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Synthesis and characterization of phosphoester linkage-containing hydrogels

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Abstract

Phosphoester linkage-containing hydrogels based on poly(ethylene glycol) (PEG) were synthesized by photo-initial cross-linking polymerization of the methacryloyl groups in the side chain of polyphosphates. These methacryloyl groups were introduced by macromolecule reaction of polyphosphite from the polycondensation of several different molecular weighted PEG chains with dimethylphosphite. The swelling property of these hydrogels was investigated. It was observed that the percentage of equilibrium swelling depended on the molecular weight of the PEG segments and the cross-link density. These hydrogels were also loaded with 5-Fluorouracil (5-FU) for the study of their drug release profiles. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Hydrogels are cross-linked polymers, which have the ability to swell in water or aqueous solvent systems but will not dissolve regardless of the solvent [1]. Wichterle and Lim developed the first synthetic polymeric hydrogels based on hydroxyethyl methacrylate, for use as biomedical materials [2]. Since then, there has been continuing interest in the development of novel types of hydrogel materials for biomedical application [3, 4]. Recently, biodegradable hydrogels have been used quite extensively in the controlled release area. The use of this biodegradable hydrogel system eliminates the removal of the drug delivery system after the entire drug is released.

Phosphoester linkages in the polymer are labile to hydrolysis in aqueous systems and polyphosphates have been investigated as biodegradable materials, especially for use in drug delivery and orthopedic applications in the past few years. It was confirmed that polymers containing phosphoester bonds in the backbone exhibit good hydrolytic degradability [5–7].

Due to the recognized biocompatibility of PEG, PEG-containing polymers have been widely explored in the biomedical material area. In the past, PEG chains were copolymerized with a variety of difunctional comonomers followed by a cross-linking reaction to synthesize hydrogels, such as those copolymerized with diisocyanates [8]. This paper deals with a series of PEG-containing hydrogels with biodegradable phosphoester bonds. These hydrogels were synthesized through photo-initial cross-linking polymerization of methacryloyl polyphosphate based on PEG chains (Fig. 1). The swelling characterization of these hydrogels was studied and 5-Fluorouracil (5-FU) was loaded into these hydrogels for the investigation of the drug release profiles.

2. Experimental

2.1. Materials and methods

Poly(ethylene glycol) samples with molecular weights of 600, 1000, 2000, and 4000 were dried at 90°C under vacuum (1.33 Pa) for 24 h. Phosphorus(III) chloride, ethylene oxide, and benzoin methyl ether were labora-

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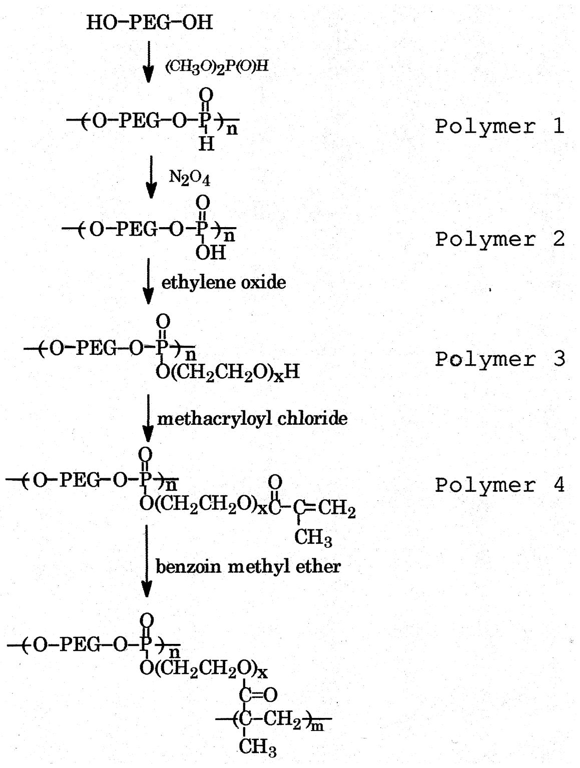

Fig. 1. Reaction scheme for the synthesis of phosphoester linkage-containing hydrogels.

tory reagents. 5-Fluorouracil (5-FU) is a crystalline power (purity > 99%). Dichloromethane was distilled twice over CaH_2. N_2O_4 is one of the decomposition products of lead(II) nitrate, and stored at −20°C in CH_2Cl_2. Triethylamine was purified under reflux in the presence of phthalic anhydride, distilled over KOH and CaH_2. Other solvents were analytically pure and used as received. Double distilled water was used.

