J. Pharm. Pharmacol. 1986, 38: 638–642
Received Fenruary 24, 1986

Mechanism of dissolution of cholesterol–calcium bilirubinate compressed discs in monooctanoin

S. C. MULLIGAN AND O. I. CORRIGAN*

Department of Pharmaceutics, School of Pharmacy, Trinity College, 18 Shrewsbury Road, Dublin 4, Eire

The dissolution of cholesterol monohydrate and calcium bilirubinate (neutral salt) mixtures in monooctanoin was investigated using the static disc method. The intrinsic dissolution rate of calcium bilirubinate was orders of magnitude (~1000 fold) lower than that of cholesterol. Cholesterol release decreased as its weight fraction in the solid decreased. In model systems containing below 50% cholesterol dissolution became negligible. The release profiles deviated from the classical model for dissolution from two component mixtures. The observed dissolution profiles of both components were greater than predicted by theory. Anomalous positive curvatures in dissolution profiles suggested that calcium bilirubinate initially reduced the surface area available for cholesterol dissolution. A model, taking into account the change in surface area, was used to fit the cholsterol dissolution data. The results were consistent with the reported relationship between human gallbladder stone cholesterol content and average stone weight loss.

Glyceryl-1-monooctanoate (monooctanoin) is effective both in-vitro (Gadacz 1979) and in-vivo (Mack et al 1981; Thistle et al 1980, Sharp & Gadacz 1982) as a dissolving agent for common bile duct stones. Successful dissolution depends largely on the chemical composition of the stones. Cholesterol monohydrate is the most common primary stone constituent in choledocholithiasis and cholecystolithiasis. The current solvent of choice is monooctanoin, which has a low in-vivo toxicity and is an excellent solvent for cholesterol. Physicochemical studies have also shown that the dissolution of cholesterol in monooctanoin is diffusion controlled (Flynn et al 1979; Bogardus 1984). Clinical reports record 50–75% partial dissolution of biliary calculi. Wosiewitz et al (1983) have recently reported that common bile duct stones have a lower cholesterol content than gallbladder stones, as many as 41% being classified as pigment stones. Therapeutic failure with monooctanoin has been linked to the presence of appreciable secondary stone components (Thistle 1983) such as calcium bilirubinate, the principal pigment in stones. The aim of the present work was to assess the possible role of secondary stone components in limiting stone dissolution. We therefore investigated the in-vitro dissolution of calcium bilirubinate–cholesterol mixtures in monooctanoin. The static disc dissolution method was employed, as compressed pellets have been used successfully as model gallstones (Higuchi et al 1973).

* Correspondence.

THEORY

Successful litholysis is not only dependent on cholesterol dissolution but also on the dissolution/disintegration of all components present in the stone. In the simple case of a non-disintegrating stone composed of two components, B and C, the classical theory for the diffusion controlled dissolution of two component mixtures (Higuchi et al 1965) predicts, for non-interacting components, that the solubilities (C_{sb}, C_{sc}), diffusion coefficients (D_b, D_c) and the relative proportions (N_b, N_c) of the two components control dissolution. Three different situations are possible at the solid-liquid interface during dissolution, depending on the relative amounts of the components present. At the critical mixture ratio defined by

$$\frac{N_b}{N_c} = \frac{D_b C_{sb}}{D_c C_{sc}} \quad (1)$$

both components coexist at all times at the solid-liquid interface and dissolution profiles of each component will be linear from a constant surface area disc under sink conditions. At all other weight ratios one or other component forms a porous layer at the interface which represents an additional barrier retarding the dissolution of the receding phase. Thus when $N_b/N_c > D_b C_{sb}/D_c C_{sc}$, the dissolution rate (dW/dt) per unit surface area (G_b) of B will be given by

$$G_b = \frac{dW_B}{A.dt} = D_b C_{sb}/h \quad (2)$$

where A is the surface area and h the diffusion layer

thickness. The dissolution rate of C decreases with time to reach a limiting value defined by

$$\frac{dW_C}{A.dt} = \frac{N_c}{N_b} G_b \quad (3)$$

When a large difference (i.e. orders of magnitude) exist between the component solubilities, deviations from this model may occur. Mixtures low in the more soluble component approximate to matrix-controlled dissolution (Higuchi 1967). Carrier-controlled dissolution may occur in mixtures low in the less soluble component (Corrigan 1985).

