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Application of Molecular Sieve Technique in Solubilization Studies of Benzoic Acid in Solutions of Cetomacrogol 1000

M. DONBROW, E. AZAZ, and R. HAMBURGER

Abstract □ The applicability of a new technique in solubilization studies, using a molecular sieve, was tested on a system consisting of benzoic acid and cetomacrogol 1000 (cetostearyl ether of polyoxyethylene) in aqueous solutions. The data obtained are in good agreement with those found by other methods. Some advantages of the method are outlined.

Keyphrases □ Benzoic acid—solubilization study □ Cetomacrogol 1000 solution—benzoic acid solubility □ Micellar solubilization, benzoic acid in cetomacrogol 1000—quantitative determination □ Molecular sieve technique—solubilization study □ Spectrophotometry, UV, visual—analysis

The importance of theoretical and pharmaceutical aspects of micellar solubilization of drugs has been well recognized in recent years (1–4). Nevertheless, only three methods are available in routine practice for quantitative investigation of the phenomenon.

The conventional solubility method has been used by almost all investigators either as a basic tool or comparatively. Its main disadvantage is that it is limited to saturated systems; hence the dependence of solubilization on the concentration of unbound solubilizate cannot be studied by this method. Another major disadvantage lies in the fact that many additives decrease the cloud point (4, 5). This means that although turbidity is often used as a criterion for saturation with liquid solubilizates, it is not necessarily an indication of maximum solubility (6–10). Furthermore, the determination of the turbidimetric end-point is subject to error.

Equilibrium dialysis was first introduced into solubilization studies by Patel and Kostenbauder (11). This method solved the problems encountered in the solubility method. Although widely accepted, it is time consuming and requires preliminary work on the selection of a proper membrane for each system. Nylon membranes used by Patel and Foss (12) are stated to swell and bind phenolic compounds; rubber membranes tried by Matsumoto *et al.* (13) varied in thickness; and methylcellulose membranes are attacked by certain surfactants (14).

Potentiometric titration was first used for solubilization studies of organic acids and bases by Donbrow and Rhodes (15–17). Although rapid and elegant enough to have been adopted for routine use (15–23), it is restricted to ionizing solubilizates in which only the unionized form undergoes micellar solubilization. It is thus unsuitable for studies on the solubilization of acids and bases of pronounced amphiphilic properties such as local anesthetics or acid derivatives of steroidal hormones, their ionized form, as well as the unionized, being solubilized (24).

In view of the increasing importance of studies on nonsaturated systems, additional methods of a more general nature and greater scope than potentiometric titration and quicker than equilibrium dialysis would be advantageous. Such methods would also be valuable for crosschecking.

A molecular sieve technique which promised to meet these needs, has in fact been applied to methyl *p*-hydroxybenzoate by Ashworth and Heard (25). Dextran gel, with a suitable degree of crosslinking, was used in a static way similar to a semipermeable membrane in dialysis. The small molecules (the solute) are distributed between the swollen gel and the external liquid, while the surfactant is unable to penetrate the internal gel phase and remains in the external liquid. The solute distribution normally follows a linear relation. (See also Eqs. 5 and 6 in *Results and Discussion*, and Eq. 1 in *Experimental*.)

The object of the present work is to broaden the applicability of this method by testing it on another

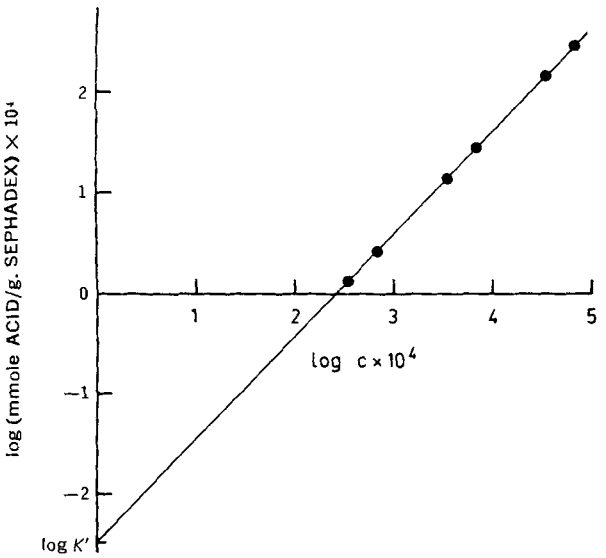

Figure 1—*Log–log plot of distribution of benzoic acid between Sephadex G25-fine and aqueous 0.005* N *HCl at 25°. (See Eq. 1 in* Experimental.)

system, benzoic acid in cetomacrogol solutions, for which data for comparison with other methods are available (18, 19, 21, 22, 26).

