as before the dissociation constant for the weak acid, HA, and X is the amount of HA formed per unit volume at equilibrium. Solving Eqs. 11 and 12 we get

$$\frac{K_{sp}^2}{C_s^2} - K_{sp} + \frac{K_{sp}(H^+)_h}{C_s} + \frac{K_a K_{sp}}{C_s} - K_A C_S = 0 \quad \text{(Eq. 12)}$$

Now substitution of Eqs. 1 and 2 with $C = 0$ into Eq. 12 will show that Eqs. 12 and 9 are again identical when all the diffusion coefficients are set equal to D.

DISCUSSION

The present analysis demonstrates that within the framework of the diffusion layer model the total solubility method and the SCRD method give the same results when all of the diffusion coefficients may be set equal to the same value. Because diffusion coefficients of solute molecules do not differ[4] much in general and other uncertainties—such as variation of dissociation constants and solubilities with ionic strength and other solute interaction effects—are frequently the overriding factors, it would be expected that the total solubility method should explain experimental results as well as the SCRD method. Nelson found this to be the case in many instances (1–3).

Where the two methods will significantly differ would be primarily those situations in which the reacting agent is a colloid, *e.g.*, micellar surfactants which solubilize the solute and nonionic polymers and polyelectrolytes which react with and bind the solute. In these instances, the relatively small diffusion coefficients of colloids will lead to much smaller dissolution rates for the SCRD theory under certain conditions.

[4] Neglecting effects of solvation, Stokes-Einstein law predicts the diffusion coefficients vary approximately as the cube root of the molecular weight for materials of the same density.

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Use of Solubility Analysis in Pharmaceutical Stability Studies

By J. P. COMER and L. D. HOWELL

Purity determined by solubility analysis was used to evaluate the efficacy of other analytical methods for measuring thermal degradation. Techniques for filtration and a method for calculation of confidence interval for solubility analysis are described.

The validity of a stability assay procedure may be confirmed in different ways. If the assays on samples stored at elevated temperatures decrease with time, the method is confirmed. The method of analysis may also be checked by a comparison with a method which is known to measure stability. Several tests of purity are available to the analysts in such cases. Those commonly used include vapor phase, thin layer, and paper chromatography. Garrett (1) reviewed other tests for solvolytic stability of drugs. The purpose of this study was to investigate the possibility of using solubility analysis as a reference assay to evaluate a proposed assay procedure on thermally degraded drug substances. It is not practical to use solubility analysis as the stability assay because of cost, time, and the fact that solubility analysis is only applicable to the drug substance free of excipients. If the proposed or conventional assay procedure is confirmed by the purity test, the procedure may then be used with a fair degree of certainty on the finished pharmaceutical if thermal instability is the main consideration.

EXPERIMENTAL

Three newly developed and two older drug substances were stored at varying temperatures and were periodically withdrawn and assayed by solubility analysis and by an alternate procedure.

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Solubility Analysis Procedure (2)

Varied amounts of the material being tested were allowed to equilibrate at 25° with 2 ml. of an appropriate solvent. After equilibrium was reached, the solution was filtered with a Swinny filter, using S and S No. 740 E filter pads. The filtrate was transferred to a previously tared drying flask and weighed. (The drying flasks were prepared by Ace Glass Co. with an average weight of 6.0 Gm.) The solvent was removed under vacuum at 40°. All weights were recorded to the nearest 0.02 mg. Ratios of mg. solute per Gm. solvent and mg. residue per Gm. solution were calculated for each amount of solute. The data were plotted as mg. residue per Gm. solution (ordinate) *versus* mg. solute per Gm. solvent (Fig. 1). The per cent purity and 95% confidence interval about this purity were calculated by a variation of the method of least squares (3).

Calculations for per cent purity measured by solubility analysis and the 95% confidence limits about this purity are % purity $= 100 - 100\theta$

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

Maximum and minimum values for per cent purity corresponding to a level of confidence of 95% are

$$\text{max. \% purity} = 100 - 100 \tan\left[\arctan\theta - \frac{\arcsin\phi}{2}\right]$$

$$\text{min. \% purity} = 100 - 100 \tan\left[\arctan\theta + \frac{\arcsin\phi}{2}\right]$$

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

where x = mg. solute/Gm. solvent, y = mg. residue/Gm. solution, λ = 1.0 for linear bivariant data, and t values (student t) were chosen to correspond to a level of confidence of 95% and (n-2) degrees of freedom.

