THE QUANTITATIVE DETERMINATION OF ALKALOIDAL SALTS USING THE STRONGLY BASIC ANION EXCHANGER DOWEX 2 AND ITS APPLICATION IN THE ASSAY OF TABLETS

By Finn Otto Gundersen, Rolf Heiz* and Rolf Klevstrand

From the Institute of Pharmacy, University of Oslo, Blindern, Norway

Received May 8, 1953

In recent years a number of papers have been published on the determination of alkaloidal salts[1,2,3,4,5] and salts of local anæsthetics[6] by the use of anion exchange resins. In an earlier method aluminium oxide was used for the same purpose[7]. Although the latter method has given good results, anion exchange resins have great advantages over aluminium oxide, primarily because they can easily be regenerated.

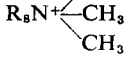
In the use of anion exchangers, the choice of resin is of great importance. Jindra and co-workers[1,2,3] and Levi and Farmilo[5] used the weakly basic Amberlite IR–4B. Baggesgaard Rasmussen, Fuchs and Lundberg[4] preferred to use the strongly basic Amberlite IRA–400, and the same resin was used by Jindra and Rentz[6] for the determination of salts of local anæsthetics.

In some preliminary experiments with the use of Amberlite IR–4B in the carbonate form, as prescribed by Jindra[1,2,3], we got unsatisfactory results in the assay of some alkaloidal salts. We therefore changed to Amberlite IRA–400, and the results were quite satisfactory, but this resin was not found to be suitable for this kind of analyses, as its regeneration was extremely time-consuming. This is a great inconvenience and limits its use in analytical practice.

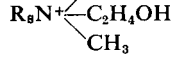
Samuelson and Schramm[8] analysed some inorganic salts using strongly basic anion exchangers. For the reason mentioned above, they found Amberlite IRA–400 to be unsuitable for this special purpose. Dowex 2, on the other hand, was found to be suitable. When we later changed to this resin, we arrived at the same conclusion.

The hydroxyl ion possesses a higher affinity than other anions for a weakly basic anion exchanger[9]. For the strongly basic Amberlite IRA–400, hydroxyl is one of the weakest replacing ions[10]. This is quite analogous to the position of the hydroxonium ion in cation exchange equilibria. Accordingly, the regeneration of a weakly basic exchanger is effected much more easily than the regeneration of a strongly basic exchanger.

Wheaton and Bauman[11] studied the properties of the strongly basic anion exchangers Dowex 1 and Dowex 2, which are polystyrene-divinylbenzene resins, containing quaternary ammonium groups. The formulæ may be written[12]:



Dowex 1



Dowex 2

* Present address: Institute of Chemistry, University of Zürich, Switzerland.

Dowex 1 is somewhat more basic than Dowex 2. This is important, as it determines the relative ease of regeneration of the resins into the hydroxide form. The position of the hydroxyl ion in the exchange potential series for Dowex 2 is more favourable than in the series for Dowex 1. Therefore, Dowex 2 seems to be especially suitable for analyses involving the free base form of the resin. It has the same salt-splitting ability as other strongly basic anion exchangers, but its regeneration is effected more easily.

In this connection a list of anion exchange resins is reprinted from a table of equivalent ion-exchange materials[13]. The resins in the same horizontal line are equivalent and generally behave in a similar manner.

TABLE I

EQUIVALENT ANION EXCHANGERS

Manufacturer ..	The Permutit Co., Ltd., London	Rohm and Haas, U.S.A.	Chemical Process Co., U.S.A.	Dow Chemical Co., U.S.A.
Weakly basic exchangers	De-Acidite E	Amberlite IR-4B Amberlite IR-45	Duolite A-2	Dowex 3 (Nalcite WBR)
Strongly basic exchangers	De-Acidite F De-Acidite FF	Amberlite IRA-400 Amberlite IRA-410		Dowex 1 Dowex 2 (Nalcite SAR)
Porous anion exchangers	Decolorite		Duolite S-30	

As will be seen, Amberlite IRA-410 is equivalent to Dowex 2, and therefore is assumed to possess the same favourable relationship between hydroxide and chloride. We have, however, been unable to find any reports on this point.

This paper describes the use of Dowex 2 for the determination of a number of salts of alkaloids and other organic bases and for the assay of some tablets.

