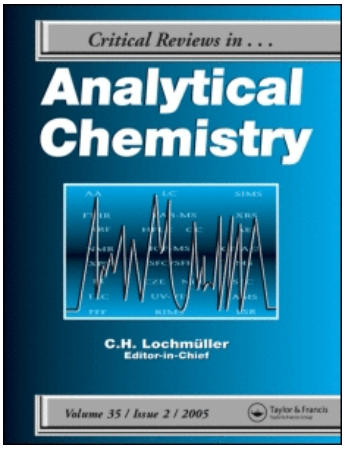

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William J. Mader; Takeru Higuchi

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PHASE SOLUBILITY ANALYSIS

Author: **William J. Mader**
Drug Standards Laboratory*
Washington, D.C.

Referee: Prof. Takeru Higuchi
School of Pharmacy
University of Kansas
Lawrence, Kansas

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I. INTRODUCTION

This review will be limited to the solubility technique[1] known as phase solubility analysis and defined elsewhere[2] as the application of precise solubility measurements to the determination of the purity of a substance. As the name implies, the method is based on the precise Phase Rule as derived by Gibbs.[3]

Constancy of a physical property, such as melting point or boiling point, has for years been used as a criterion of the purity of a compound. In like manner, constancy of equilibrium solubility usually indicates that a material is pure in composition and free from foreign admixture; conversely, variability of solubility indicates the material to be impure.

Phase solubility analysis is a nondestructive physical method for the quantitative determination of the composition of substances and is applicable to all classes and species of molecules. It requires simple, inexpensive equipment commonly available in laboratories. By this technique, one may determine simultaneously the total amount and number of impurities in a substance and the solubilities of these impurities as well as that of the main substance.

Phase solubility analysis does not require any reference standard of precisely known composition for purpose of comparison. Furthermore, neither the exact structure of the compound nor the identity of the impurities need be known. Because it is an absolute method, phase solubility analysis is directly applicable to stability determination.

The standard phase solubility analysis method consists of six distinct steps: (1) mixing, in a series of separate vessels, increasing quantities of material with measured, fixed amounts of a solvent; (2) establishment of equilibrium for each system at identical constant temperature and pressure; (3) separation of the solid phase from the solutions; (4) determination of the concentration of the material dissolved in the various solutions; (5) plotting the concentration of the dissolved materials per unit of solvent (y-axis or solution compositon) against the weight of material per unit of solvent (x-axis or system composition); and (6) extrapolation and calculation.

Although phase solubility analysis is in general use in many laboratories there have been very few publications on this subject other than brief outlines of the method in several reference works and textbooks. This is due to the very practical nature of the applications of this technique. In correspondence and discussions with scientists using the method it was evident that in some instances the method was being misapplied and misinterpreted. For this reason it was felt desirable to prepare a critical review of the method and to stress those points which may have been overlooked by some laboratories in their use of the method.

II. THE PHASE RULE

For an understanding of phase solubility analysis one must be familiar with Gibbs' Phase Rule[3] which defines the condition of equilibrium in a system by the relation between the number of coexisting phases and components. Mathematically, this rule, $F = C + 2 - P$, relates the number of components, C, the degrees of freedom (temperature, pressure, and concentration), F, and the phases, P. A phase is a homogeneous, physically distinct, and mechanically separable portion of matter. In phase solubility analysis,

temperature and pressure are maintained constant and, therefore, there is just one degree of freedom, the concentration. Hence, $F^\circ = F - 2$, and substituting in the Gibbs equation we obtain $C = P + F^\circ$, or the number of components is equal to the number of phases plus the remaining compositional degrees of freedom.

When a pure solid is brought into contact with a liquid in which it is soluble, a certain amount of it passes into solution and this process continues until the concentration reaches a definite value independent of the amount of solid present. A condition of equilibrium is established between the solid and the solution; i.e., the solution becomes saturated with the solute. For a pure solid in solution, one phase is present (two components) $F^\circ = 1 = (2 - 1)$ and one degree of freedom is possible as the concentration varies from zero to saturation. In Figure 1 this is represented by the line AB.

The condition of saturation of a solution can be defined only with respect to a solid phase; if no undissolved solid is present, the system is undefined. At any given temperature, solutions varying in concentration are possible, each containing less dissolved material than it would if the component were present in the solid form; conversely, solutions can exist which contain more of the component than corresponds to the equilibrium when the solid is present. In the former case the solutions are unsaturated, and in the latter case they are supersaturated. For a pure solid in contact with its saturated solution at equilibrium (Figure 1, BC), two phases are present, solid and solution; there can be no variation in concentration, $F^\circ = 0 = (2 - 2)$, and there are two components, solute and solvent.

Phase solubility diagrams of the type ABC indicate either that the solid is a pure material or that it is a mixture of two or more materials present in the unique ratio of their solubilities. The latter case was previously considered to be unusual but has arisen often enough that the classification no longer seems appropriate. It is treated more completely in Section VA of this discussion; except for racemic 1:1 mixtures, compounds of D- and L-isomers, or mixtures of isotopic isomers, it may be detected by changing the solvent or the temperature.

In the case of a mixture of two materials, a second type of solubility diagram is obtained (Figure 1, ADEF). With increasing amounts of solute per unit of solvent, the solution first becomes saturated with one component (Figure 1, DE) and, finally, with both components (Figure 1, EF). The slope of the line DE can be shown mathematically and by the phase rule to reflect the amount of one of the components,

FIGURE 1

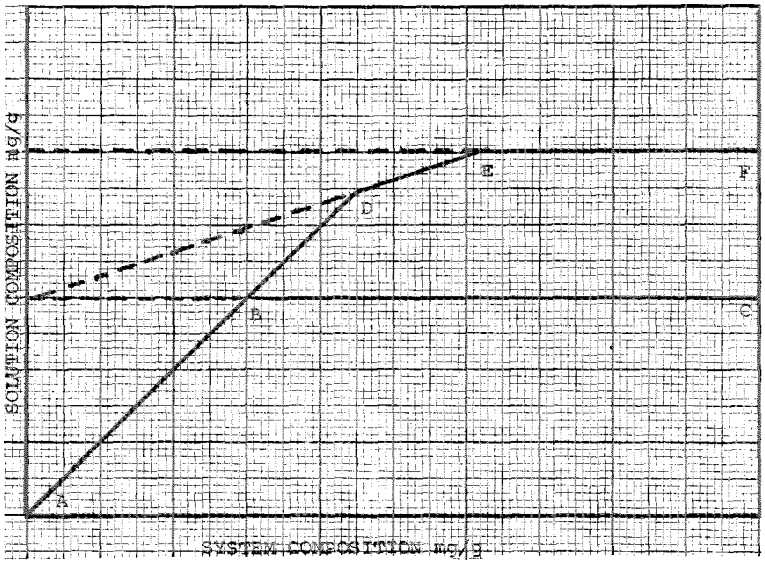
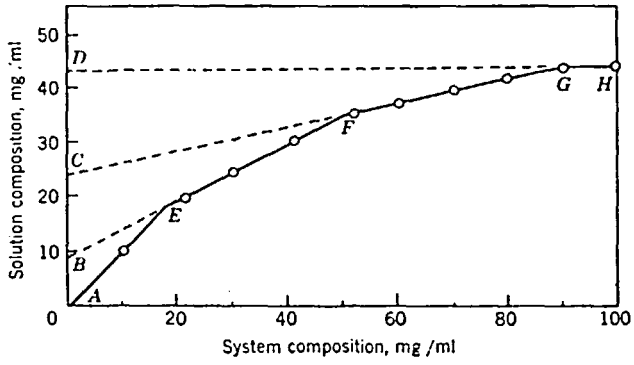


FIGURE 2



Solubility curve at 30°C for synthetic mixture of 50% DL-leucine (9.8 mg/ml), 25% DL-norleucine (12.4 mg/ml), and 25% DL-isoleucine (21.4 mg/ml) in water.

usually, but not always, the minor component. When the solvent is saturated with both components one obtains line EF. In this case, three phases are present, the solution saturated with respect to components A and B being one phase, while the solids A and B are the two other phases. Again, no variation in concentration is possible because there is no degree of freedom. A diagram of three-component mixture is illustrated in Figure 2[2] and follows the same reasoning as in the previous illustrations.

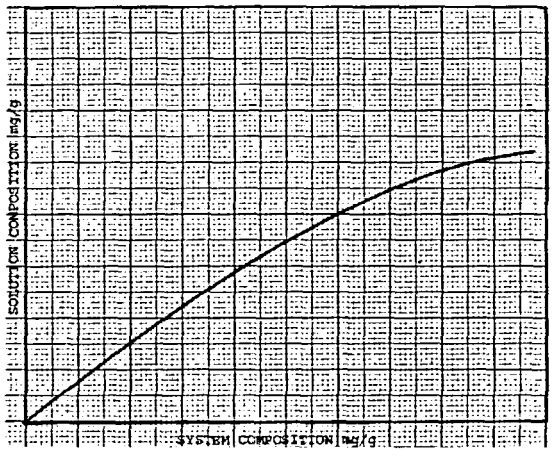
Solid solutions are those in which the solubility varies continuously as the amount of solute per unit of solvent is increased (Figure 3). In these instances no quantitative analysis by phase solubility is possible except that frequently a change of solvent may prove helpful.

