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Variability in Dissolution Rates of Cholesterol Pellets in Bile Acid Solutions

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Abstract □ Large variations in dissolution rate behavior of cholesterol monohydrate pellets may result from small changes in experimental procedures. For example, when cholesterol monohydrate pellets were stored overnight prior to a dissolution run, the initial dissolution rates varied by more than a factor of 2. It is well known that cholesterol monohydrate is converted to anhydrous cholesterol; cholesterol may be unstable toward light, heat, and other radiation in the presence of air, leading to its decomposition. To determine the cause of the variable dissolution rates, experiments were conducted with pellets "aged" under various conditions. The data show that the probable cause of the variations is the pellet surface conversion of the monohydrate to anhydrous cholesterol, which may take place during pellet storage. The combined effects of temperature and humidity seem to be important. A uniform experimental procedure is needed if investigators hope to reproduce results within their own laboratories as well as reproduce the findings of others.

Keyphrases □ Cholesterol—dissolution rate behavior in bile acid solutions □ Dissolution—rate behavior of cholesterol in bile acid solutions □ Gallstones—cholesterol, dissolution rate behavior in bile acid solutions

Cholesterol monohydrate has been used as a model substance in gallstone dissolution research (1). In earlier work in this laboratory, it was noticed that small changes in the experimental procedure resulted in significant variations in the dissolution rate behavior of cholesterol pellets. In some experiments when the pellet was stored overnight at 35° prior to a dissolution run, the initial dissolution rate was two to three times greater than the dissolution rate of freshly made pellets. Since it is desirable to limit the variations to ~10–15% in many mechanistic studies on cholesterol dissolution kinetics (1–4), the cause of dissolution rate variability was investigated.

EXPERIMENTAL

Initially, the static-disk dissolution method was used, while the more quantitative rotating-disk dissolution method was used in the later analysis. The experimental procedures were described previously (5). All dissolution rate experiments were run at 37° in 5% sodium cholate and 0.1 *M* phosphate buffer at pH 8.00.

Pellets of radiolabeled cholesterol were prepared as described previously (5). Pellets placed in the dissolution cell without solvent for varying times at ambient (room) humidity or humidity of <100% at 35° will be referred to as aged pellets. The pellets kept at 100% humidity at ambient temperature for 24 hr will be referred to as normal pellets. This latter treatment was shown to give the same dissolution rate as that of freshly made pellets.

One hundred percent humidity containers were prepared by adding water to a desiccator until it completely filled the area under the plate. The other constant-humidity chambers were prepared by making saturated aqueous solutions (6) of 80% sodium bromide and of 40% calcium chloride which then were placed in an oven at 35°.

RESULTS AND DISCUSSION

Static-Disk Apparatus—When small changes in the normal experimental procedure were introduced, significant differences in dissolution behavior resulted (Fig. 1 and Table I). Figure 2 shows the effects of exposing the pellets to ambient humidity at 35° for varying periods (aging). The normally treated pellet and the pellet aged for 1 hr gave the same dissolution rate as well as the same dissolution pattern (linear). However, aging the pellet for 12 or 24 hr markedly increased the initial dissolution rate (Table II). After a few hours of dissolution, these pellets showed a definite curvature in their dissolution profiles.

Figure 3 shows the effects of varying the humidity as well as varying

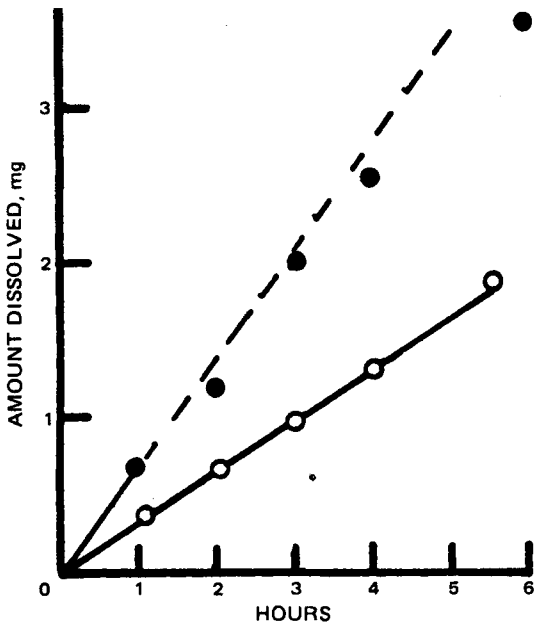

Figure 1—*Effect of pellet handling on the dissolution of cholesterol pellets at 150 rpm in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *normally prepared pellets; and* ●, *aged pellets.*

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Table I—Dissolution Rates of Cholesterol Pellets as a Function of Treatment in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00 [a]

Prerun Humidity, %	Prerun Temperature	$J/A \times 10^4$, mg/sec-cm^2
100	Ambient	0.73
Ambient	35°	1.40

[a] The prerun time in the setup was 24 hr, and there was no subsequent treatment.

