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Note

Adsorption of inorganic anions on Sephadex gels. Part II

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In a previous paper[1], we described the adsorption on Sephadex of a selected number of anions which we knew were adsorbed on cellulose. All of these anions were "salted out", desorbed by organic solvents (ethanol–water) and adsorbed least on low cross-linked Sephadex, more strongly on highly cross-linked Sephadex and greatest on hydrophobic Sephadex. We feel that these results show clearly that the retention can be termed a "hydrophobic interaction". This kind of interaction can also be observed on ion-exchange resins[2] and cellulose[3].

In this paper, we describe the use of Sephadex chromatography to establish the "hydrophobic" sequence of a series of monovalent anions, halo acids, halo oxyacids and pseudohalo acids.

EXPERIMENTAL

Sample solutions (0.1 *N*) were prepared from suitable amounts of reagents (Carlo Erba, Milan, Italy). The sample of perbromate was a gift from Prof. E. H. Appelman (Argonne National Laboratory, U.S.A.).

Sephadex G-25, G-200 superfine and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were prepared as thin layers in the TLC chamber (Pharmacia) as described previously[1]. Blue Dextran 2000 and $Co(NH_3)_6^{3+}$ were used as reference substances.

RESULTS

Sephadex G-200

There was no measurable adsorption of any of the anions examined with 0.01 *N* nitric acid as eluent. A typical chromatogram is shown in Fig. 1.

Sephadex G-25

There were very slight adsorption effects when the anions were eluted with 0.01 *N* nitric acid, the most notable being with CNS^-. When eluted with 3 *N* lithium nitrate or acetate solution, there was a measurable adsorption of $CNSe^-$ and CNS^- as well as slight differences between Cl^-, Br^- and I^-, but no differentiation or adsorption of the oxyhalo acids. On eluting with 0.01 *N* nitric acid–ethanol (1:1) all of the anions moved with the speed of $Co(NH_3)_6^{3+}$, *i.e.*, there was no adsorption effect.

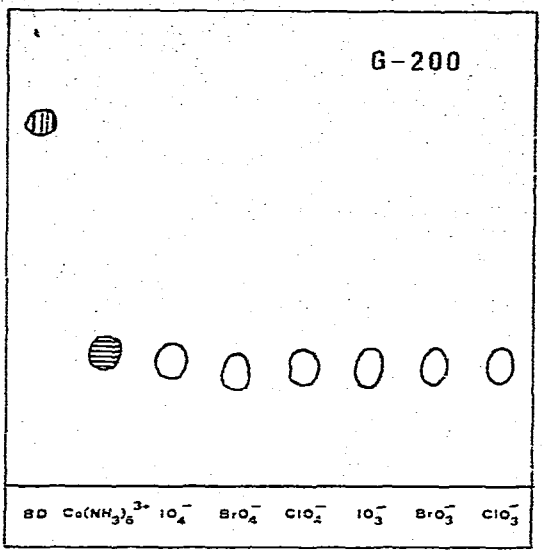

Fig. 1. Thin-layer gel filtration chromatogram on Sephadex G-200 with 0.01 *N* nitric acid as eluent. BD = Blue Dextran 2000.

Sephadex LH-20

As in the previous study[1], the most marked effects were obtained on Sephadex LH-20. Fig. 2 shows the movement of all of the anions (on two chromatograms) in 0.01 *N* nitric acid. There was a small but measurable difference between Cl^-, Br^- and I^- and a rather strong adsorption for CNS^-, ClO_4^- and BrO_4^-; $CNSe^-$ decomposed in 0.01 *N* nitric acid. When eluted with 3 *N* lithium nitrate or acetate solution,

