Phase Solubility Analysis Employing Solubility Product Relationships: Purity Determination of Monobasic Amines and Their Salts

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Abstract □ The application of phase solubility analysis has been extended to monobasic amines and their salts. The analyses are carried out in aqueous buffered solutions by *in situ* formation of slightly soluble picrate salts, whose solubility is determined by a solubility product relationship. Both a theoretical discussion and experimental testing of the method are presented. The experimental results indicate that the method is at least comparable in both sensitivity and accuracy to currently employed phase solubility techniques. In addition, the new method is applicable to systems that are not amenable to the widely used current method.

Keyphrases □ Amines, monobasic, and salts—phase solubility analysis using solubility product relationships, theory, method □ Picrate salts—phase solubility analysis using solubility product relationships, theory, method □ Phase solubility analysis—monobasic amines and their salts in aqueous solution, theory, method

Phase solubility analysis is a widely used technique for the absolute determination of purity. The general method and its application, theory, and limitations were the subjects of a recent review (1). Surprisingly, the wide use of the method has not stimulated much in the way of reported modifications or extensions. The technique, therefore, remains virtually unchanged since its inception in 1920. Some of the problems (1–4) associated with the method as now practiced are:

1. The solvent system must be chosen specifically for the substance being analyzed.
2. Liquid samples are poorly suited to the method.
3. Solids that form solid solutions are not suitable.
4. Acids, bases, and their salts that may be involved in either general acid–base equilibrium or irreversible solvolysis reactions with a solvent may not be analyzed in that solvent.
5. Mixtures of compounds that are present in a unique ratio which is identical to their solubility ratios in a particular solvent cannot be analyzed in that solvent.
6. Mixtures of enantiomers often are not suited to the method.

Two reports in the literature (4, 5) describe a modification of the technique and/or interpretation of the results which has allowed the analysis of materials that would not normally be suited to phase solubility because of one or more of the problems listed above. In both cases, the investigations were involved with solubility product relationships, but there was virtually no attempt to use such relationships to extend and modify grossly the method of phase solubility analysis.

The approach we are advocating should be applicable to certain classes of compounds such as amines, acids, and their salts, which under suitable conditions will exist as ions in aqueous media. The approach consists of utilizing the solubility product relationship which applies for slightly soluble salts in aqueous solutions. The proposed approach may alleviate many problems associated with phase solubility analysis and, in so doing, add a new dimension to the application of phase solubility analysis in the determination of purity. This work is a preliminary report on the theory and application of the method for the analysis of monobasic amines and their salts.

EXPERIMENTAL[1]

Chemicals—The chemicals used were of reagent or USP grade. Except for benzylamine and picric acid, all were used without further purification. In the case of benzylamine, the liquid was distilled prior to use. Picric acid was recrystallized from water three or more times, dried, and analyzed by differential scanning calorimetry (6). The purity of the picric acid thus prepared was calculated to be greater than 99.8% pure. In all cases, the melting or boiling points of the materials used were in good agreement with literature values.

Methods—*Preparation of Buffers*—The 0.2 *M* acetate buffer was prepared by taking 10.23 g. (0.17 mole) of glacial acetic acid and 2.48 g. (0.03 mole) of sodium acetate and dissolving them in enough water to make 1 l. of solution. The 0.5 *M* acetate buffer was prepared by using 25.0 g. (0.420 mole) of glacial acetic acid and 7.04 g. (0.086 mole) of sodium acetate. The pH of both buffers was about 3.9.

Determination of Molar Absorptivity of Picric Acid in Aqueous Solutions—Solutions of picric acid in known concentrations were prepared in the acetate buffers, and the absorbance of the picrate ion at 357 nm. was recorded. A plot of absorbance *versus* concentration yielded a linear relationship, from which the molar absorptivity, ϵ_{357}, was found to be 14,450 l./mole-cm. This value was the same in both the 0.2 and the 0.5 *M* acetates. There was no apparent change in absorptivity as a function of pH at pH > 3 in the 0.2 *M* acetate solution. The molar absorptivity of the picrate salts of pilocarpine, benzylamine, and *N,N*-diethylaniline was the same as that for picric acid when measured at 357 nm. in the 0.2 *M* acetate buffer, pH 3.9.

Method for Determination of Purity of Amines—The general method consisted of adding increasing and known weights of picric acid (Q_a) and the sample to be analyzed (W) to each of a series of clean, dry, 200-ml. volumetric flasks. Generally, the ratio of the weights of picric acid to sample was chosen to approximate the ratio of the species anticipated in the precipitate. For example, in the analysis of benzylamine, the expected precipitate was benzylamine picrate, for which the ratio of weights of picric acid to benzylamine is approximately two. Thus, in the preparation of each flask the weight of picric acid added was approximately twice as great as that of the benzylamine added.

[1] Absorbance measurements were made using either the Cary model 14 or 15 spectrophotometer. Volumetric glassware was of the class A (National Bureau of Standards) type. Equilibration was carried out by agitation of samples on a Burrell wrist-action shaker in a constant-temperature bath at 25 ± 0.1°. Weighings were made on a Mettler type H16 balance. The differential scanning calorimetry was done on a Perkin-Elmer model DSC1B. Measurements of pH were made using a Corning model 12 pH meter. All water used was distilled from acid permanganate solution in an all-glass apparatus.

Generally, the first flask in the series contained 0.05–0.10 g. of picric acid, and each succeeding flask contained increasingly greater amounts of picric acid. Usually, an amount of 0.5–1.0 g. was used in the last flask in the series. The quantity of the sample to be analyzed was increased in each flask in a similar manner.