Table II—Dissolution Rates of Cholesterol Pellets as a Function of Treatment in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00 [a]

Prerun Time in Setup, hr	Prerun Humidity, %	Prerun Temperature	$J/A \times 10^4$, mg/sec-cm^2
24	100	Ambient	0.73
1	Ambient	35°	0.73
12	Ambient	35°	1.40
24	Ambient	35°	1.40

[a] There was no subsequent treatment.

the exposure time. All pellets exposed to <100% humidity at 35° for 12 or 24 hr had an initial dissolution rate greater than that of the normally treated pellet. Table III summarizes the dissolution rates as a function of time and humidity.

At this point, it was felt that these results could be due to either dehydration of cholesterol monohydrate or to chemical decomposition (oxidation) of cholesterol. Bergstrom and Wintersteiner (7) showed that cholesterol in colloidal aqueous dispersions is attacked easily by oxygen. Weiner and coworkers (8–10) showed that monomolecular films also undergo autoxidation under different conditions. Dauben and Payot (11) showed that ^{14}C-labeled cholesterol is unstable when stored in the presence of air. They found that the decomposition required both radiation and oxygen since unlabeled cholesterol was stable in air when stored under the same conditions and labeled material was stable when stored *in vacuo*. Thus, several factors are involved: oxygen concentration, wavelength of light, storage temperature, and surface area of the cholesterol sample. Cholesterol monohydrate is known to be converted to anhydrous cholesterol (12), although this conversion generally occurs at elevated temperatures (80–100°).

To determine the effects of temperature, humidity, and light on cholesterol dissolution, a series of experiments was run in the static-disk apparatus. By varying temperature and humidity and running the experiments in the presence and absence of room light, the contribution of each variable to the change in dissolution behavior was assessed. The effects of the variables at 100% humidity were relatively small, if any (Table IV). However, at ambient humidity, the effects on the dissolution rate of raising the temperature to 35° were large (dissolution rates varied

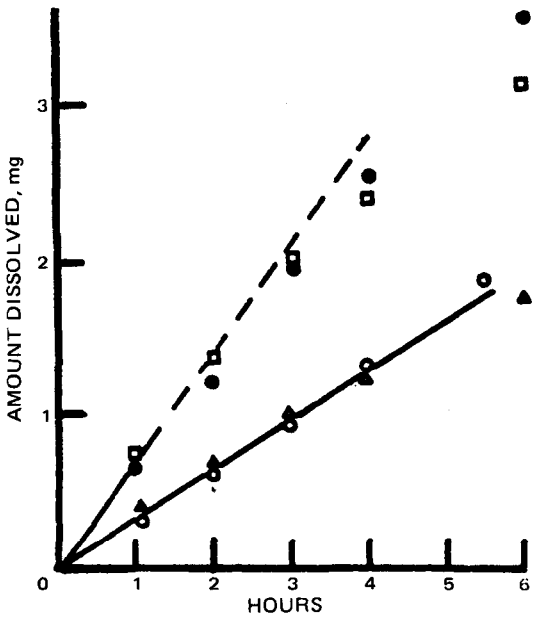

Figure 2—*Effect of pellet handling on the dissolution of cholesterol pellets at 150 rpm in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *normally prepared pellets;* ▲, *aged pellets for 1 hr;* □, *aged pellets for 12 hr; and* ●, *aged pellets for 24 hr.*

