molecular pharmaceutics

Article

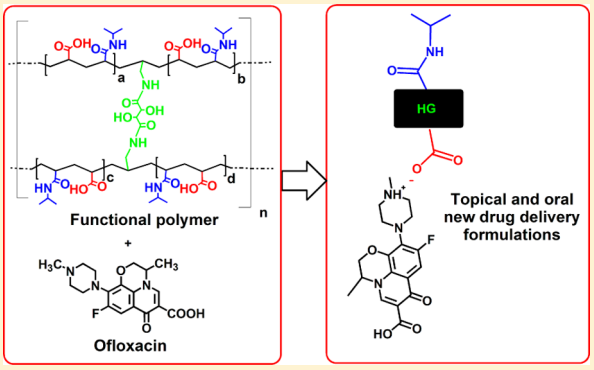
pubs.acs.org/molecularpharmaceutics

Novel Poly(NIPA-*co*-AAc) Functional Hydrogels with Potential Application in Drug Controlled Release

Julio César Cuggino,[†] Cintia Belén Contreras,[†] Alvaro Jimenez-Kairuz,[‡] Belkys Angélica Maletto,[§] and Cecilia Inés Alvarez Igarzabal*[,†]

[†]IMBIV-CONICET, Departamento de Química Orgánica, [‡]UNITEFA-CONICET, Departamento de Farmacia, [§]CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Edificio de Ciencias II, Ciudad Universitaria, Córdoba X5000HUA, Argentina

ABSTRACT: The synthesis, characterization and properties of pH/thermosensitive hydrogels based on acrylic acid (AAc) and *N*-isopropylacrylamide (NIPA) using (+)-*N*,*N*′-diallyltartramide (DAT) as cross-linking agent and water as solvent, are presented in this article. Subsequently, the incorporation of ofloxacin (OFL) as model drug to evaluate the drug load capacity of hydrogels and the *in vitro* release from OFL-polymer conjugate are presented in order to define potential pharmaceutical applications. Interestingly, the incorporation of AAc diversified the properties of NIPA-based hydrogels allowing ionic interaction of these new materials with drugs of opposite charge and produced different release profiles at pH 1.2 and 6.8 simulated physiological media.



KEYWORDS: *biomedical polymers, drug delivery, hydrogels, polymeric gels, smart formulations*

1. INTRODUCTION

Hydrogels are tridimensional networks composed by polymeric chains and water, which present excellent properties as soft nature, high water holding, and network porosity for biomedical utilization. All of these intrinsic properties of hydrogels can additionally be easily changed according to their posterior utilization. Since Wichterle and Lim have published in 1960 the first results about poly(2-hydroxyethyl methacrylate) hydrogels and their derivatives for biomedical applications such as contact lenses,[1] researches on the preparation of these materials for various other bioapplications have increased until today. Undoubtedly, one of the most intense areas of interest from 1960 was the study of utilization of these polymeric materials in drug delivery formulations.[2−6] Control release of bioactive molecules has been achieved by the utilization of macroscopic hydrogels as carriers in oral,[7,8] dermatological,[9,10] ophthalmic,[11] and odontological,[12] among other pharmaceutical formulations.[13] Carbopol derived materials, as a commercial available family of acrylic acid based polymers from Lubrizol Company, are evident examples of the importance of this kind of materials as pharmaceutical ingredients in drug delivery applications.[14,15] However, the formulation technologies in drug delivery are always looking for new carrier materials with innovative properties that can enhance or improve the control over the release profiles of any active pharmaceutical ingredient. So, stimuli sensitive hydrogels are actually coming as a new generation of carriers for biomedical uses.[16] Stimuli sensitive hydrogels are a kind of materials like gel, which can change their network structures in response to any stimulus like temperature, pH, light, electric fields, etc. So, after drug encapsulation into these sensitive gel networks, the drug diffusion through the network and the release could be controlled or activated through an applied stimulus.

Actually, temperature and/or pH sensitive gels are the most investigated for drug delivery applications because both stimuli are produced in the mammalians body.[17−22] The most investigated thermoresponsive gel polymers are based in *N*-isopropylacrylamide (NIPA). Poly(*N*-isopropylacrylamide) [poly(NIPA)] hydrogels show an expanded network structure below 32 °C, which is the lower critical swelling temperature (LCST),[23] while the collapse of these networks produced above this temperature. However, cross-linked and derivatives of poly(acrylic acid) [poly(AAc)] hydrogels are the most pH sensitive materials studied because they can exhibit much more expanded networks at pH values above the pK_a of acrylic acid (pK_a = 4.35) and the changes of network expansion are in the range close to pH of the body.

Several materials based only on acrylic acid (as Carbopol derivatives) are widely used to prepare pharmaceutical drug delivery formulations by their known properties such as good mucoadhesion, ionic drug load possibility, high swelling, and biocompatibility.[14,15] However, the use of this monomer in combination with NIPA can supply polymer-drug based

Received: October 22, 2013
Revised: April 17, 2014
Accepted: May 16, 2014
Published: May 16, 2014

formulations with some additional advantages such as (a) slower rate of drug release at body temperatures compared with polymers totally based on AAc due to their partially collapsed state at body temperature and (b) improvement of mechanical properties of the formulations by major polymer−polymer interactions. So far, although many publications have presented the preparation of dual responsive poly(AAc-*co*-NIPA) based systems, their potential application in oral or topical drug delivery formulations has not yet been considered.