After addition of the picric acid and the sample, 100 ml. (0.1 l. = V_1) of acetate buffer was added by buret and the flasks were sealed and placed in a constant-temperature water bath at 25° and agitated with a wrist-action shaker until equilibrium was achieved. This was usually accomplished within 48 hr. Then an aliquot of the supernatant liquids was removed from each flask in which there was a precipitate by use of a pipet whose tip was wrapped with glass wool to filter out any particulate matter. The contents of the pipet (V_2) were emptied into a volumetric flask of appropriate size, and this was brought to volume (V_3) with fresh acetate buffer. The absorbance (A_{357}) of the resulting solution was measured at 357 nm. in a 1-cm. cell, and the gram quantity of picrate in solution (Q_s) in each flask at equilibrium was calculated according to the following equation:

$$(Q_s) = \frac{(A_{357})}{\epsilon_{357}} \times \frac{V_3}{V_1 V_2} \times M_Q \qquad \text{(Eq. 1)}$$

The values Q_a, W, and Q_s for each flask were then used to calculate the values of $(Q_a - Q_s)/W$ and $1/Q_sW$. A plot of $(Q_a - Q_s)/W$ (as the ordinate) *versus* $1/Q_sW$ (the abscissa) was then prepared, and the slope and intercept were determined by the method of least squares. The percent purity was then obtained by multiplying the intercept by 100 M_B/M_Q, where M_B and M_Q are the molecular weights of the amine being analyzed and picric acid, respectively. The value of K_{sp}' (defined below) is calculated from the relationship $K_{sp}' = (-M_B/M_Q)$ (slope).

Under certain conditions, it was necessary to modify the general procedure to some extent. For example, in those cases where mixed samples, such as the mixture of pilocarpine nitrate and benzylamine, were prepared and analyzed, it was necessary to dissolve the materials in acetate buffer to obtain a uniform sample. In such cases, stock solutions were accurately prepared and aliquots containing the desired weight of sample were added to the various flasks containing the picric acid. Additional acetate buffer was then added such that the total volume of buffer solution added was 100 ml. For example, if 5 ml. of stock solution containing the amines was added to a flask, only 95 ml. of acetate buffer was then added to give a total of 100 ml. The rest of the procedure remained unchanged.

Another problem was encountered when liquid amines were used. Upon adding the liquid amine to a flask containing picric acid, the materials partially reacted to form a mixture of salt and unreacted materials. Such systems did not achieve equilibrium rapidly after addition of the buffer and the usual agitation. This problem was easily overcome by employing a magnetic stirring bar and vigorous stirring for about 12 hr. After such stirring the flasks were then agitated in the usual fashion for an additional 48 hr. in accordance with the general procedure.

THEORETICAL CONSIDERATIONS

At constant ionic strength, the equilibrium between a solid salt, $B_z^{+y}Q_y^{-z}$, and its saturated aqueous solution may be expressed as:

$$K_{sp(BQ)} = [B^{+y}]^z[Q^{-z}]^y \qquad \text{(Eq. 2)}$$

where $[B^{+y}]$ and $[Q^{-z}]$ are the solution molar concentrations of the ions and $K_{sp(BQ)}$ is a constant for the salt considered under the conditions employed. Although Eq. 2 completely describes the relationship between the ionic species involved, it does not necessarily express the relationship between the total concentrations or amounts of B and Q in solution. This is due to the fact that in the cases of acids and bases and their salts, in aqueous solution, the species involved in the solubility product relationship are subject to acid–base equilibria as well (8). The variation in concentration of such hydrolysis products as a function of pH and their dissociation constants is well understood (8) and will not be considered in detail here. Suffice it to say that if the pH of the solution is maintained at least 3 pH units above the pKa of the acid and 3 pH units below the pKa of the protonated base, and there are no other ionization processes involving the interacting species, the value of $K_{sp(BQ)}$ calculated on the basis of the total acidic and basic species would differ from the true value as expressed in Eq. 2 by about 0.5%, at maximun, for a 3:2 salt such as calcium phosphate $[Ca_3(PO_4)_2]$. This difference, however, falls to only about 0.2% for a 1:1 salt such as silver chloride (AgCl).

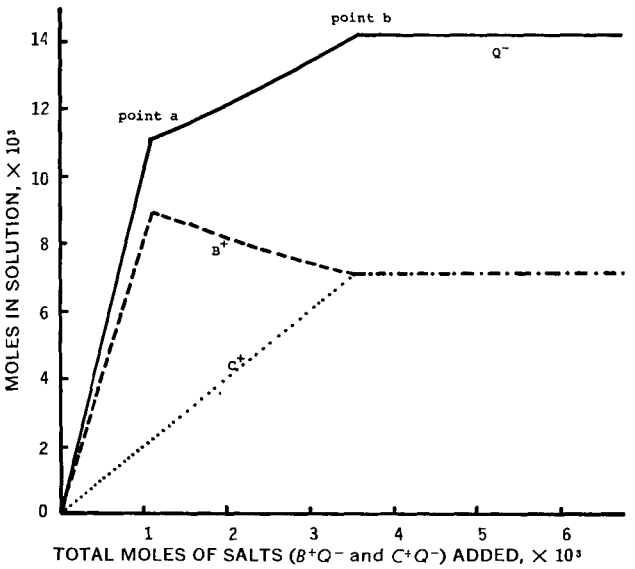

Figure 1—*Theoretical plot of the concentration of ionic species* Q^-, B^+, *and* C^+ *in solution as a function of the total moles of a mixture of salts* B^+Q^- *and* C^+Q^-, *when the solubility of all species is determined by solubility product relationships. The conditions arbitrarily chosen for the calculation were:* $K_{sp(BQ)} = K_{sp(CQ)} = 10^{-6}$ *(moles/l.)*^{2}; *the mole fractions of* B^+Q^- *and* C^+Q^- *in the mixture are 0.8 and 0.2, respectively.*

If we now consider a solid with a fixed composition of two univalent salts, B^+Q^- and C^+Q^-, where B^+Q^- is present in excess and the solubility products are identical, the concentrations of the various species in solution as a function of the salt mixture added, at a suitable pH and at constant ionic strength and temperature, is shown in Fig. 1. The curvature of the curves of $[B^+]$ and $[Q^-]$ between points a and b is due to the presence of the common ion Q^-, which is furnished by both salts as dissolution occurs. Initially, all salt added dissolves. At point a, the magnitude of $[B^+][Q^-] = K_{sp(BQ)}$; yet $[C^+][Q^-] < K_{sp(CQ)}$, so more solid CQ dissolves. The resulting increase in $[Q^-]$ causes $[B^+]$ to fall by precipitation as the salt B^+Q^- in order to satisfy the solubility product relationship. At point b, the solution is saturated with respect to both salts and further addition of the solid does not alter the composition of the solution.

