International Journal of Pharmaceutics 427 (2012) 320–327

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A mathematical model for pulsatile release: Controlled release of rhodamine B from UV-crosslinked thermoresponsive thin films

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ARTICLE INFO

Article history:
Received 9 September 2011
Received in revised form 13 February 2012
Accepted 15 February 2012
Available online 23 February 2012

Keywords:
Pulsatile release
Controllable drug delivery system
Thermoresponsive polymer
Mathematical model

ABSTRACT

A controlled drug delivery system fabricated from a thermoresponsive polymer was designed to obtain a pulsatile release profile which was triggered by altering the temperature of the dissolution medium. Two stages of release behaviour were found: fast release for a swollen state and slow (yet significant and non-negligible) release for a collapsed state. Six cycles of pulsatile release between 4 °C and 40 °C were obtained. The dosage of drug (rhodamine B) released in these cycles could be controlled to deliver approximately equal doses by altering the release time in the swollen state. However, for the first cycle, the swollen release rate was found to be large, and the release time could not be made short enough to prevent a larger dose than desired being delivered. A model was developed based on Fick's law which describes pulsatile release mathematically for the first time, and diffusion coefficients at different temperatures (including temperatures corresponding to both the fully swollen and collapsed states) were estimated by fitting the experimental data with the theoretical release profile given by this model. The effect of temperature on the diffusion coefficient was studied and it was found that in the range of the lower critical solution temperature (LCST), the diffusion coefficient increased with decreasing temperature. The model predicts that the effective lifetime of the system lies in the approximate range of 1–42 h (95% of drug released), depending on how long the system was kept at low temperature (below the LCST). Therefore this system can be used to obtain a controllable pulsatile release profile for small molecule drugs thereby enabling optimum therapeutic effects.

1. Introduction

Most local drug delivery systems aim to maintain the drug concentration at some appropriate therapeutic level for a specified period of time, and this objective is frequently achieved using sustained release dosage forms. However, for some drugs, an optimum therapeutic effect comes from a periodically fluctuating drug concentration (Kikuchi and Okano, 2002). To realise such behaviour, pulsed or pulsatile drug release systems have been developed (Bae et al., 1991; Coughlan et al., 2004; Ishino et al., 1992; Lowman and Peppas, 1999; Mundargi et al., 2010; Siegel and Pitt, 1995; Vertommen et al., 2008). This type of release system possesses a cycle with two distinct release stages; off/slow release and on/fast release. Usually, the release duration time for the slow release stage is much longer than that for the fast stage, and the release rate is much smaller.

The majority of existing pulsatile release systems can be classified into two categories (Kikuchi and Okano, 2002); time-controlled systems (Intra et al., 2008; Kashyap et al., 2007; Liu et al., 2007; Makino et al., 2000) and stimuli-induced systems (Li and D'Emanuele, 2001; Mohamad and Dashevsky, 2006; Satarkar and Hilt, 2008; Schellekens et al., 2008). Time-controlled release systems can only release at pre-programmed time points, whereas stimuli-induced pulsatile release systems are more easily manipulated. Stimuli-induced systems have been developed based on thermal, chemical, and electrical stimuli. However, systems based on thermal stimuli are particularly convenient since they can be designed and operated without significantly affecting other critical parameters of the system.

Thermoresponsive polymers undergo dramatic changes in conformation in response to a small change in temperature. They

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0378-5173/$ – see front matter.
doi:10.1016/j.ijpharm.2012.02.024

possess a lower critical solution temperature (LCST) in aqueous solution, below which they are hydrophilic and absorb water to become swollen, and above which they are hydrophobic and expel water to become dense and dry. The thermoresponsive polymer, poly(*N*-isopropylacrylamide) (pNIPAm) has been extensively studied. Its LCST (Heskins and Guillet, 1968) is near physiological temperature (37 °C), and the highly temperature-sensitive transition between the hydrophobic and hydrophilic states (also known as the volume phase transition) is independent of other factors, such as pH (Pei et al., 2004). This polymer has been used in the construction of many pulsatile drug release systems that are triggered by altering the temperature, such as hydrogel matrices (Caykara et al., 2006; Coughlan et al., 2004), microspheres (Fundueanu et al., 2009b; Mundargi et al., 2010; Wei et al., 2009), membranes (Li and D'Emanuele, 2001), porous systems (Fundueanu et al., 2009a; Vertommen et al., 2008) and thin films (Doorty et al., 2003; Kavanagh et al., 2005). The release time for a single cycle for these various delivery systems ranged from approximately 20 min (Fundueanu et al., 2009b) to 15 h (Coughlan et al., 2004). In the current study, the release profiles indicate that the behaviour is diffusion dominated, although some behaviour characteristic of zero-order release was also observed (Fundueanu et al., 2009b). The parameters that can be used to control drug release from the system include the initial drug loading concentration, the geometrical dimensions of the system (such as thickness and surface area), and the durations the device is left switched on/off; the governing mathematical model described here incorporates all of these quantities.

