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Diffusional properties of zein membranes and matrices

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Abstract

Introduction: Zein is a hydrophobic corn protein, rich in leucine, proline, and alanine, that has been investigated as an excipient in pharmaceutical formulations, maybe used as a biodegradable/biocompatible material for controlled release formulations. Zein's swelling behaviors under different conditions (e.g., pH, ionic strength, and permeant's charge) were investigated in a previous study and it showed that swelling indeed influenced zein's permeation. *Methods:* The diffusional behavior of model drugs through or from zein systems was investigated. Diffusional parameters such as effective diffusion coefficients, porosities, and tortuosities were calculated from release and membrane diffusion profiles for zein matrices and membranes. In addition, an alternative method of calculating porosity and tortuosity was proposed. *Results:* The diffusional properties of zein systems appear to be primarily through aqueous channels that form during hydration and swelling in aqueous media. Permeation and release results show that the amount of diffused or released drug depends on permeant solubility and matrix/membrane permeability parameters (i.e., porosity and tortuosity). *Discussion:* Calculated diffusional parameters for zein matrices and membranes support the aqueous channel model. *Conclusions:* Zein membranes and matrices hydrate and swell to form aqueous channels that are the dominant diffusional pathways.

Key words: *Diffusion; matrix release; membrane permeability; zein*

Introduction

Zein is a prolamine protein of molecular weight of 38,000 obtained from corn (*Zea mays*)[1,2]. It is characterized by its ready solubility in aqueous alcohol solutions, its insolubility in water, and its hydrolytic stability to dilute acids and alkalis. The nonpolar nature of zein results from its amino acid composition that includes an abundance of hydrophobic and uncharged amino acids (i.e., leucine, proline, and alanine) and relative lack of charged residues[3]. It possesses the following characteristics that may be of utility in oral drug delivery systems: (a) insoluble in aqueous media, (b) degradable by proteases, (c) hydrophobic, (d) mucoadhesive, (e) GRAS (generally recognized as safe) listed, (f) and a compendial substance (United States Pharmacopoeia)[4].

There are four types of zeins (α, β, γ, and δ) with the most common being, α-zein. A structural model for α-zein has been proposed by Argos and coworkers based on its amino acid sequence that accounts for its unusual solution properties compared to the more usual water-soluble proteins[5]. The film-forming properties of zein have been of interest for food and commercial applications because of environmental concern about the use of nonbiodegradable plastics[6]. Interest in development of edible and biodegradable films has driven research on new formulation methods for zein films[7,8,9]. Films prepared from zein are known to be tough, but also hard and brittle, thus requiring plasticizers, such as fatty acids, to improve flexibility[10–12]. In addition, zein films function as oxygen, lipid, and moisture barriers[13,14,15]. Some investigations have focused on improving its water resistance[16] and the relationship between fatty acid binding and its antioxidative effects[17,18].

Zein is used primarily as a tablet binder in wet granulation processes or as a tablet-coating agent[19]. There are

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few reports of zein's use for drug formulation or the preparation of sustained or controlled release formulations. Solutions of zein and drug have been co-spray dried and the resulting granules compressed to produce sustained release tablets[20]. Also, an aqueous zein pseudolatex for film coating was developed and investigated to study the relationship between dissolution medium pH and zein coating permeability[21,22]. A number of patents report zein microspheres prepared by various methods[23–26]. None of these studies have investigated the release mechanisms for zein formulations. In our previous study, we have investigated zein membrane's swelling behavior in various conditions such as ionic strengths and permeants' charge and reported that these conditions altered the swelling behavior[27]. Using methyl *p*-aminobenzoate as a permeant, we found that swelling significantly affects the release profiles of the permeant.

Two general mechanisms for drug diffusion through a membrane are recognized. The first is the partition mechanism in which solute permeation occurs by dissolution of solute into the membrane material itself. The second is through aqueous channels that exist in the membrane[28,29]. Our previous finding also supports that permeants diffuse through a zein membrane via aqueous channels because the higher swollen membrane showed the higher permeability because of providing more aqueous channels for permeants[27]. To investigate the relationship of permeant solubility/partition through diffusional characteristics of zein, alkyl *p*-aminobenzoate esters were used as a homologous series of permeants and expected to elucidate the diffusion mechanism in zein systems. The solute transport rate through polymer membranes may vary depending upon the physicochemical properties of the membrane and permeant. The contribution of these two mechanisms to the overall transport through a zein membrane was investigated. These same permeants were used for the membrane permeability studies for zein matrix release studies as well.

Our ultimate goal was to determine whether the same permeability parameters (i.e., diffusion coefficient, partition coefficient, porosity) apply to zein membrane permeation and matrix release. These studies will assist in determining the general utility of this proteinaceous material for controlled release formulations.

Materials and methods

Materials

Purified α-zein was purchased from CBC America Co. (New York, NY, USA). Methyl *p*-aminobenzoate was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). The ethyl and butyl *p*-aminobenzoate esters were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Propyl *p*-aminobenzoate was obtained from Pfaltz & Bauer (Waterbury, CT, USA). Hexyl *p*-aminobenzoate was synthesized previously[30].

Analytical procedures

All analyses for alkyl *p*-aminobenzoate esters were conducted by UV spectroscopy on a Hewlett Packard 8450A diode array spectrophotometer (Palo Alto, CA, USA) at 285 nm.

Solubility determination

Aliquots (5 mL) of water were added to screw-cap scintillation vials and ~5 mg increments of a particular alkyl *p*-aminobenzoate were added to each vial until no further compound dissolved, when visually monitored by the naked eye. The solutions were equilibrated at 37°C in an incubator shaker for 72 hours. Excess solute was removed by filtration through a 0.45-μm filter (Nylon Acrodisc® 13; Gelman, Ann Arbor, MI, USA). Saturated solution concentrations were measured by UV spectroscopy after appropriate dilution in water.