It should be emphasized at this point that a similar type of curve would be expected for all compositions of salt mixtures composed of B^+Q^- and C^+Q^- and for any values of their solubility products, except when the molar ratio of B^+Q^-/C^+Q^- in the salt mixture is identical to $K_{sp(BQ)}/K_{sp(CQ)}$. In such a case, points a and b would be identical and no nonlinear relationships would be observed. In addition, if the ratio of B^+Q^- to C^+Q^- in the mixture is less than the ratio of $K_{sp(BQ)}$ to $K_{sp(CQ)}$, the salt reaching saturation first would be C^+Q^-.

This discussion indicates many important aspects of the solubility product relationship but offers little in the way of an analytical procedure. Ionizable compounds that one may wish to analyze may be present in their neutral form or in the form of salts which are very soluble in aqueous solution. Furthermore, the impurities may be similar to the major component or they may be completely different.

The development of a useful analytical technique should be able to cope with these variables in the sample to be analyzed. Suppose one wishes to analyze a sample whose primary component is a species, B, which may be present either as a salt or as a neutral molecule; but under suitable conditions in aqueous solution, B^+ is formed. In addition, suppose that there is available a second chemical species, Q, which also ionizes under the conditions used to form Q^-, which then reacts with B^+ to form a slightly water-soluble salt, B^+Q^-, whose solubility with respect to species B^+ is much less than

the original solubility of B in water. If these two suppositions are realized, the quantity of B^{+} in solution will be determined by a solubility product relationship.

The chemical species Q may be referred to as a precipitating agent or "common-ion" species. The selection of such an agent would be made on the basis of the availability of high purity material and its ability to form salts having only slight aqueous solubility with compounds of the type to be analyzed. In addition it would be highly desirable that the analysis of the agent in aqueous solution be sensitive and precise.

If one has available a precipitating agent that meets these criteria for a given class of compounds such as acids and their salts or bases and their salts, it should be possible to develop a precise analytical procedure for the determination of purity of a member of that class with no concern for the nature of any impurities or whether or not the material to be analyzed is a salt or a neutral compound.

Since the purity of substances is normally expressed on a weight basis, one should rewrite the molar solubility product relationship described in Eq. 2. This may be accomplished by expressing the molar quantities $[B^{+y}]$ and $[Q^{-z}]$ in Eq. 2 in terms of their molecular weight, the volume of solution, and the weight in grams of each dissolved species. Rearrangement of the resulting expression yields Eq. 3:

$$K'_{sp(BQ)} = (B_s^{+y})^z(Q_s^{-z})^y \qquad \text{(Eq. 3)}$$

where $K'_{sp(BQ)} = K_{sp(BQ)}M_BM_QV^2$ is a constant[2]; (B_s^{+y}) and (Q_s^{-z}) are the equilibrium amounts in grams of B and Q dissolved in a solution of V liters, respectively; and M_B and M_Q are the molecular weights of species B and Q, respectively.

Let us now consider the analysis of a bulk sample composed of two components, B and C, which are members of a class of components, all of which exist as ions at a suitable pH and form slightly soluble salts with a species, Q, which is also ionized but of opposite charge. Furthermore, let us confine this discussion to the case where the salts formed are of the 1:1 type, *i.e.*, $B_z^{+y}Q_y^{-z}$, where $y = z = 1$. Consider further a series of flasks containing equal volumes of an aqueous buffer of suitable pH and ionic strength. To each succeeding flask are added accurately weighed and increasing amounts of both pure Q and the bulk sample containing B and C. If the composition of the solid and the ratio of $K'_{sp(BQ)}$ and $K'_{sp(CQ)}$ is such that after equilibration of flasks 1 to n at constant temperature, $(B_s^{+})(Q_s^{-}) = K'_{sp(BQ)}$ and $(C_s^{+})(Q_s^{-}) < K'_{sp(CQ)}$, then only $B^{+}Q^{-}$ is precipitated in those flasks. In flasks $n + 1$ to z, $(C_s^{+})(Q_s^{-}) = K'_{sp(CQ)}$ and the precipitate of $C^{+}Q^{-}$ will also be present. In those flasks (1 to n) containing only two phases (solid $B^{+}Q^{-}$ and its saturated aqueous solution), the equilibrium is defined by Eq. 3[3].

By using Eq. 3 and known or measurable parameters for the contents of each flask, Eq. 4 can be derived (see *Appendix*):

$$[Q_a - (Q_s)]/W = xM_Q/M_B - [M_QK'_{sp(BQ)}/M_B][1/(Q_s)W] \qquad \text{(Eq. 4)}$$

where Q_a and Q_s are the weights of the precipitating agent added and that found in solution at equilibrium, respectively; W is the weight of the bulk sample added; M_Q and M_B are the molecular weights of the precipitating agent Q and the species B, respectively; and x is the weight fraction of species B in the sample.

