ANALYTICAL APPLICATIONS OF ION EXCHANGE CHROMATOGRAPHY

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In addition to ion exchange processes nowadays already considered as classical methods (determination of total salt content, eliminating of interfering ions, desalting etc.) ion exchange chromatography has become one of the most important field of modern analytics regarding both its wideranging applicability and its capacity.

Ion exchange chromatography and modern instrumental analytics complete each other. Small quantities of ions and compounds having similar behaviour and interfering effect to one another, by rapid and accurate determination with optical, electrochemical and radiochemical methods, can be easily and most quickly separated by ion exchange chromatography.

Principle of ion exchange chromatography is the following: A solution containing ions to be separated is poured on a column made of ion exchange resin in the upper part of which the ions will be bound. (In case of cations separation is performed on a cation exchange column.) Thereafter a solution of electrolyte containing from point of view of the determination possibly indifferent ions is poured on the column. Dependent on the composition of poured solution there are two cases: The ions of poured electrolyte bind themselves on the column either stronger or less strongly than the ions to be separated. In the first case ions of the poured solutions displace the ions to be separated in succession due to the strength of binding. (Displacing chromatography.) In the other case the ions to be separated move likewise on the effect of the eluent but with varying rate. (Elution chromatography.) Bands developing on the column are passing with various rate towards the end of the column, and stepping out of it ions appear one after the other. While in the first case quantitative separation cannot be attained in principle, in the second case the bands are separating completely by the use of adequate long column. Formation of bands depends on two important factors. In one respect on the distribution coefficient known already at other chromatographic methods whose mathematical interpretation is as follows:

$$D_A = \frac{(A)}{[A]} \; ; \qquad D_B = \frac{(B)}{[B]}$$

(Round brackets designate the concentration in resin phase, square ones, however, the concentration in the solution.) On the other hand the formation of fronts of the bands is dependent on the column performance, or rather on the "number of theoretical plates" of the column, a conception already well known from the distillation technique. Theoretical plate-number of a given column is depending on the grain size of the resin, on the flow rate etc. It is easy to realize: the smaller the grain size, the slower the flow rate, resp. the equilibrium conditions setting in the column, the more approach the ideal states, the better the goodness of the column is.

At elution procedures the rate of passing of some ions — as already previously described — is not the same, and is approximately proportional to the reciprocal value of the distribution coefficient [1]:

$$\left(\frac{\Delta x}{\Delta V}\right)_A = \frac{1}{D_A + a} \qquad \left(\frac{\Delta x}{\Delta V}\right)_B = \frac{1}{D_B + a}$$

With Δx designated that segment volume of the column, on which the absorption band pushed forward on the effect of ΔV, the eluent volume. a void fraction of the column. (Volume of solution in the column divided by the whole volume of the column.) The greater the proportion of distribution coefficients of ions A and B to be separated — the so-called *"separation factor"* — is, the more effective separation can be reached. The fading of the front of bands depends on the column performance, on the number of theoretical plates. The larger the number of theoretical plate of the column, or rather the smaller one plate height is, the sharper the fronts and narrow the bands, and — in case of separation — the better the effectiveness of separation is. The column performance has a more important role when the separation factor is not high enough. Relation between the purity of separation and theoretical plate number of the column gives a possibility to calculate the experimental and working conditions of chromatographic separations for analytical purposes [1], [2], [3].

If, for instance, the distribution coefficient of three ions to be separated and of similar charge, ions A, B and C, in case of a given eluent composition is the following in turn: $D_A = 1$, $D_B = 10$ and $D_C = 100$, separation factors are large enough, so the separation can be made without any special conditions — i. e. with normal column dimension, flow rate etc. — with adequate purity. In practice the above mentioned distribution coefficients also indicate, that 1 column volume eluent is necessary until the first, 10 until the second, and 100 until the third ion reaches the end of the column. As the chromatographic procedure would be very long lasting by this method, we can also proceed so that we modify the composition of the eluent after the complete elution of the first ion (for instance by concentration raise) in such a way,

that the distribution coefficient of the following B ion be 1, and that of the last be 10. In this way by the "stepwise" changing of the composition of eluent, by so-called *stepwise elution*, we can separate with better efficiency. According to calculations the best results can be attained if the composition of eluent is continuously changed during the whole time of chromatographic procedure [4], [5]. Latter procedure is named as *gradient elution*.

