Elution of Alkaloids from Cation Exchangers

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Elution of tertiary and quaternary amines of a high molecular weight from a strong cation exchanger can be performed with 1 N hydrochloric acid if a resin with a very low degree of crosslinking is used. Usually alcohol will considerably improve the elution rate and for some compounds is in fact necessary.

Organic cations of high molecular weight, *e.g.* alkaloidal ions, can usually be adsorbed on strong cation exchange resins. However, once the amines are adsorbed they are often difficult to elute. Bregman¹ states that molecular adsorption is negligible for all univalent organic cations for Dowex 50, all active sites of the exchanger thus being available.

For organic bases the situation is more complicated. Some authors have been able to elute tertiary alkaloidal ions with alcoholic ammonia and Büchi and Furrer² also tried aqueous 10 % hydrochloric acid for the elution of quinine but very large eluates were necessary. The difficulties of the elution can be avoided by using an inorganic ion exchanger, *e.g.* Decalso, as suggested by the present authors³. Although this method gives good results it is somewhat inconvenient because of the low capacity.

When working on resins with cations of high molecular weight the importance of the degree of crosslinking has been emphasized by Moore and Stein⁴, Davey⁵, Balenović and Štefanac⁶, Jacobs, Work and Kressman⁷, Büchi and Furrer², van Etten⁸, Kunin and Myers⁹, Sjöström and Nykänen¹⁰ and Hale, Packham and Pepper¹¹. Briefly it may be stated that with a low degree of crosslinking the resin swells to a higher extent and becomes more porous. This causes the resin to take up and give off big ions more easily.

Most experiments performed on ions of moderate size have been restricted to aqueous solutions but with bigger ions it would seem necessary to modify the solvent. Bodamer and Kunin¹² have shown that ion-exchange in non-aqueous media is possible although the rate of the exchange is lower than in water. The main condition is that the resin swells to such an extent that the pores can hold the ions. Gemant¹³ investigated ion exchange in hydrocarbons and Drèze and Reith¹⁴ used 20 % alcohol as a solvent. Rückert and Samuelson¹⁵ studied the influence of alcohol on the shrinkage and showed that more glucose is adsorbed from alcohol than from water. Sjöström and Nykänen¹⁰ used alcoholic media at the elution from anion exchangers.

Table 1. Compounds eluted with 1 N hydrochloric acid in 25 ml eluate.

Per cent recovery for varying strengths of alcohol

Compound	Per cent alcohol	0			30			50			70			90		
	Eluate	1st	2nd	sum	1st	2nd	sum	1st	2nd	sum	1st	2nd	sum	1st	2nd	sum
Papaverine ethyltosylate	0	0	0					100.5 99.9		100.5 99.9				102.7		102.7
Ethyl quinolinium bromide	95.5	4.5	100.0								99.7	0.6	100.3			
Hyoscine methylnitrate	98.6		98.6								101.4	0.0	101.4			
	98.6		98.6													
	101.8		101.8													
	104.3		104.3													
Oxaditon ¹	99.6		99.6								103.1		103.1			
Quinine ethiodide											99.3	0.0	99.3			
	20	20	40								98.7	0.0	98.7			
	85.5	11.3	96.8					95.4	1.9	97.3	100.7	0.0	100.7			
Codeine ethiodide	97.8	2.4	100.2					98.3	2.3	100.6	100.7	1.5	102.2			
											100.0	0.0	100.0			
Berberine sulphate											99.8	0.0	99.8			
Strychnine ethiodide	69.3	22.5	91.8								99.2	0.0	99.2	98.3		98.3
	59.2	30.4	89.6								99.6	0.0	99.6	98.1		98.1
Benzethonium chloride ²	0		0								99.6	1.7	101.3	99.9	0	99.9
DMABF ³											97.2	1.7	98.9	99.3	0.9	100.2
	0	0	0 at 80°		11.5	20.3	31.8	91.0	7.6	98.6	99.2	1.2	100.4	98.5	3.2	101.7
								92.9	5.3	98.2	99.2	0.9	100.1			

¹ N,N,N,N¹-tetraethyl-N¹,N¹-dimethyl-3-oxapentane-1,5-diammonium dimonohydrogen tartrate² the elution was performed with 1 N sulphuric acid in 10 ml eluate³ the elution was performed with 2 N hydrochloric acid

Previously we ¹⁶ have found that both tertiary and quaternary amines can easily be eluted with acids if *i.* the degree of crosslinking of the resin is low, *ii.* the solvent contains ethanol, methanol, or acetone in varying amounts.