Zein membrane fabrication and diffusion studies

Zein (1 g) was dissolved in 10 mL of an 80% (v/v) ethanol/water solution. This zein solution (~8 mL) was poured into polymethylpentene Petri dishes (10 × 15 mm). Cast films were obtained by drying at 50°C for 8 hours and storing in a vacuum desiccator for 24 hours to obtain transparent films having an average thickness of 130 μm (±10 μm). Dried membranes were cut into circular disks (1.6 cm dia.). Zein films were mounted in Side-by-Side® diffusion cells (Crown Glass Co., Fremont, MI, USA) with 8 mL of saturated aqueous solutions (C_s) of permeant (alkyl *p*-aminobenzoate esters) on the donor side and water (8 mL) on the receptor side at 37°C. The receptor solution was completely removed at particular time intervals, assayed for alkyl *p*-aminobenzoate content, and replaced by an equal volume of water (8 mL) to maintain sink conditions. Both donor and receiver solutions were well stirred with a magnetic stirrer.

Zein matrix fabrication and release studies

Zein powder and powdered alkyl *p*-aminobenzoate esters were mixed in a 4:1 weight ratio (zein:ester) and compressed with a stainless steel punch and die on a hydraulic press (Model P21; Pasadena Hydraulics, Inc., City of Industry, CA, USA) at 2000 lb (total force) for 15 seconds into flat cylindrical disks (1.3 cm (dia.) × 0.15 cm, 150 ± 1.5 mg). Disks containing various loadings (10–40%, w/w) of methyl *p*-aminobenzoate were also

prepared by the same procedure. All matrices were embedded in paraffin wax by coating the edge and one face to leave one surface exposed for drug release.

All release studies were conducted in triplicate by the USP paddle method[31] at 50 rpm in 500 mL of water (37°C) with a sinker attached to the matrix so that the matrix would remain at the bottom of the dissolution vessel. The matrix releasing surface faced upward toward the paddle. The paddle was placed at 25 ± 2 mm from the bottom of the vessel. Samples (5 mL) were removed at regular intervals and replaced with the same volume of water. Released alkyl *p*-aminobenzoate ester was measured by UV spectroscopy at 285 nm. The absorbance from a blank zein control matrix release study was deducted from all absorbance values to correct for background UV effects.

Liquid leaching matrix studies were conducted by a matrix from which the drug has been extracted that contained various loadings of methyl *p*-aminobenzoate ester (10–40%, w/w). These leached zein disks were then immersed in a saturated methyl *p*-aminobenzoate ester solution (2 mL) for 72 hours to fill the pores. The methyl *p*-aminobenzoate concentrations in the loading solution were measured frequently during this process until the solution concentration did not change and the last concentration was recorded. These reloaded disks were transferred from the loading solution, blotted with filter paper to remove excess solution, and used for release studies.

Sample preparation for scanning electron microscopy

Each dry or wet film and each matrix were cut into small pieces and placed directly on stubs with double-coated carbon-conductive tape (Ted Pella, Inc., Redding, CA, USA). These samples were dried in a vacuum desiccator (3 days), coated with gold in a sputter coater for 1 minute and stored in a vacuum desiccator for 1 day before observing in the scanning electron microscope. Visually no collapse of the membrane or matrix was observed upon drying. All scanning electron micrographs (SEMs) were taken with a Hitachi S-4000 SEM (Tokyo, Japan).

Results and discussion

Membrane permeability

When diffusion is confined to transport through a membrane and steady state $(\mathrm{d}C/\mathrm{d}t = -D(\mathrm{d}^2c/\mathrm{d}x^2) = 0)$ exists, the diffusion equation becomes

$$J = \frac{D(C_1^m - C_2^m)}{h} = \frac{DK(C_1 - C_2)}{h}, \qquad (1)$$

where, h = membrane thickness
A = membrane area
J = flux
D = diffusion coefficient
K = partition coefficient
C_1^m = membrane surface concentration of the permeants on the donor solution side
C_2^m = membrane surface concentration of the permeants on receiver solution side
K = partition coefficient
C_1 = donor solution concentration
C_2 = receiver solution concentration.

As $K = C_1^m/C_1 = C_2^m/C_2$, the flux can be described with bulk solute concentrations on either side of the membrane. It can be clearly seen from Equation (1) that the concentration gradient is time independent at steady state. The diffusion coefficient can be calculated from the steady-state flux using Equation (1).

The mechanism governing solute diffusion through polymer membranes varies depending upon the physicochemical properties of the membrane. Several mechanisms can contribute to the diffusion process in a given membrane. The nature of the diffusion mechanism can also be influenced by the permeant's physical properties and the solvent. Solutes come in a wide range of sizes and degrees of hydrophobicity. However, two general mechanisms for drug diffusion through a membrane are the partition mechanism in which solute permeation occurs by dissolution of solute into the membrane material itself and aqueous channels that exist in the membrane[28].

One-dimensional steady-state flux of a solute through a membrane with contributions due to both mechanisms, partitioning and pores, is given by

$$J = \frac{\mathrm{d}Q}{A\mathrm{d}t} = \frac{D_{\mathrm{aq}}\,\varepsilon/\tau(C_1 - C_2)}{h} + \frac{D_m(1-\varepsilon)K(C_1 - C_2)}{h}, \qquad (2)$$

where, $\mathrm{d}Q/\mathrm{d}t$ = diffusion rate
A = membrane area
J = flux
D_{aq} = aqueous diffusion coefficient
D_m = diffusion coefficient in zein membrane
ε = porosity
τ = tortuosity
K = partition coefficient
h = membrane thickness
C_1 = donor solution concentration (mg/cm^3)
C_2 = receiver solution concentration (mg/cm^3).

