

The Elution of Alkaloids from SE-Sephadex

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Experiments show some properties of the new cation exchange gel, SE-Sephadex. Alkaloidal and similar ions, for example, are more readily eluted from the gel than from ordinary cation exchange resins. This may be used in analytical separations.

Organic ions of medium or high molecular weight are often difficult to elute, once they have been taken up by a cation exchange resin with a normal network. It has been shown (Refs.^{1,2}) that the elution is easier if the degree of crosslinking is low and if the eluting acid contains 70 % of an organic solvent, *e.g.* ethanol, methanol, or acetone instead of water alone. However, even then the volume of the elutriant is large compared with the volume of the resin bed and still larger than the volume calculated necessary from stoichiometry. The resin holds the great ion not only by ionic forces but also by non specific adsorption.

Now, a new kind of ion exchanger shows a different mode of action. It is SE-Sephadex, a strong ion exchanger which is a sulphoethyl ether derivative of Sephadex recently introduced by Pharmacia (Ref.³). Three examples may serve as illustrations of the properties of different ion exchange columns.

1. If you let 10 mg of an alkaloidal ion with the molecular weight 300 pass a 4×40 mm column of an ordinary uncharged Sephadex gel it is only slightly retarded. The cation appears in the effluent after 0.2 ml, *i.e.* immediately after the void volume, and it has left the bed entirely after 0.5 ml.

2. If you substitute the Sephadex for a sulphoethyl dextran gel (SE-Sephadex) with the same degree of crosslinking the alkaloidal ion is retained quantitatively but it can be eluted with 5–15 ml of 0.1 N hydrochloric acid, aqueous or, in some cases in 70 % alcohol. The minimum theoretical value is 3 ml of the acid.

3. If you make the experiment with ion exchange *resins* the elution proceeds more slowly. Dowex 50×1 , *e.g.*, requires 10–25 ml of 1 N hydrochloric acid in 70 % alcohol¹, and from the types of Dowex, *e.g.* 50×16 , 50×12 , or 50×8 , which have a denser network, the elution is still slower or even incomplete.

Most alkaloids can also be eluted with 0.1 N sodium chloride solution from the SE-Sephadex column which is seldom possible for the resin exchangers.

Kinetic experiments have shown that alkaloidal cations are released from the SE-Sephadex quite rapidly at room temperature even by very weak hydrochloric acid or sodium chloride solution. With decreasing excess the elution slows down and the equilibrium yield decreases from 100 %, when the excess of the elutriant is larger than 20–30 times, down to a few per cent when the elutriant is equivalent to 40 % of the alkaloid.

EXPERIMENTAL

Material. Sulpho-ethyl dextran gel (SE-Sephadex C-25, 865/1 B op. 104) was allowed to swell in 0.1 N hydrochloric acid for 30 min and was freed from fine particles by decantation. The gel was washed free from chloride and used in columns 4×40 mm. The capacity of the air dried material was 2.0 mequiv./g. Many of the experiments were performed with DMABF, 9-(4-dimethylamino-benzylidene)-fluorene ethyl tosylate, which is a salt of a quaternary base with a fairly great molecule (mol.wt. of the cation 326.44). The capacity of the column for DMABF was 140 mg which corresponds to 0.28 mequiv.

Elution. DMABF is eluted very slowly with aqueous solutions. 5 ml of 0.1 N or 0.2 N hydrochloric acid released only 0.4 % from the saturated column, and five more volumes yielded the same small percentage. However, all DMABF was eluted in a single eluate of 5 ml of 0.1 N hydrochloric acid in 70 % ethanol. Thus, here the nature of the solvent is decisive. Figs. 1 and 2 show how DMABF is eluted by different electrolytes dissolved in water or aqueous ethanol.

