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Effect of FITC-dextran molecular weight on its release from floating cetyl alcohol and HPMC tablets

Guojie Xu and Michael J. Groves

Abstract

The release mechanism of high molecular weight fluorescein isothiocyanate dextrans (FITC-dextrans) from HPMC hydrogel matrices was studied.

An anomaly was noted in the release behaviour of a series of high molecular weight FITC-dextrans from a tablet formulation designed to float in stomach contents. The tablets contained sodium bicarbonate and hydroxypropylmethyl cellulose (HPMC) in a cetyl alcohol matrix. When hydrated in an acid medium, this tablet consisted of a mixed solid with a viscous surface layer containing carbon dioxide bubbles through which the active ingredient (FITC-dextran) was released into the aqueous environment. However, it was observed that, above a critical molecular weight (approx. 65 kDa), the FITC-dextran was only released into the medium by an erosion-type mechanism, whereas, below this value, both diffusion and erosion processes took place. The key constraint appeared to be the apparent gel pore-size of the hydrated HPMC that was approximately 12 nm in diameter, irrespective of the molecular weight of the HPMC samples evaluated.

It was concluded that FITC-dextran release was controlled by both FITC-dextran molecular weight and the HPMC hydrogel structure.

Introduction

Intragastric floating systems have been used pharmaceutically to deliver active compounds for sustained release and targeting (Zhu & Tu 1990; Yang et al 1999). This technique has recently been well reviewed by Fell et al (2000). Usually, it consists of two compartments. One is responsible for controlling drug release. Hydrogel polymers, such as hydroxypropylmethyl cellulose (HPMC) or alginates, are best for this purpose. Another compartment is for providing buoyancy to extend gastric retention; low-density additives (e.g. fatty acid and fatty alcohol) and gas-generating agents (such as bicarbonate) are suitable for this purpose. Baumgartner et al (2000) reported that a tablet of this type floated for over 8 h and resided in the stomach for over 4 h. The feasibility of taking a similar approach was considered to deliver high molecular weight antineoplastic glycans (Wang et al 1995). Unfortunately, these highly active immunostimulants obtained from attenuated *Mycobacterium bovis* (Bacillus Calmette Guérin (BCG) vaccine), PS1, (Wang et al 1995) and *Mycobacterium vaccae*, PS4, (Li et al 1997; Tian et al 1999) are currently only available in small quantities. Therefore, a homologous series of high molecular weight water-soluble fluorescein isothiocyanate (FITC) dextrans have been used initially for calibration purposes as they are readily available and easily assayed model compounds with molecular weights encompassing the range of PS1 and PS4 (60–2200 kDa).

Institute for Tuberculosis Research, College of Pharmacy (M/C964), University of Illinois at Chicago, 2201 W. Campbell Park Drive, Chicago, IL 60612, USA

Guojie Xu, Michael J. Groves

Correspondence: M. J. Groves, Institute for Tuberculosis Research, College of Pharmacy (M/C964), University of Illinois at Chicago, 2201 W. Campbell Park Drive, Chicago IL 60612, USA. Email: groves@uic.edu

A simple floating tablet formulation was devised consisting of cetyl alcohol, sodium bicarbonate and HPMC. The HPMC was initially used because it is reputedly a mucoadhesive (Langer & Peppas 1987), and should control the release of materials incorporated into the matrix. When immersed in an acidic aqueous medium the tablet was found to float, in part because of the low density of the cetyl alcohol (0.81 g cm^{-3}), and in part because of the small bubbles of carbon dioxide trapped in the swollen HPMC gel around the surface of the tablet. Evaluation demonstrated that incorporated FITC-dextrans were released into the medium surrounding the floating tablet, but an anomaly was also observed; samples with molecular weights greater than approximately 65 kDa were released at substantially similar release rates, whereas, samples with molecular weights less than 65 kDa were released at various rates. Accordingly, this anomaly was investigated in greater depth to determine the mechanism of drug release from the hydrogel matrix.

Materials and Methods

Hydroxypropylmethyl cellulose (HPMC; Methocel K4M, K15M and K100M) was received from Dow Chemical Company (Midland, MI). Sodium bicarbonate, sodium phosphate monobasic, sodium phosphate dibasic and sodium chloride were all from Fisher Scientific (Fair Lawn, NJ), and HCl (6 M) was from Labchem Inc. (Pittsburgh, PA). Cetyl alcohol, glucose and FITC-dextrans (FD-4, 10S, 20S, 40S, 70S, 150S, 250S, 500S and 2000S) were purchased from Sigma Chemical Co. (St Louis, MO).