By plotting the left-hand portion of Eq. 4 as a function of $1/(Q_s)W$ for the contents of the various flasks, a linear relationship should be obtained for which the slope is $-M_Q/M_BK'_{sp(BQ)}$ and the intercept is xM_Q/M_B. In those flasks ($n + 1$ to z) when both $B^{+}Q^{-}$ and $C^{+}Q^{-}$ are present as precipitates, the equilibrium is a function of the solubility product constants for both salts[4], and Eq. 5 may be derived (see *Appendix*):

$$[Q_a - (Q_s)]/W = M_Q/M_C[y + xM_C/M_B] - M_Q/M_C[K'_{sp(CQ)} + M_CK'_{sp(BQ)}/M_B][1/(Q_s)W] \qquad \text{(Eq. 5)}$$

where M_C is the molecular weight of species C, and y is the weight fraction of species C in the sample. Thus, a plot of $[Q_a - (Q_s)]/W$ *versus* $1/(Q_s)W$ for flasks 1 to z in this case would yield a plot with two distinct parts, both of which are linear but exhibiting different slopes. A theoretical example of such a system is shown in Fig. 2. For purposes of simplicity, Fig. 2 was constructed using $K'_{sp(BQ)} = K'_{sp(CQ)} = 0.1$ g.^{2}l.$^{-2}$, $M_Q = M_B = M_C = 100$ g./mole, $x = 0.8$, and $y = 0.2$. The line segment ST of the plot represents flasks 1 to n where only $B^{+}Q^{-}$ is precipitated. The slope for this line $= K'_{sp(BQ)} = -0.1$. The extrapolation of the line to R yielded an intercept $= 0.8 = x$.

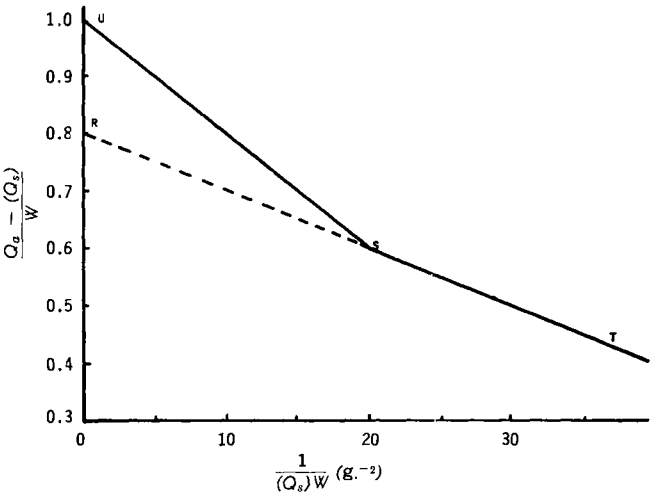

Figure 2—*Theoretical phase solubility diagram utilizing solubility product relationships as shown in Eqs. 4 and 5. The conditions chosen for this calculation were:* $K'_{sp(BQ)} = K'_{sp(CQ)} = 10^{-2}$ g.^{2}, $M_B = M_C = M_Q = 100$ *g./mole,* $x = 0.8$, *and* $y = 0.2$. *See text for further discussion.*

The left-hand portion (line segment US) of Fig. 2 represents those flasks $n + 1$ to z when both precipitates are present. In this case the slope $= -0.2 = -K'_{sp(BQ)} + K'_{sp(CQ)}$ and the intercept $= 1.0 = x + y$.

Let us now consider the behavior of a system where the bulk sample again contains two components. Suppose the major component, B, is again a species whose solubility is determined by a solubility product relationship involving the precipitating agent. The second component, D, however, is a species whose solubility is not affected by such a relationship[5]. The relationship between $[Q_a - (Q_s)]/W$ and $1/(Q_s)W$ for such a system would be expected to result in a linear relationship, as shown for the line RST of the curve in Fig. 2. There should only be one linear segment whose intercept and slope would be equal to xM_Q/M_B and $(-K'_{sp(BQ)}M_Q/M_B)$, respectively. In this case, where component D is not involved in a solubility product relationship with Q, the system behaves in the same manner as the previously described system (containing components B and C) in flasks 1 to n. Whether D is insoluble, soluble, or volatile, its effect on the system should be the same since the presence of D represents only the absence of an equivalent amount of B in the bulk sample. Since the plot obtained would be a simple linear relationship, it would not be possible to tell how many nonreactive components of the D type were present as impurities. However, it should be stressed that, in most instances, only the purity with respect to the major component is of concern.

RESULTS AND DISCUSSION

The studies thus far have been primarily concerned with the analysis of monobasic amines and their salts. The selected precipitating agent was picric acid, which meets the criteria outlined previously with regard to a good precipitating agent for amines. Picric acid is a fairly strong acid with pKa = 0.38 in water (9). Thus, at pH 3.4 and above, virtually all of the acid would be in the ionized form. In addition, picrate offers the added advantage that

[2] The units of $K'_{sp(BQ)}$ are g.$^{y+z}$ and the magnitude of the value will be dependent upon the value of solute used. In the experimental work which follows, a value of 0.1 l. was used in all cases.

[3] These conditions, *i.e.*, only one precipitate, are comparable to the conditions in Fig. 1 between points a and b.

[4] These conditions are comparable to those at and beyond point b in Fig. 1.

[5] Species of the D type would be expected to include neutral compounds such as esters and hydrocarbons, which do not form precipitates with the precipitating agent.

Table I—Data for Analysis of Pilocarpine in Pilocarpine Nitrate (Brand A) in 100 ml. of 0.2 *M* Acetate Buffer, pH 3.9, at 25°

Flask Number	W (Grams of Pilocarpine Nitrate)	Q_a (Grams of Picric Acid Added)	Q_s (Grams of Picric Acid in Solution)	$\frac{Q_a - (Q_s)}{W}$	$\frac{1}{(Q_s)W}$ (g.$^{-2}$)
1	0.0706	0.0642	0.0476	0.235	297.57
2	0.1396	0.1173	0.0449	0.518	159.54
3	0.2804	0.2335	0.0442	0.675	80.69
4	0.4188	0.3490	0.0451	0.726	52.94
5	0.5579	0.4655	0.0449	0.754	39.92
6	0.7000	0.5816	0.0442	0.768	32.32
7	0.9928	0.8658	0.0670	0.805	15.03

its analysis can be carried out directly in aqueous solution by absorption measurements at about 357 nm. Due to its high molar absorptivity (ϵ_{357} = 14,450 l./mole-cm.) at this wavelength, most other species do not interfere in the analysis. The aqueous solutions used were acetate buffers at pH 3.9.