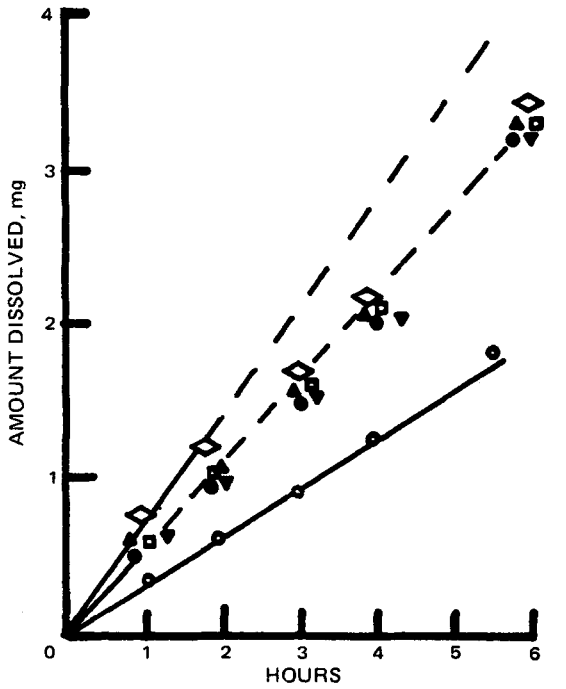

Figure 3—*Effect of pellet handling on the dissolution of cholesterol pellets at 150 rpm in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *normally treated pellets;* □, *aged pellets for 12 hr at 40% humidity;* ▲, *aged pellets for 24 hr at 40% humidity;* ▼, *aged pellets for 12 hr at 80% humidity;* ●, *aged pellets for 24 hr at 80% humidity; and* ◇, *aged pellets for 24 hr at 0% humidity. All pellets were aged at 35°.*

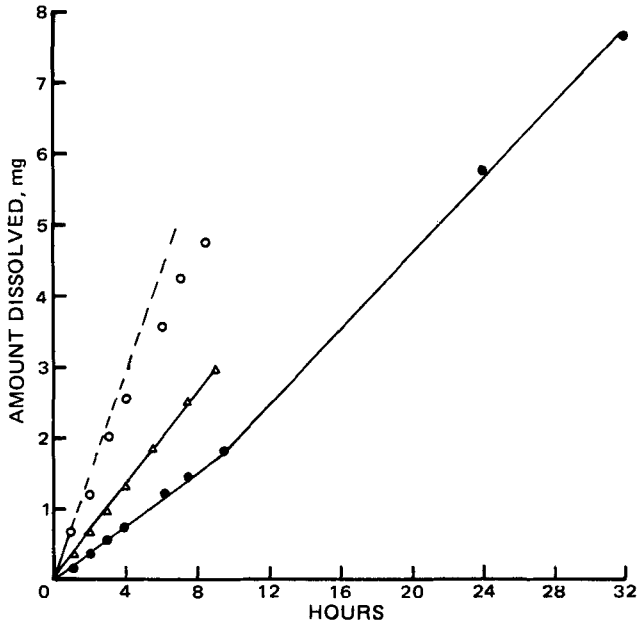

Figure 4—*Effect of pellet treatment on the dissolution rate of cholesterol in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* △, *normal pellet;* ○, *aged pellet (ambient humidity at 35° for 24 hr); and* ●, *soaked pellet. (Pellets were aged, followed by soaking in double-distilled water at 37° for 24 hr.)*

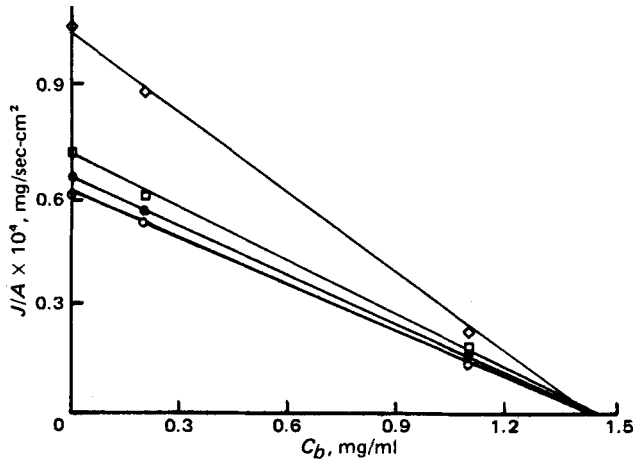

Figure 5—*Dissolution rate* versus *bulk concentration for normal pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *20 rpm;* ●, *50 rpm;* □, *150 rpm; and* ◇, *450 rpm. Each* J/A *value is an average of two experiments. Since the typical variations are ±5–~10% for these results, which are too small to be seen, only the average values are presented here and in Figs. 6–10.*

by approximately two times). Therefore, the combined effect of humidity and temperature appeared to be the primary cause for the aging effect.