Gradient elution can be made with a simple equipment. On Fig. 1 the device of EULITZ [6] can be seen. If on starting we pour saturated ammonium
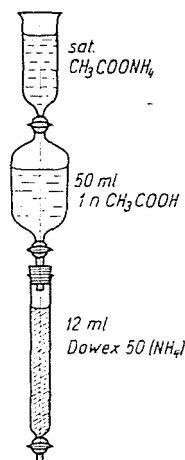

Fig. 1. A simple device for gradient elution separation of alkali earth ions according to Eulitz [6]

acetate into the upper vessel of the apparatus and into the lower one diluted acetic acid, so on the cation exchange column placed under them the alkali earth metal ions absorbed on the column can be eluted with solutions of gradually rising pH. In the solution the concentration of ammonium acetate changes exponentially according to the relation

$$c = c_0 \; (1 - e^{-b})$$

where c_0 is the original concentration of ammonium acetate solution, b is the quotient of the volume of eluent and that of the volume of the lower mixing vessel. For the construction of automatic fraction-collector serving for the gathering of the solution dropping out of the column in small quantities, we can find a lot of designs in the latest literature [7—10].

Ion exchange chromatographic separations can be utilized in wide range both in inorganic and in organic chemical analysis. The chromatographic method, however, primarily presents itself for the separation of inorganic ions, as the pore sizes of common ion exchange preparations are chiefly most suitable for exchange of inorganic ions of nearly similar size.

3*

Inorganic analytical applications

Elution chromatographic separation of metal cations absorbed on a cation exchange column can be most easily, but not always most effectively accomplished by elution with diluted hydrochloric acid. A case in point, and today it can be considered as classical method, the separation of alkali ions from one another [11—14]. The method can be used for determination of sodium contamination in sodium salts, too [15].

Hydrochloric elution is also suitable for separation of alkali and alkali earth, or alkali and heavy metal ions. Recently we have developed a procedure in our Institute for the determination of sodium oxide content of red clay, the principle of which is as follows [16]:

Dissolve the sample in hydrochloric acid, filter the solution, and poure it on a H-form cation exchange column. On the column all metal ions (iron, aluminium, calcium, sodium etc.) are bound, or rather changed by hydrogen ions. Then elute — free from interfering ions — with 0.5 N hydrochloric acid the sodium ions bound on the column, and determine flame photometrically or gravimetrically in the gathered solution. With hydrochloric elution berillium can be separated from the accompanying metal ions [17], with diluted nitric acid metal traces from small quantities of iron [18] etc. STRELOW [19], later MANN and SWANSON [20] determined in hydrochloric acid solutions. FRITZ, GARRALDA and KARAKKER [21] in hydrofluoric acid solutions the distribution coefficient of numerous metal ions for investigation of possibilities of separations on cation exchange column.

The possibility of the use of diluted mineral acid as eluent is rather limited. The formation of the separation factors namely in this case arises chiefly from the own selectivity of the resin, and the complex formation effect of acid-anions becomes prevalent only in more concentrated acidic solutions [20], [21], [22]. The elution with solutions containing complex formation ions is much more effective. In the presence of complex formation ions the distribution coefficient of the ion in question will namely be determined partly by the selectivity of the resin and partly by the complex stability. FRITZ and KARAKKER [23] carry out the elution with 0.1 N and 0.5 N ethylendiamin perchlorate. According to their investigations the separation of numerous bi- and multivalent ion pairs can be performed.

