frigerated overnight and a yellow product was collected, washed with water, dried, and recrystallized.

2-Aryl-7-hydroxy-6-(*p*-substituted phenylazo)-5*H*-1,3,4-oxadiazolo [3,2-*a*]pyrimidin-5-ones (X*b–d*, Table III)—The appropriate sulfonamide (1 mmole) was dissolved in acetic acid (20 ml) and the solution was cooled to 5°. A cold solution of sodium nitrite (1.1 mmole) in water (5 ml) was added and the resulting diazonium salt solution was filtered into a stirred solution of the proper VIII (1 mmole) in acetic acid (50–100 ml) at 10°. The reaction mixture was then worked up as described for X*a*, and the yellow crude product crystallized from the suitable solvent.

Reaction of VIII*a* with Bis(2,4,6-trichlorophenyl)benzylmalonate. (VII*e*)—Equimolar amounts of VIII*a* (0.23 g, 1 mmole) and VII*e* (0.55 g, 1 mmole) were refluxed in bromobenzene (25 ml) for 3 hr. The product (XI-A) that separated out on cooling was filtered, washed with light petroleum, and dried. It was recrystallized from a large volume of boiling ethanol, mp 306–308°. The yield was 72%; IR (KBr): 3650–2900 (OH), 1750 (C=O, α-pyrone), 1690 (C=O, amide), a band split at 1620 and 1605, 1585, 1560, 1505 (C=N, C=C, and aromatics), 1255, and 1025 (C–O–C) cm^{-1}; mass spectrum: *m/z* (relative abundance, %) 387 (69) (M^+).

Anal.—Calc. for $C_{21}H_{13}N_3O_5$: C, 65.1; H, 3.4; N, 10.85. Found: C, 65.3; H, 3.7; N, 10.3.

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Influence of Benzalkonium Chloride on the Dissolution Rate Behavior of Several Solid-Phase Preparations of Cholesterol in Bile Acid Solutions

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Abstract □ Cholesterol dissolution rate accelerators, such as benzalkonium chloride, function by reducing the interfacial barrier that exists between the negatively-charged bile acid micelle and the negatively-charged cholesterol surface. It has been proposed that this reaction is accomplished by the binding of the positively-charged accelerator to the negatively-charged micelle. An earlier report showed that different solid preparations of cholesterol give different dissolution rates under the same conditions and these differences can be primarily accounted for by variations in the interfacial transport constant (*P*). By using the rotating disk dissolution apparatus and the Levich theory it has been possible to study the dissolution behavior of different cholesterol solid phases as a function of the benzalkonium chloride concentration. It was shown that the ratios of *P* values for the different phases are relatively constant over the range of the accelerator concentrations. This suggests that the accelerators act primarily on the micelle to enhance dissolution rate.

Keyphrases □ Benzalkonium chloride—effect on dissolution of cholesterol preparations in bile acid solutions □ Dissolution—cholesterol preparations, effect of benzalkonium chloride, bile acid solutions □ Cholesterol—effect of benzalkonium chloride on dissolution, bile acid solutions □ Gallstone kinetics—effect of benzalkonium chloride on dissolution of cholesterol preparations in bile acid solutions

Physicochemical studies of gallstone (cholesterol) dissolution kinetics have been performed in these laboratories (1–7). Theoretical treatment (2) showed that *in vivo* dissolution of cholesterol gallstones occurred at much lower rates than expected if dissolution were totally diffusion controlled. It was proposed that interfacial factors may be

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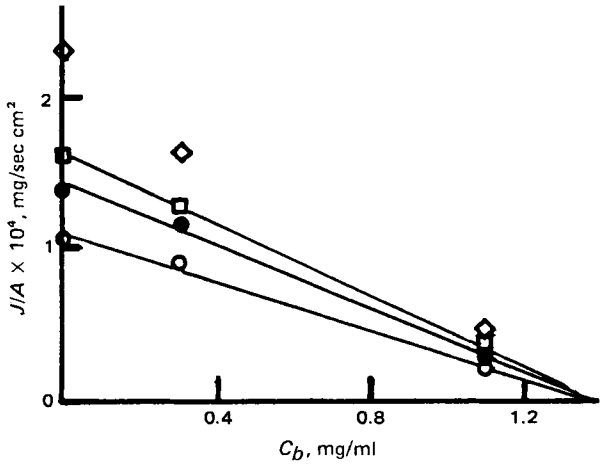

Figure 1—*Dissolution rate* versus *bulk concentration for the normal (I) pellets in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.25% benzalkonium chloride at pH 8.0. Key:* ○, *20;* ●, *50;* □, *150; and* ◇, *450 rpm.*

important *in vivo*. It was also shown (1–3) that *in vitro* dissolution of cholesterol gallstones in simulated and in human bile was dominated by an interfacial barrier at the crystal-solution interface. Kwan *et al.* (6) studied the interfacial process as a function of bile acid type and concentration, bile acid/lecithin ratio, and electrolyte type and concentration. He found that the interfacially-controlled dissolution rate increased with increasing bile acid concentration, increasing bile acid/lecithin ratio, and increasing electrolyte concentration.

