International Journal of Pharmaceutics, 91 (1993) 235–240

IJP 03038

A practical approach to evaluate the effect of the aqueous stagnant layer on drug release from polymeric devices

Richard I. Senderoff and Theodore D. Sokoloski [1]

The Ohio State University, College of Pharmacy, 500 W. 12th Ave, Columbus, OH 43210, (USA)

(Received 27 May 1992)
(Modified version received 10 September 1992)
(Accepted 13 September 1992

Key words: Drug delivery; Fibrin sheet; Serial barrier; Release dependence; Aqueous stagnant layer; Methodology

Summary

The present study describes an approach to characterize drug release from a sustained drug delivery system composed of the natural fibrin polymer. The biochemical reaction between fibrinogen and thrombin has been used to produce potentially natural, biodegradable, biocompatible, sustained drug delivery systems. One of these is a promising implantable sheet containing entrapped drug. A model was used which described the release of drug entrapped within a fibrin matrix in which data were treated assuming that flux follows a model consistent with movement through two barriers in series: diffusion in the matrix (path length varying with time) and diffusion through an aqueous stagnant layer. The experimental design permitted the measurement of rate of transport as a function of time. The methodology made it possible to determine the contribution of each barrier to the overall transport from a single experiment. This represents a practical approach to evaluate and compare sustained drug delivery systems and demonstrates the need to account for the involvement of barriers that are highly dependent on the way data are collected, since they can impact the conclusions that are made relative to the matrix affects.

Introduction

Sustained release of drugs which have been entrapped within a polymeric matrix has been shown to follow physical models in which diffusion of drug occurs through pores within the matrix structure or through the polymeric phase itself. However, the adjacent aqueous boundary layer and/or an adjacent lipid barrier can provide additional resistance which can affect the interpretation of in vitro release data or in vivo performance (Tojo et al., 1985; Diez-Sales et al., 1991). For example, passive drug diffusion across a lipidic membrane involves both the intrinsic resistance of the membrane itself and that of the adjacent aqueous boundary layers, which can provide a substantial contribution to the overall diffusion process (Stehle and Higuchi, 1972; Lovering and Black, 1974). For highly lipophilic compounds which readily permeate a lipoidal barrier,

Correspondence to (present address): R.I. Senderoff, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., 170 Tabor Road, Morris Plains, NJ 07950, U.S.A.
[1] *Present address:* SmithKline Beecham Pharmaceuticals, Pharmaceutical Sciences, UW2913, P.O. Box 1539, 709 Swedeland Road, King of Prussia, PA 19406-0939, U.S.A.

diffusion across aqueous layers could become rate-limiting, whereas for hydrophilic, easily water-diffusional compounds, diffusion in the lipoidal barrier could be rate-limiting.

These concepts can also be applied to drug release from polymeric devices. Therefore, it is necessary to identify, separate, and quantitate intrinsic matrix effects from procedure dependent hydrodynamic effects, in order to evaluate the properties of the delivery system per se.

These factors became apparent when characterizing a promising drug delivery system, in which the biochemical reaction between fibrinogen and thrombin was used to entrap drugs within the matrix of the fibrin polymer. The unique properties of the fibrin matrix make these systems viable for drug delivery (Senderoff et al., 1991). One of these is a potentially implantable fibrin sheet containing entrapped drug. In order to optimize the delivery of drugs trapped in a fibrin matrix, the kinetics of drug release had to be quantified and a model identified that was consistent with the release behavior. During the course of the studies it became apparent that aqueous stagnant layer effects had to be separated from transport contributions associated with the matrix. In this paper, the approach used to isolate the aqueous layer affect is addressed.

