Solubility of Acetanilide and Several Derivatives in Sucrose Solutions

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The solubilities of acetanilide, acetoacetanilide, and the *p*-methyl, *p*-ethoxy, *p*-hydroxy, and *p*-amino derivatives of acetanilide were determined in aqueous sucrose solutions. The solubilities of all solutes were found to change significantly with changes in the concentration of sucrose solutions. The solubility curves indicate the changes probably involve solvent polarity, as indicated by dielectric constant of the solvent, and decrease in the activity of water. The solubility changes obtained for acetanilide and the several *para* derivatives were found to show a fair correlation with respect to the magnitude of solubility in water. This indicates a possibility of predicting solubility changes for the remainder of the compounds if the solubility change for one of the compounds is known.

SEVERAL STUDIES have been carried out in these laboratories to investigate solubility phenomena and their implications in the formulation of pharmaceutical dosage forms. Previous studies indicated the solubility of a given solute in syrup could be significantly different from that in water. This was demonstrated for sulfanilamide, quinine, phenobarbital, and *p*-aminobenzoic acid (1), for xanthines, antipyrine and derivatives (2), and for the *p*-hydroxybenzoic acid esters (3). The fact that increased as well as decreased solubilities were noted and the nature of the solubility curves obtained indicated these changes were probably of complex mechanism. It was thought that the mechanism involved changes in solvent polarity, as indicated by the dielectric constant, and decreased activity of water due to an additive, sucrose, with strong dependence also on the nature of the solute and solvent.

This is the third in a series of investigations of sucrose solutions as solvents of considerable pharmaceutical interest. Solutes were selected for this study to yield information for predicting solubility changes in addition to the solubility curves obtained in the conventional manner. Solubilities of antipyrine and its 4-amino and 4-dimethylamino derivatives (2) in water and in various sucrose solutions indicated a possibility that solubility changes might be predictable.

EXPERIMENTAL

Materials.—The compounds used in this study were as follows: acetanilide N.F. (Mallinckrodt); acetoacetanilide (Eastman No. 1239); *p*-acetotoluidide (Eastman No. 425); phenacetin U.S.P. (Nepera Chemical Co.); acetaminophen N.F. (Nepera Chemical Co.); 4-aminoacetanilide (Eastman No. 13); sugar, granulated U.S.P. These materials were used without further purification.

Solubility Determinations.—Solubility determinations of each material were made in water and in the following sucrose solutions: 18.6, 31.6, 46.0, and 63.4% w/w sucrose. The 63.4% sucrose solution corresponds to syrup U.S.P. Deionized water was used throughout this study. The dielectric constants of these solutions ranged from 78.5 for water to 58.5 for syrup U.S.P. The solubility determinations were made as described previously (4–6). Equilibration time was 72 hr. All determinations were made at 25°. Sample volumes diluted for analysis were 1 ml. for 63.4% sucrose solutions and 5 ml. for all other samples. After appropriate dilutions, all samples were analyzed spectrophotometrically. The final dilutions and absorbance maxima for each compound were as follows: 1:1000 dilution at 237 mμ for acetanilide, 1:100 dilution at 241 mμ for *p*-acetotoluidide, 1:100 dilution at 245 mμ for phenacetin, 1:1000 dilution at 241 mμ for acetaminophen, 1:2000 dilution at 250 mμ for 4-aminoacetanilide, 1:1000 dilution at 240 mμ for acetoacetanilide.

Dielectric Constant.—The dielectric constants of saturated solutions of the compounds investigated in this study were measured on a WTW Multidekameter, model DK-06 (Kahl Scientific Instrument Corp.), as previously described (2). Previously determined values of the dielectric constants of the sucrose solutions were used, as these were in agreement with the values reported by other workers (7, 8).

RESULTS AND DISCUSSION

The solubility data of the solutes investigated in this study are shown in Table I. The values represent an average of three determinations in all cases, except phenacetin, where two values were determined.