MATERIALS AND METHODS

Materials

Cholesterol monohydrate was prepared by recrystallizing anhydrous cholesterol (USP) from 95% ethanol as described by Flynn et al (1979). Crystals thus prepared were stored under 100% humidity and protected from light.

Calcium bilirubinate was prepared from commercial bilirubin (Porphyrin Products, Logan, Utah 84321 USA) by a modification of the method of Sutor & Wilkie (1977). Bilirubin was suspended in deionized water and 1 M sodium hydroxide added dropwise until all material dissolved, thus forming sodium bilirubinate solution. This solution was constantly bubbled with argon. The form of calcium bilirubinate prepared is dependent on the strength and pH of the buffer used (Sutor & Wilkie 1977). To ensure formation of the neutral salt, sodium bilirubinate solution was added at a steady rate of 5 ml min^{-1} to $3{\cdot}5 \times 10^{-3}$ M calcium chloride in 0·87 M triethanolamine buffer adjusted to pH 8·0 with HCl and the pH constantly monitored. The resulting precipitate was centrifuged at 3000 rev min^{-1}, the supernatant decanted and the precipitate repeatedly washed with deionized water and twice with both methanol and chloroform. Samples were stored in a vacuum desiccator and protected from light. Powder X-ray diffraction (Sutor & Wilkie 1977), infra-red spectroscopy and calcium estimation, by the atomic absorption method, confirmed formation of the neutral salt. Glyceryl-1-monooctanoate (monooctanoin), available commercially for gallstone dissolution as Capmul (8210), Stokely-Van Camp. Inc. Indiana, was used as received.

Methods

Solubility determinations. The solubilities of cholesterol monohydrate and calcium bilirubinate at 37 °C were determined by equilibrating excess solid monooctanoin in screw-capped test tubes, agitation being provided by a wrist action shaker (Su et al 1981). Equilibrated samples were filtered through 0·45 μm disposable filters (Acrodisc; Gelman) and an accurate weight diluted with monooctanoin. Calcium bilirubinate in monooctanoin was assayed from the UV absorbance at 450 nm. Cholesterol concentrations in monooctanoin were estimated by a modification of the CHOD-PAP enzymic colorimetric method (Boehringer Mannheim GmbH Diagnostica). Monooctanoin was immiscible with the enzymic assay reagents. Therefore it was necessary to mix samples intimately for 15 s using a whirl mixer. Reproducible calibration curves were obtained by this method provided test solutions were measured within 5 min. This time constant was necessary as monooctanoin enhanced the degradation of the colour complex.

Dissolution studies. Approximately 100 mg of solid was compressed (7616 kg cm^{-1}) to form a 1·3 cm diameter disc. The disc was mounted in a stainless steel die which allowed exposure of one surface of the disc, flush with the upper surface of the die. The under surface of the pellet was sealed with hard paraffin. If disc fracture occurred on mounting, the disc was rejected. Dissolution profiles at 37 °C in 10 ml of monooctanoin were obtained using the static disc method as previously described (Kwan et al 1977; Gupta et al 1985).

Agitation was provided in the water-jacketed dissolution vessel by a paddle 0·2 cm above the disc surface, rotating at 150 rev min^{-1} (Gupta et al 1985). Components were assayed as described above under solubility determinations. Samples (1 ml) of dissolution medium, taken by pipette for assay, were replaced with pre-equilibrated fresh monooctanoin and appropriate corrections made for the dilution. At least three replicate discs of each composition were determined.

Before compression, cholesterol monohydrate and calcium bilirubinate were ground for 5 min using an agate mortar and pestle to ensure uniformity of mixing.

All experiments involving calcium bilirubinate were performed under darkroom conditions and solvents degassed using argon (Carey & Koretsky 1979).

RESULTS AND DISCUSSION

The equilibrium solubilities of cholesterol monohydrate and calcium bilirubinate in monooctanoin are summarized in Table 1. The cholesterol monohydrate solubility of 125 mg ml^{-1} at 37 °C is in

Table 1. Solubilities (C_s) and intrinsic initial dissolution rates (G) of cholesterol monohydrate and calcium bilirubinate (neutral salt) in monooctanoin at 37 °C.