The first term of Equation (2) $((D_{\mathrm{aq}}\,\varepsilon/\tau(C_1 - C_2))/h)$ represents pore diffusion and the second term $((D_m(1-\varepsilon)K(C_1 - C_2))/h)$ represents permeation through

the membrane material (i.e., zein). When the aqueous channel mechanism is dominant, partitioning mechanism only contributes a negligible amount to the total permeability. Therefore, the second term, partitioning mechanism, can be ignored in Equation (2). The above general equation is widely used for diffusional analysis as long as the condition, $(C_1–C_2)$ = constant, holds over a diffusion run. Here $C_1 = C_s$ (drug solubility in the release medium (mg/cm^3)) and $C_2 = 0$ (fresh medium replacement) or very low comparing to the C_s (the diffused amount is very limited between the sampling periods).

In Figure 1, diffusion profiles for alkyl *p*-aminobenzoate esters through zein membranes are shown. The steady-state permeation parameters are listed in Table 1. The rate of diffusion increased as benzoate alkyl chain length decreased, suggesting a solubility dependence on permeation rate as the donor solution was saturated with the ester (Figure 2). This result implies that (1) the contribution of partitioning mechanism to the permeation rate (dQ/dt) is negligible to the contribution of aqueous channel mechanism and (2) the partitioning did not play a role in esters diffusing through zein membrane. If

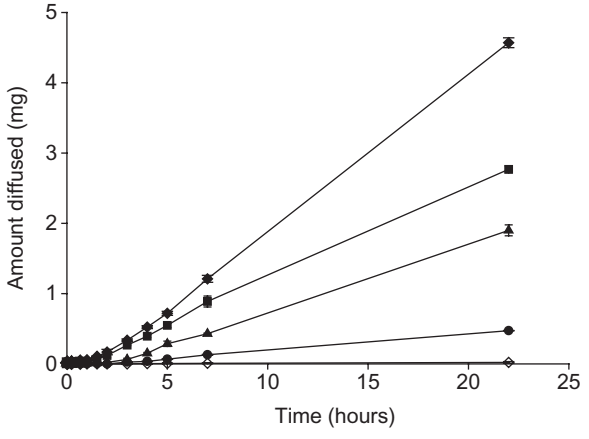

Figure 1. Diffusion profiles of alkyl *p*-aminobenzoate esters through zein membranes in water at 37°C. Key: ◆, methyl; ■, ethyl; ▲, propyl; ●, butyl; ◇, hexyl.

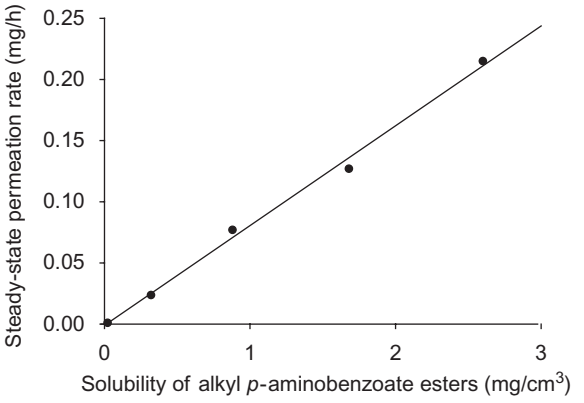

Figure 2. Solubility dependency of permeation rate for alkyl *p*-aminobenzoate esters through zein membranes.

it did, a random releasing-rate-order with increasing alkyl chain length is expected as the partition coefficients increase as benzoate alkyl chain length increases, which will result in a random order after multiplying with solubility, showing a opposite tendency as the chain length increases[32]. In either situation, it is believed that the esters diffused through zein membrane via aqueous channels.

All permeation profiles show similar lag times (t_L). For example, methyl *p*-aminobenzoate has a lag time of ~1–2 hours, which includes the time to form aqueous channels as zein hydrates and swells. For diffusion in a homogeneous polymer membrane, diffusion coefficients can be calculated using the t_L obtained from extrapolation of the steady-state permeation data to the time axis. However, since swelling, which forms aqueous channels, is a factor in this apparent lag time, the calculation of diffusion coefficients from lag times would have little meaning for zein membranes.

Table 1 tabulates the steady-state permeation rates, solubilities, and diffusion coefficients for the alkyl *p*-aminobenzoate esters along with calculated permeation parameters (D_{eff} and τ/ε) through a zein

Table 1. Steady-state membrane permeation parameters for alkyl *p*-aminobenzoate esters through zein membranes in water at 37°C.

Alkyl group	Permeation rate (mg/h)	Solubility[a] (mg/cm^3)	D_{aq}[b] $10^6 \times$ (cm^2/s)	D_{eff}[c] $\times 10^7$ (cm^2/s)	D_{eff}[d] $\times 10^7$ (cm^2/s)	t/ε[e]	t/ε[f]
Methyl	0.219	2.60 ± 0.13	7.0	1.51	2.90	45.2	24.2
Ethyl	0.129	1.68 ± 0.14	6.4	1.41	2.64	45.4	24.3
Propyl[g]	0.072	0.74 ± 0.06	6.2	1.37	2.56	42.7	24.2
Butyl	0.024	0.33 ± 0.01	6.0	1.33	2.49	45.1	24.3
Hexyl	0.0016	0.022 ± 0.002	5.7	1.28	2.40	44.5	23.8