The solubility ratios, *i.e.*, solubility in 63.4% sucrose solution relative to that in water, are shown in Table II. It is noted that significant solubility changes were obtained in going from water to 63.4% sucrose solution. For drawing solubility curves, the solubility is plotted against both sucrose concentration and dielectric constant of the corresponding sucrose solution used as solvent.

In general, the dielectric constant scale squeezes in the *x*-axis. The solubility curves are shown in Figs. 1–6.

Acetoacetanilide shows the largest relative change in solubility, with a solubility ratio of 0.5. Its solubility curve is linear in relation to both sucrose concentration and dielectric constant (DEC) of the solvent. The decrease in solubility is 0.25 mg./ml./DEC unit. The solubility curves of acetanilide and the *p*-methyl derivative (*p*-acetotoluidide) are smooth, nonlinear functions. However, it is interesting to note that for the *p*-ethoxy derivative (phenacetin) and the *p*-hydroxy derivative (acetaminophen), there is a considerable change in solubility going from 46.0 to 63.4% sucrose. The solubility ratio of the *p*-aminoacetanilide is 0.88, showing the least change in this series of solutes.

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TABLE I.—SOLUBILITY OF ACETANILIDE AND SEVERAL DERIVATIVES IN SUCROSE SOLUTIONS

Solvent	Solubility, mg./ml. Acetanilide	*p*-Methyl-acetanilide	Phenacetin	Acet-aminophen	*p*-Amino-acetanilide	Aceto-acetanilide
Water	6.38	1.05	0.93	13.85	15.98	9.87
18.6% Sucrose	5.74	0.95	0.87	13.08	16.04	8.34
31.6% Sucrose	5.41	0.90	0.82	12.62	15.68	7.59
46% Sucrose	4.63	0.81	0.76	11.63	14.74	6.17
63.4% Sucrose	4.25	0.77	0.59	9.80	14.02	4.96

TABLE II.—SOLUBILITY RATIOS FOR ACETANILIDE AND SEVERAL DERIVATIVES

Solute	Solubility Ratio: (mg./ml. in 63.4% w/w Sucrose Soln.)/(mg./ml. in Water)
Acetanilide	0.66
p-Methylacetanilide	0.73
p-Ethoxyacetanilide	0.63
p-Hydroxyacetanilide	0.71
p-Aminoacetanilide	0.88
Acetoacetanilide	0.50

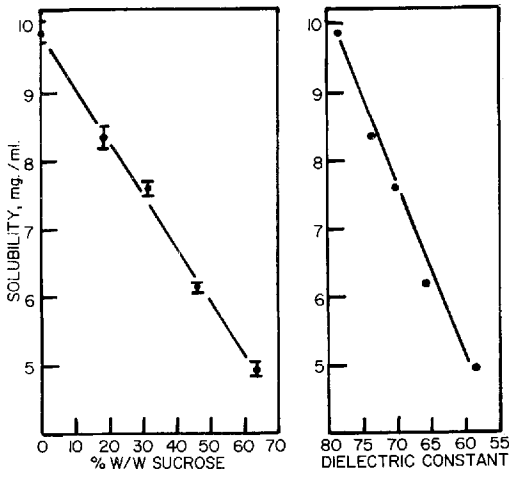

Fig. 1.—Plot of solubility of acetoacetanilide, mg./ml. at 25°, as a function of sucrose concentration and dielectric constant of solvent.

This series of solubility curves is generally in agreement with previous findings (2, 3). The curves indicate the observed changes in solubility probably involve complex mechanisms.

The decrease in solubility, going from water to 63.4% sucrose solution, is shown in Table III, for acetanilide and the *p*-methyl, *p*-ethoxy, *p*-hydroxy, *p*-amino, and the aceto derivatives. It is seen that the solubility change is fairly constant relative to the solubility in water for the first four compounds. These results indicate the possibility of predicting solubility and are in agreement with similar observations made for antipyrine and two derivatives (2). Thus, knowing the solubility change that occurs for acetanilide, the expected change for the derivatives could be approximately calculated.