BACKGROUND

The elimination of this interfacial barrier by appropriate measures is expected to be clinically significant. Studies by Prakongpan *et al.* (4, 5) showed that when quaternary ammonium compounds were added to the bile acid–lecithin solutions, dissolution rates were enhanced tremendously and approach the diffusion-controlled rates at high concentration. Previous studies (8) on the acceleration effect of primary, secondary, and

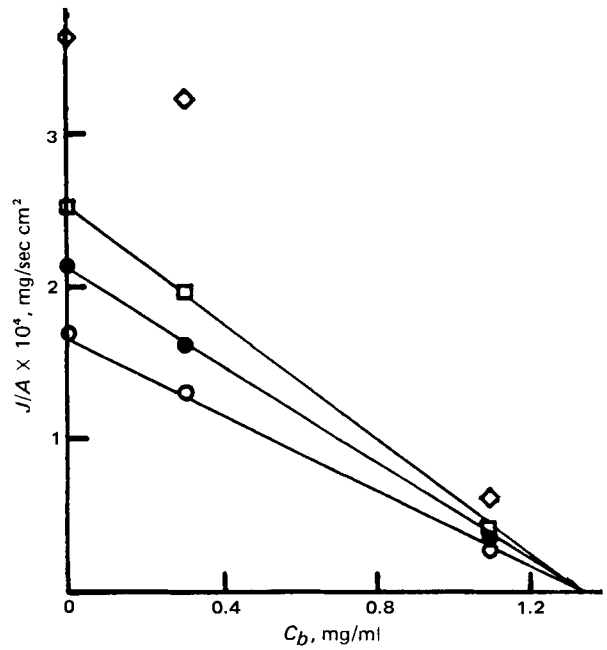

Figure 2—*Dissolution rate* versus *bulk concentration for the rapid (II) pellets in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.25% benzalkonium chloride at pH 8.0. Key:* ○, *20;* ●, *50;* □, *150; and* ◇, *450 rpm.*

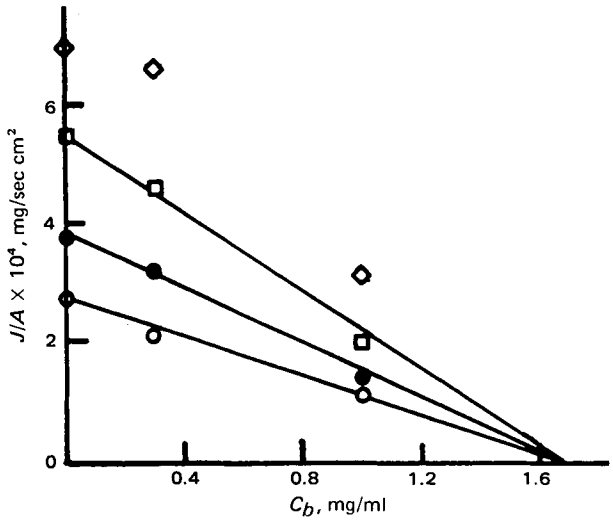

Figure 3—*Dissolution rate* versus *bulk concentration for the melt (III) pellets in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.25% benzalkonium chloride at pH 8.0. Key:* ○, *20;* ●, *50;* □, *150; and* ◇, *450 rpm.*

tertiary amines, quaternary ammonium compounds, steroidal amines, and other surfactants, found that the cationic surfactants and the steroidal amines were effective, but the nonionic and anionic surfactants were not.

Other work (8) led to the following interpretations regarding dissolution mechanisms. Since the surface reaction rate increases with increasing micelle concentration (increasing bile acid concentration) and since the dissolution rate is very sensitive to electrolytes, it was concluded that the micelle is the primary carrier of cholesterol in the rate-limiting step. It was proposed that increasing the counterion concentration results in the reduction of the electrical repulsion which exists between the negatively-charged bile acid micelle and the negatively-charged cholesterol surface. The observation that only cationic surfactants were effective cholesterol dissolution accelerators was interpreted as: (*a*) adsorption of aliphatic long-chain cations on the cholesterol surface aids reduction of the electrical repulsion between the negatively-charged cholesterol surface and the negatively-charged micelle, and/or (*b*) the formation of mixed micelles which reduce the net negative charge on the micelle, thus easing the approach of the micelles to the charged surface.

Patel and Higuchi (9, 10) carried out a systematic study of cholesterol dissolution rate acceleration mechanisms. Using membrane transport

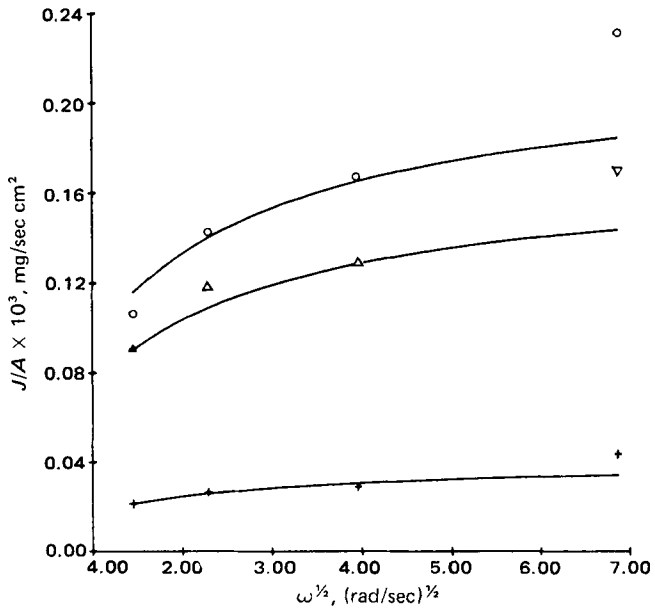

Figure 4—*Best-fit analysis of the normal pellets (I) in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.25% benzalkonium chloride at pH 8.0. Key:* C_b = ○, *0.0;* △, *0.3; and* +, *1.1 mg/ml.*

Table I—Dissolution Rates of the Normal (I) Cholesterol Pellets under Partial Saturation Conditions in 5% Sodium Cholate, 0.1 *M* Phosphate Buffer, and Benzalkonium Chloride at pH 8.0

