photothermally degraded by irradiating with intense UV light[13] for 30 min. A 100-mg sample of partially degraded I was accurately weighed, quantitatively transferred into a 50-ml volumetric flask, and dissolved in methanol. Then 20.0- and 15.0-ml aliquots were removed, the solvent was evaporated, and the sample was reassayed (Fig. 3). The recovery was 101% by the official NF XIV assay but only 33% by HPLC. The system selectivity is demonstrated further in Fig. 4, where I is resolved from II. These compounds are very similar in structure, size, and polarity.

Statistical Evaluation—The linearity of typical standard curves is summarized in Tables II and III. The response factor ratios, defined as the area ratio (active ingredient/fluorene) times the concentration ratio (fluorene/active ingredient), were quite constant for all concentrations. Precision was demonstrated by a relative standard deviation of 0.4% for nine replicate injections. Analysis of active–placebo mixtures demonstrated the accuracy of the proposed method with an average 99.1% recovery.

The experimental data shown in Table IV were obtained on randomly selected commercial tablet samples. Each sample was analyzed by the HPLC method and by the official NF XIV procedures. The results from both methods are comparable, but since the HPLC method has been shown to be a stability-indicating assay, it is more selective than the NF method and more accurately reflects true tablet potency.

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Solubility Determination of Barely Aqueous-Soluble Organic Solids

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Abstract □ Solubility determination of organic molecules having very low solubilities is hampered by such problems as slow equilibration during measurement, influence of impurities, and inherent heterogeneity in the energetic content of the crystalline solid. Three approaches to meeting these problems are presented. The first approach involves enhancing the dissolution rate by the addition of a water-immiscible solvent in which the organic solute is more soluble, thereby increasing the surface area available for dissolution. The second method is a combination of experimental data with a group contribution approach that allows the estimation of extremely insoluble solids. This approach involves measurement of the solubility in an organic solvent and calculation of the aqueous solubility from the estimated partition coefficient and the organic solvent data. The third approach is based on using a large excess of the solid and a highly specific analytical determination of the main component. The first two approaches were explored in detail and tested using norethindrone, norethindrone acetate, methyltestosterone, and methyltestosterone acetate.

Keyphrases □ Solubility determinations—organic solids, barely aqueous soluble, group contribution, partition coefficient □ Organic solids—solubility determinations, barely aqueous soluble, group contribution, partition coefficient □ Aqueous solubility—organic solids, barely soluble, solubility determination

The solubilities of solids are very useful parameters in the daily practice of chemistry and pharmacy. Solubility information is particularly vital in formulating products, developing analytical methods, chemical processing, predicting ecological impact, and assessing drug transport and distribution problems.

Solubility determination of solids that are moderately soluble, *i.e.*, ≥ 0.2%, normally poses no serious problem. Direct solubility measurement of solids having very low solubilities involves special difficulties, which can lead to large discrepancies in reported values. For example, the reported solubility of cholesterol in water ranges from 0.025 to 2600 μg/ml (1). This paper is concerned with an analysis of these problems and offers several approaches for overcoming them, particularly for determinations carried out in water.

BACKGROUND

Equilibration Kinetics—The low equilibration rate during solubility measurement of slightly soluble species presents a serious problem which has not been adequately addressed from an experimental standpoint. The solubility of a solid in a given solvent is the amount that goes into solution at equilibrium at some selected temperature. Since it is impossible to attain a true equilibrium state in real life, in practice, results are reported for systems that are reasonably close to equilibrium. Such situations are not particularly difficult to attain for moderately soluble substances. An aqueous suspension of benzoic acid crystals, for example, probably reaches a near equilibrium state with moderate stirring at 25° within a few hours. The exact rate depends on the fineness of the crystals, the amount of solid added, and the degree of agitation.

According to various studies on dissolution kinetics, the classical equation (Noyes–Whitney) (2, 3) appears to be an empirically useful relationship. A simplified form of the equation for situations where the various geometric factors are essentially held constant can be written as:

$$\frac{dc}{dt} = k(C_s - C) \qquad \text{(Eq. 1)}$$

where dc/dt is the rate of increase in the solute concentration, k is a constant, C_s is the solubility, and C is the solute concentration in solution. Upon integrating, one obtains:

$$\ln \frac{C_s - C}{C_s} = -kt \qquad \text{(Eq. 2)}$$

Under these conditions, the kinetics suggest an approximately first-order approach to saturation, with a given degree of saturation being achieved somewhat independently of the solid being studied. Under experimental conditions, however, unless a substantial excess of the solid is used, the effective surface area will decrease significantly during the dissolution process, with a corresponding delay in the attainment of equilibrium.