	C_s		G	
	mg ml^{-1}	(s.e.)	μg cm^{-2} h^{-1}	(r^2)
Cholesterol monohydrate	125	(4·7)	15590	(0·998)
Calcium bilirubinate	0·140	(0·008)	12·33	(0·988)

agreement with the value of 117 mg ml^{-1} reported by Flynn et al (1979), given the sensitivity of cholesterol monohydrate solubility to the content of di- and triglyceride and water content of commercial samples (Thistle et al 1980; Bogardus 1982). Calcium bilirubinate, neutral salt, was nearly 900 times less soluble in monooctanoin than cholesterol. The difference in dissolution rate between these two stone constituents is of the same order of magnitude (Table 1). Since Flynn et al (1979) have shown that the dissolution rate of cholesterol monohydrate in monooctanoin is diffusion-controlled, it is reasonable to assume on the basis of the data in Table 1 that the dissolution of calcium bilirubinate is also primarily diffusion controlled. Consequently equation 1 predicts that calcium bilirubinate will form the surface layer controlling dissolution in mixtures containing up to approximately 99·8% cholesterol, the critical mixture percentage. Furthermore the dissolution profiles of calcium bilirubinate over this range of compositions were expected to be linear (equation 2) while those of cholesterol monohydrate should be non-linear, reaching a limiting dissolution rate given by equation 3.

The dissolution profiles of cholesterol monohydrate from compressed discs containing varying proportions of calcium bilirubinate are illustrated in Fig. 1. Calcium bilirubinate retarded the dissolution rate of cholesterol monohydrate from constant surface area discs containing both components, the effect increasing with increasing calcium bilirubinate content. The dissolution profiles of mixtures containing greater than 50% cholesterol monohydrate had positive curvatures and the limiting rates approached that of pure cholesterol monohydrate.

The corresponding release profiles of calcium bilirubinate from mixed discs are shown in Fig. 2. The 0·9 calcium bilirubinate weight fraction disc was the only system whose release was similar to that of pure calcium bilirubinate. The other systems examined gave release profiles having a positive curvature. Mixtures having the lowest calcium bilirubinate contents showed the steepest increase in release rate with time. Leaching of particulate calcium bilirubinate into the dissolution medium was observed in these systems. Thus the amount of calcium bilirubinate released exceeded its solubility.

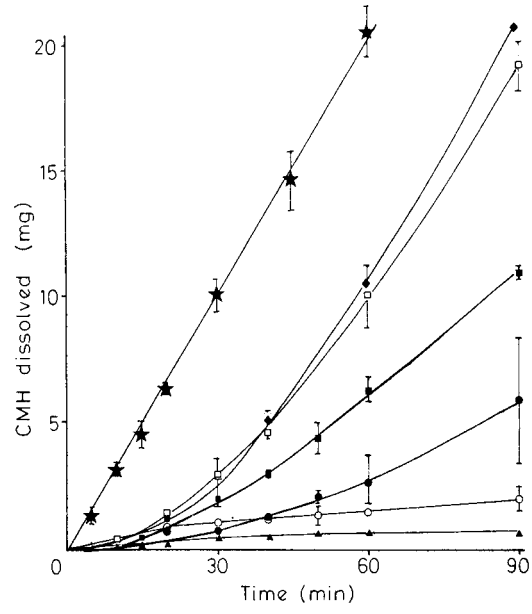

FIG. 1. Dissolution profiles of cholesterol monohydrate (CMH) from compressed discs containing varying proportions of calcium bilirubinate and cholesterol monohydrate (37 °C). Key: ★, 100%; ♦, 90%; □, 80%; ■, 70%; ●, 50%; ○, 40%; ▲, 10% cholesterol monohydrate.

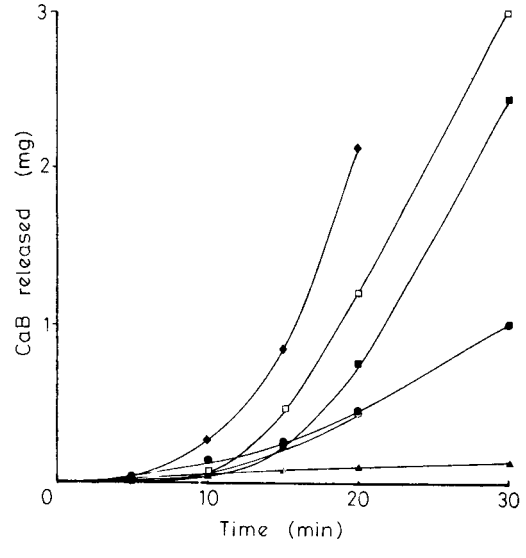

FIG. 2. Dissolution profiles of calcium bilirubinate (CaB) from compressed discs containing varying proportions of cholesterol monohydrate and calcium bilirubinate (key as in Fig. 1).