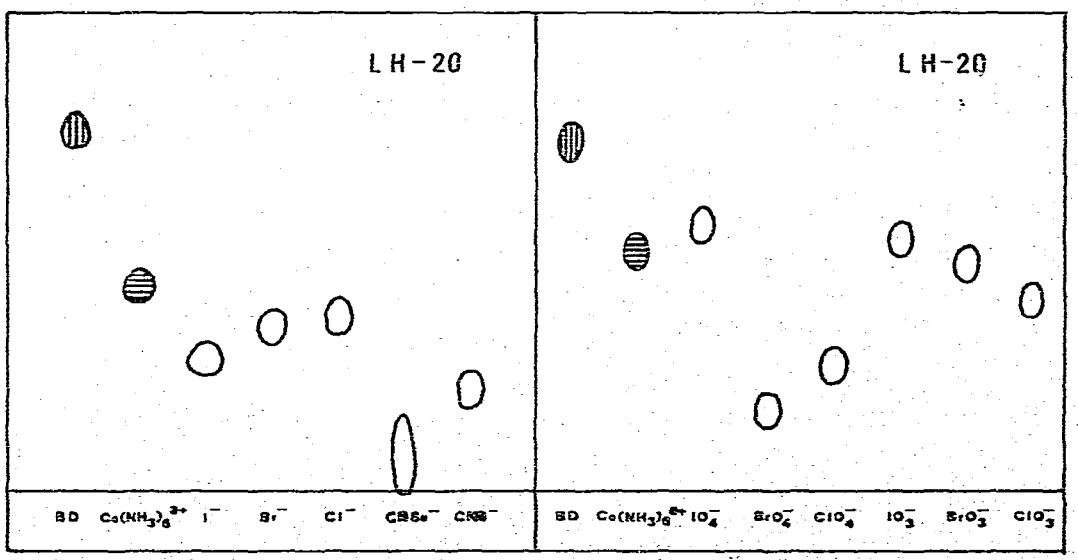

Fig. 2. Thin-layer gel filtration chromatograms on Sephadex LH-20 with 0.01 *N* nitric acid as eluent.

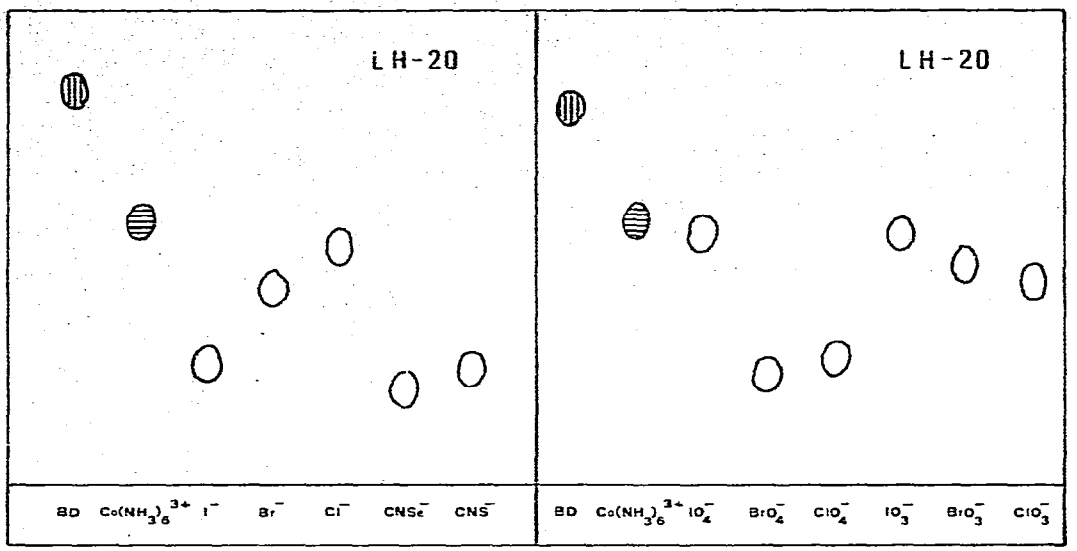

Fig. 3. Thin-layer gel filtration chromatograms on Sephadex LH-20 using the eluents 3 *N* lithium acetate solution, pH 6 (left) and 3 *N* lithium nitrate solution (right).

the differences between Cl^-, Br^- and I^- were more accentuated, as also was the adsorption of $CNSe^-$, CNS^-, ClO_4^- and BrO_4^- (Fig. 3).

In a 1:1 mixture of 0.01 *N* nitric acid and ethanol (Fig. 4), the adsorption effects were weaker but all of the effects mentioned above were still discernible and not completely suppressed by the organic solvent.