EXPERIMENTAL

The apparatus used (Fig. 1) has been described by Samuelson[12]. The tube containing the resin bed is equipped with a funnel and an outlet capillary tube with a stopcock. The opening of the outlet tube is somewhat above the upper level of the resin bed, so that the ion exchanger is always covered with liquid. It is very important that air should never enter the resin bed, since this would cause channelling, which would decrease the efficiency considerably. The diameter of the resin bed is 10 mm. and the height 130 to 140 mm. It is kept in place by two small plugs of glass wool.

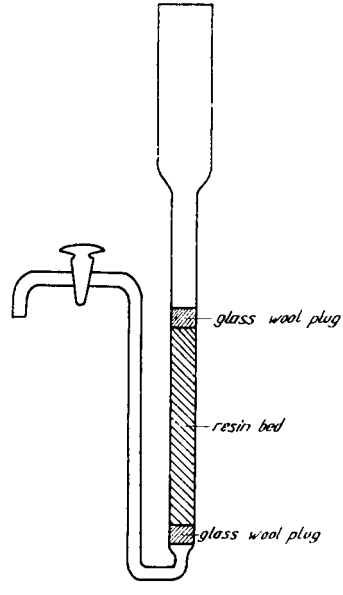


FIG. 1. Apparatus used.

This apparatus possesses the same advantage as that of Levi and Farmilo[5], and is more practical in use than those described by Jindra[1] and Baggesgaard Rasmussen, Fuchs and Lundberg[4].

The resin was used in the commercially available particle size. It is swelled in water and poured into the apparatus. The resin is supplied in chloride form and must be converted to the hydroxide form before use. This is done with about 250 ml. of N sodium hydroxide (until the chloride reaction is negative). The column is then washed with carbon dioxide-free distilled water until the washings are colourless on the addition of phenolphthalein. The water in the column is displaced by ethanol (70 per cent.), and the column is ready for use.

Standard procedure for analysis of alkaloidal salts. The salt (about 0·5 milli-equivalent) is dissolved in 10 ml. of ethanol (70 per cent.) in a small beaker. The solution is introduced into the column, and washing is performed with successive 5-ml. quantities of the same solvent, until a total volume of 50 ml. is used (in some cases more). Flow rate: about 3 ml./minute. The effluent is collected in an Erlenmeyer flask. The opening of the outlet tube is rinsed with a few ml. of solvent, and the contents of the Erlenmeyer flask are titrated with 0·1N hydrochloric acid standardised by titration of borax in ethanol (70 per cent.), using bromophenol blue as indicator.

50 ml. of ethanol (70 per cent.) is percolated through the column and titrated with 0·1N hydrochloric acid (bromophenol blue). This blank is subtracted from the value found by titration of the alkaloidal base. The completeness of the washing may be controlled by determination of the blank value immediately after the analysis. If this value is equal to the standard value for the volume used (0·04 ml. for 50 ml. of ethanol (70 per cent.)), the washing has been complete.

As the column has a total capacity of about 11 milli-equivalents (about 10 ml., capacity 1·1 milli-equivalents per ml.), it can be used several times without regeneration.

The results of a number of analyses of some salts of organic bases are summarised in Table II.

Special precautions are necessary in the analysis of amphetamine sulphate and atropine sulphate. The quantitative elution of the amphetamine base required a greater volume of solvent and a low flow rate. The admixture of 0·1 per cent. of tween 20 (non-ionic surface active agent) to the washing liquid promoted the elution. A smaller resin particle size would also have been advantageous.

For the elution of the atropine base, ethanol (96 per cent.) must be used.

On account of the strong yellow colour of the solution of the mepacrine base, a potentiometric titration was necessary. Christophers[14] has evaluated its dissociation constants. They are recorded as $pK_1 = 3{\cdot}88$, $pK_2 = 6{\cdot}47$, and a possible third value above pK 11·0. The titration curve was therefore expected to have two equivalence points. It had, however, only one sharp potential drop (in ethanol (70 per cent.)). The volume of acid used corresponded to the neutralisation of two basic nitrogen atoms.