Dimethyl phosphite and methacryloyl chloride were synthesized according to the literature [9–11].

^{1}H and ^{31}P(^{1}H)-NMR spectra were recorded on a JOEL FX-90Q and Bruker ARX-500 spectrometer. Fourier transform infrared spectroscopy (FT–IR) was performed on a Nicolet 170SX spectrophotometer. Molecular weights were determined by a Knauer vapor pressure osmometer.

2.2. Syntheses of hydrogels

The reaction scheme is shown in Fig. 1. The intermediate polymers are represented as polymer 1, 2, 3 and 4, and polymers called *X*-1, *X*-2, *X*-3, and *X*-4 (*X* = 1, 2, 3, and 4) represent polymers *X* which were synthesized from PEG segments with molecular weights of 600, 1000, 2000, and 4000, respectively.

2.2.1. Syntheses of polymer 1

Polyphosphite (polymer 1) was obtained from polycondensation of dimethyl phosphite with a corresponding PEG segment according to the literature [10]. Briefly, PEG and a 20% excess of dimethylphosphite over stoichiometry were put in a round bottom flask attached to the vacuum line. The reaction was first carried out at 120°C to move 90% of the theoretical amount of the methanol, and then the temperature and vacuum were increased slowly. The reaction was kept for 12 h at 160°C under high vacuum (0.667 Pa) and 180°C for another 12 h. The polyphosphites were obtained after the reaction products were cooled slowly.

2.2.2. Preparation of polymer 2

Polymer 2 was prepared by oxidation of polymer 1. A 10% solution of polymer 1 in CH_2Cl_2 and excess N_2O_4 solution in CH_2Cl_2 were mixed at −20°C. The mixture was kept for 48 h at room temperature. Polymer 2-1 precipitated gradually from the solution. The precipitate was washed with CH_2Cl_2 and diethyl ether in turn, then dried under vacuum. Polymers 2-2, 2-3 and 2-4 did not precipitate from the reaction solution. They were obtained by the precipitation of the solution into petroleum ether.

2.2.3. Preparation of polymer 3

Polymer 2 and a concentration of ethylene oxide that was two times higher than that of the P-OH group, were suspended or dissolved in CH_2Cl_2. The mixture was allowed to react for 48 h at 25°C. At the end of the reaction, CH_2Cl_2 and unreacted ethylene oxide were removed in vacuum to get polymer 3.

2.2.4. Preparation of polymer 4

Polymer 3 was dissolved in CH_2Cl_2 and the desired amount of methacryloyl chloride and triethylamine (5% excess) were added to the flask dropwise, maintaining the temperature below 5°C. The reaction was then allowed to proceed at room temperature for 24 h. The precipitated triethylamine hydrochloride salt was filtered off. The filtrate was washed with cold aqueous $NaCHO_3$ solution and then with distilled water until it became neutral. Residual water was removed by drying over anhydrous $MgSO_4$. It was then filtered and the solvent was removed in vacuum to get the product.

2.2.5. Preparation of photo-cross-linked hydrogels

Polymer 4 (1 g) was dissolved in 1,4-dioxane (10 ml). Benzoin methyl ether (2 mg, 0.2% wt) was added as a photoinitiator. The solution was flushed with nitrogen and poured into a glass mold. A medium pressure mercury lamp (long wave, 200 W) was placed 20 cm above the mold. The mold was irradiated for 1 h. Then the residual solvent was removed in vacuum to obtain the hydrogels.

