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Note

Drug-delivery study and estimation of polymer–solvent interaction parameter for bisacrylate ester-modified Pluronic hydrogels

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ABSTRACT

In this study, Pluronic F127 hydrogels were characterised as an injectable system for the controlled release of drugs with variable molecular weights (FITC-Dextran at 70 and 40 kDa). In addition, the polymer–solvent interaction parameter (χ) was successfully estimated. Pluronic hydrogels (10–25 wt.%) were redox cured and their swelling behaviour investigated in PBS (pH 7.45) at 37 °C. After swelling to equilibrium, the hydrogels were compressed and the rubber-elasticity theory was applied to evaluate χ. Tensile tests proved the hydrogels were elastic and their χ values ranged between 0.50 and 0.53. The full drug load could be delivered over a period of ~15 h suggesting that redox cured Pluronic F127 hydrogels can function as injectable systems for controlled and sustained release of macromolecules.

1. Introduction

Copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO, commercially known as Pluronics) are biocompatible water-soluble polymers that have found widespread use among biomedical applications. These include coatings for medical devices (Wesenberg-Ward et al., 2005), cell encapsulation (Terada et al., 2005), and matrices for drug-delivery systems (Grassi et al., 2005). The main feature of these polymers is their low critical solution temperature, which facilitates their injection as a liquid into the body followed by instantaneous gelling at body temperature. In addition, their success for drug delivery is due to their amphiphilic nature that allows solubilisation of hydrophobic drugs in an aqueous environment.

Of particular interest is Pluronic F127, $PEO_{99}PPO_{65}PEO_{99}$, which subsequent to its approval by the U.S. Food and Drug Administration (FDA), has been widely investigated as a thermo-responsive hydrogel for drug-delivery systems (Kabanov et al., 2002a,b; Marsh et al., 2003; Pisal et al., 2004). However, Pluronic hydrogels formed merely by physical gelation have a rapid rate of dissolution in the order of hours; this is a significant disadvantage when long-term delivery of the drug is required.

In this study, Pluronic F127 was modified with acrylate end groups (Sosnik et al., 2003) (Fig. 1) and polymer aqueous solutions were redox cured to fabricate hydrogels of variable solid contents that allowed the release of active drugs over longer period of time. The hydrogel swelling behaviour was also determined as function of the polymer concentration in the network. To further characterise Pluronic hydrogels as drug-delivery systems, the solvent interaction parameter (χ) was estimated by compression tests. The rubber-elasticity theory has been used previously (Migliaresi et al., 1980; Huang et al., 2002; Emami and Salovey, 2003) to evaluate χ of other polymeric networks but more experimental data are needed to determine the polymer–solvent interaction parameter of Pluronic hydrogels. This parameter indicates thermodynamically favourable interactions between the polymer and the solvent, thus it plays an important role in understanding the swelling behaviour and drug-delivery rate of hydrogels.

2. Method

All chemicals were purchased from Sigma and used without further purification, unless stated otherwise.

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2.1. Synthesis and fabrication of Pluronic F127 hydrogels

The hydroxyl end groups of Pluronic F127 were modified with methacryloyl chloride to attach bisacrylate ester groups to the polymer chain as described in literature (Sosnik et al., 2003). Briefly, Pluronic F127 (1.6 mmol, 20.0 g) was dissolved in dried chloroform (40 mL). The solution was cooled down to 0 °C and TEA (13.0 mmol, 1.3 g) was added. A solution of methacyrloyl chloride (23.15 mmol, 1.325 g) in chloroform (10 mL) was added drop-wise to the polymer solution under nitrogen. The reaction was left overnight. The solution was concentrated under reduced pressure and toluene (50 mL) was added. The solution was then filtered and the modified Pluronic 127 was obtained by precipitation in ether. The product was colleted as white powder and dried under vacuum at room temperature. The yield was 15.6 g. The modified polymer was dissolved in deuterated chloroform and analysed by ^{1}H NMR (Bruker DPX 300). The attachment of bisacrylate end groups was verified by the presence of peaks at 5.6 and 6.2 ppm corresponding to the acrylate groups.

