

THE DETERMINATION OF ALKALOIDS BY EXCHANGE OF IONS

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THE development of research on substances serving for the exchange of ions has been going on for about a century. Research workers were at first dependent only on substances of natural origin, but in the years 1910 to 1930 many experiments were made towards preparing suitable synthetic products. In 1935, Adams and Holmes¹ prepared a new type of artificial resin, and thus, with this new group of substances, started development along new lines. Griessbach² examined in detail the composition, action and theory of ion-exchange substances, also their preparation and the determination of their activity, and explained their wide use for metallurgical and chemical purposes, especially as water softeners and salt-removers. In principle we are concerned with two groups of artificial resins. The first includes the condensation products of formaldehyde with aromatic acids, chiefly polyhydric phenols, and natural products of the tannin type, these condensation products serving as exchangers of cations, or eventually of hydrogen ions. The second group is formed by the condensation products of aromatic amines with aldehydes, these products serving for the exchange of anions, including hydroxyl ions. Hesse³ deals, under the heading of chromatographic determination, with the exchange of ions in some detail, from both theoretical and practical standpoints. Similarly, Myers⁴ sees in the exchange of ions only one of the methods of chromatographic determination (see also Zechmeister⁵).

Samuelson⁶ has contributed much to our knowledge of the use of these resins for the exchange of ions, especially in inorganic analysis. The *Encyclopædia of Chemical Technology 1947* gives information of the use of synthetic resins in obtaining alkaloids of natural origin, such as quinine and nicotine, and details of the literature are given.

Ungerer⁷ has studied the adsorption of salts of organic bases, and to some extent alkaloids, on calcium permutite, and has ascertained that certain alkaloids, which are not easily soluble in water, are strongly adsorbed from aqueous solutions of their salts, due to the action of the slightly alkaline calcium permutite in precipitating the free base. In an alcoholic medium the adsorption proceeds in the normal way.

The idea of using the chromatographic adsorption method for the quantitative determination of alkaloids was realised soon after the revival of Tswett's method by Valentin, Franck and Merz^{8,9,10,11}, who describe the technique of adsorption on a column of aluminium oxide, and the working methods for determining the strength of various galenicals containing alkaloids. They have continued their work for several years, and give preference to the chromatographic method over

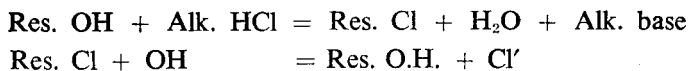
those used as standard in the pharmacopœias, the advantages lying chiefly in the smaller quantities of material required for analysis, the simpler technique, and the shorter time required for the analysis.

The most recent workers to direct their attention to chromatographic analysis of alkaloidal salts and to the suitability of aluminium oxide for such analyses are Reimers, Gottlieb and Christensen¹². They passed an ethyl alcohol solution of alkaloidal salts through a column of 10 g. of aluminium oxide, in a tube of 10 mm. diameter and 22 cm. height. The alkaloids were then washed out of the adsorbent with 25 ml. of ethyl alcohol (86 per cent.), and determined by direct titration in ethyl alcohol, using a suitable indicator in accordance with the experience of Kolthoff¹³ and Baggesgaard-Rasmussen and Reimers¹⁴.

The object of the work now to be described was first to ascertain whether artificial resins can be used as adsorbents for the determination of alkaloids, and then, if successful results were obtained, to investigate whether they could be applied to the determination of the alkaloidal content of galenical preparations. In the experimental part these questions are answered, the first very definitely in the affirmative, and the second with certain reservations. Nevertheless, even here certain definite results have been obtained, and the way indicated for further work.