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Table I—Measured Molar Solubilities and Isooctane–Water Distribution Coefficients for Steroids [a]

Substance	Molar Solubility in Isooctane[b]	Molar Solubility in Water[b]	Molar Solubility in Water in Contact with Isooctane	Isooctane–Water Partition Coefficient
Norethindrone	$6.90 \pm 0.13 \times 10^{-5}$ (5.85×10^{-5})[c]	$2.36 \pm 0.06 \times 10^{-5}$	$2.13 \pm 0.05 \times 10^{-5}$ (1.67×10^{-5})[c]	3.9 ± 0.17 (4.4)[b]
Norethindrone acetate	$2.52 \pm 0.04 \times 10^{-3}$ (2.53×10^{-3})[c]	$1.57 \pm 0.01 \times 10^{-5}$	$1.76 \pm 0.01 \times 10^{-5}$ (1.65×10^{-5})[c]	210 ± 5 (183)[c]
Norethindrone enanthate	0.0232[b]		($7 \pm 3 \times 10^{-8}$)[c]	(3.3×10^{5})[c]
Methyltestosterone	$1.3 \pm 0.03 \times 10^{-3}$	$1.12 \pm 0.02 \times 10^{-4}$ (7.47×10^{-5})[d]	$1.11 \pm 0.01 \times 10^{-4}$	16.0 ± 0.5
Methyltestosterone acetate	$5.3 \pm 0.05 \times 10^{-3}$	$1.43 \pm 0.03 \times 10^{-5}$ (5.20×10^{-6})[d]	$8.16 \pm 0.06 \times 10^{-7}$	3300 ± 173

[a] Uncertainties are expressed as standard errors of the mean. [b] Obtained by conventional method. [c] R. E. Enever, University of London, London, England, personal communication. [d] Literature value from D. B. Bowen, K. C. James, and M. Roberts, *J. Pharm. Pharmacol.*, **22**, 518 (1970).

The final rate of approach to saturation is approximately directly proportional to the excess of solid present—the larger the excess, the faster the saturation rate. A slightly soluble solid of equivalent surface area would require many times greater excess for saturation than would be needed for a moderately soluble solid. Thus, the terminal rate of attainment of equilibrium in water for benzoic acid (solubility in water = 3.4 mg/ml) (4) containing initially twice the amount necessary to saturate the solution will be equal to that of norethindrone (solubility in water $\cong$ 6 μg/ml) only if approximately the same amount (the same surface area) is left undissolved at the end. In this situation, the initial amount necessary to leave the same surface area undissolved as a onefold excess for benzoic acid would be an ~500-fold excess for norethindrone.

Although a relatively rapid equilibration even for a rather insoluble system would seem possible with a very large excess of the test solid, this process is not feasible for many real situations because the effects of impurities and energetic heterogeneity of crystals are magnified greatly when solute is present in a large excess.

The influence of impurities on the apparent solubility of crystalline solids is well known and can be visualized from a typical phase solubility diagram (Fig. 1) for a solid containing 10% of a soluble impurity. Typically, the apparent solubility increases with the amount of sample used until the system becomes saturated with respect to the impurity. Although in this example the indicated true solubility corresponds to 4.5 mg, the apparent solubility continues to increase with an increase in sample size. The increase corresponds to onefold for each 10-fold increase in the amount added for the situation shown. In the case of norethindrone with a 500-fold excess, each 1% of total soluble impurities in the sample will produce a fivefold increase in the apparent solubility of the material, that going into solution being largely the impurities present rather than the main compound.

Thus, direct solubility determinations on relatively insoluble species are difficult. If a large sample is used to obtain a fairly short equilibration time (hours to weeks), the impurities may pose a problem. On the other hand, if a small excess of sample is used to minimize the impurity effect, the equilibration time may extend to months or years. Use of only a onefold excess of norethindrone, for example, will require an ~500-fold greater equilibration time than for the described benzoic acid system, the corresponding time being on the order of months.

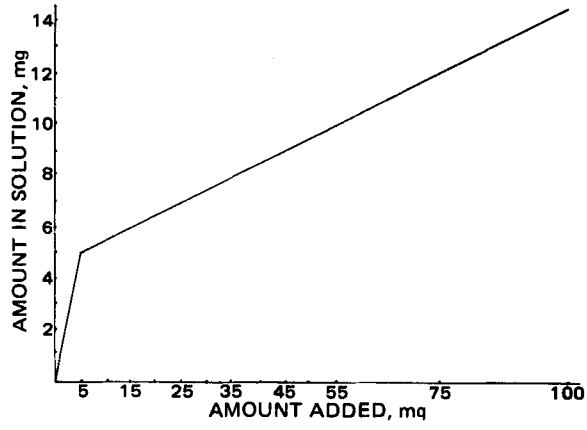


Figure 1—*Phase solubility diagram of a crystalline solid containing 10% soluble impurity.*