Materials and Methods

Dexamethasone (stock no. 104502) was supplied by Merck Sharp & Dohme. Prednisolone (no.P-6004), bovine fibrinogen (no.F-4753), and bovine thrombin (no.T-6634) were obtained from Sigma Chemical Co. All compounds were used as received, except for fibrinogen which was desalted by dialysis and subsequently freeze-dried (Virtis Co., Inc., Model no. 10-010). Flow-through diffusion cells were obtained from Vangard International, Inc. The flow of release medium, composed of Tris buffer (pH 7.5) and propylene glycol (USP/FCC), was maintained with a Rainin Rabbit peristaltic pump. Analysis of dexamethasone utilized the Rainin Rabbit Gradient HPLC system having a Perkin-Elmer LC-235 Diode Array detector and a Perkin-Elmer LCI-100 Laboratory Computing integrator. A Water's μ-Bondapack C-18 chromatography column was used.

Fibrin sheets with dexamethasone entrapped were prepared using the following general procedure. 50 mg of desalted, freeze-dried fibrinogen was dissolved in 1.0 ml of citrate buffer. A known weight of dexamethasone was dispersed in the fibrinogen solution. The fibrinogen/drug dispersion was then mixed with 2.5 units of thrombin and injected onto an 8 μm hydrophilic support

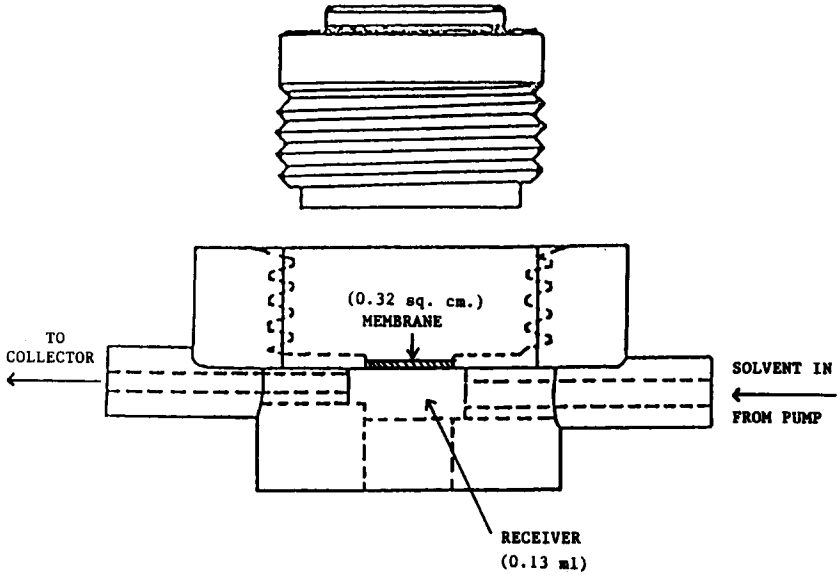

Fig. 1. Schematic of the flow-through diffusion cell used in in vitro studies.

membrane (Millipore SC). After a 30 min incubation, the fibrin gel dispersion was pressed between two glass plates for an additional 30 min. The fibrin sheet was then dried and stored in a desiccator. The supporting membranes were used to assist handling of the matrix.

Transport was characterized by following the release of drug as a function of time using a flow-through diffusion cell (Fig. 1). It was assumed that flow maintained by the pump provided a relatively constant aqueous stagnant layer. A previously dried fibrin sheet was hydrated in a 100% humidity chamber for 24 h prior to release experiments to minimize lag times and the effects of solvent sorption. Dissolution medium (37°C) containing 1 μg/ml prednisolone as internal standard was pumped across the surface of the sheet to a fraction collector (ISCO Model no.1850). The release medium consisted of Tris buffer (pH 7.5) and 0–75% propylene glycol. Propylene glycol was used as a cosolvent to vary solubility. In separate studies, the solubility of dexamethasone was logarithmically related to cosolvent fraction and found to be 40-times greater in 75% propylene glycol than in water at 37°C. The release medium was pumped at a constant rate, usually 10 ml/h. Thus, the amount of drug found in each volume collected, when divided by the collection interval, gave the rate of drug release over the time interval. In this way, the data could be evaluated in terms of both the cumulative amount released and the rate of drug released as a function of time. The amount of drug in each sample was determined by an isocratic HPLC assay using 63% methanol/water, a pumping rate of 1.2 ml/min, and a detection wavelength of 240 nm. Unreleased drug was determined by dissolving the exposed portion of the fibrin sheet in 6 M urea (containing 1 μg/ml prednisolone) and analyzing by HPLC.

