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Tunable thermo-responsive hydrogels: Synthesis, structural analysis and drug release studies

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ABSTRACT

Thermo-responsive hydrogel films, synthesized by UV-initiated radical polymerization, are proposed as delivery devices for non-steroidal anti-inflammatory drugs (Diclofenac sodium and Naproxen). N-isopropylacrylamide and N,N′-ethylenebisacrylamide were chosen as thermo-sensitive monomer and crosslinker, respectively. Infrared spectroscopy was used to assess the incorporation of monomers into the network, and the network density of hydrogel films was found to strictly depend on both feed composition and film thickness. Calorimetric analyses showed negative thermo-responsive behaviour with shrinking/swelling transition values in the range 32.8–36.1 °C. Equilibrium swelling studies around the LCST allowed the correlation between the structural changes and the temperature variations. The mesh size, indeed, rapidly changed from a collapsed to a swollen state, with beneficial effects in applications such as size-selective permeation or controlled drug delivery, while the crosslinking degree, the film thickness, and the loading method deeply influenced the drug release profiles at 25 and 40 °C. The analysis of both 3D-network structure, release kinetics and diffusional constraints at different temperatures was evaluated by mathematical modelling.

1. Introduction

Hydrogels are a class of materials containing large volumes of water in their swollen 3D-structure without dissolution [1]. This feature was carried out to the development of highly hydrated and biocompatible networks showing a quite similarity to the body tissues. The biocompatibility, biodegradability, non-toxicity, high drug-loading capacity, release rate control, and pharmaceutical stability make this class of hydrogels suitable for biomedical application, such as drug delivery and tissue engineering [2].

Generally, hydrogels can be divided into two categories, namely conventional and smart. The latter devices are able to change their shape and volume in response to external stimuli, such as pH [3], temperature [4], electric and magnetic fields [5], or by coupling more of them [6]. The design of new polymeric systems plays a key role in the development of stimuli-responsive biomaterials and represents an interesting item for researchers.

Temperature is one of the most widely used trigger for pulsatile drug delivery, since the body temperature often deviates from the physiological value (37 °C) in the presence of pathogens or pyrogens. Sometimes this deviation can act as useful stimulus activating the release of therapeutic agents from various temperature-responsive delivery systems. Thus, thermo-responsive hydrogels have recently attracted extensive interest due to their potential and promising applications in different fields, such as protein–ligand recognition [7], on–off switches for modulated drug delivery [8], artificial organs [9], and enzyme immobilization [10,11].

Among temperature-responsive smart polymers, those based on N-isopropylacrylamide (NIPAAm) are the most widely studied for pharmaceutical applications [12,13]. In literature, indeed, key examples of the preparation of NIPAAm-based thermo sensitive hydrogel films, synthesized by different approaches, and proposed as drug delivery vehicles, have been reported.

Abdon Pena-Francesch et al. describe a series of thermo-responsive thin hydrogel films prepared by initiated chemical vapour deposition of NIPAAm and ethylene glycol diacrylate in the presence of hydrophilic co-monomers in order to modulate the LCST [14]. Via in-situ polymerization technique involving magnetite, NIPAAm and hydrophilic acrylamide crosslinked with N,N′-methylenebisacrylamide, Korotych et al. developed hydrogel films sensitive to both physiological temperature and external magnetic field [15]. The synthesis of interpenetrating polymer networks by one-step polymerization of NIPAAm and calcium alginate has been reported by Petrusic et al. [16]. Minghonga et al. proposed the use of polypropylene as substrate for grafting of NIPAAm onto its surface [17].

In this work, the correlation between temperature, hydrogel films swelling properties, releasing profile and network parameters, such

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as the number-average molecular weight between cross-links, $\overline{M_c}$, the network mesh size, ξ, and the equilibrium weight swelling ratio, q, is extensively evaluated by employing suitable mathematical models [18–21]. For this purpose, we prepared thermo-responsive hydrogel films by UV-initiated radical polymerization of NIPAAm and N,N′-ethylenebisacrylamide (EBA), acting as functional monomer and cross-linker, respectively. To evaluate the influence of hydrogel film composition on the network parameters and releasing feature, four different hydrogel films were prepared combining two NIPAAm to EBA molar ratio with two thickness values.

Furthermore, the influence of drug loading procedure (e.g. soaking after polymerization and incorporation during crosslinking) was investigated by employing Diclofenac sodium (DC) and Naproxen (NPX) as model drugs. DC and NPX are strong non-steroidal anti-inflammatory drugs with analgesic effects and may cause side effects [22], thus, the identification of strategies to reduce toxicity and to increase their pharmacological effect may be highly relevant [23]. The release profiles were first evaluated at 25 and 40 °C; subsequently, pulsatile drug release experiments were performed by temperature cycling around the LCST.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAAm), N,N′-ethylenebisacrylamide (EBA), Naproxen (NPX), Diclofenac sodium (DC), glacial acetic acid, and ammonium acetate were provided from Sigma-Aldrich (Sigma Chemical Co, Milan, Italy). Irgacure 2959 (1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, with a maximum absorption at around 275 nm, from Ciba) was used as photoinitiator. Acetonitrile, ethanol, methanol and water were from Carlo Erba Reagents (Milan, Italy) and all are of HPLC grade.

