A METHOD OF DETERMINING BINARY MIXTURES BY DISTRIBUTION MEASUREMENTS, AND ITS APPLICATION TO THE ASSAY OF STRYCHNINE IN THE PRESENCE OF QUININE

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THE combined determination of strychnine and quinine in various pharmaceutical preparations, and the estimation of strychnine in the presence of brucine, are typical examples of the analysis of binary mixtures. In the assay of alkaloidal mixtures, it is customary to rely upon repeated extractions with immiscible solvents, followed by further treatment of the residue obtained by extraction, as a means of separating the components of the mixture. The labour and cumulative losses incurred in the separation are often considerable, and it has been suggested that extraction processes should be replaced by instrumental methods of analysis. Infrared and ultra-violet spectroscopy have been used in the estimation of mixtures of strychnine and brucine[1-3], and an ultra-violet spectrophotometric assay process for the combined determination of strychnine and quinine has been described[4]. In general, however, spectrophotometric determinations of binary mixtures suffer from the drawback that tedious calculations involving either the solution of simultaneous equations or the use of successive approximations are necessary in order to evaluate the results of the absorption experiments.

The distribution coefficient of a substance between suitably chosen immiscible solvents resembles its extinction coefficient at a given wavelength in being a specific physical property, characteristic of the substance. It is readily deduced from the Distribution Law that, when a mixture of two substances is dissolved in a suitable solvent and shaken with an immiscible solvent, distribution takes place in such a manner that the total weight of mixed solutes in each phase at equilibrium is a linear function of the composition of the original mixture. Thus if the weight of solutes in either phase is determined, the relative proportions of the components in the original mixture may be read on a linear graph connecting the weight of residue with the per cent. composition. For example, a rapid and accurate combined determination of strychnine and quinine may be carried out merely by dissolving a known weight of the mixed alkaloids in N hydrochloric acid and determining the weight of solutes obtained by one extraction with chloroform.

GENERAL PROCEDURE

It is shown in the theoretical section of this communication that the weight W of residue obtained by extracting a solution of 1 g. of a mixture of two substances A and B with an immiscible solvent is related to the percentage A_0 of the component A in the mixture by the expression

$$A_0 = K_1 W - K_2 \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \quad (1)$$

where K_1 and K_2 are constants. If the distribution coefficients of the substances A and B between the selected solvents are known, K_1 and K_2 may be calculated, and a linear graph connecting the weight W of the residue with the composition of the mixture may be constructed with the aid of equation (1). A more reliable method is to construct an empirical graph from data obtained by using mixtures of known composition approximating to that of the samples for analysis; by this means, errors due to such sources as deviations from the Distribution Law, mutual solubility of the solvents, or the presence of traces of impurities in the solvents, may be eliminated.

Success in the attempt to apply this method to a given determination depends upon the practicability of selecting solvents with which a favourable ratio of the distribution coefficients of the two substances is obtained. The conditions which must be satisfied in order that high accuracy may be attained are discussed in the following sections. The method is capable of extension to mixtures of three or more components, but the requirements are then more exacting; in order to evaluate completely a mixture of m components, it is necessary to make use of (m − 1) pairs of solvents.

THEORY

The theoretical basis of the method is as follows. Let x g. of a mixture of the substances A and B be dissolved in V_1 ml. of a solvent S_1, and let the solution be extracted with n successive volumes, each of V_2 ml. of an immiscible solvent S_2. Let the amounts of A and B remaining in the solvent S_1 after the first, second and nth extraction be a_1, a_2, a_n and b_1, b_2, b_n respectively, and let the partition coefficients of A and B between solvent S_1 and solvent S_2 be k_a and k_b respectively. If $V_2/V_1 = r$ and the weights of A and B initially present in solvent S_1 are a and b respectively, then after the first extraction we have $k_a = a_1 r/(a - a_1)$ and $k_b = b_1 r/(b - b_1)$ whence

$$a_1 = aK_a \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \quad (2)$$

and

$$b_1 = bK_b \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \quad (3)$$

where

$$K_a = k_a/(k_a + r) \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \quad (4)$$

and

$$K_b = k_b/(k_b + r) \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \quad (5)$$