A possible solution to this dilemma is to eliminate the complication introduced by any impurity by utilizing a highly specific method for determining the amount of principal component in solution. If only the principal component is measured, the concentration in solution in Fig. 1, for example, would become independent of the amount. Since, however, the minor components present in the solid sample are usually not eliminated during typical purification procedures, they would probably be similar to the main component and lead to analytical complications. In solubility ranges below 1 ng/ml, highly exquisite and specific analytical methods would be required since at these solubilities the main component concentration may be one-millionth of the concentrations of the main impurities to obtain a reasonable equilibration time. Nevertheless, this approach can probably be used effectively in many situations.

Another factor that affects the solubility is the energetic heterogeneity that results from the spectrum of particle sizes and individual crystals with cracks and other energetic defects in a real sample. This factor would be self-correcting under normal conditions. When large excesses are permitted, however, the observed solubility will correspond to the thermodynamic activity of the most energetic component and may be supersaturated with respect to the most stable crystal form. Because of the very low solute concentration in solution, the recrystallization rate, which brings the supersaturated solution back to normal, will be extremely slow.

Proposed Approaches—There are several possible ways to approach this dilemma. One is a direct, experimental approach in which a minimal amount of solid sample is used to minimize the effect of impurities yet allow a near equilibrium state to be attained in a reasonable time. A combination of an experimental procedure with a group contribution approach allows solubility estimation of extremely insoluble solids with some accuracy. Another possibility is that suggested earlier based on a very large excess of the solid and highly specific determination of the main component. The first two approaches will be discussed in some detail.

Dissolution Facilitation by Immiscible Solvent Addition—As already discussed, the saturation rate of a solution by a relatively insoluble solid becomes extremely slow if only a small excess of the solid is used due to the limited surface area of the solid exposed to the dissolutive process. Generally, one would expect the available area to parallel the amount of solid used. Thus, for the benzoic acid system cited earlier, a onefold excess

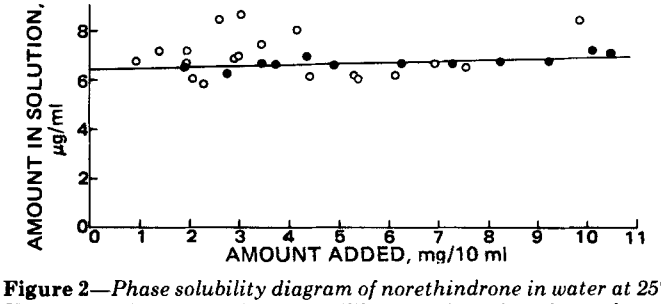


Figure 2—*Phase solubility diagram of norethindrone in water at 25°. Key: ○, 10 ml of water with an equilibration time of 120 hr; and ●, 10 ml of water plus 0.5 ml of isooctane with an equilibration time of 12 hr.*

Table II—Isooctane–Water Partition Coefficients and Standard Free Energy of Transfer for Organic Molecules [a]

Compound	Partition Coefficient	$\Delta G°$, cal/mole
2-Cyclohexen-1-one	0.46 ± 0.01	460
1-Ethynylcyclopentanol	0.36 ± 0.01	580
1-Ethynylcyclopentyl acetate	36.5 ± 1.4	−2100
1-Methylcyclopentanol	0.46 ± 0.01	460
1-Methylcyclopentyl acetate	227 ± 11	−3200

[a] Uncertainties are expressed as standard deviations.

corresponds to 6.8 mg/ml or a surface area of ~4 cm^2/ml of water at the beginning and a little more than 2 cm^2/ml at saturation if the initial average diameter of the benzoic acid is ~100 μm. Similar calculations for norethindrone acetate would be 6×10^{-3} and 4×10^{-3} cm^2/ml, much smaller values.

One way of increasing the interfacial area across which the solute is allowed to diffuse is to add a limited amount of a second immiscible solvent to the system in which the solute is significantly more soluble. The solubility of norethindrone acetate in isooctane at 25° is 860 μg/ml, 200 times that in water. If the solvent phase consists of 100 ml of water and 2 ml of isooctane with 5 mg of norethindrone acetate added to it, ~40% of the initial solid will go into solution. The isooctane phase will contain at near equilibrium ~1.8 mg and the aqueous phase will contain 0.5 mg.

If all of the hydrophobic steroid remains with the organic solvent, there will be 3 mg of the solid in contact with 2 ml of the isooctane solution or 1.0 cm^2/ml of solution. This surface area–solution volume ratio can be expected to yield a reasonable equilibration rate in the organic phase. The equilibration rate from isooctane to water will be determined effectively by the interfacial area between the two and will be independent of the amount of solid present. Since any reasonable configuration will correspond to a fairly high interfacial area–water volume ratio, a relatively rapid equilibration rate would be expected under even mild agitation. The equilibration rate in the organic phase will depend, of course, on the surface area of the solid available per milliliter of solvent used.

