On the Study of Indomethacin Release from PNIPAM-g-PEO Vesicular Nanoparticles

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Abstract: *A study of indomethacin release from poly(N-isopropylacrylamide)-graft-poly(ethylene oxide) PNIPAM-g-PEO vesicular nanoparticles was performed. In vitro release studies were conducted in distilled water containing 10% and 20% (v/v) ethanol added with different rate and using the dialysis tube method. The nano-sized polymeric vesicles were characterized with respect to morphology, drug loading content and in vitro drug release kinetics. A mathematical model for indomethacin release, recently proposed by the authors, was validated under the obtained experimental results. It was used for numerical simulation of indomethacin release from nanoparticles of the considered vesicular type in the solution, neglecting the presence of a membrane, within a period of 24 hours.*

Keywords: *Indomethacin release, Vesicular nanoparticles, Mathematical model, Numerical simulation.*

Introduction

In recent years, polymeric nanoparticles (NPs) with a core-shell structure have attracted much attention for their potential application in drug delivery. The small size and stealth properties ensure their prolonged blood circulation and access to desired organs and tissues [2]. Important advantages of core-shell NPs are high stability and drug-loading capacity, feasibility of incorporation of hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation [8, 9, 13].

A challenge in the drug targeting is the development of polymeric drug delivery carriers, which possess thermally responsive fragments. Among the temperature responsive polymers, poly(N-isopropylacrylamide) (PNIPAM) has attracted special interest because it undergoes a sharp coil-to-globule transition in water at around 32°C (the lower critical solution temperature, LCST) changing from a hydrophilic state below the LCST to a hydrophobic state above it [14]. The self-association of PNIPAM copolymers can afford core-shell NPs with PNIPAM chains incorporated into either a core or a shell [13]. The formation of core-shell structures above the phase transition temperature of double-hydrophilic block copolymers consisting of PNIPAM as a thermo sensitive segment and poly(ethylene oxide) (PEO) as corona-forming block is well established [5, 11, 15, 16]. The number and distribution of PEO grafts, the length of PNIPAM backbone as well as the copolymer concentration have a pronounced effect on the performance of such copolymers. The lower copolymer concentration and higher degree of PEO grafting lead to the formation of particles smaller in

size. Smaller but less dense NPs can be produced by fast heating of dilute solutions of the copolymers [5]. This fact is important as the larger free volume inside the PNIPAM core not only provides opportunity for efficient loading of larger amounts of compatible drugs but also can be a reason for significant acceleration of the drug release rate.

Drug incorporation into PNIPAM-g-PEO NPs has not been reported so far, except for our recent study on the self-association behavior of similar copolymers below the LCST, triggered by no covalent interactions between PNIPAM and the hydrophobic drug indomethacin (IMC) [10]. In the present work, we extend these studies to the formation of IMC-loaded PNIPAM-g-PEO vesicular NPs in mixed solutions of ethanol and water. The influence of the quantity and addition rate of ethanol on the IMC release are tested using a dialysis tube release assembly, which ensures a constant membrane area for the diffusing drug molecules. The drug release profiles are analyzed by a recently proposed mathematical model [4] considering drug-polymer interactions and dialysis membrane permeability to the tested drug. The model is validated by fitting with the obtained experimental results and can be used as an effective simulation tool in the design of NPs of the considered vesicular type.

