+}, Cu^{2+})[57]. In addition, it was proposed to apply chelating resins in metal ion forms to synthesize acetylacetonate complexes of lanthanoids and iron(III)[105,106].

(g) Unsaturated and aromatic compounds

The method of separation of unsaturated compounds based on the formation of π-complexes came into use in liquid chromatography later than in gas chromatography. Bradford *et al.*[45] were the first (1955) to apply silver nitrate to separate unsaturated from saturated hydrocarbons. Later a series of experiments were carried out in which alumina or silica gel impregnated with silver nitrate ("argentated" sorbents) was used as the stationary phase. Chapman and Kuemmel[107] applied argentated alumina to remove unsaturated impurities from paraffins. Octadecene was strongly held by this sorbent, but could be eluated with a pentene–diethyl ether mixture, the *trans*-isomer emerging from the column before *cis*-isomer.

This method can be used to separate the methyl esters of unsaturated higher acids, including the separation of their *cis*- and *trans*-isomers. For example, by elution of columns filled with argentated silica gel with light petroleum it is possible to achieve a complete separation of the methyl esters of isomers of C_{18}–C_{22} higher acids[108]. Using silver ions which are not bound to the solid phase, but move with the eluent, it is also possible to achieve the complete separation of the methyl esters of *cis*- and *trans*-isomers of a number of polyene and unsaturated carboxylic acids [109].

Solid rhodium(II) acetate or silver nitrate applied to Corasil II allows one to separate a mixture of butene isomers by high-performance liquid chromatography. The butenes are eluted in the following order: butene-1, *trans*-butene-2, isobutene and *cis*-butene-2[110].

Aromatic compounds, like non-aromatic unsaturated compounds, are also capable of forming complexes at the expense of π-electrons. Aromatic hydrocarbons were sorbed from pentane on the carboxylic resin Bio-Rex 70 (Cu^{2+} and Ag^{+})[69]. Hydrocarbons containing several aromatic rings (biphenyl, 1,2-diphenylethane, naphthalene and alkylnaphthalenes) were sorbed more strongly than other hydrocarbons. Tetrahydronaphthalene, benzene and alkylbenzenes were sorbed more weakly, which agrees with the lower electron donor capacities of these compounds.

C. Ligand-exchange chromatography in biochemistry

(a) Physiologically active amines and alkaloids

Systematic research into ligand-exchange separations of a number of physiologically active amines (phenethylamine, amphetamine, metamphetamine, norephedrine, ephedrine, benzylamine, caffeine, adrenaline and dopamine) was described by Walton and co-workers[87,111]. Sulphonated and carboxylic cation exchangers loaded with Cu^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+} ions were used as sorbents. The best results were obtained on Bio-Rex 70 (Cu^{2+}). On this resin, a method for analysing these amines at concentrations up to 0.1 mg/ml was developed. Chelex 100 (Cu^{2+}), a chelate-forming sorbent with iminodiacetic groups, showed a large affinity towards caffeine. The symmetric shape of its elution curve in 3.0 *M* aqueous ammonia favoured the successful development of a rapid method for determining the content of caffeine in both natural products (coffee, tea, cola) and medicinal drugs[112].

Walton and co-workers[113,114] studied the behaviour of alkaloids (morphine, codeine, strychnine, atropine, papaverine, narcotine, cocaine, etc.) on Chelex-100 and Bio-Rex 70 (Cu^{2+}) and pointed to the low efficiency of chromatography owing to the non-specific interactions of ligands with the hydrocarbon parts of the sorbent frameworks. Considerably better results were obtained with the help of a specially synthesized resin, Bio-Rex PC-20 of the polyacrylic type, and especially with the help of the highly polar carboxylic resin Poragel PT. An example of an efficient separation of a mixture of morphine, codeine and strychnine is shown in Fig. 8. Cocaine is partly hydrolysed under the chromatographic conditions used.

A mixture of alkaloids was separated from nicotine, which is sorbed very strongly on columns that contain titanium arsenate[115]. LEC can also be applied to alkaloids on paper impregnated with titanium arsenate[115] and zirconium phosphate[116].

