

Salting-out chromatography of water-insoluble alcohols and ketones

Salting-out chromatography^{1,2}, a method by which water-soluble non-electrolytes have been separated by elution through ion-exchange resins with aqueous salt solutions as eluents, has been applied to mixtures of low molecular weight alcohols³, amines⁴, ethers⁵, acids^{5,6}, aldehydes⁷, and ketones⁷. This note describes the behavior of various hydrophobic alcohols and ketones when eluted through ion-exchange resins with aqueous solutions containing both a salt and an organic solvent in order to study the salting-out chromatography of water-insoluble solutes.

The experimental procedures and conditions which proved most successful in earlier studies of salting-out chromatography were used throughout this work³. Each of the solutes studied was eluted through the resin several times, each time with an aqueous solution of an organic solvent (fixed concentration) containing a different concentration of an inorganic salt. Small fractions of effluent were analyzed for alcohol content by mixing the fraction with 5 ml of 0.1 *N* sodium dichromate in concentrated sulfuric acid, diluting with 25 ml of water and measuring the absorbance of the resulting Cr (III)⁸. The concentration of ketone in the effluent was determined by a modification of the differential pH method of ROE AND MITCHELL^{7,9}. The results of these elutions were interpreted by calculating the *C* value (distribution ratio)² for each solute in each elution from equations based on the plate theory^{10,11}, and plotting log *C* vs. molarity of salt in the eluent for each series of elutions with a given solute.

Hexanone-2, heptanone-2, and octanone-2 were eluted through columns of Dowex 50-X8 cation-exchange resin, 200-400 mesh, with 8 *M* methanol containing varying concentrations of magnesium sulfate, lithium chloride, and ammonium nitrate. The results of these elutions are shown in Table I. Plots of log *C* vs. molarity of salt were straight lines, as was found in the salting-out chromatography of low molecular weight ketones⁷, only in the case of magnesium sulfate. With lithium chloride and ammonium nitrate, the lines were wavy; salting out of the ketones from the liquid outside the resin phase into the resin (increase in *C*) accompanied an increase in molarity of salt only in the former case.

To test the effect of the organic portion of the eluent, 4 *M* propanol-2 was substituted for 8 *M* methanol with the salts lithium chloride and ammonium nitrate; Table II contains the resultant distribution ratios. As with these two salts above,

TABLE I
VALUES OF DISTRIBUTION RATIO, *C*, ON DOWEX 50-X8

Molarity of salt in 8 <i>M</i> methanol	<i>MgSO</i> ₄			<i>LiCl</i>				<i>NH</i> ₄ <i>NO</i> ₃				
	0	1	1.5	0	1	2	4	0	1	2	4	6
Hexanone-2	1.04	2.53	4.26	1.27	1.73	2.78	3.48	1.40	1.27	1.35	1.45	1.67
Heptanone-2	1.57	3.49	5.11	1.65	2.09	3.80	5.53	1.84	1.85	1.83	1.71	2.27
Octanone-2	2.13	4.92	7.71	2.45	3.06	5.78	*	2.75	2.76	2.44	2.78	2.75

* Severe tailing.

TABLE II
VALUES OF DISTRIBUTION RATIO, C , ON DOWEX 50-X8

Molarity of salt in 4 M propanol-2	$LiCl$				NH_4NO_3			
	0	1	2	4	0	1	2	4
Hexanone-2	0.94	1.18	1.29	1.28	1.05	1.16	0.85	0.90
Heptanone-2	0.94	1.15	1.19	1.05	1.05	0.97	0.85	0.98
Octanone-2	1.22	0.93	1.04	0.93	1.05	0.89	0.89	0.74

plots of $\log C$ vs. molarity of salt are not linear. There is evidence of a salting-in effect (decrease in C) on the ketones at higher concentrations of salt in each case.

To test the effect of the resin phase, Dowex 1-X8 anion-exchange resin, 200-400 mesh, was substituted for Dowex 50-X8 with the lithium chloride-8 M methanol eluents (Table III). The results of these elutions are somewhat similar to those with the same eluent on the cation-exchange resin: C values steadily increase for a given ketone as the molarity of salt increases, and plots of $\log C$ vs. M are again not linear; the plots tend to diverge at the higher salt concentrations for this resin, however. As is expected from the fact that Dowex 1 has more carbon atoms per functional group than Dowex 50, Dowex 1 is a better absorbent for a given ketone (higher C value) in a given eluent than Dowex 50.