III. HISTORICAL

The beginnings of phase solubility analysis can be traced back to Cohn[4] who, in 1921, suggested that the solubility of an individual protein might be taken as a criterion of chemical purity and this observation was followed in 1923 by the important work of Landsteiner and Heidelberger[5] on oxyhemoglobins. They reasoned that the solubilities of substances which do not react with each other are additive. As a consequence, if samples of two oxyhemoglobins were different, each would dissolve in a saturated solution of the other as in pure water. By this method they demonstrated that there were differences between the oxyhemoglobins of the horse, dog, rat, and guinea pig, while those of the donkey and horse were similar but probably isomorphic. During the period 1925 to 1927 Sørensen and coworkers[6] found that proteins which had been recrystallized many times, and which would have been considered "pure" by other criteria, contained several components since the solubility increased with the quantity of solid present. Their fractions may be regarded as solid solutions.

Northrop and Kunitz,[7] in 1930, presented the first theoretical discussion of solubility curves

FIGURE 3



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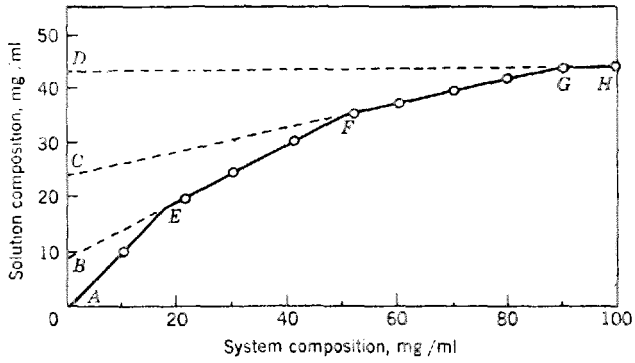

Solubility curve at 30°C for synthetic mixture of 50% DL-leucine (9.8 mg/ml), 25% DL-norleucine (12.4 mg/ml), and 25% DL-isoleucine (21.4 mg/ml) in water.

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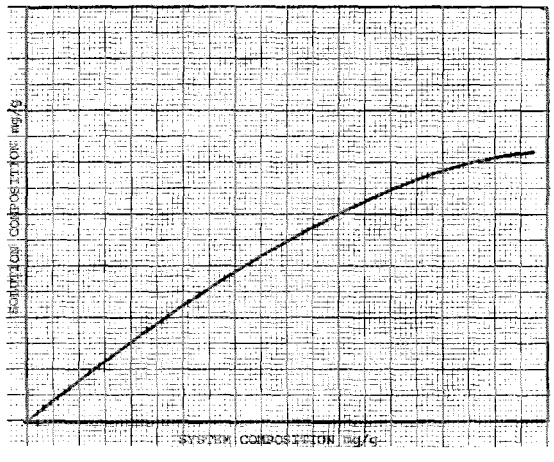


of mixtures and solid solutions of two components, which was followed by experimental analysis on chymotrypsinogen, trypsin, artificial mixtures of α and β chymotrypsin which gave a true solubility diagram and for α and β chymotrypsin which formed a "solid solution." Butler[8] restudied the solubility of chymotrypsinogen and established the conditions for applying the phase rule to proteins. By phase solubility analysis, Herriott, Desreux, and Northrop[9] showed that crude pepsin consisted of more than one protein. Purity could be determined for such proteins as swine pepsin,[10] salmon pepsin,[11] ribonuclease,[12] the luteinizing hormone of swine, metakentrin,[13] the lactogenic hormone of beef and sheep,[14] the oxytocic, pressor, and antidiuretic hormone from beef pituitary,[15] and diphtheria antitoxin.[16]

The literature during the period of 1921 to 1948, which was cited above, shows that phase solubility analysis is a very useful tool to the biochemists. However, in 1948 Webb,[17] of Merck and Company, and Herriott,[18] of the Rockefeller Institute for Medical Research, brought the general utility of the method to the attention of chemists other than biochemists. Both authors presented highly theoretical discussions of the applicability of the method. Webb was interested primarily in the application of phase solubility analysis to determinations of the purities of new substances.

Since the publications of Webb and of Herriott there have been only five papers on solubility analysis reported in the journals since 1953. Tarpley and Yudis[19] reported on the Determination of the Purity of Steroids, Outch[20] published on Phase Solubility Analysis in Quality Control, Commer & Howell[21] published on the use of Solubility Analysis in Pharmaceutical Stability Studies, and Garratt, Johnson, and King[39] reported on Phase Solubility Analysis: an Evaluation of the Technique.

The technique has been described in several reference books; namely, *Organic Analysis,* Volume 2, published by Interscience; *Advances in Analytical Chemistry and Instrumentation,* Volume 4, published by Interscience; *Physical Methods of Organic Chemistry,* Volume 1, Part 1, Interscience; a *Textbook of Pharmaceutical Analysis* published by John Wiley; and *Treatise on Analytical Chemistry,* Part 1, Volume 7, published by Interscience. In addition, the method is described in detail in the National Formulary XIII and the United States Pharmacopeia XVIII. There have been several oral presentations on phase solubility analysis which have not been published.

IV. TECHNIQUE

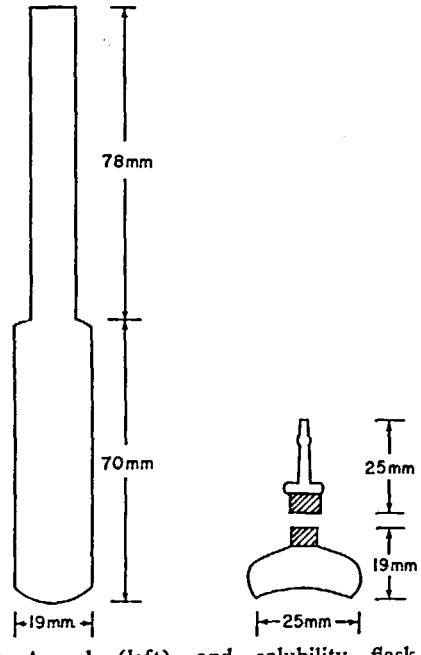
Phase solubility analysis is so simple a technique that one would expect all laboratories would adequately apply the method, but, unfortunately, some laboratories use techniques which are improper and indicate a lack of understanding of the basic principles involved. For the purpose of this review, the uniformly successful procedure will be given (from National Formulary XIII) and then each step will be discussed.

a. Standard Procedure

Note: Make all weighings within ±10 mcg)

System Composition—Weigh accurately, in grams, not less than 7 scrupulously cleaned, 15-ml ampuls (Figure 4). Weigh accurately,

FIGURE 4



Ampul (left) and solubility flask (right) used in phase solubility analysis.

in grams, increasingly larger amounts of the test substance into each of the ampuls. The weight of the test substance is selected so that the first ampul contains slightly less material than will go into solution in 5 ml of the selected solvent, while the second and subsequent ampuls contain slightly more than the indicated solubility. Pipet 5.0 ml of the solvent into each of the ampuls, cool in a dry ice-acetone mixture, and seal, using a double jet air-gas burner and taking care to save all glass. Allow the ampuls plus their contents to come to room temperature, and weigh the individual sealed ampuls with the corresponding glass fragments. Calculate the system composition, in mg per g, for each ampul by the formula $1000 (W_2 - W_1)/(W_3 - W_2)$, in which W_1 is the weight of the empty ampul, W_2 is the weight of the ampul plus test substance, and W_3 is the weight of the ampul plus test substance, solvent, and separated glass.

Equilibration—Effect equilibration by rotating the ampuls end-over-end at about 25 rpm in the constant-temperature bath. The time required for equilibration in the rotating procedure varies from 7 to 14 days, depending upon the nature of the substance being tested. In order to determine if equilibration has been effected, one ampul (the next to the last in the series) may be warmed to 40° to produce a supersaturated solution. Equilibration is assured if the solubility obtained on the supersaturated solution falls in line with the samples which approach equilibrium from an undersaturated solution.

Solution Composition—After equilibration, place the ampuls vertically in a rack in the constant-temperature bath, with the necks of the ampuls above the water level, and allow the contents to settle. Open the ampuls and remove a 2.0-ml portion from each by means of a pipet equipped with a small pledget of cotton to serve as a filter. Remove the cotton, transfer the aliquot of clear solution from each ampul to a marked, tared solubility flask (Figure 4), and weigh each flask plus its solution to obtain the weight of the solution. Cool the flasks in a dry ice-acetone bath, and then evaporate the solvent in a vacuum. Gradually increase the temperature to 70° to 100°, and dry the residue to constant weight. Calculate the solution composition, in mg per g, by the formula $1000 (F_3 - F_1)/(F_2 - F_3)$, in which F_1 is the weight of the solubility flask, F_2 is the weight of the flask plus solution, and F_3 is the weight of the flask plus residue.

Calculation—For each portion of the test substance taken, plot the solution composition as the ordinate and the system composition as the abscissa. The points for those containers which represent a true solution should fall on a straight line (Figure 1, AB) with a slope approaching 1, passing through the origin; the points corresponding to saturated solutions should fall on another straight line (Figure 1, BC), the slope, S, of which represents the fraction of impurity or impurities present in the test substance. *Failure of points to fall on a straight line indicates that equilibrium has not been achieved.* Calculate the per cent purity of the test substance by the formula $100 - 100S$. The slope may be calculated by the formula $(Y_2 - Y_1)/(X_2 - X_1)$, in which Y_2 and Y_1 represent solution compositions, and X_2 and X_1 represent the respective system compositions, at convenient points on the second straight line.

The solubility of the main component is obtained by extending the solubility line (BC) through the Y-axis. The extrapolated solubility, obtained in mg per g, should be constant for a given compound.

b. Solvents

Care must be exercised in the selection of a proper solvent. Mader[2] listed the following criteria in selecting a proper solvent:

(1) The solubility of the sample should be in the range 0.4 to 2.5% (4–25 mg/g) and not outside the range 0.1 to 7.5% (1–75 mg/g).