Data for the analysis of a commercially available sample of pilocarpine nitrate are shown in Table I. An inspection of these data indicates that in preparing the contents of the various flasks, the ratio of the weights of the sample (W) and the picric acid added (Q_a) to each flask was nearly the same. This ratio corresponds to the ratio of the amine and picric acid in the expected salt. While this is not a requirement of the procedure, it is expedient since this approach results in similar concentrations of picrate in solution in all flasks at equilibrium, as indicated by column Q_s. Since all concentrations of picrate in solution are about the same, the spectrophotometric determination of picrate concentrations in all samples may then be made using identical dilution and measuring procedures, which would be expected to result in similar precision and accuracy for such measurements in all solutions. If the ratio of W and Q_a are significantly different than their ratio in the salt, there is a considerable change in Q_s in the series of flasks, which may range over more than an order of magnitude.

The variation of Q_s in the various flasks depends on the magnitude of W and Q_a as well as the ratio Q_a/W. For example, if 25% more picric acid had been used in the experiment whose results are shown in Table I, the value of Q_s would have ranged from 0.057 g. for flask 1 to 0.261 g. for flask 5. On the other hand, use of 25% smaller amounts of picric acid would have resulted in values of Q_s ranging from 0.040 to 0.010 g. From a practical standpoint, such variation would have required that some samples be diluted more than others.

The data from Table I were used in constructing curve I, Fig. 3. The relationship between $[Q_a - (Q_s)]/W$ and $1/(Q_s)W$ in the various flasks appears to be linear. The values of the slope and intercept were found to be −0.0201 and 0.8347 g.^{2}, respectively, when calculated by the least-squares best fit (10). Multiplying these values by the ratio $-M_Q/M_B$ [where M_Q and M_B are the molecular weights

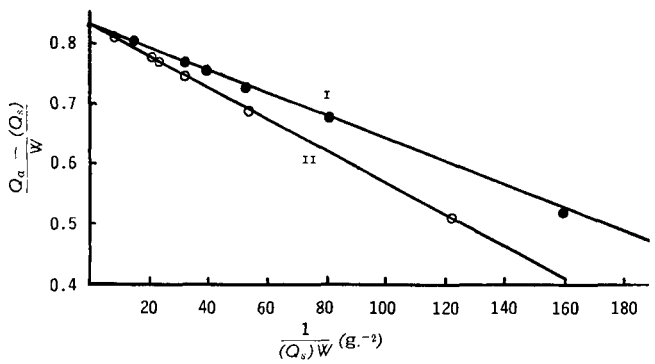

Figure 3—*Plot of the data obtained for the analysis of pilocarpine in a pilocarpine nitrate sample (Brand A) at 25°, pH 3.9, in 0.2* M *acetate (curve I) and 0.5* M *acetate (curve II) buffer, utilizing picric acid as the precipitating agent. Although seven experimental points were used in the least-squares calculation of the slope and intercept (10) for curve I, only six points are shown on this figure.*

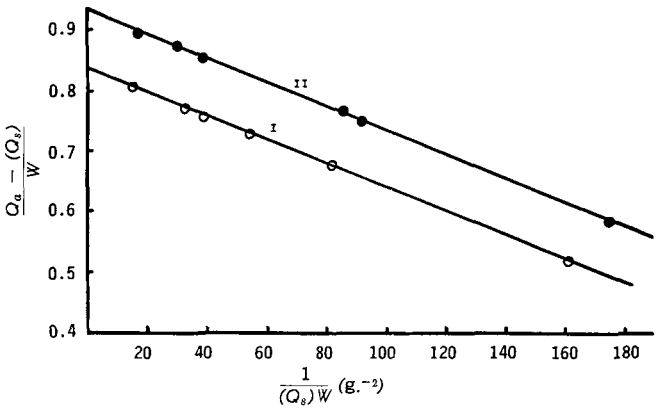

Figure 4—*Plot of the data obtained for the analysis of the pilocarpine content in samples of pilocarpine hydrochloride (curve II) and pilocarpine nitrate (curve I) (Brand B) in 0.2* M *acetate buffer, pH 3.9, at 25°. Picric acid was used as the precipitating agent.*

of picric acid (229.11 g./mole) and pilocarpine (208.26 g./mole), respectively] yields values of $K = 18.27 \times 10^{-4}$ g.^{2} and $x = 0.7587$. Thus, the purity by weight is $100x = 75.87\%$ with respect to pilocarpine in the bulk sample. The 95% confidence intervals for the purity and K_{sp}' were calculated (10) to be 75.87 ± 0.33% and (18.27 ± 0.25) × 10^{-4} g.^{2}, respectively.

Curve II in Fig. 3 shows the results from an analysis of the same sample of pilocarpine nitrate in 0.5 *M* acetate buffers, pH 3.90. The purity, as calculated for the intercept, was 75.68%, which agrees within 0.2% of that value found in 0.2 *M* acetate. The solubility product of pilocarpine picrate in the 0.5 *M* acetate system, as indicated by the slope, was 24.0 × 10^{-4} g.^{2}, which was about 35% greater than that found in 0.2 *M* acetate. These values indicate that the results relative to purity of pilocarpine in the sample are independent of ionic strength as long as it is maintained relatively constant. If this condition is not met, the solubility product will change with ionic strength and thus the results will be meaningless.

Figure 4 shows the analysis of a second sample of pilocarpine nitrate and a sample of pilocarpine hydrochloride in 0.2 *M* acetate buffer. The slope in both analyses was found to be virtually the same, while the intercept values indicate a purity of 76.32% with respect to the pilocarpine in the pilocarpine nitrate sample and 84.95% in the pilocarpine hydrochloride sample. These results substantiate the premise that the counterion of the species being analyzed has little or no effect on the analytical method, as shown by the nearly identical slopes.