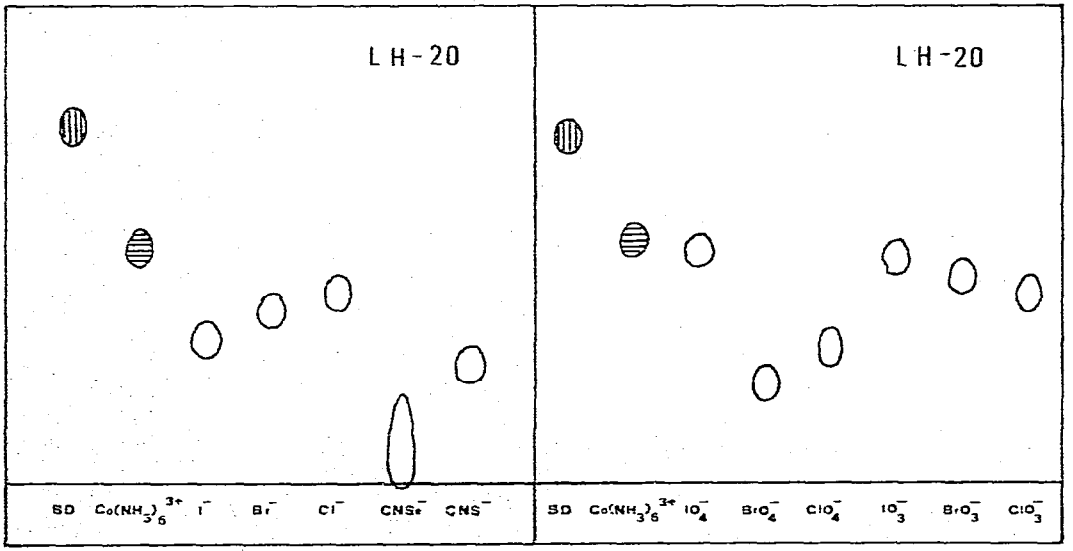

Fig. 4. Thin-layer gel filtration chromatograms on Sephadex LH-20 with ethanol–0.01 *N* nitric acid (1:1) as eluent.

DISCUSSION

The results can be summarized as follows. The halides and pseudohalides adsorb in the order of their atomic weights; chloride and bromide are hardly adsorbed, iodide is measurably adsorbed and thiocyanate and selenocyanate more so. The group comprising chlorate, bromate and iodate is not adsorbed to any appreciable extent, while perchlorate and perbromate are rather strongly adsorbed, the latter always more than the former.

These results agree in general with the adsorption observed from ammonium sulphate solutions on cellulose paper[4] (except that in the work cited there are no data for perchlorate and perbromate) (see Table I). If these anions are examined on cellulose paper in the same solvents as we used for gel filtration, different results are obtained, as shown in Table I. In 0.01 *N* nitric acid, none of the anions adsorb to any extent. In 3 *N* lithium nitrate solution, the salting-out effect does differentiate slightly between the "adsorbed" and the non-adsorbed anions, but by no means as much as on Sephadex or on paper with ammonium sulphate. Finally, in 50% ethanol there is a sequence on cellulose that can be interpreted in terms of a partition system, while on Sephadex LH-20 there is clear-cut desorption.

TABLE I

R_F VALUES OF INORGANIC ANIONS ON WHATMAN 3MM CELLULOSE PAPER

Solvents: 1, 1 *M* ammonium sulphate solution; 2, 0.01 *N* nitric acid; 3, 3 *N* lithium nitrate solution; 4, ethanol–0.01 *N* nitric acid (1:1).

Anion	*Solvent*			
	1[a]	*2*	*3*	*4*
I^-	0.76	0.92	0.87	0.82
Br^-	0.84	1.0	1.0	0.76
Cl^-	0.87	1.0	1.0	0.74
IO_3^-	0.86	1.0	1.0	0.51
BrO_3^-	0.89	1.0	1.0	0.76
ClO_3^-	0.83	1.0	1.0	0.86
IO_4^-	—[b]	0.93	0.95; 0.12*	0.55
BrO_4^-	—	1.0	0.88	0.88
ClO_4^-	—	0.95	0.94	0.86
$CNSe^-$	—	0.88	0.84	0.88
CNS^-	0.76	0.91	0.85	0.85

[a] Gives two spots.

Although both Sephadex and cellulose have a carbohydrate skeleton, their properties as adsorption media are very different owing to hydrophobic cross-linking on Sephadex and the additional hydrophobic groups on Sephadex LH-20. This increase in hydrophobic nature not only permits a stronger adsorption at lower salting-out concentrations but also a different effect on addition of ethanol, namely that the ethanol–water mixture desorbs without partitioning into a water-rich stationary phase and a water-poor mobile phase.

A comment is necessary on the pair CNS^-–$CNSe^-$. These ions were not separated in any of the experiments on Sephadex yet they could be separated on

cellulose by using ammonium sulphate solution of high concentration as eluent[5]. Clearly, an increase in hydrophobic nature does not give the same effect as a higher salting-out developing solution.

REFERENCES

1 M. Sinibaldi and M. Lederer, *J. Chromatogr.*, 107 (1975) 210.
2 M. Sinibaldi and M. Lederer, *J. Chromatogr.*, 51 (1970) 556.
3 T. J. Beckmann and M. Lederer, *J. Chromatogr.*, 3 (1960) 498.
4 M. Lederer and L. Ossicini, *J. Chromatogr.*, 15 (1964) 514.
5 M. Lederer and R. Marini-Bettòlo, *J. Chromatogr.*, 35 (1968) 213.