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COUNTER-ION EFFECTS IN PARTITION CHROMATOGRAPHY

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SUMMARY

The inorganic counter-ion of an ion-exchange resin affects retention of organic solutes in several ways, such as the formation of co-ordination complexes, ion–dipole interaction, ionic hydration and hydrogen bonding. To study these effects the retention of sugars and sugar alcohols, amino acids and hydroxy acids has been measured on a column of polystyrene-based cation-exchange resin, carrying these counter-ions: Li^+, K^+, Ca^{2+}, La^{3+}. The eluent was water or, in some cases, a salt solution. To correlate retention with complex stability the solubility of calcium sulfate was measured in solutions of the ligands mannitol, sorbitol, glycine, α- and β-alanine. We concluded that the calcium complexes are about as stable in the ion-exchange resin as in aqueous solution.

INTRODUCTION

The inorganic counter-ion affects the retention of organic compounds on ion-exchange resins in various ways. Most studies have been made with cation exchangers, where the most obvious effect is the formation of co-ordination complexes between the metal ions and organic ligands; this is the basis of ligand-exchange chromatography. Other interactions affect retention too, and one of these is ionic hydration. We have reported[1] on the partition of polar organic compounds between aqueous solutions and cation-exchange resins of the polystyrene type, containing alkali-metal and alkaline-earth cations, and we noted two effects that act in opposite directions. For many solutes, retention increases in the order K < Na < Li. For phenolic compounds the opposite order was observed. We attributed the effects to the "free" water in the resin polymer, that is, the imbibed water that is not attached to the cation as water of hydration. The proportion of "free" water apparently increases in the order Li < Na < K. "Free" water makes the polymer gel a poorer solvent for organic compounds, decreasing their absorption, but on the other hand it can form hydrogen bonds with phenols and other hydroxylic compounds and so increase their absorption.

If this theory is correct, the retention of compounds that contain many hydroxyl groups, like sugars and sugar alcohols, should increase with the counter-ion in the order Li < Na < K. We have found this to be the case.

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Cation-exchange resin columns carrying potassium ions[2,3] have been used for the chromatography of carbohydrates, and so have sodium-form resin[4], but today, one of the common methods for analyzing carbohydrate mixtures is chromatography on a calcium-loaded ion-exchange resin with water as the eluent[5–10]. Calcium-form resins retain polyols relatively strongly, and the mechanism of retention is undoubtedly the formation of co-ordination complexes. If the orientation of hydroxyl groups is favorable to complex formation, retention is strong[11], and it is stronger with lanthanum counter-ions than with calcium[11,12]. The retention of all sugars and sugar alcohols in such systems is increased by adding alcohol or acetonitrile to the mobile phase, and there is a considerable body of literature on their chromatography in alcohol–water mixtures[13].

The chromatography of sugars and sugar alcohols on a calcium-form resin is an example of ligand-exchange chromatography with weak complexes. Complex formation is not the only mechanism that influences retention. We know that the stability of metal–ligand complexes may be very different in the resin from what it is in solution[14]. The Cu(II)–ethylenediamine complexes, for example, are ten times more stable in a sulfonated polystyrene cation exchanger than in aqueous solution[15].

In this investigation we measured the retentions on a cation-exchange resin of a number of sugars and sugar alcohols, as well as other alcohols and amino acids and hydroxy acids, substances that are commonly associated with sugars in nature. We compared four counter-ions, Li^{+}, K^{+}, Ca^{2+} and La^{3+}. At each run we injected a small volume of dilute salt solution, for example $LiNO_3$ with the lithium-loaded column, to mark the interstitial volume of the column and fittings; it was assumed, and verified by using different salt concentrations, that the salt was excluded from the resin by the Donnan equilibrium. To find the total volume of water in the column, inside and outside the resin, we injected deuterium oxide as a tracer. The difference between the deuterium oxide peak and the salt peak gave the internal water volume, the volume of water inside the resin beads. We estimated the stability constants of certain complexes by measuring the solubility of calcium sulfate, first in pure water, then in solutions of amino acids and sugar alcohols. One aim of the investigation was to see whether these complexes were more stable in the resin than in solution.