Hydrogels were fabricated by dissolving the modified polymer (see Fig. 1) in deionised water (DI-H_2O) at 0 °C to final concentrations ranging from 10 to 25 wt.% polymer. Ammonium persulfate (APS) was added to the macromer solution at a ratio of 3 wt.% under agitation followed by the addition of 1 μg/g *N*,*N*,*N*′,*N*′-tetramethylethylene diamine (TEMED). Polymer solutions were sucked into 1 mL syringes and immersed in a water bath at 37 °C until gelation occurred. Gelation time was determined by the test tube inverting method (Zhu and Ding, 2006). Briefly, the syringes were inverted and gelation was regarded to occur when no flow of liquid was observed. The hydrogels were then cut into cylindrical shape of ~2.4 mm radius and ~5 mm length.

To further characterise the cross-linking efficiency of the redox system, the sol fraction of hydrogels was determined by soxhlet extraction in methylene chloride. Cured hydrogels were weighed at synthesis (m_i), dried under vacuum and reweighed (m_{d1}). The dried hydrogels were extracted in methylene chloride for 48 h, redried under vacuum and then reweighed (m_{d2}). The % sol fraction was determined according to Eq. (1):

$$\%\,\text{sol fraction} = \frac{(m_{d1} - m_{d2})}{m_{d1}} \times 100 \quad (1)$$

2.2. Swelling studies

Wet weight of Pluronic hydrogels (n = 6, for each polymer concentration) was measured at fabrication (m_i), immersed in phosphate buffered saline (PBS, pH 7.4) at 37 °C and left to swell until equilibrium was reached. Wet weight of swollen samples ($m_{s.t}$) was determined at different time points after gently blotting the hydrogels with filter paper. When the swollen samples reached a constant equilibrium weight ($m_{s.eq.}$), they were subjected to compression tests (Section 2.4) and then lyophilised to get their dry weight (m_d). Their volumetric swelling ratio (Q_e) at equilibrium was calculated according to Eq. (2) (Peppas, 1987):

$$Q_e = 1 + \frac{\rho_{\text{dry.polymer}}}{\rho_{\text{solvent}}}(q_e - 1) \quad (2)$$

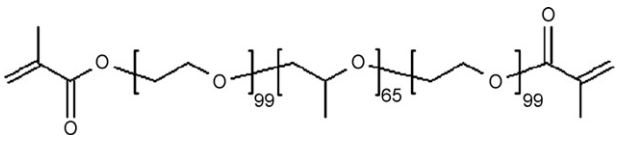

Fig. 1. Chemical structure of Pluronic F127 modified with dimethacrylate end groups.

where $\rho_{\text{dry.polymer}}$ = 1.05 g/cm^3 (Gu and Alexandridis, 2004), ρ_{solvent} is the density of water (1 g/cm^3) and q_e is the mass swelling ratio calculated as:

$$q_e = \frac{m_{s.eq}}{m_d} \quad (3)$$

2.3. Mechanical testing

The polymer–solvent interaction parameter (χ) was determined for hydrogels swollen to equilibrium according to the rubber-elasticity theory (Flory, 1953). Pluronic polymeric chains were assumed to be Gaussian as previously reported (Lam and Goldbeck-Wood, 2003). Hydrogels (n = 6 for each polymer concentration) were subject to compression tests by using a computer-interfaced tensiometer (Instron Mini 55, MA, USA). Cylindrical samples of the swollen networks were compressed up to 20% strain at 37 °C in an isothermal chamber. This strain percentage was chosen as the upper limit since below 20% strain, hydrogels are described to have an elastic behaviour and hence the rubber-elasticity theory is valid to describe the structural properties of the network (Peppas et al., 2006).