Preparation of a floating tablet

Optimizing the buoyancy and drug release enabled a standardized formulation consisting of 22 mg HPMC, 98 mg cetyl alcohol, 15 mg sodium bicarbonate and 2.5 mg FITC-dextran to be established in the preliminary experiments. To obtain content uniformity, all ingredients were first milled and separately passed through a 120-mesh screen. Components equivalent to five tablets were put into a plastic bag and mixed manually by inverting the bag 50 times. The mixture was separated into five parts by accurately weighing and filled into five preheated hard plastic tubes (inner diam. 6.0 mm). These were then heated in an oven at 70°C until the cetyl alcohol had completely melted. After cooling under flowing nitrogen, the tablet was removed from the tube with a plastic rod. The ratio of height to diameter of tablet was 3:4. The melting and hardening were finished in less than 2 min. One batch of five tablets was used to test the weight uniformity and content uniformity. The weight of tablets ranged from 99.51 to 100.08 % of the labelled tablet. The contents of FITC-dextrans were from 98.47 to 101.04 % of labelled tablet. All the operations were carried out in subdued lighting given the highly light-sensitive properties of FITC-dextran. Tablets were stored in the dark overnight before further testing.

Release of FITC-dextran

A bath shaker was used to measure the release of FITC-dextran. Tablets were individually placed into 20-mL glass vials with 10 mL 0.1 M HCl (pH 1.2) and agitated at 50 oscillations min^{-1} in the bath shaker at 37°C. Samples (2 mL) were drawn at 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h respectively, and were replaced with the same amount of fresh medium. Samples were filtered using a 0.65-μm membrane (Durapore PVDF membrane; Millipore, Bedford, MA), and 0.5 mL of the prefiltrate was discarded to eliminate the effect of membrane absorption. All the operations were carried out in the dark. The concentration of FITC-dextran was determined spectrophotometrically at 452 nm (linear range: 50–500 g mL^{-1}). The removal of FITC-dextran in samples and the dilution by replacement of media were accounted for in the calculations. All experiments were carried out in triplicate.

Determination of the molecular weight of FITC-dextran and HPMC samples

An on-line light scattering system was used to measure the molecular weight of both the FITC-dextran and the HPMC samples. This system consisted of an HPLC pump (Waters 600 Multisystem; Waters, Milford, MA), with a gel permeation column (SEC 125 or 400 column; BioRad. Hercules, CA, or Ultrahydrogel Linear column; Waters, Milford, MA), a refractive index detector (Waters 410 differential Refractometer; Waters, Milford, MA), and a light-scattering detector (Mini Dawn; Wyatt Technology Corporation, Santa Barbara, CA). Phosphate buffer solution containing 0.05 M Na_2HPO_4, 0.05 M $NaH_2PO_4.H_2O$ and 0.15 M NaCl, pH 6.8, filtered with a 0.1-μm membrane filter (Nitro-

Table 1 Molecular weight measured using the light scattering technique (n = 3), overall mass transfer resistance (R_T), and apparent diffusion coefficient (D_p) measured using the diffusion cell method (n = 4) for FITC-dextrans and HPMC.

Sample	Labelled MW[a] (kDa)	Measured MW (kDa)	MW/Mn	R_T ($s\ cm^{-1} \times 10^{-4}$)	D_p ($cm^2\ s^{-1} \times 10^7$)
FD-4	4.4	5.7	1.27	3.82	6.89
FD-10S	12.0	12.5	1.19	4.93	4.90
FD-20S	19.5	16.5	1.35	6.33	3.58
FD-40S	42.0	45.3	1.34	9.04	2.36
FD-70S	77.0	72.3	1.36	11.04	1.89
FD-150S	148.0	176	1.84	20.67	0.96
FD-250S	260.0	282	2.50	22.89	0.86
FD-500S	464.0	444	1.75	31.63	0.61
FD-2000S	2000	2050	3.65	48.42	0.40
HPMC K4M	–	279	1.52	47.33	0.41
HPMC K15M	–	348	2.19	77.23	0.25
HPMC K100M	–	421	1.65	161.39	0.12

[a]Data from Sigma.

cellulose membrane; Millipore, Bedford, MA) was used as the mobile phase. The flow rate of the mobile phase was 0.5 mL min^{-1}. Data collection and analysis were performed with ASTRA for Windows 4.5 and RICAL for Windows 2.0 software (Wyatt Technology Corporation, Santa Barbara, CA). All analyses were in triplicate.