Materials and methods

Materials

Preparation and characterization of IMC-loaded PNIPAM-g-PEO vesicular NPs

PNIPAM-g-PEO vesicular NPs containing IMC were prepared by the nanoprecipitation technique [3, 7] at 20°C. In this technique, hydrophobic drugs are dissolved into a water-miscible organic solvent and the resulting solution is injected into an aqueous solution of a copolymer. As the organic solvent diffuses through the dispersing aqueous phase, small monodisperse nanostructures are formed as a result of interfacial hydrodynamic phenomena [7, 12]. The incorporation of IMC into PNIPAM-g-PEO NPs was performed in mixtures of ethanol and water (1:9 and 2:8, v/v) at a drug-to-polymer ratio of 1.5:1 (w/w) according to the following procedure. The copolymer was dissolved in water and to this IMC solution in ethanol was added either quickly in one shot (fast injected, 0.2 $ml \cdot s^{-1}$) or at a slower rate (slowly injected, 0.015 ml/s) under moderate magnetic stirring. The obtained clear solution having a final polymer concentration of 0.09 mg/ml was allowed to equilibrate for 1 h, then filtered through 0.80 μm membrane filter (Sartorius AG, Germany) to remove precipitating drug (if any) and the filtrate was immediately analyzed.

IMC loading content was determined by UV-vis spectrometry (Hewlett Packard 8452A) at 310 nm using the standard method. First, the total IMC concentration was assayed after appropriate dilution (25-times) of the filtered sample with ethanol. Then, to obtain the quantity of encapsulated drug, the NPs were separated from the aqueous phase by ultrafiltration (15 min, 6000 g, Amicon Ultra, 100 000 MWCO) and the amount of dissolved, non-encapsulated IMC (the free part) was determined in the resulting filtrate. The IMC loading content was calculated from the difference between the total drug concentration in the nanoparticle solution and the free part of IMC.

The morphology of PNIPAM-g-PEO vesicular NPs were investigated using HRTEM (high resolution transmission electron microscope, JEOL JEM 2100, Japan) operated at 200 kV. The samples were prepared by placing a drop of the sample solution on a carbon-coated copper grid followed by drying at room temperature. No staining was used for the TEM observations because of the good electron contrast of the tested objects. A TEM image of PNIPAM-g-PEO vesicular NPs produced by fact addition of ethanol is presented in Fig. 1.

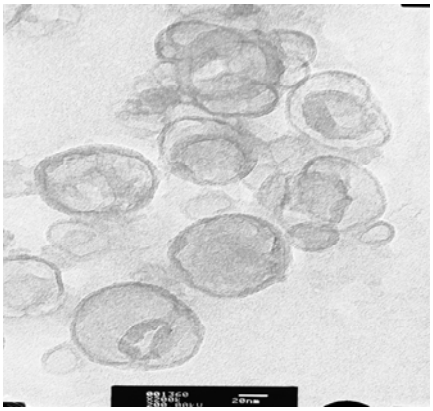

Fig. 1 TEM image of IMC-loaded PNIPAM-g-PEO vesicular NPs prepared by fast injection

In vitro IMC release studies were conducted in distilled water containing either 10% or 20% (v/v) ethanol using the dialysis tube method [6]. In the release experiment, 5 ml of the polymer some solution were transferred into a dialysis tube closed at one end with a dialysis membrane (7.065 cm^2 exposed area, MWCO 12 000-14 000, Sigma-Aldrich, Germany). The dialysis tube was immersed into an outer vessel containing 100 ml of release medium whose ethanol concentration was the same as that of the tested NPs solution. During the experiment the temperature of the entire assembly was maintained at 37°C ± 0.5°C, using a circulating water jacket (Huber, Germany). At appropriate intervals, 3-ml aliquots were withdrawn from the outer aqueous solution and replaced with an equal volume of fresh release medium. The released IMC was quantified by UV spectroscopy (Hewlett Packard 8452A) at 320 nm, using a standard calibration curve. Each release experiment was repeated four times.

Numerical modeling

The following assumptions for the experimentally observed drug release were accepted [4]:

(1) Two main coupled physicochemical processes control IMC release. The first one is the release of the bound IMC from the vesicular NPs included in the formulation. Diffusion of the free drug from the inner container into the outer aqueous phase realizes at the same time;
(2) The concentrations of the free drug and the bound one are uniformly distributed in the inner container;
(3) The predominant mechanism of the drug release kinetics from the NPs is overcoming the polymer-drug interaction;
(4) A perfect sink condition at the boundary from the outer membrane side exists.

