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Synthesis, characterization and slow drug delivery of hydrogels based in N-acryloyl-tris-(hydroxymethyl) aminomethane and N-isopropyl acrylamide

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ABSTRACT

In this study, new hydrogels in rod shape were prepared from N-isopropyl acrylamide (NIPA), N-acryloyl-tris-(hydroxymethyl) aminomethane (NAT) and N,N′-methylenebisacrylamide (BIS). The effect of the incorporation of NAT into poly(N-isopropyl acrylamide) (PNIPA) structures for which the monomer composition was varied from NIPA 100% to NAT 100% was explored. The rheological studies were used to test their viscoelastic properties. Swelling experiments were used to test the capacity of water absorption, the modification of the network parameters, the swelling kinetics, the temperature and pH swelling response and *n* (number that determines the type of diffusion of water). NAT-containing hydrogels showed values of *n* between 0.5 and 1, therefore the diffusion of water into the hydrogels was found to have a non-Fickian character. The elastic moduli and the equilibrium water content (EWC) measurements suggest that these materials may have a potential application as biomaterials. The structure of shrunken NIPA 100 at high temperature that maintains the drug and hinders the release was controlled by the introduction of NAT into the network, to allow a slow drug release of ibuprofen at 37 °C and pH 7.4.

1. Introduction

A hydrogel is a kind of polymeric three-dimensional network that exhibits ability to absorb and to swell in water, maintaining its form until attaining a certain equilibrium balance. In the hydrogels, the process of water absorption is reversible and in numerous cases dependent on the environmental conditions to which the hydrogel is exposed pH [1,2], temperatures [2–6], light [7], ionic concentration [8], pH and temperature [9], etc. This important characteristic to control the swelling process depending of the environment conditions allows to yield hydrogels to be used in controlled drug release systems [10–16] and other important applications such as immunoassays [17], separation processes [18–20] and immobilization of enzymes [21].

It is well known that poly(N-isopropyl acrylamide) (PNIPA) undergoes a rapid and reversible hydration–dehydration change in response to small temperature cycles around its lower critical solution temperature (LCST) in aqueous media at 32 °C. Below this temperature, the NIPA-containing hydrogels are swollen and hydrated, whereas above this temperature, they collapse and the water is expulsed. These have been the most frequently thermo-sensitive hydrogels studied over the past few years for academic and industrial interest [22]. In general, the control of the swelling–deswelling properties of this kind of hydrogels with the temperature has been used as on–off pulsatile control drug delivery systems [23,24]. In this system, the drug is released bellow of LCST (32 °C) of PNIPA and retained at 37 °C [25–27]. However, there are no so many jobs that use the PNIPA collapsing properties to obtain a constant release of hydrophobic drug at 37 °C [28] which could be relevant applications in control drug delivery. In this sense, the copolymerization of NIPA with hydrophilic monomers like N-acryloyl-tris-(hydroxymethyl) aminomethane (NAT) or macromonomers could permit the design of slow drug release system at 37 °C for diverse applications.

On the other hand, NAT is a hydrophilic monomer not commonly employed in the synthesis of hydrogels. It was previously used and reported as a component of multifunctional polyacrylamides [29] and in gels for electrophoresis and isoelectric focusing [30]. Therefore, this monomer was utilized to form a quasi-interpenetrating network with poly(vinylpyrrolidone), and the product was employed as a separation matrix for double and single-stranded DNA fragments by capillary electrophoresis [31]. We reported studies [32,33] in which different hydrogels in rod shape were prepared. Alvarez and Arrua [32] synthesized hydrogels using NAT and 2-hydroxyethyl methacrylate, acrylamide or acrylic acid as co-monomers, in all cases crosslinked with BIS. Some of the final products were selected to perform urea release assays, conducted through swelling-controlled release. Cuggino et al. [33] prepared

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hydrogels from NAT using three different crosslinking agents: poly(2-methyl-2-oxazoline) bismacromonomer (MC); ethylene glycol dimethacrylate (EGDMA) and BIS. Several of the assays performed on these materials suggested that they may have potential applications as biomaterials.

The present study focuses on the preparation of new hydrogels that were obtained by radical co-polymerization from two monomers: NIPA and NAT, and BIS as crosslinking agent. The monomer composition was varied from NIPA 100% to NAT 100%. The new hydrogels incorporated two kinds of monomers, each one performing a different role: monomer NIPA allowed the gel to swell and to deswell reversibility in response to small changes in temperature, and monomer NAT introduced hydroxyl functional groups, efficient for a planned post-modification for a possible immobilization of biological agents. The effect of the incorporation of NAT into PNIPA structures on the rheological properties, the modification of network parameters, the kinetics of swelling, the determination of the type of diffusion of water into the hydrogels, the temperature and pH swelling response and the controlled of IBU release using the new materials were studied. Then, it was demonstrated that NIPA 100%-containing structure that shrinks or collapses at high temperatures maintaining the drug and impeding the release can be slightly modified by the introduction of a monomer as NAT into the network, to allow a slow controlled release of a hydrophobic drug like IBU.

