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Note

Selection of a marker in ion-exchange chromatography using dioxane-water as the eluent

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In earlier work¹ on ion-exchange chromatography through a SP-Sephadex C-25 column using dioxane-water as the eluent, a difficulty was encountered in selecting a marker for determining the void volume. Blue dextran 2000 could not be used, because its retention volume changed with the dioxane content of the eluent. The use of $\text{NH}_4[\text{Co}(\text{EDTA})]^-$ as a marker was tried because it contains a coloured complex anion, but it also proved to be unsuitable because its retention volume in a solvent with a high dioxane content sometimes exceeded that of the metal complex cation. Hence a criterion for selecting a marker suitable for use with dioxane-water as the eluent needed, and this paper describes such a criterion.

EXPERIMENTAL

The sample complexes used were $(\text{NH}_4)_3[\text{Cr}(\text{NCS})_6]$, $\text{NH}_4[\text{Co}(\text{EDTA})]$, $\text{NH}_4[\text{Co}(\text{NCS})_4(\text{NH}_3)_2]$ and *cis*- $[\text{Co}(\text{NCS})_2(\text{en})_2]\text{Cl}$, which were prepared according to the literature²⁻⁴ and identified by elemental analyses and from UV absorption spectra.

The eluents used were 0.1 M ammonium chloride in pure water and in 70% (v/v) dioxane-water. SP-Sephadex C-25 was packed to a length of 43 cm in a column of I.D. 11 mm; the length of the packing was decreased to 17 cm when working with the dioxane-water eluent. Sample solutions were prepared by dissolving 25 mg of the complex salt in a few millilitres of eluent. After injecting the sample solution, elution was carried out at a flow-rate 0.6 ml/min for aqueous solutions and 0.3 ml/min for dioxane-water solutions.

RESULTS AND DISCUSSION

As SP-Sephadex is a cation exchanger that contains SO_3^- groups at a high density, it exerts a co-ion exclusion effect on the complex anion. Fig. 1 shows the chromatograms of three complex anions, $[\text{Co}(\text{NCS})_6]^{3-}$, $[\text{Co}(\text{EDTA})]^-$ and $[\text{Co}(\text{NCS})_4(\text{NH}_3)_2]^-$, and a complex cation, $[\text{Co}(\text{NCS})_2(\text{en})_2]^+$, eluted with 0.1 M aqueous ammonium chloride solution. There are differences among the retention volumes of

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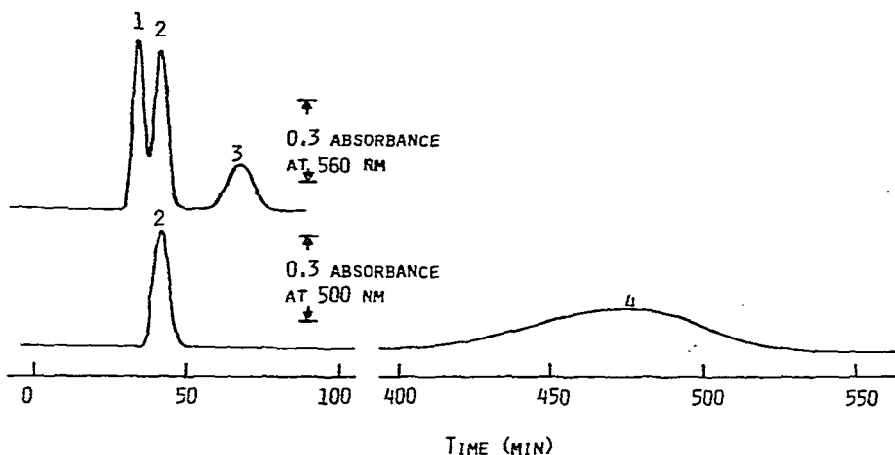


FIG. 1.