Diffusion coefficient determination

Diffusion experiments were performed using Valia-Chien side-by-side diffusion cells (Crown Glass Company, Somerville, NJ) in the dark. The membrane (Cellulose ester microporous membrane, 0.22 μm pore size, 80% porosity and 150 μm thickness; Micron Separations Incorporation, Westboro, MA) was clamped between the two identical chambers. The volume of each chamber was 3.8 mL, and the exposed membrane area 0.664 cm^2. The temperature was maintained at 37°C with circulating water. The stirring speed was 600 rev min^{-1}. The donor chamber was filled with 1 mg mL^{-1} FITC-dextran solution, and the receptor chamber with distilled water. Samples of 0.1 mL were drawn periodically for analysis from both donor and receptor chambers and replaced with 0.1 mL distilled water. Because the FITC-dextran concentration in the receptor chamber was too low to be detected spectrophotometrically at 452 nm, a more sensitive phenol–sulfuric acid method was used (Dubois et al 1956). In this method, 0.1 mL of the sample was added to 0.5 mL 5% phenol solution and 2.5 mL sulfuric acid, and the colour was measured spectrophotometrically at 480 nm after standing for 2 h at room temperature. The linear range for this method was 50–500 μg mL^{-1}. All the diffusion tests were carried out in quadruplicate.

Results and Discussion

Molecular weight of FITC-dextran and HPMC

The light scattering technique can be regarded as an absolute method for the characterization of molecular weight (Wyatt 1993). The refractive index increment, dn/dc, was required for accurately determining the polymer molecular weight using this method. The measured values of dn/dc in these experiments were 0.1564 for FITC-dextran and 0.1928 for HPMC. Measured molecular weights of standards were close to labelled values and are shown in Table 1.

Diffusion coefficient of FITC-dextran and HPMC

The concentration data from the diffusion experiment obeyed the following equation (Lebrun & Junter 1994):

$$\ln[C_0/C_0 - 2C_R] = 2At/VR_T \quad (1)$$

where C_0 is the initial solute concentration in the donor chamber of diffusion cell, C_R is the solute concentration in the receptor chamber of diffusion cell, A is the effective diffusion area, V is the chamber volume, and R_T is the overall mass transfer resistance.

$$R_T = R_m + 2R_b, \quad \text{and} \quad R_m = 1/\epsilon D_p \quad (2)$$

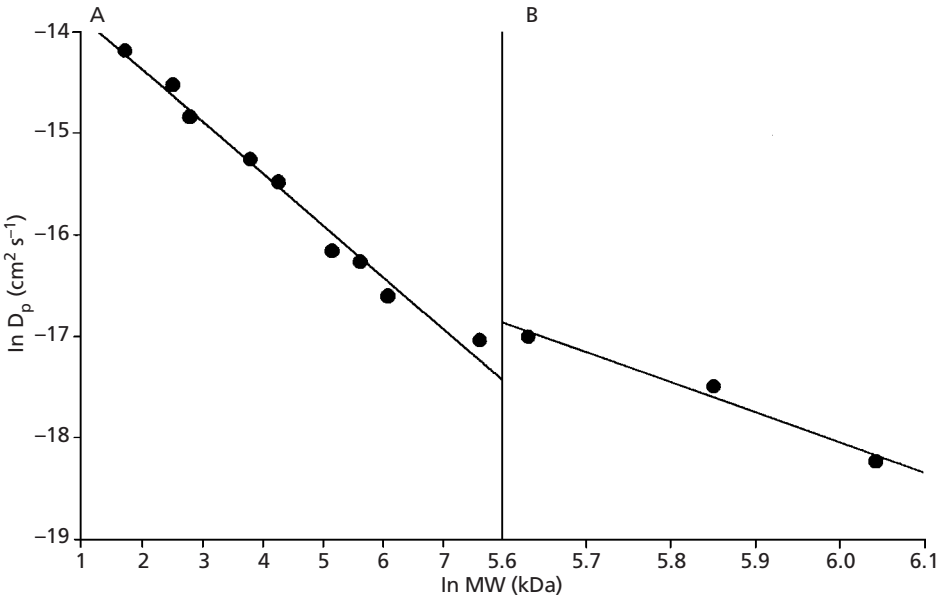

Figure 1 Relationship between molecular weight and diffusion coefficient of FITC-dextran (A) and HPMC (B).