2.3. Determination of the methacryloyl contents

The contents of the methacryloyl groups of polymer 4 were determined by a method of bromine titration [12]. A methacryloyl group-containing polymer was dissolved in 50 ml water, and 10 ml 0.2 N bromine solution (0.8 g Br_2 and 6 g KBr in 50 ml water) was added. The mixture was allowed to react in the dark for 2 h. Then 10 ml of 10% KI was added and the iodine formed from unused bromine was titrated with 0.1 N sodium thiosulphate solution. A blank experiment without a sample was carried out at the same time.

2.4. Equilibrium swelling

Disc samples of different polymer gels (0.4 ± 0.02 mm in thickness and 10.8 ± 0.2 mm in diameter) were prepared in a mold. After being immersed in a 50 ml of solvent each at 25°C, the discs were patted dried and weighed at regular intervals until equilibrium was attained. The discs were then dried under vacuum over phosphorus pentoxide to achieve constant weight. The percentage equilibrium swelling was calculated from the swollen and dry weights of the gel accordingly. The percentage equilibrium swelling was equal to $[(W_2 - W_1)/W_2] \times 100$ where W_1 is the weight of the dry gel, and W_2 is the weight of swollen gel.

2.5. Drug loading and release from hydrogels

Hydrogel disc samples were immersed in 10 mg/ml of 5-FU aqueous solution in the absence of light, until equilibrium was obtained (1 wk), and then dried at ambient temperature for at least 3 days. Total drug loading was determined after the completion of the release experiments. The drug loading percent was calculated as:

$$\text{Load\%} = \left[W_{\text{drug}}/\left(W_{\text{sample}} + W_{\text{drug}}\right)\right] \times 100.$$

Drug release from the hydrogel was carried out at 37°C. The drug loaded discs were each immersed into 50 ml of distilled water, and duplicate samples were included for each gel. At various times, aliquots of 0.4 ml were removed from the medium and 0.4 ml of distilled water was added to maintain the total volume of the medium. The 5-FU concentration was determined by UV spectroscopy. The drug release always maintained "sink" conditions, namely, the amount of 5-FU released did not exceed 15% of its solubility in water. The highest load of 5-FU was 17.4%, which corresponds with 10.8 mg of 5-FU, so the total drug released into the medium did not exceed 0.22 mg/ml. The solubility of 5-FU was determined to be 12.8 mg/ml by UV spectroscopy, so the total drug released was less than 15% of the 5-FU solubility.

3. Results and discussion

3.1. Synthesis of hydrogels

Polyphosphites with the reactive P-H (polymer **1** series) were obtained by a two-step polycondensation process [10]. A 20% molar excess of dimethylphosphite was used to react with the poly(ethylene glycol), followed by removal of the methoxyphosphonyl end groups in the oligomers at high temperature and high vacuum. These polyphosphites were highly viscous at high temperature (~200°C), solidifying into amorphous products upon cooling to room temperature. The polyphosphites became unmixable even at 200°C, beyond which they started to decompose. This high viscosity of the polymers limited their final molecular weights.

The average molecular weights of these polyphosphites were between 1×10^4 and 3×10^4. The result was similar to that observed by Pretula [10]. The P-H bond in the polymers was confirmed by the presence of ^{1}H-NMR and ^{31}P(^{1}H)-NMR spectra. A typical polyphosphite based on PEG 600 (polymer **1-1**) showed distinctive phosphite P-H doublet at δ6.8 ppm with a $J_{\text{P-H}} = 697$ Hz in the ^{1}H-NMR spectrum. It also gave a single peak at δ9.85–9.94 ppm in a ^{31}P(^{1}H)-NMR spectrum [Fig. 2(a)]. The NMR data of polymers **1-1**–**1-4** are shown in Table 1.