EXPERIMENTAL

As an ion-exchanging adsorbent material, Amberlite IR-4B, manufactured by the Resinous Products and Chemical Company of Philadelphia, U.S.A., was selected, this being suitable for anion exchange. Attempts to find a basic exchanger suitable for quantitative work proved a failure, though such adsorbents gave excellent results when used for the isolation of alkaloids. The reactions in the adsorbing column were as follows:



The technique of the method has already been described in detail. Here only a brief description of the preparation of the adsorption apparatus is necessary since the whole was of the simplest type.

A tube of ordinary glass of 0.5 cm. diameter was used, which, at some 20 cm. from its end, was drawn out into a short narrow outlet tube, which was fitted into a rubber stopper, and the whole tube was thus attached to a suction flask.

It was necessary to crush the adsorption material, and it was found best to crush it and then leave it standing under water in a beaker for a day or two. During this period it was several times stirred up, and, when it had settled again, the water was changed. The light yellow powder was thus removed, and a material obtained of constant grain size, which plays a certain role in obtaining a perfect exchange of ions.

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This prepared material was poured, together with a little water, into the adsorption tube, whose lower end was closed with a small wad of cotton wool, so as to ensure that the adsorbent material settled firmly in place, and did not tend to form layers when subjected to a steady flow of water. When a sufficient amount of material was in the tube, it was further washed with water, and secured by a second plug of cotton wool. The adsorbent was thus evenly distributed in a column about 5 cm. high, water flowing through it at a speed of about 100 to 120 drops per minute, under its own pressure.

Such a column has to be regenerated before and after each analysis. With Amberlite IR-4B this is carried out by means of a 4 per cent. solution of sodium carbonate. In determining alkaloids about 50 ml. of such a solution was sufficient for each regeneration. The excess of sodium carbonate was removed by washing with boiled distilled water until the washings gave no reaction with phenolphthalein. Usually about 300 to 400 ml. of water was sufficient. It was necessary always to maintain a layer of a few ml. of water above the adsorbent column in order to avoid the drying and packing of the material. When ceasing work the upper end of the tube was closed with a rubber stopper. If working with adsorption in a medium other than water, e.g. in alcohol, the adsorption tube and column should be washed out before the experiment with a few ml. of the solvent used.

The principal condition for success in the analysis of alkaloidal salts is the use of a solvent in which both the alkaloidal salt and the free base are readily soluble. Numerous experiments showed that it was not possible to obtain satisfactory results under any other conditions. Thus, for example, when working with strychnine nitrate in aqueous solution, the free base is precipitated on the adsorbent, and although strychnine is sufficiently soluble in ethyl alcohol, it was never possible, even using hot ethyl alcohol, to wash it out of the column quantitatively; and the results were always some 10 per cent. too low, even when using a considerable excess of solvent. This case is interesting since workers using aluminium oxide as adsorbent obtained quantitative extraction of the base from the adsorbent using comparatively small quantities of alcohol. For the examples used in the course of the present work, ethyl alcohol was found to be suitable, and in the case of morphine, methyl alcohol. In most cases the speed of flow of the liquid through the column is not important, and good results may be obtained with a speed of flow of 90 to 120 drops per minute. Only in the analysis of atropine sulphate did we obtain results rather lower than according to theory, and by reducing the speed of flow to 30 to 40 drops per minute we obtained satisfactory results. The speed of flow may be readily controlled by means of a rubber stopper in the adsorption tube in the neck of the Erlenmeyer flask in which the liquid is collected. After the adsorption is completed the tube and vessel are washed out with about 50 ml. of warm solvent (ethyl or methyl alcohol). This quantity of solvent may be regarded as sufficient for the alkaloids analysed, and

it was not thought necessary to ascertain the minimum quantity of solvent needed, since we were using a quantity appropriate to the amount of substance being determined.

Adsorption from galenicals was carried out in exactly the same manner, since the first experiments showed that it was unnecessary to add inorganic acids in advance, in order to prepare salts of the alkaloids with strong acids. The question of a suitable solvent again plays the same important role. The alcoholic solution of the galenicals lost a small amount of its colour in passing through the adsorbing column together with some of the accompanying substances. In regenerating the adsorbent material after each analysis we remove the colouring matter and accompanying matter retained by the adsorbent, so that it is not necessary to change the contents of the column after each analysis.