2. Materials and methods

2.1. Materials

The following chemicals were purchased and used as received: N-isopropyl acrylamide (NIPA) (Aldrich), N-acryloyl-tris-(hydroxymethyl) aminomethane (NAT) (Aldrich), N,N′-methylenebisacrylamide (BIS) (Mallinckrodt), benzoyl peroxide (BPO) (Fluka) and ibuprofen (IBU) (Aldrich). Dimethylformamide (DMF) (Cicarelli) was purified through vacuum distillation (84 °C/90 mm Hg).

2.2. Physico-chemical characterization

Fourier Transform Infrared (FT-IR) spectra were obtained on a Nicolet 5-SXC FT-IR spectrometer on KBr discs. A rotational rheometer Anton Paar Physica MCR 301 was used to characterize the hydrogels. The UV–visible spectra were recorded with a Shimadzu recording spectrophotometer UV-260.

2.3. Synthesis of the hydrogels

The hydrogels were prepared by free-radical solution crosslinking polymerization. A typical procedure for co-polymerization is described as follows: the monomers (NIPA and NAT) and the crosslinking agent (BIS) were dissolved in DMF in a glass tube (14 mm internal diameter and 15 cm long) and used as a polymerization reactor and stirred for 20 min with an ultrasonic bath. Nitrogen was bubbled through the solution for 15 min. The initiator (BPO) was then added to each solution and left to react for 2.5 h at 80 °C. Table 1 summarizes the experimental conditions. The products were referred to as NAT 100; NIPA:NAT (30:70); NIPA:NAT (50:50); NIPA:NAT (70:30) and NIPA 100. After breaking the tubes, the matrices obtained in long cylindrical shapes were cut into pieces and immersed twice in a large excess of distilled DMF at room temperature for 24 h. Afterwards, they were exhaustively washed with water and dried under vacuum at 25 °C to constant weight for 2 days. The dry discs (100–150 mg) were 7–8 mm in diameter and between 2.5 and 3 mm thick.

2.4. Network characterization

2.4.1. The network parameters

The polymer volume fraction of the swollen state (v_{2s}) in water was calculated as Eq. (1) [4], where W_e is the weight of swollen hydrogel at equilibrium, W_d is the weight of dry hydrogel, ρ_2 is the density of the dry polymer and ρ_1 is the density of liquid of swelling (water). To determine W_e, weighted dried samples were placed into distilled water and kept at 25 °C for swelling. The swollen samples removed from the water bath at regular intervals were superficially dried with tissue paper, weighted by an electronic balance and placed in the same bath. The measurements were carried out until a constant weight was achieved.

$$V_{2s} = |1 + \{[(W_e/W_d - 1)\rho_2]/\rho_1\}|^{-1} \quad (1)$$

The determination of the density of the hydrogels was performed by measuring the mass, radius and thickness of the dry discs. The volume was calculated as a cylinder.

The molecular weight between crosslinks M_c was calculated as Eq. (2) [8], where Φ is the functionality of the crosslinking agent ($\Phi = 4$), V_1 is the molar volume of the solvent (18 mL mol^{-1}), χ is the polymer solvent interaction parameter calculated using Eq. (3) [18] and v_{2r} is the volume fraction of the hydrogel after its preparation calculated as Eq. (4) [4,8], in which C_0 is the initial monomer concentration (mol mL^{-1}) and V_r is the average molar volume of the polymer repeat units [Eq. (5)]. In Eq. (5), M is the molecular weight of monomers and f is the mole fraction of monomers.

$$M_c = [(1 - 2\Phi^{-1})V_1\rho_2 v_{2r}^{2/3} v_{2s}^{1/3}]/[\ln(1 - v_{2s}) + v_{2s} + \chi v_{2s}^2] \quad (2)$$

$$(\chi = 1/2 + v_{2s}/3) \quad (3)$$

$$v_{2r} = C_0 V_r \quad (4)$$

$$V_r = (M_{NIPA} f_{NIPA} + M_{NAT} f_{NAT})/\rho_2 \quad (5)$$

In addition, the network pore size ξ was calculated as Eq. (6), in which C_n is the Flory characteristic ratio of the linear polymer (9.15 was used, corresponding to PNIPA) [34] and l is the length of the bond along the polymer backbone (1.54 Å for vinyl polymers) [35]. This parameter was determined at 25 and 37 °C.

$$\xi = v_{2s}^{-1/3}\left\{[(2C_n M_c)/V_r]^{1/2}\right\} l \quad (6)$$

2.4.2. Swelling measurements

The degree of swelling in water at time t (DS_t), at equilibrium (DS_e) and equilibrium water content (EWC) were determined according to Eq. (7)–(9) [36], where W_t is the swollen mass of hydrogel at time t. For the swelling curves, DS_t values at each time were plotted vs. time (min).