A few models have previously been developed to describe the release behaviour from swelling delivery systems (Brazel and Peppas, 2000; Crank, 1975; Fujita, 1961; Grassi and Grassi, 2005; Kikuchi and Okano, 2002; Lee, 1985; Siegel and Pitt, 1995; Siepmann et al., 1998; Siepmann and Siepmann, 2008), and diffusion from thin films has been well studied (Cooke and Chen, 1995; McCaig et al., 2000; Sanches Silva and Cruz, 2007; Wang et al., 2007). However, in this paper, the first model to incorporate release from a system that alternates between a swollen hydrophilic state and a film-like hydrophobic state is described.

In this work, a fabrication procedure is described for thin hydrogel films loaded with rhodamine B, and the drug release behaviour from these films is analyzed. The objective of the study is to develop a new controllable drug delivery system based on thin UV-crosslinked thermoresponsive films, which can be characterised and tuned with the aid of a mathematical model.

2. Modelling pulsatile release

A one-dimensional model is formulated for drug diffusion in the film, and the release behaviour is considered for the case in which the temperature of the film is quickly and repeatedly switched between a value above the LCST and a value below the LCST. The motion of the drug molecules through the film is assumed to be governed by Fick's law and we denote by $c(x, t)$ the concentration of drug at penetration x and time t in the film. When the film is held at a temperature above the LCST, it is in a condensed state, and we denote by H_c, D_c the constant thickness and diffusivity of the condensed film, respectively. If the film is held at a temperature below the LCST, it is in a swollen state, and we denote by H_s, D_s the constant thickness and diffusivity of the swollen film, respectively. The lateral dimensions of the film are fixed because they are constrained by the wall of the containing well, and swelling/collapsing can only occur in the x direction. We shall find, as would be expected, that $D_c \ll D_s$, so that alternating the temperature between values above and below the LCST results in a release profile with an on/off pulsatile character.

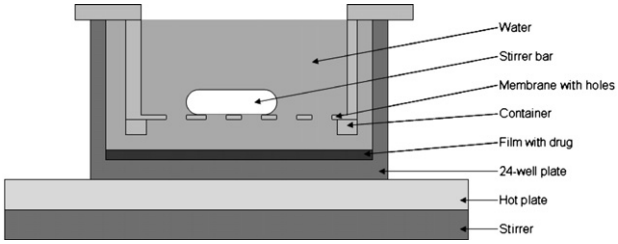

Fig. 1. The experimental setup for the drug release experiment at the start.

The time the polymer was left in either the fully swollen or fully collapsed state was typically of the order of minutes. However, in the experimental work for this paper, the time it took for the thin polymer film to either fully swell or fully collapse was considerably shorter than a minute. Hence, in the current model, we assume that the swelling and collapsing processes occur instantaneously. This assumption simplifies the problem considerably since incorporating the detail of the swelling or collapsing behaviour in the model would require the tracking of moving boundaries (Siepmann and Siepmann, 2008). This would lead to a much more challenging mathematical problem, from which an analytical expression for the release profile could not be in general obtained.

We suppose that at time $t = 0$ the film is at the temperature above the LCST and is fully collapsed. At time $t = t_1$ the temperature of the film is taken to instantaneously switch to the value below the LCST and the film is fully swollen; at time $t = t_2$ the film instantaneously reverts to the collapsed state, and so on (collapsed → swollen → collapsed → ...).

If $H(t)$, $D(t)$ denote the thickness of the film and the drug diffusivity at time t, respectively, then under the assumptions stated above, the concentration $c(x, t)$ of drug in the film is governed by:

$$\frac{\partial c}{\partial t} = D(t)\frac{\partial^2 c}{\partial x^2} \quad \text{for} \quad 0 < x < H(t), \tag{1}$$

where

$$D(t) = \begin{cases} D_c, 0 \le t < t_1, \\ D_s, t_1 \le t < t_2, \\ D_c, t_2 \le t < t_3, \\ \vdots \end{cases} \quad \text{and} \quad H(t) = \begin{cases} H_c, 0 \le t < t_1, \\ H_s, t_1 \le t < t_2, \\ H_c, t_2 \le t < t_3, \\ \vdots \end{cases} \tag{2}$$

Eqs. (1) and (2) are solved subject to the following conditions.

(i) The film is initially uniformly loaded with drug, so we take $c = c_0$ at $t = 0$ in $0 < x < H_c$ where c_0 is constant.
(ii) The bottom of the film (see Fig. 1), $x = 0$, is attached to a plastic cover slip substrate, which is taken to be impermeable to the drug, and so we impose $-D(\partial c/\partial x) = 0$ on $x = 0$.
(iii) Perfect sink conditions are assumed for the drug at the top surface of the film which is in contact with the eluting medium, and so we set $c = 0$ on $x = H(t)$.