It was found that 5–50 μ equiv. of the compounds investigated were quantitatively retained by Dowex 50 X1 in a column 20–30 $\times$ 4 mm and could not be eluted by the usual solvents. In Table 1 the elution of different substances has been made with 1 N hydrochloric acid in water or alcohol of different strengths. Although some compounds may be eluted with aqueous acid this medium is without effect for papaverine ethyltosylate, benzethonium chloride and DMABF [9-(4-dimethylamino-benzylidene)-fluorene ethyltosylate]. If the acid is made alcoholic its elution capacity is increased. 50–70 % of alcohol seems to be sufficient and generally 15–20 ml eluates gave quantitative recoveries. By taking small eluates of 5 ml this phenomenon can be demonstrated and some typical curves are shown in Fig. 1 and 2. The effect of alcohol is not correlated to the molecular weight of the substance. It is interesting to note that DMABF which is soluble in hot water cannot be eluted by aqueous hydrochloric acid even at 80°.

Fig. 1. Elution at different alcohol strengths with 1 N hydrochloric acid.

- A Quinine ethiodide in water
 B Strychnine ethiodide in water
 C DMABF in 50 % alcohol
 D Quinine ethiodide in 70 % alcohol
 E DMABF in 70 % alcohol
 F DMABF in 90 % alcohol
 G Strychnine ethiodide in 70 % alcohol

Fig. 2. Elution of tertiary amines and their corresponding quaternary compounds with 1 N hydrochloric acid in water.

- A Quinine ethiodide
 B Quinine sulphate
 C Strychnine nitrate
 D Strychnine ethiodide

From Fig. 2 may be seen that there is hardly any difference between the tertiary alkaloid and the corresponding quaternary compound in the two cases investigated. The elution curves were obtained with aqueous 1 N hydrochloric acid as alcoholic acids eluted both types of compounds too rapidly to obtain any reliable values.

Table 2. DMABF eluted with perchloric acid of different concentrations in alcohol of varying strengths.

Acid concentration	Per cent recovery for varying concentrations of perchloric acid											
	0.2 N			0.5 N			1 N			2 N		
	1st	2nd	sum	1st	2nd	sum	1st	2nd	sum	1st	2nd	sum
Alcohol % 30	0	0	0	18.7	21.0	39.7	27.7	28.3	56.0	93.6	3.6	97.2
» » 50	82.0	14.8	96.8	96.8	2.0	98.8	98.6	0.9	99.5	92.9	7.1	100.0
» » 70	95.3	1.1	96.4	98.5	0	98.5	98.8	0	98.8	99.9	0	99.9
» » 90	95.0	1.5	96.5	98.8	0	98.8	99.0	0	99.0			

Table 3. The effect of the degree of crosslinking on adsorption and elution.