[a]$N = 3$. [b]D_{aq} = aqueous diffusion coefficient[2,7]. [c]D_{eff} = effective membrane diffusion coefficient with dry membrane thickness (~130 mm). [d]D_{eff} = effective membrane diffusion coefficient with wet membrane thickness (~250 mm). [e]$t/\varepsilon = D_{aq}/D_{eff}$ with dry membrane thickness. [f]$t/\varepsilon = D_{aq}/D_{eff}$ with wet membrane thickness = 1.87 × dry thickness. [g]For membrane area = 2.55 cm^2 (disk diameter = 1.8 cm) only for this permeant.

membrane. The aqueous diffusion coefficients (D_{aq}) are literature values[33,34]. The calculated parameters (D_{eff} and τ/ε, $D_{eff:}$ effective membrane diffusion coefficient, $\tau/\varepsilon = D_{aq}/D_{eff}$) were obtained using Equation (2) (assuming aqueous channel mechanism is dominant). Thus, τ/ε values of ~45 using the dry membrane thickness or ~25 using the wet membrane thickness are obtained. The same wet membrane τ/ε values can be obtained from the slope (0.82 cm^3/h) of the line in Figure 2, where the steady-state permeation rate is plotted versus alkyl *p*-aminobenzoate solubility. For this plot, the slope is $D_{aq}\varepsilon A/\tau h$ for which we use D_{aq}~7×10^{-6} cm^2/s, A = 2.01 cm^2, and h = 0.025 cm (wet thickness) and obtain the same τ/ε estimate of ~25. As zein SEM did not show highly porous structure in later work, ε may be assumed to be less than 0.5, then τ would be ~2.5–12.5. Thus, we conclude that zein membrane permeation is under pore diffusion control, which are moderately tortuous pores.

Other investigations have observed permeation through a zein membrane. O'Donnell et al. studied acetaminophen release from a tablet coated with a zein pseudolatex[21]. The release rate was higher in 0.1 N HCl and decreased with increasing pH. Higher permeation at low pH may be due to protonation of acetaminophen making it more water-soluble. Increasing the zein coating thickness decreased the acetaminophen release rate. It was not clear whether drug was released through pores forming in the zein coating or whether acetaminophen diffused through the zein phase. Based on our work, pore formation is quite likely in such coated formulations.

Matrix release

Solid matrix (Higuchi) and liquid leaching matrix (Desai) studies

Higuchi proposed that the rate of drug release from a planar surface of an insoluble matrix containing drug in excess of its solubility is governed by the following relationship[35]:

$$Q = \sqrt{2D_{aq}(\varepsilon/\tau)C_s W t}, \qquad (3)$$

where Q = drug release per unit area at time t (mg/cm^2)
t = time (s)
D_{aq} = aqueous diffusion coefficient (cm^2/s)
ε = matrix porosity
C_s = drug solubility in the release medium (mg/cm^3)
τ = matrix tortuosity
W = drug loading (mg/cm^3).

This equation predicts a linear relationship between Q and $t^{1/2}$ with the slope being $(2D_{aq}(\varepsilon/\tau)\ C_s W)^{1/2}$. Desai et al.[36–38] used Equation (4) for leached porous matrices reloaded with a solution at a concentration, C_0:

$$Q = 2C_0\varepsilon\left(\frac{D_{aq}t}{\tau\pi}\right)^{1/2}, \qquad (4)$$

where C_0 = reloaded solution concentration after solid leaching matrix study (mg/cm^3). This equation predicts linear Q versus $t^{1/2}$ profiles with a slope of $2C_0\varepsilon$ $(D_{aq}/\pi\tau)^{1/2}$ for ~60% of the release profile[36].

Solid matrix release studies with zein matrices (Equation 3)

Figure 3 shows the release profiles (Q versus $t^{1/2}$) for alkyl *p*-aminobenzoates from compressed zein disks loaded with excess solid ester at a 4:1 weight ratio (i.e., 20%, w/w, loading). The slopes of release profiles ($dQ/dt^{1/2}$) increased as benzoate alkyl chain length decreased, suggesting a solubility dependence for release rate. The linear release profiles (Q versus $t^{1/2}$) allowed the calculation of matrix diffusion parameters. Table 2 gives the diffusional parameters for alkyl *p*-aminobenzoate ester release from these zein matrices with Equation (2). Effective diffusion coefficients (i.e., $D_{aq}(\varepsilon/t)$) and tortuosity/porosity ratios (τ/ε) for each alkyl *p*-aminobenzoate were calculated from the Q versus $t^{1/2}$ slopes. Values of τ/ε (3–4 for dry matrix or 4–5 for wet matrix) for zein matrices for different esters are similar, indicating that these values are matrix-dependent despite benzoate alkyl chain length and solubility differences for the esters used.

Figure 4 shows the release profiles (Q versus $t^{1/2}$) for methyl *p*-aminobenzoate from compressed zein disks

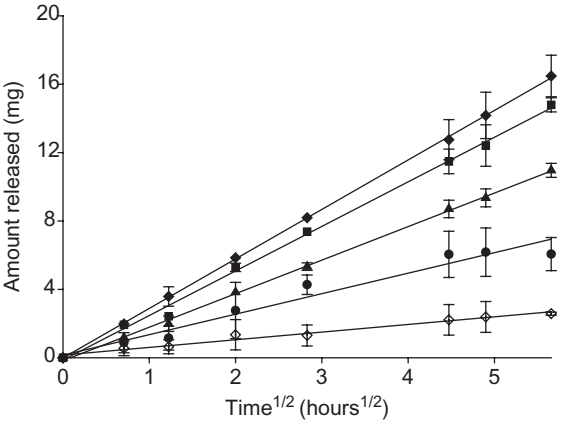

Figure 3. Release profiles of alkyl *p*-aminobenzoate esters from compressed zein disks (20%, w/w) into water at 37°C. Key: ◆, methyl; ■, ethyl; ▲, propyl; ●, butyl; ◇, hexyl.