The experimental conditions where flux of drug in the absence of the fibrin matrix was measured were identical to the matrix studies. In these studies a layer of drug was deposited by evaporation from an isopropanol dispersion added drop wise onto the 8 μm hydrophilic Millipore support membrane that had been mounted in the flow-through cell.

Results and Discussion

A typical release profile for dexamethasone is shown in Fig. 2. A simple approach to describe solute release behavior from such systems has been proposed by Ritger and Peppas (1987), using an empirically derived exponential relation of the form

$$\frac{M_t}{M_\infty} = kt^n \tag{1}$$

where M_t is the cumulative amount released in time t, M_∞ denotes the total amount that can be released, k is a constant incorporating characteristics of the macromolecular network system, and n represents a diffusional exponent which can provide insight to the apparent transport mechanism; e.g., pure Fickian diffusion from thin polymer slabs with drug dispersed give a value for n of 0.5. On the other hand, a zero-order release mechanism gives $n = 1.0$. Drug transport resulting from a combination of two or more of these processes has a diffusional exponent of $1.0 > n > 0.5$. Thus, the value for n indicates whether drug transport follows classical diffusion models or if a more complicated process is operative. Changes in an apparent release mechanism brought about by alterations of the formulation or experimental conditions can also be characterized by noting changes in the diffusional exponent. According to

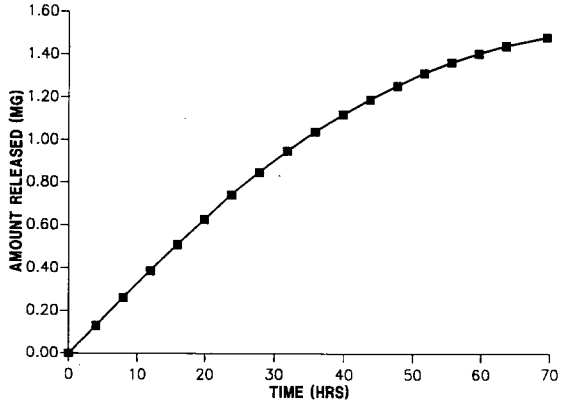

Fig. 2. Cumulative amount of dexamethasone released from a fibrin sheet into 50% propylene glycol/Tris buffer (pH 7.5, flow rate 10 ml/h, 37°C).

Ritger and Peppas, Eqn 1 is generally valid for the first 60% of fractional release, for which the assumption of one-dimensional diffusion under perfect sink conditions is valid. The diffusional exponent is obtained by plotting the logarithm of fraction transported as a function of the logarithm of time and determining the slope. Analyzing the release of dexamethasone dispersed in fibrin sheets in this way resulted in diffusional exponents ranging from 0.8 to 0.9, which were independent of formulation parameters such as pH and ionic strength of reaction solutions, or on experimental conditions used such as fraction of cosolvent in the release medium (Senderoff et al., 1991). These values for n are consistent with a near zero-order release dependence, but clearly not purely zero-order.