(b) Nucleosides, nucleic acid bases and nucleic acids

Thymine, cytosine, adenine and guanine are separated on Chelex 100 (Ni^{2+}) by

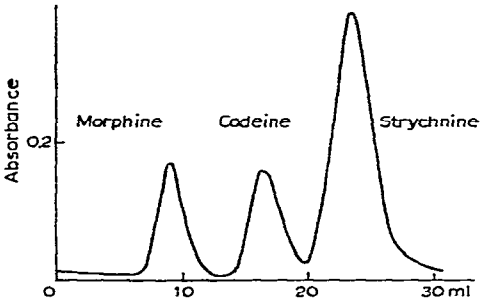


Fig. 8. Separation of a mixture of morphine (0.012 mg), codeine (0.12 mg) and strychnine (0.05 mg) on Poragel PT (Cu^{2+}). Eluent: 0.2 *M* ammonia solution in 33% aqueous ethanol[113].

elution with 0.5 *M* ammonia solution, emerging from the column in the above order[32]. However, adenine is eluted last if the same resin is loaded with Cu^{2+} ions[111].

Goldstein and co-workers[117,118] used LEC for the separation and analysis of nucleic acid components. Nucleotides are not retained on the chelating resin (Cu^{2+}) and are therefore easily separated from nucleosides and nucleic bases. Nucleosides of low basicity are separately eluted from the resin when it is further washed with water. To desorb nucleosides of higher basicity, it is necessary to use 1.0 *M* ammonia solution, while nucleic bases are eluted with 2.4 *M* ammonia solution. Analysis of the nucleoside mixtures obtained by alkaline hydrolysis of RNA was performed with an accuracy of 3–4%. The dephosphorylation preceding the alkaline hydrolysis of t-RNA allows the end nucleosides to be determined.

Nucleic acid molecules possess a very complicated structure, but nevertheless they are all known to be capable of forming complexes with transition metal ions. Kothari[119] used this property to separate DNA mixtures on the sulphonic cation-exchange resin Amberlite IR-120 (Al^{3+}). Later, an RNA extract derived from bovine liver was fractionated into six portions on the same resin by elution with EDTA[120]. The absence of any separation on the resin in the Na^+ form is an evidence in favour of the ligand-exchange mechanism[121].

(c) Amino acids, peptides and proteins

Amino acids strip M^{2+} metal ions from conventional ion-exchange resins to a significantly greater extent than other mobile ligands owing to the presence of the carboxylic group in their molecule and the formation of uncharged bis-amino acid complexes. Therefore, only chelate-forming resins that strongly retain metal ions are used to separate α-amino acids.

Hering and co-workers[75,122] applied a resin that was synthesized from sarcosine (H_3CNHCH_2COOH) and chloromethylated crosslinked polystyrene. This resin (Cu^{2+} and Ni^{2+} forms) made it possible partially to separate some α-amino acids. The separation, in this instance, was poor because of the unusually low chromatographic efficiency.

Using the exceptionally high selectivity of ligand-exchange processes, Siegel and Degens[103] effected the sorption of α-amino acids derived from sea water on Chelex 100 (Cu^{2+}). The amino acids absorbed were then eluted from the column with 3.0 *M* ammonia solution and analysed. Not a single amino acid was destroyed, with the

exception of cysteine, which was oxidised to cystine. They also applied the same resin (Fe^{3+}, Co^{2+}, Ni^{2+} forms) and observed significant alterations in the sorption properties of the resin, particularly the high affinity of Fe^{3+} ions towards acidic and hydrophobic amino acids[103].

Buist[123] separated α-amino acids from oligopeptides on Chelex 100 (Cu^{2+}). Having systematically studied the behaviour of α-amino acids and peptides on the same resin in the presence of ammonia at pH 10.3, Boisseau and Jouan[71,124], established that acidic and neutral peptides and also acidic amino acids are eluted readily under these conditions. Neutral α-amino acids and the basic peptides were eluted with 1.5 *M* ammonia solution, while tryptophan, hystidine and arginine required 6.0 *M* ammonia solution. Further investigations[72,73,125,126] confirmed these observations.

Hitachi and Perkin-Elmer tested the LEC method for analysing amino acids of protein hydrolysates[127] by applying sulphonic cation-exchange resins in a weakly acidic buffer containing transition metal ions. The complete analysis of amino acids in the presence of Zn^{2+} ions could be performed in 4.0 h. Pyridoxal reagent[128], Xylenol Orange and Pyrocatechol Violet[127] were proposed instead of ninhydrin as the colour reagent for the detection of amino acids in this method.

Wagner and Liliedahl[129] developed a rapid method for the analysis of the content of α-aminobutyric acid in rat tissue homogenate with the help of LEC.