From the slope of the stress–strain plot, the effective network chain density (υ_e/V_0) was calculated based on the following relation (Ruiz et al., 2003; Bell and Peppas, 1996):

$$\tau_s = \left(\frac{\upsilon_e}{V_0}\right) RT\upsilon_{2s}^{1/3}\upsilon_{2r}^{2/3}(\alpha - \alpha^{-2}) \quad (4)$$

where τ_s is the applied force per unit area of the swollen hydrogel, R is the gas constant (8.315 Pa m^3/K mol), T is absolute temperature (310 K), α is the deformation ratio, υ_{2s} is the polymer volume fraction in swollen hydrogels and is the inverse of the volumetric swelling ratio ($1/Q_e$), υ_{2r} is the polymer volume fraction in the hydrogel in its relaxed state that is prior to swelling, and was calculated according to the following equation:

$$\upsilon_{2r} = \frac{m_d/\rho_{\text{dry.polymer}}}{m_i/\rho_{\text{polymer.solution}}} \quad (5)$$

where $\rho_{\text{polymer.solution}}$ is the density of the macromer solution, and was determined experimentally at 37 °C by a pycnometer. Table 1 lists the values of υ_{2r},υ_{2s} and $\rho_{\text{polymer.solution}}$ as function of the polymer concentration.

From υ_e/V_0, the polymer–solvent interaction parameter (χ) was calculated according to Eq. (6) (Flory, 1953):

$$\ln(1 - \upsilon_{2s}) + \upsilon_{2s} + \chi\upsilon_{2s}^2 + V_1\left(\frac{\upsilon_e}{V_0}\right)\left(\upsilon_{2s}^{1/3}\upsilon_{2r}^{2/3} - \frac{\upsilon_{2s}}{2}\right) = 0 \quad (6)$$

where V_1 is the molar volume of the solvent water (18 cm^3/mol).

2.4. Release studies

For release studies, FITC-Dextran (40 and 70 kDa) was used as a model macromolecule. FITC-Dextran (1 wt.%) was added to Pluronic

Table 1
Density of Pluronic F127 solution in a range of concentration (10–20 wt.%) determined by a pycnometer at 37 °C (mean ± standard deviation, n = 6)

[Polymer] wt./wt.%	$\rho_{\text{polymer.solution}}$ (g/mL)	υ_{2r}	υ_{2s}
10%	1.004 ± 0.001	0.058	0.045
15%	1.014 ± 0.001	0.082	0.055
20%	1.023 ± 0.003	0.114	0.075
25%	1.033[a]	0.158	0.102

Polymer volume fraction of hydrogels in its relaxed (υ_{2r}) and swollen state (υ_{2s}).

[a] *Note*: The rapid physical gelation of the 25 wt.% solution prevented an accurate measure of the density. For this reason, the average density at 25 wt.% was extrapolated from the line intercepted by the density values at 10, 15 and 20 wt.%.

solutions (15 and 20 wt.%) and redox cured as described above. Loaded-F127 cylinders were incubated in 50 mL PBS (pH 7.4) at 37 °C on an orbital shaker. At different time points, 1 mL samples were taken and replaced by same volume of fresh PBS. All samples were stored frozen and then analysed using a fluorescence microplate reader at λ = 490–520 nm to determine the amount released (M_t). M_t was calculated from a standard curve of known concentrations of FITC-Dextran solutions in the range of 0–10 μg/mL.

Fraction released of the drug from the network (M_t/M_{inf}), where M_{inf} is the total amount released, was plotted against square root of time ($t^{1/2}$). Assuming only radial release, the diffusion of solute from a cylinder can be described by Crank's diffusion model as (Crank, 1975):

$$\frac{\partial C}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD\frac{\partial C}{\partial r}\right) \quad (7)$$

with the boundary conditions being:

$C = f(r)$ if $0 < r < a$ and $t = 0$
$C = 0$ if $r = a$ and $t \geq 0$

where r is the inner radius of the cylinder, a is the radius of the cylinder (~0.24 cm), $f(r)$ is a function of the distribution of the concentration (C) of the solute dispersed in the cylinder, t is the release time and D is the diffusion coefficient. The solution for Eq. (7) under the specified boundary is:

$$\frac{M_t}{M_{inf}} = \frac{4}{\pi^{1/2}}\left(\frac{Dt}{a^2}\right)^{1/2} - \frac{Dt}{a^2} - \frac{1}{3\pi^{1/2}}\left(\frac{Dt}{a^2}\right)^{3/2} + \cdots \quad (8)$$

For early time release, Eq. (8) can be reduced to

$$\frac{M_t}{M_{inf}} = 4\sqrt{\frac{Dt}{\pi a^2}} \quad (9)$$

for $M_t/M_{inf} < 0.6$ (Bajpai and Rajpoot, 2001).

Three gels were used for each drug at 15% and 20% polymer concentrations. Data were statistically analysed using two-way ANOVA.

3. Results and discussion

3.1. Fabrication of Pluronic hydrogels

The sol fraction exhibited by F127 hydrogels was 1–2%. The low sol fraction suggests that the hydrogels were cured successfully by APS/TEMED. However, gelation time varied as the macromer concentration changed. The 20 and 25 wt.% solutions gelled in ~10 min after incubation at 37 °C, whereas 10 and 15 wt.% solutions were allowed to incubate for 4 h before curing was complete. Typically, Pluronic solutions of polymer concentrations higher than 20 wt.% exhibit sol–gel transition even at room temperature (Lee et al., 2004), a mechanism described by micelles formation. As the temperature of these solutions is brought up to 37 °C, hydrophobic interactions between the PPO segments become more prominent. Hence, a more compact network comprised of tighter micellar structure should form in a short period of time explaining the rapid gelation observed for the 20 and 25 wt.% solutions. These hydrophobic interactions are however weaker in more dilute Pluronic solutions, which do not exhibit a sol–gel transition at room temperature. Lower concentration solutions may require more time to cure for this reason.

3.2. Swelling studies

Studies were conducted on F127 hydrogels to investigate their swelling behaviour. Monitoring the wet weight over time, it was observed that Pluronic hydrogels reached equilibrium within 2 h of incubation. Fig. 2 represents the volumetric swelling ratio (Q_e) at equilibrium as a function of the solid content in the network. The swelling ratio decreased as the solid content of the network increased. This result may be related to the closely packed micellar structures, which form in more concentrated solutions and allow for less water to diffuse into the network. Similar trends for the decrease of swelling ratio as a function of polymer concentration in networks have been reported in literature (Lee et al., 2004).

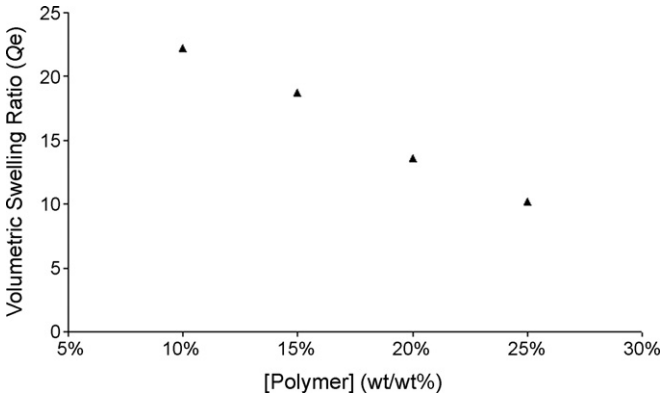

Fig. 2. Volumetric swelling ratio (Q_e) of hydrogels at equilibrium as a function of polymer concentration in the network.