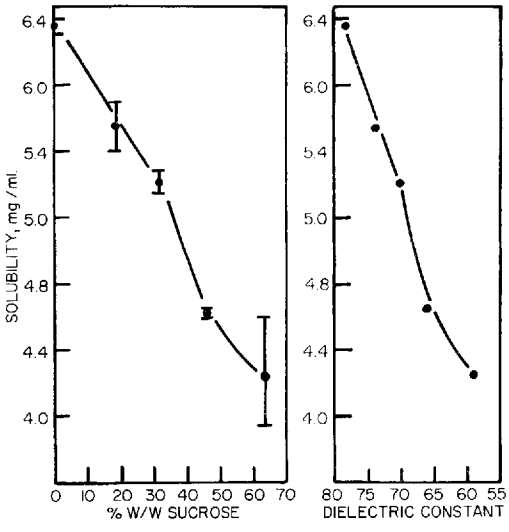

Fig. 2.—Plot of solubility of acetanilide, mg./ml. at 25°, as a function of sucrose concentration and dielectric constant of solvent.

The *p*-amino derivative differs from the rest of the solutes in this regard. This may be due to the fact that the amine substitution may cause a much stronger change in the polarity of the molecule.

It should also be noted that the *p*-amino derivative has the highest magnitude of solubility in this series of solutes. Acetoacetanilide, showing about a 50% decrease in solubility, was not expected to correlate since the substitution was directly on the functional group of the parent compound.

It again indicates the dependence on the nature of the solute and solvent. However, the apparent correlation noted in the change in solubility with two sets of derivatives may be of value if it is further substantiated.

The dielectric constants of the saturated solutions followed, in general, the shape of the dielectric constant curves of the solvents. There was no indication of any specific correlation. This may partly be due to the low solubilities of the solutes.

SUMMARY AND CONCLUSIONS

The solubilities of acetanilide and five related compounds were determined in water and in sucrose solutions containing from 18.6 to 63.4% sucrose. The solubility of all solutes changed significantly in going from water to syrup. The solubility curves obtained in this study again indicate that observed changes are of complex mechanism probably in-

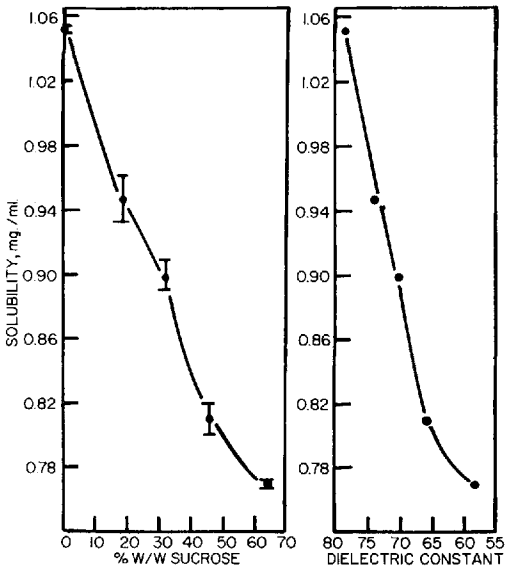

Fig. 3.—Plot of solubility of *p*-methylacetanilide, mg./ml. at 25°, as a function of sucrose concentration and dielectric constant of solvent.

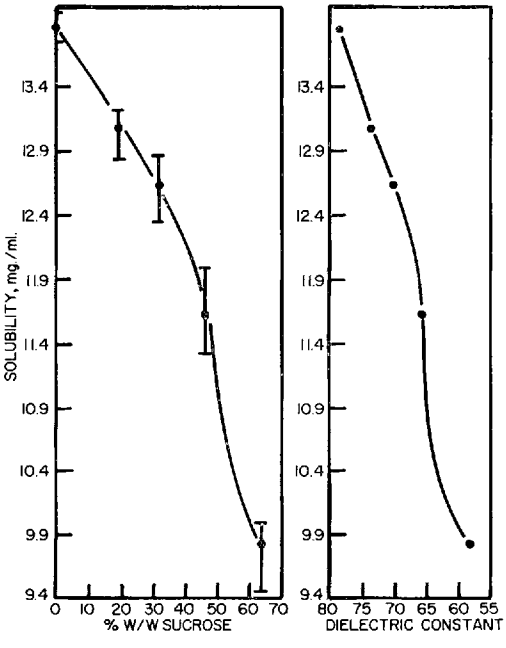

Fig. 4.—Plot of solubility of *p*-hydroxyacetanilide, mg./ml. at 25°, as a function of sucrose concentration and dielectric constant of solvent.