$$DS_t = (W_t - W_d)/W_d \quad (7)$$

$$DS_e = (W_e - W_d)/W_d \quad (8)$$

$$EWC = (W_e - W_d)/W_e \quad (9)$$

To determine the process of diffusion of water into the matrices at 25 °C, the Fick's law model was used as in Eq. (10) [4,18], where M_t is the amount of water diffused into the matrix at time t, M_∞ is the amount of water diffused into the matrix at equilibrium, k is a constant related to the structure of the network, and exponent n is a number that determines the type of diffusion of water.

$$F = M_t/M_\infty = kt^n \quad (10)$$

Eq. (10) is applied to the initial stages of swelling (60%). Plots of ln F versus ln t were drawn using the kinetics of swelling, and n and k values were calculated from the slopes and intercepts of the lines, respectively.

Table 1
Experimental reaction conditions used in the synthesis of the products.

Product[a]	NIPA (mmol)	NAT (mmol)	Type of product	DS_e[b]	DS_e[c]	EWC	*n*
NAT 100	–	9.89	Soluble	–	–	–	–
NIPA:NAT (30:70)	2.86	7.02	Insoluble	3.37	2.91	0.77	0.58
NIPA:NAT (50:50)	4.94	4.96	Insoluble	3.59	3.26	0.78	0.56
NIPA:NAT (70:30)	6.98	2.85	Insoluble	4.45	3.32	0.82	0.61
NIPA 100	9.89	–	Insoluble	6.08	3.57	0.86	0.99

[a] All products were yielded using 0.77 mmol of BIS, 0.04 mmol of BPO and 5 mL of DMF and left 2.5 h at 80 °C.
[b] Determined in water at 25 °C.
[c] Determined in buffer phosphate pH = 7.4 at 25 °C.

2.4.3. Study of swelling at pH 7.4

For this study, the hydrogels were swollen at equilibrium in buffer phosphate, pH = 7.4 for 24 h. Dry weighted samples (100 mg approximately) were placed into a bath within a beaker containing 15 mL of the buffer at 25 °C. Then, they were superficially dried with tissue paper and weighted with an electronic balance. DS_e values were calculated according to Eq. (8) for this pH.

2.4.4. Studies of swelling temperature-response

For the studies of swelling in response to changes in temperature, the hydrogels were swelled at equilibrium in water in a range of temperatures between 22 and 45 °C for 24 h. Dry samples (100 mg approximately) were placed into the bath with 15 mL of distilled water at each temperature for 24 h, then removed from the bath, superficially dried with tissue paper, weighted with an electronic balance and re-equilibrated into the same bath at a different temperature. DS_e was calculated according to Eq. (8) for each temperature.

2.4.5. Studies of swelling pH-response

For the studies of swelling in response to the changes in pH, the hydrogels were swollen at equilibrium in a range of pH between 3 and 10 for 24 h. Dry weighted samples (100 mg approximately) were placed into a bath within a beaker containing 15 mL of each liquid (Britton–Robinson buffer [37]) at 25 or 37 °C. Then, they were superficially dried with tissue paper, weighted by an electronic balance and re-equilibrated in another solution of different pH. DS_e was calculated according to Eq. (8) for each pH. Then, DS_e was plotted versus pH.

2.4.6. Rheological studies

For the rheological studies, the hydrogels were swelled at equilibrium in water at 25 or 37 °C. Dry samples were placed into a bath containing 15 mL of distilled water at each temperature for 24 h, after which they were removed from the bath, superficially dried with tissue paper and cut in 1.5 mm of thickness. The elastic, G', and the viscous, G'', moduli of the matrices were measured in a rotational rheometer using parallel plates of 8 mm in diameter and gaps of approximately 2 mm. The dynamic moduli were measured in a small-amplitude oscillatory shear flow at 25 and 37 °C. The stress sweeps at a constant frequency of 10 s^{-1} were performed on each sample at 25 °C to determine G' values and the linear viscoelastic region profiles of each material at this temperature by shearing them until the structure breakdown. In addition, the same assays were analyzed for NIPA 100 and NIPA:NAT (70:30) at 37 °C. Then, frequency sweeps at a constant stress were applied to the samples over a wide range of frequencies (0.1–1000 s^{-1}) to study the viscoelastic performance of the hydrogels. No evidence of dehydration was found during the tests.

2.5. Drug loading and pulsatile temperature release

2.5.1. Drug loading

Samples of hydrogels NIPA 100 and NIPA:NAT (70:30) (100–150 mg) were swollen with 2 mL of IBU buffer phosphate (pH = 7.4) solution (5.2 mg/mL), allowing enough time for the drug to diffuse into the gel (48 h). The samples were dried until constant weight under vacuum to entrap the drug. One milliliter of each remaining solution was diluted to 25 mL and the absorbance was measured by UV–visible spectrophotometry at 264 nm. The amount of drug in the remaining solutions was determined and calculated using the extinction coefficient obtained from an appropriate calibration plot. The amount of drug entered into each hydrogel was calculated by difference.