EXPERIMENTAL

Materials—Cetomacrogol 1000 BPC[1] was used (n_D^{60}, 1.451; n_D^{25} for 20% w/v solution, 1.360). The material, dried at 60° in vacuum, gave by combustion analysis the following results: C, 58.66; H, 9.62; O, 31.72. Assuming a molecular weight of 1300, this ratio fits the formula: $CH_3(CH_2)_{15}(OCH_2CH_2)_{24}OH$. Benzoic acid, analytical grade; dextran gel;[2] and Sephadex G25-fine[2] were also used. Water regain equalled 2.5 ± 0.2 g./g. dry gel. Particle size, 20–80 μ, was confirmed microscopically.

Procedure—*Determination of Internal Solvent Volume*—Ten milliliters of 0.1% solution of dextran blue 2,000,000[2] was added to about 3.5-g. samples of the gel swollen in 15 ml. 0.05 *N* NaCl. After the samples were shaken for 1 hr. at 25°, they were decanted and centrifuged. The concentration of the dye was determined by measuring the absorbance at 620 mμ directly, without further dilution. The water regain was measured on three samples of the gel. The mean value of a given batch was found to be 2.33 g./g. dry gel (± 0.05 g./g. dry gel).

Determination of K' *(Distribution Coefficient of Benzoic Acid between Aqueous 0.005* N *HCl and Sephadex G25-Fine)*—Assuming a linear distribution of benzoic acid between the gel and the external phase (Eq. 1) and aiming at equal amounts of benzoic acid outside and inside the gel (Eq. 2), the optimal weight of dry gel for a given external volume was calculated from Eq. 3:

$$M = CK' \qquad \text{(Eq. 1)}$$

$$MW = CK'W = CV \qquad \text{(Eq. 2)}$$

or

$$W = \frac{V}{K'} \qquad \text{(Eq. 3)}$$

where M = mmoles of benzoic acid bound to 1 g. gel; C = concentration of the free acid in the external phase in mmoles; W = weight of the dry gel in grams; K' = the distribution coefficient in (mmoles acid/g. gel)/(mmoles acid/l.); and V = aqueous external volume in liters = $V_t - 2.33\ W$ (where V_t is total aqueous volume and 2.33 g./g. dry gel is water regain of the gel).

Substituting K' of 0.0045 l./g., as approximated from the preliminary experiment, and external volume (V) of 0.002 l., suitable quantities of Sephadex (W) were about 0.6 g. The samples were weighed accurately from weighing bottles into known volumes of aqueous 0.005 *N* HCl (thus minimizing moisture uptake from the air). This concentration of HCl was maintained in all subsequent stages of the work to suppress the ionization of benzoic acid. The gel was allowed to swell at room temperature for 3 hr. Solutions of benzoic acid of varying concentration were added and made up to constant volume of 0.004 l.(V_t). The samples were shaken at 25° for 1 hr.

After equilibration, aliquot portions of the external phase were decanted, diluted suitably with 0.005 *N* HCl, and centrifuged to remove any fine particles present. The absorbance was read spectrophotometrically at the 230 or 273-mμ maximum. Blanks were prepared using the same procedure but omitting the benzoic acid (Sephadex was found to release traces of UV absorbing materials.)

Distribution of Benzoic Acid between Cetomacrogol 1000 and Aqueous Solutions of 0.005 N *HCl*—The procedure and amounts were identical with those used in the previous stage, except that cetomacrogol 1000 as well as benzoic acid was added from stock solutions to the conditioned gel. The concentration of cetomacrogol in the samples was 2.3–2.6% (varying with the weight of dry gel). Equilibration and analysis were carried out as previously. At the dilutions of surfactant studied, Beer's law was observed. The $\lambda_{max.}$ and $\epsilon_{max.}$ were as in water.