Oxidation of the P-H bonds in the polyphosphites by N_2O_4 [13] yield the corresponding polyacids, polymer **2** series, which are elastic solid polyphosphates. Polymer **2-1** was insoluble in CH_2Cl_2 but **2-2**, **2-3** and **2-4** were soluble in CH_2Cl_2. This transformation led to the complete disappearance of the P-H bond, which was confirmed by the absence of the distinctive phosphite P-H doublet, in the ^{1}H-NMR spectra. In the ^{31}P(^{1}H)-NMR spectrum of polymer **2-2** [Fig. 2(b)], the signal at δ1.596 ppm also confirmed the transformation of P-H to P-OH. The FT–IR spectrum of polymer **2-3** showed, in Fig. 3(a) for example, a weak absorption band at 2210–2622 cm^{-1} due to the P(O)-OH group, a strong P=O stretch at 1256 cm^{-1} and a P-O-C stretch at 1099 cm^{-1}, which also confirmed the presence of the phosphoester bond in the polymer.

Polymer **2** with a P-OH bond became insoluble in all of the solvents including a cooled NaOH aqueous solution, after being kept at room temperature for over 30 days in a desiccator over P_2O_5. This result might be attributable to the condensation between the polymer chain when forming cross-linkages.

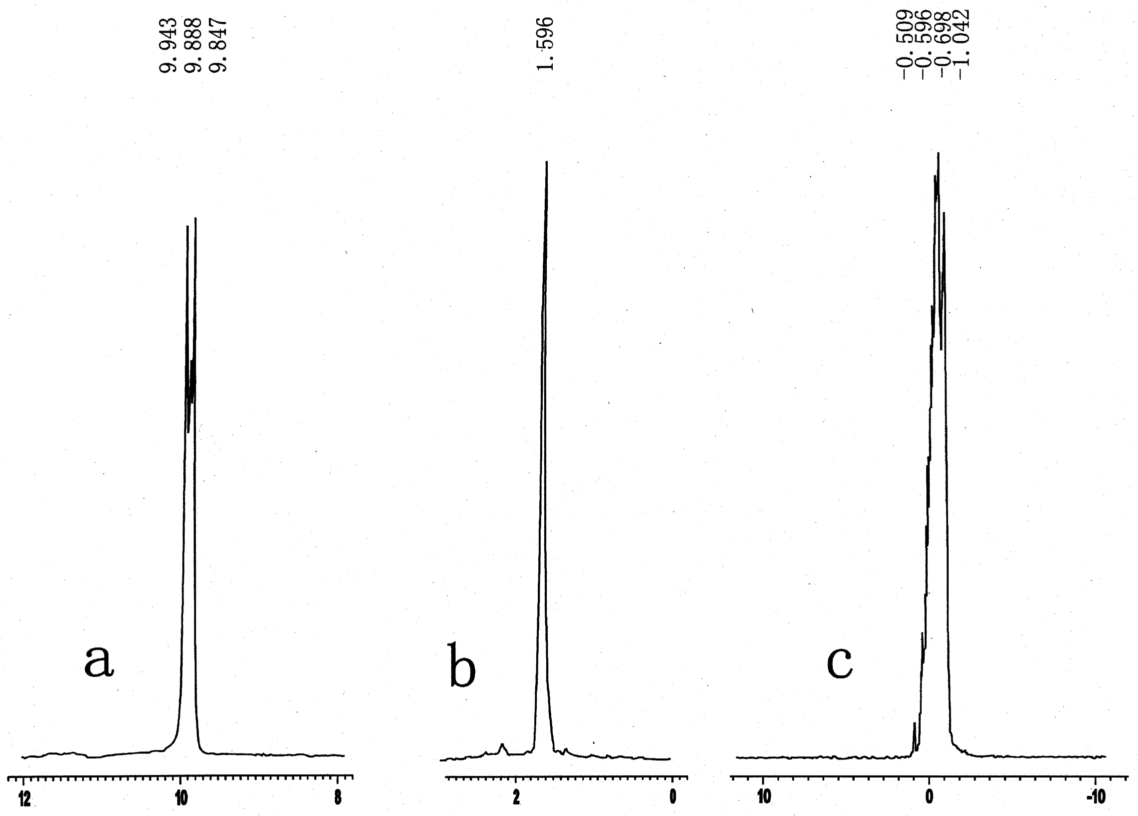

Fig. 2. ^{31}(^{1}H)-NMR spectra of polymer: (a) **1-1**; (b) **2-2**; and (c) **3-1**.