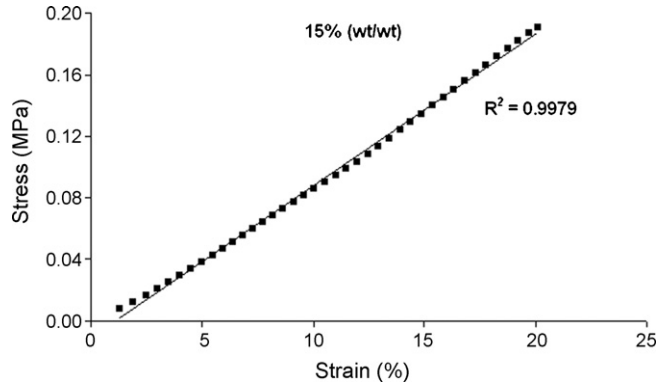

Fig. 3. Typical stress–strain dependence for Pluronic hydrogels at 37 °C after swollen to equilibrium. The hydrogels showed a linear dependence ($R^2 > 0.98$) between stress and strain at all concentrations.

3.3. Mechanical testing

Pluronic F127 hydrogels swollen to equilibrium were subjected to compression tests. All hydrogels showed a linear dependence between strain and stress ($R^2 > 0.98$, Fig. 3) even though Pluronic solutions undergo phase transition with temperatures increase (Gu and Alexandridis, 2004; Zhu and Ding, 2006). Values of χ ranged between 0.50 and 0.53 for all networks suggesting that water is not a very good solvent for Pluronic F127 (Table 2). Hydrogels like Pluronic are thus expected to have a relatively low degree of

Table 2
Experimental estimation of the hydrogel polymer–solvent interaction parameter (χ) (mean ± standard deviation, $n = 6$) at various F127 Pluronic concentrations

[Polymer] wt./wt.%	χ
10%	0.50 ± 0.01
15%	0.51 ± 0.01
20%	0.51 ± 0.01
25%	0.53 ± 0.01

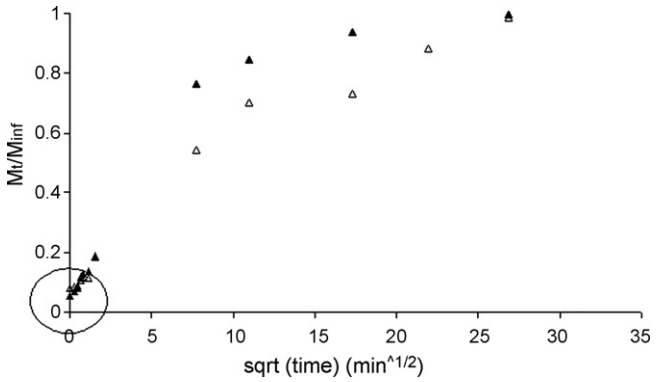

Fig. 4. Fraction release of FITC-Dextran (FD40) from: ▲ 15 wt.% and △ 20 wt.% hydrogels. The encircled data denotes the burst effect observed at the beginning.

swelling, in agreement with our results (Fig. 2). The hydrophobic segment (PPO) of the Pluronic backbone is likely responsible for the poor water interaction.

It should be noted that the χ values of F127 Pluronic hydrogels are close to the χ value of Pluronic solutions ($\chi = 0.55$), which was previously measured with osmotic stress techniques (Gu and Alexandridis, 2004). However, no data were reported by these authors for χ of Pluronic hydrogels. In agreement with theory (Flory, 1953; Lee and Yuan, 2002), a trend of increasing values for χ was noted as the polymer fraction enlarged. This trend is also in agreement with the behaviour of the swelling ratio that decreased for networks of higher solid content.

3.4. Release studies

Two sizes of FITC-Dextran, 40 kDa (FD40) and 70 kDa (FD70), were chosen as model drugs to assess the function of Pluronic hydrogels as delivery systems for macromolecules. In particular, the effect of the drug molecular weight and the polymer concentration in the network was studied in order to achieve networks of variable and gauged release properties.

