ratios permit the evaluation of $(k_{HAc})_1$ or $(k_{HAc})_{-1}$ as the intercepts and $(k_{Ac})_1$ or $(k_{Ac})_{-1}$ as the slopes. Such plots of Eq. 17 in Fig. 8 demonstrate general base-catalyzed dehydration and rehydration due to acetate ion. General acid catalysis due to acetic acid is negligible since the intercepts are zero. Thus, the apparent first-order rate constant for acetate buffer region dehydration of cycloheximide can be expressed

$$k_1 = (k_{Ac})_1[Ac^-] + (k_o)_1 \qquad \text{(Eq. 18)}$$

where at 80° $(k_{Ac})_1 = 1.36 \times 10^{-3}$ L./mole/second and $(k_o)_1 = 1.0 \times 10^{-6}$ second$^{-1}$ and is largely due to solvent catalysis. An apparent hydroxyl ion-catalyzed dehydration is also indicated from the increasing values of the intercepts in Fig. 7 and should become highly significant at pH values greater than the pH range of the acetate buffer region.

Similarly, the apparent first-order rate constant for acetate buffer region rehydration of anhydrocycloheximide can be expressed

$$k_{-1} = (k_{Ac})_{-1}[Ac^-] + (k_o)_{-1} \qquad \text{(Eq. 19)}$$

where at 80° $(k_{Ac})_{-1} = 1.43 \times 10^{-3}$ L./mole/second and $(k_o)_{-1} = 2.1 \times 10^{-6}$ second$^{-1}$ and is largely due to solvent catalysis. An apparent hydroxyl ion catalyzed rehydration is also indicated from the increasing values of the intercepts in Fig. 7 and should become highly significant at pH values greater than the pH range of the acetate buffer region.

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# Solubility Studies on Certain Barbiturates

By RICHARD L. SEDAM, ALFONSO R. GENNARO, and ARTHUR OSOL

**The solubilities in water at 25° of five barbiturates were determined by the application of liquid scintillation counting of $C^{14}$-tagged compounds to the technique of phase solubility analysis. The effects of several added solutes on these solubilities were also determined, and over-all salting-out constants were obtained from graphs based on the empirical Setschenow equation.**

THE SOLUBILITIES in water of many of the barbiturates are reported in such general terms as "slightly soluble" or "very slightly soluble" (1, 2). Even when solubilities are defined more precisely, based on quantitative measurements on saturated solutions, the data often differ (3–6). The technique of phase solubility analysis apparently has not been applied to barbiturates.

Solubility data for barbiturates have special utility in preparing liquid pharmaceutical dosage forms; in developing these formulations, a knowledge of the effect of other solutes on barbiturate solubility is highly desirable. In general, the solubility in water of a nonelectrolyte is altered by the addition of an electrolyte. Inorganic electrolytes commonly cause salting-out to occur, although there are exceptions to this general statement. Nonelectrolytes such as sucrose also may cause salting-out. Salting-in is frequently caused by the salts of various organic acids and by organic-substituted ammonium salts. Long and McDevit (7) have summarized the many theories advanced to explain salt effects; unfortunately, none of the theories explains completely the available experimental data. It appears that an empirical or semiempirical approach must be employed at the present time.

In the present investigation, the solubilities in water at 25° of five barbiturates were determined by the application of liquid scintillation counting of $C^{14}$-tagged compounds to the technique of phase solubility analysis. The effects of four added solutes on these solubilities were determined also.

Phase solubility analysis was described first by Northrop and Kunitz (8) as a criterion of the chemical purity of a substance. These workers also demonstrated that the technique can be used to obtain the exact solubility of the pure substance. This can be accomplished without the

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experimental material itself being pure. Many solubility studies are conducted by equilibrating an excess of solute with the solvent and measuring the concentration of the resulting solution. Any soluble impurity present will be measured as pure substance if it is detected by the analytical method employed. The resulting possibility of erroneously high values is obviated by phase solubility analysis. If there is any impurity present, a separation is effected so that material of a high degree of purity can be obtained from initially impure samples. One can recover the entire sample or a pure fraction.

Provided tagged compounds are available or can be synthesized without undue difficulty, radiometric analytical methods can be made highly sensitive by manipulation of the specific activities of the compounds. In addition, nonradioactive materials present will not interfere. Chase and Rabinowitz (9) have presented an excellent discussion of liquid scintillation counting and the use of the instrument employed in these studies.