Reaction of polyphosphate **2** with ethylene oxide, was carried out in a heterogeneous or homogeneous system, namely in CH_2Cl_2 at 25°C, with one fold excess of the ethylene oxide. Since this reaction was catalyzed by the P(O)OH group itself [14], it led to polytriphosphoesters with a hydroxyl oligo ethoxy group in the side chain (polymer **3**). When all of the P-OH groups were consumed, oxyethylation also stopped in the absence of other external acids as catalyst.

The transformation of P-OH into P-O$(CH_2CH_2O)_x$-H were observed from FT–IR [Fig. 3(b)]. A strong stretch absorption at 3340–3386 cm^{-1} of C-OH replaced the stretch at 2210–2622 cm^{-1} due to the P-OH group. In the ^{31}P-NMR spectra of polymer **3-1** [Fig. 2(c)], the signal at $\delta-$ 0.509$--$ 1.024 ppm also confirmed the polyphosphotriester structure. The average x value may be 1–2 according to the literature [14, 15].

The side chain hydroxyl groups in polyphosphate **3** could be partially or completely converted to methacryloyl groups by the reaction with the desired amount of methacryloyl chloride. The C-OH absorption at 3340–3386 cm^{-1} is absent in the FT–IR spectrum of polymer **4-1** [Fig. 3(c)]. A strong absorption at 1717 cm^{-1} confirmed the C=O of unsaturated ester. The methacryloyl contents were determined by bromine titration and results were summarized in Table 2. These polymers can be dissolved in water, CH_2Cl_2,

Table 1. Molecular weights and NMR data of polymer **1** series

			^{1}H-NMR data		^{31}P(^{1}H)-NMR data
Polymer	PEG	$\bar{M}n$	$\delta_{\text{P-H}}$ (ppm)	$J_{\text{P-H}}$ (Hz)	$\delta_{\text{P-H}}$ (ppm)
1-1	600	13,600	6.7	697	9.8–9.9
1-2	1000	14,800	6.7	704	9.7
1-3	2000	26,700	6.8	705	8.9
1-4	4000	28,200	6.7	713	8.8

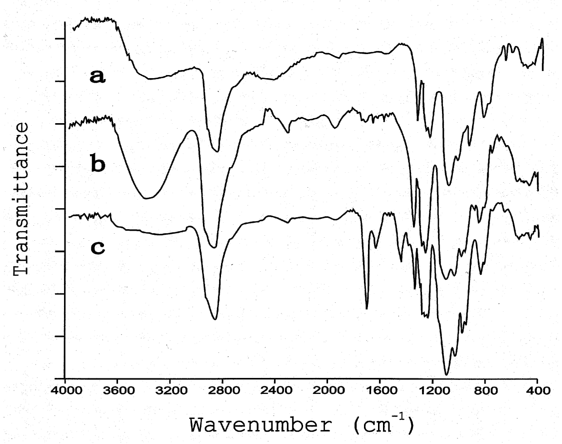

Fig. 3. FT–IR spectra of polymer: (a) **2-3**; (b) **3-2**; and (c) **4-1**.

$CHCl_3$, alcohols, 1,4-dioxane, tetrahydrofuran (THF), N,N-dimethylformamide (DMF).

Photo-initialized polymerization was chosen to crosslink the side chain methacryloyl groups in the macromers. Probably due to the low density of methacryloyl groups in reaction mixture, initial attempts of photo-crosslinking in water or CH_2Cl_2 failed to get a membrane with reasonable strength. However, the success of the cross-linking reaction was observed in 1,4-dioxane. An increase in the crosslinking rate was observed as the initiator concentration or the intensity of UV irradiation increased. In this study, the polymerization was carried out in 1,4-dioxane, with 0.2 wt.% benzoin methyl ether, under 1 h irradiation (20 cm below a 200 W medium pressure mercury lamp).