The actual determination of the alkaloidal bases was carried out by direct titration with hydrochloric acid. In determining alkaloidal salts, i.e., in colourless solutions, the indicator used was a mixture of methyl red and methylene blue, giving at the appropriate dilution with water (about 100 ml.) a sharp change from green to violet. When working with galenicals it was necessary to ascertain the equivalence point electrometrically, using very sensitive measuring instruments. The potential drop is masked in these cases by the accompanying substances (the more intensely coloured the solution, the more difficult is the determination of the equivalence point). It is interesting to compare the titration curve of strychnine in ethyl alcohol with that of the same quantity of strychnine in tincture of nux vomica, in which the masking effect under the conditions prevailing in the tincture is clearly evident. There are possibilities here for working with minimum quantities, and also of using micro-methods for the determination of alkaloids in various galenicals, which are being further investigated by the present author. The cause of the double result obtained with tincture of nux vomica as compared with the theoretical result (the equivalent used was the mean of the molecular weights of the two alkaloids) is also being investigated.

The time required to carry out the analysis is about the following:— filling of apparatus, 15 minutes; regeneration of adsorbent, 30 to 45 minutes; adsorption and washing out, 15 minutes. The total time required is from 1 hour to 1½ hours, in addition to the time required for the titration.

(a) DETERMINATION OF ALKALOIDAL SALTS

An accurately weighed quantity of the alkaloidal salt (0.1 to 0.2 g.) was dissolved in 20 ml. of ethyl alcohol (96 per cent.), and adsorbed on the prepared column of Amberlite IR-4B synthetic resin. The vessel was then washed out several times with a total of 50 ml. of warm (50°C.) ethyl alcohol (96 per cent.), and the column washed out with the same solvent. The dissolved base in the alcoholic solution was then determined by titration with N/10 hydrochloric acid ($F = 1.029$), using

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as indicator a mixture of 10 drops of solution of methyl red with 2 drops of solution of methylene blue. To ascertain the equivalence point the mixture was diluted to 100 ml. with distilled water. The colour change from green through blue to violet is sharp.

For quinine and cinchonine the alkaloidal base was used for analyses, being dissolved by the addition of an equivalent quantity of hydrochloric acid and 10 ml. of water, the solution being made up to 100 ml. with ethyl alcohol. For the determination of morphine and quinine methyl alcohol solutions were used. Strychnine nitrate was dissolved in ethyl alcohol (75 per cent.). The percentage content of alkaloids in the salts was ascertained experimentally by the Danish Pharmacopœia methods. This was necessary because in many cases impure compounds were used whose content differed considerably from theory. The results are shown in Table I.

TABLE I
DETERMINATIONS OF ALKALOIDAL SALTS

| Alkaloidal Salt | Weighed Out | N/10 Hydrochloric Acid ($f=1.029$) | Found by Adsorption Method | Found by Danish Pharmacopœia Method |
|-------------------------------|-------------|--|----------------------------------|---|
| | g. | ml. | per cent. | per cent. |
| Strychnine nitrate | 0.0950 | 2.17 | 83.36 | 84.14 |
| | 0.0950 | 2.16 | 82.92 | |
| | 0.1420 | 3.27 | 84.04 | |
| Atropine sulphate | 0.1691 | 2.23 | 41.74 | 40.40 |
| | 0.2184 | 2.73 | 39.48 | |
| | 0.2267 | 3.00 | 41.79 | |
| Morphine hydrochloride | 0.1395 | 3.37 | 75.24 | 75.91 |
| | 0.1395 | 3.38 | 75.46 | |
| | 0.1395 | 3.35 | 74.79 | |
| Bucine hydrochloride | 0.1261 | 2.47 | 84.37 | 84.29 |
| | 0.1261 | 2.48 | 84.71 | |
| | 0.0966 | 1.89 | 84.23 | |
| Ephedrine sulphate | 0.1556 | 5.05 | 58.52 | 58.86 |
| | 0.1556 | 4.98 | 57.70 | |
| Quinine | 0.1344 | 3.74 | 98.52 | |
| | 0.1344 | 3.78 | 99.57 | |
| Cinchonine | 0.0976 | 3.14 | 98.46 | |
| | 0.0976 | 3.13 | 98.15 | |