Similarly, after the second extraction $k_a = a_2 r/(a_1 - a_2)$ and $K_b = b_2 r/(b_1 - b_2)$ or $a_2 = a_1 K_a$ and $b_2 = b_1 K_b$. It follows from equations (2) and (3) that $a_2 = aK^2_a$ and $b_2 = bK^2_b$. In general terms, the amounts of the substances A and B remaining in solvent S_1 after n extractions are $a_n = aK^n_a$ and $b_n = bK^n_b$ respectively, and the total weight of mixed solutes recoverable from the solvent S_2 after n extractions is

$$w = (1 - K^n_a)a + (1 - K^n_b) b \quad .. \qquad .. \qquad .. \qquad .. \quad (6)$$

Substituting $b = x - a$ in this equation we obtain $100a = wK_1 - K_2x$

where $K_1 = 100/(K^n_b - K^n_a)$ .. .. (7)

and $K_2 = K_1(1 - K^n_b)$ .. .. (8).

The percentage of the substance A in the original mixture, viz.,

$A_0 = 100a/x$, is thus given by $A_0 = K_1W - K_2$ .. .. (1)

where $W = w/x$ is the weight of mixed solutes recovered from 1 g. of the original mixture.

When values of A_0 are plotted as abscissae against values of W as ordinates, a linear graph is obtained which, when produced, cuts the A_0 axis. The intercept on this axis gives K_2, and the slope of the graph is equal to $1/K_1$. When the sample for analysis consists merely of the component B, i.e., when $A_0 = O$, the weight of residue obtained from 1 g. of the sample is $1 - K^n_b$; similarly, it is readily shown that if only the component A is present ($a = x$) a residue $1 - K^n_a$ is obtained. As n and r are included in the terms K_1 and K_2, it follows that, throughout a given determination, the total number of extractions, and the ratio r of the volumes of the two phases, must be constant. In the following analysis of the conditions which must be satisfied in order that high accuracy may be attained, a ratio r of unity has been assumed for convenience; under these conditions, equations (4) and (5) simplify to $K_a = k_a/(k_a + 1)$ and $K_b = k_b/(k_b + 1)$ respectively.

Accuracy

Evidently, the highest accuracy is attained when the increment in the weight of residue due to a change of 1 per cent. in the proportion of the substance A is large; in other words, the solvents should be so chosen that the slope of the W/A_0 graph, viz.,

$dW/dA_0 = 1/K_1 = (K^n_b - K^n_a)/100$ .. .. .. (9)

is as steep as possible. When $k_a = k_b$, the slope dW/dA_0 is zero, and the weight of residue obtained is independent of the composition of the mixture. On the other hand, if k_a is very small and k_b is large, K^n_a and K^n_b approach zero and unity respectively, and the graph attains a maximal slope $dW/dA_0 = 1/100$, i.e., an increment in the weight of residue of 10 mg. per g. of mixture is obtained for a change in composition of 1 per cent. Assuming that the weight of residue obtained from 1 g. of the mixture is determined with an accuracy of $\pm y$ mg., the error in the determination of the substance A is $y/10(K^n_b - K^n_a)$; for example, when $y = \pm 1$ mg., $k = 10.0$, and $k_a = 0.5$, the error amounts to ± 0.17 per cent., if the determination is based upon a single extraction ($n = 1$) or ± 0.14 per cent. for $n = 2$. Thus whilst the highest accuracy is obtained when the solvents are so chosen that the ideal condition $k_b \gg k_a$ is satisfied, the requirements in this respect are by no means exacting. It is also clear that, in general, no substantial gain in accuracy is obtained by increasing the number of extractions. Indeed, calculation shows

that when $k_b < 5 \cdot 0$, the highest accuracy is attained by basing the determination upon a single extraction with the immiscible solvent.