The model equation describing the first process is the following [1]:

$$M_b(t) = M_{b0}\left(1 - \frac{1}{(1+at)^{3/2}}\right), \tag{1}$$

where M_b and M_{b0} are the current value of the decreasing mass of the bound drug within the inner container and its initial value; t is the time. The model parameter a is a rate constant referred to overcoming the interaction between the polymer and the embedded drug in the NPs.

The equation of the diffusion of the free drug mass from the inner container, denoted by M_f, is derived on the basis of the Fick's law and the assumptions (2) and (4) as follows [4]:

$$\frac{dM_f(t)}{dt} = -\frac{K}{H} M_f(t), \tag{2}$$

where $K = \frac{DP}{h}$ is the permeability constant; D is the drug diffusivity; h is the membrane thickness; P is the distribution coefficient.

The total mass of the drug released in the outer tube within a period of t hours is denoted by $\overline{M}(t)$, referred to the initial total drug mass and the main model equation for fractional drug release under zero initial condition is obtained:

$$\frac{d\overline{M}}{dt} = \frac{K}{H}\left(\frac{M_{fo}}{M_o} + \frac{M_b(t)}{M_o} - \overline{M}(t)\right), \quad \frac{M_b}{M_o} = \left(1 - \frac{M_{fo}}{M_o}\right)\left(1 - \frac{1}{(1+at)^{3/2}}\right), \tag{3}$$

where $M_{fo} = M_o - M_{bo}$ is the initial value of the free drug mass in the solution given from the experiment and H is the height of the solution in the inner tube.

The following procedure for numerical evaluation of the main model parameters (the drug permeability K and the rate parameter a) was proposed:

1st step. The case of drug pure solution (i.e. $M_{fo} = M_o$) in the inner container is considered in order to evaluate the permeability K of IMC by fitting the model equations (2) to the obtained experimental data.

2nd step. Evaluation of the rate parameter a under the determined permeability K fitting the model equations (3) to the experimental data for fractional IMC release from PNIPAM-g-PEO NPs assembly.

To evaluate the goodness of fit the determination coefficient is calculated at each of the above steps, as follows:

$$R^2 = 1 - \frac{\sum_{n=1}^{N}\left(R_{num}^n - R_{exp}^n\right)^2}{\sum_{n=1}^{N}\left(R_{arithm}^n - R_{exp}^n\right)^2}, \tag{4}$$

where R_{arithm}^n is the arithmetic mean of the experimental data of the considered fractional drug release; R_{num}^n and R_{exp}^n, n = 1, …, N are the numerical results and the experimental data respectively.

3rd step. Numerical simulation of IMC release from the considered NPs following (1) under the evaluated value of the model rate parameter a.

Numerical results

The proposed procedure for numerical evaluation of the model parameters was applied under 37°C for the cases: IMC release in the medium of 10% ethanol of the obtained solution prepared by slow and fast injection; IMC release in the medium of 20% ethanol under the above conditions. Fitting the model to the experimental data for IMC release from pure solution under 10% and 20% ethanol the model parameter K was found as follows:

$K = 0.12$ cm·h^{-1} and $K = 0.1$ cm·h^{-1} ($R^2 = 0.995$).

Finite difference method was applied when evaluating the fractional drug release $\overline{M}(t)$. Standard least square method was used when fitting the model equations with the experimental data.

Fig. 2 and Fig. 3 represent the model fitting to experimental data for IMC release from PNIPAM-g-PEO NPs assembly under slow and fast injection for 10% and 20% ethanol, respectively, when $R^2 = 0.98$ on average. The obtained values of the model rate parameter a are not significantly different (especially for 10% ethanol). It is observed a tendency of drug release increase with ethanol content decrease as well as with increase of the rate of injection.