(b) DETERMINATION OF ALKALOIDAL CONTENT OF GALENICALS

The determination was carried out according to the same principles as for the alkaloidal salts. Tincture of ipecacuanha and tincture of nux vomica were adsorbed direct, while fluid extract of cinchona and tincture of opium had the weighed quantity diluted with 25 ml. of ethyl alcohol (96 per cent.). The further procedure was the same as that detailed above. The colouring matter is adsorbed in the column only in small measure, and the solutions of the alkaloidal bases obtained are coloured. The equivalence point must be determined electrometrically with the aid of the glass and calomel electrodes. With tinctures of nux vomica and ipecacuanha the potential change is, however, masked by the accom-

panying substances, but is none the less perceptible, provided that sensitive apparatus is used. Amounts corresponding to theory have been found in all experiments, except in the case of tincture of nux vomica

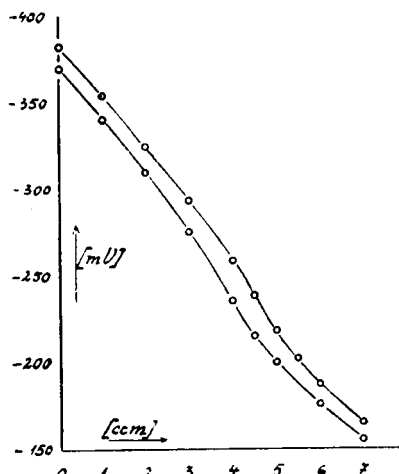


FIG. 1. Tincture of Ipecacuanha

when the result obtained was invariably double the theoretical figure. Figures 1, 2, 3 and 4 show the course of the titrations. In carrying out the titrations the ethyl alcohol solutions were diluted with water to 100 ml. In working the fluid extract of cinchona much depends upon the careful washing out of the adsorbed column, since cinchonine is far less soluble than quinine. In the case of opium a larger alkaloidal content was found that would correspond to morphine alone since the total content of morphine and other alkaloids was determined. The results are shown in Table II.

SUMMARY

1. A method has been worked out for the determination of salts of alkaloids by an adsorption method involving exchange of ions, on the synthetic resin known as Amberlite IR-4B which was used with the substances specified in Table I.

2. It is shown that this method is also suitable for determining the alkaloidal content of the galenicals specified in Table II.

TABLE II
DETERMINATIONS OF ALKALOIDS IN GALENICALS

| Preparation Used | Weighed Out | N/50 Hydrochloric Acid (f=1.092) | Found by | Found by |
|------------------------------|-------------|--|----------------------|---|
| | | | Adsorption Method | Czechoslovak Pharmacopoeia Method |
| | g. | ml. | per cent. | per cent. |
| Tincture of Ipecacuanha ... | 48.80 | 4.00 | 0.107 | 0.095 |
| | 48.80 | 4.50 | 0.120 | |
| | 48.80 | 4.25 | 0.114 | |
| | 30.00 | 3.50 | 0.109 | |
| | | | | |
| Fluid Extract of Cinchona... | 0.9452 | 2.65 | 4.733 | 4.450 |
| | 0.9756 | 2.65 | 4.586 | |
| | 0.9692 | 2.65 | 4.586 | |
| | 0.8315 | 2.25 | 4.568 | |
| Tincture of Opium ... | 2.9170 | 3.45 | 1.842 | 1.11 |
| | 1.3434 | 1.65 | 1.912 | |
| | 1.9841 | 2.20 | 1.727 | |
| Tincture of Nux Vomica ... | 35.27 | 10.00 | 0.563 | 0.247 |
| | 35.27 | 9.40 | 0.530 | |
| | 30.00 | 7.00 | 0.464 | |