Fig. 1. Chromatograms of electrolyte complexes with 0.1 *M* aqueous NH_4Cl solution as the eluent. Peaks: 1 = $[\text{Cr}(\text{NCS})_6]^{3-}$; 2 = $[\text{Co}(\text{EDTA})]^-$; 3 = *trans*- $[\text{Cr}(\text{NCS})_4(\text{NH}_3)_2]^-$; 4 = *cis*- $[\text{Co}(\text{NCS})_2(\text{en})_2]^+$.

the three complex anions; the trivalent anion, $[\text{Cr}(\text{NCS})_6]^{3-}$, shows the smallest retention volume, which reflects the largest co-ion exclusion effect. Thus, it became clear that an anion with a high negative charge should be used as a marker with aqueous solutions. The difference between univalent anions, $[\text{Co}(\text{EDTA})]^-$ and $[\text{Cr}(\text{NCS})_4(\text{NH}_3)_2]^-$, may be attributed to the different degrees of hydration of these ions. The retention volume of the complex cation, $[\text{Co}(\text{NCS})_2(\text{en})_2]^+$ is, of course, much larger than those of the complex anions.

The order of elution of these complexes is significantly different with dioxane-water as the eluent. As shown in Fig. 2, $[\text{Cr}(\text{NCS})_6]^{3-}$ and $[\text{Cr}(\text{NCS})_4(\text{NH}_3)_2]^-$ have

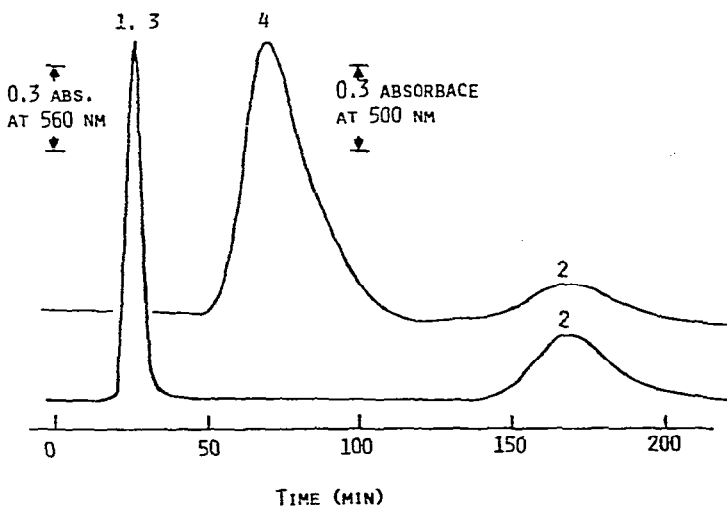


Fig. 2. Chromatograms of electrolyte complexes with 0.1 *M* NH_4Cl in dioxane-water (70:30) as the eluent. Peaks as in Fig. 1.

the smallest retention volumes, but $[\text{Co}(\text{EDTA})]^-$ has a large retention volume that is greater than that of the complex cation, $[\text{Co}(\text{NCS})_2(\text{en})_2]^+$.

These results can be interpreted in the following way. Generally, complexes with an NCS^- ligand have a strong affinity toward organic solvents. This is expected from the method of preparation of these complexes. Therefore, they have a tendency to solvate dioxane selectively in the dioxane–water mixed solution. On the other hand, Sephadex contains many hydrophilic functional groups and therefore, when it is in contact with dioxane–water, it adsorbs water preferentially so that the stationary phase becomes water-rich in comparison with the mobile phase and the mobile phase becomes dioxane-rich in comparison with the stationary phase. Consequently, the complex with NCS^- as a ligand tends to be attracted to dioxane in the mobile phase. This tendency, together with the co-ion exclusion effect, is considered to result in the smallest retention volumes being shown by $[\text{Cr}(\text{NCS})_6]^{3-}$ and $[\text{Cr}(\text{NCS})_4(\text{NH}_3)_2]^-$. On the other hand, the complex anion $[\text{Co}(\text{EDTA})]^-$ is considered to be hydrophilic because it contains many carboxylate groups, and is kept in the water-rich stationary phase against the co-ion exclusion effect. As $[\text{Co}(\text{NCS})_2(\text{en})_2]^+$ is lipophilic owing to the presence of two NCS^- groups, it tends to be attracted to dioxane in the mobile phase against the attractive force by the SO_3^- group of the stationary phase. Thus, the order of elution of these two ions is reversed.

It is concluded that complex anions containing the NCS^- ligand, such as $[\text{Cr}(\text{NCS})_6]^{3-}$ and $[\text{Cr}(\text{NCS})_4(\text{NH}_3)_2]^-$, have the smallest retention volumes and are suitable markers when dioxane–water is used as the eluent.

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