The optimum amount of dry gel (W) for a given external volume (V') in this case may be calculated from Eq. 4, aiming to equalize the amount of benzoic acid outside and inside the gel:

$$CWK' = CV' + CGK'' \qquad \text{(Eq. 4a)}$$

or

$$W = \frac{1}{K'}(V' + GK'') \qquad \text{(Eq. 4b)}$$

where C, K', V, and W are as defined for Eq. 1; V' = external volume corrected for micelle partial volume (27) = $V - 0.84\ G$; G = weight of micelles in sample in grams; and $K'' = K_1K_2$ = apparent distribution coefficient of benzoic acid between cetomacrogol and

Table I—Change in Concentration of Benzoic Acid Solution Shaken with Sephadex G25-fine at 25° with Time[a]

Time, hr.	Benzoic Acid Concentration, mmoles
0	0.145[b]
0.25	0.115
1	0.115
24	0.115
96	0.115
120	0.101
144	0.068
168	0.064
192	0.050
264	0.000

[a] Total aqueous volume 50 ml., Sephadex about 3.5 g. [b] Hypothetical, calculated from total acid added.

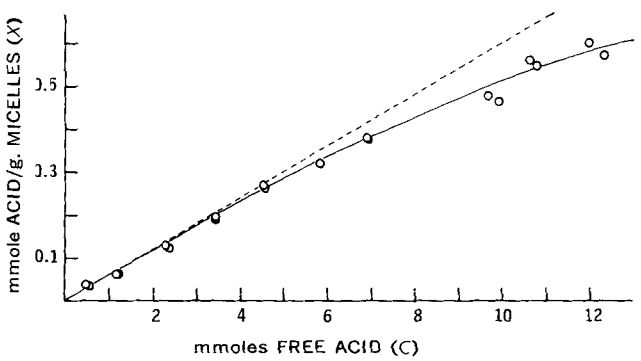

Figure 2—*Adsorption isotherm of benzoic acid in 2% w/v cetomacrogol and 0.005* N *HCl at 25°. (See text for description.) Key:* ——, *experimental isotherm,* X = $(K_1K_2C)/(1 + K_1C)$; *and* - - -, *slope of the initial portion of the isotherm* X = K_1K_2C.

[1] Marketed as "Texofor AIP," by Glovers Chemicals Ltd., Wortley Low Mills, Leeds, England.
[2] Pharmacia, Uppsala, Sweden.

water in l./g., neglecting its decreasing value with rising concentration (see *Results and Discussion*).

RESULTS AND DISCUSSION

From Fig. 1, plotted in log–log form for convenience, it is evident that the uptake of benzoic acid by Sephadex follows a linear relation over the range studied, and that the assumption made in Eq. 1 is valid.

The value of 4.46×10^{-3} l./g. found for K', the distribution coefficient, was reproducible over a wide concentration range ($1 \times 10^{-2} - 1M$), provided that the equilibration time was limited. If the period of contact was extended above 96 hr. (Table I), benzoic acid reacted further with the gel. Such reactions have been observed for other aromatic and heterocyclic compounds (28).

With regard to the solubilization of benzoic acid in cetomacrogol 1000, some typical results are plotted in Fig. 2. These results clearly demonstrate that, as already reported (19, 22), there is a deviation from linear distribution.

The values of C and X (Fig. 2) were calculated from Eqs. 5 and 6, respectively:

$$C_0V = C_fV + K'CW \qquad \text{(Eq. 5}a\text{)}$$

$$C = \frac{(C_0 - C_f)V}{K'W} \qquad \text{(Eq. 5}b\text{)}$$

$$C_fV = CV' + GX \qquad \text{(Eq. 6}a\text{)}$$

$$X = \frac{C_fV - CV'}{G} \qquad \text{(Eq. 6}b\text{)}$$

where C, G, K', V, V', and W are as defined earlier (Eqs. 1–4*b*); C_0 = initial concentration of benzoic acid in mM; C_f = final total concentration of benzoic acid outside the gel in mM; and X = mmoles of benzoic acid bound/1 g. micelles.