Ofloxacin (OFL) is a fluoroquinolone antibacterial agent, which has a broad antimicrobial spectrum against both Gram-positive and Gram-negative bacteria.[24] It is approved for use by both, oral route, in the treatment of gastrointestinal infections, respiratory tract infections, and urinary tract infections, and topical administration, in the treatment of ophthalmic, otic, and intravaginal infections.[25−29]

The OFL is a zwitherionic molecule (Figure 1) that exhibits pH-dependent solubility. It is more soluble in acidic pH and

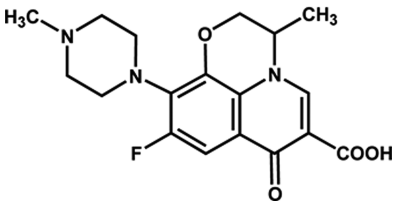


Figure 1. Chemical structure of ofloxacin molecule.

slightly soluble at neutral or alkaline pH conditions (intestinal environment).[29] Additionally, this drug has been chosen as a model of basic drug due to the presence of ionizable ternary piperazine amine group with capacities of acid−base interaction with the carboxylic groups of poly(NIPA-*co*-AAc). It is known that such interaction yields a high degree of ionic pairs between a drug and a polyelectrolyte, acting as a drug reservoir. Similar hydrogels obtained by the partial neutralization of Carbomer 934P, 940, and 971 with ciprofloxacin, norfloxacin, and OFL, showed appropriate physicochemical and release properties to be used in topical bioadhesive dosage forms of controlled release.[30]

So, in this article we presented the preparation and properties of hydrogels with different NIPA/AAc composition and the evaluation of their possible utilization as functional/smart carrier in the preparation of oral and topical drug delivery systems using OFL as a basic drug model.

2. EXPERIMENTAL SECTION

2.1. Materials. The following chemicals were used as purchased: acrylic acid (AAc, Aldrich 98%); *N*-isopropylacrylamide (NIPA, Aldrich 97%); ammonium persulfate (APS, Aldrich 98%); tetramethylenethylendiamine (TEMED, Aldrich); *N*,*N*′-diallyltartradiamide (DAT, Aldrich 99%), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma), Carbomer 974P (Noveon), and ofloxacin (OFL, Parafarm 97%).

2.2. Synthesis of Hydrogels. The hydrogels were prepared by free radical polymerization in aqueous solution using NIPA and AAc as monomers, DAT as cross-linking agent, and the redox couple APS/TEMED as initiator. In a typical procedure, the monomers, cross-linking agent, and initiator were dissolved in 5 mL of water in a tube and shaken for 10 min in an ultrasonic bath, and then, nitrogen was bubbled for 5 min to deoxygenate the system. Finally, an aqueous solution of 0.32 M TEMED was added, and the reaction mixture was rapidly transferred to a disposable polypropylene hypodermic syringe, which served as polymerization reactor. The reactions were carried out for 24 h at 25 °C. After reaction, each product was extracted from the syringe, and the polymer was cut in regular discs of 1 cm in diameter by 3 mm thick. The discs were washed extensively (several changes of solvent per day for 3 days) with distilled water to remove residual unreacted monomers. Finally, the hydrogels in form of discs were dried for 3 days at room temperature and then in an oven for 2 days at 30 °C until constant weight. All polymers were prepared using a total monomer concentration (NIPA + AAc) of 1.4 M with 2 and 1 mol % of DAT and APS, respectively. One equivalent of TEMED with respect to APS was added from the 0.32 M solution. The products obtained were named NIPA 100; NIPA:AAc (70:30); NIPA:AAc (50:50); NIPA:AAc (30:70); and AAc 100 depending on the molar monomer composition. The experimental conditions to yield the products are summarized in Table 1.

Table 1. Experimental Conditions to Yield Hydrogels

HG[a]	NIPA (mmol)	AAc (mmol)	polymeric mass[b] recovery (%)
AAc 100		7.08	85
NIPA:AAc (30:70)	2.13	5.01	98
NIPA:AAc (50:50)	3.52	3.66	99
NIPA:AAc (70:30)	5.01	2.13	97
NIPA 100	7.08		98

[a]All the HG were prepared using a total monomer concentration (NIPA + AAc) of 1.4 M using 2 and 1 mol % of DAT and APS, respectively. One equivalent of TEMED with respect to APS was added from the 0.32 M TEMED solution. [b]Determined from the dry weight of the final products after purification and compared with the total mass of monomers before polymerization.

