OXIDISED CELLULOSE IN ION EXCHANGE
A PRELIMINARY NOTE

By F. M. Freeman

From the Laboratories of E. R. Squibb & Sons Ltd., Speke, Liverpool, 19

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The product, resulting from the oxidation of a large portion of the primary hydroxyl groups of cellulose to carboxyl, by nitrogen dioxide, is a polyanhydroglucuronic acid referred to as oxidised cellulose. In physical appearance oxidised cellulose differs little from ordinary cellulose. It will, however, dissolve readily in alkaline solutions such as sodium or ammonium hydroxides, and it is also soluble in sodium bicarbonate solutions, certain organic amines and quaternary ammonium bases, but is insoluble in water and common organic solvents. Salts can be formed by controlled neutralization with base or by treatment with solutions of metallic acetates[1,2]. The property of ready formation of metallic salts and the reported adsorption of ACTH from solution[3], indicate that oxidised cellulose should serve as a useful ion exchange medium in analytical separations by functioning as a carboxylic cation exchange medium. This note will describe the application of oxidised cellulose as an ion exchange resin to alkaloidal analysis.

Experimental

Reagents. Oxidised cellulose in powder form with a carboxyl content of from 16 to 22 per cent., atropine sulphate, quinine sulphate, codeine sulphate and strychnine sulphate. 0·1 and N hydrochloric, sulphuric and acetic acids. Glass or cotton wool (washed), ethanol 95 per cent., chloroform B.P. and sodium hydroxide 3 per cent. aqueous solution.

Apparatus. Unicam SP 500 or similar spectrophotometer. Polyethylene or pyrex tubes 12 cm. long, 1 to 1·2 cm. in diameter.

Preparation of columns. Columns 5 cm. in length and 1 cm. in diameter were prepared by slurrying approximately 2 g. of oxidised cellulose with water and transferring to a polyethylene or pyrex tube plugged at one end with glass or cotton wool and fitted with a stopcock. After allowing the columns to settle, no pressure or packing being used, a small plug of cotton or glass wool was pressed on to the top of each column.

Adsorption. An aliquot of the alkaloidal solution of from 2 to 5 ml. was transferred to the top of the cellulose column and gently forced in by air pressure, this was followed by 5 ml. washes of distilled water until the pH of the effluent had returned to initial value or gave a negative sulphate reaction.

Factors Influencing the Adsorption Step

Solvent. Of the four alkaloids investigated all were quantitatively adsorbed from aqueous solutions as well as from solutions in 50 per cent. and 90 per cent. ethanol.

Rate of flow. When using 5 ml. aliquots containing 20 mg. of alkaloid and a flow rate of from 0·5 ml. per minute to 2·0 ml. per minute, there was quantitative adsorption on columns 5 cm. in length and 1·2 cm. in diameter.

Presence of other salts. 5 ml. aliquots of a solution of quinine sulphate containing varying amounts of sodium chloride were transferred to columns 5 cm. in length and 1·2 cm. in diameter and adsorbed in the usual manner. The columns were washed with a total of 50 ml. each of distilled water. The adsorbed alkaloid was then eluted with 0·1N sulphuric acid and after dilution measured spectrophotometrically at 315 mμ.

Table I shows that for a given length of column the adsorption of alkaloid is dependant both on the total salt concentration of the solution and on the alkaloid to salt ratio.

TABLE I

DEGREE OF ADSORPTION FROM SOLUTIONS CONTAINING ALKALOID AND VARYING AMOUNTS OF SODIUM CHLORIDE

Aliquots of 5 ml. containing		Per cent. alkaloid retained on column
mg. of Quinine	mg. Sodium chloride	
20	60	78
	40	96
	20	99
	10	100
10	60	90
	40	100
2·5	100	93·5
	80	97·5
	60	99·0
	40	97·0
	20	98·0
	10	97·0
	5	98·0

Elution

The two possible methods for the recovery of the adsorbed alkaloids were (*a*) dissolution of the cellulose in alkali which resulted in depolymerisation and liberation of the absorbed alkaloid which could then be extracted and (*b*) elution with acids of which hydrochloric or sulphuric acids at the strength of N or 0·1N were found to be effective, little difference in elution volumes between 0·1 and N acids was found. Both methods gave satisfactory results. The second, however, was more economical, easier to manipulate, and was the preferred method. After elution of alkaloids, columns washed free from acid could be used again. As many as 16 successive adsorptions and elutions have been performed on the same column over a period of one month without any apparent decrease in adsorption properties.

Removal of Impurities

If a spectrophotometric method was to be used for the final determination of the alkaloids in the eluate, unused cellulose was washed with a volume of 100 ml. of 0·1N sulphuric acid followed by distilled water until the washings were acid free. This treatment was necessary to remove most of the impurities which absorbed in the region 220 to 280 mμ, and which would otherwise interfere with spectrophotometric measurements in this region. Allowances must, however, sometimes be made for small residual absorption in this region by either using as a blank, the alkaloid free eluent or by the use of a predetermined figure.