The model (1) and (2) is readily solved subject to (i)–(iii) by separating variables (Crank, 1975) and the amount of drug released per unit area from the film by time t, $M(t) = H_c c_0 - \int_0^{H_c} c(x, t)dx$,

is easily calculated. The fraction of drug released from the film by time t is found to be:

$$\frac{M(t)}{M(\infty)} = \begin{cases} 1 - \displaystyle\sum_{n=1}^{\infty} \frac{2}{\lambda_n} \exp\left(-\lambda_n \frac{D_c t}{H_c^2}\right), & 0 \le t < t_1, \\ 1 - \displaystyle\sum_{n=1}^{\infty} \frac{2}{\lambda_n} \exp\left(-\lambda_n \left(\frac{D_c t_1}{H_c^2} + \frac{D_s(t-t_1)}{H_s^2}\right)\right), & t_1 \le t < t_2, \\ 1 - \displaystyle\sum_{n=1}^{\infty} \frac{2}{\lambda_n} \exp\left(-\lambda_n \left(\frac{D_c t_1}{H_c^2} + \frac{D_s(t_2-t_1)}{H_s^2} + \frac{D_c(t-t_2)}{H_c^2}\right)\right), & t_2 \le t < t_3, \\ \vdots \end{cases} \tag{3}$$

where $\lambda_n = ((2n-1)^2\pi^2)/4$. For the case in which the temperature in the film is held fixed for all time (non-pulsatile), the appropriate result can be obtained by simply setting $D_c/H_c^2 = D_s/H_s^2 = D/H^2$ (constant) in Eq. (3) to obtain:

$$\frac{M(t)}{M(\infty)} = 1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp\left(-\frac{(2n-1)^2\pi^2 Dt}{4H^2}\right) \quad \text{for all} \quad t > 0. \tag{4}$$

Eq. (3) gives an analytical expression for $M(t)$, the total drug released from the pulsatile system at time t. The development of such an expression is of significant practical value in both the design and operation of a pulsatile release device. In the design stage, it can be used to predict the thickness, surface area, and initial drug load to be used so that the resulting device can deliver doses of appropriate strength over an appropriate time-scale. In the operation stage, it can be used to devise a detailed schedule for when the device should be switched on and off so as to deliver the required doses at the desired time intervals.

To see how Eq. (3) might be used in practice, a hypothetical example is considered. Suppose it is required to deliver n equal doses of a drug, with each dose delivering an amount of drug d. It is further supposed that there is a fixed time interval T between the deliveries of each dose. In this example, it shall be assumed that some mechanism has been devised that quickly switches the device on and off and that the parameters D_c/H_c^2 and D_s/H_s^2 are known; a procedure for estimating these parameters from experimental data has been given in this paper.

The total drug loaded onto the device, $M(\infty)$, which of course must exceed nd here, can be controlled by varying either the volume of the device or the initial drug concentration. It is supposed that the device is deployed in the off state at time $t = 0$ and that it is first switched on at $t = t_1$. To determine the time $t = t_2$ at which the device should be switched off, we solve the equation:

$$M(t_2) - M(t_1) = d \tag{5}$$

for t_2 where $M(t)$ is given by Eq. (3); notice that this ensures that the required dose d is delivered over the time interval (t_1, t_2). Although Eq. (5) gives rise to a rather complicated expression for t_2 that cannot be solved analytically, t_2 can be readily estimated numerically with the aid of mathematical packages such as MATLAB® or MAPLE®. The device is switched on for the second time at $t = t_2 + T$, and then switched off again at $t = t_3$ where t_3 is determined by numerically solving: $M(t_3) - M(t_2 + T) = d$.

Proceeding in this way, it is clear how a schedule may be devised for switching the device on and off so as to deliver the required doses at the desired intervals.

The appropriate release formula for an isothermal system which does not swell or collapse is given by Eq. (4). This simpler form is very familiar and has been used on numerous occasions previously to describe diffusion from a planar sheet (Crank, 1975). We now obtain an estimate for the time it takes for a release device with a single constant diffusivity to empty its drug load; more precisely, we estimate the time it takes for there to be only a small fraction $r \ll 1$ of the initial drug load left remaining in the device. This time is such that $Dt/H^2 \gg 1$ and from Eq. (4) the following approximation is obtained:

$$\frac{M(t)}{M(\infty)} \approx 1 - \frac{8}{\pi^2} \exp\left(-\frac{\pi^2 Dt}{4H^2}\right) \quad \text{for} \quad \frac{Dt}{H^2} \gg 1.$$

This expression is used to solve for t_r, in $M(t_r)/M(\infty) = 1 - r$ to obtain

$$t_r \approx \frac{4H^2}{\pi^2 D} \ln\left(\frac{8}{\pi^2 r}\right) \tag{6}$$

as the estimate for the time at which there is a fraction $r \ll 1$ of the initial drug left in the device. Eq. (6) can be used to estimate the effective life-span of the pulsatile device. Clearly the device will have its minimum effective life-span, $t_{\min}$, if it is continually left on, so that $t_{\min} = ((4H_s^2)/(\pi^2 D_s)) \ln(8/(\pi^2 r))$. The device will have its maximum effective life-span, $t_{\max}$, if it is continually left off, and $t_{\max} = ((4H_c^2)/(\pi^2 D_c)) \ln(8/(\pi^2 r))$. The effective life-span of the pulsatile device then lies in the range $[t_{\min}, t_{\max}]$, and these useful quantities are readily calculated; for the system described in this paper, $t_{\min}$ is approximately 1 h and $t_{\max}$ is approximately 42 h for 95% of drug released ($r = 5\%$).