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3. The advantage of the method is its rapidity, simplicity and the use of only small quantities of solvents, together with the determination of all the alkaloids present without any of the possibilities of loss arising from more complicated present experimental procedures.

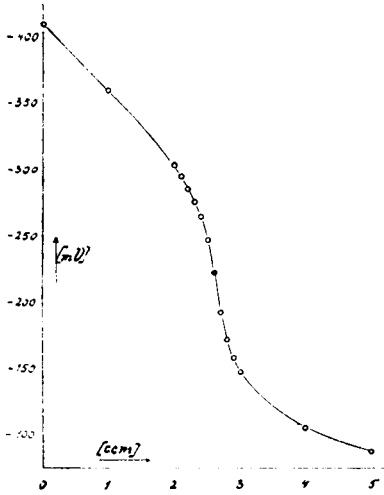


FIG. 2 Fluid Extract of Cinchona

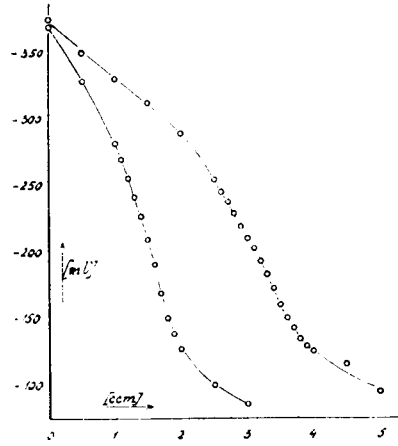


FIG. 3 Tincture of Opium

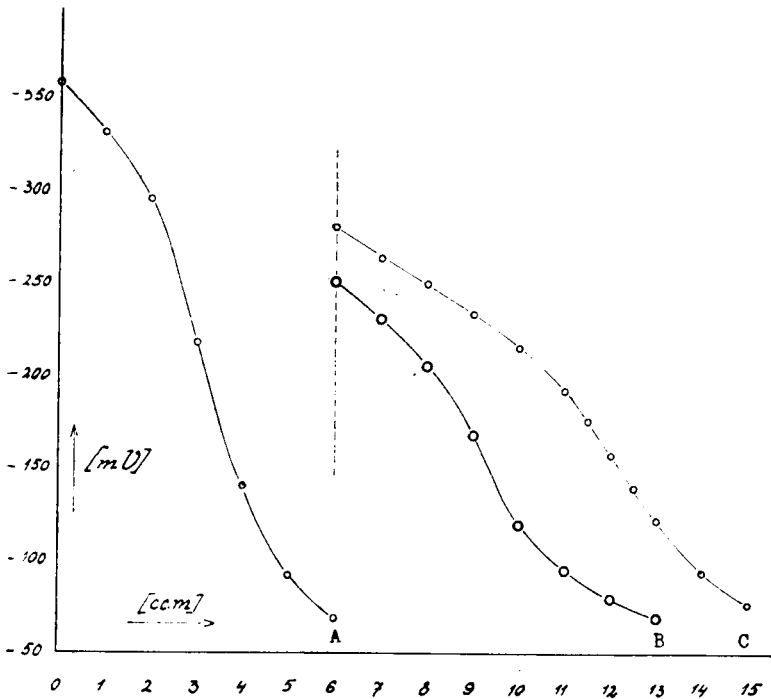


FIG. 4. A. 0.05 g. of strychnine in alcohol. B. Tincture of nuxvomica. C. 0.05 g. of strychnine in tincture of nuxvomica.

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