EXPERIMENTAL

The resin and the column

The resin was Benson BCx6 (Benson, Reno, NV, U.S.A.) a sulfonated polystyrene, 6% cross-linked, particle size 7–10 μm. It was packed into a glass column (Glenco, Houston, TX, U.S.A.) 30 cm × 6.3 mm I.D., water jacketed with adjustable bed supports, rated to 1000 p.s.i. internal pressure. Column temperature was maintained at 60°C by a Lauda heater-circulator pump (Brinkman Instruments, Westbury, NY, U.S.A.); the flow-rate was generally 0.5 ml min^{-1}. The ionic capacity of the column was found by converting it into the hydrogen form with 0.5 *M* nitric acid, then washing it with water and displacing the hydrogen ions with calcium nitrate; the displaced acid was titrated with sodium hydroxide. Also, the calcium-loaded column was flushed with dilute nitric acid to displace the calcium ions, which were then titrated with EDTA. The two measurements agreed closely; the ionic capacity was 16.6 milliequivalents, or 8.30 mmol Ca^{2+}.

After each change of counter-ion the column was washed with water, and the bed supports were adjusted to leave no void space at the ends of the resin bed. The bed length was *ca.* 23 cm, but varied from one counter-ion to another. The retention volume of deuterium oxide tracer, noted below, indicates the swelling and shrinkage of the resin.

Chromatographic system

This consisted of an M-45 pump and R-401 refractive index detector (Waters Assoc., Milford, MA, U.S.A.), a six-port injector (Rheodyne, Cotati, CA, U.S.A.) and the glass column described above.

Solubility measurements

The solubility of calcium sulfate dihydrate was measured in water and in solutions of five ligands in order to estimate the stabilities of the calcium–ligand complexes. A buret with a glass-wool plug at the bottom was packed with small crystals of calcium sulfate dihydrate to give a column *ca.* 10 cm long, and the aqueous solution was allowed to flow through the column at *ca.* 1 ml min^{-1}; it was recirculated until the calcium-ion concentration, measured by EDTA titration, became constant; two passes through the column were generally enough. The experiments were carried out at room temperature, 21 $\pm$ 1°C; closer temperature control seemed unnecessary, since the solubility of calcium sulfate changes by only 0.5% per degree centigrade. To obtain the desired particle size, the calcium sulfate was prepared by dissolving commercial calcium sulfate in hot 1 *M* hydrochloric acid, filtering and neutralizing the excess acid by the hydrolysis of urea added to the boiling solution (the method of "precipitation from homogeneous solution").

RESULTS

Retention volumes

The retention volumes are given in Table I. They are shown as multiples of the retention volume of deuterium oxide, because the bed volume changed as one counter-ion was substituted for another. The footnote to Table I shows the measured retention volumes of deuterium oxide. The internal volume of the resin changed from one counter-ion to another, being least with La^{3+}, greatest with Li^{+}.

For La^{3+} the salt peak does not truly represent the interstitial volume, for it emerges at a greater volume than sucrose. There is some penetration of the resin by lanthanum nitrate. The reason for this penetration is presumably ion association.

All the sugars gave a single, symmetrical peak, indicating that mutarotation was complete and rapid at the column temperature.

Sugars, sugar alcohols

Table I shows the trends between the counter-ions. The increase in retention of sugars and sugar alcohols on going from Li^{+} to K^{+} can be ascribed to an increase in "free" water available for hydrogen bonding, as we discussed in the Introduction. In the calcium-form resin there is less total water and much less "free" water than in the potassium-form resin, for Ca^{2+} is strongly hydrated; hence the drop in retention of glucose and sucrose. The retention of the sugar alcohols, mannitol and sor-

TABLE I

COMPARISON OF RETENTIONS WITH DIFFERENT COUNTER-IONS

Deuterium oxide = 1.00; column temperature, 60°C except where marked. Volume retentions of deuterium oxide were lithium, 5.81 ml; potassium, 5.62 ml; calcium, 5.58 ml; lanthanum, 4.95 ml.

Counter-ion	Li^+	K^+	Ca^{2+}	La^{3+}
Salt peak	*0.385*	*0.47*	*0.50*	*0.72*
Sucrose	0.52	0.605	0.57	0.60
Lactose	–	–	0.56	0.60
Glucose	0.58	0.76	0.67	0.65
Sorbose	–	–	0.80	0.71
Fructose	0.59	0.83	0.82	0.77
Arabinose	–	–	0.90	0.77
Xylose	–	0.82	0.77	0.71
Ribose	0.72	0.94	1.43	2.05
Glycerol	0.77	0.82	0.96	1.10
Pentaerythritol	0.75	0.73	0.90	1.08
Mannitol	0.64	0.73	1.10	1.44
Sorbitol	0.665	0.74	1.36	2.60
Malic acid	0.37	0.46	1.20	> 12*
Tartaric acid	–	0.45	1.52	> 12*
Citric acid	–	0.45	–	> 12*
Glycine	1.49	1.02	1.81	6.25*
α-Alanine	1.43	0.94	1.55	5.56*
β-Alanine	1.72	1.29	2.19	> 12*

* Column temperature, 72°C.

bitol (glucitol), rises sharply, as does that of ribose. Complex formation with Ca^{2+} is clearly indicated. The four hydroxyl groups of ribose in its pyranose form are oriented on the same side of the ring in such a way as to favor co-ordination; a potentiometric study[16] showed that ribose associates with calcium ions with a stability constant of 1.6 l mol^{-1}, while the association of the pentoses xylose and arabinose was much weaker.