To further characterize this release behavior, it was assumed that the diffusional exponent was the result of a combination of Fickian diffusion through the pores of the fibrin matrix, path length varying with time, and a zero-order diffusion through a stagnant aqueous layer on the surface of the sheet. Fig. 3 depicts the model used where amount transported is the result of movement through the two barriers (matrix and stagnant layer) in series. Roseman and Higuchi (1970) have previously described a similar model which takes into account additional resistance to mass transfer by an unstirred diffusion layer at the surface of a monolithic device. However, the resultant equations for the rate and the cumulative amount of drug released are inconvenient to use to isolate and quantitate the relative contributions of matrix and stagnant layer effects. The approach used here is to further constrain the model by assuming that at pseudo-steady state the concentration at the interface between the matrix and the stagnant layer (C_b, Fig. 3) remains constant (or does not change appreciably) and then verify the validity of this assumption through independent experimentation. This assumption allowed the model to be developed in terms of the individual resistances of each barrier (matrix and stagnant layer) in series. For a serial diffusion process, the total resistance to movement (R_T) is the sum of the resistances in each barrier (m, matrix; a, aqueous stagnant layer),

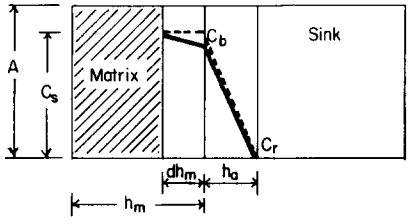

Fig. 3. Model used for drug flux through matrix (m) and aqueous (a) resistance layers in series. Dexamethasone is dispersed in a fibrin matrix at a total (solid and solution) concentration of A. C_s is the solubility of dexamethasone in solvent. The effective path length within the matrix is h_m, and h_a is the effective aqueous layer length. The concentrations at the interface between the two barriers is C_b, and the receiver concentration is C_r. The dashed line is the concentration-distance profile at time, $t = 0$, and the solid line depicts a time, $t > 0$, at pseudo-steady state where the matrix has been depleted of drug over a distance dh_m.

$$R_T = R_m + R_a \tag{2}$$

Under pseudo-steady state conditions the resistance in the matrix pores should follow the Higuchi relationship for drug dispersed in a matrix having a slab geometry

$$R_m = \left[\frac{2 \cdot \Delta C_m \cdot t}{A \cdot D_m}\right]^{0.5} \tag{3}$$

where ΔC_m is the membrane concentration gradient (C_s–C_b, Fig. 3), t denotes time, A is the initial total amount of drug per unit volume of sheet, and D_m represents the effective matrix diffusion coefficient. For simplification, the tortuosity and porosity constants are grouped into the effective matrix diffusion coefficient. Since the fibrin matrix consists of a network of rigid fibers (Seegers, 1967) it is assumed that drug movement does not include partitioning and transport through the matrix itself.

Resistance to transport in the stagnant layer (R_a) is

$$R_a = \frac{h_a}{D_a} \tag{4}$$

where h_a is the effective aqueous layer thickness and D_a denotes the effective aqueous diffusion

coefficient. Substituting these resistances into the steady state flux per unit area expression

$$\frac{dQ}{dt} = \frac{1}{R_T} \cdot \Delta C \tag{3}$$

gives Eqn 6 after rearrangement with $\Delta C = C_s$ (solubility) under sink conditions.

$$\frac{dQ}{dt} = \frac{D_a \cdot C_s}{D_a\left[\frac{2 \cdot \Delta C_m}{A \cdot D_m}\right]^{0.5} \cdot t^{0.5} + h_a} \tag{6}$$

Eqn 6 reduces to the Higuchi equation (matrix-controlled kinetics) when

$$D_a\left[\frac{2 \cdot \Delta C_m}{A \cdot D_m}\right]^{0.5} \cdot t^{0.5} \gg h_a$$

since, in the absence of significant resistance by the aqueous stagnant layer, $C_b = C_r = 0$. Likewise, Eqn 6 reduces to a zero-order rate expression when h_a is large (aqueous stagnant layer-controlled kinetics).