2.5.2. Drug release assays

For the release studies, the loaded dry samples were placed into a tubular recipient containing 5 mL of phosphate buffer (pH = 7.4), under stirring. The samples were first kept at 25 °C by 1 h. Aliquots (1 mL) were removed periodically at this temperature from the released solution and the IBU amount determined using UV–visible measurements at 264 nm. The final drug concentration from aliquots was then calculated using the extinction coefficient previously measured. The discs were then transferred to an identical bath maintained at 37 °C for 3 h. The assay to determine the drug released was the same as that explained above for 25 °C. The experience was carried out at 25 °C again (for 3 h) and finally at 37 °C for 11 h.

In all cases, each aliquot (1 mL) removed periodically (to determine the amount of IBU released) was replaced by 1 mL of phosphate buffer (pH = 7.4). So the volume in the solution was always 5 mL. The actual values of IBU were corrected in all cases according to the IBU removed in all aliquots.

3. Results and discussion

3.1. Synthesis of the hydrogels

In this study, new hydrogels were synthesized by free radical co-polymerization of mono-vinyl monomers as NIPA and NAT, di-vinyl monomer BIS as crosslinking agent (Fig. 1), DMF as solvent and BPO as initiator at 80 °C for 2.5 h. Table 1 summarizes the experimental reaction conditions. Every product was obtained in rod shape, except NAT 100, which in these conditions kept soluble in the reaction medium.

3.2. Network characterization

3.2.1. FT-IR

For the confirmation of inclusion of both monomers into the hydrogels, they were characterized by FT-IR. Fig. 2 shows the infrared spectra of the hydrogels NIPA:NAT (30:70) to NIPA 100. The

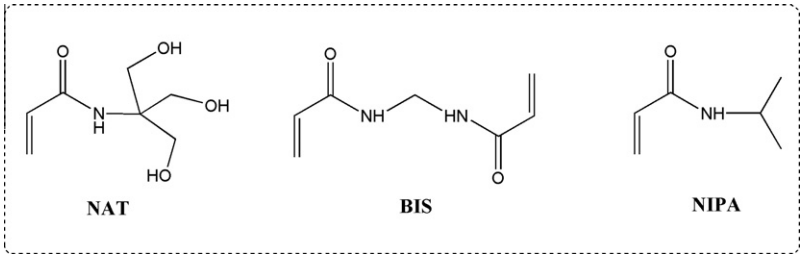

Fig. 1. Monomers and crosslinker used in the synthesis of the products.

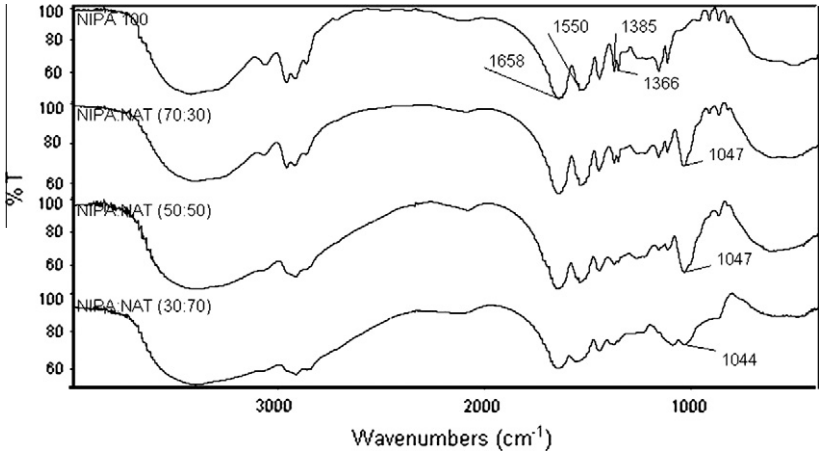

Fig. 2. FT-IR of dry hydrogels.

absorption band present in the spectra of NIPA:NAT (70:30); NIPA:NAT (50:50) and NIPA:NAT (30:70) at 1047–1044 cm^{-1} was assigned to the C–O vibration belonging to NAT. The broad band at 1658 cm^{-1} in all the spectra corresponds to the carbonyl of the amide group. The band at 1550 cm^{-1} and the double band at 1366 and 1385 cm^{-1} can be assigned to the N–H vibration and to the C–H deformation of the ter-butyl group of NIPA, respectively, which relatively increases from NIPA:NAT (30:70) to NIPA 100. Thus, the presence of the characteristic bands in the spectra confirms the reaction of co-polymerization between NIPA and NAT in the products.