2.2. Synthesis of thermo-responsive polymers

For the synthesis of hydrogel films, NIPAAm and EBA were dissolved in a suitable volume of water (Table 1) and the solution was purged with gaseous nitrogen for 20 min. Irgacure 2959 was finally added as photoinitiator. For the polymerization reaction, two 10 × 10 cm^2 glass plates, separated with a Teflon spacer (0.8 or 1.6 mm), were brought together using binder clips and the polymerization was initiated by a high pressure mercury lamp (Philips HPK 125, 500 mW cm^{-2}, wavelength 275 nm, irradiation time 10 min). Finally, the obtained films (code # P_1, P_2, P_3 and P_4) were extensively washed with water to remove unreacted species and, then, dried for 12 h in an oven under vacuum at 40 °C.

2.3. FT-IR spectroscopy

Fourier transform infrared (FT-IR) spectra of monomers and hydrogel films were obtained from pellets in KBr with a FT-IR spectrophotometer (model Jasco FT-IR 4200) in the wavelength range of 4000–400 cm^{-1}. Signal averages were obtained from 100 scans at a resolution of 1 cm^{-1}.

2.4. Shape and surface morphology

Morphological analyses (top views and cross sections) were performed by a Leica LEO 420 scanning electron microscope. Samples were placed on appropriate stubs and, then, sputtered with gold (thickness ~ 100 Å) under argon atmosphere to achieve the necessary conductivity. To analyse film cross sections, samples were cryo-fractured after immersion in liquid nitrogen. The accelerating voltage was 15 kV under high vacuum conditions.

2.5. Thermo-behaviour of hydrogel films

DSC thermograms of hydrogel films were recorded on Netzsch DSC200 PC, and the obtained LCST values are reported in Table 1. In a standard procedure, samples were immersed in distilled water at room temperature for at least 2 days to reach the swollen state. About 10 mg of the swollen samples was placed in aluminium pans and then hermetically sealed with aluminium lids. Thermal analyses were performed from 25 °C to 55 °C under a dry nitrogen atmosphere with a flow rate of 25 ml min^{-1} and a heating rate of 3 °C min^{-1}. The onset point of the endothermal peak, determined by the intersecting point of two tangent lines from the baseline and slope of the endothermic peak, was used to determine LCST [24].

2.6. Swelling studies

Hydrogel films cross-linked structure was investigated by the method of Peppas and Meadows [18]. Specimens of ~1 cm^2 were cut from each sample and weighed in air and in a non-solvent by placing the sample in a stainless-steel mesh basket suspended in *n*-heptane. Then samples were placed in a PBS buffer (pH 7.0) at 25 °C and 40 °C for 3 days to reach swelling equilibrium, and again weighed in air and in *n*-heptane, after being blotted with a tissue to remove surface moisture.

2.7. Loading of drugs into hydrogel films by soaking procedure

Incorporation of drugs (NPX or DC) into hydrogel films was performed as follows: 200 mg of empty polymer was wetted with 2.0 ml of a concentrated drug solution (10 mg ml^{-1}). After 3 days under slow stirring at 37 °C, the polymer was filtered and dried at reduced pressure in presence of P_2O_5 to constant weight. The loading efficiency percent (LE%) was calculated by HPLC analysis of filtered solvent according to Eq. (1):

$$LE(\%) = \frac{C_i - C_0}{C_i} \times 100 \quad (1)$$

Table 1
Synthesis and physical and structural characterization of thermo-responsive hydrogels.

Code	Chemical composition		Physicochemical parameters						
	NIPAAm (g/mmol)	EBA (g/mmol)	Molecular weight between cross-links M_c (g mol^{-1})		Mesh size ξ (Å)		Equilibrium swelling ratio, q		LCST (°C)
			25 °C	40 °C	25 °C	40 °C	25 °C	40 °C	
P_1^*	1.45/12.8	0.108/0.64	1140	325	25.0 ± 1.2	9.8 ± 0.2	35.4 ± 0.2	15.6 ± 0.2	32.8 ± 0.1
P_2^*	1.69/14.9	0.250/1.49	678	201	18.6 ± 0.4	9.1 ± 0.2	32.1 ± 0.3	12.3 ± 0.3	35.7 ± 0.1
P_3^{**}	1.69/13.5	0.108/0.64	1265	352	27.8 ± 1.1	9.9 ± 0.3	35.1 ± 0.2	13.3 ± 0.5	34.8 ± 0.1
P_4^{**}	1.53/14.9	0.250/1.49	801	230	18.8 ± 0.9	9.4 ± 0.2	33.6 ± 0.1	10.5 ± 0.7	36.1 ± 0.1

Water volume = 7.0 ml; thickness film = 0.8 mm* and 1.6 mm**; NIPAAm = N-isopropylacrylamide; EBA = N,N′-ethylenebisacrylamide; LCST = Lower Critical Solution Temperature.