C_b, mg/ml	rpm	0.0% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2	0.25% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2	0.5% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2
0.0	20	0.58 ± 0.01	1.07 ± 0.08	1.34 ± 0.02
	50	0.67 ± 0.04	1.43 ± 0.01	1.78 ± 0.05
	150	0.72 ± 0.01	1.68 ± 0.08	2.10 ± 0.02
	450	1.07 ± 0.03	2.31 ± 0.01	3.43 ± 0.05
0.2	20	0.52 ± 0.01	—	—
	50	0.54 ± 0.01	—	—
	150	0.59 ± 0.01	—	—
	450	0.88 ± 0.01	—	—
0.3	20	—	0.91 ± 0.03	1.09 ± 0.01
	50	—	1.18 ± 0.03	1.40 ± 0.04
	150	—	1.29 ± 0.03	1.76 ± 0.04
	450	—	1.68 ± 0.04	2.57 ± 0.04
1.1	20	0.14 ± 0.01	0.22 ± 0.01	0.27 ± 0.02
	50	0.15 ± 0.01	0.27 ± 0.01	0.32 ± 0.01
	150	0.16 ± 0.01	0.29 ± 0.01	0.36 ± 0.01
	450	0.22 ± 0.01	0.44 ± 0.04	0.62 ± 0.02

[a] Each J/A value is an average of two experiments and the range refers to the high and low values.

methods, the concentrations of bound and unbound accelerators were determined in the simulated bile acid solutions at low concentrations of accelerators. From the determinations of bound and unbound amine in simulated bile and from data on cholesterol dissolution rate acceleration, it was shown that the amount of bound amine correlated with the efficacy of cholesterol monohydrate dissolution rate acceleration and that the charged form of the amine was important. The electrophoretic mobility of chenodeoxycholate micelles was measured as a function of the amine concentration using the moving boundary electrophoresis method. In addition, particle microelectrophoresis studies showed that there was no significant surface charge variation on cholesterol particles as a function of amine concentration. From an analysis of these data, it was proposed that the predominant mechanism by which the amines enhanced the dissolution rate involved reducing the electrical charge of the bile acid micelles.

The present investigation studied the action of dissolution rate accelerators upon different solid cholesterol phases and attempted to determine the extent to which the donor phase may influence interfacial transfer acceleration kinetics.

THEORY

The rotating disk dissolution rate equation described previously (5) is appropriate for the present study. The following may be used to describe dissolution rate (11):

$$J = \frac{A(C_s - C_b)}{1.612D^{-2/3}\,\nu^{1/6}\,\omega^{-1/2} + 1/P} \qquad \text{(Eq. 1)}$$

where:

J = dissolution rate
A = apparent surface area of the pellet (cm^2)
D = diffusion coefficient (cm^2/sec)
ν = kinematic viscosity (cm^2/sec)
ω = angular velocity of rotation (radians/sec)
C_s = saturation solubility (mg/ml)
C_b = bulk concentration (mg/ml)
P = permeability coefficient (interfacial transport constant, cm/sec)

EXPERIMENTAL

Solutions—Sodium cholate test solutions were prepared by adding equivalent portions of cholic acid with sodium hydroxide. The pH of the solutions was then adjusted to 8.0. Dissolution rate accelerators (benzalkonium chloride) and buffers were added as needed. The solutions were allowed to equilibrate for 1 day before use.

Materials—Commercially obtained cholesterol[1] was recrystallized three times from 95% ethanol. Radioactive cholesterol monohydrate was prepared by mixing 100 μCi of [4-^{14}C]cholesterol with a given amount of the purified cholesterol at 60° in 400 ml of 95% ethanol. The solutions were then allowed to stand undisturbed for 2 days at room temperature. The crystals were filtered and dried *in vacuo* for 24 hr. The purified crystals were stored in the dark and in a container saturated with water vapor at room temperature.

[1] J. T. Baker Co., Phillipsburg, N.J.

Table II—Dissolution Rates of the Rapid Cholesterol (II) Pellets under Partial Saturation Conditions in 5% Sodium Cholate, 0.1 *M* Phosphate Buffer, and Benzalkonium Chloride at pH 8.0

C_b, mg/ml	rpm	0.0% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2	0.25% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2	0.50% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2
0.0	20	0.85 ± 0.02	1.70 ± 0.00	1.94 ± 0.02
	50	0.96 ± 0.05	2.14 ± 0.17	2.59 ± 0.09
	150	1.18 ± 0.03	2.48 ± 0.07	3.33 ± 0.01
	450	1.44 ± 0.04	3.68 ± 0.06	4.51 ± 0.01
0.3	20	0.59 ± 0.02	1.32 ± 0.01	1.43 ± 0.07
	50	0.77 ± 0.00	1.61 ± 0.00	1.99 ± 0.12
	150	0.88 ± 0.02	1.95 ± 0.01	2.59 ± 0.07
	450	1.08 ± 0.10	3.23 ± 0.04	3.62 ± 0.08
1.1	20	0.15 ± 0.02	0.28 ± 0.03	0.36 ± 0.02
	50	0.14 ± 0.01	0.30 ± 0.03	0.42 ± 0.02
	150	0.19 ± 0.02	0.30 ± 0.00	0.51 ± 0.00
	450	0.26 ± 0.01	0.54 ± 0.00	0.82 ± 0.07

[a] See Table I.