TABLE II

DETERMINATION OF SALTS OF ALKALOIDS AND OTHER ORGANIC BASES USING DOWEX 2

About 0·5 milli-equivalent of alkaloidal salt

The base is eluted with 50 ml. of ethanol (70 per cent.) unless otherwise specified

Flow rate: about 3 ml./minute

Salt	Recovered per cent.	Remarks
Amphetamine sulphate	98·3	100 ml. of ethanol (70 per cent.)
	98·7	225 ml. " "
	99·9 99·7	Dissolved in 3 ml. of water, rinsed with 3 ml. of water and ethanol (70 per cent.) to a total volume of 100 ml. Flow rate: about 0·5 ml./minute
	99·3 98·7	50 ml. of ethanol (70 per cent.) with 0·1 per cent. of tween 20
Atropine sulphate	83·4	
	99·0 99·8	Dissolved in ethanol (70 per cent.) (10 ml.), rinsed with ethanol (96 per cent.) to a total volume of 50 ml.
	98·6 98·7	
Cocaine hydrochloride	99·1 99·6 98·8	
Codeine phosphate	100·0 99·8 100·3	
Diphenhydramine hydrochloride	99·3 99·9 100·3	
	99·8 99·7	
Ephedrine hydrochloride	99·8 99·1 100·8	
	99·2 99·1 99·3	
Hyoscine hydrobromide	101·0 100·3 100·3	
Lobeline hydrochloride	100·4 101·4	
Mepacrine hydrochloride	99·2 99·6 99·9	Potentiometric titration
Methadone hydrochloride	99·4 100·0 98·7	
	98·7	
Pethidine hydrochloride	99·8 100·0 99·8	
Procaine hydrochloride	100·5 100·6 100·6	
	100·5 100·3	
Strychnine nitrate	100·2 100·2 101·8	
	100·1	

Salts of morphine and other phenolic bases cannot be analysed with this resin, as they are retained as anions. This may, however, be utilised for the separation of morphine from other alkaloids. In a single experiment we found that ephedrine hydrochloride (0·2515 g.) could be quantitatively determined in the presence of morphine hydrochloride (0·1505 g.) by percolation through the column.

Owing to its strongly basic character, Dowex 2 cannot be used for the analysis of some bases containing easily hydrolysable groups. We found that neostigmine (synstigmine), pilocarpine and yohimbine were in part retained by the resin. This must be due to the formation of acidic groups as a result of the hydrolysis, which is followed by sorption as anions to the resin.

Like Baggesgaard Rasmussen, Fuchs and Lundberg[4] we found that carbacholine chloride could not be analysed by this method.

Standard procedure for assay of tablets. An amount of pulverised tablets containing about 0·5 milli-equivalent of alkaloidal salt is suspended with 5 to 10 ml. of ethanol (70 per cent.) in a small beaker. The suspension is filtered into the funnel, and beaker, filter and funnel are rinsed with 5-ml. quantities of ethanol (70 per cent.). A total volume of 50 ml. of effluent (in some cases more) is collected and titrated with 0·1N hydrochloric acid (bromophenol blue). The blank is determined.

Four tablet species of the Danish Pharmacopœia have been assayed by the official methods and by ion exchange. The tablet powders were made with an accurately known content of alkaloidal salt. The proportions of starch, lactose, talc and gelatin (powder) were much the same as in the official tablets. The results are given in Table III.

TABLE III

ASSAY OF TABLETS USING DOWEX 2 AND BY OFFICIAL METHODS (PH.D.)

Tablet powder corresponding to about 0·5 milli-equivalent of alkaloidal salt

The base is eluted with 50 ml. of ethanol (70 per cent.) unless otherwise specified

Flow rate: about 3 ml./minute

	Content of salt given per cent.	Found		Remarks
		Ion exchange per cent.	Official method per cent.	
Tablettæ amphetamini Ph.D.	6·39	6·32 6·35 6·36 6·35 6·31 Mean: 6·34	6·22 6·38 6·33 6·53 Mean: 6·37	By the ion exchange method 100 ml. of ethanol (70 per cent.) is used
Tablettæ codeini Ph.D.	30·3	29·7 30·0 29·8 30·3 29·9 30·3 Mean: 30·0	28·2 29·0 27·5 28·3 28·8 29·3 Mean: 28·5	
Tablettæ ephedrini Ph.D.	25·1	25·2 25·1 25·2 25·1 25·4 Mean: 25·2	26·1 26·1 26·0 26·0 26·0 Mean: 26·0	
Tablettæ pethidini Ph.D.	11·11	11·13 11·19 11·09 11·11 11·14 Mean: 11·13	11·02 11·02 11·18 11·12 Mean: 11·09	

DISCUSSION

Our work on ion exchange analysis of pure salts confirms the results found earlier by others[1,2,3,4,5]. Two salts, lobeline hydrochloride and mepacrine hydrochloride (atebrin), seem not to have been analysed in this way previously. This series of papers illustrates the usefulness of the ion exchange method in pharmaceutical analysis.