By using complexing agents we succeeded in certain cases in making one of the ion pairs to be separated into a complex without charge or with opposite charge respectively, to such a degree, that they do not absorb at all on the ion exchange column. In such cases the ratio of distribution coefficients — i.e. the separation factor — can also reach very high values. If a complexing agent is given to the solution containing ions to be separated prior to pouring on it is called *selective sorption* ; if the ions bound on the column are eluted

with a solution of a complex forming substance it is called *selective elution*. An example for the first is the method of OLIVER and FRITZ [24]:

Several metal ions (iron, aluminium, uranium, thorium, zirconium) form with sulphonic salicylic acid a complex of negative charge, the divalent copper, cadmium, zinc, nickel ions, however, form with ethylendiamin ions positive charged complexes. Out of the solution containing complex forming agents the positive charged complex ions can be selectively absorbed by a cation exchange column, and by the anion exchange the negative charged ions. An example is for selective elution the method used by ROZANOVA and KATA-JEV [25] for determination of copper, iron contaminations in metal arsenic: The sample is dissolved in acid, thereafter the solution is poured on a cation exchange column, on which all metal ions will be absorbed. The copper and iron ions can be separated selectively from arsenic by elution with Seignette-salt solution and determined photometrically. In a similar way selectively can be separated from numerous accompanying ions by selective elution: lead by ammonium carbonate [26], antimon by tartaric acid [27], nickel by diethyl dithiocarbamat [28] solution.

If complex stability of metal ions to be separated and according to this its distribution coefficient deviates only a slightly, so separation of ions will be made with real chromatography. With the help of organic complexing agents (citrate-, tartarate-, ethylendiamin tetraacetate etc. ions) not only alkali earth metal ions [6], [29—32], rare earth metal ions [33], [34], but fission product mixtures containing 30—40 components [35], even the trans-uranium elements [34], [36] can be separated and determined. Recently the chromatographic separation of calcium and strontium ions has been parti-cularly significant, with the help of which rapid determination of the stron-tium content of bones can be accomplished [32].

In order to increase the own selectivity of the resin investigations have been made since long to produce such ion exchange products, whose active groups has a great specificity for some ions. In this field lately significant results have been achieved by WOLF and HERING by creating ion exchange resins containing aminoacidic acid, imino-diacetic acid [37] and nitrilo-triacetic acid [38] active groups. Ion exchange resins containing 8-hydroxi-quinoline groups are suitable for the selective binding of copper, nickel, cobalt [39], resin prepared of fluoron derivatives for germanium [40] and for the separation from the accompanying ions respectively. Dowex A 1, being avail-able also on the market (Dow Chemical, USA) contains as active groups imino diacetic acid groups. With its help, e. g. separation of cobalt-nickel, or deter-mination of calcium in lithium salts [41] can be accomplished.

Metal ions can be separated also on anion exchange column. In the pre-sence of organic complexing agents metal-complexes of anion character can be chromatographed on anion exchange column. With oxalate, citrate, tar-

tarate, ethylendiamin tetraacetate ions build several metal ions depending on pH, complexes of various stability. With eluent containing oxalate and citrate ions e. g. zirconium, titanium, niobium, tantalum, tungsten and molibden ions can be separated [42]. According to the investigations of NELSON, DAY and KRAUS [43] using ethylen diamin tetraacetate containing eluent, separation of several metal ions can be performed as well. Similarly in the presence of malonate ions on an anion exchange column tin ions can be separated from antimon and lead ions. The method is suitable for analysis of bearing metals [44]. With the help of ascorbinate ions thorium [45] and zirconium [46] ions can be separated from the accompanying, interfering elements.

Partly KRAUS and co-workers [47], partly JENTZSCH and co-workers [48] have earlier developed separation methods using complex-forming halogen ions for the separation of metal ions on an anion exchange column. Namely most part of metal ions form with halogen ions (chloride, bromide, iodide, fluoride), but also with other inorganic ions (sulphate, nitrate, carbonate, phosphate etc.) ion association complex or complexes affectable by various ion concentrations and by the concentration of ligand ions. KRAUS and co-workers have investigated the behaviour of ions of nearly all elements in hydrochloric acid solutions, and plotted the distribution coefficients in relation with the concentration of hydrochloric acid. According to the dates of their table constructed on the basis of the periodical system, very large number of determinations can be performed with the solely use of hydrochloric acid as eluent. It is not difficult to find such hydrochloric concentration for numerous ion pairs, where distribution coefficient is larger than 1.5. As distribution coefficients are in most cases over 10 or rather over 100, separations can be made very quickly with selective sorption or selective elution. The method is applicable among others for the separation and rapid determination of components of silver solder [49], steel [50], aluminium alloys [51], zinc ore [52], platin metals [53], ferrites [54] etc.

