SOME ASSAY PROCESSES INVOLVING THE USE OF ION EXCHANGE RESINS

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Received October 2, 1953

In a recent review[1] one of the authors has outlined the possible applications of ion exchange resins in assay processes of pharmaceutical interest. This paper outlines experimental details of several such processes and gives a summary of the results obtained by them. A valuable book[2] dealing with the application of ion exchange resins to analytical chemistry in general, has now become available in this country.

I. Estimation of Quinine in Ethanolic Solution

These experiments have been carried out with a solution of pure quinine in ethanol (absolute) with the object of investigating whether the alkaloid can be quantitatively absorbed on to and displaced from an ion exchange column, so as to provide a reasonable method of assay. A weak cation exchanger in the hydrogen form was used so that the displacement could be carried out with as small a volume of liquid as possible. Saunders and Srivastava[3] have shown that this type of resin absorbs quinine rather slowly and that quantitative displacement of quinine from the resin column can be effected by means of ammonia solution in ethanol.

Owing to the slow absorption of quinine, a fairly long resin column is required to hold about 0·1 g. of quinine, if the solution is put on to the column at a reasonably rapid rate. Most of the quinine is held at the top of the column, and in the subsequent displacement the alkaloid has to be driven through the comparatively fresh resin in the lower half of the column. This involves the use of a large quantity of displacing liquid. In order to speed up the experiment and to reduce this volume, we have used a split column consisting of two short tubes containing resin placed one above the other. The quinine solution is put on to the upper column, which takes up over 95 per cent. of the base, and the effluent flows directly into the lower column. Both columns are then washed by putting ethanol on to the upper column and allowing it to flow through both quantities of resin. This washing can be continued, without loss of quinine, so as to remove any non-basic impurities in the quinine if the original solution is not pure. For displacement, the columns are separated and ammonia solution is passed separately through the two columns, in parallel. The two effluents can be collected together in a suitable vessel for evaporation and the total quinine recovered can be determined either by evaporation to dryness and weighing, or by evaporation to small bulk, diluting to a definite volume with ethanol, and observing the optical rotation of the solution. Throughout these processes, the columns should not be allowed to dry out and about 1 cm. head of liquid above the resin in each tube, should be maintained.

Experimental Details. The resins were contained in glass tubes 25 cm. long and 9 mm. internal diameter, drawn down to 4 mm. diameter at their lower ends and fitted with glass wool plugs and seasoned rubber tubes, screw clips and glass jets with a tip diameter of 1 mm.

To prepare the resin columns, the tubes were half filled with the solvent to be used and a slurry of resin which had previously been well soaked in solvent was washed into the tube until a 10 cm. column of resin was obtained. In this experiment the resin used was a weak cation exchanger. The resin was then thoroughly washed with solvent, maintaining a head of 1 cm. of liquid above the resin to avoid any drying-out of the latter.

10 ml. of an approximately 1 per cent. solution of quinine in ethanol was put on to the upper column and the liquid issuing from this was allowed to flow on to the lower column at a rate of 1 drop per second. The flow from the lower column was adjusted to a similar speed. When most of the solution had passed through the resin, 10 ml. of ethanol was added to the upper tube and allowed to pass through the columns in series at the same flow rate of 1 drop per second; this was repeated twice, and the effluents from the lower column were rejected. If the original quinine solution had been impure the washing could have been continued, without loss of quinine.

The two columns were then separated and placed side by side so that their further effluents could be collected in a single vessel. Each was eluted with 6 quantities, each of 10 ml., of a saturated solution of anhydrous ammonia in ethanol at the same flow rate. On completion of the displacement, the combined effluents were evaporated to dryness for estimation by weighing or to small volume (about 2 to 3 ml.) for polarimetric determination. In the latter case, the residual solution with ethanol washings of the container was made up to 25 ml. with ethanol and the rotation was determined in a 2 dm. polarimeter tube.

An experiment carried out by the authors, in which estimation by weighing was used, gave a 96·5 per cent. recovery from the upper column, the total recovery being 100·4 per cent.

The assay was carried out by a number of analysts, each of whom made a single polarimetric determination without previous experience of the method. Discounting results in which definite mishaps occurred, such as accidental drying-out of the column, the mean of 17 results was found to be a 98·8 per cent. recovery of quinine (standard deviation 2·1 per cent.); owing to the small rotation of the final solution (— 1·30° for 100 per cent. recovery) it was considered that ± 2·5 per cent. represented a reasonable experimental error. 13 of the 17 results had an error within these limits and these had a mean of 99·5 per cent. recovery and a standard deviation of 1·2 per cent. The low values of the means are to be expected since apart from random error in the polarimetric determination, all manipulative errors lead to a loss of quinine. The results indicate that a quantitative uptake and displacement of quinine can be achieved in a reasonably short time, 3½ hours for the polarimetric method and about 5 hours for the estimation by weighing. The latter is more accurate and an experimental error of less than 0·5 per cent. should be obtainable.