Because it was felt that pellet aging might lead to cholesterol decomposition, samples of bile acid–cholesterol solutions were spotted onto TLC plates. These samples were obtained during the first 30 min of actual dissolution experiments; if decomposition products were causing the large rate of dissolution, it would be possible to determine this by TLC. The plates then were placed into a tank with acetic acid–carbon tetrachloride–isopropyl ether–isoamylacetate–*n*-propanol–benzene (5:20:30:40:10:10) as the solvent (13). Good separation of the bile acids and cholesterol was obtained. For both normal and aged pellet samples, one spot for cholesterol was found (R_f 0.71). To confirm that the samples obtained for TLC were from differently dissolving pellets, another sample was drawn from each dissolution cell and counted on a liquid scintillation counter[1]. The radioactivities for the aged and normal pellets were 2346.2 and 1235.2 cpm, respectively. It can be concluded that the differences in pellet behavior were not due to cholesterol decomposition during aging.

To check the dehydration hypothesis, aged pellets then were soaked in double-distilled water at 37° for 24 hr. Figure 4 shows the comparison of normal pellets, aged pellets, and aged pellets followed by soaking. Soaking an aged pellet lowered the dissolution rate, but this decrease was much greater than expected and will be discussed later.

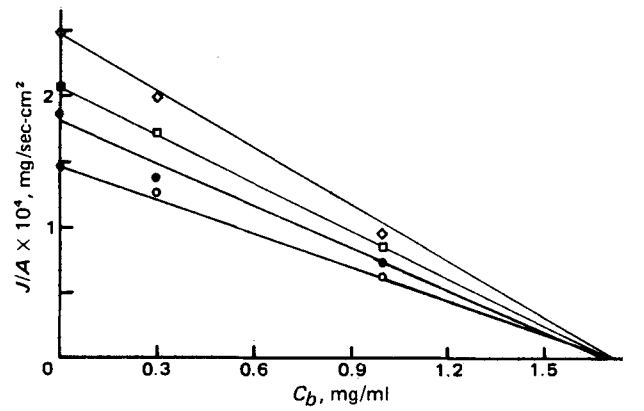

Figure 6—*Dissolution rate* versus *bulk concentration for aged pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *20 rpm;* ●, *50 rpm;* □, *150 rpm; and* ◇, *450 rpm.*

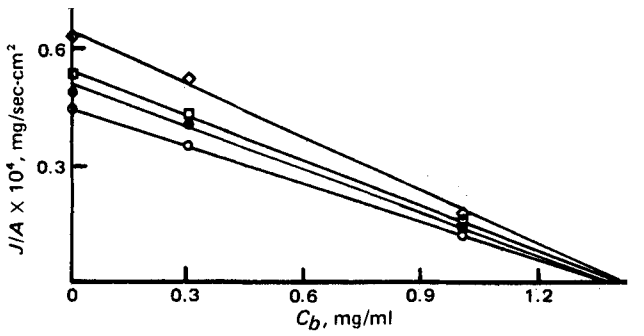

Figure 7—*Dissolution rate* versus *bulk concentration for soaked pellets in 5% sodium cholate, and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *20 rpm;* ●, *50 rpm;* □, *150 rpm; and* ◇, *450 rpm.*

Table III—Dissolution Rates of Cholesterol Pellets as a Function of Treatment in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00 [a]

Prerun Time in Setup, hr	Prerun Humidity, %	Prerun Temperature	$J/A \times 10^4$, mg/sec-cm²
24	100	Ambient	0.73
12	40	35°	1.20
24	40	35°	1.20
12	80	35°	1.20
24	80	35°	1.20
24	0	35°	1.39

[a] There was no subsequent treatment.

Table IV—Dissolution Rates of Cholesterol as a Function of Humidity, Light, and Temperature [a]

Number of Experiments	Humidity, %	Light	Temperature	Prerun Time in Setup, hr	$J/A \times 10^4$, mg/sec-cm²
2	100	None	Ambient	0	0.71
3	100	None	Ambient	24	0.69–0.73
3	100	None	35°	24	0.79–0.86
2	100	Present	Ambient	24	0.78
3	100	Present	35°	24	0.86–0.91
2	Ambient	None	Ambient	24	0.78
2	Ambient	None	35°	24	1.40
2	Ambient	Present	Ambient	24	0.86
1	Ambient	Present	35°	24	1.40

[a] Ambient = room conditions.