A simple and rapid method for the quantitative isolation of chymopapaine derived from papaya latex (*Carica papaya*) was proposed by Joshi *et al.*[130]. Using Amberlite IR-120 (Hg^{2+}) they obtained homogeneous protein. They also reported[130] similar work on the separation of enzymes[131,132].

In 1975, Porath *et al.*[133] published a paper entitled "Metal chelate affinity chromatography, a new approach to protein fractionation", which described a successful sorption and fractionation of a number of proteins of human serum on columns with biscarboxymethylaminoagarose (Zn^{2+} and Cu^{2+}), followed by stepwise elution with phosphate and acetate buffers, and with EDTA solution. Considering the mechanism of the sorbate–sorbent interaction, they assumed that the chromatographic behaviour of a protein is largely governed by its number or density of exposed surface imidazole and thiol groups that are able to form metal complexes. The separation mechanism is consequently a typical ligand exchange, the mobile ligands being high-molecular-weight proteins as in the above case of nucleic acids and enzymes.

Porath *et al.*[133] developed an important new type of sorbent for the LEC of high-molecular-weight and conformationally labile natural biopolymers, the stationary iminodiacetic ligands of the sorbent having the following long-chain link to the sorbent framework:

$$\text{agarose–OCH}_2\text{CH(OH)CH}_2\text{O(CH}_2)_4\text{–OCH}_2\text{CH(OH)CH}_2\text{–N(CH}_2\text{COOH)}_2$$

They concluded that the capacity and the separation power of the method seem to make it suitable for large-scale fractionations.

(d) Carbohydrates and amino sugars

Carbohydrates (saccharose, glucose, mannitol, sorbitol and pentaerythritol) are sorbed on the macroporous Amberlyst 15 (Fe^{3+}) from methanolic solutions. The elution of these carbohydrates is performed with anhydrous methanol; water must be

excluded as it removes the carbohydrates from the column within the free volume[58]. This method permits one to separate sugars from the lower ketones and alcohols, which are held more weakly. No carbohydrate sorption was observed when the same sulphonic resin in the Cu^{2+} form was used.

Amino sugars can be sorbed from aqueous solutions on the carboxylic cation exchanger Bio-Rex 70 (Cu^{2+})[134]. As no sorption of carbohydrates takes place under such conditions, this technique is suitable for the separation of sugars from amino sugars. Aminohexoses (glucosamine, galactosamine and mannosamine) were separated on the above sorbent by elution with aqueous ammonia solution containing Cu^{2+}.

(e) Steroids, unsaturated acids and esters

Column chromatography on argentated silica gel and alumina was first proposed by De Vries[135,136], who separated cholesterol from cholestenol. Knights and Brooks[137] separated monounsaturated sterol acetate from the diunsaturated derivative by elution with diethyl ether in light petroleum in the same way. Steroids from orange juice were isolated on argentated sorbents[138].

Houx *et al.*[139] separated components including isomers of a synthetic attractant mixture consisting of acetate esters of saturated and monounsaturated long-chain alcohols.

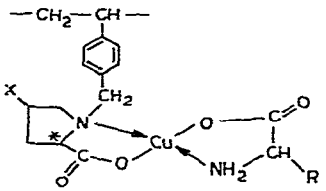
A cation-exchange resin (Ag^+ form) was applied to the separation of esters of oleic and linoleic acids by elution with aqueous methanol or a butene solution in methanol[140]. *Cis*- and *trans*-isomers of natural unsaturated acid esters were separated on the macroporous resin Amberlyst XN-1005 (Ag^+)[141]. Gibberellin and dihydrogibberellin were partly separated on Sephadex (Ag^+)[35]. Methyl *cis*-15-octadecenoate was separated[142] from a mixture on a sulphonic cation exchanger (Ag^+).

D. Ligand-exchange chromatography of racemic compounds

Stringent requirements that are placed on the geometry of the coordinating ligands by the central metal ion are responsible for the efficient separation of different isomers by LEC. One of the most difficult tasks that has been successfully solved by this technique is the separation of optical isomers.