The constant terms in Eqn 6 can be grouped to give

$$\frac{dQ}{dt} = \frac{A'}{B' \cdot t^{0.5} + C'} \tag{7}$$

Integrating Eqn 7 gives

$$Q = \frac{2A' \cdot t^{0.5}}{(B')} - \frac{2 \cdot A' \cdot C'}{(B')^2} \cdot \ln\left[\frac{B'}{C'} \cdot t^{0.5} + 1\right] \tag{8}$$

Eqn 8 relating the cumulative amount transported is cumbersome to fit for estimates of the constants A', B', and C'. However, the reciprocal of the rate expression (Eqn 7) can be taken,

$$\frac{dt}{dQ} = \frac{B'}{A'} \cdot t^{0.5} + \frac{C'}{A'} \tag{9}$$

Since release rate is measured experimentally, it is possible to take its reciprocal and plot it as a function of $t^{0.5}$. The slope of the linear plot is

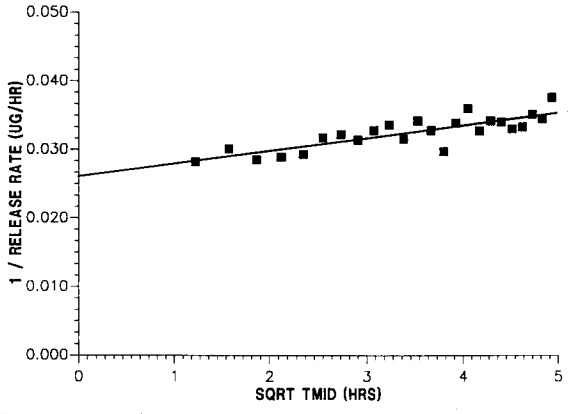

Fig. 4. Reciprocal rate of release vs square root of time for dexamethasone release from a fibrin sheet into 50% propylene glycol/Tris buffer pH 7.5 (flow rate 10 ml/h, 37°C). Intercept = $h_a / D_a \cdot C_s$.

B'/A' and its intercept is C'/A'. In a comparison of transport networks, changes in the intercept reflect changes due to the aqueous layer ($C'/A' = h_a/D_a \cdot C_s$), whereas changes in the slope are due to either a drug carrier and/or aqueous layer effect. Fig. 4 shows compliance of release rate for dexamethasone embedded in a fibrin sheet analyzed via Eqn 9.

Verification of the magnitude of the hydrodynamic layer existing in series with the fibrin matrix was evaluated through independent dissolution studies using the flow through system; dexamethasone was cast as a disc on the surface of a large pore hydrophilic membrane placed in the diffusion cell. In the simplest case, the measured dissolution rate, dQ/dt, should follow a zero-order relationship (Eqn 10).

$$\frac{dQ}{dt} = \frac{D_a \cdot C_s}{h_a} = \frac{A'}{C'} \tag{10}$$

Fig. 5 shows the Q vs t relationship for the dissolution of dexamethasone in the flow-through system. The slope of such a plot (Eqn 10) should be the same as the reciprocal intercept obtained in the matrix release studies, if the release of dexamethasone embedded in a fibrin sheet complies with the two-barrier diffusion model.

Table 1 lists the results of studies where such comparisons are made under different experi-

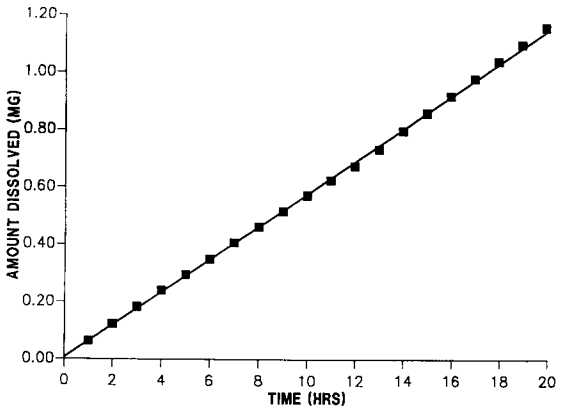

Fig. 5. Dexamethasone dissolution into 50% propylene glycol/Tris buffer pH 7.5, (flow rate 10 ml/h, 37°C). Slope = $D_a \cdot C_s / h_a$.