In the preceding discussion, it has been assumed that the solutions behave ideally; complications due to such phenomena as association in the ethereal phase, dissociation in the aqueous solvent, or the influence of the solutes on the miscibility of the two layers have not been considered. In practice, errors due to deviations from the Distribution Law may be avoided by constructing the graph connecting the weight of residue with the composition of the mixture empirically from data obtained by using mixtures of known composition approximating to that of the samples for analysis.

The Assay of Strychnine in the presence of Quinine

Strychnine and quinine can be separated by extraction with chloroform from a strongly acid aqueous solution of the alkaloids. Evers[5] stated that strychnine can be removed completely from a solution in 2N hydrochloric acid by five extractions with chloroform, whereas quinine is only extracted to a very slight extent under these conditions. Haddock and Evers[6] used 0·5N hydrochloric acid semi-saturated with sodium chloride as the aqueous solvent, and subjected the residue obtained by five extractions with chloroform to further treatment in order to remove traces of quinine. Herd[7] separated the strychnine from a solution of the alkaloids in 7N sulphuric acid by five extractions with a 1 per cent. solution of dichloroacetic acid in chloroform. In the present work it was found that satisfactory results could be obtained by a single extraction with chlorofrom from a solution of the alkaloids in N hydrochloric acid.

EXPERIMENTAL

Preliminary experiments showed that when a solution of strychnine in N hydrochloric acid is shaken with chloroform, the weight of residue recovered by evaporating the chloroformic layer decreases by about 1 per cent. for 1° C. rise in the temperature of the solvents. In the experiments described below, solutions of strychnine and quinine were shaken with chloroform in a round bottomed flask immersed in a thermostat at 25° C. Equilibrium was attained after stirring the contents of the flask mechanically for one hour, a rubber sealed stirrer being used to prevent evaporation. The liquid was then allowed to separate into two layers. The chloroform was removed from the aqueous phase as quickly as possible, filtered through dry filter paper, evaporated to dryness, and the residue dried to constant weight at 105° C. All measurements of volume were made at 25° C.

Solutions of strychnine and quinine (0·2 per cent. w/v) in N hydrochloric acid were mixed in suitable proportions to produce a series of solutions in which the percentage of strychnine present in the total alkaloids varied between the limits of 0·0 and 100. A volume of 50 ml. of each solution was shaken with 100 ml. of chloroform as described above, and the weight of residue obtained by evaporating 75 ml. of the chloroformic solution was determined. A linear graph was constructed by plotting the weight W

of solutes obtained from 1 g. of the mixed alkaloids against the corresponding percentage A_0 of strychnine in the mixture, and from this the constants $K_1 = \delta A_0/\delta W$ and $K_2 = K_1 W - A_0$ of equation (1) were evaluated. The weight a (in g.) of strychnine in each mixture was calculated from the weight w of residue obtained from x g. of total alkaloids by means of the relationship $a = k_1 w - k_2 x$ where $k_1 = 0.01\ K_1$ and $k_2 = 0.01\ K_2$. The results are recorded in Table I.

TABLE I

$K_1 = 180.0$ $\quad$ $K_2 = 2.0$ $\qquad\qquad$ $k_1 = 1.80$ $\quad$ $k_2 = 0.02$

Volume of 0.2 per cent. w/v quinine solution ml.	Volume of 0.2 per cent. w/v strychnine solution ml.	x = weight of total alkaloids taken g.	w = weight of residue from x g. of mixture g.	W = w/x = weight of residue from 1 g. of mixture g.	Ao = per cent. of strychnine in the mixture	Weight of strychnine taken g.	Weight of strychnine found = a = k₁w-k₂x g.
50	0	0·0917	0·0008	0·0087	0	0	−0·0004
45	5	0·0925	0·0066	0·0713	10·81	0·0100	0·0100
40	10	0·0934	0·0121	0·1296	21·42	0·0200	0·0198
30	20	0·0950	0·0234	0·2463	42·09	0·0400	0·0400
25	25	0·0959	0·0294	0·3067	52·16	0·0500	0·0508
20	30	0·0967	0·0347	0·3589	62·06	0·0600	0·0601
10	40	0·0983	0·0447	0·4546	81·35	0·0800	0·0780
5	45	0·0992	0·0503	0·5072	90·75	0·0900	0·0880
0	50	0·1000	0·0565	0·5650	100·0	0·1000	0·0990