Figure 5 shows the results of analyses of freshly distilled benzylamine (curve I) and a mixture of benzylamine and aniline (curve II). The amines were dissolved in acetate buffer, and aliquots of these solutions were used in the analytical procedure. It would have been equally easy to add the amines as a neat liquid since their volatility is not great. In the analysis of the benzylamine sample, the purity was found to be 99.82%. When the analysis of the mixture containing 81.00% by weight of redistilled benzylamine and 19% by weight of reagent grade aniline was carried out, the benzylamine content as calculated from the intercept value was 80.79%, which was in excellent agreement with the theoretical content. These results clearly illustrate that the presence of the second amine does not significantly affect the results obtained.

Figure 6 shows the results of analyses of different samples containing mixtures of pilocarpine nitrate with some other components. Curve I was obtained for the analysis of a mixture of the nitrate salts of isopilocarpine and pilocarpine. The theoretical pilocarpine content of the mixture, based on the supposition that the nitrate salts of each diastereoisomer were pure, was 72.89%. The content of pilocarpine found in the analysis was 72.79%, while the solubility product for pilocarpine picrate was 18.13 × 10^{-4} g.^{2}, which is in general agreement with results from other analyses of pilocarpine.

The analysis of a mixture of pilocarpine nitrate and benzylamine, containing (theoretically) 69.82% by weight of pilocarpine, gave the results shown in curve II of Fig. 6. The pilocarpine found on the basis of experimental points c, d, and e was 69.53% of the sample, which once again was in good agreement with the added amount of alkaloid. The analyses of those samples represented by curves I and

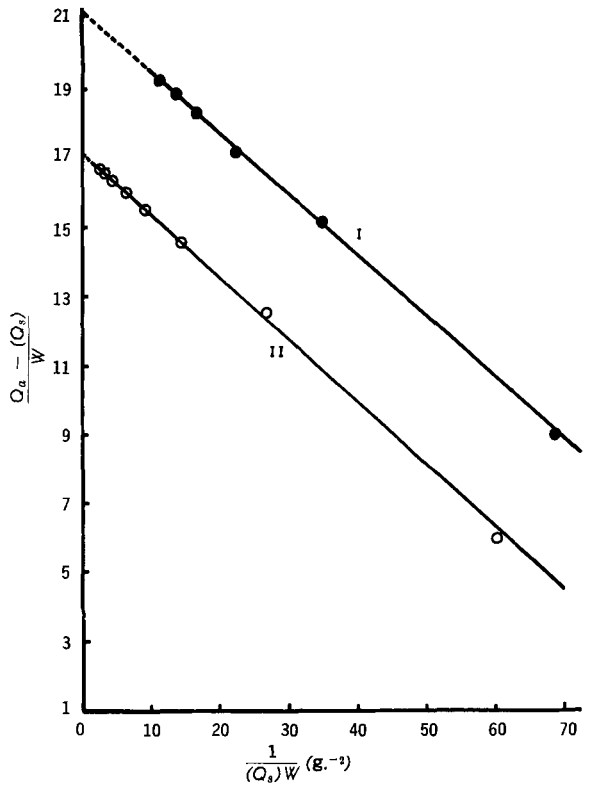

Figure 5—*Plot of the data obtained for the analysis of benzylamine in samples of freshly distilled benzylamine (curve I) and a mixture of benzylamine and aniline containing 81.00% benzylamine by weight (curve II). The analyses were carried out in 0.2* M *acetate buffer, pH 3.9, at 25° with picric acid as the precipitating agent.*

II were run in identical buffer solutions; under these conditions there was excellent agreement between the solubility products of pilocarpine picrate found in each analysis (Table II).

Curve II of Fig. 6 shows the appearance of a second precipitate (benzylamine picrate) at a high concentration of sample and picric acid (*i.e.*, as $1/Q_sW \rightarrow 0$). Although there appears to be a linear relationship between points a, b, and c, as shown by the dotted line, calculations of the benzylamine content and the K_{sp}' based on those points suggest that point c is not on the same line with points a and b. When all three points were used, the content of benzylamine in the sample was calculated to be only 7.67% compared with the expected value of 9.05%. Similarly, the K_{sp}' was calculated to be 68.60×10^{-4} g.^{2}, which was lower than the value of about 85×10^{-4} g.^{2} found in other experiments involving benzylamine (Table II). These low results are what would be expected if point c corresponds to values of $(Q_a - Q_s)/W$ and $1/Q_sW$ where the product of the quantities of benzylamine and picrate in solution is less than the K_{sp}' of benzylamine picrate.

Curve III in Fig. 6 was obtained from the analysis of a mixture of pilocarpine nitrate, benzylamine, and methylsuccinic acid in which the expected purity was 38.25% with respect to pilocarpine. The calculated value obtained from curve III was found to be only 37.33. The reason for the significant difference between these values is not clear. However, considering the complexity of the system and the relatively low concentration of pilocarpine in the samples, this result is not altogether surprising.

Also in curve III, Fig. 6, it may be seen that there is evidence suggesting that a second precipitate was present in the flask containing the largest quantity of picric acid and sample. Since only pilocarpine and benzylamine among the components present are capable of forming picrate salts, it seems reasonable to assume that the second precipitate was benzylamine picrate.

Although all of the above-mentioned analyses yielded results that were close to those expected, the accuracy of the method was still in doubt. Therefore, the analysis of mecamylamine hydrochloride was carried out. Analysis for the mecamylamine content of mecamylamine hydrochloride was carried out in both the 0.5 and 0.2 *M* acetate buffers at pH 3.9. The results are shown in Table II. Duplicate analyses were also done by normal phase solubility as described elsewhere (11). Values obtained by the USP method (11) for each analysis indicated the mecamylamine hydrochloride content of the sample to be 99.57 and 99.82%. Correcting these values to the mecamylamine content of the sample yielded values of 81.78 and 81.99%, which are in good agreement with those obtained by the new method reported here and thus substantiate in part the accuracy of the solubility product approach.