where R_m is the membrane diffusion resistance, R_b is the boundary layer diffusion resistance on each side of membrane, l is the membrane thickness, ϵ is the membrane porosity, and D_p is the apparent diffusion coefficient of solute in the membrane pore.

Because R_b can often be significant and may not be neglected, 1 % glucose, for which the diffusion coefficient was known (Bohrer 1983), was used to measure R_b. The overall mass transfer resistance R_T was obtained from the slope of the line of $\ln[C_0/(C_0-2C_R)]$ vs time. The membrane diffusion resistance (R_m) for glucose could be calculated because the membrane parameters, chamber volume and glucose diffusion coefficient were all known. The calculated boundary resistance, $2R_b$, was 1.1×10^4 s cm^{-1} according to equation 2, in which the glucose diffusion coefficient in water, D_∞, was substituted for the diffusion coefficient in membrane pores.

The measured overall mass transfer resistances and diffusion coefficients of all FITC-dextrans and HPMC samples are also listed in Table 1.

Linearity was established when plots of molecular weight measured by light scattering vs diffusion coefficient were made on a logarithm scale for these two different polymers (Figure 1). This gave the relationship between diffusion coefficient and molecular weight as:

$$D_p = 5.53 \times 10^{-5}\ M_w^{-0.51} \qquad r^2 = 0.985 \text{ (FITC-dextran)} \quad (3)$$

$$D_p = 7.15 \times 10^{-5}\ M_w^{-2.98} \qquad r^2 = 0.977 \text{ (HPMC)} \quad (4)$$

FITC-dextran release

The dissolution profiles are shown in Figure 2A–C. Two points are worthy of comment here. Firstly, the matrix structure of the tablet was evidently maintained by the HPMC system, since a transparent surface layer was visible during dissolution. However, formulations with FITC-dextran molecules greater than 45 kDa showed a biphasic release pattern, that is, a slow release phase followed by a rapid release phase. For formulations with FITC-dextran molecules less than 45 kDa, the biphasic patterns were not always obvious because of the small amount of drug remaining in the second release stage. These biphasic release patterns were caused by an apparent change in the matrix structure. Over the first phase, hydrated HPMC remained at the surface of the solid core and the apparent gel structure did not change significantly. At this stage the release appeared to be due to the diffusion of FITC-dextran through the hydrated HPMC gel layer, occurring together with surface erosion of the matrix. Over the second phase, the solid core disappeared and no new HPMC gel was formed. As the HPMC swelled and disentangled, the density and strength of gel layer weakened, resulting in a rapid erosion. The molecular weight of HPMC evidently determined the length of time the drug could be sustained in the device. HPMC K4M, having the lowest molecular weight, provided the fastest drug release profile and the shortest controlled release period. The lengths of the sustained release phase for the three grades of HPMC were approximately 12–14 h, 16–18 h and 16–18 h, re-