Recently a method has been developed in our Institute for the determination of zinc traces in condensed water. In the condensed water, containing in significant quantity copper ions too, direct determination of zinc could not be performed neither photometrically, nor polarographically. Principle of the method is as follows [55]:

To of ca. 1 litre volume water zinc ion free hydrochloric acid is given, that its concentration to hydrochloric acid will be just 1 N. Thereafter the solution is poured on a chloride-form anion exchange column, on which the zinc-chlorocomplex-anions of high stability bound on the resin column, and the less complex forming copper- and iron ions pass through the column. Finally the zinc ions enriched on the column can be eluted with 50 ml 0.01 N nitric acid, and polarographed [55].

FARIS [56] investigated systematically the behaviour of various ions on anion exchange column using as eluent hydrogen fluoride of various concentration. Results of his measurements are presented similarly to Kraus and Nelson on periodical table. With hydrogen fluoride as eluent the separation of numerous ions can be performed.

FRITZ and PIETRZYK [57] on one hand, WILKINS and SMITH [58], on the other, develop further KRAUS' hydrochloric methodology by adding to the hydrochloric acid eluent various organic solvents. Through these solvent-
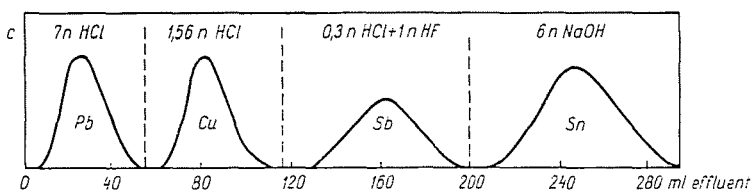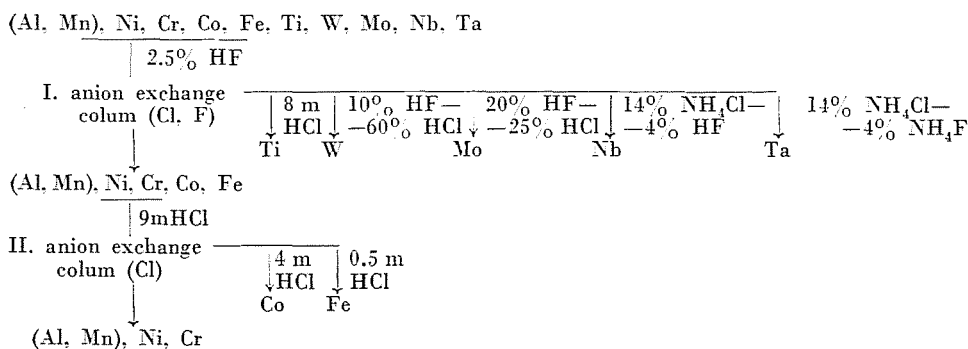

Fig. 2. Anion exchange separation of constituents of bearing metall according to Ariel and Kirowa [62]

combinations the applicability of the method is widened to a large extent. KRAUS and co-workers [59] investigated the possibilities of the use of hydrochloric acid and hydrogen fluoride together. The methods can be combined with one another. Combined methodology has very great importance in the analysis of metals and its effect is enormous. By its aid analysis of large variety of alloys, the most complicated separations can be performed [60], [61]. On Fig. 2 separation of components of bearing metal using hydrochloric and hidrogen fluoric acid as eluent are to be seen [62].