In 1968, Rogozhin and Davankov[143] proposed a method for the resolution of racemates by LEC on asymmetric complex-forming sorbents. The method is based on the enantioselectivity effects in the formation of labile complexes[144,145]. On the basis of "macronet isoporous" styrene copolymers (via the stages of their chloromethylation), a whole series of sorbents with optically active bifunctional[146–148] and trifunctional[149–151] α-amino acid groups were synthesized. As the stability of the mixed sorption complexes $\bar{R}MA$ which were formed by these stationary ligands, metal ion and mobile ligands depend upon the steric configuration of the mobile ligands, the optical isomers of the mobile ligands possess dissimilar affinities towards the stationary phase.

A series of papers[149,152–155] were devoted to the resolution of α-amino acid racemates on asymmetric sorbents in the presence of Cu^{2+}, Ni^{2+} and Zn^{2+}. Sorbents containing L-proline[18,68–70,152] and L-hydroxyproline functions[152] have been studied in great detail. The elementary unit of these sorbents, together with the amino acid molecule (mobile ligand) coordinated by means of Cu^{2+}, has the following structure:



As can be seen from the results in Table 4, these resins efficiently resolve racemates of many α-amino acids and some α-hydroxy acids, β-amino acids, amino alcohols and other classes of compounds[152]. Usually, one of the enantiomers is eluted from the column with water, dilute ammonia solution or buffer, whereas the second enantiomer is eluted with a stronger ammonia solution. The column is regenerated by washing it with water. Davankov and co-workers studied the dependence of the LEC process on such parameters as the degree of saturation of the sorbent with metal ions[69], temperature and concentration of the displacing ligand[18].

TABLE 4

RESOLUTION OF RACEMIC AMINO ACIDS AND MANDELIC ACID BY STEPWISE ELUTION ON SORBENTS CONTAINING GROUPINGS OF L-PROLINE (I) AND L-HYDROXYPROLINE (II)[152]

Sorbent	M^{2+} *ion*	*Racemate*		*Degree of resolution (%)*	
		Substance	*Amount (g)*	L-*Isomer*	D-*Isomer*
I	Cu	Proline	0.10	98	100
II		Proline	0.15	76	96
I		Valine	0.10	47	62
II		Valine	0.15	79	90
II		Threonine	0.16	100	100
II		Serine	0.09	76	81
II		Isovaline	0.16	74	100
II		Mandelic acid	0.16	28	37
I		Isoleucine	0.10	38	48
II	Ni	Proline	0.15	15	17
II		Threonine	0.15	10	17
II		Isoleucine	0.12	23	25
II		Isovaline	0.15	22	22
II		Mandelic acid	0.16	3.0	3.6

Comparison of the results of the resolution of a large number of racemates on different asymmetric resins using different metal ions led Davankov *et al.*[152] to the conclusion that the separation of optical isomers becomes most efficient when the sum of the dentations of the stationary and mobile ligands is equal to the coordination number of the central metal ion. In this instance the mobile ligand can coordinate only according to a definite pattern, the sorption complex having a single structure. This regularity is probably also true for other LEC processes.

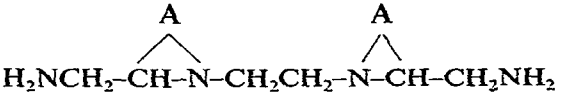
Angelichi and co-workers[156,157] synthesized a sorbent on the basis of a chloromethylated styrene–divinylbenzene copolymer with N-carboxymethyl-L-valine as the

tridentate stationary ligand. Isoleucine, valine and alanine were partially resolved in the presence of copper ions on this sorbent.

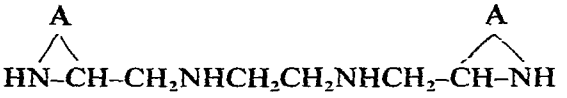
Sorbents produced as a result of interactions between amino acids and a sulphochlorated styrene copolymer[158] as well as a sorbent with α-aminobenzylphosphonic acid in a polystyrene matrix[159] have been used to resolve racemates.

In the experiments of Bernauer and co-workers[33,78], the stationary optically active complex of Fe^{3+} with N-(β-hydroxyethyl)-D-propylenediaminotriacetic acid was bound to the polymeric carrier (Dowex 1-X2 anion exchanger) not by a covalent, but by an electrostatic bond. The acetic groups of this complex can probably be replaced temporarily with a mobile ligand. The enantioselectivity of such a replacement process by mobile ligands of the type of acetyl and benzoyl derivatives of leucine, alanine, phenylalanine and methionine appeared to be insignificant, and the ratio between the sorbed D- and L-ligands was 1.08–1.03. Nevertheless, this difference appeared to be sufficient for the racemates to be partially resolved. Better results were obtained with the help of Cu^{2+}, Ni^{2+} and Zn^{2+} complexes with (—)-propylenediaminetetraacetic acid as stationary complexes in the anion exchanger[79,80]. The degree of separation of α-phenylethylamine antipodes on these sorbents reached 60%.