3.2.2. The network parameters

In order to evaluate the possibility of using a hydrogel like a controlled drug delivery material, it is very important to know the network parameters that define its structure at a particular temperature. Basically, to characterize the structure of hydrogels, three important parameters were evaluated at 25 and 37 °C, the polymer volume fraction in the swollen state (v_{2s}), the effective molecular weight of the polymer chain between crosslinking points (M_c), and the network pore size (ξ) (Table 2).

Table 2
Network parameters of hydrogels at 25 or 37 °C in water.

Product	25 °C			37 °C		
	v_{2s}	M_c (g/mol)	ξ (Å)	v_{2s}	M_c (g/mol)	ξ (Å)
NAT 100	–	–	–	–	–	–
NIPA:NAT (30:70)	0.22	2909	47.17	0.21	3115	49.10
NIPA:NAT (50:50)	0.21	3076	50.90	0.26	1546	34.03
NIPA:NAT (70:30)	0.20	4075	63.15	0.31	715	22.81
NIPA 100	0.17	6516	90.75	0.57	44	4.93

From Table 2, the influence of both the composition of monomers in the medium of reaction and the temperature of swelling in the network parameters of the hydrogels can be observed. At 25 °C, v_{2s} decreases slightly but M_c and ξ increase in hydrogels with an increase in the amount of NIPA in the products according to their swelling capacity in water (DS_e, Table 1).

At 37 °C v_{2s} increases but M_c and ξ decrease with an increase in the amount of NIPA in the products. In general, the network parameters of the products are influenced by temperature although this influence is almost depreciable for NIPA:NAT (30:70). In this sense, it can be seen that v_{2s}, M_c and ξ for NIPA:NAT (30:70) did not suffer changes in varying the temperature since it did not show deswelling or collapse with temperature (37 °C). However, more noticeable modifications can be found in the parameters v_{2s}, M_c and ξ for the hydrogels with a large amount of NIPA. This behavior can be explained by the NIPA properties caused by changes in the intermolecular interactions with temperature. At 25 °C, the hydrogel NIPA 100 (without NAT) swells because the amido groups form intermolecular hydrogen bonds with the surrounding water at low temperature. As soon as the temperature increases, these interactions weaken and the hydrophobic interactions by the isopropyl groups become predominant, and consequently a collapsed state is produced at 37 °C.

3.2.3. Swelling parameters

When dry gels are placed into contact with any fluid, the fluid starts to penetrate into the polymeric network at certain rate and decreases until reaching the equilibrium. This equilibrium is achieved when both, the force generated by the molecules of fluid to enter the polymer, and the elastic forces generated by the polymeric chains, are equilibrated.

Table 1 shows the results of the different swelling equilibrium parameters in water, and Fig. 3 exhibits the swelling kinetics of

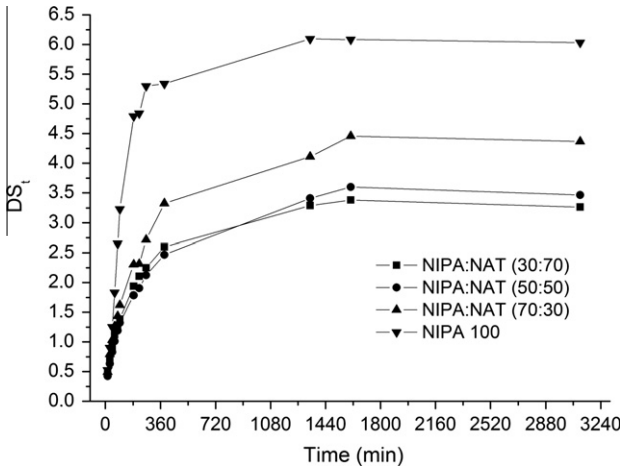

Fig. 3. Swelling kinetic of the hydrogels in water.

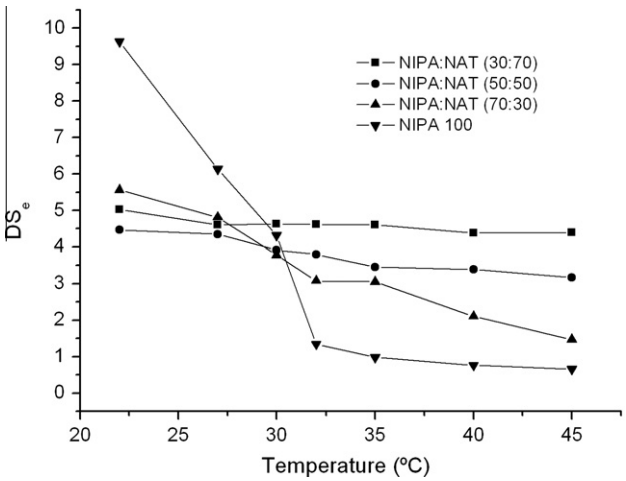

Fig. 4. Effect of temperature on DS_e.

the hydrogels at 25 °C. The degree of swelling at equilibrium, DS_e and equilibrium water content, EWC, increases when the amount of NIPA increases in the polymer (Table 1). At 25 °C, NIPA:NAT (70:30) swelled but in a low proportion with respect to NIPA 100, possibly due to the formation of intermolecular hydrogen bonding between amide and hydroxyl groups [38], decreasing the number of the hydrophilic group of the hydrogel for which the affinity of the gel towards water decreases. The NAT monomer could also cause high intra molecular hydrogen bonding interaction by the presence of the three hydroxyl groups [29]. Therefore, the interaction with water molecules and the amount of water absorbed on the matrix might be low.