Lanthanum ions not only form stronger complexes than calcium[17], but discriminate better between mannitol and sorbitol[12], and between ribose and other sugars.

Returning to the lithium- and potassium-form resins, we see that sugars and sugar alcohols are partially excluded from the resins, that is, that the ratio of sugar to water inside the resin is less than that outside. Steric exclusion may be a factor, but a more likely reason is the limited opportunity for hydrogen bonding within the resin.

Amino acids, hydroxy acids

Table I shows that the three amino acids tested are attached to the resin and that retention by lithium-form resin is stronger than retention by potassium-form resin. Again we can invoke the concept of "free" water. Hydrogen bonding is not a factor here, but rather the hydrophobic interaction with the resin matrix, which is

stronger with lithium-resin because there is less "free" water. Hydrophobic interaction is also indicated by these results, not included in Table I: on calcium-form resin, retention volumes of valine, 1.87; serine, 1.71; threonine, 1.57 (deuterium oxide = 1). Further, 1-butanol was retained at volume 1.52 (deuterium oxide = 1), while methanol, ethanol and 2-propanol all eluted close to deuterium oxide.

In the bonding of amino acids to the resin, the obvious effect is their strong retention by the calcium- and lanthanum-resins. In previous papers[18] we have noted the strong absorption of dipolar ions by rssins loaded with Ca^{2+} and La^{3+} and suggested an interaction between the dipoles and strong electrostatic fields existing between polyvalent metal ions and unoccupied fixed ions of the resin. If this mechanism is important, amino acid complexes in the resin should be much stronger in the resin than in aqueous solution. Results described below indicate that calcium complexes are *not* more stable in the resin than in solution, but the lanthanum complexes may well be more stable.

The retention of amino acids could be occurring by cation exchange, even though the eluent was water and the amino acids were close to their isoelectric pH. There was evidence that β-alanine might be retained by cation exchange, with accompanying displacement of calcium ions. When repeated injections were made, the retention volumes increased and the peaks became very asymmetrical. This condition was cured by an injection of calcium nitrate solution. If 0.1 *M* calcium nitrate was used as the eluent instead of pure water, stable, reproducible retention volumes were obtained with all the amino acids. These are the volumes listed in Table I. Volumes in pure water were *ca.* 5% greater than in 0.1 *M* calcium nitrate, and those in 0.25 *M* calcium nitrate were *ca.* 10% less. We attempted to use these data to calculate the stability of the calcium-ligand complexes in solution.

The retention of hydroxy acids was also measured in 0.1 *M* calcium nitrate, and the results clearly indicated complex formation. On lanthanum-form resin the retention of hydroxy acids was very strong indeed.

Calculations of stability constants

From the solubility of calcium sulfate. The solubility data are shown in Fig. 1. The manner of plotting was suggested by the work of Kirkwood[19] on the activities of solutions of dipolar ions. Complex formation raises the solubility, and the order of solubilities shown in Fig. 1 is the same as the order of retention of the five ligands on calcium-form resin. The graphs are almost linear at low ligand concentrations, and if we ignore activity coefficients or (more legitimately) assume them to cancel, we can calculate stability constants as follows:

Let s and s_0 be the solubilities of calcium dihydrate in the presence and absence of ligand; let L represent the ligand, and K_{sp} be the solubility product of calcium sulfate dihydrate; then,

$$[Ca^{2+}] + [CaL^{2+}] = s = [SO_4^{2-}] = K_{sp}/[Ca^{2+}];\ K_{sp} = s_0^2;$$

therefore: $[Ca^{2+}] = s_0^2/s$, and $[CaL^{2+}] = \dfrac{s^2 - s_0^2}{s}$

$$\text{Stability constant of } CaL^{2+} = \frac{s^2 - s_0^2}{s_0^2} \times \frac{1}{[L]}$$