Three preparations of cholesterol were used. Normal cholesterol (sample I) was prepared by crystallizing 5 g of cholesterol in 400 ml of 95% ethanol. Rapidly crystallized cholesterol (sample II) was prepared by

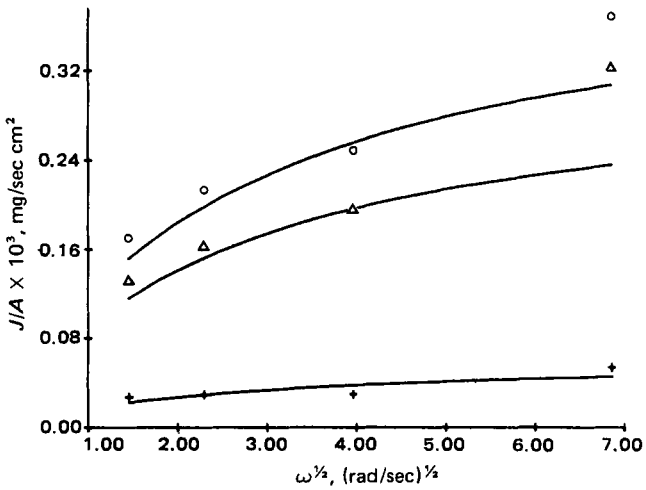

Figure 5—*Best-fit analysis of the rapid (II) pellets in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.25% benzalkonium chloride at pH 8.0. Key:* C_b, ○, *0.0;* △, *0.3; and* +, *1.1 mg/ml.*

Table III—Dissolution Rates of the Melt (III) Pellets under Partial Saturation Conditions in 5% Sodium Cholate, 0.1 *M* Phosphate Buffer, and Benzalkonium Chloride at pH 8.0

C_b, mg/ml	rpm	0.0% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2	0.25% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2	0.5% Benzalkonium Chloride $J/A \times 10^4 \pm$ Range[a], mg/sec cm^2
0.0	20	2.41 ± 0.00	2.74 ± 0.00	3.07 ± 0.00
	50	3.24 ± 0.06	3.73 ± 0.01	3.98 ± 0.14
	150	3.67 ± 0.05	5.48 ± 0.08	6.66 ± 0.15
	450	4.39 ± 0.00	6.91 ± 0.33	10.52 ± 0.22
0.3	20	2.09 ± 0.10	2.17 ± 0.03	2.35 ± 0.05
	50	2.75 ± 0.13	3.21 ± 0.03	3.66 ± 0.19
	150	2.94 ± 0.09	4.60 ± 0.00	5.37 ± 0.11
	450	3.85 ± 0.23	6.53 ± 0.06	8.31 ± 0.46
1.0	20	1.17 ± 0.03	1.13 ± 0.04	1.40 ± 0.08
	50	1.34 ± 0.02	1.45 ± 0.07	1.54 ± 0.02
	150	1.64 ± 0.01	2.00 ± 0.08	2.49 ± 0.02
	450	1.92 ± 0.06	3.11 ± 0.04	3.68 ± 0.06

[a] See Table I.

crystallizing 10 g of cholesterol in 400 ml of 95% ethanol. A melt preparation (III) was obtained by heating sample I at 160° for 10 min. Pellets of each sample were prepared and experiments were run as described previously (5). Cholic acid was recrystallized three times from 95% ethanol and the purity was checked and confirmed by TLC. Benzalkonium chloride[1] was used as received.

Differential scanning calorimetric thermograms were obtained for the three cholesterol samples. Thermograms of samples I and II showed endotherms at 80°, corresponding to the release of water from cholesterol monohydrate. This endotherm was absent from thermograms of sample III. Samples I–III showed endotherms at 150° for melting. In general, these thermograms agree with those obtained previously (12).

Assessment of the Experimental Systems Based on the Solid Preparation Study—Test solutions of 5% sodium cholate, 0.1 *M* phosphate buffer, and 0.0, 0.25, and 0.5% benzalkonium chloride at pH 8.0 were used. These conditions were chosen because the diffusion control/interfacial control ratios would be small to moderate and thus allow for reliable determinations of P values. If a test solution with 1.0% benzalkonium chloride were used, the melt pellets would dissolve ~100% diffusion controlled and the uncertainty in the P value determination would be too great.

RESULTS AND DISCUSSION

The dissolution rates for the various cholesterol pellets were determined in partially saturated solutions at various rotational speeds at 37°. Tables I–III show the dissolution rates as a function of rotational speed and partial saturation for samples I–III. As can be seen, there are significant differences in the dissolution rates among pellet types.

Dynamic solubilities were determined by plotting dissolution rate (J/A) against bulk concentration (C_b). Figures 1–3 show the results of the experiments carried out in 5% sodium cholate, 0.1 *M* phosphate buffer, and 0.25% benzalkonium chloride solutions at pH 8.0. By extrapolation of the data to zero J/A the dynamic solubilities can be determined. This was done with the data in Figs. 1–3 except for the 450-rpm experiments where deviations from Eq. 1 were noted (the possible cause of these deviations is discussed later). Figures 1–3 show a linear decrease in dissolution rate with increasing partial saturation. This indicates that the dissolution kinetics are proportional to ΔC over the entire range of saturation. These figures show that the dynamic solubility is ~1.35, 1.30, and 1.65 mg/ml for the sample I, II, and III pellets, respectively. The tabulated dissolution rates for the three solid preparations in this system are shown in Tables I, II, and III, respectively.

Figures 4–6 show the Levich plots of the described data. The deviations from linearity clearly show the presence of the interfacial barrier in the dissolution process. If cholesterol dissolution were completely diffusion controlled, a plot of J/A *versus* $\omega^{1/2}$ would give a straight line through the origin.