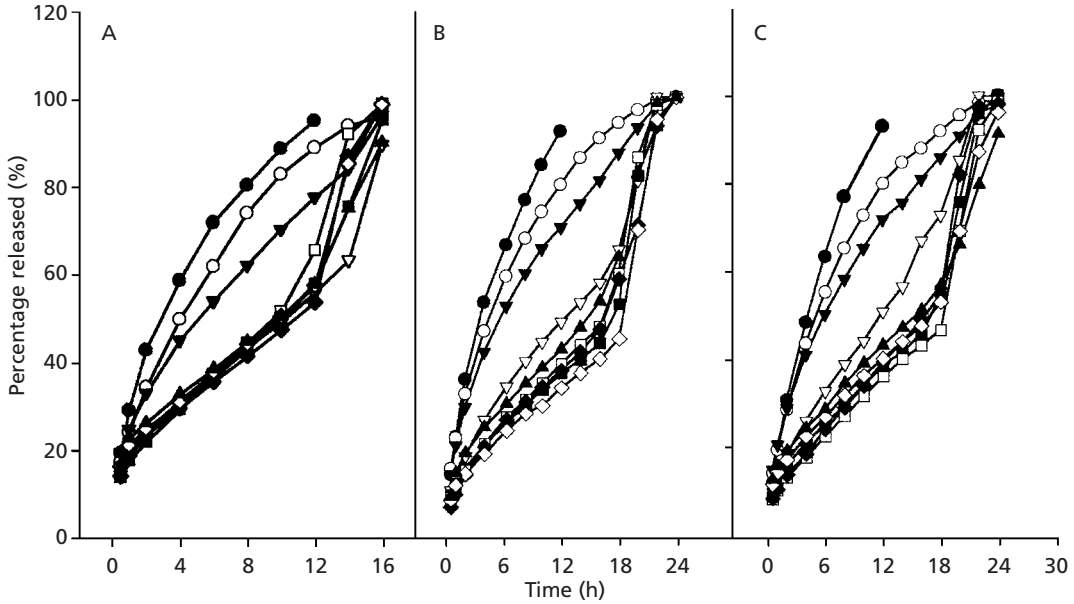

Figure 2 Percentage release vs time for FITC-dextran (●, 4S; ○, 10S; ▼, 20S; ▽, 40S; ■, 70S; □, 150S; ◆, 250S; ◇, 500S; ▲, 2000S). A. HPMC K4M tablets; B. HPMC K15M tablets; C. HPMC K100M tablets.

spectively. These times reflected the influence of HPMC molecular weight on the overall matrix erosion. Secondly, the drug release depended on its own molecular weight. During the controlled release phase, the release of FITC-dextran decreased to a limiting value with increasing molecular weight of FITC-dextran (Figure 2A–C).

Release profiles of FITC-dextran 4S, 10S, 20S, and of the first phases of all other FITC-dextrans, could be fitted using a square-root-of-time equation similar to the Higuchi equation (Higuchi 1962, 1963), although the latter was derived from different models.

$$W_t/W_\infty = k_1(t - t_{lag})^{\frac{1}{2}} \quad (5)$$

Where W_t/W_∞ is the fraction of drug released, t_{lag} is the lag time, and k_1 is the drug release rate constant. The $t_{100\%}$ could be calculated by extrapolating to complete release, and the overall release rate k could be obtained by using the following equation.

$$k = 100/(t_{100\%} - t_{lag}) \quad (6)$$

The relationship between the overall drug release rate, k, and overall drug release rate constant, k_1, is $k = k_1^2/100$.

The anomaly noted earlier is shown in Figure 3A–C in which overall release rates are plotted as a function of FITC-dextran molecular weight. Initially, the overall release rate was a direct function of molecular weight, but became independent at a critical molecular weight of 65.2, 66.5 and 64.1 kDa (mean 65.3 kDa), respectively, for the three HPMC grades. This abrupt change could be explained by referring to the gel structure surrounding the dry core of the tablet. Although it evidently forms the rate-controlling structure in the tablet, the details of the mechanism are presently unclear. Diffusion of an incorporated drug is generally thought to involve movement between hydrated polymer molecules or between cracks or imperfections in the gel layer. In the case of a water-swollen polyethylene glycol hydrogel, Iza et al (1988) concluded that diffusion depended on the porous structure of the hydrogel. Thus, the gel layer seemed to act effectively as a filter. It would therefore be anticipated that the pore size and porosity would depend on polymer molecular weight, concentration of polymer and other components in the formulation.

There was excellent linearity between the logarithm of the diffusion coefficient and the logarithm of the molecular weight over the range of FITC-dextran studied (Figure 1). This suggested that the divergence between overall release rate and molecular weight was not due to FITC-dextran molecules themselves but rather the porous structure of the HPMC gel formed around the tablet surface. This critical molecular weight could be used to calculate the mean limiting pore diameter of HPMC gels, assuming FITC-dextran to be rolled into a rigid sphere. The molecular diameter, D, is a function of molecular weight according to:

$$D = 0.0471M^{0.5} \quad (7)$$

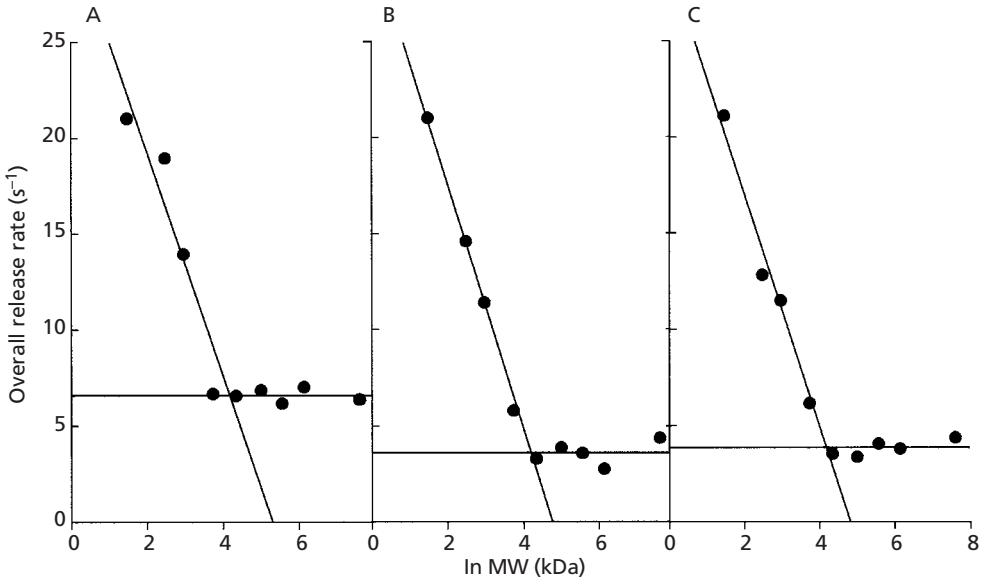

Figure 3 Relationship between overall release rate and FITC-dextran molecular weight. A. HPMC K4M tablets; B. HPMC K15M tablets; C. HPMC K100M tablets.

Table 2 Overall release rates and diffusion rates of FITC-dextrans, below the apparent critical molecular weight (65 kDa[a]), from floating tablets (n = 3).

Sample	Overall release rate ($\times 10^4$ s^{-1})			Diffusion release rate ($\times 10^4$ s^{-1})		
	HPMC K4M	HPMC K15M	HPMC K100M	HPMC K4M	HPMC K15M	HPMC K100M
FD-4	21.11 ± 1.08	20.28 ± 1.17	21.11 ± 0.97	6.03 ± 0.39	7.14 ± 0.69	7.31 ± 0.64
FD-10S	18.89 ± 2.86	14.72 ± 0.78	12.81 ± 0.31	3.42 ± 0.14	4.28 ± 0.50	4.08 ± 0.31
FD-20S	13.89 ± 0.33	11.39 ± 0.69	11.47 ± 0.75	1.22 ± 0.31	2.78 ± 0.89	2.50 ± 0.36
FD-40S	6.67 ± 0.19	5.83 ± 0.69	6.17 ± 1.47	0.00	0.28 ± 0.14	0.28 ± 0.14

[a]Above this molecular weight the mechanism was predominately by erosion release.

giving mean diameters of the pores in the gel layers of 12.0, 12.2 and 12.0 nm for HPMC K4M, K15M and K100M gels, respectively.

Thus, it appears that if the FITC-dextran sample was larger than this limiting size, the release rate was controlled by a matrix surface erosion process, quite independently of FITC-dextran molecular weight. The release rates for FITC-dextrans (FD-70S, 150S, 250S, 500S and 2000S), which are larger than the limiting size, were taken as erosion release rates. The matrix erosion release rates of FITC-dextrans estimated using these release data were $(6.58 \pm 0.33) \times 10^{-4}$, $(3.56 \pm 0.61) \times 10^{-4}$ and $(3.81 \pm 0.39) \times 10^{-4}$ s^{-1} for HPMC K4M, K15M and K100M, respectively. If the FITC-dextran sample was smaller than this limiting size, the release would be due to a combination of both diffusion through the gel rate-controlling layer and surface erosion. Subtracting the erosion release data from the overall release data provided a figure for the diffusion process. For a limited number of samples, the diffusion data could also be fitted by equation 5. Diffusion release rates were also calculated using equation 6. The relationship between overall release rate, erosion release rate and diffusion release rate is:

$$\sqrt{k_{overall}(t - t_{lag.overall})} = \sqrt{k_{erosion}(t - t_{lag.erosion})} + \sqrt{k_{diffusion}(t - t_{lag.diffusion})} \quad (8)$$