Suggested Applications. This type of extraction and displacement could be applied to the determination of a number of alkaloids in mixed solutions and preparations. It can be used for very weak bases, for example, Saunders and Srivastava[4] have shown that the weak cation exchanger, Amberlite 1RC50, will absorb quite large quantities of caffeine from solution.

The method cannot be applied directly to the determination of alkaloids in their salts since it is essential to have an alkaline feed to the weak cation exchanger column if a reasonable capacity is to be achieved. A modification of the method suitable for this analysis is described below.

2. Estimation of Quinine in Quinine Salts

If a solution of quinine sulphate is put directly on to a weak cation exchanger column, some quinine is removed, leaving an acid solution, but since the resin is only a weak carboxylic acid, it is unable to compete with the sulphuric acid in the solution so as to effect quantitative removal of the alkaloid. This difficulty can be overcome by first passing the alkaloidal salt solution through a strong anion exchanger, the effluent consisting of a solution of the alkaloid is then fed on to the two weak cation exchanger columns in series, where quantitative removal of quinine is achieved. Since all 3 columns can be run in series, the time required for the determination is not much greater than that for the previous experiment.

Experimental Details. 10 ml. of an approximately 1 per cent. solution of quinine sulphate in ethanol (some water can be added to the solvent to facilitate solution) was put on to a 10 cm. column of strong anion exchange resin in the hydroxyl form, prepared with ethanol in a glass tube as already described (the resin was first extracted and washed with warm ethanol). The effluent from this column (flow rate 1 drop per second) passed on to the upper of the 2 weak cation exchanger columns. The 3 columns in series were then washed with 10 successive quantities, each of 10 ml., of ethanol (1 drop per second) in order to ensure complete removal of quinine from the anion exchanger tube; the effluent from the bottom column was rejected. The 2 weak cation exchanger columns were then separated and each eluted with 60 ml. of ethanol saturated with ammonia, as in the previous experiment. The combined effluents were concentrated, made up to 25 ml. with ethanol and their quinine content determined polarimetrically.

The result of an assay of a sample of quinine sulphate by this method gave a percentage of quinine in the original sample of 77·0 per cent., a somewhat low figure which was confirmed by potentiometric titration, which gave a mean quinine content of 77·1 per cent. 2 repeat estimations of quinine in quinine hydrochloride gave a mean result of 81·9 ($\pm$ 0·2) per cent., the value given by potentiometric titration of this salt was 82·5 per cent.

Suggested Applications. This assay could be applied to most solutions containing alkaloidal salts, including salts of very weak bases, when the final estimation would be made by weighing. It has an advantage over

the Jindra method (see section 3 below) in that mineral salts in the alkaloidal salt will not interfere.

3. Direct Estimation of Ephedrine Hydrochloride

A direct determination of alkaloidal salts by passing the salt solution through an anion exchange resin column and titrating the liberated alkaloid with acid, has been developed by Jindra and Pohorsky[6] and has been applied to a number of salts of organic bases[7,8]. In order to apply this to salts of strong bases such as ephedrine, a strong anion exchanger must be used, with the consequent difficulty that any other salts present, e.g., sodium chloride, will be converted to alkalis which will also titrate. This problem can be solved either by ashing the alkaloidal salt and finding the titre of the extracted ash after passage through the column or by carrying out the 3-column process described above for quinine salt in which the liberated alkaloid is taken up on and then displaced from a weak cation exchanger column.

Experimental Details. A glass tube of the type previously used was filled wet with a 10 cm. column of strong anion exchanger in water. The column was washed with demineralised water and the colour given by the final washings with universal indicator, was noted. 10 ml. of an approximately 1 per cent. solution of ephedrine hydrochloride, prepared from repeatedly recrystallised material, was put on to the column and allowed to flow through at a rate of 1 drop per second. The column was then washed with demineralised water until the effluent *p*H, determined by spotting on a white tile with dilute universal indicator solution, was the same as that obtained in the initial washing of the column with water (80 to 100 ml. of washing water required). The total effluent with washings, was titrated with 0·1N sulphuric acid contained in a microburette, using bromophenol blue as indicator. The end-point was taken as the disappearance of the blue colour of the indicator, i.e., the change from pale blue to pale green. The acid was standardised with sodium carbonate solution to this end-point.