In Eq. (6), it is seen that there is a strong quadratic dependency on the thickness of the film, but that the dependence on the diffusivity is weaker, being inversely proportional to only the first power. The diffusivities D_c and D_s are determined largely by the nature of the material being used, although there is some flexibility in material choice and preparation. The thickness of the film, however, is a quantity that can be readily varied in practice, and given that the release time-scales have a strong dependence on film thickness, it is probably the most effective parameter to vary when designing a release device.

3. Materials and methods

3.1. Materials

N-Isopropylacrylamide (NIPAm, Aldrich) was re-crystallised from hexane and acetone and dried at room temperature under vacuum. Acrylamidobenzophenone was prepared from acryloyl chloride and 4-aminobenzophenone by a standard amidation reaction, with triethylamine as the acid scavenger. 2,2′-Azobisisobutyronitrile (AIBN, Phase Separation Ltd.) was re-crystallised from methanol. Benzene was dried under sodium-wire and distilled before use. All other solvents were reagent grade and purified by conventional methods before use. Rhodamine B was purchased from Aldrich and used as received.

3.2. Preparation of crosslinkable pNIPAm

N-Isopropylacrylamide (NIPAm) and the UV sensitive crosslinker, acrylamidobenzophenone (ABzPh) (molar ratio is 98.8 mol%: 1.2 mol%, 5 g of total monomers) were copolymerised by radical polymerisation using AIBN (0.7 mol%) as an initiator in benzene (30 ml) under argon to form poly(NIPAm-*co*-ABzPh). After polymerisation at 60 °C for 24 h, the mixture was precipitated in n-hexane. Precipitation was repeated three times using acetone as a solvent and n-hexane as a non-solvent. The polymer was dried at 45 °C in a vacuum oven, and after three precipitations the yield was 70%.

3.3. Lower critical solution temperature LCST of p(NIPAm-co-ABzPh)

The LCST was determined by the cloud point measurement obtained on a Cary 100 UV-VIS spectrophotometer equipped with a temperature controller, and twelve-position sample holder. Polymer aqueous solution (3.5 ml of 1 mg/ml) in a cuvette was heated at a rate of 0.1 °C/min whilst obtaining the absorbance at 500 nm wavelength. The solution temperature was determined by the internal temperature probe with a resolution of 0.5 °C and an accuracy of ±0.1 °C, were monitored.

3.4. Preparation of films

For experiments, crosslinkable copolymer solution was prepared in dry methanol (2% (w/w) polymer/methanol). Aliquots (50 μl and 125 μl respectively) of the solution were applied evenly to the wells of 24-well polystyrene tissue culture plates and polyethylene plastic cover slips (diameter 25 mm). The resulting films (5 μm thick) were allowed to dry at room temperature in a methanol atmosphere overnight. The films were then dried at 40 °C for 4 h under vacuum, and crosslinking initiated by exposure of covered plates to UV light at an intensity of 400 mW/cm^2 for 20 min, followed by inversion of the plates and exposure for a further 20 min. During irradiation, the polystyrene served as a long pass filter for UV light. All loading and release experiments were carried out on copolymer films cast on 24-well tissue culture plates. Films used for characterisations were prepared as above on polyethylene plastic cover slips (diameter 25 mm) unless stated otherwise.

3.5. Drug distribution in the film

An aliquot of copolymer solution (38.5 μl) was applied to glass bottom culture dishes (P35G-0-14-C, MetTek). The resulting films (5 μm thick) were dried and crosslinked as previously described. Rhodamine B-methanol solution was added to the surface of each film and dried in the methanol atmosphere at room temperature and then dried at 40 °C for 4 h under vacuum. Exposure to light was minimised. Images were recorded at room temperature using a Leica TCS SL confocal system and z-stack technique, which is described elsewhere (Kavanagh et al., 2005) in order to confirm the thickness and observe the rhodamine B distribution in the film.

3.6. Drug loading

Rhodamine B (50 μl, 0.4 mM) solution in dehydrated methanol was cast on top of crosslinked dry films of thickness (5 μm), which were deposited in a 24-well plate. The samples were dried in ethanol atmosphere at room temperature and then dried at 40 °C for 4 h under vacuum. Exposure to light was minimised. In order to obtain the loading efficiency, the solution used for rinsing off the rhodamine B on the surface of films before the release experiment and the solutions used for extracting the remaining rhodamine B after the release were collected and the rhodamine B concentration was determined by plate reader (FLx800, Bio-tek, Ex: 548 nm/Em: 590 nm). The drug loading efficiency was calculated by one minus the ratio of the number of moles of rhodamine B rinsed to the number of moles of total rhodamine B coated (composed of rinsed, controlled released and extracted).

3.7. In vitro drug release kinetics

In vitro drug release kinetic studies were performed, at different temperatures, by soaking the samples in distilled water (2 ml). The drug loaded samples were first rinsed twice with warm distilled water (40 °C) to remove the rhodamine B on the surface of the films. At regular time intervals 1 ml of the dissolution medium was withdrawn and analysed by plate reader. The same volume of fresh distilled water was added to replace the volume of the extracted samples. Any residual drug remaining in the samples was then extracted by injecting distilled water at 4 °C into the wells (30 min). A thermal plate (IC22XT, Torry Pines Scientific) and hotplate combined with magnetic stirrer (Fig. 1) were used to help maintain the film at the desired temperature. All experiments were carried out in triplicate. The release profile was obtained with and without stirring.

