Table I—Comparison of Serum Creatinine Levels Using Three Different Methods

HPLC, mg%	Automated Picrate Method[a], mg%	Deviation from HPLC, %	Modified Picrate Method, mg%	Deviation from HPLC, %
0.44	0.5	13.6	—	—
0.42	0.5	19.0	0.72	71.4
0.41	0.5	22.0	0.65	58.5
0.52	0.5	−3.8	0.75	44.2
0.59	0.5	−15.3	0.98	66.1
0.48	0.5	4.2	0.89	85.4
0.60	0.5	−16.7	0.88	46.7
0.47	0.5	6.4	0.80	70.2
0.57	0.6	5.3	0.91	59.6
0.47	0.6	27.7	0.95	102.1
0.48	0.6	25.0	0.83	72.9
0.48	0.6	25.0	0.20	−58.3
0.51	0.6	17.6	0.86	68.6
0.56	0.6	7.1	0.87	55.4
0.55	0.6	9.1	1.26	129.1
0.38	0.6	57.9	1.02	168.0
0.52	0.6	15.4	0.90	73.1
0.62	0.6	−3.2	0.54	−12.9
0.61	0.6	−1.6	0.84	37.7
0.49	0.6	22.4	0.86	75.5
0.64	0.7	9.4	0.92	43.8
0.62	0.7	12.9	1.11	79.0
0.51	0.7	37.3	1.10	115.7
0.61	0.7	14.8	0.88	44.3
0.61	0.7	14.8	0.46	−24.6
0.66	0.7	6.1	0.84	27.3
0.42	0.7	66.7	0.71	69.0
0.67	0.8	19.4	1.05	56.7
0.68	0.8	17.6	0.71	4.4
0.67	0.8	19.4	0.49	−26.9
Mean		15.2		55.2
±*SD*		±17.5		±47.2

[a] Only one significant figure was provided for concentrations <1.0 mg% by the printout of the instrument.

by an average of 32% in serum samples with a wider range of creatinine levels (0.62–18.5 mg% based on the HPLC method). However, for samples (n = 3) with serum levels in the range of 0.6–0.7 mg% (10) the mean overestimation was 88.2%. Thus, both of the modified picrate methods (12, 13, 15) resulted in consistently much higher creatinine values. The reason for the apparent discrepancy between their claimed specificity (12, 15) and the present as well as the previous (10, 16) findings remains to be investigated.

The results of the present study indicate that one should be cautious in using the automated and modified picrate methods for creatinine determinations. Since the HPLC method is generally considered to be more specific (3), its use should be preferred when accurate determinations are required.

It has been recently reported that creatinine might be extensively secreted and reabsorbed by renal tubules in both humans (17, 18) and animals (18, 19), and there might also be a significant nonrenal elimination in normal humans (19, 20) and normal rabbits (19). The full implications of these findings in the use of creatinine remain to be explored.

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*Yih-Chain Huang**
Win L. Chiou[x]

Departments of Pharmacodynamics and Pharmaceutics
College of Pharmacy
University of Illinois at Chicago
Chicago, IL 60612

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* Present address: College of Pharmacy, Rutgers–The State University, Piscataway, NJ 08854.

Crystalline Anhydrous–Hydrate Phase Changes of Caffeine and Theophylline in Solvent–Water Mixtures

Keyphrases □ Phase changes—in crystals, anhydrous to hydrate, caffeine, theophylline, solubility studies □ Caffeine—crystalline phase changes, anhydrous to hydrate, solubility studies □ Theophylline—crystalline phase changes, anhydrous to hydrates solubility studies

To the Editor:

The extended Hildebrand solubility approach (1–3) was recently developed and evaluated for calculation of solubilities in solvent–water systems that are not adequately described by the regular solution theory (4). The approach is based on regression analysis of solubility data to calculate solute–activity coefficients as a function of the solubility parameter of the solvent mixture.

A problem exists with several of the solutes used in the evaluation of the extended Hildebrand approach: crystalline anhydrous–hydrate phase transformation as the water content of the solvent mixture is changed. In the dry solvent it is clear that only a nonhydrated (anhydrous or solvated) form can be present at equilibrium (5); but in aqueous solvent mixtures the equilibrium form may be anhydrous solvated or hydrated crystals. This was recently demonstrated for cholesterol in water–glyceryl-1-monooctanoate solutions (6). The transition between anhydrous and monohydrate forms occurred at 5% water (37°) and was temperature dependent. In this system the max-

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imum cholesterol solubility coincided with the anhydrous–hydrate crystalline phase change.