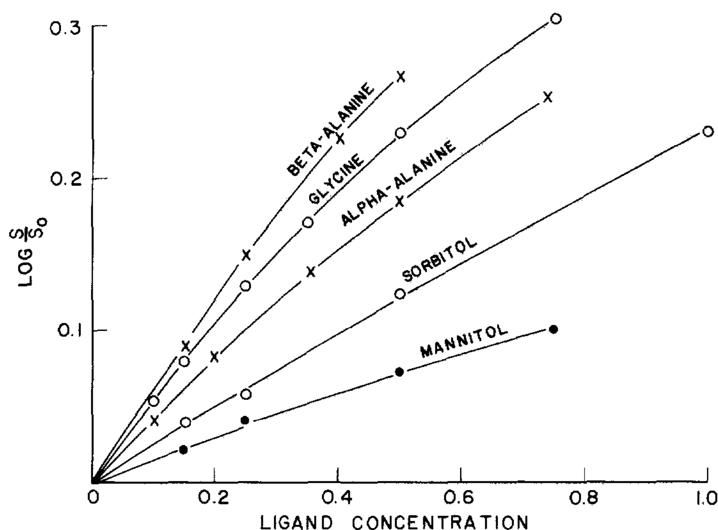

Fig. 1. Effect of dissolved ligands on the solubility of calcium sulfate dihydrate at 21°C: s = solubility in ligand solution; s_0 = solubility in water; ligand concentrations in molarities.

The stability constants thus deduced are (l mol^{-1}): mannitol, 0.7; sorbitol, 1.2; α-alanine, 2.1; glycine, 2.6; β-alanine, 3.2. The measured solubility, s_0, was 0.0153 *M*. The fraction of ligand bound to calcium is small, and in this calculation, [L] was taken as the concentration of ligand added.

We showed the net charge of the ligand as zero, even for the amino acids. At high pH, Ca^{2+} and other metal ions displace a proton from amino acid molecules and yield a singly charged complex ion. Stability constants for such complexes are well documented, but we were working at neutral pH, and to show that a proton was not displaced, we added calcium nitrate crystals to solutions of amino acids around a glass electrode; only minimal pH changes were observed, from 6.50 to 6.55 with 0.25 *M* glycine, 6.55 to 6.10 with 1 *M* β-alanine. We thus conclude that the amino acid complexes are ion–dipole pairs.

From the effect of calcium nitrate solutions on chromatographic retention. The retention volumes of amino acids and sorbitol are reduced if calcium salt is added to the water eluent, because complexes are now formed in solution as well as in the resin, and we hoped to use this effect to measure stability constants. However, the effect is small, and its interpretation is complicated by the shrinkage of the resin beads when the salt is added. For the calcium–glycine complex we calculated a stability constant between 0.7 and 1.4 l mol^{-1}, depending on whether we did or did not allow for the change in resin volume. All we can say about these estimates are that they are of the right magnitude.

Stability within the resin. From Table I retention volume of sorbitol on calcium-form resin is 1.36 (deuterium oxide = 1), and the inter-particle void volume is 0.50. Let us suppose that if sorbitol did not form a complex, its retention would be the same as that of glucose, namely 0.67. The corresponding capacity factors, k', are: sorbitol, 1.72; glucose, 0.34. We may infer that the internal concentrations of complexed and uncomplexed sorbitol are in the ratio (1.72 − 0.34) to 0.34, or 4.0:1. The

internal concentration of calcium ions is 8.3 mmol in 2.8 ml (see above), or 3.0 molal. The stability constant is thus 1.33. Thus the complex is about as stable in the resin as it is in solution. Similar calculations for amino acids are more complicated, for we cannot estimate what the retention would be without complexation, but they lead to the same conclusion, namely, that the complexes in the resin are not markedly more stable nor less stable than they are in aqueous solution.

CONCLUSIONS

Retention data are presented that have analytical interest. They confirm in general, but not in detail, the results of other workers[11,12] and show that calcium is the best counter-ion for separating pentose and hexose sugars, while lanthanum is better for sugar alcohols or polyols. They offer the possibility of analyzing mixtures of sugars with fruit acids (hydroxy acids) and amino acids on the same column without previously removing the acids from the sample. Hydroxy acids are bound very strongly to the lanthanum-loaded column, but if the proper eluent is found, a lanthanum column may be useful for analyzing such acids.

On the theoretical or mechanistic side, we have found no great differences between complex stabilities in the resin and those in water, though such differences may well exist in lanthanum-loaded resins. Ion–dipole association is shown to exist between calcium ions and amino acids in solution and in the resin.

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