All of the sampling solution was removed into black 96-well plate, and then placed in a plate reader to obtain the drug concentration. The resulting data was subsequently analyzed with the aid of Microsoft® office Excel® and the final curves were produced using Originlab®.

3.8. Pulsatile release

The effect of thermal cycling on rhodamine B release from UV-crosslinked thermoresponsive polymer films was investigated. The drug loaded samples were first rinsed twice with warm distilled water (40 °C) to remove the rhodamine B on the surface of the films. Then the films were soaked into distilled water (1 ml, 37 °C) and at pre-designed time intervals, all the dissolution medium was withdrawn and replaced by distilled water (1 ml, 4 °C). After a pre-calculated time period, all of dissolution medium was replaced by same amount of distilled water (37 °C). Any residual drug remaining in the samples was then extracted by injecting distilled water at 4 °C into the wells (30 min). A thermal plate (IC22XT, Torry Pines Scientific) and hotplate combined with magnetic stirrer were used to help maintain the film at the desired temperature. The results of this experiment were compared with simulations of the mathematical model for values of the diffusion coefficients obtained from the fixed temperature release experiments at 4 °C and 40 °C, 25 °C and 37 °C, 28 °C and 37 °C, 30 °C and 37 °C were chosen to minimise the difference between high temperature and low temperature whilst pulsatile release profile still is obtainable.

4. Results and discussion

4.1. Drug loading

The drug loading efficiency was found by calculating (one minus rinsed drug/total drug), expressed as a percentage (Table 1). The total drug was composed of three parts: the rinsed fraction prior to release, the fraction released during the experiments, and the fraction extracted upon completion of the experiments.

4.2. Drug distribution

The thickness of the films was measured using confocal microscopy, with images of the film being acquired as z-stacks through the film depth. The measurement was carried out at six random locations on the film and in each case the thickness of the dry films was found to lie in the range 5.0 μm ± 0.2 μm. z-Stack images were used to determine the three dimensional distribution of the fluorescent drug in the dry film; the results (Fig. 2) confirmed that the loaded drug was uniformly distributed initially.

4.3. Fixed temperature release

The fraction of total drug released from the system as a function of time was measured for various temperatures, ranging from 4 °C to 40 °C. It is emphasised that the temperature is kept fixed for

Table 1
Calculation of drug loading efficiency.

The desired loading amount (mg)	Rinsed off (mg)	Total released (mg)	Loading efficiency (%)
0.01	0.0007 ± 0.0003	0.009 ± 0.002	92 ± 6

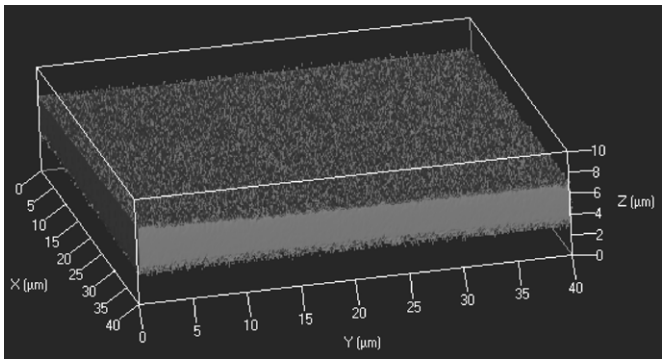

Fig. 2. 3D-fluorescent images of rhodamine B distribution in a dry film. The grey dots represent the fluorescent signal of rhodamine B.

each release experiment; the pulsatile release behaviour in which the temperature is varied in the experiment is discussed separately below. It is clear from this data that the rate at which drug releases from the system decreases significantly as the temperature is increased through the range from 27 °C to 34 °C. However, the release rate is less sensitive to changes in temperature for ranges below 27 °C or above 34 °C.

In Fig. 3, a selection of the experimental release profiles is displayed, together with theoretical curves obtained from our mathematical model fitted to the experimental data. The selection includes examples where the film is fully collapsed, fully swollen, and in an intermediate state between these two extremes. For each of these release experiments, the temperature is held fixed so that the appropriate theoretical release profile is given by Eq. (4) above. This expression was fitted to the experimental data using the method of least squares, with D/H^2 being the unknown parameter to be estimated. Once the estimate for D/H^2 has been obtained, D follows immediately if H is known. The nonlinear equation for