(2) The solvent should not affect the sample adversely. Solvents which cause decomposition or react with the sample cannot be used. Solvents which solvate or form salts have been used, although such solvents should be avoided if possible.

(3) The solvent should be of known purity and composition. Such solvents as petroleum ether, higher acetates, etc. are to be avoided due to variability in composition. Trace impurities may affect the solubility

greatly.[40] Carefully prepared mixed solvents are permissible.

(4) A very volatile solvent is difficult to measure and a high-boiling solvent offers difficulty in removal. The desirable boiling range is between 65° and 100°.

Although the boiling point of the solvent should preferably be between 65° and 100°, solvents with boiling points outside this limit have been used successfully. Table 1 lists some commonly used solvents.

TABLE 1

Some Solvents Commonly Used in Phase Solubility Analysis

Acetone and aqueous mixtures
Ethanol and aqueous mixtures
Methanol and aqueous mixtures
Isopropanol and aqueous mixtures
n-Propanol and aqueous mixtures
Pyridine and aqueous mixtures
Chloroform and alcoholic mixtures
Ethyl acetate
Benzene
Dioxane
Dimethylformamide (DMF)
Hexane
Cyclohexane
Water

The purity of a solvent is extremely critical for some compounds: the lower the solubility, the greater the likelihood that the purity of the solvent will be important. A compound may not be adversely affected by a pure solvent but could be changed considerably by an impurity in the solvent. For example, a phase diagram of a compound which forms a hydrate could become quite complicated in the presence of insufficient water in the solvent to completely change the compound to a hydrate.

There is some difference in statements regarding the preferred solubility range. Mader[2] suggested 4 to 25 mg/g of solvent; MacMullan[22] recommends 1 to 20 mg/g; Trenner[23] suggests 10 mg/g. The upper suggested limit of 75 mg per gram of solvent is based on the difficulty of properly agitating or mixing some compounds in a tube containing 75 or 100 mg of solid per gram of solvent. Good phase diagrams have, however, been obtained at the higher concentrations; for example, D- or L-arabinose in 75% ethanol at 75 mg/ml, DL-valine in water at 74.8 mg/ml, and L(-)hydroxyproline in 50% ethanol at 70.5 mg/ml.

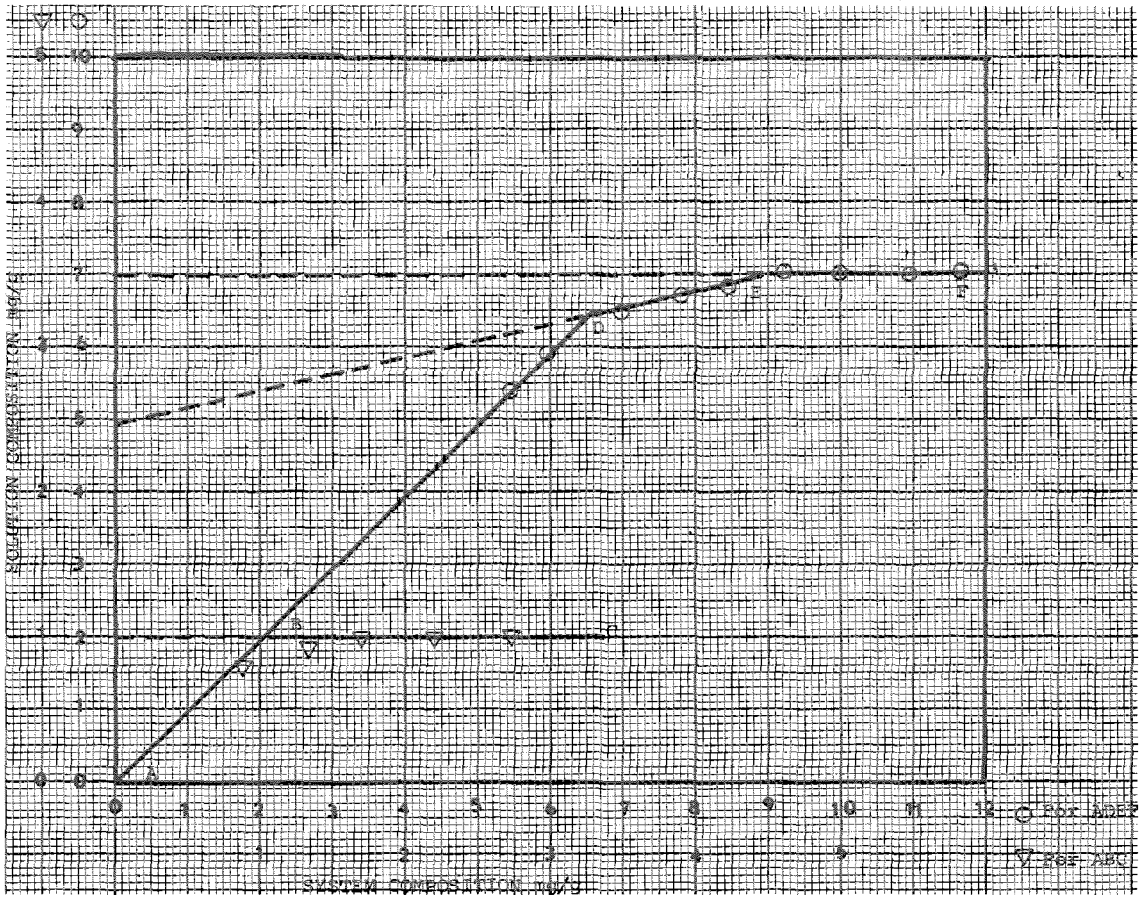
In selecting the solubility range to work in, one must take into consideration the sensitivity and accuracy of the balance being used and the experience of the analyst. Figure 5 illustrates the dangers of selecting a solvent in which the main component exhibits a low solubility, as compared to one in which the solubility is greater. The curve ABC represents the analysis of a pharmaceutical substance in which acetone was used as a solvent, the solubitity being slightly less than 1 mg/g. The curve ADEF was obtained using 90% ethanol, in which the solubility of the compound is 5 mg/g. The slope of DE is 20%, representing a purity of 80%. The best one can say about the system using acetone is that additional points close to the intersection of AB and BC might possibly define the product qualitatively as impure. No confidence in numerical estimates would be possible. The curve ADEF indicates two components, the major one with a solubility of 5 mg/g and the minor one having a solubility of 2 mg/g.

It can be shown mathematically that a two-component system containing 95% A and 5% B, each with a solubility of 1 mg/g, will have a coefficient of variation of ±20% for calculated values of purity, even if the error in weighing is only ±10 micrograms and if all other factors are equal.

c. Particle Size

The solubility one obtains for a solute in a solvent at equilibrium is independent of particle size (colloidal suspensions excepted). The rate of attaining equilibrium, however, is a function of several factors, including particle size. Noyes and Whitney[28,29] gave this equation for dissolution rate: $dc/dt = k\ (C_s - C)$, where C is the concentration of the solute in the solvent at time t, C_s is the solubility of the solute in the solvent, and k is a constant with the dimension of reciprocal time. Bruner and

FIGURE 5



St. Tolloczko[30] included the surface area so that the equation is:

$$dw/dt = k_1S(C_s - C)$$

Here S is the surface area of the solute, dw is the mass of the solute entering solution, and the first-order rate constant k has the dimension mass area $^{-1}$ time $^{-1}$. It has been stated that small particles[31,32] have greater solubility than those having plane surfaces. This has been discussed by others[33-35] and the conclusion is that, given sufficient time for equilibration, both small particles and large ones will reach a normal solubility value. This is obtained by the process of small particles going into solution and increasing the size of insoluble particles. Ostwald ripening and lattice-distortion effects are transient. With very fine particles spurious results may be obtained because colloidal suspensions are formed and make it impossible to separate the phases completely prior to analysis.

The solubility diagram in Figure 8 illustrates the effect of super-solubility of fine particles: supersaturation is obtained in the first two tubes equilibrated for two days. This is possibly due to a lack of nuclei on which the crystals form and again illustrates the necessity of allowing sufficient time for equilibration. It has been suggested that since equilibrium is more difficult to obtain at or near the saturation point this area should be avoided. On the contrary, it is this area which will indicate in many instances whether a compound is pure or impure.

d. Weight of Samples

Normally, five to ten ampuls containing increasing portions of sample are used in a determination. The National Formulary recommends seven points. Most workers use one unsaturated point and the remaining saturated. It is highly desirable that as many points as possible be in the region of first saturation. The need for this is evident from the study of a pure (Figure 6) and an impure (Figure 7) sample of 2-phenylazo-*p*-cresol. Without points in the section AB on the diagram of the impure compound, one would not be able to define the purity of this compound; further, if one did not know the true solubility (14 mg/g) of the pure compound, one might be led to assign a purity of 94.8% to the compound which, in fact, is only 82% pure.

For a relatively pure compound sample weights representing 90 to 150%, in increments of 10%, of the true equilibrium solubility will normally give a reasonable distribution of points. It is always desirable to cluster as many points as possible in the region of first saturation. This is based on the assumption that most processes of isolation and purification would have impurities which, when taken with proportions, are relatively more soluble than the main component.

e. Equilibration

Solubility is an equilibrium phenomenon and, unless equilibrium has been established, the analysis is valueless.