The suggested approach presupposes that: (*a*) the solid phase effectively stays with the organic phase, (*b*) the solubility in water is sufficient for measurement after equilibration, and (*c*) the organic solvent is sufficiently immiscible in water not to affect the solubility significantly. Although some of these conditions may not be met in all instances, the method offers a solution to many situations where equilibration may be a problem. This approach would be contraindicated for solids containing impurities that are significantly more soluble in water than in the organic phase as compared to the main component.

Estimation of Aqueous Solubility of Extremely Insoluble Hydrophobic Solids—For systems that are significantly less soluble than a few nanograms per milliliter, the equilibration rate as well as the measurement of the solute concentration presents serious experimental problems, requiring methods other than direct measurement.

One possible approach is based on the fact that hydrophobic compound solubilities are generally sufficient in a water-immiscible organic solvent to allow direct measurement. Once the solubility in some selected organic solvent is known, the solubility in water can be calculated from the directly measured or, more usually, the estimated partition coefficients.

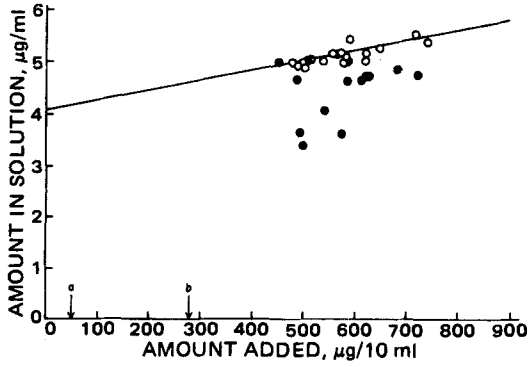


Figure 3—*Phase solubility diagram of norethindrone acetate in water at 25° with an equilibration time of 51 hr. Key: ○, 10 ml of water and 0.25 ml of isooctane; ●, 10 ml of water; a, amount of drug that saturated 10 ml of water; and b, amount of drug that saturated 10 ml of water and 0.25 ml of isooctane.*

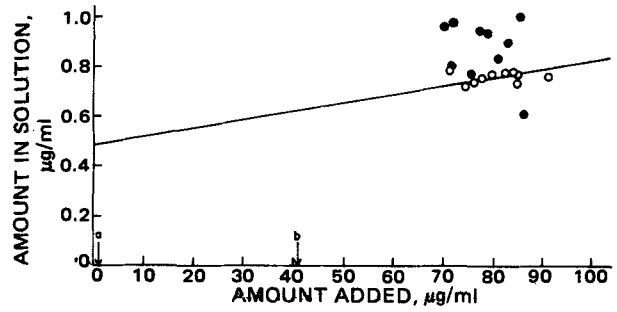


Figure 4—*Phase solubility diagram of methyltestosterone acetate in water at 25° with an equilibration time of 34 hr. Key: ○, 10 ml of water and 0.2 ml of isooctane; ●, 10 ml of water; a, amount of drug that saturated 10 ml of water; and b, amount of drug that saturated 10 ml of water and 0.2 ml of isooctane.*

At present, prediction of solubility is difficult because it depends on cooperative interactions within the crystal structure of the molecular components. However, changes in partition coefficients with alterations in molecular structure can be predicted with reasonable confidence since partitioning is, from a practical standpoint, a noncooperative operation (Scheme I).

The solubility of a crystalline solid in the organic solvent is measured accurately by the phase solubility technique. Its partition coefficient between water and the organic phase is estimated by the usual group contribution approach based on experimental results obtained on the nearest appropriate analog. Aqueous solubility is calculated from these two numbers.

Data are presented and analyzed to illustrate the utility of the two proposed approaches.

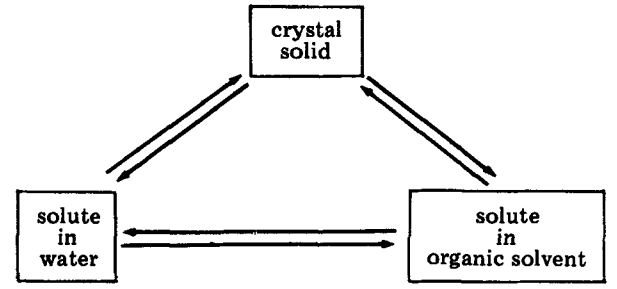
EXPERIMENTAL

Materials—Norethindrone[1] (I), norethindrone acetate[1] (II), methyltestosterone[1] (III), 2-cyclohexen-1-one[2], 1-ethynylcyclopentanol[2], and 1-methylcyclopentanol[2] were used without further purification.