3.2. *Swelling and drug release properties of hydrogels*

The general swelling behavior of these hydrogels was measured. The results are shown in Figs. 4 and 5. All polyphosphite-derived hydrogels swelled in solvents rapidly. The swelling of hydrogel discs reached equilibrium within 2 h. The percentages equilibrium swelling of polymer **4-1**–**4-4** gels were 68–90%, 49–80%, 47–69% in water, THF, and benzene, respectively. Partially cross-linking hydrogels based on PEG 600

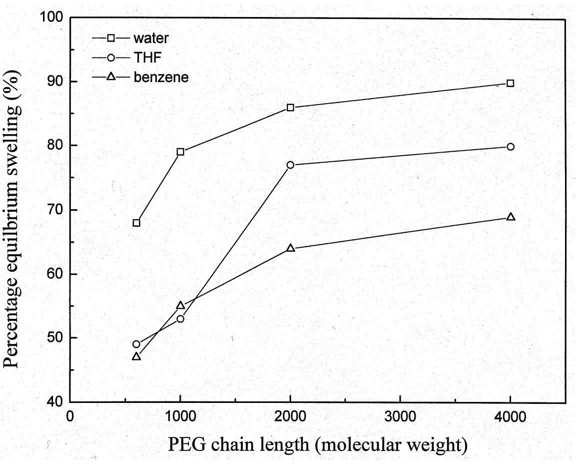

Fig. 4. Equilibrium swelling of hydrogels of cross-linked polymer **4-1**, **4-2**, **4-3** and **4-4**.

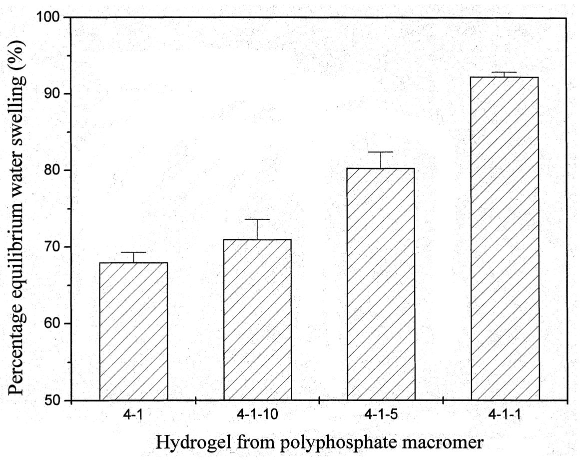

Fig. 5. Equilibrium swelling of hydrogels of cross-linked polymer **4-1**, **4-1-10**, **4-1-5** and **4-1-1** in water.

Table 2. Methacryloyl contents of polyphosphate macromers

Polyphosphate macromer code*	Methacryloyl chloride (wt %)	Methacryloyl content by titration (wt %)
4-1-1	1	0.58
4-1-5	5	2.51
4-1-10	10	5.16
4-1	20 (excess)	8.32
4-2	15 (excess)	5.71
4-3	10 (excess)	3.02
4-4	6 (excess)	1.43

*Y in polyphosphate macromer code (4-1-Y) represents the weight ratio of methacryloyl chloride added to polymer **3-1** in the synthesis

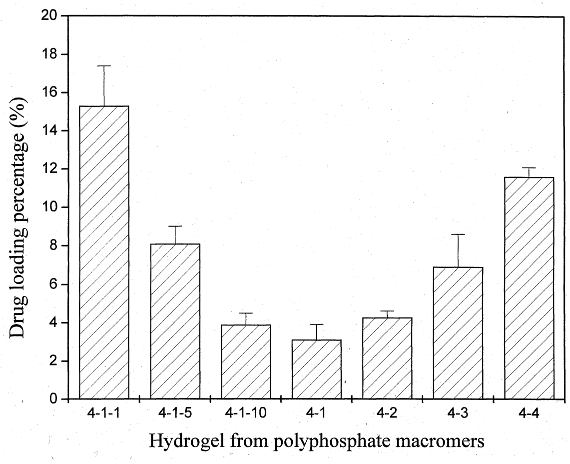

Fig. 6. 5-FU loading percentage of hydrogels of cross-linked macromers.