It was suggested recently[160] that columns packed with metal complexes of optically active tetramines of the structure



or



(A = C_3–C_4 alkylene with or without substituents) could be used to separate enantiomers of amino acids. Here too, ligand exchange is undoubtedly responsible for the enantioselectivity of the sorption process.

It has been long known that the chromatographic separation of optical isomers of kinetically inert octahedral complexes of Cr^{3+}, Co^{3+} and some other metals on asymmetric sorbents (cellulose, starch, lactose, optically active quartz and optically active ion exchangers) takes place more readily than the separation of enantiomers of conventional organic compounds [161]. In the light of present knowledge, we believe it more logical to explain such behaviour of inert complexes in terms of the enantioselectivity of the exchange of ligands in their second, external coordination sphere. Thus it is possible to relate such chromatographic processes, for example, on lactose to the LEC of complexes the internal coordination sphere of which does not participate in the exchange of ligands. This assumption agrees with the enantioselectivity of solvation processes of optically active complexes now under intensive investigation[162]. In this connection, mention should be made of the attempt to use an asymmetric sorbent which is, in fact, a carboxylic cation exchanger bearing optically active trisethylenediaminocobalt cations[163]. In this instance the separation of antipodes probably takes place as a result of the LEC of racemates, only the external coordination sphere of the stationary complex participating in the ligand exchange.

E. Ligand-exchange chromatography as a method of investigation of labile complexes

In 1965, Latterell and Walton[30] first suggested that it might be possible to obtain information on the stability of mixed complexes containing two different ligands, *e.g.*, ammonia and amines, through the study of ligand-exchange equilibria. In general, the study of the formation of mixed complexes, when the solution contains several different ligands, is especially complicated in kinetically labile systems, as it is impossible to isolate and study the individual complex species. However, if one of the ligands is bound to a stationary phase, and the remainder are transported with the mobile phase, the order of elution of these ligands from the chromatographic column must correspond to the increasing stability of the mixed complexes being formed by these ligands with the stationary ligand[145,164].

If the different ligands have similar compositions and structures, this method of studying mixed complexes is simple and sensitive. However, the comparison of ligands of different natures can lead to erroneous results. For instance, it is known[30] that a sulphonic polystyrene cation exchanger (Ni^{2+}) retains dimethylamine more strongly than ethanolamine, although the formation constants of Ni^{2+} complexes for the latter are higher ($K_1 = 950$ compared with 50 for dimethylamine). The most reliable results are obtained by the LEC of isomers, as the side effects of interactions with the sorbent framework for the isomers under comparison are the same.

The efficiency of this method of studying labile mixed complexes was demonstrated by Davankov and Rogozhin[145,164] when they detected enantioselectivity effects in copper–amino acid complexes. Earlier, it was believed that the stability of copper complexes with bidentate amino acids is independent of whether they are racemic or optically active. Using the LEC of racemic amino acids on sorbents with optically active amino acid stationary ligands, we showed the presence of enantioselectivity effects in a large number of complexes of transition metals with bifunctional[152] and trifunctional[149–151,153,155] amino acids. Thus the sorbents produced by interaction between chloromethylated crosslinked styrene copolymers with L-proline or L-hydroxyproline in the presence of Cu^{2+} ions bound D-amino acids* more strongly than their L-antipodes[152]. The conclusion that the copper(II) mixed complexes of ligands of the type N-benzyl-L-proline and N-benzyl-L-hydroxyproline with D-antipodes of amino acids are more stable was confirmed by independent methods[165–168]. All of the examples on the resolution of racemates by LEC described in the previous section indicate the occurrence of enantioselectivity in the corresponding mixed complexes.

As LEC in the presence of corresponding sorbents can also be used in non-aqueous solutions, the method will undoubtedly find wider application in the study of mixed π-complexes.