The model parameter a was evaluated under the experimentally measured initial partial free drug quantity $\frac{M_{f0}}{M_0}$ equal to 13.54%, 8.48%, 25.57% and 12.6% respectively.

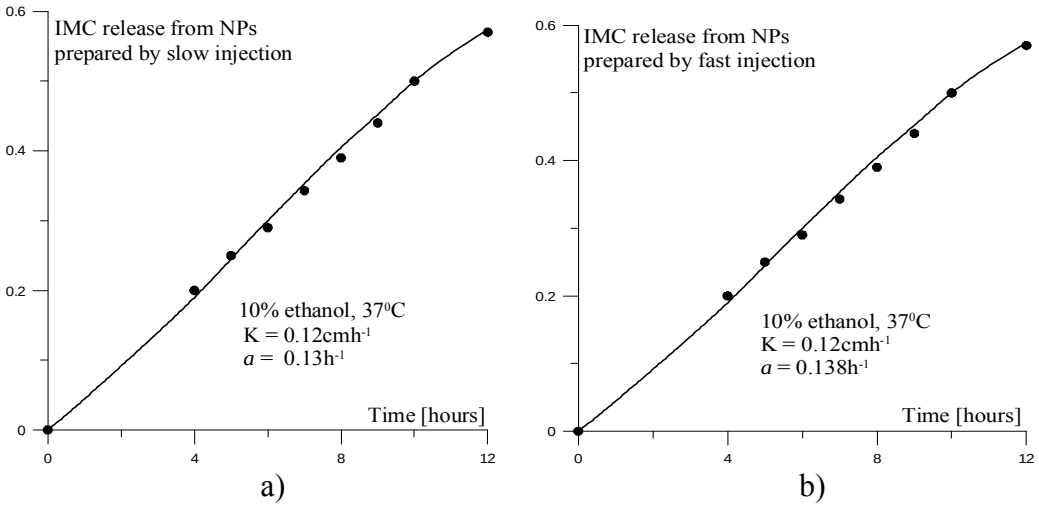

Fig. 2 Validation of the model for IMC release from NPs at 10% ethanol prepared by: (a) slow injection and (b) fast injection

In order to obtain a more reliable picture of IMC release from NPs in the solution we performed numerical simulation of the fractional release under evaluated model rate parameter a for each of the cases. It was assumed that the unique process is to overcome the interaction between the polymer and the embedded drug in the NPs described by Eq. (1).

In Fig. 4 it is shown the comparison of the model numerical curves of the fractional drug release for 10% and 20% ethanol under solution obtained by slow injection as well as for fast and slow injection under 20% ethanol. In the other cases significant differences between the numerical results were not obtained.