(polymer **4-1-10**, **4-1-5**, **4-1-1**) showed 71–92% of equilibrium swelling. Macromers with a higher content of methacryloyl groups tended to give higher cross-linking density, which lowered the equilibrium swelling. It was also observed that the equilibrium swelling increased with the increasing of molecular weight of PEG segments in the macromers.

5-FU was loaded into the hydrogels by immersing them in 10 mg/ml of 5-FU aqueous solution. Fig. 6 shows the effect of polymer composition on the equilibrium drug loading in these hydrogels. The equilibrium drug loading content in the hydrogel decreased with increasing the methacryloyl group contents and decreasing the molecular weight of PEG segments in the macromers. A higher swelling ratio and lower cross-linking density induced by a lower content of methacryloyl groups in the macromers enhanced the drug loading.

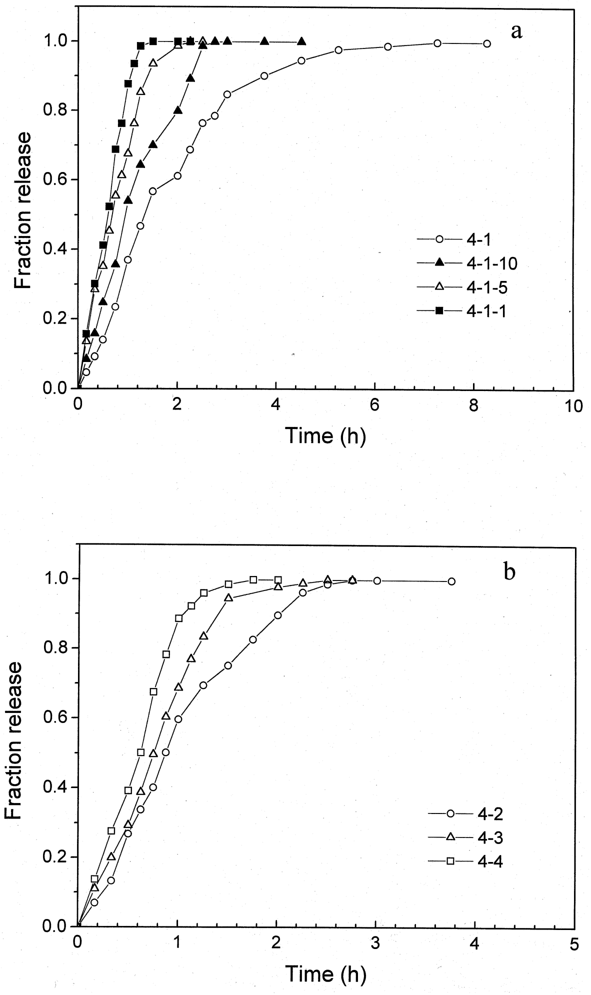

Fig. 7. 5-FU release from drug loaded hydrogel samples in water at 37°C.

The drug release study was performed in double distilled water at 37°C. Initially, dry 5-FU loaded hydrogel discs with a 0.4 ± 0.02 mm thickness and a 10.8 ± 0.2 mm diameter were used in the drug release experiments. The fraction release of the drug is shown in Fig. 7. The release profile depended on the PEG composition and cross-linking density. The release rate decreased as the cross-linking density increased and the PEG segment molecular weight decreased, which can be seen by comparing the 50% drug release time

Table 3. Release profiles of 5-FU from hydrogel discs

Polymer	$t^{a}_{1/2}$ (min)	n	t'^{b} (min)	M_t/M^{c}_{∞} (%)	r^2 (%)
4-1	92	1.06	150	76.4	98.7
4-2	53	1.10	90	75.2	98.6
4-3	46	1.04	68	76.9	99.3
4-4	38	1.02	45	67.6	99.3
4-1-10	61	1.00	90	69.9	99.5
4-1-5	42	0.89	68	76.0	99.3
4-1-1	34	0.96	53	76.3	99.4

[a]Time for 50% 5-FU release.[b]The end time to get the exponent n value.[c]The fraction release at $t\prime$. r is the correlation coefficient

($t_{1/2}$) summarized in Table 3. It was found that the hydrogel with a higher equilibrium water swelling had a shorter half drug release time which was similar to what was observed by McNeill et al. [17].