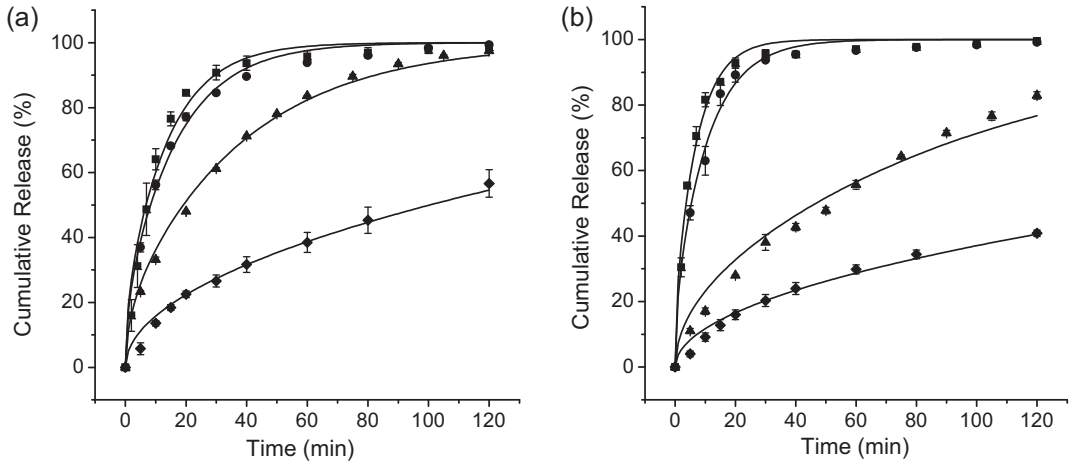

Fig. 3. Influence of temperature (■, 4 °C; ●, 25 °C; ▲, 32 °C; ♦, 37 °C) and stirring on the release of rhodamine B (20 nmol/film) from 5 μm thick UV-crosslinked polymer films (16 mm of diameter) in distilled water for 120 min. (a) Release profile with stirring. (b) Release profile without stirring. The continuous line curves correspond to solutions (4) of the mathematical model which have been fitted to the experimental data.

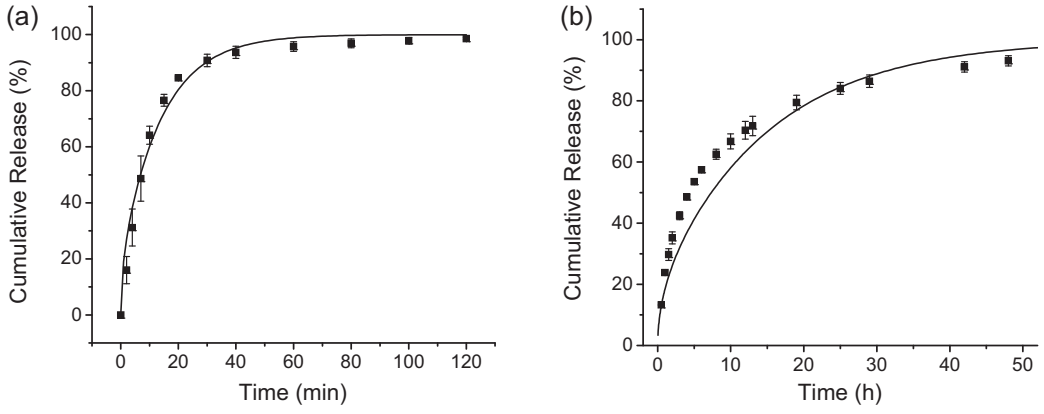

Fig. 4. Lifetime of the system. (a) Release profile of rhodamine B at 4 °C over 2 h. (b) Release profile of rhodamine B at 37 °C over 48 h.

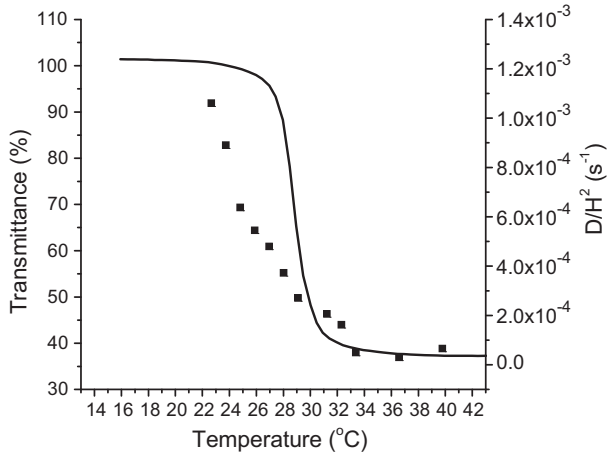

Fig. 5. Transmittance curve for the uncrosslinked polymer in aqueous solution together with D/H^2 values for the crosslinked film. The D/H^2 values (■) were obtained by fitting a theoretical curve to the data and the transmittance (continuous line) was obtained using the UV-Vis spectrophotometer of p(NIPAm-*co*-ABzPh) used to construct the thin films. The polymer concentration was 1 mg/ml in double distilled water and the heating ratio was 0.1 °C/min.

D/H^2 arising from this fitting procedure was solved numerically using the mathematical package MAPLE®, and the estimates for D/H^2 obtained from the fitting procedure are given in Fig. 5. In Fig. 3, it is observed that in all cases the fit between the experimental and theoretical curves.

By comparing the release profile of rhodamine B at 4 °C, 25 °C, 32 °C and 37 °C with and without stirring, it can be seen that the release profiles were very similar when the temperature is below LCST (4 °C and 25 °C). The release was slightly slower without stirring when the temperature is above the LCST (37 °C). At 32 °C, it is clear from Fig. 3 that stirring has dramatically increased the drug release rate. However 32 °C is close to the polymer's LCST, and the composition of the polymer is uncertain in this regime. We could not obtain reproducible experimental results close to the LCST, and the model described here is not appropriate for this regime.