At this point, mention should be made of the possibility of studying the external coordination sphere of coordinationally-saturated inert complexes of Co(III) and Cr(III). At present there is sufficient evidence that all cationic complexes of these metals have the capacity to coordinate stepwise different anions[169] and molecules that have donating properties[170,171] in their external sphere. For example, Co(III) complexes are capable of an external sphere binding of molecules of ammonia and

* Recently it has been shown that hystidine and allohydroxyproline are exceptions to this rule.

ethylenediamine[171] and other ligands[172]. Many analogous interactions have been studied, mainly by spectroscopic methods[173], but not by chromatography. Nevertheless, LEC could contribute to the solution of a number of problems, in particular that of the enantioselectivity of external sphere exchange by exceptionally simple and precise methods.

F. Gas ligand-exchange chromatography

LEC in its gas version was first applied in 1955 by Bradford *et al.*[45]. They achieved the complete separation of ethylene and ethane by adding silver nitrate to the liquid stationary phase (ethylene glycol). Subsequently a great deal of work was carried out on gas chromatography in which ligand sorption and ligand-exchange processes were used on different occasions, but gas LEC developed separately from achievements in liquid chromatography. Although Helfferich[15] pointed out in 1965 the most important common features in liquid and gas chromatography, there seem to be only two papers in which attempts were made to associate one method with the other[54,55]. Such a situation had an adverse effect on the development of LEC in general.

DuPlessis and Spong[174] calculated the dissociation energy of silver–ammonia complexes using gas chromatographic experimental data.

Gil-Av and Herling[175] were the first to describe the chromatographic determination of the stability constant of silver–olefin complexes. Later, the formation constants of silver complexes[176–179] with a large number of unsaturated and aromatic hydrocarbons were determined in the same way. On the basis of these results, some general conclusions were drawn on the influence of the structure of hydrocarbons on the stability of complexes.

A series of investigations was performed by Vitt *et al.*[180] to determine the thermodynamic parameters and equilibrium constants of reactions of displacement of a weak ligand (octadecyl bromide) from the coordination sphere of metal bromides (Ca^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Co^{2+}, Cu^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}) by oxygen-containing organic compounds.

Duffield and Rogers[47] studied the dependence of the separating capacity of a column on the amount of silver salts ($AgNO_3$, AgCl, Ag_2SO_4) applied to Chromosorb and determined the heat of sorption of hexene isomers on the stationary phase. In all instances the heat of sorption of the *cis*-isomer was higher than that of the *trans*-isomer, which always eluted from the column first. The retention time of the hexenes was always higher than that of benzene, but lower than that of cyclohexene.

The application of silver salts as complex-forming materials in the stationary phase involves a number of inconveniences, the main one being its rapid reduction, which, in turn, makes it impossible to use higher temperatures. In attempts to increase the stability of the column and to improve the separation, Cook and Givand[181] applied different Ag^+ complexes as the stationary phase. Here there was no sorption of olefins when 2,6-dimethylpyridine, quinoline, isoquinoline, 2,2-bipyridyl, 2,2-biquinoline and 1,10-phenanthroline were used. However, the sorption of olefins took place on AgR_2NO_3 complexes where R = pyridine or 4-, 2- or 3-methylpyridine.

Further research has considerably expanded the area of application of this method[22]. Stearine salts of divalent transition metals (Mn, Co, Ni, Cu, Zn) have been proposed as additives to the liquid hydrocarbon phase (Apiezon). The retention time on stearates compared with that on pure Apiezon L increased in the following series

of compounds: aliphatic hydrocarbons, aromatic hydrocarbons, aliphatic ketones, cyclic ketones, secondary alcohols containing a methyl group in the α-position, normal alcohols and, finally, amines. The latter had the longest retention time and in some instances they were sorbed irreversibly.

Pecsok and Vary[53] applied phthalocyanine complexes of divalent Cu, Zn, Fe, Co, Ni and trivalent Al and Cr as the stationary phase. The determination of the retention times for 15 ligands and their summation for the separate phases made it possible to arrange the divalent metal ions in the following order of activity with respect to ligands: Fe > Ni > Co > Zn > Cu. The order of activity with respect to nitrogen-containing ligands was as follows: Fe > Co > Ni > Zn > Cu. They also noted the strong influence of steric factors on complex formation, *e.g.*, methanol was held more strongly than ethanol and dimethyl ether more strongly than diethyl ether. Fig. 9 illustrates the influence of iron phthalocyanine additives on the resolution capacity of the column.