Alternate Assays

α - d - 4 - Dimethylamino - 1,2 - diphenyl - 3-methyl-2-propionoxy Butane Hydrochloride.—Sixty-five milligrams of the sample was dissolved in distilled water, and the free base was precipitated with 4 drops of 50% NaOH. The free base was extracted with four 25-ml. portions of chloroform; the chloroform was evaporated to dryness. The residue was dissolved in exactly 2 ml. of chloroform; the absorbance of this solution was measured on a Beckman I.R. spectrophotometer at 5.8 μ in 0.1 mm. rocksalt cells at a slit width of 0.3 mm. These absorbances were compared with those obtained in the same manner on 65 mg. of standard material.

α - 1 - 4 - Dimethylamino - 1,2 - diphenyl - 3-methyl-2-propionoxy Butane-N-oxide Hydrochloride.—The alternate assay for this compound was the same as the one for α-*d*-4-dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane hydrochloride.

Ergonovine Maleate.—This material was assayed per the directions for the standard in the procedure for ergonovine maleate injection in the U.S.P. XVI.

Ascorbic Acid.—The U.S.P. XVI method was used to analyze 100-mg. samples.

N-(p-Acetylphenylsulfonyl)-N-cyclohexyl Urea.—The absorbance of the sample and standard were measured at 249 mμ, at a concentration of 0.01 mg./ml., on a Beckman DU spectrophotometer. The absorbances were compared with those obtained on the original starting material in the same manner as the sample.

The data obtained by solubility analysis and by the alternate procedure were plotted as ln concentration *versus* time (Fig. 2). The results in Table II were obtained from these plots. By plotting the data in this way the assumption is made that the reaction occurring is first order. This was found to be true for the compounds used over the range investigated. The slope of the best fitting line for this type of plot will be the reaction rate constant (k). The k values and the confidence intervals about these values were calculated by the method of least squares.

t values (student t) were chosen to correspond to a level of confidence of 95% and (n-2) degrees of freedom.

One of the major advantages in using a technique such as solubility analysis as a reference assay was that the values obtained were figures of absolute purity. Table II lists the k values and the 95% confidence intervals about these values on five different drug substances. The k values are shown for both solubility analysis and for an alternate procedure. The 95% confidence interval appears to be quite large compared with the k values. The major consideration, however, is to observe the difference between the two methods. If the confidence intervals of the two methods do not overlap, it is apparent that the results of the two methods are significantly different.

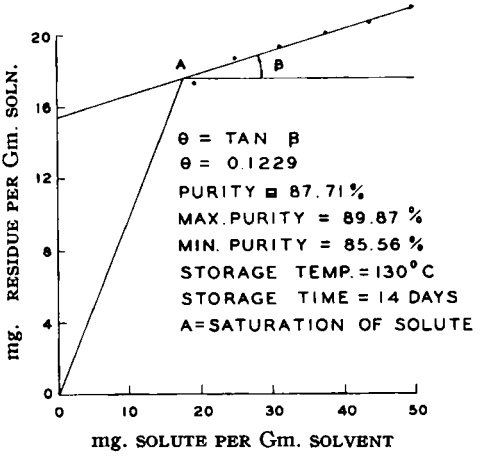

Fig. 1.—α-*d*-4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane hydrochloride.

RESULTS

Neither solubility analysis nor the I.R. spectrophotometric assay detected degradation of α-*d*-4-dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane hydrochloride. The solubility analysis and the I.R. spectrophotometric data showed approximately the same amount of degradation on α-1,4-

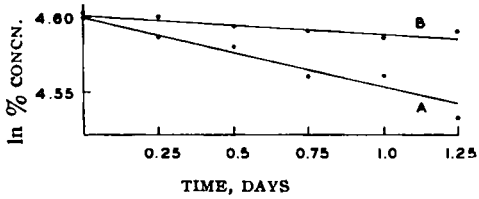

Fig. 2.—Ascorbic acid storage temperature, 130° C. Key: A, solubility analysis data; B, U.S.P. data. Slope A = −0.051 ± 0.021; slope B = −0.0158 ± 0.0079.