From a best fit analysis (using a kinematic viscosity of 6.99×10^{-3} cm^2/sec), based on a 15% correction (5) of Eq. 1, it was possible to obtain values for C_s, P, and D, where P is the interfacial transport constant and D is the diffusivity. The calculated dissolution rates using the best fit values were compared to the experimental dissolution rates and Figs. 4–6 show the good agreement between the two. The same analysis was done for the three pellet types in 0.0 and 0.50% benzalkonium chloride solutions. Figures 7–9 show the Levich plots for the 0.50% benzalkonium chloride system. Again, a good fit between experiment and theory is seen. It should be noted that the points at 450 rpm ($\omega^{1/2} = 6.86$) were not weighted in this analysis.

A possible explanation for the high J/A value at 450 rpm in some of the experiments is the following. Microscopic examination of individual crystals of cholesterol monohydrate in bile acid solutions showed that

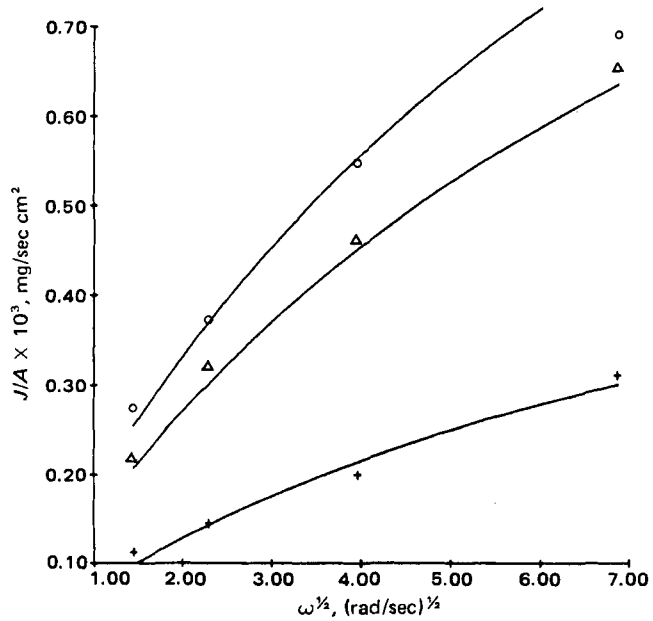

Figure 6—*Best-fit analysis of the melt pellets (III) in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.25% benzalkonium chloride at pH 8.0. Key:* C_b, ○, *0.0;* △, *0.3; and* +, *1.0 mg/ml.*

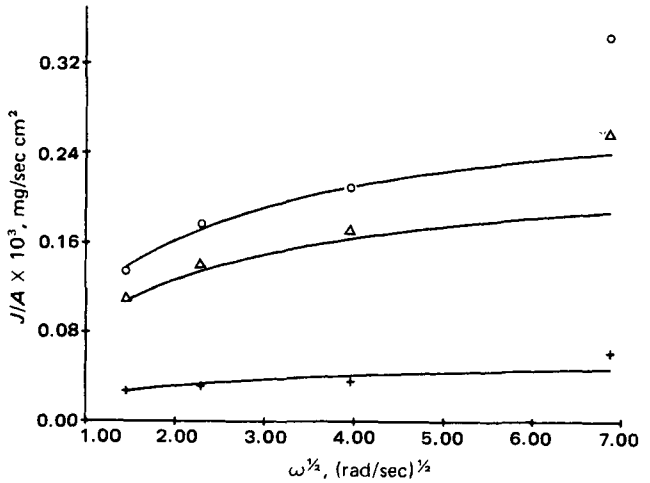

Figure 7—*Best-fit analysis of the normal pellets (I) in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.5% benzalkonium chloride at pH 8.0. Key:* C_b, ○, *0.0;* △, *0.3; and* +, *1.1 mg/ml.*

Table IV—Summary of the Partial Saturation Experiments and the Best-Fit Analysis for the Three Pellet Types in 5% Sodium Cholate, 0.1 *M* Phosphate Buffer, and 0.0% Benzalkonium Chloride at pH 8.0

Pellet	C_s from Extrapolation of Partial Saturation Data	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
I	1.45 (20 rpm)			
	1.45 (50 rpm)			
II	1.45 (150 rpm)	1.45	0.51	1.25
	1.38 (20 rpm)			
	1.36 (50 rpm)			
III	1.37 (150 rpm)	1.37	0.96	1.15
	1.76 (20 rpm)			
	1.72 (50 rpm)			
	1.76 (150 rpm)	1.79	3.32	1.16

surface etching occured. Within 2 hr, a definite saw-tooth pattern developed along the crystal edge. At high rotational speeds in the rotating disk experiments it is possible that the effective surface area might be increased or that some shearing of the peaks along the crystal surface takes place. This reaction, along with normal micellar dissolution, could result in a positive deviation in the apparent dissolution rate from that expected from theory.

Tables IV–VI summarize the results of the partial saturation experiments and the least-squares analysis. It can be seen that the C_s values obtained for the normal pellets (sample I) and the rapid pellets (sample II) are nearly equivalent, while the C_s values obtained for the melt pellets (sample III) was ~20% higher in all cases. It should be noted that the solubilities for samples I and II obtained from the partial saturation experiments agreed with the saturation solubilities of cholesterol (13).

The D values reported here are somewhat smaller than those obtained earlier (14, 15) for bile salt–lecithin systems. Since cholesterol dissolution is essentially surface controlled, the error in determining D values from the present dissolution-rate would be rather large; therefore, significances should not be attached to differences between the D values reported here and those determined using conventional methods (see *Appendix*).