TABLE III
VALUES OF DISTRIBUTION RATIO, C , ON DOWEX 1-X8

Molarity of salt in 8 M methanol	$LiCl$			
	0	1	2	4
Hexanone-2	2.68	3.77	4.68	5.62
Heptanone-2	5.32	8.44	9.63	13.0
Octanone-2	12.03	18.24	*	*

* Severe tailing.

n -Hexyl, n -heptyl, and n -octyl alcohols were studied on an acetate-form Dowex 1 resin with various concentrations of ammonium acetate and sodium acetate in 7 M acetic acid; the results are listed in Table IV. The acetate system was chosen in this case because the dichromate-oxidation method is the only convenient means for determining small amounts of alcohols in effluent fractions, and acetic acid is one of

TABLE IV
VALUES OF DISTRIBUTION RATIO, C , ON DOWEX 1-X8

Molarity of salt in 7 M acetic acid	NH_4 acetate					Na acetate		
	0	1	3	5	7	0	1.5	3.0
n -Hexyl alcohol	0.73	1.07	1.35	2.32	2.86	0.73	1.10	2.06
n -Heptyl alcohol	0.76	1.23	1.67	2.91	3.80	0.76	1.55	2.73
n -Octyl alcohol	0.94	1.52	2.07	3.88	5.63	0.94	1.88	4.09

the few organic compounds not oxidized by dichromate. In each case, increasing M increases the distribution ratio of the alcohols (salts them out of the aqueous phase). Plots of $\log C$ vs. M for ammonium acetate resemble those for lithium chloride in 8 M methanol in that they are non linear. The plots for sodium acetate resemble those for $MgSO_4$ in 8 M methanol in that they are linear but not divergent at higher values of M .

Based on the results cited above, the following comments on the salting-out chromatography of water-insoluble alcohols and ketones are in order:

1. When comparing the ability of salts to salt out small amounts of solute from the eluent phase to the resin phase, the salt which is least soluble in the eluent is most effective. It was found that the order of solubility of the salts in the systems studied was $NH_4NO_3 > LiCl > MgSO_4$, and NH_4 acetate $>$ Na acetate. Tables I and IV show that magnesium sulfate and sodium acetate were the most powerful salting-out agents used as shown by their effect on the C values of a given alcohol. Also, only these two salts gave plots of $\log C$ vs. M which were linear.

2. Changing the organic constituent of the eluent from methanol to propanol-2 caused a given salt to have less salting-out ability. In some cases, a decrease in C or salting-in effect was noted. This indicates that the salt is causing the concentration of either the solute and/or the propanol-2 to be decreased in the resin phase and increased in the eluent phase.

3. In the cases in which straight lines were obtained for plots of $\log C$ vs. M (magnesium sulfate and sodium acetate), these lines do not diverge sufficiently at higher values of M to allow the method to be used to separate the solutes studied. Although non-linear plots were obtained for lithium chloride with Dowex 1, the curves diverge so that a separation would be possible with 4 M lithium chloride except for the severe spreading of the elution graphs at this high salt concentration.

It is obvious that the process of salting-out chromatography of water-insoluble non-electrolytes in partially non-aqueous solvents is much more complicated and empirical than the salting-out chromatography of water-soluble non-electrolytes in water. This is due to the fact that in the former case one of the constituents of the eluent is free to interact with the resin phase as well as with the solute. Solvent-solute, solvent-resin, or solute-resin interactions are of greater magnitude than the salt-solute interaction; *i.e.*, the solubilizing effect of the organic constituent of the eluent is more powerful than the salting-out effect of the salt. This is indicated by the following observations which differ from the earlier results of salting-out chromatography. Plots of $\log C$ vs. molarity of salt are not straight lines for all salts; when straight lines do occur, they do not diverge, indicating non-selective salting out. In some cases, salting-in was noted. Also, the curves obtained do not retain their identity when the nature of the resin phase is changed. BREYER⁷ found C_0 values (C values obtained with water as eluent) to be lower with ammonium-form resin than with magnesium-form resin for a given ketone. C values obtained in the absence of salt with 8 M methanol for a given ketone in this work show the opposite result, the value on the ammonium-form resin being higher than the value on the magnesium-form resin (Table I).

The present work indicates that solubilization chromatography^{12,13}, a method in which varying concentrations of organic solvents are used as eluents, is superior to salting-out chromatography for the separation of water-insoluble alcohols and ketones in columns of ion-exchange resin.

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BOOK REVIEWS

Comprehensive Analytical Chemistry, Volume 1B, *Classical Analysis*, edited by CECIL L. WILSON AND DAVID W. WILSON, Elsevier Publishing Company, Amsterdam, 1960, xxii + 878 pages, price £ 7.15.0; Dfl. 82.00.

The first half of this book (398 pages) deals with inorganic titrimetric analysis. The theory and principles are dealt with by E. BISHOP from a very mathematical point of view. It is adequate in most of its aspects but ignores consistently the more recent results: for example it is inadmissible today to consider the solubility product of

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