In Table 1, however, separating scheme of nickel-, chromium-, cobalt-, iron-, titanium-, tungsten-, molibdenum-, niobium-, tantalum- etc. components

Table 1

Chromatographic separation of constituents in a high temperature alloy according to WILKINS [63]

(Al, Mn), Ni, Cr, Co, Fe, Ti, W, Mo, Nb, Ta

| 2.5% HF

I. anion exchange column (Cl, F) ——— | 8 m | 10% HF— 20% HF— | 14% NH$_4$Cl— | 14% NH$_4$Cl—
↓ HCl ↓ —60% HCl ↓ —25% HCl ↓ —4% HF ↓ —4% NH$_4$F
Ti W Mo Nb Ta

(Al, Mn), Ni, Cr, Co, Fe

| 9mHCl

II. anion exchange column (Cl) ——— |4 m | 0.5 m
↓ HCl ↓ HCl
Co Fe

(Al, Mn), Ni, Cr

of an alloy with high melting point and of special composition is to be seen. Determination of separated metal ions can be carried out partly photometrically, partly polarographically or complexometrically.

Separation of metal ions can also be performed on an anion exchange column transformed by precipitateforming ions. KEMULA and co-workers [64] determined silver content of copper ores by pouring the nitric acid solution of a sample to be investigated on a chloride-form anion exchange column. On the column silver ions are bound in form of silver chloride precipitate, and they can be later eluted with ammonium hydroxide solution, and determined nephelometrically free from interfering ions. Similarly anion exchange column containing sulphate ions can be used for the selective binding of lead ions [65].

Among the chromatographic separations of ions of non-metallic elements on an anion exchange column must be mentioned the improved separation method of GRANDE and BEUKENKAMP [66], worked out for various phosphates (ortho-, pyro-, tri-, trimetha-, tetra-, tetrametha-phosphate). By utilization of this method an automatic analyzer is constructed recently [67]. The device, suitable for the total analysis of wash-materials, polyphosphate mixtures, after hydrolysation into orthophosphate ions determines photometrically the phosphate ions separated by potassium chloride elution, and registers their quantity.

Organic analytical applications

At the applications of ion exchange chromatography in organic analysis must be taken into consideration the followings:

1. As a rule organic ions are of larger volume than inorganic ones, therefore ion exchange resins of common pore size are not suitable in every case, so products with higher pore size are necessary.

2. Most part of organic compounds does not dissolve in water, so the organic solvents mixible with water play more important role at chromatographic separations.

3. One part of organic compounds inclines to adsorption. Adsorption accomplished on the resin in some cases is very disadvantageous, because it makes difficult the elution of organic ions, but it can be often advantageous too, because by deliberate use a way is found for the use of other (salting out, solubilization etc.) chromatographic methods, too.

Chromatographic separation of organic acids can be performed on an anion exchange column. For the purpose of eluting diluted acid solution (hydrochloric acid, formic acid) can be used. RHEINBOTE [68] accomplishes chromatographic separation of non-volatile plant acids with formic acid, then the eluate is gathered in fractions after evaporating the formic acid and determination of acids is performed by acidimetric titration. With its method

succinic acid, malic acid, malonic acid, tartaric acid etc. can be separated from one another. For the separation of acids a micro method is recently developed [69].

Similarly to the acids in non-aqueous medium the chromatographic separation of compounds of acidic character [71], phenol derivatives [72] can be accomplished on anion exchange column. Oxy acids similarly to poly-oxy compounds can be chromatographed with buffer solutions containing borate ions [71]. Separation of basic compounds (amines [73—75], cyanide derivatives [76]), however, can be accomplished on a cation exchange column. HELFFERICH [77] advises previously anion exchange column saturated with heavy metal ions for the chromatographic separation of amines and other complex forming substances. On the basis, that complex forming liability of ligands are different, very large distribution factors can be reached.

Chromatographic separation of some non-ionic compounds can be accomplished on anion exchange column transformed by complex forming ions On anion exchange column of bisulphiteform aldehydes, ketons [78], on anion exchange column saturated with borate ions, however, sugars [79], [80] and other polyoxi compounds [72] can be separated.

Separation of amino acids of amphoteric character on ion exchange column is possible. As already known, principle of separation is the following: Amino acids bound on cation exchange column are eluted with buffer solutions of various pH. Distribution coefficients of amino acids can be influenced only by the pH of the solution, and therefore large distribution coefficients can not be reached. It is necessary to make use of the total capacity of the performance of the column, for the purpose, that separations in praxis would be accomplished adequately [81], [82]. Recently such automatic devices are put in circulation which perform by securing the most adequate experimental conditions and by photometric measurement of chromatograms in 24 hours the total analysis of an proteinhydrolysate. Chromatographic separation of amino acids is recently developed in form of semi-micro [85] and ultra-micro methods [86].