Fig. 3 shows the swelling kinetics of the hydrogels in water at 25 °C. Interestingly, the swelling equilibrium of the products was achieved at 25 h. The number that determines the type of diffusion of water, *n*, ranged between 0.5 and 1 in NAT-containing hydrogels (Table 1). These results indicate that the mechanism of penetration of water is controlled, in these cases, by diffusion and chain polymer relaxation (Non-Fickian) [39]. NIPA 100 presented a value of *n* close to 1 (Case II transport) indicating that the dominant mechanism for drug transport was due to polymer relaxation as the hydrogel swells, for which the water penetration was independent of the time [39].

Table 1, however, shows the DS_e values of the products in buffer phosphate (pH = 7.4) at 25 °C. As shown, these DS_e values decreased with respect to those determined in distilled water. The values in buffer phosphate at pH 7.4 were determined because the loading of the drug and temperature pulsatile release were performed at that pH.

In addition, the EWC values of the hydrogels (0.77–0.86, Table 1) were higher than those found in most human tissues (about 0.6). This suggests that the hydrogels may have biomedical applications [33,36].

3.2.4. Studies of swelling temperature-response in water

To establish the approximate LCST of the products, the degree of swelling at equilibrium, DS_e, was determined and plotted versus the temperature. Fig. 4 shows the experimental results. As expected, NIPA 100 showed a noticeable transition of phase at around 32 °C. Interestingly, the incorporation of a low proportion of NAT monomer into the hydrogels produces a slight increase in the LCST probably due to the hydrophilic character of this monomer. These results were in agreement with other studies, where hydrophilic monomers were co-polymerized and combined with NIPA. The transition of phase was progressively less marked when more NAT was incorporated. For NIPA:NAT (30:70), this effect was not evidenced. This could derive from a decrease in the hydrophobic aggregation of NIPA sequences. The incorporation of the hydrophilic co-monomer like NAT, disrupts the NIPA 100 sequence resulting in the decreased aggregation force of dehydrated NIPA 100 chains at higher temperatures [4,10,40].

3.2.5. Studies of swelling pH-response in buffer

Fig. 5 shows the experimental results of the studies related to the effect of pH (using the Britton–Robinson buffer) on DS_e at 25 or 37 °C. As it can be noted, from slight amount of NAT incorporated into the structures, there is a notorious dependence of the swelling in NAT-containing hydrogels on solutions of pH higher than 7. It could be explained by a possible ionization of the hydroxyl groups on the main network chains. This effect was previously observed for poly(2-hydroxyethyl methacrylate) gels at different pH values [41].

3.3. Rheological characterization

Fig. 6 displays the storage modulus (G') of the hydrogels obtained during dynamic shear stress sweeps at 25 °C for all products and at 37 °C for NIPA 100 and NIPA:NAT (70:30) using a frequency of 10 s^{-1}. This test determines the shear range of the linear response of polymeric materials [42].

At 25 °C, Fig. 6 shows that NIPA 100 presents wider range of linear viscoelastic behavior than those of NAT-containing hydrogels. Beyond the linear viscoelastic region, the storage modulus

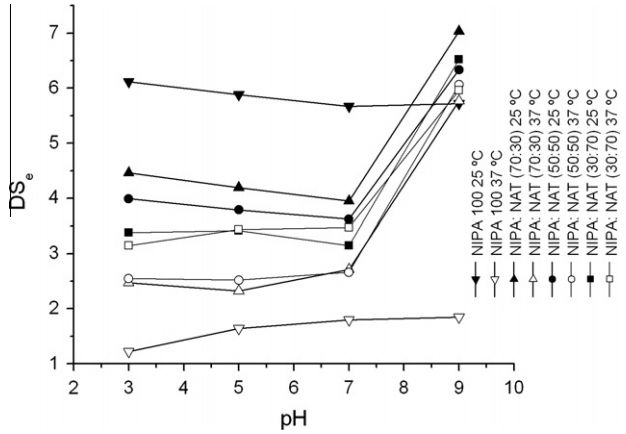

Fig. 5. Effect of pH on DS_e.