Table 2. Steady-state matrix release parameters for solid alkyl *p*-aminobenzoate ester release from zein matrices in water at 37°C (20%, w/w, loading).

Alkyl group	Slope (mg/s$^{1/2}$)	Initial loading[a] (mg/cm^3)	D_{eff}[b] $\times 10^6$ (cm^2/s)	D_{eff}[c] $\times 10^6$ (cm^2/s)	D_{aq}[d] $\times 10^6$ (cm^2/s)	t/ε[e]	t/ε[f]
Methyl	0.050	151.4	1.80	1.36	7.0	3.9	5.1
Ethyl	0.042	151.8	1.96	1.48	6.4	3.6	4.8
Propyl	0.031	160.3	2.32	1.75	6.2	3.1	4.5
Butyl	0.019	154.2	2.07	1.56	6.0	3.4	4.0
Hexyl	0.0053	159.0	2.27	1.72	5.7	3.1	4.1

$N = 3$. [a]Initial loading = amount of drug in matrix/matrix volume (mg/cm^3). [b]D_{eff} = effective membrane diffusion coefficient with dry matrix area. [c]D_{eff} = effective membrane diffusion coefficient with wet matrix area. [d]D_{aq} = aqueous diffusion coefficient[2,7]. [e]$t/\varepsilon = D_{aq}/D_{eff}$ for dry matrix with an area of 1.33 cm^2. [f]$t/\varepsilon = D_{aq}/D_{eff}$ with wet matrix with an area of 1.53 cm^2.

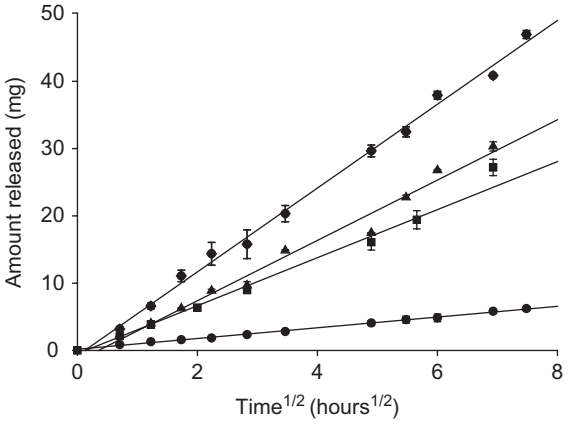

Figure 4. Release profiles of methyl *p*-aminobenzoate ester from compressed zein disks with various loadings (% w/w) in water at 37°C. Key: ●, 10%; ■, 20%; ▲, 30%; ◆, 40%.

at different loadings (10–40%, w/w). The release profiles show that the release rate ($dQ/dt^{1/2}$) increased with loading. Table 3 gives the diffusional parameters for methyl *p*-aminobenzoate from zein matrices of various loading. Effective diffusion coefficients (i.e., $D_{aq}(\varepsilon/t)$) and tortuosity/porosity ratios (τ/ε) for each matrix were calculated. Effective diffusion coefficients and tortuosity/porosity ratios (τ/ε) are dependent on loading as expected with D_{eff} increasing and τ/ε decreasing with loading. From these results (Tables 2 and 3), it was confirmed that D_{eff} of zein matrices are constant despite permeant's chain length and only proportional to drug loading %, suggesting zein system's diffusional mechanism does not depend on permeant's properties (e.g., partitioning) but aqueous channels. Diffusion through zein occurred mainly through aqueous channels formed by swelling. Therefore, it can be concluded that release from zein matrices is by a porous channel mechanism.

Liquid leaching matrix studies using previously used porous zein matrices (Equation 4)

Equation (4) gives linear Q versus $t^{1/2}$ profiles with a slope of $2\varepsilon C_0 \cdot (D_{aq}/\tau\pi)^{1/2}$ for ~60% of release profiles. They reported that release rates from plastic matrices were significantly changed when (a) different plastics were used for the matrix, (b) the amount of drug in the matrix was varied, (c) drug solubility was changed, (d) soluble additives were incorporated, and (e) different solvents were used[36–38]. Analysis of release results showed that the above factors not only altered release rate directly as predicted by the Higuchi equation, but also indirectly by altering apparent porosities and

Table 3. Steady-state matrix release parameters for various loadings of solid methyl *p*-aminobenzoate from zein matrices in water at 37°C.

Loading % (w/w)	Slope (mg/s$^{1/2}$)	Initial loading[a] (mg/cm^3)	D_{eff}[b] $\times 10^6$ (cm^2/s)	D_{eff}[c] $\times 10^6$ (cm^2/s)	D_{aq}[d] $\times 10^6$(cm^2/s)	t/ε[e]	t/ε[f]
10	0.021	75.5	0.64	0.48	7.0	11.0	14.5
20	0.050	151.4	1.80	1.36	7.0	3.9	5.1
30	0.063	220.1	1.97	1.49	7.0	3.2	4.7
40	0.102	273.4	4.16	3.14	7.0	1.7	2.2

$N = 3$. [a]Initial loading = amount of drug in matrix/matrix volume (mg/cm^3). [b]D_{eff} = effective membrane diffusion coefficient with dry matrix area. [c]D_{eff} = effective membrane diffusion coefficient with wet matrix area. [d]D_{aq} = aqueous diffusion coefficient[2,7]. [e]$t/\varepsilon = D_{aq}/D_{eff}$ for dry matrix with an area of 1.33 cm^2. [f]$t/\varepsilon = D_{aq}/D_{eff}$ with wet matrix with an area of 1.53 cm^2.