volving solvent polarity, as indicated by the dielectric constant of the solvent, and decrease in the activity of water due to the additive sucrose. A strong dependency on the nature of the solute and the solvent is also indicated.

The decrease in solubility relative to the solubility in water, in going from water to syrup, was found to be fairly consistent for several of the derivatives studied. This further indicated the possibility of predicting solubility changes. These conclusions should be considered within the type and range of systems investigated.

This and previous studies show that the formulator, in considering solubility or applying solubility data, needs to take into account the solvent as represented by the total system of interest. It is the authors' view that an appropriate recognition of solubility phenomena is necessary if the formulator is to use solubility differences of the type reported in this and other studies either to his ad-

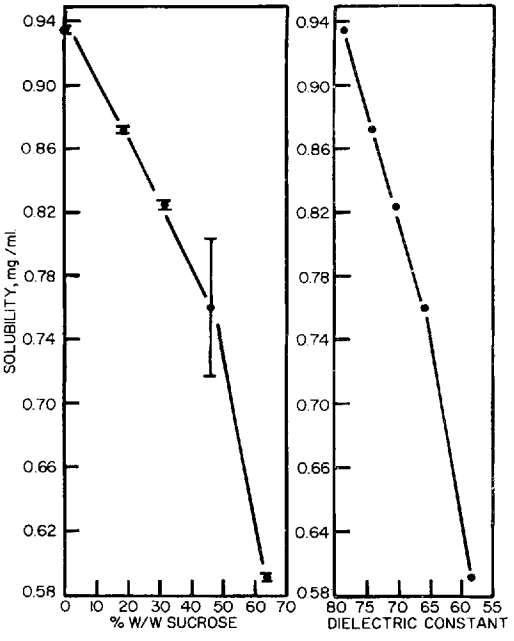

Fig. 5.—Plot of solubility of *p*-ethoxyacetanilide, mg./ml. at 25°, as a function of sucrose concentration and dielectric constant of solvent.

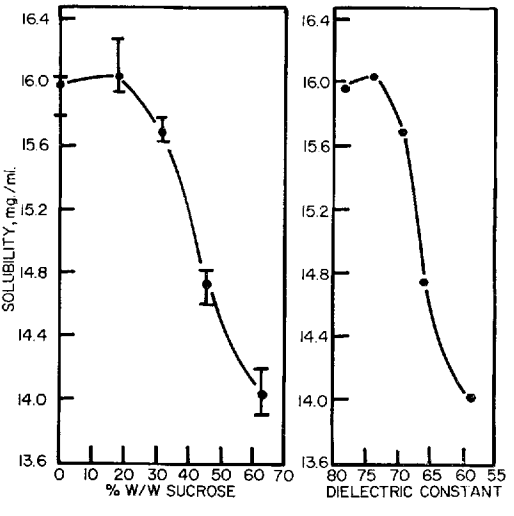

Fig. 6.—Plot of solubility of *p*-aminoacetanilide, mg./ml. at 25°, as a function of sucrose concentration and dielectric constant of solvent.

TABLE III.—SOLUBILITY CHANGE, PER CENT DECREASE RELATIVE TO SOLUBILITY IN WATER, FOR ACETANILIDE AND SEVERAL DERIVATIVES, IN GOING FROM WATER TO 63.4% SUCROSE SOLUTION

Solute	Solubility in Water, mg./ml.	Solubility in 63.4% Sucrose Soln., mg./ml.	Decrease, %
Acetanilide	6.38	4.25	33
p-Methylacetanilide	1.05	0.77	27
p-Ethoxyacetanilide	0.93	0.59	36
p-Hydroxyacetanilide	13.85	9.80	29
p-Aminoacetanilide	15.98	14.02	12
Acetoacetanilide	9.87	4.96	50

vantage in achieving solubility or in avoiding problems due to increased or decreased solubility.