Ion exchanger	Per cent recovery														
	Ethyl quinolinium bromide Elution with 1 N hydrochloric acid in 70 % alcohol in 25 ml eluate					Benzetonium chloride Elution with 1 N sulphuric acid in 70 % alcohol in 10 ml eluate					DMABF Elution with 1 N hydrochloric acid in 70 % alcohol in 25 ml eluate				
	not adsorbed	Eluate No.				not adsorbed	Eluate No.				not adsorbed	Eluate No.			
		1	2	3	Sum		1	2	3	Sum		1	2	3	Sum
50 X1	0.0	99.7	0.6		100.3	0.3	99.6	1.7		101.6	0.0	99.2	0.9		100.1
						0.0	97.2	1.7		98.9	0.0	99.2	1.2		100.4
50 X2	0.0	97.5	0.7	0.0	98.2	3.0	96.0	0.0		99.0	0.0	87.8	8.4	0.7	96.9
						0.0	99.8	0.0		99.8	0.0	85.7	11.4	1.7	98.8
50 X4	0.0	99.3	0.0	0.0	99.3	8.4	83.3	9.7	0.0	101.4	0.0	48.3	26.4	12.8	87.5
						0.0	95.2	4.9	0.0	100.1	0.0	52.6	24.2	11.5	88.3
50 X8	0.0	92.5	7.0	0.0	99.5	83.8	12.0	4.4	0.0	100.3	0.0	35.9	13.8	9.8	59.5
						59.9	22.4	11.0	5.2	98.5	0.0	45.5	12.5	8.1	66.4
50 X12	0.0	85.1	8.4	2.6	96.1	96.1	3.8	0.0		99.8	0.0	64.1	12.6	5.2	81.9
						88.5	7.7	3.9	0.0	99.9	0.0	74.0	9.3	2.8	86.1
50 X16	0.0	79.0	12.2	6.3	97.5	95.1	5.0	0.0		100.1	0.0	73.8	10.8	2.8	87.3
						86.7	10.0	2.6	0.0	99.3	0.0	66.6	11.4	4.8	82.8

In Table 2 perchloric acid solutions of different concentrations and of various alcoholic strengths have been tested for their elution capacity towards DMABF. If the acid concentration is higher than 0.5 N and the alcohol strength more than 50 % the elution is quantitative with 25 ml. Other experiments have shown that hydrochloric acid is somewhat inferior to perchloric acid and that sulphuric acid has a markedly lower elution power. As alcoholic solutions of perchloric acid are disagreeable we have chosen 1 N hydrochloric acid as the general elutriant.

Substitution of ethanol for methanol or acetone gave for DMABF the same result. Thus, elution with 1 N hydrochloric acid in 70 % methanol gave 100.6 % recovery in 25 ml eluate and in 70 % acetone 100.3 % recovery.

For a few compounds alcoholic potassium hydroxide is a good elutriant. Thus 0.5 N potassium hydroxide in 90 and 50 % alcohol, respectively, gave quantitative recoveries of papaverine ethyltosylate in 25 ml eluate. On the other hand 1.0 and 0.5 N potassium hydroxide in 70 % alcohol gave only about 70 % recoveries in 25 ml eluate. As many compounds are unstable in alkaline medium and as column blanks are liable to become high these media were not further investigated.

Some experiments have been made with alcoholic solutions of sodium bromide, calcium chloride, barium bromide, and aluminium chloride. In 0.5 M solutions in 50–70 % alcohol the salts will elute DMABF but the elution is rather slow and seems generally to be inferior to that of hydrochloric acid.

In order to investigate the effect of crosslinking, cation exchangers from the same manufacturer with varying degrees of crosslinking were used in the same manner as before. Table 3 shows the results obtained on three different compounds. Of these molecules the ethyl quinolinium bromide is comparati-

Fig. 3. Equilibrium between DMABF and Dowex 50 X1 at varying concentrations of alcohol and hydrochloric acid.

- A 0.05 N hydrochloric acid in 30 % alcohol
- B 0.05 N hydrochloric acid in 50 % alcohol
- C 0.05 N hydrochloric acid in 70 % alcohol
- D 0.05 N hydrochloric acid in 90 % alcohol
- E 0.1 N hydrochloric acid in 70 % alcohol
- F 0.1 N hydrochloric acid in 70 % alcohol

ABCD and F: Dowex 50 X1 with DMABF adsorbed was shaken with the corresponding acid.

E: Dowex 50 X1 was shaken with DMABF dissolved in the corresponding acid.