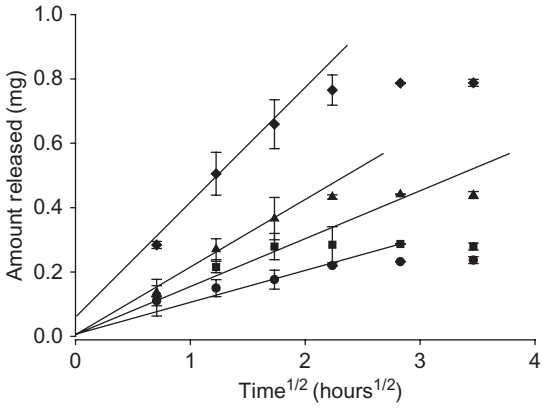

Figure 5. Release profiles of methyl *p*-aminobenzoate esters from compressed zein disks reloaded with a saturated solution into water at 37°C. Key: ●, 10%; ■, 20%; ▲, 30%; ◆, 40%.

tortuosities. Therefore, this equation requires obtaining porosity and tortuosity values if Equation (3) was to be applied quantitatively.

Further matrix release studies were conducted with leached zein matrices, which were reloaded with a saturated solution (2.6 mg/cm^3) of methyl *p*-aminobenzoate (Figure 5). The release from liquid reloaded matrices was 10-fold less than with the solid-loaded matrices. Only a few early points (3–4) in Figure 5 plots were used to calculate permeability parameters using Equation (4). Table 4 gives release parameters from the reloaded matrices. Using Equation (3), we obtained values of τ/ε^2 from the slopes of Q versus $t^{1/2}$ plots (Table 4).

Alternative calculation method of permeability parameters using combined two studies

Although Higuch and Desai's experiments have been performed individually, these two methods can be used together to calculate permeability parameters, ε, of zein matrices.

Rearranging Equations (3) and (4) to Equation. (5) and (6) gives expressions τ/ε and τ/ε^2, respectively:

$$\frac{\tau}{\varepsilon} = \frac{(D_{aq})_1 \cdot 2W \cdot C_s \cdot A^2}{(\text{slope}_1)^2} \tag{5}$$

$$\frac{\tau}{\varepsilon^2} = \frac{4C_0^2 \cdot (D_{aq})_2 \cdot A^2}{\pi(\text{slope}_2)^2}. \tag{6}$$

Dividing Equation (5) by Equation (6) gives:

$$\begin{aligned}\varepsilon &= \frac{W \cdot C_s \cdot \pi \cdot (\text{slope}_2)^2 (D_{aq})_2}{2C_0^2 \cdot (\text{slope}_1)^2 (D_{aq})_1} \\ &= \frac{W \cdot C_s \cdot \pi}{2C_0^2}\left(\frac{\text{slope}_2}{\text{slope}_1}\right)^2\left(\frac{(D_{aq})_2}{(D_{aq})_1}\right).\end{aligned} \tag{7}$$

As $(D_{aq})_1 = (D_{aq})_2$ and $C_0 = C_s$, Equation (6) becomes

$$\varepsilon = \frac{W\pi}{2C_s}\left(\frac{\text{slope}_1}{\text{slope}_2}\right), \tag{8}$$

where slope_1 = slope of Q versus $t^{1/2}$ for a liquid leaching release profile;

slope_2 = slope of Q versus $t^{1/2}$ for solid matrix release profile.

Thus, Equations (7) and (8) represent an alternative method for calculating matrix porosity. To evaluate Equations (7) or (8) compared to the typical ε calculation (Q_{tot}/C_0), the data of Desai et al.[37] were used. For 20% loaded sulfanilamide, the calculation is shown below:

$(D_{aq})_{sulf} = 1.29 \times 10^{-5}$ cm^2/s
$C_s = 10.8$ mg/mL
$W = 182$ mg/mL

Table 4. Steady-state matrix release parameters for methyl *p*-aminobenzoate release from various zein matrices reloaded with a saturated solution of methyl *p*-aminobenzoate in water at 37°C: loading concentration of methyl *p*-aminobenzoate (C_0) = 2.6 mg/cm^3.

Initial loading (% w/w)	Slope[a] (mg/h$^{1/2}$)	Q_{tot}[b] (mg)	Actual C_0 (mg/mL)	V_{pore} (cm^3)	ε[c] from Q_{tot}/C_o	t/ε^{2d}	t/ε^{2e}
10	0.07	0.26	2.50	0.104	0.26	72.4	95.9
20	0.16	0.28	2.48	0.113	0.28	13.6	18.1
30	0.21	0.44	2.39	0.184	0.46	7.4	9.7
40	0.37	0.78	2.24	0.348	0.87	2.1	2.8

[a]Initial three points were used to calculate these slopes. [b]Q_{tot} = total amount released (mg). [c]$\varepsilon = V_{pore}/V_{matrix}$ and $V_{pore} = Q_{tot}/C_o$, V_{matrix} = ~ 0.4 cm^3. [d]Equation (4) with dry matrix area (1.33 cm^2). [e]Equation (4) with wet matrix area (1.53 cm^2).

C_0 = 9.5 mg/mL
Slope$_1$ (from solid-loaded matrices) = 0.033 mg/s$^{1/2}$
Slope$_2$ (from liquid leaching) = 3.03×10^{-3} mg/s$^{1/2}$.

$$\varepsilon = \frac{W \cdot C_s \cdot \pi \cdot (\text{slope}_2)^2 (D_{aq})_2}{2C_o^2 \cdot (\text{slope}_1)^2 (D_{aq})_1}$$

$$= \frac{182 \times 10.8 \times \pi \times (3.03 \times 10^{-3})^2}{2 \times 9.5^2 \times 0.033^2} = 0.288$$

This value of ε gives τ = 13.3,[37] ε = 0.233, and τ = 9–11 for the same set of data that are in reasonable agreement. This comparison demostrated that this alternative method is applicable to obtain separated porosity and tortuosity.