Possible Meaning of *P* in Relation to the Dissolution Process—In general, the solubilization process involves contact of the solvent with the solid surface, where an interaction occurs; this is followed by the transport of the solute or product molecules away from the interface into the bulk. The dissolution of cholesterol in bile acid solutions can be regarded as following this mechanism. The slow or rate-determining step in this process is the approach of the micelle to the cholesterol surface and the subsequent transport of cholesterol molecules into the micellar phase. This process is associated with the interfacial transport constant P.

To understand how the benzalkonium chloride acceleration effects may be dependent on the solid phase of cholesterol, a more detailed consideration of the process is necessary. The model in Fig. 10 is proposed.

Step 1: The micelle moves from the bulk solution through the aqueous diffusion layer to a region close to the pellet surface.

Step 2: Molecules from the solid surface may disengage as a function of their configurations and energies. These molecules remain close to the solid surface and can be thought of as part of the adsorbed layer.

Step 3: Solubilization of these relatively free molecules occurs at the crystal-solution interface as a result of micelle surface "collisions." This solubilization process is a function of a steady-state concentration of the free cholesterol near the surface. The larger the k_1 value, the greater the concentration of free cholesterol and the greater the dissolution value.

Table V—Summary of the Partial Saturation Experiments and the Best-Fit Analysis for the Three Pellet Types in 5% Sodium Cholate, 0.1 *M* Phosphate Buffer, and 0.25% Benzalkonium Chloride at pH 8.0

Pellet	C_s from Extrapolation of Partial Saturation Data	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
I	1.35 (20 rpm)			
	1.35 (50 rpm)			
II	1.33 (150 rpm)	1.35	1.62	1.04
	1.32 (20 rpm)			
	1.30 (50 rpm)			
III	1.28 (150 rpm)	1.29	3.28	1.04
	1.64 (20 rpm)			
	1.65 (50 rpm)			
	1.64 (150 rpm)	1.63	10.62	1.04

Table VI—Summary of the Partial Saturation Experiments and the Best-Fit Analysis for the Three Pellet Types in 5% Sodium Cholate, 0.1 *M* Phosphate Buffer, and 0.50% Benzalkonium Chloride at pH 8.0

Pellet	C_s from Extrapolation of Partial Saturation Data	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
I	1.41 (20 rpm)			
	1.38 (50 rpm)			
II	1.36 (150 rpm)	1.36	2.19	1.08
	1.36 (20 rpm)			
	1.33 (50 rpm)			
III	1.32 (150 rpm)	1.33	4.85	1.08
	1.72 (20 rpm)			
	1.66 (50 rpm)			
	1.66 (150 rpm)	1.63	15.87	1.12

A large k_1 would essentially mean a high concentration of accessible cholesterol for solubilization by the micelles during collision.

In context of the present experiments, k_1 may be related to several factors. A low intrinsic activation energy for molecular disengagement would contribute to a large k_1. A large microscopic surface area at the pellet surface or a high density of active sites may also lead to a large effective k_1. The results of the present study suggest that the activational free energy (or the effective activation free energy) for the cholesterol molecule disengagement from the surface of the melt preparation is lower than that for the monohydrate preparations. This might have arisen from the possibly greater general state of disorganization of the surface molecules in the anhydrous melt, or from other factors such as crystalline defects which may have been more preponderant in the melt preparations.

This description of the dissolution process, together with earlier studies (9) on the influence of long-chain alkyl ammonium ions upon cholesterol dissolution rates in sodium chenodeoxycholate solutions, may be used to examine the present results with benzalkonium chloride. Table VII summarizes the P values and their ratios for all of the experiments. The P_{II}/P_I and the P_{III}/P_I ratios are relatively constant (~2.0 and 7.0, respectively) over the benzalkonium chloride concentration range, up to 0.5%. The ratios change only ~10–15% over a four- to fivefold range of rates.

Constancy in the P_{II}/P_I and the P_{III}/P_I ratios would be consistent with the following analysis. If the relevant surface charge densities for all three cholesterol preparations were essentially the same, and if the addition of benzalkonium chloride to the system did not significantly influence the surface charges, then the mechanism proposed earlier (9, 10) (*i.e.*, the partial neutralization of the negative micellar charge) may dominate and would predict that k_2 (Fig. 10) would be essentially the same in the experiments with all cholesterol samples. Then, if the k_1 values are not influenced by benzalkonium chloride or if k_1 values are affected the same

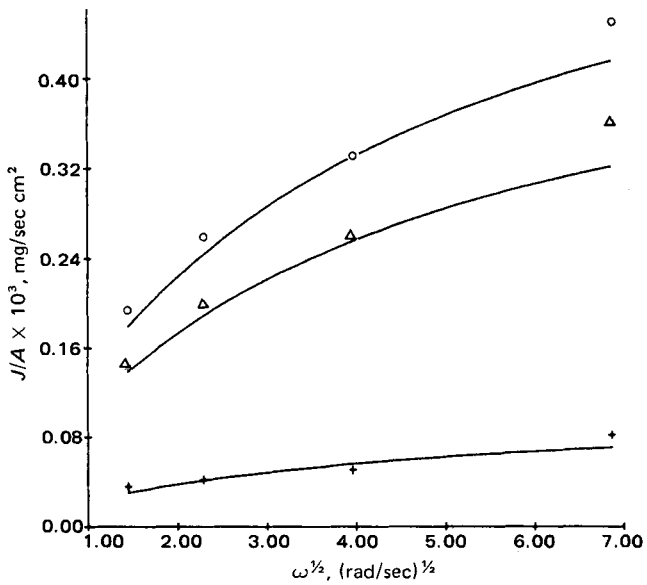

Figure 8—*Best-fit analysis of the rapid (II) pellets in 5% sodium cholate, 0.1* M *phosphate buffer, and 0.5% benzalkonium chloride at pH 8.0. Key:* C_b, ○, *0.0;* △, *0.3; and* +, *1.1 mg/ml.*