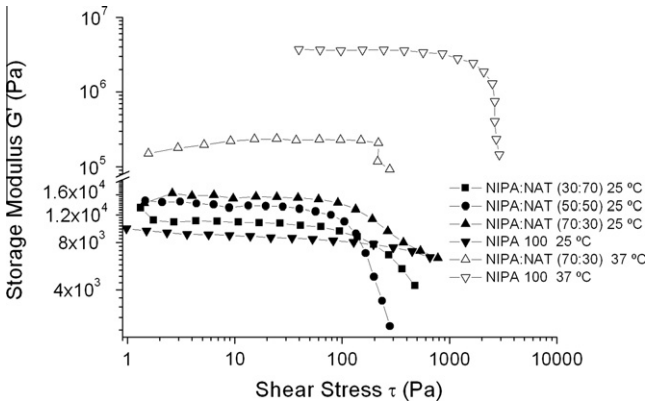

Fig. 6. Dynamic amplitude sweep of hydrogels.

decreased indicating (as critical shear stress) that the structure breakdown occurred as a consequence of the large deformation imposed. NIPA 100, with the same concentration of crosslinking agent as other hydrogels, has a critical shear stress (limit of linear response) several times larger than that of other hydrogels.

At 37 °C, an increase can be found in the critical shear stress and the extension of the linear viscoelastic region. These effects are much more pronounced in the product NIPA 100 in which the collapse resulting from the hydrophobic interactions by isopropyl groups is more noticeable. In addition, a considerable increase in the G' value [for NIPA 100 and NIPA:NAT (70:30)] is observed as it represents the elastic component of the material deformation correlated with the increase in crosslinks by the collapse at 37 °C.

The rheological characterization was completed with (dynamic) frequency sweeps performed at 25 °C for all hydrogels and at 37 °C only for NIPA 100 and NIPA:NAT (70:30) to analyze their performance at different frequencies. The strains used were smaller than the critical one for each material (range 0.1–1% at 25 °C and 0.01–0.1% at 37 °C depending on the hydrogel). The storage moduli are displayed in Fig. 7 as a function of frequency. The results showed that the storage moduli are practically not influenced by the frequency of oscillation for NIPA:NAT (50:50) and NIPA:NAT (30:70). Nevertheless, products NIPA 100 and NIPA:NAT (70:30) showed an increase in the G' value at higher frequencies at 25 °C, since at this temperature the large chains with few crosslinkings tend to re-arrange themselves and stiffen up, assuming a more "solid-like" behavior characterized by a more sharp increase in G'. This explanation correlates with M_c values at 25 °C of products NIPA 100 and NIPA:NAT (70:30). At this temperature, M_c values were higher for NIPA 100 and NIPA:NAT (70:30) than for NIPA:NAT (50:50) and NIPA:NAT (30:70), leading to a longer relaxation time with a consequent increase in G. At 37 °C, the effect above explained is practically not found since the G' values for NIPA 100 and NIPA:NAT (70:30) are merely influenced, even at high frequencies. At that temperature, the network collapses leading to an increase in the crosslinking joints, reducing the relaxation times and causing almost no change in the G' values. This explanation correlates with that for the M_c values at 37 °C.

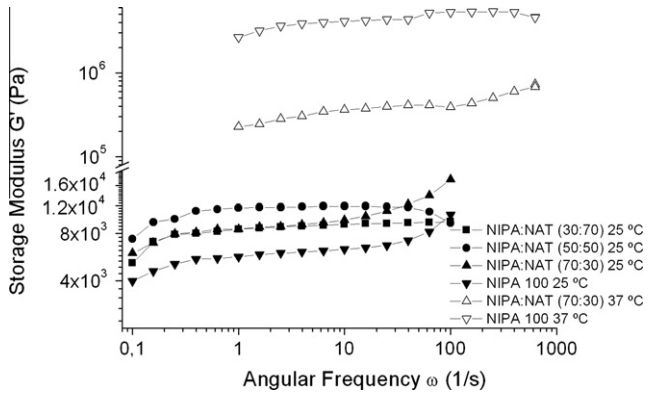

Fig. 7. Dynamic angular frequency sweeps for hydrogels.

3.4. Drug loading and temperature pulsatile release

3.4.1. Drug loading

NIPA 100 presented higher power of load for IBU (43.3 mg IBU/g of polymer) than NIPA:NAT (70:30) (32.0 mg IBU/g of polymer). This could be caused by the slightly higher capacity of NIPA 100 to absorb the buffer solution at 25 °C at equilibrium (Table 1).

3.4.2. Drug release assays

Poorly water-soluble drugs as ibuprofen are candidates for incorporation into polymer dispersion (physical gels) for topical administration or into hydrogels that release them at the small intestine, minimizing the adverse gastric drug effects after oral application [43,44]. In this part, IBU was employed as a model drug in order to investigate drug release behavior at pH 7.4.