The acetylation of 1-ethynylcyclopentanol was accomplished by combining the alcohol with acetic anhydride and stirring for 2 hr at 55°, using pyridine as the solvent. 1-Methylcyclopentanol and methyltestosterone were acetylated by the method of Steglich and Höfle (5) with 4-*N*,*N*-dimethylaminopyridine as a catalyst. Product purity was checked by IR and NMR spectra. The melting point of methyltestosterone acetate (IV) was 167.5°.

Distilled water was obtained by distilling previously deionized water[3]. Isooctane[4] was distilled from potassium permanganate and filtered through neutral alumina. Methanol[5] and isopropyl alcohol[6] were used without further purification.

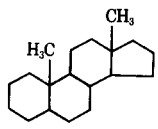
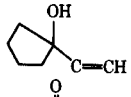
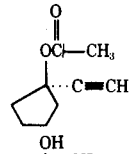
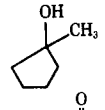
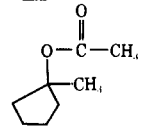
Procedure—Solubilities in each solvent were determined by the phase solubility technique (6). Seven to 15 15-ml glass, polytef-lined, screw-capped tubes were scrupulously cleaned. To each tube, increasingly larger amounts of the test substance were added. A constant volume (10 ml) of solvent (water or isooctane) was added to each tube. If the approximate



Scheme I—Determination of solubility of extremely water-insoluble hydrophobic solids.

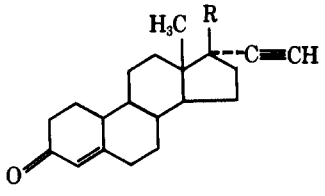
[1] Sigma Chemical Co.
[2] Aldrich Chemical Co.
[3] Model AG-1a distillation apparatus, Corning Glass Works.
[4] Analytical grade, Fisher Scientific Co.
[5] Spectral grade, Fisher Scientific Co.
[6] Analytical grade, Mallinckrodt Chemical Works.

Table III—Calculation of the Free Energy of Transfer and Partition Coefficients for Norethindrone, Methyltestosterone, and Their Acetates

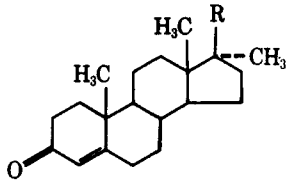
Functional Group	Structure	$\Delta(\Delta G°)$, cal/mole	Added or Subtracted I	II	III	IV
17-Androstyl		−10,700[a]	+	+	+	+
Methyl		−900[a]	−	−		
Cyclohexyl		−4400[a]	−	−	−	−
2-Cyclohexen-1-one		460[b]	+	+	+	+
Cyclopentyl		−3700[a]	−	−	−	−
1-Ethynylcyclopentanol		580[b]	+			
1-Ethynylcyclopentyl acetate		−2100[b]		+		
1-Methylcyclopentanol		460[b]			+	
1-Methylcyclopentyl acetate		−3200[b]				+

	I	II	III	IV
$\Delta G_t°$	−660	−3340	−1680	−5340
Partition coefficient (calculated)	3.05	281	17.0	8200
Partition coefficient (measured)	3.9	230	9.4	3300

[a] Reference 7. The relative surface area used for the 17-androstyl group was 2.91; for the methyl group, it was 0.51 where the *tert*-butyl group has a value of 1. [b] Table II.



I: R = OH
II: R = $OCOCH_3$



III: R = OH
IV: R = $OCOCH_3$

solubility was reasonably large, the tubes were prepared so that the contents would be completely dissolved in at least one tube.

The tubes were sealed with parafilm and fixed on a rotating shaft in the constant-temperature bath. They were equilibrated at 25° for ≥7 days, depending on the system.

After equilibration, the tubes were taken out of the bath and centrifuged. The sample was filtered immediately under pressure through a sintered-glass filter, which was presaturated with the tested compound. A desired volume of filtrate was diluted with the solvent, which was used to construct the calibration curve. The diluted solutions were analyzed spectrophotometrically[7].

In cases of facilitated dissolution, an additional isooctane aliquot was added to the top of the aqueous layer. The solutions were allowed to equilibrate in the same manner as before. The isooctane layer was removed and discarded before filtration under pressure. The filtrate was measured spectrophotometrically either directly or after dilution.