In Table 5, porosity values were calculated by Equation (8) and hence these porosity values, τ, were obtained using Equations (5) or (6). Porosities increase with drug loading but tortuosities did not change significantly. This is expected because drug loading alters matrix porosity, which were produced after the drug is released from the matrix. In addition, these pores in the leached matrix zone are flexible enough to rearrange and can fill the void spaces, resulting in similar tortuosities; therefore, the matrix porosities depend upon the swelling characteristics of zein and the drug loading.

The advantage of using Equation (8) is that this combined equation can calculate the permeability propeties without swelling information and separate each parameter (e.g., porosity and tortuosity), which can give clear description after swelling and also be used to predict the permeability in any zein-based systems.

The typical way to evaluate matrix porosity is by dividing the total amount released (Q_{tot}) by C_0 (2.60 mg/cm^3) for the liquid leaching systems. The methyl *p*-aminobenzoate concentration in the loading solution was reduced during the matrix reloading process for liquid leaching studies, because water in the zein channels diffused out to dilute the resaturation solution. This dilution process lowered the loading solution concentration to less than saturation (C_s). So the more accurate value of C_0 is that measured at the end of the reloading process.

To confirm no adsorption of methyl *p*-aminobenzoate by zein membrane, a binding study was performed. Dry membrane (~36 mg) was placed into a methyl *p*-aminobenzoate solution (8.06 μg/mL) and no concentration change was observed. Therefore, reloading solution dilution occurs during reloading without adsorption effects. Using the measured C_0, after reloading, the porosity was calculated according to the next equations and all parameters were tabulated in Table 4:

$$V_{\text{pore}} = \frac{Q_{\text{tot}}}{C_o}$$

$$\varepsilon = \frac{V_{\text{pore}}}{V_{\text{matrix}}},$$

where V_{pore} = the pore volume in leached matrix;
V_{matrix} = the swollen matrix volume, ~0.4 cm^2 based on measurement.

This calculation gives similar porosity values to that obtained from altenative calculation with Equation (8) (i.e., ε~0.25–0.90).

To visualize the swollen zein membranes and matrices, SEM micrographs (Figures 6 and 7) of surface and cross sections for zein membranes and pure matrices (no drug loading) were taken before and after exposure to water. Before exposure to water, a zein membrane appears as a dense and fairly uniform material without pores even at high magnification. After exposure to water, the membrane becomes porous and exhibits distinct pores as a result of hydration and swelling. The zein matrix also shows minimal initial porosity that becomes much higher after swelling. At the same magnifications, the matrix appears more porous than the membrane, as expected. These SEMs support the proposal that swollen zein generates channels through which diffusion occurs.

Table 5. Calculated porosity and tortuosity values for zein matrices in water at 37°C.

Loading (% w/w)	ε[a]	t[b]	t[c]
10	0.15	1.7	2.2
20	0.29	1.1	1.5
30	0.48	1.7	2.3
40	0.81	1.4	1.8

[a]Calculated by Equation (7). [b]Equation (4) or (5) with dry matrix area (1.33 cm^2). [c]Equations (4) or (5) with wet matrix area (1.53 cm^2).

Conclusions

Zein membranes and matrices hydrate and swell to form aqueous channels that are the dominant diffusional pathways. Permeation and release results show that the amount of diffused or released material depends on permeant solubility and matrix/membrane permeability