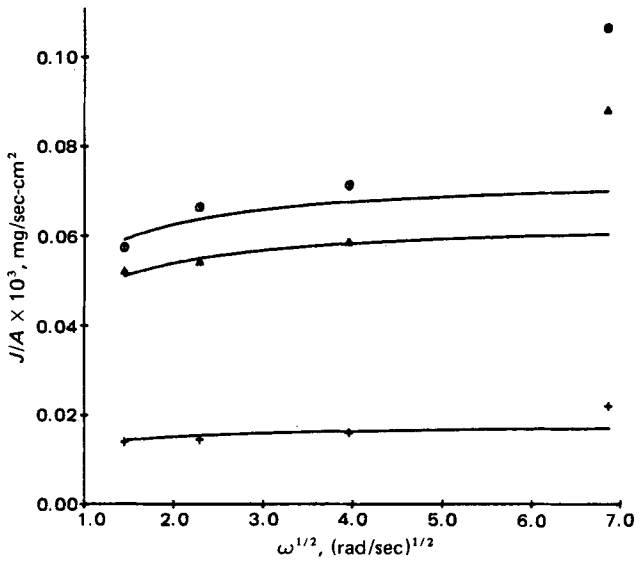

Figure 8—*Best-fit analysis of the normal pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key (*C_b *in milligrams per milliliter):* ○, *0.0;* ▲, *0.2; and* +, *1.1.*

[1] Beckman Instruments, Irvine, Calif.

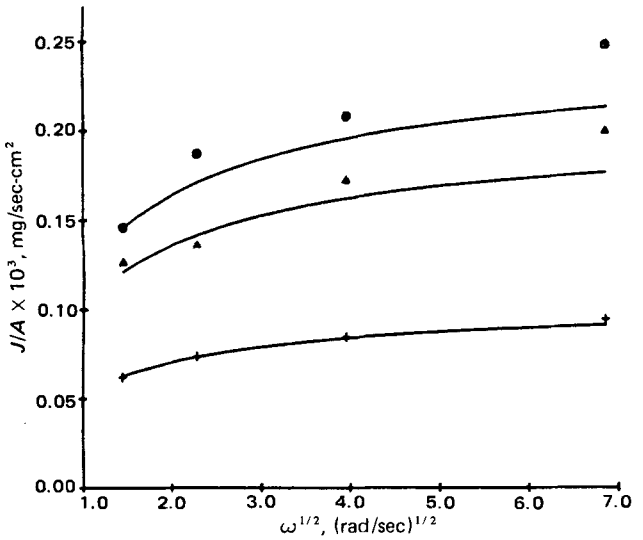

Figure 9—*Best-fit analysis of the aged pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key (C_b in milligrams per milliliter): ●, 0.0; ▲, 0.3; and +, 1.0.*

Rotating-Disk Method—Since minor procedural changes resulted in significant differences in the dissolution behavior of cholesterol pellets, an in-depth study was undertaken to determine if these differences were due to changes in solubility and/or interfacial transport. It was hoped that a better understanding of the changes would be gained. The pellets chosen for study were those shown in Fig. 4. As already seen, major differences were present in the dissolution rates.

To determine the dynamic solubility, initial dissolution rates were determined (5) and plotted against bulk concentration. Figures 5–7 show the results of the experiments carried out in 5% sodium cholate and 0.1 *M* phosphate buffer at pH 8.00. By extrapolating to a dissolution rate (J/A) of zero, it was possible to obtain the dynamic solubility. Figures 5–7 show a linear decrease in the dissolution rate as partial saturation is increased. This decrease indicates that the dissolution kinetics are proportional to the change in concentration (ΔC) over the entire saturation range. From these figures, it can be seen that the solubility for the normal and soaked pellets was ~1.40 mg/ml while that for the aged pellet was ~1.73 mg/ml. The solubility obtained for the aged pellet was the same as that previously obtained (5) for the anhydrous pellets, which indicates that dissolution from the aged pellet occurs from the same phase as from the anhydrous pellets.