On ion exchange column consisting of resin of large pores — similarly as in case of chromatographic separation of amino acids —, or rather recently on column made of cellulose containing ion exchange groups — peptides, proteins [87], biological active substances (enzimes [88], viruses etc.), on anion exchange column polysacharides [88] has been separated with good results.

In organic analysis besides common ion exchange chromatography there are other chromatographic methods, too. These have extended in high degree the applicability of ion exchangers. In methods suitable to such purposes high absorption ability of organic compounds plays an important role. A procedure already known as chromatographic method is based on the phenomenon of

ion-exclusion [90], recently on the called *salting-out* and *solubilization* chromatography.

According to the investigations of SARGENT and RIEMANN [91] absorption of organic substances soluble in water can be effected by salt concentration of the solution. Relation between logarithm of distribution coefficient and salt concentration of solution they found as linear:

$$\log D = \log D^\circ + k\,[\mathrm{M}]\,.$$

In the equation D is the distribution coefficient if salt solution of $[\mathrm{M}]$ concentration and D° if only water is present, k is the salting-out coefficient. By selecting a salt solution of suitable concentration it is possible to separate
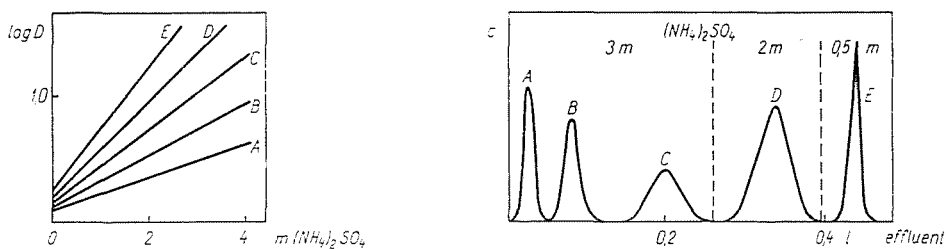

Fig. 3. The distribution coefficients of ether homologs and their separation by salting out chromatography using ammonium sulphate solution as eluent according to Sargent and Riemann [92]

on the ion exchange column homologous compounds of similar chemical feature: alcohols [91], esters, ethers [92], ketons [93], amines (the latters do not dissociate in weakly alkalic solutions). As in this case binding is not ionic, for the purpose of chromatographic separation both cation and anion exchange resin products are suitable. Elution must be performed with salt solutions of stepwise decreasing concentration. On Fig. 3 formation of distribution coefficients of aetherhomologs (carbon atomic number from 2 to 8) and the elution curves of the separation are to be seen.

The so-called solubilization chromatographic method is suitable for the separation of organic compounds weakly soluble in water. In this case the substances absorbed on the ion exchange column can be eluted by aqueous mixture of soluble organic solvents. (E. g. with acetic acid-, alcohol-water mixture.) [94] The method is suitable for the separation of alcohols of higher carbon-atomic number, ketons and some hydrocarbons. On similar principles as above described are based the methods of SEKI [95] worked out for the separation of acid- and phenol-derivatives.

For the separation of electrolytes and non-electrolytes a special amphoteric ion exchange resin product was recommended. In the pores of the special

resin cation- and anion-active groups are opposite each other. On the column made of this resin both cations and anions of the electrolyte will be bound, the non-ionic compounds, however, pass through. With more intensive aqueous washing electrolytes can be washed out. The phenomenon is called "ionretardation". The special resin is brought into the market by the firm Dow Chemical.

Ion exchange paper chromatography

Ion exchange chromatography can be performed not only on ion exchange resin column, but also on paper impregnated with ion exchange resin, or on paper made of cellulose containing ion exchange groups, by paper chromatographic methodology. On ion exchange paper rapid chromatographic separation of inorganic ions [96], [97] organic compounds [98], drugs [99], amino acids can be accomplished chiefly for qualitative analytical purposes.

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