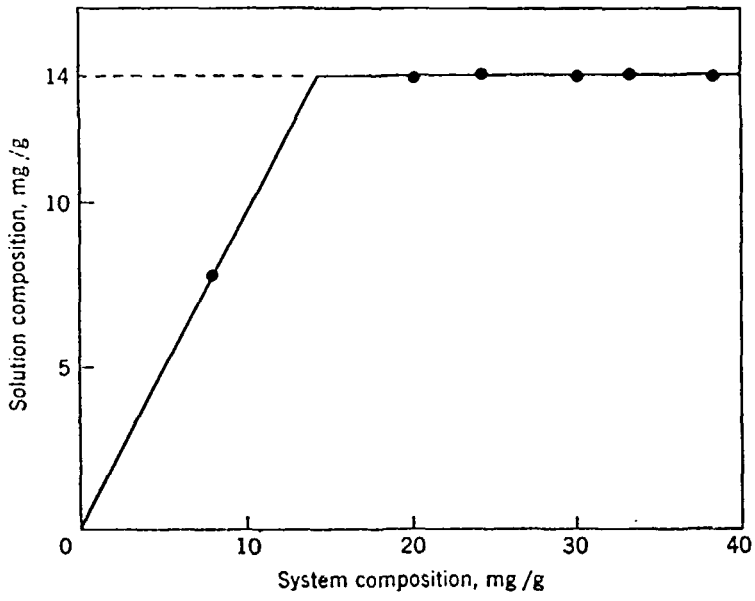
Scattering of the points in a solubility diagram is a clear warning that equilibrium has not been reached (Figure 8). This will also be reflected in unusually broad confidence ranges after least-squares calculation.

Two methods of equilibration are in general use, the vibrational procedure and the end-over-end rotational method. Shaking, stirring, and ultrasonic agitation have also been suggested, but practically all phase solubility analyses are carried out by the vibrational or rotational methods. Those who use the vibrational method feel very strongly that it has advantages over the rotational one, and vice versa.

The proponents of the vibrational method claim that the method is more rapid. Outch[20] claims that equilibration is achieved in 8 to 16 hours as compared to 2 to 14 days for the rotational method; MacMullan[22] claims that it is achieved uniformly in approximately 24 hours. Actually, the time required for equilibration is of secondary importance. The work involved in preparing ampuls for equilibration, removing the samples after equilibration, and calculation, i.e., the working time, is identical; only the elapsed time is different. The important question is whether one method is more reliable than the other method. Dr. Bengt Ohrner of the World Health Organization Apotekens Centrallaboratorium[24] determined the purity of chloramphenicol using the vibrational technique, "equilibrating" for 65 hours. He obtained purities of 99.66% and 99.65%. The Drug Standards Laboratory, using the identical sample but equilibrating by rotation end-over-end for 14 days, obtained a purity of 99.63%. In this instance, the agreement between the two laboratories is exceptionally good. DeAngelis and Papariello,[25] in their paper on differential scanning calorimetry, obtained solid solutions by mixing *p*-ethoxyacetanilide with *p*-chloroacetanilide and acetanilide. A mixture comprising 1.0% acetanilide and 1.0% *p*-chloroacetanilide with the remainder pure *p*-ethoxyacetanilide assayed at 98.2% when equilibrated by rotation for 14 days, whereas the same mixture equilibrated 30 hours by vibration indicated only a solid solution.

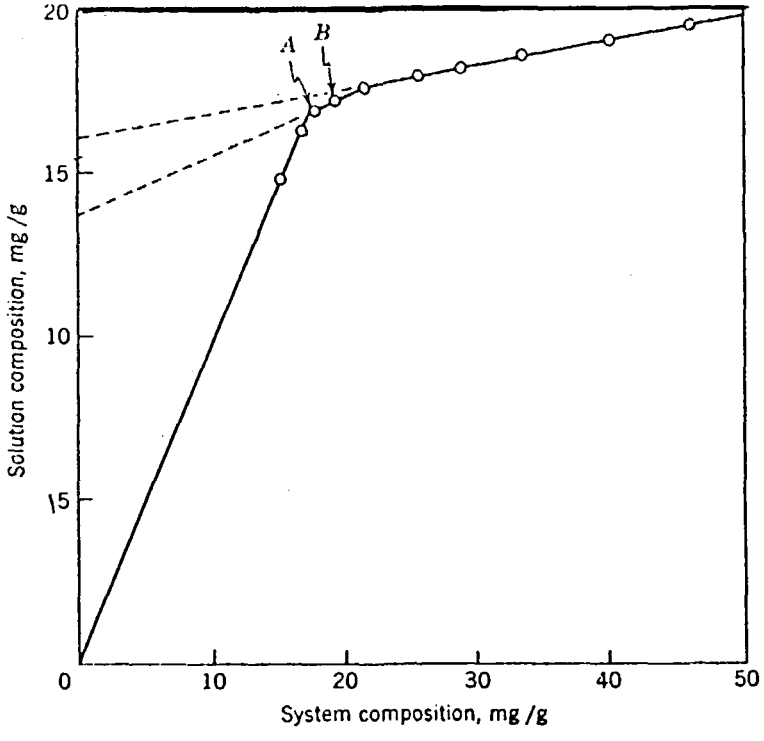
Thus, the proponents of the vibrational method claim that it gives faster equilibration and involves less chance of extensive degradation, while those favoring rotational equilibration state that the overall time may be less when the vibrator is used but that the actual working time is identical and that the greater reliability of the rotational method is well worth the extra time. As for the degradation of the compound, if it degrades in 7 to 14 days it will also degrade in the time to equilibrate by vibration. There is some question as to whether the energy supplied by the vibration may not hasten degradation. It has been observed[9] not only that the rate of solution is proportional to the rate of stirring, but also that the rate of decomposition increases with increasing stirring rate. For routine analysis and process control, the vibrational method

FIGURE 6



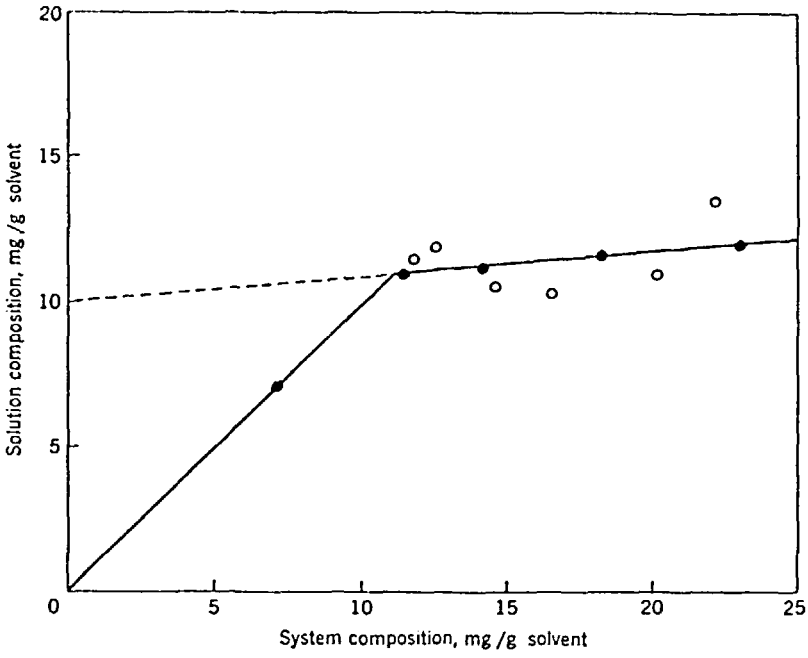
Solubility curve for 2-phenylazo-*p*-cresol in absolute ethyl alcohol at 25°C; solubility, 13.98 mg/g; slope, 0.4%.

FIGURE 7



Solubility curve for 2-phenylazo-*p*-cresol in absolute ethyl alcohol at 25°C; slope, 18%.

FIGURE 8



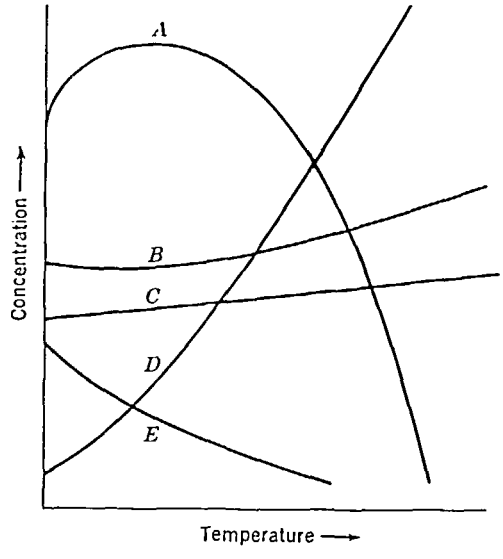
Solubility curve and equilibration time at 30°C for DL-methionine in 50% ethyl alcohol: white circles, two days; black circles, seven days; slope, 8.8%.

may be advantageous after its reliability has been proven by comparison with the rotational method.

Two methods are in general use for determining if equilibration has been reached. The first method depends upon reaching saturation from the supersaturated side and is accomplished by heating one tube, usually the next to the last tube in the series, about 10° above the temperature of saturation. Solid must be present at all times. If, after completion of the analysis, this point falls between those obtained for the preceding and following tubes, one is assured that equilibrium has been reached. MacMullan[22] has proposed that the attainment of equilibrium be established by following the solution composition at one fixed system composition as a function of time.

Various types of holders or clamps have been devised for holding the ampuls in the vibrational method. It has been observed that in those types of holders where the tubes are in a line and parallel to the shaft, the tube nearest to the shaft reaches equilibrium faster than those farthest from the shaft. For this reason, circular holders where the ampuls are equidistant from the shaft are preferred.