Although there is no sweeping involvement of the dielectric constant as either the parameter of choice or the mechanism involved, it may be a useful tool to the formulator in considering solubility problems.

REFERENCES

(1) Paruta, A. N., *J. Pharm. Sci.*, **53**, 1252(1964).
(2) Paruta, A. N., and Sheth, B. B., *ibid.*, **55**, 896(1966).
(3) *Ibid.*, to be published.
(4) Paruta, A. N., Sciarrone, B. J., and Lordi, N. G., *ibid.*, **53**, 1349(1964).
(5) *Ibid.*, **54**, 838(1965).
(6) Paruta, A. N., and Irani, S. A., *ibid.*, **54**, 1334(1965).
(7) Maryott, A. A., and Malmberg, C. G., *J. Res. Natl. Bureau Std.*, **45**, 299(1950).
(8) Akerlof, G., *J. Am. Chem. Soc.*, **54**, 4125(1932).

Metabolism of ^{14}C-Labeled Glutamic Acid and Pyroglutamic Acid in Animals

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A useful method has been developed for the paper chromatographic separation and identification of certain amino acids found in biological fluids. A synthetic route to ^{14}C-labeled pyroglutamic acid was developed. The ^{14}C-labeled glutamic acid and pyroglutamic acid were given orally to mice and rabbits. The drug concentration in various tissues was determined utilizing a chromatogram scanner or a liquid scintillation counter. Experimental data indicated that the metabolic products formed following glutamic acid therapy were pyroglutamic acid, γ amino butyric acid, and glutamine. Animals to which the labeled pyroglutamic acid had been administered showed radioactivity present as γ amino butyric acid and glutamic acid.

GLUTAMIC ACID is an important amino acid. Although it is not essential for growth, it has been known for many years to be a major constituent of body protein and to take part in many metabolic processes. In clinical therapy the monosodium salt has been used in place of the free acid because L-glutamic acid is only slightly soluble in water and is absorbed slowly by ingestion. On the other hand the monosodium salt, which is soluble to the extent of over 70% at room temperature is readily absorbed (1, 2). Monosodium glutamate has been tested in many types of neurological and psychiatric cases with positive results (3, 4) and with negative results (5). However, glutamic acid therapy is a problem from two points of view. First, large doses are required; and second, its taste is difficult to mask.

In 1944, Ratner demonstrated the formation of D-pyroglutamic acid in rats fed DL-glutamic acid (6). Wilson and Koeppe determined labeled carbon dioxide excretion, pyroglutamic acid formation, and tissue glutamic acid concentrations after the administration of D- and L-glutamic acid-2-^{14}C and DL- or D-glutamic acid-5-^{14}C (7). They found that when labeled D-glutamic acid was administered in small doses, intraperitoneally or by stomach tube, more than 50% of the radioactivity was excreted in the urine in 24 hr., most of it as D-pyroglutamic acid. There has been a great deal of investigation of the metabolism of pyroglutamic acid in animals and man. Bethke and Steenboek found that it was converted to glutamic acid and postulated that it was an enzymatic transformation (8). However, no reports of the presence of pyroglutamic acid in brain tissue were found. Thus, it is proposed to develop a micro-method which could accurately determine the distribution of glutamic acid or a metabolic product in various tissues following oral administration of glutamic and pyroglutamic acid. If the oral administration of pyroglutamic acid shows the same compound or compounds are crossing the blood–brain barrier then possibly it can be used in place of glutamic acid to give a similar therapeutic response at a lower dosage.

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METHODS AND PROCEDURES

The paper chromatographic procedure described below is based on a method reported by Clayton and Strong (9).

Paper Chromatography.—Standard solutions were prepared of various amino acids. Known quantities of the solutions were spotted on strips of Whatman No. 1 chromatographic paper. The strips were