To look at the data in a slightly different way, plots of (J/A) *versus* the square root rotational speed ($\omega^{1/2}$) were prepared. Figures 8–10 show

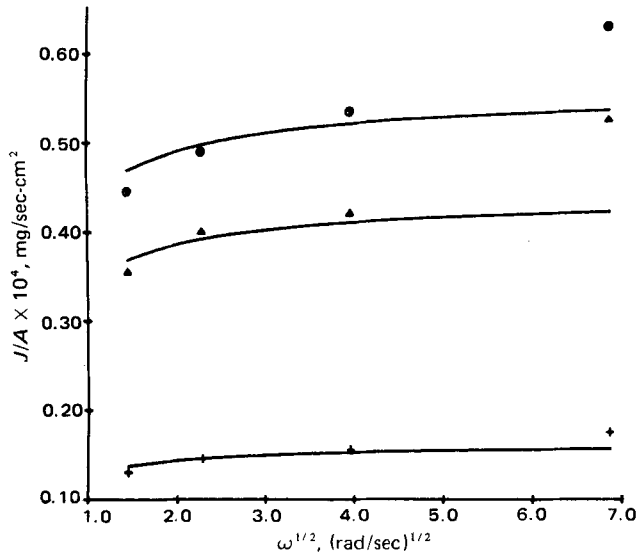

Figure 10—*Best-fit analysis of the soaked pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key (C_b in milligrams per milliliter): ●, 0.0; ▲, 0.3; and +, 1.0.*

Table V—Summary of Partial Saturation Experiments and Best-Fit Analysis for Normal Cholesterol Pellets in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s[a], mg/ml	Revolutions per Minute	Best-Fit Analysis: C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.45	20	1.45	0.51	1.25
1.45	50			
1.45	150			
1.42	450			

[a] From extrapolation of partial saturation data.

clearly the important involvement of the interfacial barrier in dissolution. If the interfacial barrier was not significant in dissolution, a plot of J/A *versus* $\omega^{1/2}$ would give a straight line through the origin.

Based on a best-fit analysis using the equation previously reported (5), $J = A(C_s - C_b)/[1.612D^{-2/3}\nu^{1/6}\omega^{-1/2} + 1/P]$, 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; there was a good fit between experiment and theory (Figs. 8–10). The points at 450 rpm ($\omega^{1/2} = 6.86$) were not weighted in this analysis (5).

Tables V–VII summarize the results of the partial saturation experiments and the best-fit analysis. The P_{aged}:P_{normal} ratio was ~2.71, while the P_{soaked}:P_{normal} ratio was ~0.78. Therefore, the differences in the dissolution rates can be accounted for primarily by significant changes in P.

Figure 11 shows a plot of the amount of cholesterol dissolved *versus* time for the normal and aged pellets at 150 rpm using the rotating-disk apparatus. The pattern of dissolution from the normal pellet was linear while that from the aged pellet showed a definite curvature after 1 hr (Figs. 2 and 4). The fact that the normal pellet showed a linear dissolution pattern indicates that dissolution occurs from a single phase (monohydrate). The curved dissolution pattern indicates that more than one phase probably takes part in the dissolution.

Because the solubilities for the aged and anhydrous pellets (5) are

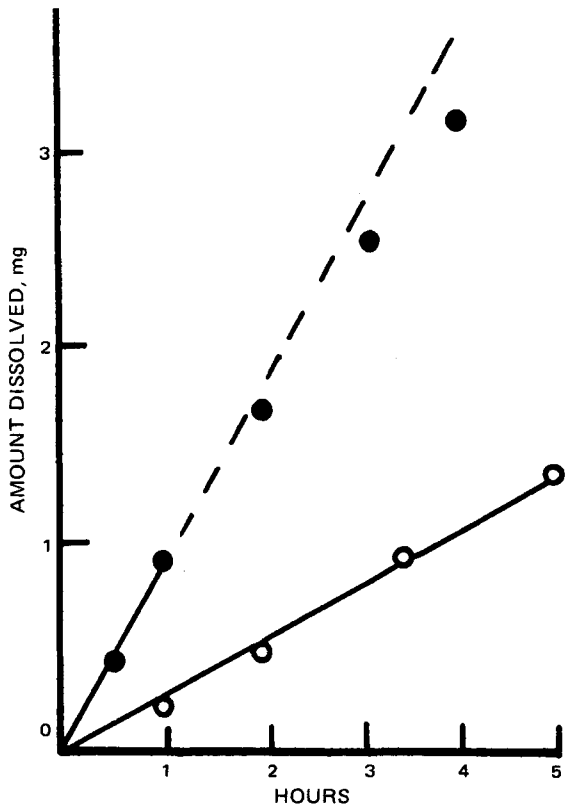

Figure 11—*Effect of pellet handling on the dissolution rate behavior at 150 rpm in the rotating-disk apparatus in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key: ●, aged pellet; and ○, normal pellet.*

Table VI—Summary of Partial Saturation Experiments and Best-Fit Analysis for Aged Pellets in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s[a], mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.73	20	1.75	1.39	1.26
1.73	50			
1.72	150			
1.73	450			

[a] From extrapolation of partial saturation data.