In Figs. 3, 4 and 5 equivalent amounts of two compounds have "competed" for the hydrogen sites of three separate SE-Sephadex C-25 columns, which could take only half the applied quantity of ions. The three pairs of substances behaved quite differently

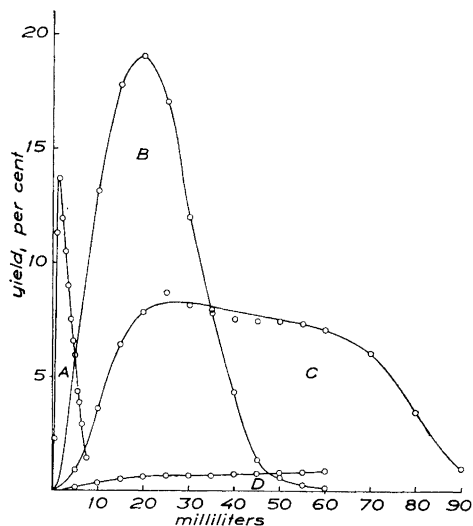


Fig. 1. 15 mg of DMABF on a column of SE-Sephadex, 4×40 mm, is eluted with hydrochloric acid: A 0.1 M in 70 % alcohol, B 0.01 M in 70 % alcohol, C 0.01 M in 50 % alcohol, D 0.01 M in 30 % alcohol.

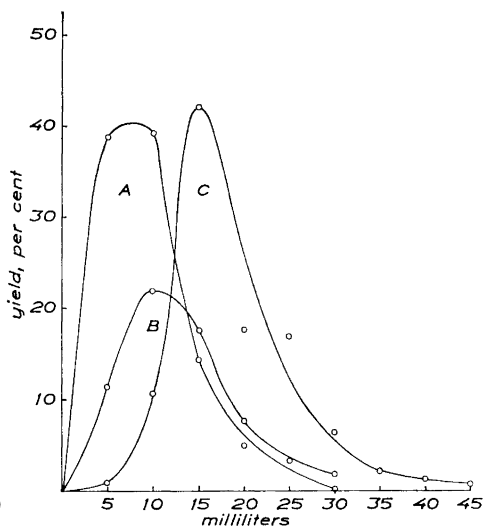


Fig. 2. 15 mg of DMABF on a column of SE-Sephadex, 4×40 mm, is eluted with 0.01 M solutions of a salt in 70 % alcohol: A. AlCl_3 , B. BaBr_2 , C. NaBr .

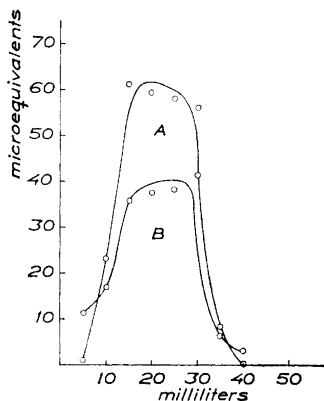


Fig. 3. 300 μ equiv. of DMABF and of sodium bromide applied to a column of SE-Sephadex, 4×40 mm. Washing with 70 % alcohol: A. DMABF in the effluent, B. Sodium bromide in the effluent.

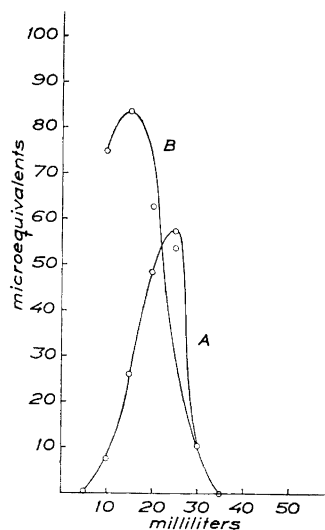


Fig. 4. 300 μ equiv. of strychnine nitrate and of sodium chloride applied to a column of SE-Sephadex, 4×40 mm. Washing with water. A. Strychnine nitrate, B. Sodium chloride.