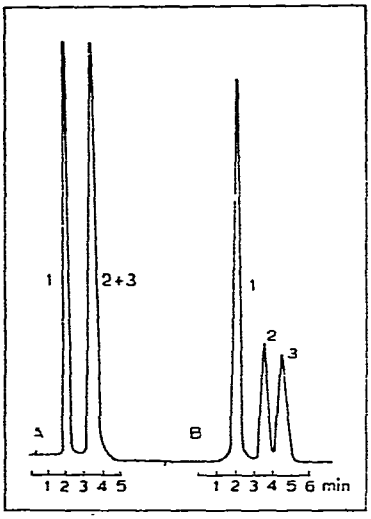
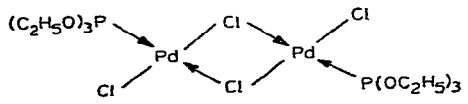


Fig. 9. Separation of *n*-hexane (1), cyclohexene (2) and pentanone-3 (3) at 60°. A, On DC 200 silicone oil on Chromosorb T; B, on 0.09 g of iron phthalocyanine dispersed in DC 200 silicone oil on Chromosorb T[53].

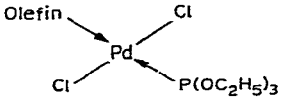
Gil-Av and Herzberg-Minrly[25] suggested characterizing the haemoglobin content of blood through the retention time of oxygen on columns filled with stationary phases processed with the blood being tested.

In the work mentioned above[23,48–53,181], a large number of transition metal complexes were tested as additives to be used in the stationary phases. In all instances it was suggested that the coordinational unsaturation of the complexes used (which have square-planar structures with the unoccupied axial positions in the octahedral coordination sphere) accounts for the selectivity of interaction of the mobile ligands with the stationary phase.

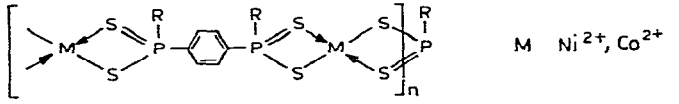
Cartoni *et al.*[51] used in the chromatographic column a bridged palladium complex with the structure



which showed a considerable affinity towards olefins, acetylenes and aromatic hydrocarbons. They consider that the complex interacts with olefins with reorganization into the structure.



The inorganic ion exchanger zirconium phosphate (Cu^{2+} and Zn^{2+}), which is widely applied in LEC, was used by Fujimura and Ando[55] to separate volatile low-molecular weight amines. They made a detailed study of the gas chromatographic behaviour of almost all of the isomers of aliphatic amines from methylamine to butylamine. Interestingly, the retention times of amines on the ion exchanger in the Cu^{2+} form are 1.3–3.0 times higher than on that in the Zn^{2+} form. A mixture of water vapour with ammonia was used as the mobile phase. A specially synthesized metal-containing polymer:



M Ni^{2+}, Co^{2+}

was thoroughly studied in both liquid[92], and gas chromatography[54]. The use of this polymer on Chromosorb made it possible to separate different amine mixtures. The derivatives of benzylamine and aniline, some alkylamines, pyridine and thiophene have also been studied.

Coordinationally saturated complexes such as $M(NCS)_2(4\text{-}MPy)_4$ and $M(NCS)_2$ (1-phenylethylamine)$_4$ ($M = Ni^{2+}$, Co^{2+}, Fe^{2+}) applied to the Chromosorb have made it possible to separate the isomers of disubstituted benzene derivatives[182–184]. These interesting separations were referred to as "clathrate chromatography", although the sorbate–stationary phase interactions have not been studied sufficiently. We believe that ligand-exchange processes may play an essential role in this instance and that "clathrate" chromatography may turn out to be a variety of LEC.

Finally, mention should be made of work[185] in which the exchange of coordinated ethylene in the complex salt $K[PtCl_3(C_2H_4)]_2 \cdot H_2O$ for propylene under dynamic conditions was studied. It was emphasized that ligand exchange proceeds at a sufficient rate only if the surface area of the solid phase is large enough.

Two reviews[4,5] were devoted to the application of complex compounds as stationary phases in gas chromatography. The latter survey also considers the application of complex-forming ions to thin-layer chromatography.

G. Ligand exchange in thin-layer chromatography

The impregnation of standard sorbents (alumina, silica gel) applied in thin-layer chromatography by means of complex-forming metal ion salts usually results in a significant change in the R_F value of the compounds being separated.