The results plotted in Fig. 2 may be represented by use of Langmuir's equation (Eq. 7):

$$X = \frac{K_1K_2C}{1 + K_1C} \qquad \text{(Eq. 7)}$$

where X = mmoles acid bound to 1 g. surfactant; C = concentration of free acid in mM; and K_1 and K_2 = adsorption parameters in l./mmoles and mmoles/g., respectively.

By means of the reciprocal form of Langmuir's equation (Eq. 8):

$$\frac{1}{X} = \frac{1}{K_2} + \frac{1}{K_1K_2C} \qquad \text{(Eq. 8)}$$

linearity was obtained over the full range (Fig. 3).

Unfortunately, the individual values of K_1 and K_2 could not be obtained by graphical methods. As can be seen from Fig. 3, the intercept ($1/K_2$) in the plot is very small and K_2 is subject to a very large error. This shortcoming has been pointed out previously (22).

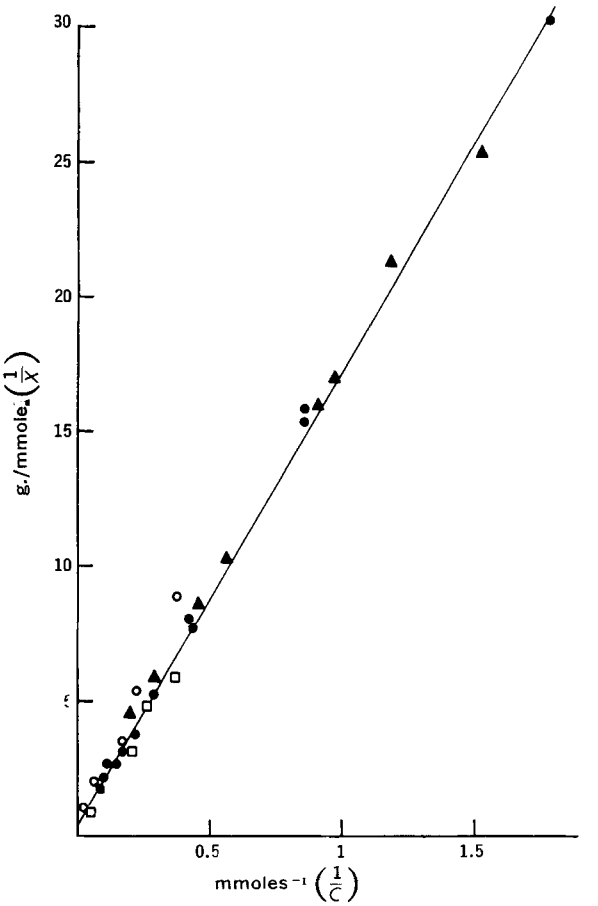

Figure 3—*Adsorption isotherm of benzoic acid in cetomacrogol at 25° from data obtained by different methods:*

$$\frac{1}{X} = \frac{1}{K_2} + \frac{1}{K_1K_2C}$$

Key: ●, *molecular sieve, present work;* ▲, *Donbrow and Azaz (21);* □, *Mitchell and Brown (18); and* ○, *Donbrow* et al. *(19, 22).*

To overcome this difficulty, a X/C *versus* X plot was tried using the rearranged form of Langmuir's equation (Eq. 9):

$$X/C = K_1K_2 - K_1X \qquad \text{(Eq. 9)}$$

The slope was, however, very small, giving a large error in K_1.