The water content at which a given compound has the potential to convert to its hydrate is not predictable. A metastable form may remain supersaturated for long periods, particularly if the degree of supersaturation is not large. In addition, little is known about the effects of such crystal changes on solubility profiles in mixed solvents. Based on these and other considerations discussed below, compounds subject to such phase changes are not appropriate for modeling by the extended Hildebrand approach. Caffeine, theophylline, and theobromine all form hydrates, although they have been used as model compounds for evaluation of the extended Hildebrand approach (1–3). The purpose of this communication is to point out that knowledge of the crystal phase present at equilibrium is essential for the study of solubility. Furthermore it is especially important to identify the solid phase at equilibrium in mixed solvents.

To illustrate these points we examined the solid phases present when caffeine or theophylline were equilibrated at 25° in 0–50% water–dioxane solutions for 3–5 days[1]. These conditions were similar to those employed previously (1, 2). To avoid compositional bias, separate samples were equilibrated which contained either the anhydrous or monohydrate forms. At equilibrium the solid phases were filtered, dried under ambient conditions, and analyzed by differential scanning calorimetry (DSC)[2]. The hydrates were prepared by aqueous recrystallization and found to be monohydrates using Karl Fischer titrimetry[3]. At ambient conditions the hydrates were stable for at least 24 hr with respect to dehydration (7), and the anhydrous forms did not react with atmospheric moisture to form the hydrates. For some samples, solubilities were measured spectrophotometrically after 0.2-μm membrane filtration[4]. When heated in the DSC at 10°/min, the presence of water crystallization was verified by the broad dehydration endotherm centered at about 80° (caffeine) and 90° (theophylline). In these experiments the heat of dehydration was not measured and, thus, samples with the hydrate peak could also contain some anhydrous material. The solubilities were consistent with the previously reported data (1–3) except as described below.

For theophylline systems above ~5% water, the hydrate was always present at equilibrium. Below this concentration the anhydrous form was isolated. These findings were independent of the solid form initially added to the solvent, indicating that equilibrium was achieved with the more stable form. With a few exceptions, samples of caffeine equilibrated with 10–50% water, however, remained in the crystal form initially added. This apparent resistance to nucleation and crystallization of caffeine monohydrate led to significant differences in solubility. For example, at 50% water the solubilities were 71 mg/ml (hydrate) and 86 mg/ml (anhydrous). As the solvent water content decreased the solubilities became more similar [49 mg/ml (hydrate) *versus* 51 mg/ml (anhydrous)]. This behavior may explain the irregularity of the reported solubility profile in water–dioxane (3). With 0–5% water, samples initially prepared with hydrate were found to be anhydrous at equilibrium. There was no correlation between maximum solubility [at ~30–50% water (3)] and the crystal form change (at 5–10% water) in these systems. This is probably related to the extensive self-association of caffeine in water (8).

The regular solution theory (4) was developed to describe the solubility of molecular crystals, *i.e.*, single component substances. For solubility calculations the heat (or entropy) of fusion and the melting point of the solute are required. In the previous work these constants were obtained for the anhydrous forms by DSC (1–3). Since the equilibrium crystal form was most often the hydrate, these values should not have been used for calculation of solubility. The different calorimetric heats of solution (25°) in water for anhydrous caffeine (3.4 kcal/mole) and the hydrate (5.0 kcal/mole) show that the forms have quite different crystal energies (8). Further work will be required to develop the appropriate equations and physical constants for solubility modeling of hydrated (or solvated) crystalline compounds.

[1] Vibromixer E1, Chemapec Inc.
[2] DSC-1B, Perkin-Elmer.
[3] Auto-aquatrator, Precision Scientific Co.
[4] Alpha Metricel, Gelman.

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Joseph B. Bogardus
College of Pharmacy
University of Kentucky
Lexington, KY 40506

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Pharmacokinetic Absorption Plots from Oral Data Alone or Oral/Intravenous Data and An Exact Loo–Riegelman Equation

Keyphrases □ Deconvolution—amount absorbed as a function of time for all common disposition models; amount of drug in peripheral compartments of mammillary model from measurement in central compartment □ Wagner-Nelson equation—drug absorption □ Loo–Riegelman equation—drug absorption

To the Editor:

The purposes of this Communication are: (*a*) to give exact absorption equations when drug disposition is described by one, two, or three exponential terms; (*b*) when disposition is described by two exponential terms to show that one of the new absorption equations is an exact Loo–Riegelman equation and simpler and easier to use than the latter; and (*c*) to describe and illustrate use of the equations in a preliminary manner only.

The models to be considered are shown as models I, II, and III below.

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