Shedding of particulate calcium bilirubinate into the dissolution medium is to be expected, given the

low calcium bilirubinate concentration present at the critical mixture ratio. The concentration of calcium bilirubinate in the disc is evidently insufficient to maintain an intact porous surface layer, as required for equation 3 to apply during dissolution (Corrigan 1985). On compression, cholesterol monohydrate became the continuous solid matrix, calcium bilirubinate forming the dispersed phase. Consequently, on cholesterol dissolution, the dispersed calcium bilirubinate is carried into solution, being released by the dissolving cholesterol monohydrate.

The positive curvatures in dissolution profiles require further explanation. The presence of calcium bilirubinate appears to inhibit cholesterol monohydrate dissolution by initially retarding access of the dissolution medium to the cholesterol monohydrate surface. Recently, Chow et al (1986) observed an increase in the dissolution rate with time for adipic acid crystals grown in the present of fatty acid derivatives and suggested that the slower initial dissolution rate may be caused by initially imperfect wetting of the crystal surfaces. Assuming that the rate of availability of cholesterol monohydrate surface is proportional to the surface remaining to be made available, then

$$\frac{dF}{dT} = k(1 - F) \tag{4}$$

where F is the fraction of surface available and k is a rate constant. On integration

$$F = (1 - e^{-kt}) \tag{5}$$

or

$$A = S(1 - e^{-kt}) \tag{6}$$

where A is the surface available at time T and S is the total surface area available at time ∞.

Combination of equations 2 and 6 gives the dissolution rate of cholesterol monohydrate

$$\frac{dW_c}{dT} = SD_cC_{sc}(1 - e^{-kt})/h \tag{7}$$

The mass of calcium bilirubinate (M_b) removed from the stone may be obtained from equation 8.

$$\frac{dM_b}{dT} = \frac{dW_c}{dT} \cdot \frac{N_b}{N_c} \tag{8}$$

Equations 7 and 8 were used to simulate cholesterol and calcium bilirubinate release and gave profiles in qualitative agreement with the experimental results. The cholesterol dissolution data were fitted to equation 7 using the non-linear regression programme NONLIN (Metzler et al 1974). The results are shown in Fig. 3. The systems containing over

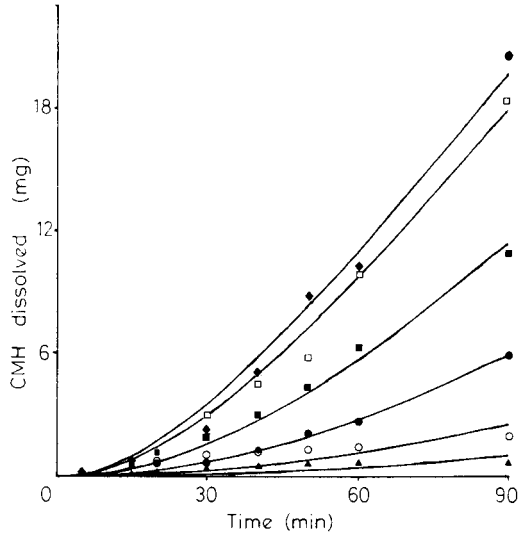

FIG. 3. Dissolution profiles of cholesterol monohydrate (CMH) from mixed discs. The solid lines represent the fitted curves determined using Equation 7 (key as in Fig. 1).

50% cholesterol monohydrate showed good agreement with theory ($r^2 > 0{\cdot}97$). The values of k decreased as the cholesterol content of the solid declined. The effect of calcium bilirubinate content on the release profiles of calcium bilirubinate was simulated using equation 8 and is shown in Fig. 4.