FIGURE 9
Temperature-Concentration Curves



f. Temperature

Phase solubility analyses can be carried out at any constant temperature. For convenience, usually 25 or 30° is used. Temperature may have so great an effect on solubility that a good thermostated bath is essential.

Generally, the solubility of a solid increases with increasing temperature (Figure 9, curve D). However, there are available examples that illustrate little or no change (curves B and C), or even a decrease (curve E), with increase of temperature. The solubility curve may exhibit a maximum (curve A) or a minimum (curve B). The relative temperature error in phase solubility analyses will reflect the temperature solubility curves of the components.

Temperature control within 0.1° is usually sufficient. For example, L-proline has a solubility of 12.8 mg/ml in absolute alcohol at 30° and 11.8 mg/ml at 25°. A variation of 0.1° will cause a change of 0.02 mg/ml in the solubility, which is well within the experimental error of the method.

g. Calculation

The standard procedure calls for plotting the solution composition as ordinate against the system composition as abscissa. In addition to determining the purity P from the slope of the line BC, one can also compute it by the least squares method with a 95% confidence limit by the formula

$$P = 100\,\frac{n\Sigma xy-\Sigma x\Sigma y}{n\Sigma x^2-(\Sigma x)^2} \pm 100\,t\sqrt{\frac{[n\Sigma x^2-(\Sigma x)^2][n\Sigma y^2-(\Sigma y)^2]-[n\Sigma xy-\Sigma x\Sigma y]^2}{[n\Sigma x^2-(\Sigma x)^2]^2(n-2)}}$$

where

- x = mg solute/g solvent
- y = mg residue/g solvent
- n = number of values above the saturation point
- t = Student's t for a level of confidence of 95% and (n — 2) degrees of freedom.

Commer and Howell[21] use a slightly different approach for calculating the purity.

$$\theta = \frac{[n\Sigma x^2-(\Sigma x)^2]-[n\Sigma y^2-(\Sigma y)^2]}{2[n\Sigma xy-\Sigma x\Sigma y]} \pm \sqrt{\left[\frac{[n\Sigma x^2-(\Sigma x)^2]-[n\Sigma y^2-(\Sigma y)^2]}{2[n\Sigma xy-\Sigma x\Sigma y]}\right]^2 + \lambda}$$

where x, y and n are as above and $\lambda = 1.0$ for linear bivariant data. Alternatively, one may write

$$\theta = \frac{\sqrt{[n\Sigma x^2-(\Sigma x)^2][n\Sigma y^2-(\Sigma y)^2]-[n\Sigma xy-\Sigma x\Sigma y]^2 t}}{\sqrt{\frac{([n\Sigma x^2-(\Sigma x)^2]-[n\Sigma y^2-(\Sigma y)^2])^2}{4} + [n\Sigma xy-\Sigma x\Sigma y]^2(n-2)}}$$

Per cent purity = $100 - 100\,\theta$

where

$$\text{Maximum Percent Purity} = 100 - 100 \tan\left[\arctan\theta - \frac{\arcsin\theta}{2}\right]$$

$$\text{Minimum Percent Purity} = 100 - 100 \tan\left[\arctan\theta + \frac{\arcsin\theta}{2}\right]$$

Breunig[26] suggested that *n* be not less than 7, at which point the values of t "level out."

V. PROBLEMS IN INTERPRETATION

a. Unique Ratio

Webb[17] has stated that phase solubility analysis becomes exceedingly simple in principle if (i) the impurity or foreign entity, i.e., the second component, is present as a distinct phase, (ii) the ratio of the two components does not coincide with a certain unique ratio, and (iii) the contaminants are present in substantial quantities, say greater than 0.1%.

Seldom does one run into instances when phase solubility analysis does not work due to the "unique ratio" of solubility to composition. Instances are available where the wrong interpretation has been applied due to the ratio of solubility to composition approaching equality. This is usually not critical since a change of solvent will change the solubility ratio, permitting logical analysis.

For a two-component mixture the ratio of the solubilities, S, of *a* and *b* can be either equal to, greater than, or less than the ratio of the concentrations, C, of *a* and *b*. A solubility analysis of a 50:50 mixture of D- and L- isomers will fail as the solubilities of the two isomers are equal in all solvents.

When $S_a/S_b = C_a/C_b$, phase analysis alone will give erroneous results, indicating a single, pure component. For other than optical isomers a change of solvent will generally change the solubility ratio sufficiently to permit analysis. Aminoacetic acid and DL-alanine have equal solubilities (27 mg/ml) in 50% ethyl alcohol at 30°, and a 1:1 mixture of the two in this solvent will assay as a single component with an extrapolated solubility of 54 mg/ml. An even better example is a mixture of DL-valine and DL-leucine. The solubility of the first is 75 mg/ml in water at 30°; that of the latter is 10 mg/ml. The ratio of these solubilities is 7.5 at 30°. A mixture of 88.5% DL-valine and 11.5% DL-leucine would have a ratio of 7.4. The phase diagram is illustrated in Figure 10. The slope of the line is zero; therefore, the mixture assays as pure and the extrapolated solubility is 85 mg/ml, the combined solubility.

In those cases where $S_a/S_b < C_a/C_b$, a normal phase diagram is obtained. As an example of this type of diagram, a mixture of 92% DL-valine and 8% DL-leucine would have a diagram illustrated in Figure 11. Here the ratio of the concentrations is 10.5, which is greater than the solubility ratio. The slope of the line is 8, and it would, therefore, be concluded that the sample contained 92% DL-valine and 8% DL-leucine, while the extrapolated solubility is 75 mg/ml, the solubility of the pure DL-valine.

Where the ratio of solubilities is greater than the ratio of concentrations the solubility diagram is reversed. For example, for a mixture containing 85% DL-valine and 15% DL-leucine, the concentration ratio is 5.7, which is less than the solubility ratio. The solubility data are plotted in Figure 12. The slope of the line BC is 85% and the extrapolated solubility is 10 mg/ml, the solubility of DL-leucine.

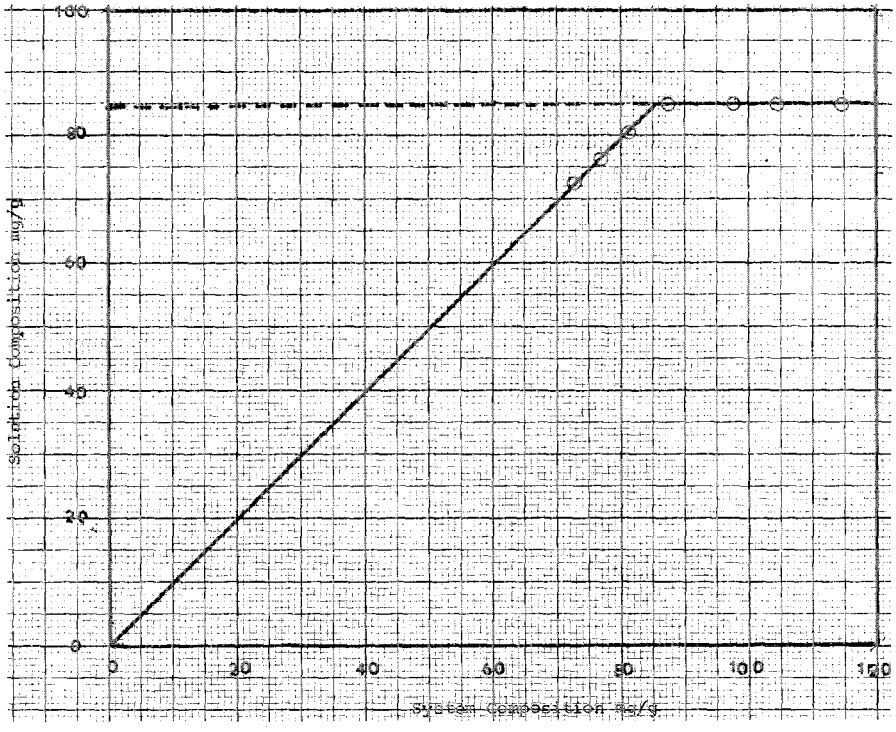
The study of Figures 10, 11, and 12 indicates clearly the necessity of obtaining points close to the saturation point of the compound and the importance of the extrapolated solubility. In these three instances the extrapolated solubilities were 10, 75, and 85 mg/ml; this is more than sufficient evidence to call the analyst's attention to the fact that something is amiss and suggest a change of solvent. A series of similar samples assayed in identical solvents should give identical extrapolated solubilities.

b. Optical Isomers

Herriott[18] states that phase solubility analysis is sufficiently sensitive to distinguish between D- and L- optical isomers although as an unknown a 1:1 mixture would behave as a single component.