Fig. 4 is an illustrative example of the FD40 released fraction from 15 and 20 wt.% hydrogels. The same trend was observed for the release of FD70. It was observed that hydrogels displayed an initial release at time zero, as the encircled data shows in Fig. 5. The burst effect is presumably due to the instantaneous release of the drug on the surface of the hydrogel. However, the burst effect had to be eliminated in order to be able to calculate the diffusion coefficient according to Eq. (9). Thus the experimental data was

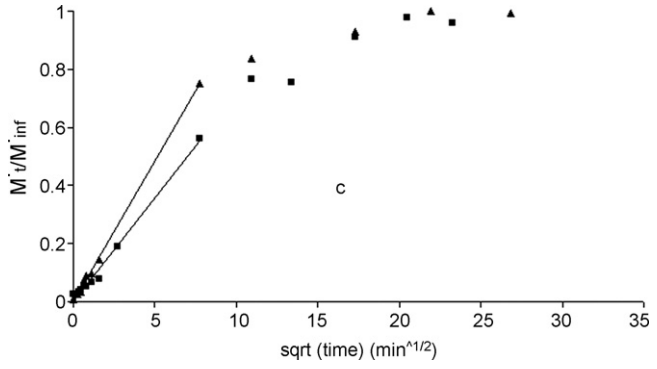

Fig. 5. Adjusted fraction release of ▲ FD40 and ■ FD70 from 15 wt.% hydrogels. The lines represent the slope from which the diffusion coefficient was calculated according to Eq. (7).

Table 3
Experimental diffusion coefficients of FD40 and FD70 (mean ± standard deviation, $n = 3$) from 15 and 20 wt.% F127 Pluronic hydrogels

[Polymer] wt./wt.%	$D_{exp} \times 10^{-7}$ (cm^2/s) of FD40	$D_{exp} \times 10^{-7}$ (cm^2/s) of FD70
15%	16.8 ± 3.3	7.8 ± 3.2
20%	10.5 ± 0.5	5.4 ± 4.0

transformed according to Eq. (10):

$$\frac{M'_t}{M'_{inf}} = \frac{M_t - i}{M_{inf} - i} \quad (10)$$

where i is the burst amount released initially and calculated by Excel as the intercept of the linear part of the curve of M_t versus square root of time. Once the data was transformed, the diffusion coefficients were calculated according to Eq. (9) from the slope of the curve of M'_t/M'_{inf} versus $t^{1/2}$, and their values are listed in Table 3.

There was a very noticeable effect of the drug molecular weight on the release rate: diffusion coefficients decreased significantly from $16.8 \times 10^{-7} \pm 3.3 \times 10^{-7}$ to $7.8 \times 10^{-7} \pm 3.2 \times 10^{-7}$ cm^2/s for gels at 15%, loaded with FD40 and FD70 respectively ($p = 0.004$, two-way ANOVA). While FD40 was mostly released in 2 days, FD70 took 5 days before reaching a plateau, having a slower diffusion rate (Fig. 5). We may speculate that FD70 diffusion is slowed down by its larger hydrodynamic radius. A similar trend was observed for gels at 20%: diffusion coefficients decreased significantly from $10.5 \times 10^{-7} \pm 0.5 \times 10^{-7}$ to $5.4 \times 10^{-7} \pm 4.0 \times 10^{-7}$ cm^2/s for gels loaded with FD40 and FD70 respectively ($p = 0.004$, two-way ANOVA).

Finally, diffusion coefficients decreased significantly with the rising of polymer concentration for gels loaded with FD40 and FD70 ($p = 0.04$, two-way ANOVA). This behaviour is to be expected since networks of higher solid content were less swollen, as shown in Fig. 2 (Miyazaki et al., 1984; Escobar-Chàvez et al., 2006).

4. Conclusion

Structural and diffusion properties of Pluronic F127 hydrogels, which were cured by redox initiation, were successfully characterised for prolonged and controlled delivery of macromolecules. The full drug load was delivered over a period of ~15 h suggesting that redox cured Pluronic F127 hydrogels can be developed to function as injectable systems for controlled and sustained release of drugs. The polymer–solvent interaction parameter of Pluronic F127 hydrogels at different concentrations (10–25%, w/w) was effectively estimated by compressing cylindrical hydrogels swollen to equilibrium. χ values ranged between 0.50 and 0.53 in agreement with the presence of a hydrophobic segment in the polymer chain.

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