In other experiments, mixtures approximating in composition to the residue obtained by complete extraction of the total alkaloids from 100 ml. of Easton's Syrup were prepared by adding amounts of strychnine varying from 0·0 to 0·05 g. to roughly equal weights (about 1·2 g.) of quinine. Each mixture was dissolved in 20 ml. of N hydrochloric acid and extracted with 50 ml. of chloroform at 25° C. The weight of residue obtained by evaporating 40 ml. of the chloroform layer was determined, and the values of k_1 and k_2 were calculated as described above. The results are recorded in Table II.

The samples of quinine and strychnine used in this work were assayed by means of moisture determinations and by titration with 0·5 N acetous perchloric acid, Oracet Blue B being used as indicator.

TABLE II

$K_1 = 215$ $\quad$ $K_2 = 1.0$ $\qquad\qquad$ $k_1 = 2.15$ $\quad$ $k_2 = 0.01$

Weight of anhydrous quinine taken g.	x = weight of total alkaloids taken g.	w = weight of residue from x g. of mixture g.	W = w/x = weight of residue from 1 g. of mixture g.	Ao = per cent. of strychnine in the mixture	Weight of strychnine taken g.	Weight of strychnine found = a = k₁w-k₂x g.
1·1130	1·1130	0·0051	0·0046	0	0	−0·0003
1·1856	1·1986	0·0116	0·0098	1·097	0·0130	0·0130
1·1421	1·1618	0·0145	0·0125	1·695	0·0197	0·0196
1·0667	1·0939	0·0194	0·0177	2·487	0·0272	0·0308
1·0815	1·1113	0·0191	0·0172	2·682	0·0298	0·0299
1·1904	1·2302	0·0246	0·0200	3·235	0·0398	0·0406
1·1573	1·2029	0·0269	0·0224	3·791	0·0456	0·0458

CONCLUSION

The weight a (in g.) of strychnine present in the weight x (in g.) of the mixture of strychnine and quinine obtained by complete extraction of the

total alkaloids from 100 ml. of Easton's Syrup may be determined by the following method:—

Dissolve the mixture of strychnine and quinine in 20 ml. of N hydrochloric acid, and shake the solution for one hour with 50 ml. of chloroform at 25° C. Separate the chloroformic layer, filter through dry filter paper, and evaporate 40 ml. of the filtrate to dryness. Determine the weight w (in g.) of the residue after drying to constant weight at 105° C. All volumes should be accurately measured.

The weight in g. of strychnine in the mixture of strychnine and quinine is given by a $= 2 \cdot 15w - 0 \cdot 01x$. Alternatively, the percentage $A_0 = 100a/x$ of strychnine in the mixture may be read on a linear graph constructed with the aid of the equation $A_0 = 215W - 1 \cdot 0$, where $W = w/x$ is the weight of residue obtained from 1 g. of the mixture.

SUMMARY

1. When a mixture of two substances is dissolved in a suitable solvent and shaken with an immiscible solvent, the weight of mixed solutes present in each phase at equilibrium is a linear function of the per cent. composition of the original mixture, provided that the Distribution Law is obeyed; thus, if, after separation of the solvents, the weight of solutes in either phase is determined, the relative proportions of the components in the mixture may be read on a linear graph connecting the weight of residue with the percentage composition.

2. Errors due to deviations from the Distribution Law may be avoided by constructing the graph empirically from data obtained by using mixtures of known composition approximating to that of the samples for analysis.

3. A mixture of quinine and strychnine may be rapidly and accurately assayed by dissolving a known weight of the mixed alkaloids in N hydrochloric acid and determining the weight of residue obtained by one extraction with chloroform.

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DISCUSSION

The paper was presented by PROFESSOR W. H. LINNELL on behalf of the authors.