## EXPERIMENTAL

### Materials

Amobarbital-2-$C^{14}$, aprobarbital-2-$C^{14}$, barbital-2-$C^{14}$, and phenobarbital-2-$C^{14}$ were synthesized by condensing the appropriate malonic ester with urea-$C^{14}$ in the presence of sodium ethoxide, using toluene as the solvent. In all cases, the sodium barbiturate produced was converted to the acid form by acidification with dilute sulfuric acid. Vinbarbital-2-$C^{14}$ was obtained by condensing the appropriate cyanoacetic ester with the urea-$C^{14}$ to produce the imino form of the barbiturate and hydrolyzing this to the oxo form. The radioactive urea[1] had an initial activity of 3 mc./m*M* and was diluted with stable urea to produce barbiturates having the desired activity. The radioactive compounds were recrystallized twice from water and had the following melting points[2]: amobarbital-2-$C^{14}$, 156–157°; aprobarbital-2-$C^{14}$, 138–138.5°; barbital-2-$C^{14}$, 187–188°; phenobarbital-2-$C^{14}$, 172–173°; vinbarbital-2-$C^{14}$, 161–163°. All other chemicals were reagent grade.

The water employed was obtained by passing distilled water through a mixed-bed ion-exchange resin column. Purity was tested with a Barnstead model PM-4 purity meter; water having a conductivity greater than 0.1 p.p.m. as sodium chloride was rejected. The water was boiled for 30 minutes immediately prior to use to expel dissolved carbon dioxide.

The liquid scintillator contained 3 Gm. of 2,5-diphenyloxazole (PPO) and 100 mg. of 2,2′-paraphenylenebis(5-phenyloxazole) (POPOP) in a mixture of 43% toluene by volume and 57% absolute ethanol by volume, to make 1000 ml.

All mass determinations were performed on a Mettler model S-6 semimicro balance.

[1] New England Nuclear Corp.
[2] All melting points are uncorrected.

### Procedures

**Phase Solubility Analysis of Barbiturates in Water at 25°.**—Mader (10) has described the technique of phase solubility analysis in detail. It consists of mixing different masses of solid sample with known masses of solvent until equilibrium is obtained and determining the concentration of solute in the solutions. This solution concentration is plotted against the total amount of sample added per unit mass of solvent (system concentration). Six to eight solute–solvent systems usually suffice. At least two of these systems should contain an amount of solute insufficient to attain saturation, and the concentrations of the systems in this region should be spaced as evenly as possible between zero and saturation concentration. In the region above saturation, the system concentrations should vary from just above saturation to several times saturation concentration to provide for the plotting of a sufficient number of points.

The slope of the phase solubility diagram in the region above saturation is an index of the purity of the material; the mass fraction of any contaminant is equal to the slope of the curve. In the region below saturation, the slope of the curve should equal unity; the degree of conformity to this value provides a check on the precision of the experimental method. The solubility of the pure material is given by the *Y* intercept obtained by extrapolation of the portion of the curve representing the region of saturation.

Ampuls of U.S.P. type I glass, having a nominal capacity of 20 ml., were employed for the equilibration. The ampuls were cleaned with steam, rinsed twice with distilled water, and dried in a vacuum oven for 8 hours at 60°. After cooling to room temperature in a desiccator, the mass of each ampul was determined. Transfer of the barbiturate was effected through a small funnel, taking care that no solid adhered to the neck of the ampul. The amount of solid added was determined by noting the increase in the mass of the ampul. Twenty milliliters of water was added to each ampul with a 20-ml. syringe fitted with a long hypodermic needle. The mass of the ampul was determined again and the mass of added solvent calculated. The ampuls were sealed and tested for leakage. The systems thus prepared were agitated by end-over-end rotation of the ampuls at 19 r.p.m. in a water bath at 25 ± 0.02° for 2 weeks, during which time equilibrium was achieved. The positions of the ampuls on the rotor were reversed during the equilibration period to change the direction of rotation and thus retard clumping of the solid material in the constricted part of the ampuls.

An aliquot of approximately 1 ml. was removed from each ampul with a syringe fitted with a long hypodermic needle. A pledget of cotton had been placed in the hub of the needle to act as a filter. After withdrawal of the aliquot, the needle and cotton filter were removed, and the aliquot was injected into a liquid scintillation spectrometer vial, the mass of which had been determined previously. The mass of the vial was determined again and the mass of the aliquot calculated. Twenty milliliters of liquid scintillator was added to each vial, and the contents were mixed thoroughly.

The samples were counted in a model 314 Tri-Carb liquid scintillation spectrometer (Packard

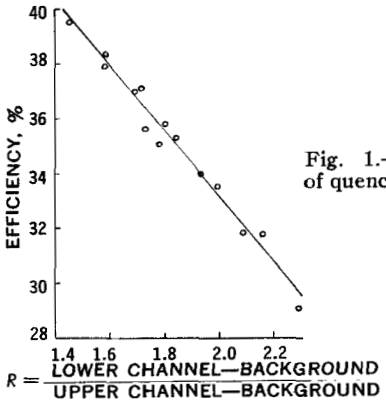


Fig. 1.—Efficiencies of quenched samples.