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

TABLE I.—SOLVENT SYSTEMS OF SOLUBILITY ANALYSIS

Compd.	Solvent System, %	Solubility at 25°C., mg./Gm.
α-d-4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane hydrochloride	100 ETOAc	7.5
α-1,4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane-N-oxide hydrochloride	65 ETOAc 35 CH_3OH	16.0
Ergonovine maleate	100 CH_3OH	10.5
Ascorbic acid	50 $(CH_3)_2CHOH$ 50 CH_3CH_2OH	15.0
N-(p-Acetylphenylsulfonyl)-N-cyclohexyl urea	40 $CHCl_3$ 60 CH_3OH	23.0

N-(p-Acetylphenylsulfonyl)-N-cyclohexyl urea was measured by an ultraviolet spectrophotometric assay and by solubility analysis. The data obtained indicated that the U.V. assay was incapable of completely measuring the thermal degradation that had occurred. The reaction rate constant (k) of the solubility analysis plot on the samples stored at 130° was ten times greater than that obtained from the U.V. data.

By using solubility analysis as the criteria for purity in the above examples, the conventional methods for measuring thermal degradation were confirmed for one compound, shown to be questionable for three, and verified the thermal stability for one drug substance.

There are many practical considerations involved in the interpretations of these data for pharmaceutical stability studies. Thermal instability may be inconsequential in reference to other modes of degradation. In such cases assay procedures must be designed and verified by other procedures. The use of a method that will measure purity—such as solubility analysis for short term accelerated aging studies—does give valuable information to the pharmaceutical analyst. It provides an estimate of the efficacy of the proposed method and also provides preliminary evaluation of the thermal stability of the drug substance.

TABLE II.—REACTION RATE CONSTANTS

Drug Substance	Storage Temp., °C.	Sol. Anal., $k \times 10^{-3}$	Conf. Int., 95% ± 1×10^{-3}	Alt. Assay, $k \times 10^{-3}$	Conf. Int., 95% ± 1×10^{-3}	Alt. Assay Method
α-d-4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane hydrochloride	80.0	−0.03	0.01	0.01	0.07	I. R.
	105.0	−0.04	0.30	−0.01	0.11	I. R.
	130.0	−0.76	6.70	0.09	3.40	I. R.
α-1,4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionoxy butane-N-oxide hydrochloride	80.0	−0.49	0.70	−0.53	1.20	I. R.
	105.0	−4.83	7.10	−5.7	7.64	I. R.
	125.0	−109.0	72.5	−105.0	57.5	I. R.
Ergonovine maleate	65.0	0.48	1.07	−0.10	1.04	Colorimetric
Ergonovine maleate	80.0	−0.50	0.67	−0.28	0.71	Colorimetric
Ergonovine maleate	100.0	−3.98	1.76	−1.61	0.47	Colorimetric
Ascorbic acid	80.0	−0.98	1.59	−0.88	0.44	I_2 titr.
Ascorbic acid	105.0	−9.10	1.93	−1.06	0.91	I_2 titr.
Ascorbic acid	130.0	−51.0	21.3	−15.8	7.88	I_2 titr.
N-(p-Acetylphenyl sulfonyl)-N-cyclohexyl urea	80.0	−0.06	0.57	−0.49	0.87	U. V.
	105.0	−4.09	1.28	−1.19	1.10	U. V.
	130.0	−123.0	14.7	−13.7	18.8	U. V.

dimethylamino-1, 2-diphenyl-3-methyl-2-propionoxy butane-N-oxide hydrochloride.

The solubility analysis on ergonovine maleate indicated that more degradation had occurred than was shown by the colorimetric assay. The colorimetric assay was therefore not capable of measuring completely the thermal degradation that had occurred.

Ascorbic acid was measured by iodometric titration and by solubility analysis. The iodometric assay was incapable of completely measuring the thermal degradation that had occurred.

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