General Method for Pure Alkaloidal Solutions.

Columns of oxidised cellulose 5 cm. in length and 1·2 cm. in diameter were prepared as previously described washing well with 0·1N sulphuric acid and water. A 5 ml. aliquot of the aqueous alkaloidal solution was transferred to the top of the cellulose column and gently forced in by air pressure. This was followed by 5 ml. washes with distilled water. The alkaloid was recovered by elution with 0·1N or 1N sulphuric acid, pressure being applied to maintain a flow rate of 1·5 to 2 ml. per minute. A volume of 50 ml. was collected.

RESULTS

Atropine

Method. Two methods were used for the estimation of atropine in the eluent from the columns. (*a*) The eluate was made alkaline with strong ammonia and extracted several times with chloroform. The chloroform was washed with water and evaporated to dryness. The alkaloidal residue was then estimated titrimetrically. (*b*) Colorimetric assay was by the following method[4]. The eluate was diluted to a concentration of 0·05 mg. of atropine per ml. A 2 ml. aliquot was evaporated to dryness and nitrated by the addition of 0·3 ml. of fuming nitric acid which was removed by evaporation on a steam bath or hot plate. The nitrated residue was transferred to a 10 ml. standard flask by means of small amounts of dimethylformamide. 0·3 ml. of tetraethylammonium hydroxide 25 per cent. aqueous solution were then added and the solution made up to volume. After allowing to stand for 5 minutes the colour produced was measured at 540 mμ. The atropine content was calculated by reference to a standard graph. Results are given in Table II.

TABLE II
RECOVERY PER CENT. FROM A 20 MG. SAMPLE OF ATROPINE ELUTED WITH 0·1N AND N HYDROCHLORIC ACID

0·1N	N
97·5	99·0
99·0	96·0
97·5	100·0
97·5	97·5
101·0	100·0
101·0	100·0
98·0	99·0
98·0	99·0
100·0	99·0
100·0	104·0
101·0	101·0
100·0	101·0

Strychnine

Method. Strychnine eluate was estimated by dilution to 250 ml. with 0·1N sulphuric acid and measured spectrophotometrically at 255 mμ using a blank consisting of 10 ml. of alkaloid-free eluent diluted to 50 ml. with 0·1N sulphuric acid to compensate for background absorption. Results are given in Table III.

TABLE III
RECOVERY PER CENT. FROM A 5 MG.-SAMPLE OF STRYCHNINE ELUTED WITH 0·1N SULPHURIC ACID

99·0	101·0	101·0
101·0	100·0	97·0
100·5	101·0	98·0
100·0	98·0	98·0
98·0		

Quinine and Codeine

Method. Quinine in the eluate was estimated spectrophotometrically after dilution with 0·1N sulphuric acid at 278 mμ or 315 mμ. Measurements at 278 mμ required the use of alkaloid-free portions of the eluent suitably diluted, as a blank. At 315 mμ 0·1N sulphuric acid sufficed as a blank. Codeine was estimated in a

similar manner after dilution with 0·1N sulphuric acid by measurements at 278 mμ. Results are given in Table IV.

Discussion

Previous work on the ion exchange analysis of alkaloids using cationic resins such as Amberlites IR-120H or IRC-50 has met with certain difficulties in that whilst alkaloids were usually readily adsorbed from aqueous solution the quantitative recovery was difficult. Decalso, a synthetic zeolite, has been used with some success in this direction, the presence, however, of small amounts of salts interferes with adsorption. Oxidised cellulose resembles the strong cationic resins such as IR-120H in its uptake of alkaloid from solution but, unlike IR-120H, recovery of adsorbed alkaloid is rapid and quantitative, and is probably due to the lack of cross linking in the material, thus enabling the free passage of alkaloid molecules to and from the sites of exchange. Oxidised cellulose appears to be superior to the resin IRC-50, and Decalso in the uptake of alkaloid from salt solution, and should therefore be useful in the analysis of alkaloidal extracts which often contain appreciable amounts of other salts. Work in this direction is proceeding.

TABLE IV

RECOVERY PER CENT. FROM 5 MG. SAMPLES OF QUININE AND CODEINE ELUTED WITH 0·1N SULPHURIC ACID

Quinine	Codeine
101·0	97·0
101·0	97·0
101·0	97·0
100·0	97·5

Summary

1. Oxidised cellulose has been successfully employed as an ion exchange material in the determination of atropine, strychnine, quinine and codeine.

2. These alkaloids were readily adsorbed from solution and recovery was rapid and quantitative.

References

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