Instrument Co.), with discriminator settings such that the lower channel recorded pulses having amplitudes between 10 and 50 v., and the upper channel pulses having amplitudes between 50 and 100 v. At least 10,000 counts were recorded in each case. The activities of the samples were compared with the activities of standard solutions of the same radioactive barbiturates in absolute ethanol. Instrumental efficiency was determined by a channels ratio method, which has been described thoroughly by Bush (11). Preparation of the efficiency graph was accomplished by using a standard benzoic-$C^{14}$ acid solution quenched with successive increments of stable benzoic acid, stable barbital, and sodium bromide.

**Effect of Other Solutes on Barbiturate Solubility in Water at 25°.**—The radioactive barbiturates previously synthesized were purified further by a technique analogous to phase solubility analysis. For each barbiturate, a large container of water with excess solute was prepared. The containers were sealed, and the systems thus prepared were subjected to end-over-end rotation in the water bath at 25 ± 0.02° for a minimum of 2 weeks. The excess solute remaining in each container was separated by filtration and dried in a vacuum oven at 60°. This process should insure purities at least as high as those observed in the phase solubility analyses.

Aqueous solutions of potassium chloride, potassium bromide, sodium chloride, and sodium bromide were prepared in concentrations of 0.1, 0.5, and 2.0 *M*. A quantity of each solution sufficient for the entire study was prepared. Specific gravities of the solutions were determined by a pycnometer method.

Systems were prepared representing duplicate samples of each barbiturate with each of the solutions described above in each of the three concentrations. Ampuls were employed as previously described, but the system concentrations were not determined. Rather, a quantity of solute sufficient to insure the presence of an excess solid phase was placed in each ampul and 20 ml. of the salt solution added. The ampuls were sealed and tested for leakage. The systems thus prepared were equilibrated in the water bath at 25 ± 0.02° during a period of 2 weeks.

The analytical technique used was the same as that described above, except that a correction was made for the natural potassium-40 present in the potassium salts by the following method. Two series of liquid scintillation spectrometer vials containing increasing amounts of potassium chloride and potassium bromide were prepared. Twenty milliliters of liquid scintillator was added to each vial, and the samples were counted. Graphs were constructed relating the quantity of salt present to the counts per minute above background observed on each of the channels; sample counts were corrected then by reference to these graphs.

## RESULTS AND DISCUSSION

**Analytical Technique.**—Quenching, a process which results in a decrease in intensity of the light flashes produced through interaction of beta particles with the liquid scintillator, is a potential source of error in measurement. If a single species of isotope is being measured, the ratio of the counts recorded on the lower channel to those recorded on the upper channel can be used to determine when changes in the degree of quenching occur. A constant ratio indicates a constant degree of quenching. A change in the ratio indicates a change in the degree of quenching, which leads to a change in instrumental efficiency with the attendant possibility of measurement error. It was observed in preliminary experiments that even the addition of a spike of benzoic-$C^{14}$ acid for the purpose of determining efficiency led to a change in the ratio. Differing amounts of barbiturate and the presence of other solutes could be expected to result in rather large changes in the ratio. Furthermore, the addition of a spike to each vial is extremely time consuming and introduces another potential source of error in measurement. It was for these reasons that the channels-ratio method of determining efficiencies was adopted.

Figure 1 illustrates the relationship between channels ratios and efficiencies. Plots of this type are curvilinear over a wide range of ratios but may be considered essentially linear over a narrow range, especially if the ratios are low.

The particular liquid scintillator adopted was chosen on the basis that it provided homogeneous systems without the necessity for evaporation of the sample aliquots to obtain samples suitable for counting. Other solvent systems demonstrated higher efficiencies and complete solubility for the barbiturates but were immiscible with the water present. Evaporation of the aliquots in the liquid scintillation spectrometer vials proved to be extremely time consuming. The possibility also existed that some of the material might be lost by spattering; this possibility was shown with preliminary experiments to be more pronounced in the presence of salts. In addition, evaporation of aliquots which contained salts resulted in the formation of large salt crystals. It was believed that barbiturate particles might be entrapped within the salt crystals and not make contact with the liquid scintillator, resulting in erroneously low values.

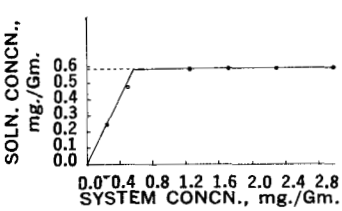


Fig. 2.—Phase solubility analysis of amobarbital at 25°.