An experiment by the authors gave a titre corresponding to a 99·9 per cent. purity of the solid ephedrine hydrochloride. The results of a group of 27 analysts, each carrying out a single determination, gave a mean of 100·7 per cent. with a standard deviation of 2·2 per cent.; 16 of these results fell within the expected experimental error of $\pm$ 1·5 per cent. and these had a mean of 100·3 per cent. and a standard deviation of 0·8 per cent.

The principal error in the determination arises from the end-point of the titration. With experience, this can be reduced to $\pm$ 0·5 per cent. and by increasing the scale of the experiment, this could be further reduced.

4. Estimation of Glycine in the Presence of Gelatin

When a gelatin sol is passed through a column consisting of mixed, activated, strong cation and anion exchangers, small ions are removed and the specific electrical resistance of the sol is increased by a factor of about 100 (Janus, Kenchington and Ward[5]), gelatin is only very slightly

absorbed by the resins. We have used this effect to develop a method of estimating an amino-acid in the presence of gelatin. This is an assay which could be useful for determining the extent of hydrolysis of gelatin and protein sols. The determination is rapid and can be completed in half an hour. In order to examine its quantitative nature, mixtures of gelatin sol (freed from small ions) and glycine, were used. Neither of these materials can be titrated directly with alkali, using an indicator, but in the presence of neutral formaldehyde (prepared by running 40 per cent. formaldehyde solution through a strong anion exchanger resin), titration is possible, using phenolphthalein. The amount of formaldehyde added must be accurately controlled.

Experimental Details. A glass tube similar to those already described, was half-filled with demineralised water and a slurry of mixed strong anion and cation exchangers in water was put into the tube to give a 10 cm. resin column.

A 2 per cent. gelatin sol made with low ash gelatin was run through the column, to remove small ions (a measurable amount of free amino-acid was present in the original sol) the effluent was collected and the column was then thoroughly washed with demineralised water. A 10 ml. sample of the purified gelatin sol was diluted to 50 ml. with water and titrated with 0·1N sodium hydroxide contained in a microburette, in the presence of an accurately measured volume of neutral formaldehyde. Phenolphthalein was used as indicator and a titre of about 0·5 ml. was obtained. A similar titration was carried out with 10 ml. of approximately 0·4 per cent. glycine solution.

10 ml. of each of the gelatin and glycine solutions were put on to the mixed resin column and allowed to flow through at 1 drop per second. The column was washed with 3 successive quantities, each of 10 ml., of demineralised water and the total effluent and washings were titrated with 0·1N sodium hydroxide, adding the same volume of neutral formaldehyde as in the previous titrations. The titre was very close to that of the gelatin sol alone, indicating that the glycine had been completely removed by the column. An experiment carried out by the authors gave the following results; gelatin titre, 0·45 ml.; glycine titre, 3·27 ml.; combined effluent titre 0·45 ml.

This experiment was carried out by a group of analysts, each making a single determination without previous experience. The results were expressed as the difference between the total gelatin and glycine titres, before and after running through the column (i.e., the estimate of amino-acid concentration determined by the experiment), divided by the known glycine titre and multiplied by 100. A 100 per cent. result therefore indicated a complete removal of glycine by the resin, with no measurable loss of gelatin. Under the conditions of the determinations an error of $\pm$ 2·5 per cent. was to be expected, mainly due to titration error. The mean of 26 results gave a percentage of 100·6 with a standard deviation of 3·1 per cent. 24 of these results were within the expected limits of error, these had a mean of 100·3 per cent. and a standard deviation of 0·9 per cent.

Suggested Applications. This method can be employed to give an estimate of the concentration of low molecular weight amino-acid in gelatin and protein sols. The use of a mixed resin column ensures that neutral salts in the original sol will not interfere. The amino-acid concentration is measured by the difference between the caustic soda-formaldehyde titres before and after running through the column, a correction being made if the initial sol has a measurable titre with caustic soda, in the absence of formaldehyde. The assay might be useful for detecting deterioration of blood and other protein preparations during storage.

Conclusions

The assay processes described are all capable of giving results with an error of $\pm$ 0·5 per cent. Further development should lead to a reduction of these limits.

The use of ion exchange columns for routine analysis has much to recommend it. The columns can be set up so as to run with very little attention and, after use, the resin can be easily regenerated without removing it from the column (except in the case of the mixed resin column). The ion exchange method for determining alkaloids in their salts and in their preparations is more convenient than the two solvent extraction method and it may also prove to be more reliable. A similar process could be developed for estimating medicinal organic acids in their salts, e.g., sodium salicylate.

The authors thank Professor Linnell for his interest and support, Mr. J. N. T. Gilbert for the quinine hydrochloride assay, and the Permutit Co. for a gift of some of the resins.

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