The kinetics implying the drug release mechanism in hydrogels can be described by the following simple exponent equation [16]

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

where M_t/M_∞ is the fraction release, t is the drug release time and k is the releasing kinetic constant. The exponent n is the indication of drug releasing characteristics. When $n = 0.5$ the release is taken to be Fickian. When $n = 1$ the release is zero order, namely, constant release with time. The release with an n value in the range between 0.5 and 1 is anomalous. The closer the n value is to 1, the closer is the release pattern to zero order.

The value of n was obtained from the slope of $\log(M_t/M_\infty)$ vs $\log t$ in the range of time from 0 to t'. The resulting values are summarized in Table 3. All the hydrogels gave basically zero-order release of 5-FU to about 70% of total content. Drug release behavior from PEG based hydrogels are influenced by several factors: polymer composition, sample dimensions and geometry, drug concentration in polymer etc. [17, 18]. Zero-order release from an initially dry slab of PEG hydrogels was also observed by McNeill et al. and the thickness of the device and surface area are important factors to influence the release profile [17]. In our experiments, initially dry hydrogel discs with 0.4 mm thickness are used. The surface of the top and bottom exceed 93% of the disc total surface, which is favorable to approach zero-order release.

In conclusion, the load and release of 5-FU can be controlled by the PEG segments molecular weight and the cross-linking density in these hydrogels to obtain the desired loading level and constant release profiles. The biodegradability of phosphate ester linkage contained in hydrogels is potentially useful for the elimination and removal of the drug delivery system after the entire drug is released.

Acknowledgements

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References

[1] Kim SW, Bae YH, Okano T. Pharmacol. Res. 1992;81:85.

[2] Wichterle O, Lim D. Nature 1960;185:177.

[3] Ratner BD, Hoffman AS. Hydrogels for medical and related applications. In: Andrade JD, editor. ACS Symposium Series. Washington DC: American Chemical Society, 1976. p. 13.

[4] Peppas NA, editor. Hydrogels in medicine and pharmacy. Boca Raton, Florida: CRC Press, I, II and III. 1987.

[5] Richard M, Dahiyat BI, Arm DM, Brown PR, Leong KW. J. Biomed. Mater. Res. 1991;25:1151.

[6] Leong KW, Mao HQ, Zhuo RX. Chin. J. Polym. Sci. 1995;13:289.

[7] Dahiyat BI, Hostin E, Posadas EM, Leong KW. 1993. J. Biomater. Sci., Polym. Edn.4. 529.

[8] McNeill ME, Graham NB. 1993. J. Biomater. Sci., Polym. Edn. 4. 305.

[9] McComble H, Saunders BC, Stacey GJ. J. Chem. Soc. 1945;380.

[10] Pretula J, Penczek S. Makromol. Chem. 1990;191:671.

[11] Stempel GH, Cross RP, Mariella RP. J. Am. Chem. Soc. 1950;72:2299.

[12] Muller K. Functional group determination of olefinic and acetylenic unsaturation. New York: Academic Press, 1975:103.

[13] Denne DZ, Chen GY, Denny DB. J. Am. Chem. Soc. 1969;91:6838.

[14] Biela T, Szymanski R, Kubisa P. Makromol. Chem. 1992;193:285.

[15] Penczek S, Pretula J. Macromolecules 1993;26:2228.

[16] Peppas NA, Ponchol G, Dechene D. J. Control. Rel. 1987;5:143.

[17] McNeill ME, Graham NB. 1993. J. Biomater. Sci., Polym. Edn. 5. 111.

[18] McNeill ME, Graham NB. 1996. J. Biomater. Sci., Polym. Edn. 7. 937.