4.4. Lifetime of system

In order to acquire the lifetime of this system, long-term release profiles of rhodamine B were obtained at 4 °C and 37 °C. The system was kept at 4 °C and 37 °C for 2 and 48 h respectively. The resulting release profiles are presented in Fig. 4. It is noted in Fig. 4(b) that there is some discrepancy between theory and experiment. This can be accounted for by noting that a non-negligible fraction of the drug has been retained by the collapsed polymer even after 40 h of release; a better fit can be obtained if the data is re-scaled so that the last data point of Fig. 4(b) corresponds to 100% release. This suggests that for the collapsed polymer, a small fraction of the drug releases extremely slowly, if at all. However, we have not attempted to incorporate this effect in the current modelling.

In 1 h, 98.6 ± 0.4% of rhodamine B was released at 4 °C and 91.1 ± 1.7% of model drug was release in 42 h at 37 °C.

4.5. Temperature dependence of the drug diffusivity across the LCST

In Fig. 5, transmittance is plotted versus temperature for the heating process of the copolymer pNIPAm-*co*-ABzPh aqueous solution, and this data shows that the transition temperature lies in the range of 28–30 °C. The scaled diffusivities D/H^2 were estimated at different temperatures by fitting the experimental data with the theoretical release profile given by Eq. (4), and the results are displayed in Fig. 5. This data reveals that the scaled diffusivity D/H^2 decreases by approximately one order of magnitude as the temperature is increased through the LCST, which conforms to our intuitive expectations since this temperature change corresponds to the transition from the swollen to the shrunken state for the film. The volume phase transition of the films used in this study was in the range of 22–30 °C. This transition temperature range is 6 °C wider than that for the uncrosslinked polymer in aqueous solution. It should be noted, however, that the LCST for the polymer in aqueous solution was found to be 3 °C higher than that for the crosslinked film (Feil et al., 1993; Zhang and Zhuo, 2001).

4.6. Pulsatile release

In Fig. 6, experimental drug release data is displayed for a system in which the temperature is repeatedly switched between 40 °C (above the LCST, slow release) and 4 °C (below the LCST, fast release). The resulting release profile has a clear on/off pulsatile character as would be expected. In the experiment, the system was held in the "off" state for a fixed 3 min in each cycle, but the time it was left in the "on" state was increased with increasing cycle number so as to achieve an approximately uniform dose for each cycle. However, for the first cycle, a larger dose was delivered. In Fig. 6, a theoretical curve based on Eq. (3) is also displayed which has been fitted to the experimental data using the method of least squares, with D_c/H_c^2, D_s/H_s^2 being the unknown parameters to be estimated. The results of the analysis reveal that D_c/H_c^2

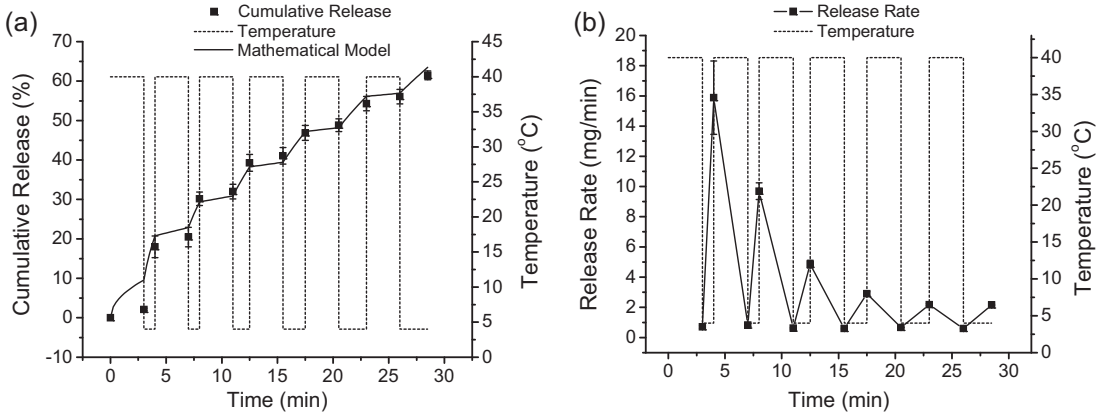

Fig. 6. (a) Pulsatile release profile between 4 °C and 40 °C. The cumulative release of rhodamine B (20 nmol/film) from 5 μm thick UV-crosslinked polymer films (16 mm of diameter) in distilled water at 4 °C and 40 °C were sampled at selected time points. The data points have been fitted to the mathematical model using Eq. (3). (b) Release rate profile corresponding to the cumulative release profile in (a).