Fig. 4. Equilibrium between DMABF and Dowex 50 X1 with 0.5 M sodium bromide in 70 % alcohol.

vely small, the benzethonium chloride contains a fairly long chain, and the DMABF is rather large. The long-chained benzethonium ion could not be completely adsorbed on ion exchangers with more than 4 % crosslinking. On the ion exchangers X12 and X16 the adsorption from water was very small. Further, there is a tendency towards a slower elution of the amount adsorbed on the more crosslinked resins. For all crosslinkings tried both the small ethyl quinolinium ion and the large DMABF ion are completely adsorbed. The elution rate is, however, greatly decreased if the crosslinking is increased. Further, the large DMABF ion is more strongly retained than the smaller ethyl quinolinium ion. It is interesting to note that the elution rate of DMABF has a pronounced minimum at X8 where the rate is only about 40 % of that of X1 and 60 % of that of X12 and X16.

In Fig. 3 the influence of the concentrations of alcohol and acid is clearly demonstrated from experiments on the equilibrium between DMABF and Dowex 50 X1. The equilibrium is rapidly attained with alcoholic hydrochloric acid. Therefore, the column experiments were most likely performed under equilibrium conditions. With 0.1 N hydrochloric acid in 70 % alcohol the equilibrium was reached from both sides, thus showing that it is a true one. With 0.5 M sodium bromide in 70 % alcohol the process is much slower and here it is possible to apply Langmuir's adsorption isotherm. This is shown in Fig. 4, where the desorption is directly proportional to the logarithm of the time.

EXPERIMENTAL

Column experiments

The ion exchangers (200–400 mesh) used were washed with ammonium citrate solution to remove traces of heavy metals and were then alternately treated several times with aqueous and alcoholic solutions of sodium hydroxide and hydrochloric acid. By this treatment the blanks became negligible.

Unless otherwise stated Dowex 50 X1 in the hydrogen form was used. The columns were 20–30 × 4 mm and could adsorb 15 to 20 mg of substance. When working with resins with a low degree of crosslinking the swelling and shrinking are often cumbersome but with the method used in this work no troubles were encountered. When changing solvents in the columns bubbles are easily formed. They can be avoided by using freshly boiled solvents. The flow rate was kept at about 1 ml/min.

The reagents were of analytical grade and the compounds investigated were of the highest quality available and recrystallized from alcohol and water. Most of the quaternary compounds were synthesized by standard methods and recrystallized from alcohol and water. The equivalent weights and elementary analyses were in good agreement with theory.

For most of the substances a standard curve was drawn at the maximum characteristic for the compound in question in the ultraviolet region and the concentrations of the solutions assayed with its aid. The solvent was usually water or alcohol-water. If acetone was used at the elution it had to be evaporated and the residue dissolved in the appropriate solvent for the final assay. Benzethonium chloride was titrated according to Carkhuff and Boyd¹⁷ and hyoscine methylnitrate was determined either according to Berggren and Nordberg¹⁸ or colorimetrically using bromothymol blue¹⁹.

Procedure 1 to 2 mg of the substance to be investigated — of benzethonium chloride, however, 10 mg and of hyoscine methylnitrate 10–15 mg — dissolved in 10 ml of water or alcohol-water was allowed to flow through the column which was then washed three times with 5 ml of the solvent. The liquids were collected and assayed. The substances on the column were eluted with different solutions, the eluates diluted to the appropriate volume, and assayed. Blanks were always run but their values were usually negligible.

Equilibrium experiments

50 ml of Dowex 50 X1 was mechanically stirred in 400 ml of 50 % alcohol and from a burette a solution of 207.4 mg DMABF in 100 ml of 50 % alcohol was slowly added during 2 h. The resin was transferred to a column and thoroughly washed with 50 % alcohol and finally dried at 50° over night. The resin so prepared contained 4.9 % of moisture determined by the Karl Fischer method.

The resin was assayed by elution with 1 N perchloric acid in 70 % alcohol. Three determinations gave 53.6, 53.1, and 53.9 mg of DMABF per g of resin.

Prior to all experiments an accurately weighed amount, about 50 mg, of the resin was allowed to swell over night in 10.00 ml of water. The rest of the elutrient, about 90.00 g, was added and the mixture shaken. At known intervals 5.00 ml was withdrawn in such a manner that no resin was removed and assayed spectrophotometrically. No special attempts were made to keep the temperature constant.

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