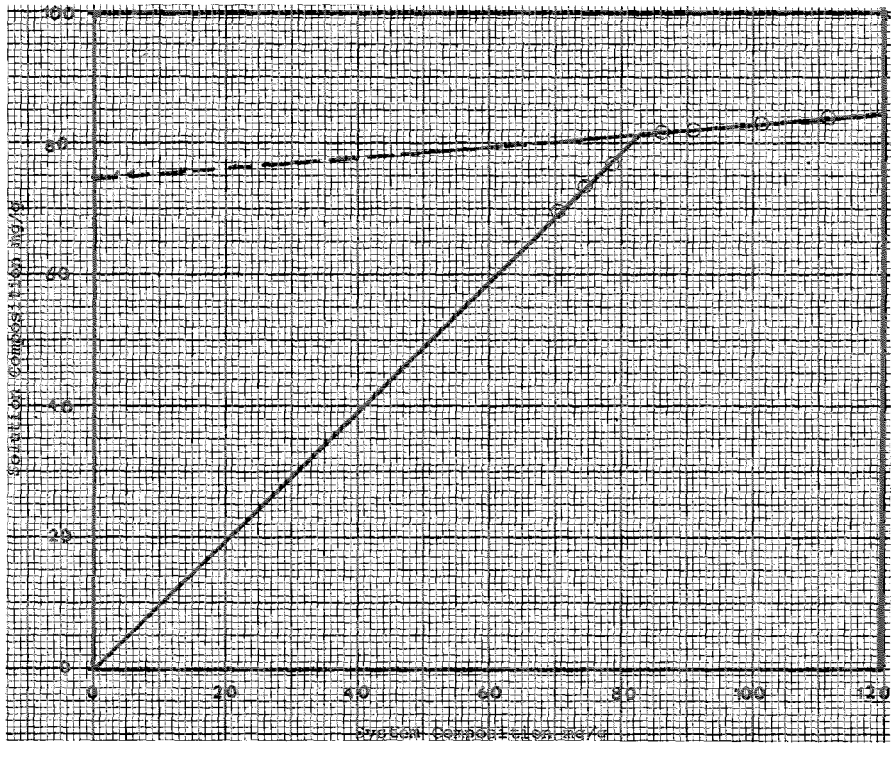
D- and L- isomers present in equal amounts may form a single inactive mixture (a DL- mixture) which is a mechanical mixture of individual crystals of the dextro and levo forms, or they may form a racemic compound in which a pair of enantiomorphs unite to form a molecular compound, all the crystals of which contain equal amounts of both isomers and are identical. In solution almost complete dissociation occurs. In the case of the optically inactive crystalline mixture, the crystals are present as pure D- isomer and pure L- isomer, and this was the basis of the classical separation of tartaric acid by Pasteur. D- and L- isomers have equal melting points and equal solubilities. A

FIGURE 10



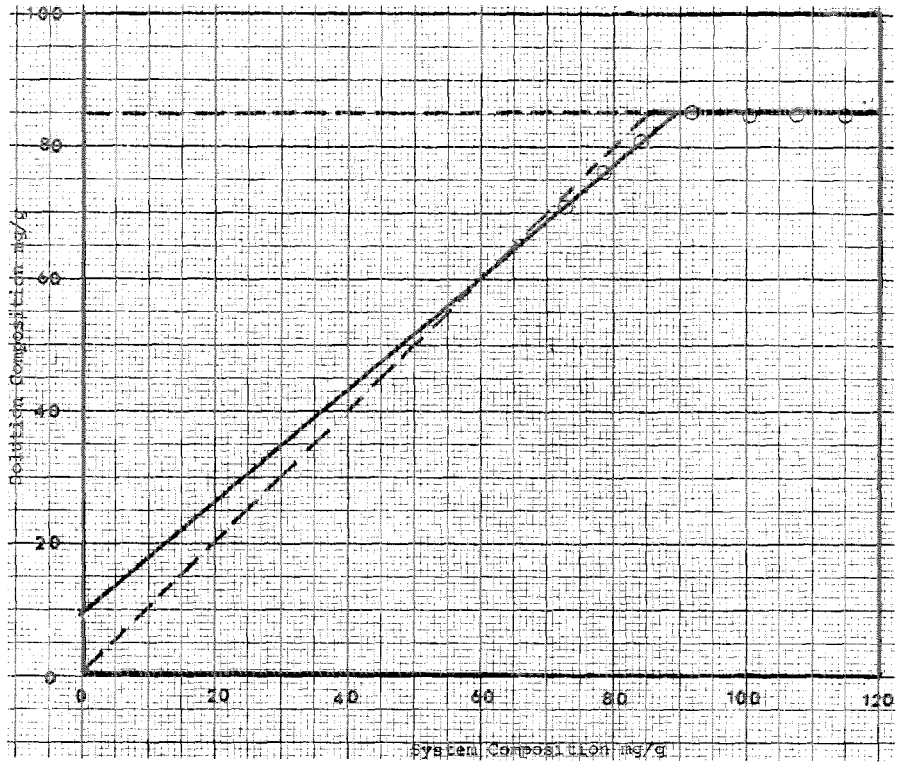
Solubility diagram for a mixture containing 88.5% DL-valine and 11.5% DL-leucine. Ratio of solubilities 7.5, ratio of concentrations 7.4.

FIGURE 11



Solubility diagram for a mixture containing 92% DL-valine and 8% DL-leucine. Ratio of solubilites 7.5, ratio of concentrations 10.5, slope 8%.

FIGURE 12



Phase diagram for a mixture containing 85% DL-valine and 15% DL-leucine. Ratio of solubilities 7.5, ratio of concentrations 5.7, slope 85%.

racemic mixture will have a lower melting point (eutectic) and the solubility will be twice that of the individual isomer, whereas a racemic compound will generally have a higher melting point and lower solubility than the individual isomer.

In either case, a racemic compound or a racemic mixture will behave in phase solubility analysis as a single pure component. Suppose one were to add D- or L- isomer to a racemic compound so that one would have, for example, 60% D- and 40% L- present. The phase diagram would indicate a two-component system, 80% DL- and 20% D-; all of the L- isomer would react with an equal amount of the D-isomer to form 80% DL- isomer, leaving 20% of the D- isomer unreacted. If the racemate, on the other hand, is a mixture, the 1:1 mixture would assay as a single component but the addition of either the D- or the L- isomer would resolve the mixture. In this case, a mixture of 60% D- isomer and 40% L- isomer would assay as a 60:40 mixture.

In 75% ethyl alcohol at 30°, D- and L-arabinose are soluble to the extent of 75 mg/ml. When a 1:1 mixture of the D- and L-isomers was analyzed by phase solubility, a diagram indicating a single pure component with an extrapolated solubility of 9.8 mg/ml was obtained. This lower solubility indicates that arabinose forms a racemic compound. When a mixture containing 80.3% D-arabinose and 19.7% L-arabinose was analyzed, the L-arabinose reacted with an equal amount of D-arabinose to form 39.4% DL-arabinose, leaving 60.6% D-arabinose. The phase diagram (Figure 13) indicated a slope of 60.6% and had a reverse slope as one would predict, since the ratio of solubilities, 98/75 = 0.13, is less than the ratio of concentrations, 39.4/60.6 or 0.66. This diagram also indicates that D- and L-arabinose form a racemic compound.

c. Volatile Impurities

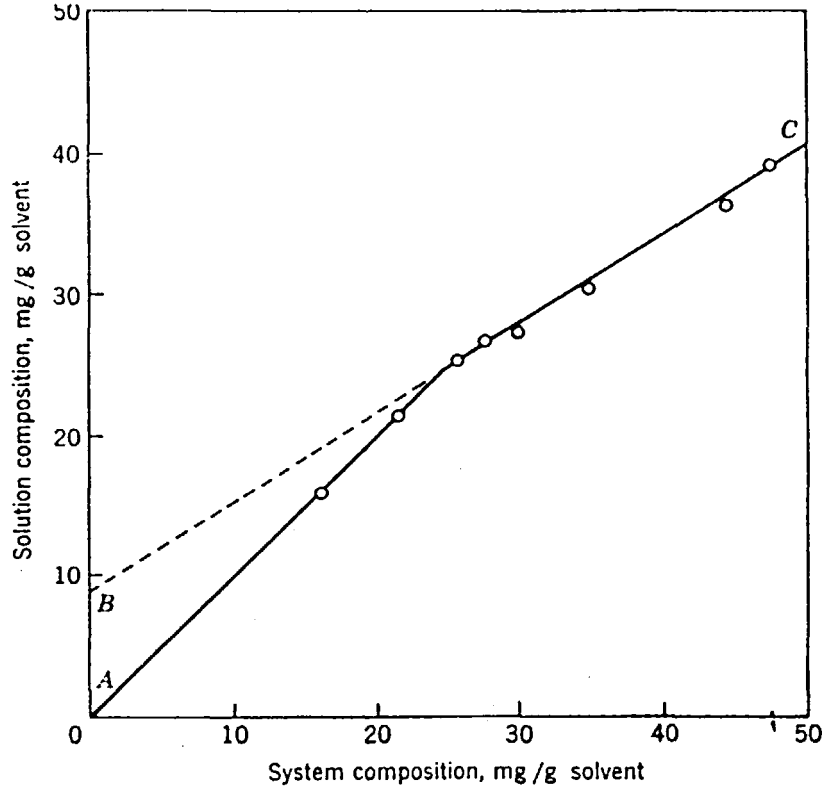
A material which contains only volatile impurities will behave as a pure compound in the saturated section of the phase diagram and will extrapolate to the solubility of the pure compound. For those systems where the amount of solid is insufficient for saturation, the solution composition points will fall below the 45° line by the percentage of volatile components. Figure 14 illustrates a phase diagram one could expect for a compound containing 10% volatile solvent and having a solubility of 10 mg/ml.