DR. F. HARTLEY (London) said if there were an appreciable quantity of alcohol present in the chloroform it would seem necessary to derive distribution figures for that particular sample, a procedure which destroyed some of the elegance and rapidity claimed for the method. To what extent was the method sensitive when up to 2 per cent. of alcohol was present in the chloroform?

DR. G. E. FOSTER (Dartford) said that the extraction method was based very largely upon work published by Dr. Evers some years ago. In that method the chloroform extract was shaken with ammonia in order to convert the alkaloids to bases. It would seem the authors evaporated the chloroform extract immediately. Was it strychnine hydrochloride or base which was weighed? He wondered why the chloroform extract was not tested with ammonia before evaporation. He also considered some of the chloroform additive compound was likely to be present.

MR. A. R. ROGERS (Brighton) said he could not agree that the fairly simple arithmetical calculations involved in spectrophotometric determinations were tedious, as was stated in the paper.

MR. R. L. STEPHENS (Brighton) asked whether the authors had considered the change of partition coefficient between the two substances with changes of temperature. The determination was carried out at 25° C. but the influence which $\pm 1°$ might have upon the precision of the results was not stated.

DR. D. C. GARRATT (Nottingham) said he felt that the method outlined was one which merited closer investigation. It would be interesting to know why it was necessary to take one hour to obtain equilibrium—one would have thought that equilibrium would be established in a short time and thereby the length of time of assay could be shortened.

PROFESSOR W. H. LINNELL, in reply, said he would refer the questions to the author.

MR. C. MORTON, in a written reply, states that the chloroform used was of Pharmacopœial quality, and the alcohol was not removed from it before use. When there are numerous samples for analysis, the most convenient procedure is to "calibrate" suitable solvents by means of standard mixtures, and reserve the solvents for use in the subsequent determinations. The linear relationship between composition and weight of residue is rigidly maintained, even when the proportion of strychnine in the mixture varies between the extreme limits of zero and 100 per cent. (Table I), and it is sufficient to use two standard mixtures in preparing the calibration graph. This method eliminates possible error due to varying alcohol content. In the absence of reliable information on the dissociation constants of strychnine, the degree of ionisation of its hydrochloride in aqueous solution, and the true distribution coefficients of strychnine and its hydrochloride between water and chloroform, it would be unwise to be dogmatic, but there can be little doubt that, under the conditions of the assay, the strychnine is present in both phases entirely as hydrochloride. It is also possible that the chloroform compound referred to by Dr. Foster is present in small amount. The observed distribution coefficient may accordingly be regarded as that of the hydrochloride rather than the free base, but is more correctly described as the apparent distribution coefficient of strychnine, i.e., the ratio of the concentration of the total alkaloid (in whatever forms it may be present) in the aqueous phase to that of the total alkaloid in the ethereal phase.

Provided that the concentration of acid in the aqueous phase is constant, the apparent distribution coefficient is also constant. Whilst it is true that, as Dr. Foster points out, the alkaloid is weighed initially as free base and finally as hydrochloride, the effect is merely to multiply the apparent distribution coefficient by a constant which, during the preparation of the calibration graph, is automatically included in the constants K_1 and K_2. Regenerating the free base after extraction would complicate the assay and necessitate redetermination of the constants K_1 and K_2 without improving the accuracy of the process. The proposed method, in which the relative proportions of the components in the mixture are read on a linear graph connecting composition with weight of residue, would appear to be less tedious than a spectrophotometric assay entailing the solution of simultaneous equations. It must also be borne in mind that the costly instruments required for infra-red and ultra-violet spectroscopy are not universally available in analytical laboratories, and that the suggested method may be applicable to determinations for which no spectrophotometric assay process can be devised. As pointed out in the paper, the weight of residue obtained by extracting a solution of strychnine in N hydrochloric acid decreases by about 1 per cent. for 1° C. rise in the temperature of the solvents. Thermostatic temperature control is accordingly necessary, and any departure from the recommended temperature of 25° C. would entail a redetermination of the constants K_1 and K_2. The length of time required to establish equilibrium obviously depends upon the efficiency of agitation, which cannot readily be standardised. The period can be considerably shortened if care is taken to ensure efficient shaking.