In Fig. 3 the competitors are DMABF and sodium bromide in 70 % ethanol. They appear in the effluent at the same time, but 90 % of the DMABF goes through and only 65 % of the sodium. In Fig. 4 strychnine nitrate and sodium chloride are applied to the column. Both cations appear in the effluent at the same time, but the recovery of the organic ion is only 50 % compared with 90 % of the sodium ions. In Fig. 5 calcium ions lag

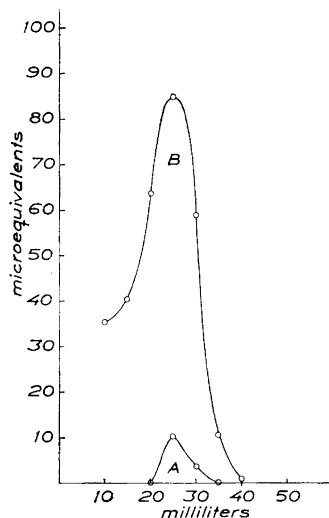


Fig. 5. 265 μ equiv. of calcium chloride and of sodium bromide applied to a column of SE-Sephadex, 4×40 mm. Washing with water. A. Calcium chloride, B. Sodium bromide.

Table 1. Elution from SE-Sephadex and Dowex 50 × 1.

Compound eluted <i>ca.</i> 15 mg	Elutriant	Solvent	From SE-Sephadex ml eluates					% Recovery			From Dowex ml eluates								
			5	10	15	20	25	30	50	SUM	25	50	SUM						
Strychnine	0.1 M HCl	70 % ethanol		95.9		2.8													
	0.1 M NaCl	water	45.1	52.8	0.9	0.6													
	1 M HCl	water													69.3	22.3	91.6		
Ethyl strychnine	1 M HCl	water																	
	1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol		99.6		0.6													
Codeine	0.1 M HCl	70 % ethanol		97.7		1.1													
	0.1 M HCl	70 % ethanol		14.1	0.5	0.2													
	0.1 M NaCl	water	85.4																
Ethyl codeine	0.1 M HCl	70 % ethanol		96.0		2.7													
	1 M HCl	water																	
	1 M HCl	70 % ethanol																	
Papaverine	0.1 M NaCl	70 % ethanol		7.9	0.3	0.0													
	0.1 M HCl	water	91.7	97.4		0.8													
	1 M HCl	70 % ethanol																	
Ethyl papaverine	1 M HCl	water																	
	1 M HCl	50 % ethanol																	
	0.1 M HCl	70 % ethanol		99.0		1.0													
Methyllyoscine	0.1 M NaCl	water	96.6	3.6															
	1 M HCl	50 % ethanol																	
	0.1 M HCl	70 % ethanol		99.2		0.8													
DMABF	0.1 M HCl	70 % ethanol		99.0		0.2													
	0.1 M HCl	70 % ethanol																	
	1 M HCl	70 % ethanol																	
Ethyl quinoline	1 M HCl	water																	
	0.1 M HCl	70 % ethanol		100.0		0.3													
	0.1 M HCl	70 % ethanol		37.7		20.3													
8-Quinolol	0.1 M HCl	70 % ethanol		57.2		18.8													
	0.1 M HCl	70 % ethanol		48.7		16.0													
	0.1 M HCl	70 % ethanol		15.6		13.2													
Quinine	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
Quinidine	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
Cincomine	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
Ethyl quinine	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
	0.1 M HCl	70 % ethanol																	
Ethyl quinone	0.1 M HCl	70 % ethanol																	
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	0.1 M HCl	70 % ethanol																	

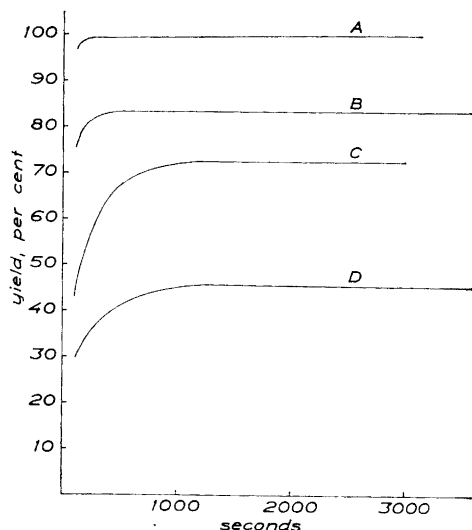


Fig. 6. SE-Sephadex saturated with strychnine shaken with 100 ml of hydrochloric acid.