It must be pointed out that an identical diagram would be obtained if the sample contained 10% of a totally insoluble impurity instead of 10% of a volatile impurity.

d. Polymorphism

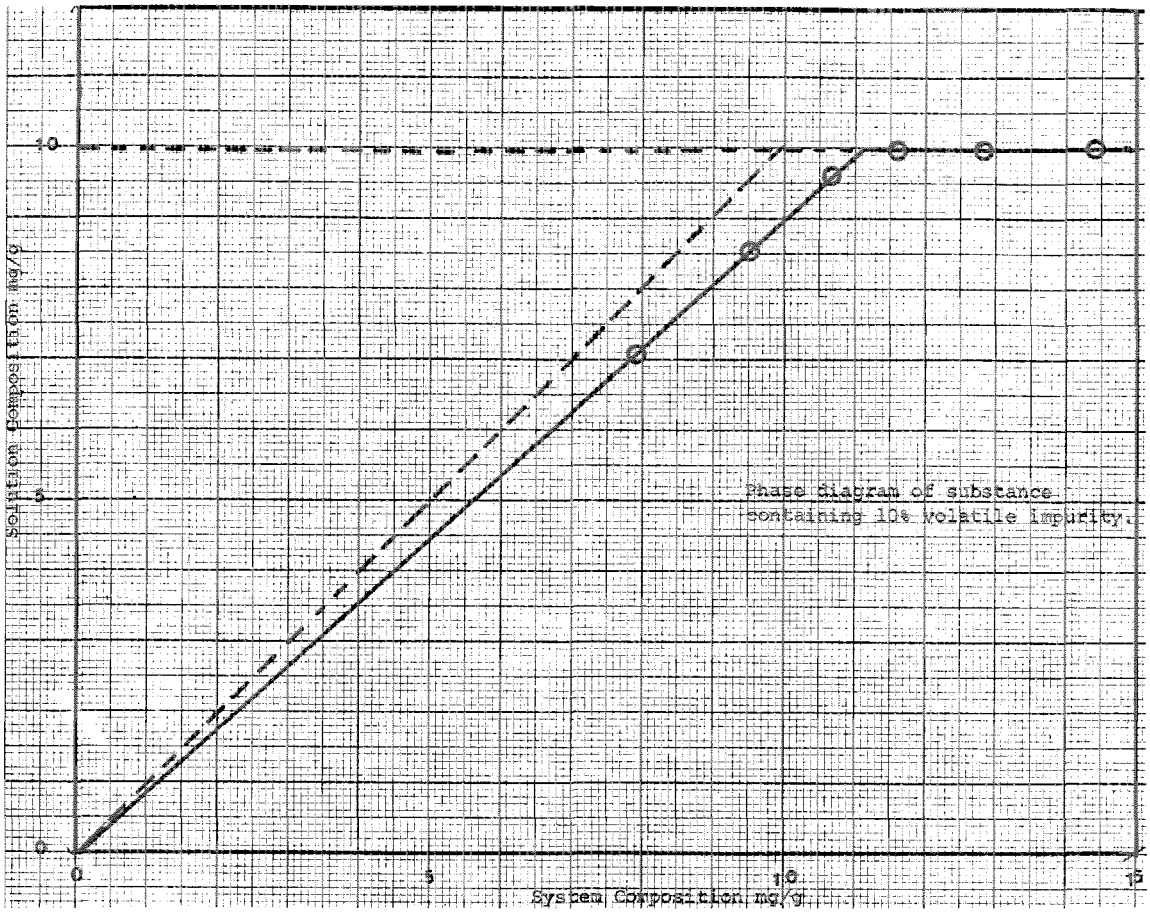
Mitscherlich[27] was the first to observe that a solid can exist in more than one crystalline form. Polymorphism is now recognized as a very common occurrence. The difference in the form of the crystal lattice or the distance of the lattice points manifests itself not only in crystalline shape, but also in the solubility. According to Gibbs' phase rule, each of these polymorphic forms constitutes a separate phase. Polymorphic forms do not exist in solution. The more stable form is the least soluble; if sufficient time is allowed, the metastable form will dissolve and crystallize out as the stable form. The transformation process can require considerably more time but is more rapid close to the saturation point and in sol-

FIGURE 13



Solubility curve and equilibration time at 30°C for a synthetic mixture of 80.3% D-arabinose and 19.7% L-arabinose in 75% ethyl alcohol for eight days; slope 60.6%.

FIGURE 14



vents in which the solubility is higher and the rate of dissolution therefore greater. Non-equilibrium due to polymorphism is indicated by a high and erratic slope. Increasing the equilibration time or changing the solvent or the temperature will correct the situation. Steroids and the sulfonamides are prone to exhibit polymorphism.

VI. MODIFICATIONS

Many modifications of the classical phase solubility technique are possible, the most obvious being methods of determining the solution composition other than the gravimetric method described here. In fact, the original method depended upon determining the solution composition of the proteins by the nitrogen content of the solution. If prior knowledge of a sample indicates that the likely impurities all have identical absorption characteristics, then one may use a spectrophotometric method; similarly, conductivity, refractivity, and titrimetry have been used, but in each such instance one must assume that the impurities present behave in exactly the same way as the major constituent. Such an assumption is not required in the standard method as mass is the variable factor being measured. For routine process-control work, modifications can be made which are more rapid and no less accurate.

a. Solubility Temperature Method

Reeve and Adams[36] have suggested the analysis of mixtures by measuring the "solubility temperature," which is the temperature at which a solid dissolves in a given amount of solvent. When properly applied the method exhibits reasonable accuracy, and once a calibration chart is available the analysis can be accomplished within one hour. Although the authors recommend establishing the solubility temperature–composition diagram using mixtures of pure components, it is preferable to analyze a series of samples of varying purity by the phase solubility method, determining the solubility temperature of each. This permits rapid determination without the necessity of first obtaining pure components. The method has been found especially useful for determining the purity of routine samples in the range of 50 to 100% purity. The method has been used for synthetic alkaloids.

b. Extraction Method

Stenger, Crummett, and Kramer[37] developed a procedure for material with a purity of 99.5% or better. These investigators increased the relative variation in solubility in two ways: (a) by using a solvent in which the compound to be tested is only slightly soluble at room temperature, and (b) by increasing the ratio of impurities to solvent through the Soxhlet extraction process.

Solvent—The solvent must be one in which the compound is soluble to the extent of only 0.5–2.0 mg/ml at room temperature. The solubility range for various solvents is determined, roughly, by preliminary experiments with small amounts of a sample which need not be pure. To be suitable, a solvent should be chemically inert and should have a moderately low boiling point (50° to 90°C, depending on the stability of the compound). Moisture should be absent and nonvolatile impurities, if any, should be removed by distillation.

Wash Solution—Warm about 1–1.5 g of the compound (using the purest material available) with 500 ml of the chosen solvent. Mix well, cool to room temperature, and allow to stand for one hour or more. Make sure that a small excess of the solute is present; if not, add more and warm again. Record the temperature and filter out the undissolved compound. Measure 50 ml of the filtrate in a graduated cylinder and pour it into a 100 ml glass evaporating dish that has been tared against a similar dish. Evaporate the filtrate nearly to dryness on the steam bath, but remove it in time so that the last of the solvent comes off at a lower temperature. Finally, dry under the conditions indicated in the procedure. Weigh the dish plus residue against the tare dish dried similarly, and calculate the solubility in grams per milliliter of solution at the recorded working temperature. Use the wash solution within one degree or so of this temperature.

Procedure

Preliminary Drying—Determine the best conditions for drying the compound by heating weighed small portions at various temperatures. If the compound sublimes readily, it may be necessary to dry at room temperature in a desiccator containing a suitable absorbent for the solvent that is to be used. In any case, the drying should be continued until the sample reaches constant weight. Dry a 10-g portion of the material under the determined conditions, cool, and weigh. Calculate the percentage of moisture plus other volatile impurities.

Extraction—Heat two clean extraction thimbles with an excess of the chosen solvent, drain, and dry, first in air, and then in an oven at 80°C. Cool in weighing bottles in a desiccator and weigh. Divide the dry 10-g sample between the thimbles and weigh them again in the bottles. Dry and weigh two Soxhlet flasks. Place each thimble in a Soxhlet apparatus and add 70 ml of the solvent. Heat on a hot plate until extraction is complete, which may require several hours or several days, depending on the solubility. Make sure that none of the compound remains in the thimble, and then dry the thimble and any residue in air and in the oven at 80°C. Cool in a weighing bottle in the desiccator as before and weigh. Any gain in weight is calculated as the percentage of insoluble impurities.

Treatment of Extract—The recrystallized compound remains in the boiler flask with 40–60 ml of solvent and the soluble impurities. Cool this mixture to, but not below, the temperature at which the wash solution is satu-

rated. It may be advisable to weigh the flask and contents, at this point, as a check on later evaporation losses. Stir well and filter, by gravity or with very gentle suction, on a coarse or medium sintered-glass crucible which has previously been tared after drying under the recommended conditions. Transfer as much of the precipitate as possible without washing and let it run fairly dry, but do not allow much time for evaporation. Measure the volume of the filtrate in a graduated cylinder, then place the filtrate in a 100-ml glass evaporating dish which has been tared as in the solubility determination. Transfer the loose remaining crystals to the filter with four 5-ml portions of wash solution, carefully measured, and follow these with 3 ml of pure solvent. Add the washings to the measured filtrate in the evaporating dish, evaporate and dry as before, and weigh. Dry the crucible and the flask in air and, finally, under recommended conditions, cool each, and weigh.

Calculation—The combined weight of crystals in the crucible and the flask is too low by the quantity required to saturate the original filtrate. Multiply the volume of filtrate (excluding washings) by the solubility (in grams per milliliter) and add this figure to the weight of the crystals. Divide the corrected crystal weight by the original dry sample weight and multiply by 100 to find the percentage of purity on the dry basis.