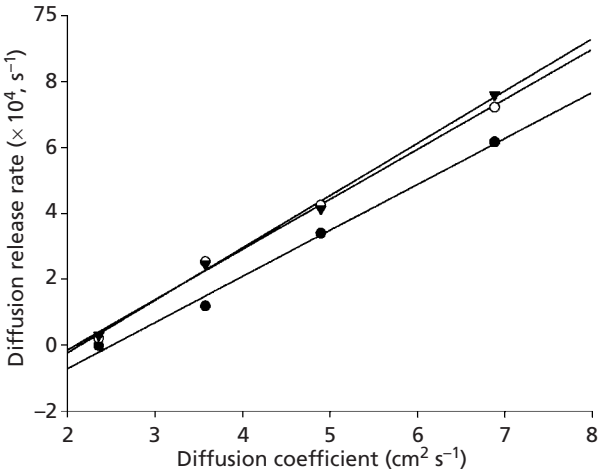

Figure 4 Relationship between the diffusion release rate of FITC-dextran from floating tablets and diffusion coefficient (D_p) of FITC-dextran in microporous membrane. ●, HPMC K4M tablets; ○, HPMC K15M tablets; ▼, HPMC K100M tablets.

where, $k_{overall}$, $k_{erosion}$ and $k_{diffusion}$ were overall release rate, erosion release rate and diffusion release rate, respectively, and $t_{lag.overall}$, $t_{lag.erosion}$ and $t_{lag.diffusion}$ were overall lag time, erosion lag time and diffusion lag time, respectively. When all the lag times were small:

$$\sqrt{k_{overall}} \approx \sqrt{k_{erosion}} + \sqrt{k_{diffusion}} \quad (9)$$

The overall release rates and diffusion release rates are shown in Table 2. Linearity between the diffusion release rates and the diffusion coefficients of the FITC-dextrans in membrane pores could be demonstrated (Figure 4).

Conclusions

The release mechanism of high molecular weight FITC-dextrans from HPMC hydrogel matrices was studied. It was found that FITC-dextran release was controlled by both FITC-dextran molecular weight and HPMC hydrogel structure. HPMC gel behaved as a filter with the pore size of 12 nm irrespective of its molecular weight. The diffusion of FITC-dextran was dependent on the molecular weight. If the molecular size of FITC-dextran was greater than the gel pore size, its diffusion through HPMC gel was too little to be noticed, and in this case, erosion was the prominent release mechanism. If the FITC-dextran molecule was less than the pore size, both erosion and diffusion contributed to the overall FITC-dextran release from HPMC matrix.

Appendix

Derivation of equations 8 and 9:

Express the total drug release, diffusion release and erosion release in the Higuchi equation as:

$$\begin{aligned} W_t/W_\infty &= k_{lag.overall}(t - t_{lag.overall})^{\frac{1}{2}} \\ &= k_{lag.erosion}(t - t_{lag.erosion})^{\frac{1}{2}} \\ &\quad + k_{lag.diffusion}(t - t_{lag.diffusion})^{\frac{1}{2}} \end{aligned} \quad (10)$$

where $k_{lag.overall}$, $k_{lag.erosion}$ and $k_{lag.diffusion}$ are the total drug release rate constant, diffusion release rate constant and erosion release rate constant.

Substituting $k_{overall} = k^2_{lag.overall}/100$, $k_{erosion} = k^2_{lag.erosion}/100$, $k_{diffusion} = k^2_{lag.diffusion}/100$ into equation (10), gives:

$$\begin{aligned} &\sqrt{k_{overall}(t - t_{lag.overall})} \\ &= \sqrt{k_{erosion}(t - t_{lag.erosion})} \\ &\quad + \sqrt{k_{diffusion}(t - t_{lag.diffusion})} \end{aligned} \quad (8)$$

If all the lag times are small, i.e., $t_{lag.overall} \approx 0$, $t_{lag.erosion} \approx 0$ and $t_{lag.diffusion} \approx 0$, equation 8 can be simplified as:

$$\sqrt{k_{overall}} \approx \sqrt{k_{erosion}} + \sqrt{k_{diffusion}} \quad (9)$$

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