Table VII—Summary of Partial Saturation Experiments and Best-Fit Analysis for Soaked Pellets in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s[a], mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.40	20	1.41	0.39	1.26
1.40	50			
1.42	150			
1.42	450			

[a] From extrapolation of partial saturation data.

similar, leaving the pellet at 35° and ambient humidity for 24 hr may effectively dehydrate the outer surface layer, thereby making the pellet anhydrous. After initial dissolution removes the outer layer, dissolution again is controlled by the more slowly dissolving monohydrate layers.

Another possible explanation for this dissolution pattern is that pellet dehydration is not just a phenomenon of the outer surface layer but that the internal portions of the pellet also dehydrate. When the pellet contacts the dissolution medium, the more rapidly dissolving anhydrous material is removed from the surface. Dissolution from the internal portions of the pellet now is controlled by both anhydrous cholesterol and cholesterol monohydrate. The deeper the dissolution goes into the pellet, the less anhydrous material is present. As the curvature becomes greater, more cholesterol monohydrate probably is exposed; thus, dissolution slows and begins to approach the dissolution rate of a normal pellet.

As shown in Fig. 4, pellets exposed to ambient humidity for 24 hr at 35° and then soaked in double-distilled water at 37° for 24 hr showed linear dissolution, which indicates that soaking of an aged pellet effectively rehydrates the pellet. This rehydration is seen in the fact that the solubilities for the soaked and normal pellets are similar. However, the reformation of the monohydrate is probably not to the original state, perhaps because of precipitation of cholesterol onto the pellet surface. The newly formed crystals might be another polymorph of cholesterol monohydrate or they might be more perfect crystals (fewer defects) with fewer effective sites available for dissolution. The lower dissolution rate seen for the soaked pellet can be accounted for by either explanation. If dissolution of the soaked pellet is studied for a long period (~24 hr), a second linear portion is seen in the dissolution profile. Its slope is closer to that of the normal pellet.

CONCLUSION

From this analysis, the need for a uniform experimental procedure can be seen. If investigators hope to reproduce results within their own laboratories as well as reproduce other investigators' findings, standard experimental procedures must be defined and used.

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High-Pressure Liquid Chromatographic Analysis of Sennosides A and B Purgative Drugs

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Received January 21, 1980, from the *Pharmaceutical Analysis Unit, Department of Pharmaceutical Chemistry, University of Ife, Ile-Ife, Nigeria.* Accepted for publication September 10, 1980.

Abstract □ Procedures are described for the analysis of the main anthraquinone glycosides of senna powder, senna fruit tablets, and sennoside tablets by high-pressure liquid chromatography (HPLC). In one HPLC analysis, TLC was used to separate the glycosides prior to elution on a strong anion-exchange column with 0.1 *M* ammonium nitrate solution (pH 9.0) as the mobile phase. In another HPLC analysis, separation was effected using a weak anion-exchange column with 0.1 *M* ammonium nitrate solution (pH 5.7) as the mobile phase.

Keyphrases □ Sennosides A and B—high-pressure liquid chromatographic analysis, separation of isomers from crude drug and pharmaceutical preparations □ Anthraquinone glycosides—high-pressure liquid chromatographic assay, separation of isomeric sennosides A and B from crude drug and pharmaceutical preparations □ High-pressure liquid chromatography—analysis, anthraquinone glycosides, separation of isomeric sennosides A and B from crude drug and pharmaceutical preparations □ Cathartics—anthraquinone glycosides, isomeric sennosides A and B, high-pressure liquid chromatographic analysis

The pods and leaves of senna as well as the pharmaceutical preparations containing sennosides A and B are widely used in medicine because of their laxative properties. An accurate, simple, and easy method is needed for estimation of sennosides A and B individually.

The most commonly used approach involves removal