Yasuda[186–189] attempted a systematic study of the influence of the impregnation of silica gel with salts of Co, Zn, Mn, Cd, Al and Cu on the separation of isomeric toluidines, nitroanilines, chloroanilines, aminophenols, aminobenzoic acids, naphthylamines, etc., in different eluting mixtures. The results of the successful separation of some *o*-, *m*- and *p*-substituted anilines on silica gel impregnated with cadmium salts are given in Table 5.

TABLE 5

THIN-LAYER CHROMATOGRAPHY ($R_F \times 100$ VALUES) ON SILICA GEL IMPREGNATED WITH Cd^{2+} SALTS IN A BENZENE–ACETIC ACID (9:1) SYSTEM[187]

1 = Unimpregnated silica gel; 2 = silica gel impregnated with cadmium sulphate; 3 = silica gel impregnated with cadmium acetate; 4 = silica gel impregnated with cadmium phosphate.

Compound	*1*	*2*	*3*	*4*
o-Toluidine	26	13	22	24
m-Toludine	17	8	12	15
p-Toluidine	12	4	8	9
o-Anisidine	19	11	18	20
m-Anisidine	18	8	13	16
p-Anisidine	5	1	6	5
o-Chloroaniline	62	64	70	60
m-Chloroaniline	44	33	34	44
p-Chloroaniline	32	15	25	30
1-Naphthylamine	33	27	35	37
2-Naphthylamine	24	18	23	27
o-Aminobenzoic acid	44	43	13	41
m-Aminobenzoic acid	14	10	3	14
p-Aminobenzoic acid	33	31	12	33

Tabak *et al.*[190] studied the behaviour of aromatic and unsaturated acids and amines on silica gel containing Ag_2O. Anthocyanines and anthocyanidines were separated on silica gel impregnated with tin salts[191]. Iron (III) chloride on kaolin absorbed nitrogen-containing compounds from oil[192].

The ability of silver ions to form adducts with unsaturated compounds has long been used in the thin-layer chromatography of natural compounds. The impregnation of silica gel and alumina with silver nitrate improves the separation of many unsaturated carbonic acids and their esters[135,193], terpenes[194,195], steroids[196–202], lipids[203], etc. Usually, this method is helpful in separating mixtures of *cis*- and *trans*-isomers. Recently, "argentated" tin foils such as Silufol UV 254 have become more widely used[204].

5. CONCLUSION

LEC has exceptionally diverse uses in many separation processes. It can be used successfully in both its liquid and gas versions, in aqueous media and organic solvents.

The possibilities of chromatography in non-aqueous solutions have not yet been fully studied, so that the work carried out so far can be regarded as only a preliminary exploration in this area. Ligand exchange is also possible in systems with two non-miscible liquid phases. For instance, $HTlCl_4$ dissolved in diethyl ether changes efficiently the chlorine ions for bromine ions extracted from the aqueous phase[185].

LEC can be used to solve many unique problems, and is particularly useful for the separation of different types of isomers, including optical isomers and isotopes. LEC can also be used to concentrate extremely diluted solutions of complex-forming products or to sorb irreversibly a number of harmful substances from vented gas and effluent water in industry.

The high selectivity of LEC has made it possible to develop a wide variety of methods for the analysis of natural mixtures.

Like the specific sorbents for affinity chromatography that are now being intensively studied, resins in the metal-ion form can sorb selectively some high-molecular-weight biopolymers that possess an affinity towards metal ions. This is particularly useful in applications with different kinds of metal-containing enzymes and co-enzymes. Moreover, LEC methods will probably be invaluable in studying the mechanism of metal–enzyme action.

LEC is a novel and powerful method for studying coordination compounds, both kinetically labile and inert, the free ligand exchange of which takes place in the external sphere. We hope that this review will serve to demonstrate the potential of LEC and will prove helpful to both chemists and biochemists working in diverse fields.

6. SUMMARY

A review is presented of the chromatographic separation of substances interacting with the stationary phase through the coordination bond formation in the coordination sphere of a transition metal present in the system, covering the literature up to the end of 1976. Advances in liquid chromatography have been considered in more detail than those in gas–liquid and thin-layer chromatography, since the former provides usually more information about the stoichiometry and mechanism of ligand-exchange processes.

The first part of the review describes theoretical questions and peculiarities of ligand-exchange chromatography, the second part deals with the practical applications of this new technique in organic chemistry and biochemistry, the study of coordination compounds, the separation of optical isomers, drugs, etc.

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