Curve	Mg of strychnine Sephadex	Molarity of acid	Quotient mequiv. acid/strychnine
A	49.62	0.05	91
B	49.02	0.01	19
C	17.41	0.001	5.3
D	23.39	0.0005	1.9

behind the sodium ions in the effluent. All the sodium is recovered there, but only 5 % of the calcium.

As in ion exchange resins the calcium ion is hard to elute with sodium ion. From 19.4 mg of calcium chloride on a column, only 18 % was eluted by 50 ml of an 0.01 M aqueous solution of sodium bromide, and 25 ml of 0.1 M sodium bromide to follow released only 28 %. (5.7 ml of 0.1 M solution is equivalent to the whole calcium quantity).

Table 1 shows results of elution experiments from SE-Sephadex columns and — for comparison taken from Ref.¹ — from Dowex 50 × 1, an ion exchange resin with a very low degree of cross-linking. From this resin the elution of big ions is easier than from other ion exchange resins. Some conclusions may be drawn from the table.

1. Some ions need alcoholic media for their elution, most do with water.

2. Elution from SE-Sephadex is by far easier than elution from the resin. In most cases 0.1 M aqueous solutions of HCl or NaCl elute rapidly in quantities which are 2–3 times of theory. Elution from Dowex 50 × 1 requires 10–20 times more elutriant, and it is not possible without using 1 M concentrations and — often — alcoholic media.

Kinetic experiments. Sulphoethyl-dextran gel C-25 was saturated with the free bases of codeine, lidocaine, strychnine, and choline by shaking. The excess alkaloids were washed away with appropriate solvents and the resulting product dried *in vacuo*. The content of alkaloids was determined after exhaustive elution with hydrochloric acid and was: of codeine 1.34, of strychnine 1.75, of lidocaine 1.94, and of choline 2.01 mequiv/g ion exchanger. The products did not lose any alkaloid on washing with water, alcohol etc.

15–70 mg of gel saturated with alkaloid was shaken with 100 ml of hydrochloric acid or sodium chloride solutions of different concentrations. At specified intervals 5.00 ml of the liquid was taken out and its alkaloidal content determined. Figs. 6 and 7 show some of the results.

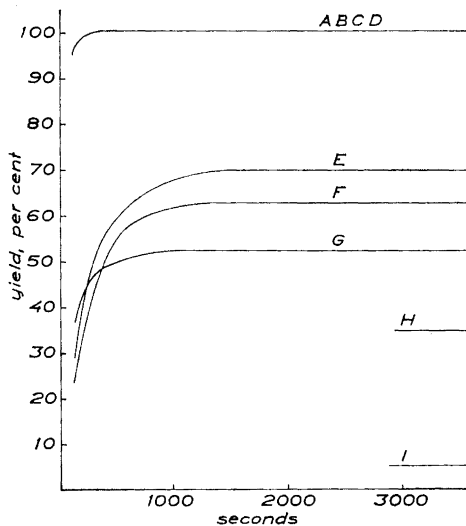


Fig. 7. SE-Sephadex saturated with codeine, lidocaine, strychnine or choline shaken with 100 ml of sodium chloride solution.

Curve	Alkaloid	Mg of alkaloid Sephadex	Molarity of sodium chloride	Quotient mequiv. NaCl/alkaloid
A	Codeine	36.07	0.025	74
B	Lidocaine	69.19	0.025	27
C	Strychnine	18.02	0.025	125
D	Choline	15.35	0.030	24
E	Strychnine	17.40	0.001	5.3
F	Codeine	34.82	0.001	3.0
G	Lidocaine	69.41	0.001	1.1
H	Choline	18.86	0.001	0.6
I	Choline	16.10	0.0005	0.4

REFERENCES

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