The importance of absolute methods of purity determination and the utility of this method are well illustrated by the results of an analysis of a sample of diisobutylamine. These authors were asked to analyze a sample of the liquid amine which had been freshly distilled and, according to results of GC and nonaqueous titration, was nearly 100% pure. However, the behavior of other physical properties suggested that the sample contained some impurities[6]. The phase solubility analysis with picric acid confirmed the presence of impurities at a level of 4.48%. On the basis of these results, further GC studies were carried out, which eventually resulted in detection of a second component. Although the secondary component was not isolated and characterized, the demonstration of its presence by an absolute method aided in the development of an instrumental method which was able to separate and detect the impurity.

The results of the analyses discussed in this article and several additional systems are summarized in Table II. In general, the results are in good agreement with the theoretical values based on the assumption that all components were pure. Since this assumption is quite probably an optimistic one, it is reassuring that all results obtained were less than the theoretical values.

SUMMARY

The use of the solubility product relationship in phase solubility analysis in aqueous solution has been shown to give reasonable

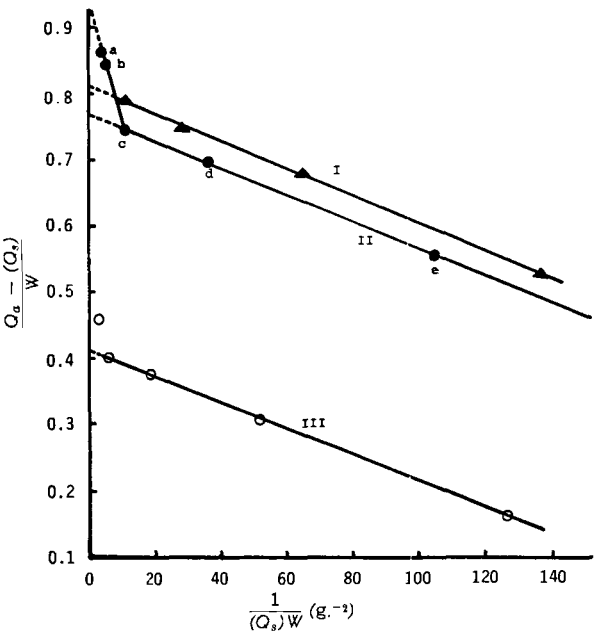

Figure 6—*Plots of the data obtained for the analysis of pilocarpine in samples containing: pilocarpine nitrate and isopilocarpine nitrate (curve I); pilocarpine nitrate and benzylamine (curve II); and pilocarpine nitrate, benzylamine, and methylsuccinic acid (curve III). All analyses were carried out in 0.2* M *acetate buffer, pH 3.9, at 25°, using picric acid as the precipitating agent. Curves II and III show the appearance of a second precipitate at low values of* $1/Q_sW$. *For the theoretical content of pilocarpine in the samples, see Table II. See text for further discussion.*

[6] I. H. Pitman and K. Uekama, these laboratories, personal communication.

Table II—Data for Analysis of Various Samples Containing Monobasic Amines

Bulk Sample	Species Analyzed	Percent Expected	Percent Found	K_{sp}' Found[a], g.^{2} × 10^4	Comment[b]
Pilocarpine nitrate (Brand A)	Pilocarpine	76.77	75.87	18.27	—
Pilocarpine nitrate (Brand A)	Pilocarpine	76.77	75.68	24.00	Used 0.5 *M* acetate buffer, pH 3.9
Pilocarpine nitrate (Brand B)	Pilocarpine	76.77	76.32	18.45	—
Pilocarpine hydrochloride	Pilocarpine	85.10	84.95	18.63	—
Pilocarpine nitrate and isopilocarpine	Pilocarpine	72.89	72.79	18.13	—[c]
Pilocarpine nitrate and benzylamine	Pilocarpine	69.82	69.53	18.12	Second precipitate formed at higher concentration
Pilocarpine nitrate, benzylamine, and methylsuccinic acid	Pilocarpine	38.25	37.33	17.89	Second precipitate formed at higher concentration
Benzylamine	Benzylamine	100.00	99.82	85.81	—[c]
Benzylamine and aniline	Benzylamine	81.00	80.79	87.13	—[c]
Atropine	Atropine	100.00	99.10	20.61	—[c]
Mecamylamine hydrochloride	Mecamylamine	82.10	81.24	23.00	—
Mecamylamine hydrochloride	Mecamylamine	82.10	81.65	31.45	Used 0.5 *M* buffer, pH 3.9
N,N-Diethylaniline	*N,N*-Diethylaniline	100.00	99.65	77.83	Neat liquid used as sample
Diisobutylamine	Diisobutylamine	100.00	95.52[d]	633.44	Neat liquid used as sample

[a] These values were obtained from experiments in which the volume of buffer was 0.1 l. To convert these values to a solubility product in terms of (moles/liter)^2, it is necessary to divide K_{sp}' by ($M_Q M_B \times 10^{-2}$). [b] All samples were weighed out except as noted. Analyses were run at 25° in 0.2 *M* acetate buffer at pH 3.9, except as noted. [c] Sample was added as a stock solution in acetate buffer. [d] This sample was known to contain some impurities of an undetermined nature (see text).

results, with an accuracy generally of the same order as seen for normal phase solubility analysis. The methodology requires further detailed investigations with respect to quantitative effects of pH, ionic strength, the effects of various impurities, and the extension of the method to other polybasic amines. These and other related studies are planned or are currently in progress in these laboratories. It appears that the reported method will solve many problems associated with regular phase solubility analysis and will complement methods presently used for the determination of purity for quality control and the development of reference standards.

APPENDIX

Derivation of Eq. 4 in the Text—Equation 3 from the text may be rearranged as shown in Eq. A1:

$$(Q_s) = \frac{K'_{sp(BQ)}}{(B_s)} \qquad \text{(Eq. A1)}$$

(B_s) may be expressed as:

$$B_s = B_a - B_p \qquad \text{(Eq. A2)}$$

where B_p is the weight of species B precipitated (as B^+Q^-) at equilibrium, and B_a is the weight of B added initially.