Partition coefficients from water to isooctane were measured using a standard procedure, which involved preparing a stock solution in isooctane (or water). Stock solution aliquots were diluted quantitatively to several concentrations, and at least two or three samples for each concentration were prepared. A suitable amount of water (or isooctane) was added to each flask, and these flasks were capped and sealed with parafilm. Each sample was shaken by hand for 2 min and allowed to stand in a water bath at 25 ± 0.5° for 8 min. The process was repeated six times, and the samples were allowed to stand in a thermostated water bath for 24 hr. The solute concentrations in each layer were measured by UV spectroscopy or GLC.

The relative surface areas needed were estimated by the method of Harris *et al.* (7) or were taken from Ref. 7.

Analytical Methods—Norethindrone, norethindrone acetate, methyltestosterone, methyltestosterone acetate, and 2-cyclohexen-1-one were determined spectrophotometrically[7]. 1-Ethynylcyclopentanol, 1-methylcyclopentanol, and their acetates were determined by GLC[8]. The column temperature was 115° for 1-ethynylcyclopentanol and 1-ethynylcyclopentyl acetate, yielding a retention time of 11.2 min for each; 1-heptanol, the internal standard, had a retention time of 7.2 min. A column temperature of 75° was used for 1-methylcyclopentanol and 1-methylcyclopentyl acetate, resulting in retention times of 9.5 and 9.6 min, respectively, compared with 10.7 min for the internal standard, 1-pentanol.

RESULTS AND DISCUSSION

Facilitated Dissolution Approach—The solubilities and isooctane–water partition coefficients at 25° for norethindrone, norethindrone

[7] Cary model 15, 16, or 118.

[8] Varian aerograph series 2100 with flame-ionization detectors using a glass column packed with 10% Carbowax 20M on Gas Chrom Q (80–100 mesh). Injection port and detector temperatures were both 145°, and the carrier gas (nitrogen) flow rate was 50 ml/min.

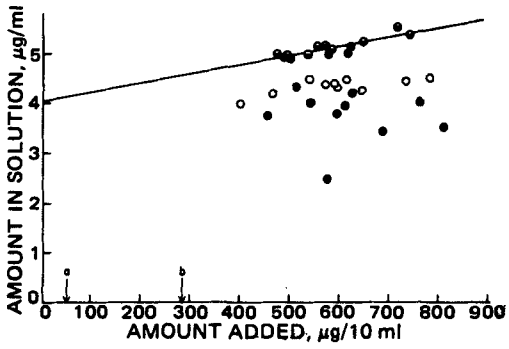


Figure 5—*Comparison of concentrations of norethindrone acetate obtained by the facilitated dissolution method and by the conventional method at the preequilibration state of 6 hr at 25°. Key: ○, 10 ml of water and 0.25 ml of isooctane; ●, 10 ml of water; and ⊖, 10 ml of water and 0.25 ml of isooctane at 51 hr (Fig. 3).*

acetate, methyltestosterone, and methyltestosterone acetate are given in Table I. Phase solubility diagrams (Figs. 2–5) for norethindrone, norethindrone acetate, and methyltestosterone acetate show equilibration both in pure water and in water to which a small amount (<5% of total volume) of isooctane had been added.

There was considerably greater scatter among the experimental points for the solubility determinations in pure water, particularly at the shorter equilibration times. The addition of an immiscible solvent definitely enhanced the equilibrium rate and yielded more precise results with at least comparable, and usually improved, accuracy.

The scatter observed in the data obtained with small excesses of solute in pure water may have resulted from particle-size heterogeneity as well as from crystalline differences. Particle-size heterogeneity is magnified greatly when small amounts are used. The facilitiated dissolution method prevented this problem even at an early stage of equilibration (Fig. 5). This procedure also reduced the effect of impurities on the apparent solubility if these impurities were more soluble in isooctane than in water. Methyltestosterone acetate, for example, was prepared containing 14% soluble impurities as calculated from the phase solubility diagram in isooctane. Attempts to extrapolate its aqueous solubility directly from its phase solubility diagram in water were not successful. The addition of 5% isooctane to the system greatly reduced the difficulty (Fig. 4).

The facilitated dissolution method offers an attractive alternative for aqueous solubility measurements where equilibration may be a problem. With radioactive labeling techniques, it should be feasible to apply this approach to solubilities at the nanogram per milliliter level.

Group Contribution Approach—As already described, substances that are extremely insoluble in water present great difficulties in direct solubility measurements. An approach suggested here consists of calculating the solubility in water from the measured solubility in an organic solvent combined with either a measured or estimated partition coefficient. This approach was tested with norethindrone, methyltestosterone, and their acetates.