Table VII—Summary of the P Values Obtained as a Function of Benzalkonium Chloride Concentration

Percent Benzalkonium Chloride in Solution	$P \times 10^4$, cm/sec				
	I	II	III	P(II)/P(I)	P(III)/P(I)
0.0	0.51	0.96	3.32	1.90	6.53
0.25	1.62	3.28	10.62	2.02	6.55
0.50	2.19	4.85	15.87	2.20	7.25

Table VIII—Summary of the C_s Values Obtained as a Function of Benzalkonium Chloride Concentration

Percent Benzalkonium Chloride in Solution	C_s, mg/ml				
	I	II	III	C_s(II)/C_s(I)	C_s(III)/C_s(I)
0.0	1.45	1.37	1.79	0.95	1.23
0.25	1.35	1.29	1.63	0.96	1.21
0.50	1.36	1.33	1.63	0.97	1.20

(percentage-wise) for all three samples, then constancy of the P ratios should be expected.

A corollary analysis might assume, for example, that there are interactions between benzalkonium chloride and the cholesterol surface to reduce the surface charge density. However, if the initial surface charge densities and the benzalkonium chloride surface effects are essentially the same for all preparations, then again k_2 would be expected to be the same for the three preparations. For this situation, if the initial k_1 values are not affected by the benzalkonium chloride or if they are affected the same, the constancy of the P ratios would again be expected.

The first of these two interpretations may easily explain the behavior difference between sample I and sample II since both of these are cholesterol monohydrates with the same differental scanning colorimetric patterns and essentially the same solubilities (Table VIII). In terms of the proposed model, sample I and sample II might differ only in that their k_1 (or their apparent k_1) values are different because of differing microscopic surface areas or crystallite sizes. The conditions associated with k_2 might be expected to be identical for these two preparations.

The constancy of the P_{III}/P_I ratio is more difficult to predict since sample III is anhydrous cholesterol that had undergone a melting step. The present data suggest, however, that the proposed mechanism may be correct even in this instance; although k_1 may be significantly greater for the melt than for the sample I monohydrate, it is relatively uninfluenced by benzalkonium chloride. The electrical characteristics of the melt pellet surface is similar to that for the monohydrate under the present dissolution kinetics conditions so that the primary effect of benzalkonium chloride is the reduction of the micellar charge and facilitating step 3 *via* the enhancement of k_2.

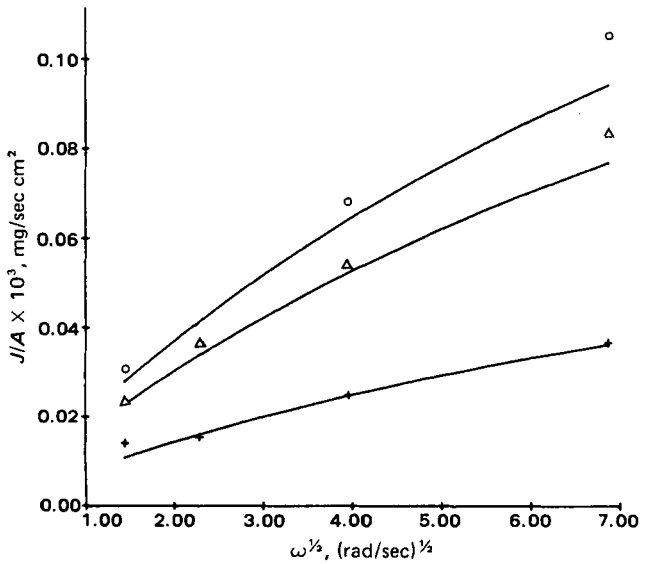

Figure 9—*Best-fit analysis of the melt (III) pellets in 5% sodium cholate, 0.1 M phosphate buffer, and 0.5% benzalkonium chloride at pH 8.0. Key:* C_b, ○, *0.0;* △, *0.3; and* +, *1.0 mg/ml.*