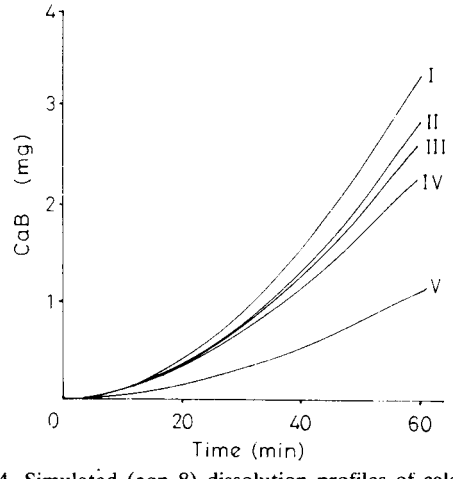

FIG. 4. Simulated (eqn 8) dissolution profiles of calcium bilirubinate (CaB) release versus time from constant surface area discs containing varying proportions of cholesterol monohydrate and calcium bilirubinate. Key: I, 10%; II, 20%; III, 30%; IV, 50%; V, 60%.

The profiles are qualitatively in agreement with the experimental results (Fig. 2), in so far that the profiles are positively curved ans the release rate increased as the calcium bilirubinate content

decreased. The direct application of equation 8 to the calcium bilirubinate data requires information on the particle size distribution of the released calcium bilirubinate particles.

The results suggest that in model systems containing below ~50% cholesterol, dissolution becomes negligible. However, in systems containing greater than 50% cholesterol, litholysis is controlled by the cholesterol surface available for dissolution and the disintegration of the minor component into the dissolution medium. These results with model systems are consistent with the gallstone dissolution results reported by Sharp & Gadacz (1981). Human gallstones incubated in monooctanoin showed negligible weight loss if the cholesterol content was less than ~50%. With increasing stone cholesterol content (over 50%) average daily weight loss increased significantly. Thistle (1983) observed that duct stones containing appreciable amounts of calcium bilirubinate often have a soft consistency, and for successful monooctanoin therapy a cholesterol content of at least 40% is a requirement.

Acknowledgements

Financial support for this work was provided by the Medical Research Council of Ireland. Capmul 8210 was generously supplied by Stokely Van Camp Inc.

REFERENCES

Bogardus, J. B. (1982) J. Pharm. Sci. 71: 370–372

Bogardus, J. B. (1984) Ibid. 73: 906–911

Carey, M. C., Koretsky, A. P. (1979) Biochem. J. 179: 675–689

Chow, K. Y., Go, J., Grant, J. W. (1986) Drug Devel. and Ind. Pharm. 12: 247–264.

Corrigan, O. I. (1985) Ibid. 11: 697–724

Flynn, G. L., Shah, Y., Prakongpan, S., Kwan, K. H., Higuchi, W. I., Hofmann, A. F. (1979) J. Pharm. Sci. 68: 1090–1097

Gadacz, T. R. (1979) J. Surg. Res. 26: 378–380

Gupta, S. L., Higuchi, W. I., Ho, N. F. H. (1985) J. Pharm. Sci. 74: 1172–1177

Higuchi, W. I. (1967) Ibid. 56: 315–324

Higuchi, W. I., Mir, N. A., Desai, S. J. (1965) Ibid. 54: 1405–1410

Higuchi, W. I., Prakongpan, S., Young, F. (1973) Ibid. 62: 945–948

Kwan, K. H., Higuchi, W. I., Molokia, A. M., Hofmann, A. F. (1977) Ibid. 66: 1094–1101

Mack, E., Patzer, E. M., Crummy, A. B., Hofmann, A. F., Babayan, V. K. (1981) Arch. Surg. 116: 341–344

Metzler, C. M., Elfring, G. L., McEwen, A. J. (1974) A users manual for NONLIN and associated programs. Upjohn, Kalamazoo, Michigan, USA

Sharp, K. W., Gadacz, T. R. (1982) Ann. Surg. 196: 137–139

Su, C. C., Park, J. Y., Higuchi, W. I., Alkan, M. H., Corrigan, O. I., Hofmann, A. F. (1981) J. Pharm. Sci. 70: 713–715

Sutor, D. J., Wilkie, L. I. (1977) Clin. Sci. and Molec. Med. 53: 101–103

Thistle, J. L. (1983) in: Paumgartner, G., Stiehl, A., Gerok, W. (eds) Cholesterol in Health and Disease. Falk Symposium 33. MTP Press Limited, Lancaster, pp. 401–405

Thistle, J. L., Carlson, G. L., Hofmann, A. F., La Russo, N. F., McCarthy, R. L., Flynn, G. L., Higuchi, W. I., Babayan, V. K. (1980) Gastroenterology 78: 1016–1022

Wosiewitz, V., Schenk, J., Schmack, B. (1983) Digestion 26: 43–52