Fig. 8 shows the cumulative IBU release from NIPA 100 and NIPA:NAT (70:30). The chemical structure of this drug is displayed in Fig. 9. In the two cases, the release of IBU occurred by diffusion at 25 °C as the hydrogels swelled allowing medium influx, and thus, drug diffusion.

For NIPA 100 load with IBU, a marked increase in temperature values above the transition temperature of NIPA 100 caused the formation of a dense, shrunken layer [25] on the gel surface (skin layer) which hindered water permeation from inside the gel into the environment and the drug release was stopped altogether from

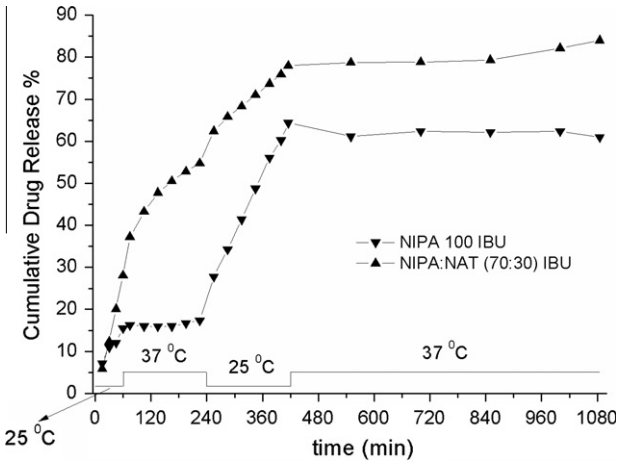

Fig. 8. Cumulative IBU release from NIPA 100 to NIPA:NAT (70:30).

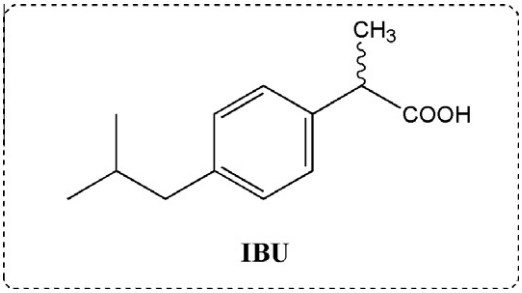

Fig. 9. Chemical structure of IBU.

the gel, achieving an on–off release regulation. At this temperature a possible interaction between the drug and the network by the presence of their isopropyl groups contributes to the formation of the skin layer by the collapsed components of the network preventing drug release from the inside [45]. As can be seen, this drug was largely kept (about 40%) by the gel until the final experimental conditions assayed (1080 min at 37 °C).

NIPA:NAT (70:30) exhibited a different behavior with respect to NIPA 100. As can be seen, the presence of NAT in the network produced less thermo-sensitivity [26] and allowed drug release at 37 °C because the hydrogel was not completely dehydrated at this temperature, and therefore the release occurs by diffusion from the network. It can thus be concluded that the NIPA 100%-containing structure that shrinks or collapses at high temperatures maintaining the drug and impeding the release, can be slightly modified by the introduction of a monomer as NAT into the network, to allow a slow controlled release of a hydrophobic drug like IBU.

4. Conclusion

New rod-shaped hydrogels were prepared by co-polymerization of NIPA, NAT and BIS. The effect of the incorporation of NAT into PNIPA structures, for which the monomer composition ranged from NAT 100% to NIPA 100%, was studied. About the results, it was concluded that both the composition of monomers in the products and the temperature of swelling influenced the network parameters of the hydrogels. At 25 °C, DS_e and EWC increased when the amount of NIPA increased in the network. At that temperature, M_c and ξ increased in general, in hydrogels with a high proportion of NIPA according with their DS_e. At 37 °C, M_c and ξ decreased with a proportional increase in NIPA. NIPA:NAT (30:70) was merely altered in M_c and ξ with some variations in temperature since it did not show collapse with the temperature.

The changes produced in the hydrogels by the presence of NAT can be summarized as follows: firstly, a little amount of NAT in the hydrogel structures produced changes in the capacity to shrinkage with the increase in temperature (25–37 °C). Secondly, the incorporation of a low proportion of NAT into the hydrogels slightly increased the LCST. Thirdly, a dependence of the swelling on high values of pH in NAT-containing hydrogels was also observed. Finally, the presence of a 30% of NAT into hydrogels NIPA:NAT (70:30) allowed a slow controlled release at 37 °C of IBU (being the controlled release rate in continuous form) which was not possible with NIPA 100. In addition, the inclusion of NAT could permit the immobilization of different drugs or biological agents like enzymes or peptides to its hydroxyl groups.

As general conclusion, NIPA 100%-containing structure that shrinks or collapses at high temperatures maintaining the drug and impeding the release can be slightly modified by the introduction of a monomer as NAT into the network, to allow a slow controlled release of a hydrophobic drug like IBU. Furthermore, the hydrogels developed in this study exhibited fluid contents (EWC) and viscoelastic properties similar to those found in soft body tissues, suggesting that they have potential biomedical applications.

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