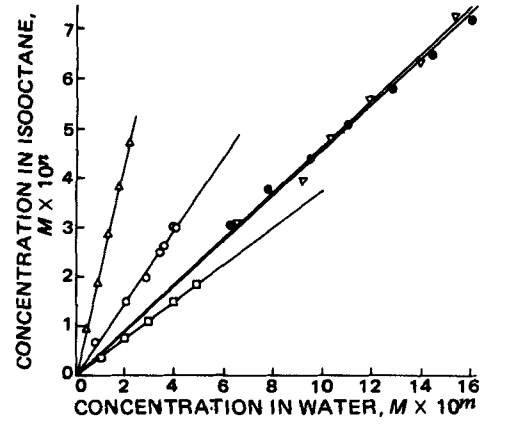


Figure 6—*Data used for the determination of isooctane–water distribution coefficients for several organic compounds at 25°. Key: ●, cyclohexen-1-one, m = 5, n = 5; △, 1-methylcyclopentanol, m = 4, n = 4; □, 1-methylcyclopentyl acetate, m = 4, n = 3; ▽, 1-ethynylcyclopentanol, m = 4, n = 4; and ○, 1-ethynylcyclopentyl acetate, m = 4, n = 3.*

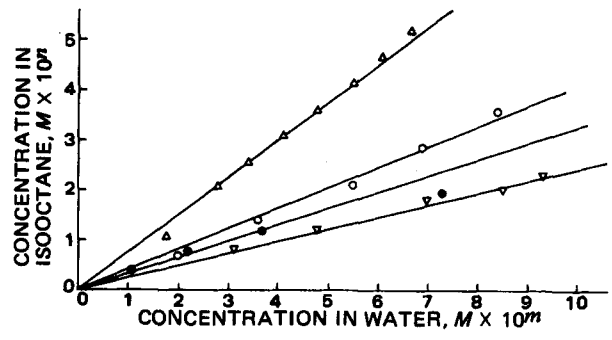


Figure 7—*Distribution of several steroidal compounds between isooctane and water at 25°. Key: ○, norethindrone, m = 6, n = 5; ▽, norethindrone acetate, m = 7, n = 4; △, methyltestosterone, m = 6, n = 5; and ●, methyltestosterone acetate, m = 7, n = 3.*

The measured solubilities and partition coefficients are given in Table I. The predicted partition coefficients were determined using a group contribution approach, which assumes that certain thermodynamic or other physical properties of a molecule can be found from the sum of the values for the different groups comprising the molecule (7, 8). Isooctane was the organic solvent, and the group contribution for transfer between water and isooctane for the saturated hydrocarbon moieties was taken from the data of Harris *et al.* (7) or determined as described by them using the relationship:

$$\Delta(\Delta G) = -4070 \text{ (relative surface area)} + 1156 \qquad \text{(Eq. 3)}$$

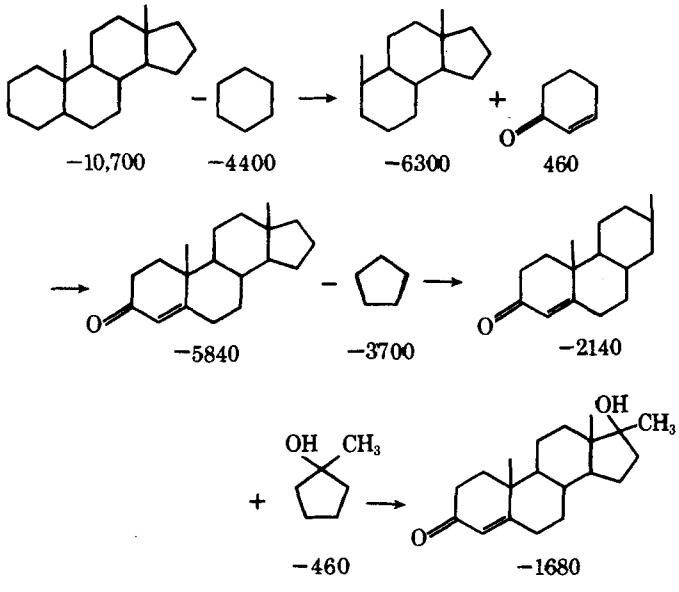
where $\Delta(\Delta G)$ is in calories per mole and the correlation coefficient is 0.992.

The partition coefficients for moieties having polar groups were measured (Fig. 6) and are reported in Table II along with the corresponding free energy of transfer from the relationship:

$$\Delta G^\circ = -RT \ln K \qquad \text{(Eq. 4)}$$

where K is the partition coefficient.

Prediction of Partition Coefficients—Based on the assumptions of the group contribution approach (8), one should be able to predict partition coefficients for a molecule when the partition characteristics of its constituent parts are known, assuming that they are additive. This approach should be helpful for highly hydrophobic compounds for which



Scheme II—Schematic diagram for calculating the free energy of transfer for methyltestosterone using the 17-androstyl group as the beginning group. The numbers represent the values for each group and intermediate used in the calculations.

actual measurement is difficult. Scheme II illustrates such a calculation for methyltestosterone starting from the 17-androstyl group.