**Solubility Studies.**—Figure 2 is a phase solubility diagram representative of those obtained in this study. The purities and solubilities of the five barbiturates investigated are given in Table I. Literature solubility values defined in somewhat precise terms are available for three of the five barbiturates at 25°. For comparison, the values in this study have been converted to similar units. In the case of barbital, the N.F. (12) reports a solubility of 1 Gm. in 130 ml.; found: 1 Gm. in 139.7 ml. The U.S.P. (13) reports a solubility of 1 Gm. in 1300 ml. for amobarbital; found: 1 Gm. in 1700 ml. Phenobarbital is reported by the U.S.P. (14) as soluble to the extent of 1 Gm. in *about* 1000 ml.; found: 1 Gm. in 783 ml. It is interesting that at least four other values for the solubility of phenobaribital at 25° have been reported: 0.108% w/v (3), 0.12% w/v (4), 0.11% w/v (5), and 0.13% w/v (6). Conversion of the value obtained in this study yields a solubility of 0.128% w/v.

**Salt Effects.**—The most widely used empirical equation for determining the extent of salt effect is that of Setschenow (15)

$$\log \frac{S_o}{S} = kC$$

where $S_o$ is the solubility of the nonelectrolyte in pure solvent; $S$ is the solubility of the nonelectrolyte in the salt solution; $k$ is a constant, dependent upon the salt, the nonelectrolyte, the solvent, and the temperature; and $C$ is the molar concentration of the salt solution. The ratio $S_o/S$ is equal to $f$, the activity coefficient of the nonelectrolyte in the salt solution. Assuming that the Setschenow equation is valid, a plot of log $f$ *versus* $C$ gives the over-all salting-out constant directly from the slope of the line; constants so obtained are perhaps more meaningful than individual constants calculated for each salt concentration. Accordingly, these over-all constants are presented in Table II; a representative plot is given in Fig. 3.

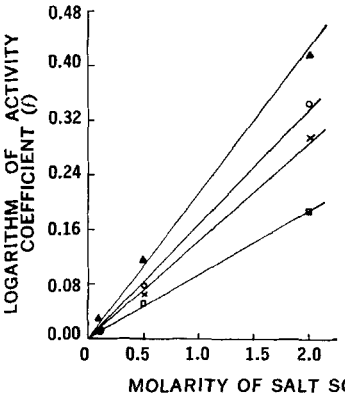


Fig. 3.—Effect of salt concentration on the activity coefficient of amobarbital at 25°. Key: ▲, NaCl; ○, KCl; ×, NaBr; □, KBr;

Generally, it is accepted that because of greater charge density, sodium ions have a greater salting-out effect than potassium ions if the anion is constant. The role of the anion is less clear, but chloride ions apparently have a greater salting-out effect than bromide ions if the cation is constant. The experimental values obtained in this study confirm these relative orders of effect in every case. The values obtained for the salting-out constants for phenobarbital agree generally with those of Eriksson (16) with respect to the order and direction of their magnitudes. However, his were determined at 20°, so a direct comparison cannot be made; this constant is temperature-dependent.

TABLE I.—SOLUBILITIES OF BARBITURATES IN WATER AT 25°

| Barbiturate | Purity, % | Solubility, mg./Gm., and 95% Confidence Limits |
|---|---|---|
| Amobarbital | 99.7 | 0.583 ± 0.012 |
| Aprobarbital | 99.9 | 4.12 ± 0.06 |
| Barbital | 99.0 | 7.18 ± 0.12 |
| Phenobarbital | 98.1 | 1.28 ± 0.11 |
| Vinbarbital | 98.3 | 0.638 ± 0.031 |

TABLE II.—SALTING-OUT CONSTANTS OF FIVE BARBITURATES AT 25°

| Salt | Salting-Out Constant, $k$ |
|---|---|
| **Amobarbital** | |
| Potassium chloride | 0.168 |
| Potassium bromide | 0.095 |
| Sodium chloride | 0.212 |
| Sodium bromide | 0.143 |
| **Aprobarbital** | |
| Potassium chloride | 0.136 |
| Potassium bromide | 0.062 |
| Sodium chloride | 0.184 |
| Sodium bromide | 0.120 |
| **Barbital** | |
| Potassium chloride | 0.092 |
| Potassium bromide | 0.042 |
| Sodium chloride | 0.136 |
| Sodium bromide | 0.088 |
| **Phenobarbital** | |
| Potassium chloride | 0.092 |
| Potassium bromide | 0.034 |
| Sodium chloride | 0.132 |
| Sodium bromide | 0.078 |
| **Vinbarbital** | |
| Potassium chloride | 0.125 |
| Potassium bromide | 0.036 |
| Sodium chloride | 0.143 |
| Sodium bromide | 0.096 |

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