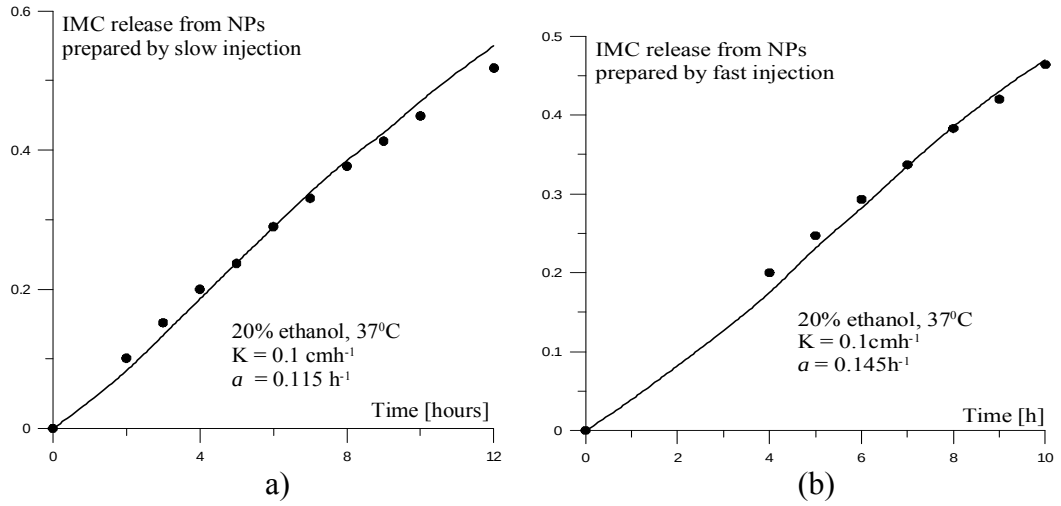

Fig. 3 Validation of the model for IMC release from NPs at 20% ethanol prepared by: (a) slow injection and (b) fast injection

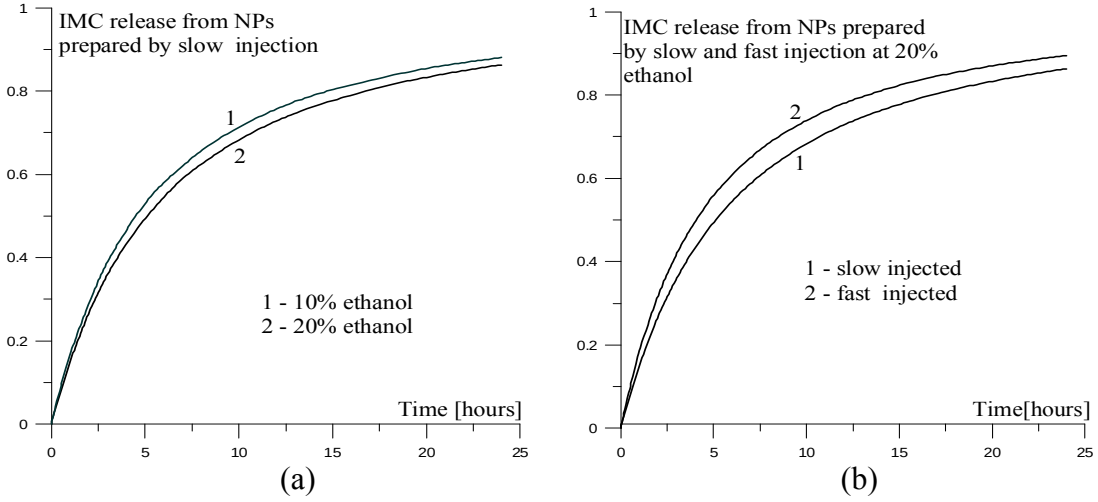

Fig. 4 Simulation of IMC release from NPs in the solution prepared by: (a) 10% and 20% ethanol at slow injection and (b) slow and fast injection at 20% ethanol

Conclusion

A study of the release of the socially important drug IMC from a new type of vesicular NPs was performed.

IMC-loaded PNIPAM-g-PEO vesicular NPs were formed in an organic solvent/water system as a result of drug-polymer physical interactions. The nano-sized polymeric vesicles were characterized with respect to morphology, drug loading content and *in vitro* drug release kinetics.

A mathematical model, recently proposed by the authors [4], was used to continue the study of IMC release kinetics from PNIPAM-g-PEO NPs. The experiments for drug release were performed in two steps in accordance with the first two steps of the numerical procedure for evaluating the model parameters. The first experiment was realized for pure drug solution whether the second one – for the NPs solution. The idea of a conditional separation of the

main processes enables the consecutive evaluation of the drug permeability and the rate constant instead of using more complicated numerical procedures.

The model equations were validated for two different values of ethanol content and two different rates of ethanol injection. It was determined a weak tendency of increase of IMC release with decrease of ethanol content and increase of the rate of injection. Numerical simulation of IMC release from the considered NPs solution was performed for all considered cases. Observable differences of IMC release were established for 10% and 20% ethanol under slow injection as well as for fast and slow injection under 20% ethanol.

The presented mathematical model and numerical procedure can be used as an effective simulation tool in a future study of NPs solutions of the considered type.

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