Table III summarizes similar calculations of the free energy of transfer of the compounds studied along with the measured partition coefficients taken from the data shown in Fig. 7.

Agreement (within a factor of 2) was obtained between the calculated partition coefficients and the measured values with the exception of methyltestosterone acetate. The measured value for methyltestosterone added to the acetyl group contribution determined from the data for 1-methylcyclopentanol and 1-methylcyclopentyl acetate gave a value of ~5000 for the partition coefficient, which is in much better agreement with the experimental value.

This study shows that in cases where experimental values are difficult to obtain, estimates based on the group contribution approach are reasonably accurate.

If one takes the measured solubility in isooctane and divides by the partition coefficient as an approximation to the solubility in water, reasonable agreement with the measured solubility values is obtained. Thus, in the special case of extremely low solubility in water, estimating the partition coefficient by the group contribution approach and combining it with the known solubility in an organic solvent provide a good estimate of the aqueous solubility. In such cases, this approach may be the only feasible method of obtaining an accurate solubility estimate.

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Determination of Phenylmercuric Nitrate by Potentiometric Titration

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Abstract □ A procedure was developed for measuring small amounts of phenylmercuric nitrate in aqueous solutions. The method depends on the formation of insoluble phenylmercuric iodide upon titration of phenylmercuric nitrate with potassium iodide. The end-point can be detected using an iodide-sensitive electrode. The method is able to measure down to 0.000125% aqueous solution of phenylmercuric nitrate with a 1% accuracy. Procedural details and descriptions of excipient effects on the assay are presented. Naphazoline hydrochloride, phenylephrine hydrochloride, fluorescein sodium, and antipyrine interfered with the method, while the common buffer systems, polyvinyl alcohol, sodium thiosulfate, edetate sodium, and chloramphenicol had no effect.

Keyphrases □ Phenylmercuric nitrate—analysis, potentiometric titration, dilute aqueous solutions, excipients □ Ophthalmic dosage forms—phenylmercuric nitrate, potentiometric titration analysis, dilute aqueous solutions, excipients □ Potentiometric titration—analysis, phenylmercuric nitrate, dilute aqueous solutions

Phenylmercuric nitrate is frequently used as a preservative for ophthalmic and other preparations. To carry out routine determinations of small amounts of phenylmercuric nitrate in aqueous solution, a simple, rapid, and sensitive assay is necessary. Published methods for determining phenylmercuric nitrate have been numerous, but many of these are tedious or lack sensitivity.

BACKGROUND

Microbiological methods (1) are time consuming and are only semiquantitative. Spectrophotometric methods take advantage of the UV absorption at 257 nm by phenylmercuric nitrate (2). Although this assay is rapid, its sensitivity is limited. Many procedures involve conversion of organomercury to mercuric ion, followed by classical thiocyanate titrimetry (3). Again, this method lacks the sensitivity required for very dilute solutions.

The British Pharmacopoeia lists the dithizone extraction method (4), which involves extraction of the mercury from an acidic solution with a solution of dithizone in chloroform. The dithizonate is then measured spectrophotometrically. Because of the extractions involved, this method is tedious for routine determinations.

Ordinary polarographic methods have been used for higher phenylmercuric nitrate concentrations (5); cathode ray polarography of phenylmercuric nitrate has been investigated (6) and is a simple, rapid, and sensitive assay for routine determinations. Furthermore, phenylmercuric nitrate has been detected satisfactorily by atomic absorption spectroscopy (7). This procedure is based on "protodemercuration" of the mercurial compound with hydrochloric acid under various heating conditions, followed by reduction of the resulting mercuric ion to elemental mercury with subsequent detection and quantitation by vapor phase atomic absorption spectroscopy. Since these procedures require instruments that may not be readily available, the technique described in this paper was developed. This relatively rapid, simple, and sensitive assay is based on the precipitation of the phenylmercuric moiety with iodide ion.

Insoluble phenylmercuric iodide, $K_{sp} = 9.7 \times 10^{-16}$ (8), is formed when an aqueous solution containing phenylmercuric ions is titrated with iodide according to Scheme I.

$$C_6H_5—Hg^+ + I^- \rightleftharpoons C_6H_5—HgI$$

Scheme I

In the developed procedure, the end-point for this reaction is detected potentiometrically using an electrode sensitive to iodide ions.

EXPERIMENTAL

Five milliliters of an aqueous phenylmercuric nitrate[1] solution was transferred to a 50-ml beaker equipped with a magnetic stirring bar. The amount of phenylmercuric nitrate in solution ranged from ~0.05 to 2.5

[1] Lot 0900030, British Drug Houses Chemicals Ltd., Poole, England.

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