Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Walton, Harold F.(1984) 'Counterion Effects in Partition Chromatography', Separation Science and Technology, 19: 11, 849 — 855
To link to this Article: DOI: 10.1080/01496398408068597
URL: http://dx.doi.org/10.1080/01496398408068597

SEPARATION SCIENCE AND TECHNOLOGY, 19(11 & 12), pp. 849-855, 1984-85

Counterion Effects in Partition Chromatography

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Abstract

Inorganic counterions may affect the partition of organic solutes between water and an ion-exchange resin in at least four ways. One is by coordination of the solute to a metal ion held in the resin; this is the basis of ligand-exchange chromatography. Another is through hydration of the counterion, which has two effects: (1) it modifies the solvent characteristics of the polymer gel; (2) it makes the water of the gel less available for hydrogen bonding. Finally, strong electric fields between fixed ions and doubly or triply charged counterions attract dipolar ions like those of amino acids.

INTRODUCTION

"Ion-exchange partition chromatography" is the name given to chromatographic analysis in which the stationary phase is an ion-exchanging polymer and the principal mechanism of retention is something other than the interaction of ionic charges. Ion-exchange resins are absorbents for organic solutes and can be used as reversed-phase packings for high-performance liquid chromatography. The solvent is usually water, but water may be mixed with an organic modifier. The solutes range from aromatic hydrocarbons at one extreme to sugars and polyhydric alcohols at the other. Between these extremes are aromatic compounds that carry polar groups, like phenols, amines, esters, and amides or caffeine and the xanthines. The main retention mechanism in these cases is the interaction of pi-electrons between the aromatic solutes and the aromatic rings of the resin polymer, which is generally cross-linked polystyrene.

A major drawback to ion-exchange resins in partition chromatography is slow diffusion and mass transfer. This drawback is partially overcome by

 0149-6395/84/1911-0849$3.50/0

using resins with very small particles, 7–10 μm. An advantage to resins over bonded silica packings is their pH resistance; another is their compatibility with water. Compared to porous polystyrene, which has similar absorptive properties, they are more uniform and give more symmetrical chromatographic bands. The presence of ionic groups makes the resins hydrophilic and makes it possible to use water as the mobile phase rather than, say, 50% acetonitrile. The nature of the exchangeable ions in the resin affects the absorptive properties, sometimes drastically, and it is this effect that is the subject of this paper.

Nearly all experimental studies of partition chromatography have been made with cation exchangers of the sulfonated polystyrene type. Anion exchangers have been studied too, but they are more complicated and harder to control.

CHARGE EFFECTS

The most obvious characteristic of the replaceable cation is its charge. Resins carrying divalent cations, like Ca^{2+}, are stronger absorbents for hydrocarbons and for most organic solutes than the same resins carrying univalent ions (*1*). At first sight this effect is explained by the reduced swelling and lower water content caused by divalent ions; the ions behave as electrostatic cross-linking agents, drawing polymer chains closer together; with less water in the resin, the resin behaves more like an organic solvent. However, the cross-linking effect is balanced by the greater hydration of divalent ions compared with univalent. In addition, cations of higher charge, and particularly transition metal cations, are likely to bind organic molecules by coordination. Another binding mechanism, ion–dipole interaction, is possible and will be discussed below.

HYDRATION EFFECTS

In an attempt to study the effects of ionic hydration and the water content of the swollen resin, with minimum perturbation by ion–solute coordination, we measured the retention of 12 polar aromatic solutes on columns of ion-exchange resins of two cross-linkings that carried as counterions Li, Na, K, Mg, and Ca. The water contents of the swollen resins were found using a centrifuge technique (*2*).

Two different patterns were seen for the effect of the counterion on retention. For solutes without phenolic hydroxyl groups, like caffeine or acetanilide, Li gave the strongest retention of the univalent ions; retention dropped in the sequence Li-Na-K, then rose, going from K to Mg. With

phenolic compounds, like phenol itself or the parabens (alkyl *p*-hydroxybenzoates), retention increased in the sequence Li-Na-K, then fell, going from K to Mg. In all cases Ca caused stronger retention than Mg.

To interpret these findings we made a distinction between "bound" and "free" water in the resin. "Bound" water was that held by the cations as water of hydration. To define water of hydration we used the estimates of Harned and Owen, based on distances of closest approach of ions in solution (*3*). Lithium-loaded resin has more total water than sodium-loaded resin and swells more, but lithium ions are much more hydrated than sodium ions, and the amount of "free" water is actually greater in the sodium-loaded resin. The "free" water has a dual role. On the one hand it dilutes the polymer chains and makes the resin a poorer solvent for the organic solutes. On the other hand, free water can enter into hydrogen bonding with phenols while bound water (we postulate) cannot. Hydrogen bonding (we postulate) is an important factor in binding phenolic compounds to the resin.