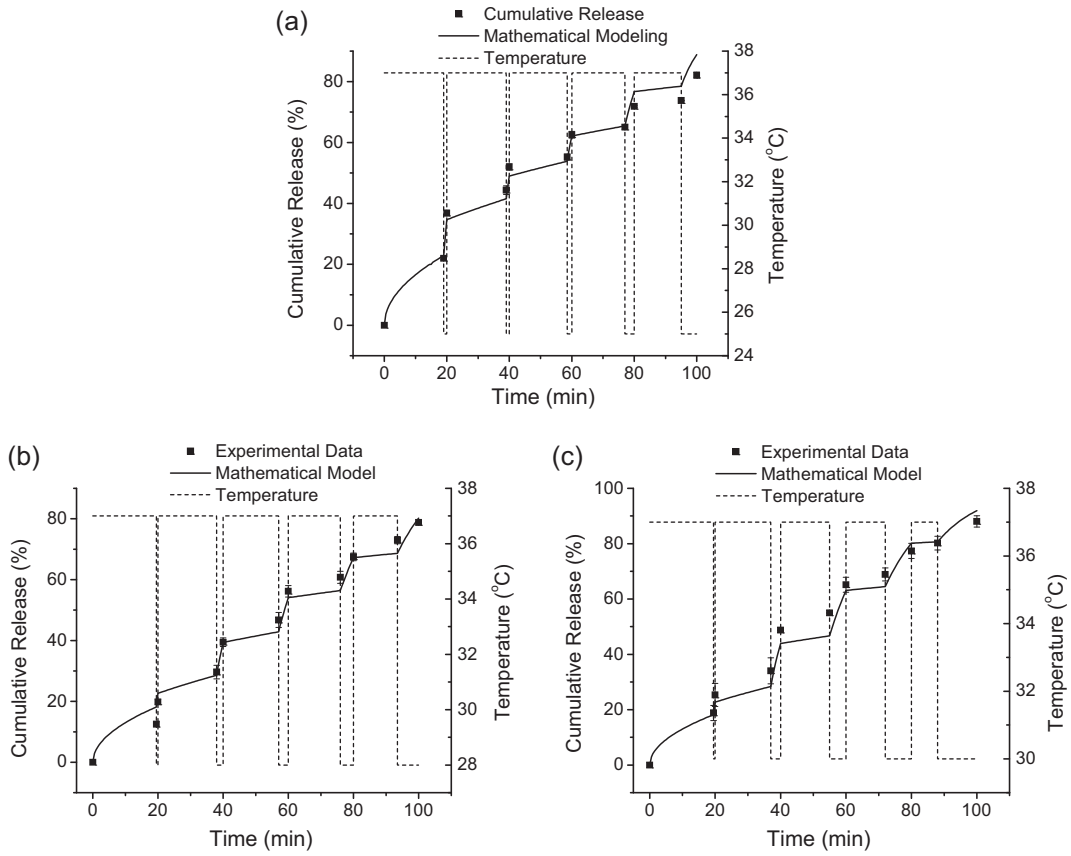

Fig. 7. Pulsatile release profiles for the UV-crosslinked polymer films (see Fig. 6 for details) where the temperature is varied between (a) 25 °C and 37 °C, (b) 28 °C and 37 °C, and (c) 30 °C and 37 °C.

is one order of magnitude smaller than D_s/H_s^2, which is consistent with the results of the fixed temperature experiments. The fit is again quite good indicating that our model, despite the simplifying assumptions made in its development, is adequate for capturing the essential features of the release behaviour. In this case, a total of six pulses were obtained. It should be noted that it is very difficult to realise a temperature transition of 36 °C (from 4 °C to 40 °C) in vivo. However, we shall show that the system is capable of successfully exhibiting pulsatile release for temperature transitions as modest as 7 °C (from 30 °C to 37 °C), which is clearly a much more realistic scenario from the point of view of potential applications.

Having established that the model adequately fits the experimental data, we used it to aid in the design of two further pulsatile release experiments. Specifically, we used the model to estimate when the temperature of the polymer should be changed in the experiments so as to ensure that there would be five cycles, each of duration of 20 min approximately, and that between 10% and 20% of the total drug would be released in each cycle. The results of the experiments are displayed in Fig. 7, and we see that these objectives were broadly achieved, except for the first temperature drop where less than 10% of the drug was released. It is also noteworthy that there is a much smaller jump in the temperature for these experiments, such as 12 °C for the pulsatile release of 25 °C and 37 °C, 9 °C for the pulsatile release of 28 °C and 37 °C, and only 7 °C for the pulsatile release 30 °C and 37 °C.

5. Conclusion

This study has shown that the copolymer of poly(NIPAm-*co*-ABzPh) is capable of incorporating and pulsatile releasing rhodamine B at designated time points. Two stages of release were observed: slow diffusion when the temperature is above the LCST range and swelling followed by more rapid diffusion when the temperature drops below the LCST. The mathematical model, which was developed based on Fick's law, has shown that the behaviour of the system can be adequately described by a time dependent diffusivity, and a formula for the drug fraction released as a function of time was constructed. Pulsatile release was observed and successfully modelled by our release formula by choosing appropriate diffusivities. As the results show, by carefully controlling the release time at low and high temperature, the release dosage of each cycle can be relatively stable at 10%. However, the minimum practicable release time is about 30 s, and this is too long to keep the dose delivered below 10% in the first release cycle. The model also provided an estimate for the effective life time of this system, and this was found to lie in the approximate range of 1–42 h. In our view, pulsatile systems such as the one developed and analysed here will form important components of future technologies that realise the goal of controlled drug release in vivo.

Acknowledgements

This publication has emanated from research work conducted with financial support from Science Foundation Ireland (08/RFP/MTR1201). The research is also supported by the Mathematics Applications Consortium for Science and Industry (MACSI, SFI Grant 06/MI/005).

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