The weight of soluble impurities found in the evaporating dish is too high by the solubility correction applied above and by the amount of compound present in 20 ml of wash solution. Subtract these corrections. Divide the corrected figure by the original, dry sample weight and multiply by 100 to find the percentage of soluble impurities on the dry basis.

Failure of the percentages of purity and impurities to total 100 may indicate a loss of impurities by volatilization or decomposition. High results may be attributed to incomplete drying of the crystallized product or to oxidation.

The authors applied this technique to recrystallized chloranil and obtained purities of 99.87 and 99.90%. To this sample, they added 1.01% pentachlorophenol and obtained purities of the mixture of 98.89 and 98.90%. A second sample of recrystallized chloranil assayed 99.96% and when 0.80% pentachlorophenol was added they obtained 99.12%. The authors have applied this method to phenothiazine, *p,p'*-isopropylidenediphenol, 1-(*o*-dephenylxyloxy)-2-propanol, 2,4,6,-tribenzyl-5-trioxane, 4-aminoantipyrine, acetyl-D,L,tryptophan and 2-(*o*-biphenylxyloxy)ethanol.

VII. APPLICATION

a. Stability Testing

Some compounds are bound to degrade during the course of phase solubility analysis. If this is suspected, it can be ascertained by several methods. First, the plotted points will be erratic and not fall on a straight line, some being above and some below. Secondly, the extrapolated solubility will not be reproducible from one run to another. Thirdly, analysis of a solution point by thin-layer chromatography (or another chromatographic technique) will yield more spots or more intense spots than a freshly prepared solution. If there is some question about the stability of a substance in the selected solvent, it is well to compare the chromatogram of the solution after equilibration with that of a freshly prepared solution.

Solubility analysis has been used by Commer and Howell[21] to determine the stability of a pharmaceutical which was subject to thermal degradation. Commer also recommends solubility analysis as a means of checking other analytical procedures used for degradation studies. For example, a greater degree of degradation was noted by phase solubility analysis on a sample of ascorbic acid stored at 130° than was obtained by the USP colorimetric method. Similarly, ergonovine maleate degrades more than was indicated by a colorimetric assay. By using solubility analysis as the criterion, the conventional methods for measuring thermal degradation were confirmed for one compound, shown to be questionable for three compounds, and verified for one drug substance.

b. The Insoluble Residue

Referring to Figure 1, the insoluble residues in the tubes between D and E will be pure

ine. In Figure 2, the insoluble resi-
e tubes between E and F will be pure
. Similarly, the insoluble residue
egion of the first slope after satura-
epresent pure material. Isolation of
naterial permits one to obtain physi-
ties such as melting point, infrared
iolet spectra, optical rotation, and
action, without interference from the
which were present in the original sample. Conversely, the residue obtained by evaporation of the tubes in this region will be more rich in the impurities than the original starting material.

c. Examples of Substances Assayed

Table 2 lists a few of the many compounds which have been analyzed by phase solubility analysis and gives the solvents used in their assay.

TABLE 2

Typical Examples of Phase Solubility Systems*

Compound	Solvent	Solubility	Ref.
xene	isopropanol	28.1 mg/g	38
e hydrochloride	absolute EtOH	33.6 mg/g	38
	$CHCl_3$-EtOH, 99-1	8.2 mg/g	38
	DMF	6.4 mg/g	38
e bromide	*n*-propanol	24.8 mg/g	38
e methylsulfate	*n*-propanol	30.0 mg/g	38
an camsylate	isopropanol	24.5 mg/g	38
id	50% EtOH	14.0 mg/g	38
romide	95% EtOH	28.6 mg/g	38
e cyprionate	70% MeOH	1.8 mg/g	38
hydrochloride	95% EtOH	13.2 mg/g	38
	Ethyl acetate	23.1 mg/g	38
coumarin	20% pyridine, 80% H_2O	15.7 mg/g	38
zine maleate	90% EtOH	15.9 mg/g	38
	30% DMF in acetone	12.0 mg/g	38
one acetate	dioxane	9.7 mg/g	38
	95% EtOH	8.5 mg/g	38
e	benzene	15.6 mg/g	38
ine hydrochloride	dioxane	7.4 mg/g	38
hydrochloride	water	9.7 mg/g	38
	$CHCl_3$-MeOH, 2-1	25.6 mg/g	38
xin	EtOH	6.1 mg/g	38
mol	Chloroform	13.0 mg/g	38
diacetate	70% EtOH	19.3 mg/g	38
nicol	35% EtOH	17.6 mg/g	38
nicol	20% EtOH	6 mg/g	24
	95% EtOH	16.1 mg/g	38
one	95% EtOH	20.2 mg/g	38
lrel	30% acetone in hexane	20.8 mg/g	38
in	95% EtOH	24.8 mg/g	38
de iodide	water	21.0 mg/g	38
hydrochloride	acetone	19.9 mg/g	38
ion hydrochloride	benzene-$CHCl_3$, 1-1	15.7 mg/g	38
e	95% EtOH	24.7 mg/g	38
thiazide	70% MeOH	17.5 mg/g	38
	40% EtOH	24.8 mg/g	38

s are at 25° unless otherwise noted.

Compound	Solvent	Solubility	Ref.
Prilocaine hydrochloride	isopropanol	19.3 mg/g	38
Dromostanolone propionate	MeOH-H_2O (1-1)	26.6 mg/g	38
Phenazopyridine hydrochloride	pyridine	10.4 mg/g	38
Glyceryl guaiacolate	2% absolute EtOH in benzene	15.4 mg/g	38
Nylidrin hydrochloride	95% EtOH	21.6 mg/g	38
Ephedrine hydrochloride	*n*-Propanol	16.3 mg/g	38
Chlorothen citrate	95% EtOH	28.4 mg/g	38
Betamethasone valerate	60% EtOH	16.7 mg/g	38
Dydrogesterone	isopropanol	19.5 mg/g	38
Niacin	water	17.6 mg/g	38
Levopropoxyphene napsylate	40% EtOH	16.0 mg/g	38
Dimethisterone	60% EtOH	11.8 mg/g	38
Oxandrolone	acetone	17.3 mg/g	38
Desipramine hydrochloride	absolute EtOH-cyclohexane, 1-1	(22-23 mg/ml)	38
Methotrimeprazine	absolute EtOH	14.4 mg/g	38
Thihexinol methyl bromide	water	14.9 mg/g	38
Ethamivan	water	14.1 mg/g	38
Haloperidol	MeOH	17.8 mg/g	38
Cyproheptadine hydrochloride	40% EtOH	22.4 mg/g	38
Cortisone acetate	benzene	1.33 mg/ml	19
	EtOH	1.6-1.7 mg/ml	19
	MeOH	15-16 mg/ml	19
	20% $CHCl_3$ + 80% Benzene	3.63 mg/g	19
Methylandrostenediol	toluene	ca. 1.2 mg/ml	19
	ethyl acetate	8.5-9.0 mg/ml	19
	chloroform	—	19
	acetone	—	19
Pregnenolone acetate	acetone	80-85 mg/ml	19
	ethyl acetate	95-100 mg/ml	19
Pregnenolone	acetone	9-10 mg/ml	19
	benzene	6-7 mg/ml	19
3-β-Acetoxy-20-hydroxy-5-cholenic acid lactone	benzene	1.5-2.0 mg/ml	19
4-Bromodehydrocortisone acetate	benzene	1-2 mg/ml	19
	ethyl acetate	10-11 mg/ml	19
Desoxycholic acid	acetone	14.6 mg/g	19
Hydrocortisone acetate	MeOH	4.6 mg/g	19
Hydrocortisone alcohol	MeOH	15.6 mg/g	19
DL-α-Alanine	50% EtOH	27.0 mg/ml @30°	2
β-Alanine	75% EtOH	47.6 mg/ml 30°	2
Aminoacetic acid	50% EtOH	26.6 mg/ml 30°	2
DL-Aspartic acid	water	8.8 mg/ml 30°	2
L-Cystine	0.5 *N* HCl	41.0 mg/ml 30°	2
L(+)Glutamic acid	water	10.3 mg/ml 30°	2
L(+)Histidine monohydrochloride	50% EtOH	30.0 mg/ml 30°	2
L(−)Hydroxyproline	50% EtOH	70.5 mg/ml 30°	2
DL-Methionine	50% EtOH	10.3 mg/ml 30°	2
DL-Norleucine	water	12.4 mg/ml 30°	2
DL-Phenylalanine	water	15.1 mg/ml 30°	2
L(−)Proline	absolute EtOH	12.8 mg/ml 30°	2
DL-Serine	water	59.0 mg/ml 30°	2
DL-Tryptophan	25% pyridine	8.7 mg/ml 30°	2
L(−)Tyrosine	0.5 *N* HCl	38.8 mg/ml 30°	2
DL-Valine	water	74.8 mg/ml 30°	2
α-d-4 Dimethylamino-1,2-diphenyl-3-methyl-2-propinoxy butane hydrochloride	ethyl acetate	7.5 mg/g	21

Compound	Solvent	Solubility	Ref.
α-1,4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane-n-oxide hydrochloride	ethyl acetate-MeOH, 65-35	16.0 mg/g	21
Ergonovine maleate	MeOH	10.5 mg/g	21
		15.0 mg/g	21
N-(*p*-Acetylphenyl-sulfonyl) -n-cyclohexyl urea	Isopropanol-EtOH, 1-1 $CHCl_3$-MeOH, 2-3	23.0 mg/g	21
Mecamylamine hydrochloride	isopropanol	22 mg/g	20
Hydrochlorothiazide	—	—	20

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