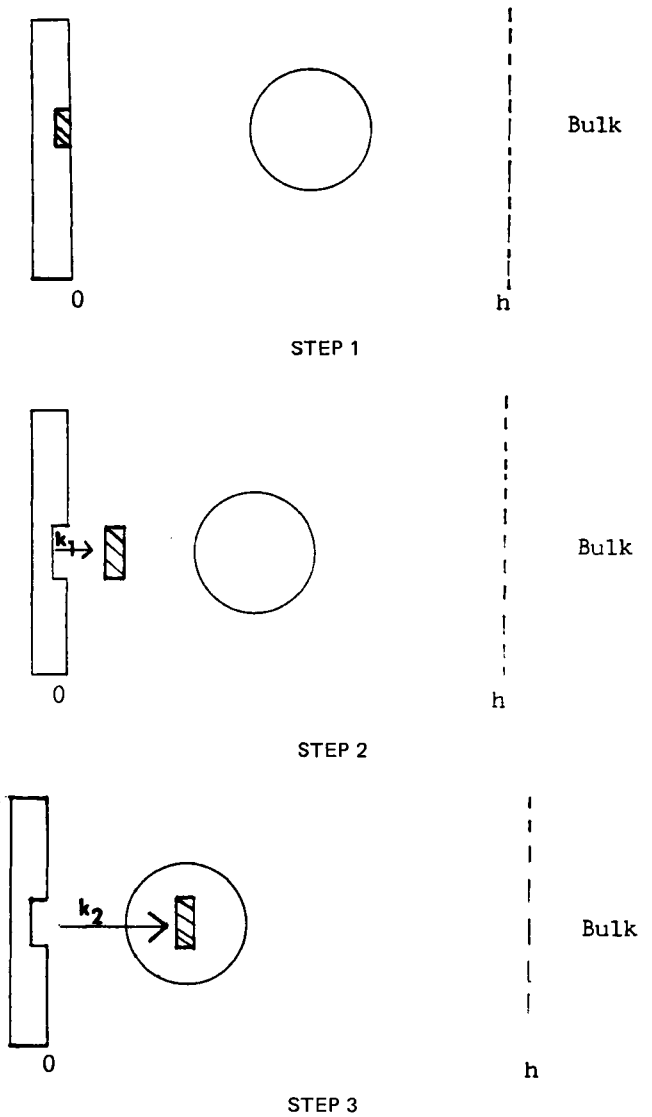

Figure 10—Step 1—*The micelle moves from the bulk solution through the aqueous diffusion layer to a region close to the pellet surface.* Step 2—*Molecules from the solid surface may disengage as a function of their configurations and energies. These molecules remain close to the solid surface and can be considered part of the adsorbed layer.* Step 3—*Solubilization of these relatively free molecules occurs at the crystal-solution interface as a result of micelle surface "collisions." The symbol* k_2 *is the rate constant for the solubilization of the (surface) free cholesterol by the micelle.*

APPENDIX

Sensitivity of the Best Fit Analysis to P—To relate the present results to their sensitivities to P, the parameters C_s and D must be considered. As already shown, there was good agreement between the solubilities obtained from the best fit analysis and those obtained from extrapolation of the partial saturation data. Diffusivities obtained from the best fit analysis were in fairly good agreement with those obtained experimentally. Diffusivity measurements were made at 37° in a small volume diaphragm diffusion cell employing a modified Keller's method (14). The D values determined experimentally in 0.0, 0.25, and 0.50% benzalkonium chloride solutions were 1.52, 1.40, and 1.28×10^{-6} cm^2/sec, respectively. These compare with the best fit D values of 1.25, 1.04, and 1.08×10^{-6} cm^2/sec.

Because of the good agreement between experimental and best fit parameters for C_s and D, an assessment of P can now be made. It must be remembered that as a system approaches total diffusion control, P becomes very large (surface equilibrium is fast) and therefore, $1/P$ becomes small. The smaller the $1/P$ ratio, the less sensitive the best fit results will be to small changes in P. Another look at the systems studied shows that the diffusion control/interfacial control ratios are small to moderate except for the melt pellets in 5% sodium cholate, 0.1 M phosphate buffer, and 0.50% benzalkonium chloride at pH 8.0 where the diffusion control/interfacial control ratio is ~90%. By making small changes in P, the sensitivity of the best fit results can be seen. It can be shown that if P is varied by ±10% there is little change in the best fit results. However, a change in P greater than 10% will result in marked deviation of the experimental results and the theoretical curves. For the case where the diffusion control/interfacial control ratio is ~90%, the uncertainty in P can be as high as 25%.

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Particle Size Reduction by a Hammer Mill I: Effect of Output Screen Size, Feed Particle Size, and Mill Speed

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Abstract □ A hammer mill is an impact mill commonly used in pharmaceutical manufacturing for reducing particle size for a variety of drugs. Commercial grade ammonium sulfate was milled as a model powder. This salt was sieved to obtain particle size fractions with average diameters of 1.3, 0.9, and 0.72 mm which were used as feed particles. The milled material was analyzed for particle size distribution (PSD) using standard sieves. At a moderate speed (~2500 rpm), the feed size did not result in a significantly changed arithmetic mean diameter, $d_{\bar{x}}$ of milled particles. A new particle size reduction constant, k, is proposed as a result of a linear relationship between $d_{\bar{x}}$ and output screen size, d_{ss}. As d_{ss} decreases, the PSD range of milled particles narrows; as mill speed increases (~5000 rpm), $d_{\bar{x}}$ decreases. The decrease is of a greater magnitude for larger d_{ss} (2 mm) than for a smaller d_{ss} (1 mm). At low speeds (~1000 rpm), the PSD is wider compared to medium and high speeds.

Keyphrases □ Particle size—reduction by hammer mill, effect of output screen size, feed particle size, and mill speed □ Pharmaceutics—particle size reduction by a hammer mill □ Hammer mill—particle size reduction, pharmaceutics

The hammer mill is an impact mill commonly used in pharmaceutical manufacturing for reducing particle size for a variety of drugs (1–3). Milling is an essential unit operation in tablet and capsule manufacture, yet very little has been reported about factors that affect milling (4, 5).

The present report examines the effect of related parameters such as output screen size, feed particle size, and mill speed on particle size reduction by a hammer mill. Commercial grade ammonium sulfate was used as a model substance due to its low cost and large particle size distribution.

EXPERIMENTAL

Equipment—A laboratory bench-type hammer mill[1], USP standard testing sieves[2], and a laboratory sieve vibrator[3] were used. The sieve sizes used were: 2.0, 1.6, 1.0, 0.8, 0.63, 0.4, 0.315, and 0.1 mm for preparation and analysis.

Particle Size Reduction—Ammonium sulfate was sieved to obtain various particle size fractions with average diameters of 1.3, 0.9, and 0.72 mm. Sieve sizes used were 1.6, 1.0, 0.8, and 0.63 mm. The particles were stored at room temperature (25 ± 3°) and at a constant humidity (60 ± 2%, to prevent agglomeration of particles) until required for milling.

[1] Model C580, micro hammer mill, Glen Creston, Stanmore, England.
[2] W. S. Tyler Inc., Mentor, OH 44060.
[3] Model 150, Derrick Inc., Buffalo, N.Y.

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