The value of B_a may be expressed in terms of W, the weight of the sample added, and x, the weight fraction of B in the sample, as shown in Eq. A3:

$$B_a = xW \qquad \text{(Eq. A3)}$$

B_p can be related to the weight of the precipitation agent which has been precipitated, Q_p, at equilibrium by use of the molecular weights of each species as shown in Eq. A4 (for a 1:1 salt):

$$B_p = \frac{M_B}{M_Q} Q_p \qquad \text{(Eq. A4)}$$

Furthermore, Q_p is related to the weight of the precipitation agent added initially (Q_a) and that in solution at equilibrium (Q_s) by Eq. A5:

$$Q_p = Q_a - Q_s \qquad \text{(Eq. A5)}$$

Using Eqs. A2 through A5 and substituting into Eq. A7, one obtains:

$$Q_s = \frac{K'_{sp(BQ)}}{xW - \frac{M_B}{M_Q}(Q_a - Q_s)} \qquad \text{(Eq. A6)}$$

which can be rearranged to yield Eq. 4 as shown in the text.

Derivation of Eq. 5 in the Text—A series of equations analogous to Eqs. A1–A3 can be written for a species C:

$$(Q_s) = \frac{K'_{sp(CQ)}}{C_s} \qquad \text{(Eq. A7)}$$

$$C_s = C_a - C_p \qquad \text{(Eq. A8)}$$

$$C_a = yW \qquad \text{(Eq. A9)}$$

where all subscripts have the same meaning as above, and y is the weight fraction of species C in the sample.

Combining Eqs. A8 and A9 and substituting into Eq. A7 gives Eq. A10:

$$(Q_s) = \frac{K'_{sp(CQ)}}{yW - C_p} \qquad \text{(Eq. A10)}$$

The quantity C_p may be expressed in a manner analogous to that used in the preceding derivation, as shown in Eqs. A11–A13:

$$C_p = \frac{M_C}{M_Q}\left(Q_p - \frac{M_Q}{M_B} B_p\right) \qquad \text{(Eq. A11)}$$

$$B_p = xW - \frac{K'_{sp(BQ)}}{Q_s} \qquad \text{(Eq. A12)}$$

$$C_p = \frac{M_C}{M_Q}(Q_a - Q_s) - \frac{M_C}{M_B}\left[xW - \frac{K'_{sp(BQ)}}{Q_s}\right] \quad \text{(Eq. A13)}$$

Combining and rearranging Eqs. A13 and A10 result in Eq. 5 as shown in the text.

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Cross-Resistance in *Pseudomonas aeruginosa* Resistant to Phenylethanol

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Abstract □ The effects of benzalkonium, chlorhexidine, and phenylmercuric nitrate on exponential phase cultures of phenylethanol-sensitive *Pseudomonas aeruginosa* NCTC 6750 growing in nutrient broth and on phenylethanol-resistant cells growing in nutrient broth plus 0.2% phenylethanol v/v were determined. The resistant cultures grown in the presence of phenylethanol were more sensitive to benzalkonium, chlorhexidine, and phenylmercuric nitrate than phenylethanol-sensitive cells grown in nutrient broth. Phenylethanol–antibacterial combinations were active against phenylethanol-resistant and phenylethanol-sensitive cultures. Survival times in solutions of benzalkonium, chlorhexidine, and phenylmercuric nitrate were determined for overnight *P. aeruginosa* cells grown in nutrient broth and for overnight *P. aeruginosa* cells trained to be resistant to phenylethanol and grown in nutrient broth plus 0.5% phenylethanol v/v. The cells grown in the presence of the phenylethanol were more sensitive to the action of the three antibacterials than the cells grown in nutrient broth alone.

Keyphrases □ *Pseudomonas aeruginosa* cultures, phenylethanol resistant and sensitive—effect of benzalkonium, chlorhexidine, phenylmercuric nitrate □ Phenylethanol-resistant and sensitive *Pseudomonas aeruginosa* cultures—effect of benzalkonium, chlorhexidine, phenylmercuric nitrate □ Benzalkonium effect—phenylethanol-resistant and sensitive *Pseudomonas aeruginosa* cultures □ Chlorhexidine effect—phenylethanol-resistant and sensitive *Pseudomonas aeruginosa* cultures □ Phenylmercuric nitrate effect—phenylethanol-resistant and sensitive *Pseudomonas aeruginosa* cultures

Phenylethanol was first recommended for use as a preservative for ophthalmic solutions in 1953 (1) following a report that it was active against Gram-negative organisms (2). Other workers found phenylethanol to have too slow an antibacterial action for use in ophthalmic solutions (3). It has been shown that phenylethanol exerts its antibacterial effects by modifying the permeability properties of the bacterial cell (4, 5).

Richards *et al.* (5) suggested that phenylethanol had a use in combination with other antibacterial agents in the preservation of ophthalmic solutions and other pharmaceutical solutions. Subsequently, the activities of a wide range of preservatives used in the preservation of ophthalmic solutions were shown to be enhanced when used in combination with phenylethanol (5–10).

Although there is widespread use of antibacterial combinations as preservative systems in pharmaceutical solutions (11), there is little or no published support for some of the combinations used. Neither has there been any enumeration of the properties required of an antibacterial combination or of the individual components of the combination. The following properties seem desirable for the combination:

1. The antibacterial combination should have a faster sterilization time against the test organism than the same concentration of either of the antibacterials used individually. For ophthalmic solutions, this sterilization time should be 1 hr. or less for an inoculum of *Pseudomonas aeruginosa* having a final concentration in the test system of not less than 10^6 cells/ml. (3, 9, 10).
2. The antibacterial combination should still be effective when the test organism has acquired a resistance to either one of the antibacterials.
3. The spectrum of activity of the combination should include pathogenic Gram-positive and Gram-negative bacteria and fungi.