Interpretations aside, the fact remains that the nature of the inorganic counterion affects separation factors and selectivity orders, and chromatographic separations can be "fine-tuned" by proper choice of the counterion. Counterions affect chromatographic band widths, too. The narrowest bands are obtained with lithium ions.

COMPLEX IONS

The most important role of the inorganic counterion is the formation of coordinate bonds with organic solute molecules that are electron donors. To be useful in chromatography, the bonds so formed must be labile; they must be formed and broken rapidly. Cations that form strong but labile coordination complexes are primarily those of transition metals such as Cu^{2+}, Zn^{2+}, Ni^{2+}, and Ag^{+}. Donor molecules that are effective in aqueous solutions are mainly those of amines and amino acids. The kind of chromatography where metal ions are fixed on a column of cation-exchange resin and different donor molecules change places on the metal ions and travel along the column at different rates is known as *ligand-exchange chromatography*. Since its introduction by Helfferich in 1961 (*4*), ligand-exchange chromatography has become an established technique, and is the subject of recent reviews (*5, 6*).

"Ligand-exchange chromatography" is a term broadly used to include many processes in which complexes are formed and dissociated and an ion exchanger is the stationary phase. It will be convenient to group these processes according to whether the complexes are very strong, moderately strong, or weak.

(a) Strong Complexes. The best known applications of ligand-exchange chromatography are in the analysis of mixtures of amines, amino acids, peptides, and proteins. The metal ions are usually Cu(II) but may be Zn(II). Formation constants of the complexes are quite high, of the order 10^6 or much more.

A column of cation-exchange resin is loaded with Cu(II), usually in the form of its complex ions with ammonia, and aqueous ammonia is used as the eluent. To counteract the displacement of Cu from the column, which must inevitably occur if ions are present in the eluent, copper ions are added in low concentrations, 0.001 *M* or less, to the eluent.

A striking feature of ligand-exchange chromatography is its selectivity. Space inside the swollen ion-exchanger is limited, and anything that tends to obstruct access of donor nitrogen atoms to the metal ions weakens the binding considerably. Primary amines are held much more strongly than secondary or tertiary amines, and substituents close to the amino group on the ligand weaken the coordination. While steric effects are important, other effects, notably pi-electron interaction of solutes with the polystyrene resin matrix, may strengthen the binding considerably. Any attempt to understand selectivity in ligand-exchange chromatography must recognize that stabilities of complexes in aqueous solution are only a rough guide; complexes may be much more or much less stable in the resin than in free aqueous solution, for reasons that are imperfectly understood (*7, 8*). A very striking example is the Cu(II)–ethylenediamine complexes, which are 10 times as stable in a cation-exchange resin, and 1000 times as stable in a montmorillonite clay, as they are in aqueous solution (*9*).

The greatest triumph of ligand-exchange chromatography in recent years has been the separation of optical isomers of amino acids (*10*). These separations are based on the stabilities of 2:1 amino acid:Cu(II) complexes in which one of the amino acid molecules is in a specified optically active form, and is usually *l*-proline. Whether the second molecule is in the *d*- or the *l*-form makes several hundred calories difference in the free energy of association, and results in separation factors up to 4 and more. In general the *ll*-combinations are more stable than the *ld*-combinations. For chromatography the first amino acid (*l*-proline) may be incorporated into the stationary phase, grafted on to cross-linked polystyrene or bonded to silica, or it may be incorporated in the mobile phase. Where the chiral discrimination occurs in the mobile phase, it is not necessary for the stationary phase to be an ion-exchange resin; it can be a hydrophobic C_{18}-bonded silica which absorbs the neutral binary complexes CuL_1L_2 (L_1 and L_2 being amino acid ligands) but not the charged complexes CuL^+ (*11*). It is doubtful whether this procedure falls under the definition of ligand-exchange chromatography.

Another striking example of the selectivity of ligand-exchange chromato-

graphy is the separation of mixtures of peptides by Foucault (*12*). Short columns of silica gel are impregnated with Cu(II) by simply passing a $Cu(NH_3)_4^{2+}$ solution; these ions are bound very tightly to the silicate ions on the surface. Eluents are water–acetonitrile solutions of ammonia, presaturated with silica. Among simple di- and tripeptides the number of possible combinations of amino acid units is very great, and typical mixtures gave over 40 sharp peaks. Applications to medical diagnosis have been suggested.

(b) Complexes of Moderate Stability. To illustrate the role in partition chromatography of complexes whose formation constants in water are of the order 10 to 100, we cite the chromatography of carboxylic acid anions on lanthanum-loaded resins (*1*).

Lanthanum ions are the largest and least hydrolyzed of the simple trivalent cations. Hydrolysis is only 1% at pH 6. We can therefore use La^{3+} in partition chromatography without being restricted to low pH values, as we are with Fe^{3+}.