Values of K_1 and K_2 were, in fact, calculated statistically from a

Table II—Comparison of Adsorption Parameters Obtained by Molecular Sieve Method with Those Obtained by Other Methods

K_1K_2, l./g.						
Statistical Mean,[a] $K_1K_2 \times 10^2$	Standard Deviation, $S \times 10^2$	Range of Individual Runs, $K_1K_2 \times 10^2$	Maximal Concn. of Free Acid, mmoles	Cetomacrogol Concn., % w/v	Methods Used	References
4.4[b]	0.046	4.2–4.8	29.2	4–12	Potentiometric titrations and solubility	19, 22
5.9[c]	0.32	4.8–6.5	20.2	1.3–13	Potentiometric titrations	18[c]
5.6[d]	0.055	4.4–6.9	10.73	19	Potentiometric titrations	21
6.05[e]	0.17	—[e]	12.5	2.2–2.6	Molecular sieve	Present work (Fig. 3)

[a] Data were treated by the least-squares method using a Control Data Corp. computer to obtain the range of the individual runs. The means reported were combined parameters obtained by using all the individual results in the least-squares calculations. [b] The published data (19) were corrected for surfactant partial volume (27), replotted, and treated sta tistically. [c] These values were calculated from the potentiometric titration data of Mitchell and Brown (18) by changing the units and substituting into Langmuir's equation (Eq. 8). A plot of $1/x$ *versus* $1/c$ gave a straight line, from the slope of which the value of K_1K_2 was found to be 5.9×10^{-2} l./g. In the original publication, results were reported as distribution coefficients. The solubility method gave the lowest distribution coefficient (4.8×10^{-2} l./g.) as would be expected. Results within the same range were obtained by these authors by pH measurements (one point titration) and the equilibrium dialysis method. [d] The experimental procedure was similar to the one used previously (19, 22). [e] All repeated experiments were included.

very large number of results and will be reported (21). For the objective of this present work, the product of the two parameters $K_1K_2 = K''$, which can be determined accurately from either type of plot (Eq. 8 or 9), is sufficient to characterize the system. This combined parameter can be regarded as a linear distribution coefficient for a limited range of benzoic acid concentration in cetomacrogol 1000 solutions (Fig. 2, straight-line extrapolation from initial points). The concept of K'' as a distribution coefficient is derived from Eq. 7 which, at low values of the binding constant K_1 or low concentrations of adsorbate ($K_1C \ll 1$), approaches $X = K_1K_2C = K''C$.

Table II shows that the value of 6.05×10^{-2} for K'' by the molecular sieve method is of the same order as values obtained by other methods; the standard deviation, which falls within the range previously obtained, is acceptable. Since the method was found to be rapid and reproducible, it should prove to be suitable for routine solubilization studies, not only for benzoic acid in nonionic surfactants such as cetomacrogol 1000 but also for other unsaturated solubilized systems.

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Some Factors Affecting Release and Availability of Drugs from Hard Gelatin Capsules

B. J. McGEE, D. R. KENNEDY, and G. C. WALKER

Abstract □ Acetylsalicylic acid, acetylsalicylic acid and lactose, and acetylsalicylic acid and dibasic calcium phosphate were administered orally in No. 3 and No. 4 hard gelatin capsules to rabbits, using a Latin square design. Plasma levels were determined at 1-, 2-, and 3-hr. intervals; an analysis of variance showed that at hour 3, there was a difference between the average for the No. 3 and No. 4 capsules, irrespective of excipient. The significantly better plasma levels obtained with the more tightly compacted No. 4 capsules may be due to the diffusion of gastric juice through the gelatin which created higher pressure within the capsules. Dissolution determinations were made using the Levy beaker method and the oscillating tube method. The mean plasma levels at the end of 1, 2, and 3 hr. were lower than the concentrations obtained at the corresponding hours from the *in vitro* study, and direct correlation of the two sets of data could not be made. Hard gelatin capsules containing acetylsalicylic acid alone broke down slowly *in vitro*.

Keyphrases □ Capsules, hard gelatin—drug release, availability □ *In vivo–in vitro* release rates—drug from capsules □ Aspirin release, capsules—excipient effect □ Gelatin capsule size effect—aspirin release rates

Encapsulation remains a popular method for administering medication because of the general view that capsules readily break down upon ingestion to release the enclosed medicament (1). It was thought desirable to investigate the effects of two different excipients and the effects of two different pressures of fill on the rate of absorption of acetylsalicylic acid (ASA) from hard gelatin capsules in the rabbit. ASA is reported to be absorbed rapidly from all parts of the gastrointestinal tract and may serve as a "marker" to assess the effect of formulation and dosage form characteristics on absorption rate (1). *In vitro* dissolution