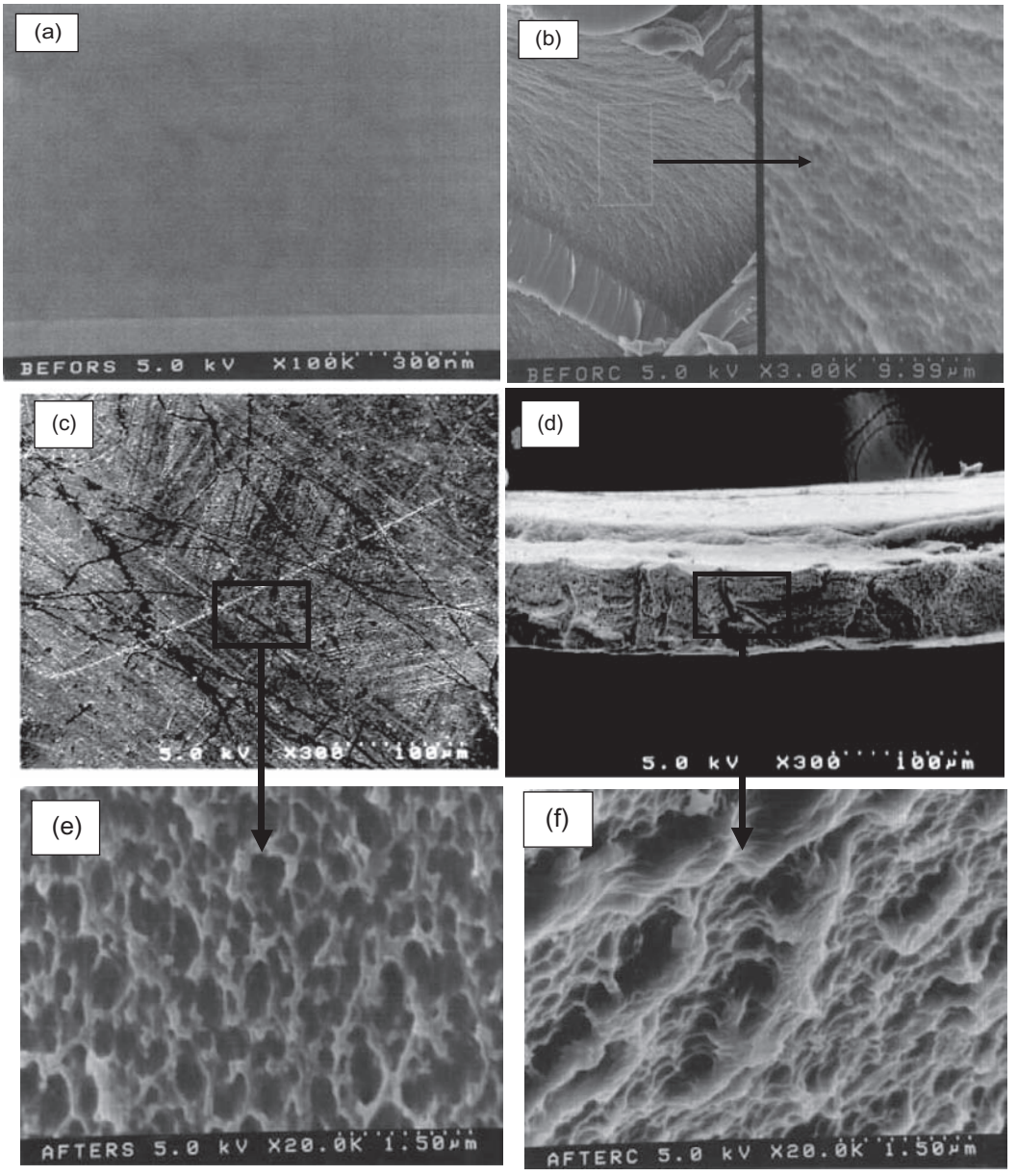

Figure 6. SEM micrographs of a zein membrane: (a) surface before swelling in water (100,000×); (b) cross section before swelling in water (3000×, 15,000×); (c) surface after swelling in water (300×); (d) cross section after swelling in water (300×); (e) surface after exposure to water (20,000×); (f) cross section after exposure to water (20,000×). Scales are in microns.

parameters (i.e., porosity and tortuosity). Porosities (ε) and tortuosities (τ) are 0.1–0.5 and 4–23 for membranes and 0.1–0.8 and 1.1–2.3 for matrices (pure and loaded), respectively. Matrix porosities increase with loading but tortuosities are not altered by loading. Therefore, loading affects the effective diffusion coefficient through the porosity. Porosity is also affected by swelling because the drug-leached void space in the zein matrix becomes partially filled with swollen zein. Additionally, we have shown that ε and τ can be calculated by combining solid matrix and liquid leaching models, which give values similar to those determined by conventional calculation methods. This alternative method provides for calculation of the permeability properties without swelling information and separate porosity and tortuosity. These porosity and tortuosity values can give a clear description about any system, even if the system is swelling.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

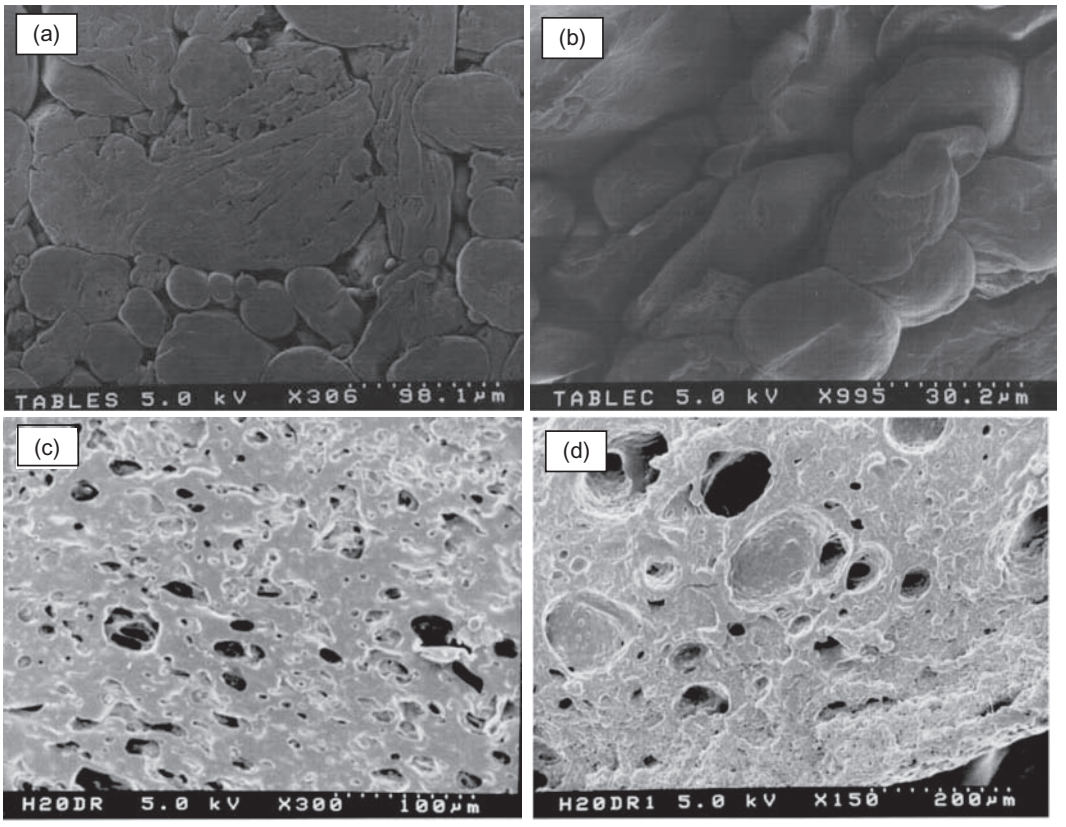

Figure 7. SEM micrographs of a zein pure matrix: (a) surface before swelling in water (306×); (b) cross section before swelling in water (995×); (c) surface after swelling in water (300×); (d) cross section after swelling in water (150×). Scales are in microns.

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