mental conditions (e.g., differing eluting solvents and flow rates). There is excellent agreement between the values for $h_a/D_a \cdot C_s$ in the two entirely separate experiments; each using a different method of data analysis. Focusing on the effects under varying experimental conditions, the results found in Table 1 are consistent with the two-barrier diffusion model. As the fraction of cosolvent in the medium is increased, there is an increase in the solubility of dexamethasone resulting in a decrease in the contribution of the aqueous stagnant layer. Similarly, increasing the flow rate would be expected to decrease the aqueous layer resistance resulting in a decrease in the role of the stagnant layer.

TABLE 1

Comparison of estimates of the aqueous stagnant layer contribution ($h_a / D_a \cdot C_s$) to dexamethasone flux from fibrin sheets and from dissolving discs under identical flow-through cell conditions

Release medium [a] (%)	Flow rate (ml/h)	$h_a / D_a \cdot C_s$ (S.E.)	
		Matrix [b] release study	Dissolution [c] study
25	10	0.0794 (0.0045)	0.0797 (0.0008)
50	10	0.0272 (0.0014)	0.0273 (0.0001)
75	10	0.0143 (0.0007)	0.0169 (0.0001)
50	20	0.0172 (0.0007)	0.0175 (0.0001)

[a] Percent propylene glycol in Tris buffer pH 7.5. [b] $h_a / D_a \cdot C_s$ from the intercept of reciprocal flux vs square root time plots. [c] $h_a / D_a \cdot C_s$ from the slope of amount dissolved vs time plots.

Conclusions

A practical approach is presented to account for additional resistance to diffusion of drug from a polymeric matrix by the stagnant aqueous layer. Appropriate equations were derived which allowed isolation and quantitation of the relative contribution of the aqueous stagnant layer to dexamethasone flux from fibrin sheets in a single experiment. Although the model is constrained by the assumption of a pseudo-steady state condition where the concentration at the interface between the matrix and the stagnant layer remains constant, the release behavior of dexamethasone embedded in a fibrin sheet was shown to be consistent with the theory through independent experimentation. What initially could have been interpreted as a zero-order release from the matrix was in reality a reflection of in vitro experimental conditions. Although the importance of the experimental system in data analysis was considered by Tojo et al. (1985), the present study shows how this easily might be accomplished.

References

Diez-Sales, O., Copovi, A., Casabo, V.G. and Herraez, M., A modelistic approach showing the importance of the stagnant aqueous layers in in vitro diffusion studies, and in vitro-in vivo correlations. *Int. J. Pharm.*, 77 (1991) 1–11.

Lovering, E.G. and Black, D.B., Diffusion layers effects on permeation of phenylbutazone through polydimethylsiloxane. *J. Pharm. Sci*, 63 (1974) 1399–1402.

Retgers, P.L. and Peppas, N.A., A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of spheres, cylinders, or discs. *J. Controlled Release*, 5 (1987) 23–26.

Roseman, T.J. and Higuchi, W.I., Release of medroxyprogesterone acetate from a silicone polymer. *J. Pharm. Sci.*, 59 (1970) 353–357.

Seegers, W.H., *Blood Clotting Enzymology*, Academic Press, New York, 1967, pp. 200–207; 487–549.

Senderoff, R.I., Sheu, M.T. and Sokoloski, T.D., Fibrin based drug delivery systems. *J. Parenter. Sci.*, 45 (1991) 1–6.

Stehle, R.G. and Higuchi, W.I., In vitro model for transport of solutes in three-phase system. II: Experimental considerations. *J. Pharm. Sci.*, 61 (1972) 1931–1935.

Tojo, K., Ghannam, M., Sun, Y. and Chien, Y.W., In vitro apparatus for controlled release studies and intrinsic rate of permeation. *J. Controlled Release*, 1 (1985) 197–203.