A general trend that we have noted is that divalent ions cause stronger retention than univalent. Trivalent ions should cause even stronger retention. With nonionic polar aromatic solutes like phenacetin (4′-hydroxyacetanilide), this is the case, but the effect is small. Much larger effects are found with compounds having carboxylate groups, including amino acids.

Let us consider carboxylic acids first. At low pH values where ionization is suppressed, aromatic acids are absorbed on resins carrying lanthanum ions, but the retention is little more than that where calcium or sodium ions are present. Above pH 4, however, when the carboxylic acids are ionized, retention rises with pH, and capacity factors become as high as 50 at pH 5.5. To account for the binding of negative carboxylate ions by a cation-exchange resin, we must assume the formation and absorption of positively charged complexes, LaA^{2+}, where A^- is the anion of the carboxylic acid. Such complexes exist in solution; a clear proof for their existence is the fact that retention of carboxylate ions on lanthanum-loaded resins is decreased by adding lanthanum salts to the mobile phase. Formation constants of ions LaA^{2+} can be measured in aqueous solutions by adding lanthanum salts to solutions of the acids HA and measuring the drop in pH. We found values of 34 for benzoate, 37 for acetate, 130 for mandelate, and 16 for trigonelline (*N*-methylpyridinium-3-carboxylate, a compound found in coffee and many vegetable products).

Speculating on the binding of LaA^{2+} to the resin, one knows that the free anions A^- are excluded from cation-exchange resins through the Donnan equilibrium, and one must conclude that the complexes are much more stable inside the resin than outside.

Speculations aside, a column of lanthanum-loaded resin is selective for

aromatic acids and as such has potential use. Unfortunately, the chromatographic bands are broad, indicating not only slow diffusion but slow formation and dissociation of the absorbed metal complexes.

(c) Weak Complexes. One of the more valuable applications of ion-moderated partition chromatography has been the analysis of sugars (*13, 14*). Columns of calcium-form resin are used, with pure water as the eluent. Sucrose, glucose, fructose, mannitol, and sorbitol are eluted in that order, and oligosaccharides come out before sucrose. With di-, tri-, and oligosaccharides the main factor in retention seems to be steric exclusion from the resin, but with monosaccharides and their corresponding alcohols the mechanism is the formation of bi- and tridentate complexes with the calcium ions in the resin; compounds with the most favorable steric arrangement of hydroxyl groups are held the most strongly (*13*). The complexes are weak; capacity factors are generally below 3. That they exist in solution as well as in the resin is shown by the fact that retention is decreased by adding calcium salt to the water eluent (*15*); this effect can be used to measure the formation constants.

The simple amino acids, glycine, alpha- and beta-alanine, are retained by a calcium-form resin to about the same extent as sorbitol. (They are retained by a lanthanum-form resin a good deal more.). Adding calcium salts to the water decreases their retention, pointing to the existence of complexes in the aqueous phase and permitting an estimate of their stability. With pure water as eluent there is no perceptible displacement of calcium ions from the resin, showing that complexation does not involve displacement of hydrogen ions from the amino acids; addition of calcium ions to aqueous solutions of the amino acids does not change the pH. One must infer that any complexes are formed between calcium ions and neutral molecules (or dipolar ions) of amino acids.

ION-DIPOLE INTERACTIONS

The amino acids just cited are held by calcium-loaded and lanthanum-loaded resins in the order alpha-alanine (weakest), glycine, beta-alanine (strongest). To explain this effect, and also the retention of trigonelline and its isomer homarine (*N*-methylpyridinium-2-carboxylate), we have postulated (*1*) that in cation-exchange resins carrying divalent or trivalent ions there exist strong local electrostatic fields. According to Coulomb's law, a divalent ion finds its position of maximum stability near one of the fixed sulfonate ions, leaving the second one bare. In a cross-linked polymer this simple picture is modified by the approach of two polymer chains to one another, the "electrostatic cross-linking" that we mentioned above. This

effect will lessen, but not eliminate, the local electrostatic fields. When such fields are present they will attract dipolar molecules like those of amino acids. The greater the dipole moment, the stronger the attraction. We do find that beta-alanine is more strongly absorbed than alpha-alanine, and trigonelline more than homarine; comparing absorption on La^{3+} resin with that on Ca^{2+} resin, the increase is greater with trigonelline than with homarine; in the experiments with La^{3+}-loaded resin, adding lanthanum ions to the mobile phase had very little effect on the retention of trigonelline, indicating that very little complexing occurred in the mobile phase. Ion–dipole binding, if it occurs, should occur mainly in the resin phase.

The role of ion–dipole binding in ion-exchange partition chromatography is still speculative. It is evident that many factors influence the partition of organic solutes between solutions and ion-exchange resins. A recent review which emphasizes practical applications of "ion-moderated partition chromatography" mentions some factors that we have not (*16*).

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