Some limitations of the method have been mentioned in the experimental part. Strongly basic anion exchangers cannot be used for the analysis of salts of phenolic bases or of bases containing easily hydrolysable groups, with formation of acidic groups by hydrolysis.

It must be assumed that atropine and hyoscine are also hydrolysed to a certain degree. In these cases, however, the moiety containing the basic nitrogen atom has no acidic groups and is consequently not retained by the resin, and the result of the analysis is not affected.

Neither Baggesgaard Rasmussen and co-workers[4] nor ourselves met with any complications in the analysis of cocaine hydrochloride. This is a little surprising, as the product of hydrolysis, ecgonine, contains a carboxylic acid group besides the nitrogen atom, and it should therefore be retained by the resin.

The adsorption of the amphetamine base must be due to the intervention of van der Waals' forces. The irregular behaviour of aromatic compounds

on ion exchange resins, which are predominantly aromatic in character, is well known from the work of others (*e.g.*, Partridge[15]). The elution of ephedrine, however, caused no difficulties, although the constitution of this aromatic base is very similar to that of amphetamine.

The 4 tablet species chosen are assayed in 4 different ways according to the Danish Pharmacopœia. The methods are as follows.

Amphetamine sulphate tablets. The tablet powder is boiled with hydrochloric acid for half an hour. The mixture is cooled and transferred to a separatory funnel. An excess of sodium hydroxide is added and the base shaken out with chloroform 5 times. The chloroform solution is shaken with 0·1N hydrochloric acid and the excess of acid titrated with 0·1N sodium hydroxide.

Codeine phosphate tablets. The tablet powder is boiled with hydrochloric acid, and after having been made alkaline, the mixture is shaken 4 times with chloroform. The chloroform solution is evaporated to dryness. The base is dissolved in ethanol and titrated with 0·1N hydrochloric acid.

Ephedrine hydrochloride tablets. The tablet powder is suspended in water, starch solution and fluorescein indicator are added, and chloride ion is titrated with 0·1N silver nitrate.

Pethidine hydrochloride tablets. To the tablet powder nitric acid and 0·1N silver nitrate are added. The mixture is boiled, water and ferric ammonium sulphate are added, the mixture is filtered, and the excess of silver nitrate in an aliquot is titrated with 0·1N ammonium thiocyanate.

As will be seen from Table III, these tablet powders are accurately assayed by the ion exchange method. The results correspond very well to those found by the official methods.

Summary

1. A number of alkaloidal salts and salts of other organic bases have been assayed by use of the anion exchange resin Dowex 2. This resin is especially suitable for analyses of this kind.

2. As examples of the application of the method for the assay of alkaloid-containing galenicals, 4 tablet species have been assayed. The results agree very well with the values found by official methods. The ion exchange method should be of general value for the assay of tablets containing alkaloidal salts, as other salts as a rule are not present. It is in general more accurate, rapid and convenient than older methods.

References

1. Jindra, *J. Pharm. Pharmacol.*, 1949, **1**, 87.
2. Jindra and Pohorský, *ibid.*, 1950, **2**, 361.
3. Jindra and Pohorský, *ibid.*, 1951, **3**, 344.
4. Baggesgaard Rasmussen, Fuchs and Lundberg, *ibid.*, 1952, **4**, 566.
5. Levi and Farmilo, *Can. J. Chem.*, 1952, **30**, 793.
6. Jindra and Rentz, *J. Pharm. Pharmacol.*, 1952, **4**, 645.
7. Reimers, Gottlieb and Christensen, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 99.
8. Samuelson and Schramm, *Svensk Kem. Tidskr.*, 1951, **63**, 307.
9. Kunin and Myers, *J. Amer. chem. Soc.*, 1947, **69**, 2874.
10. Kunin and McGarvey, *Indust. Engng Chem.*, 1949, **41**, 1265.

11. Wheaton and Bauman, *ibid.*, 1951, **43**, 1088.
12. Samuelson, *Ion Exchangers in Analytical Chemistry*, Stockholm and New York, 1952.
13. Kressman, *Mfg. Chem.*, 1952, **23**, 241.
14. Christophers, *Ann. Trop. Med.*, 1937, **31**, 43; cit. *Chem. Abstr.*, 1937, **31**, 8110.
15. Partridge, *Biochem. J.*, 1949, **44**, 521.