2.3. ^{1}H NMR Characterization. Chemical characterization of the products was realized by ^{1}H NMR using a nuclear magnetic resonance spectrometer BRUKER 700 MHz NMR. Approximately 5 mg of fine powder of each polymer was swelled in 0.8 mL of D_2O for 24 h before each measurement, in order to permit the polymer swelling.

2.4. Swelling Studies. For the studies of swelling in response to the changes in pH, the hydrogels were swollen at equilibrium in aqueous solutions of different pH (values between 3 and 6) for 24 h. Dried weighed samples (100 mg approximately) were placed within a beaker containing 15 mL of each liquid (Britton−Robinson buffers[31] of pH = 3.0; 4.0; 5.0; and 6.0) in a bath 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. Degree of swelling (DS_e) was calculated according to eq 1 for each pH.

$$DS_e = (W_e - W_d)/W_d \quad (1)$$

where W_e is the weight of the swollen polymer at equilibrium and W_d is the weight of the dry polymer. DS_e values were determined in triplicate. Then, DS_e was plotted versus pH. Britton−Robinson buffers were prepared by dissolving 2.3 mL of glacial CH_3COOH, 2.7 mL of H_3PO_4, and 2.5 g of H_3BO_3 in

1000 mL of distilled water. With aliquots of 100 mL, pH of each solution was adjusted with 2 M NaOH solution.

2.5. Rheological Characterization. The rheological characterization of NIPA 100 and NIPA:AAc (70:30) was carried out using an Anton Paar Physica MCR 301 controlled-strain rheometer. Dry disc of polymer was swollen for 24 h in simulated gastric fluid (SGF, pH = 1.2) or phosphate buffer solution (PBS, pH = 6.8). The swollen discs were cut in order to obtain regular discs of 1.5−2 mm thick and 25 mm diameter. A 25 mm plate−plate (PP25) geometry and 1.5−2 mm gap was used for all experiments. First, amplitude sweep studies (strain 0.1−20% at constant angular frequency of 10 Hz) on each disc of NIPA 100 in SGF; NIPA 100 in PBS; NIPA:AAc (70:30) in SGF; and NIPA:AAc (70:30) in PBS, were performed at 37 °C with the objective to determine the linear viscoelastic region (LVR) profiles and the critical strain region (CSR) of each formulation. Then, frequency sweep from 0.1 to 1000 Hz at a fixed strain (0.1−1% depending on each hydrogel) was also performed at 37 °C on each disc to determine the storage modules (G') and the loss modules (G'') of the swollen materials in simulated media at different frequencies.

2.6. DSC Studies. The DSC method was the technique used for examination of the phase transition phenomenon exhibited by the thermosensitive gels. The analyses were performed using a DSC 2920 Modulated-DSC and processed with specific software Universal Analysis 2000 V3.9A (TA Instrument). The LCST of samples was determined using samples between 4 and 6 mg (previously swelled in water). Samples were run in a hermetic sealed aluminum pans at a heating rate of 10 °C/min in the range of 10−50 °C under nitrogen atmosphere. The transition temperature has been defined as the maximum of the endothermic transition peak.

2.7. SEM Study. The microstructure of AAc 100, NIPA 100, and NIPA:AAc (70:30) was analyzed by scanning electron microscopy (SEM) using LEO1450VP equipment. Samples were mounted on an aluminum sample holder. The SEM images were obtained under VP mode (variable pressure) at low vacuum (70 Pa) at 150×.

2.8. Cytotoxicity Studies. The *in vitro* cytotoxicity evaluation of NIPA:AAc (70:30) and Carbomer 974P hydrogels was carried out by direct-contact assay with fibroblast cells line according to ISO standards.[32] Fifty milligrams of NIPA:AAc (70:30) or Carbomer 974P in powder form were swollen in 2.2 mL of RPMI 1640 medium (Sigma-Aldrich) for 24 h at 37 °C. Briefly, L929 cells were subcultured from stock culture by trypsinization and seeded into 96-well flat-bottom culture plates. Cells were fed with RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum (Natocor, Argentina), 2 mM GIBCO Glutamax, 100 U/mL penicillin, and 100 μg/mL streptomycin (all from Life Technologies) and incubated at 37 °C in 5% CO_2 atmosphere. When the cells attained approximately 80% confluence, 5 and 10 μL of the swollen samples (previously sterilized for 30 min by UV exposition) were kept in contact with the cells, in triplicate, for direct-contact assay. In parallel, we used culture medium as blank control and 2% NaCl solution as positive control. In this step of the assay, 2.5 mM HEPES buffer is added to the culture medium. After incubation of the samples in contact with cells for 24 h at 37 °C, the cytotoxicity was quantitatively assessed by MTT assay, which measures the metabolic reduction of MTT to formazan by viable cell.[33]

2.9. Preparation of NIPA:AAc (70:30)-OFL. *2.9.1. Preparation and Characterization in Disc form.* For the determination of OFL loading efficiency, preweighed dry discs were placed to swell in 5 mL of OFL solutions of different concentrations (1.27×10^{-4}; 4.90×10^{-4}; 7.73×10^{-4}; 1.24×10^{-3}; and 6.97×10^{-3} M) in phosphate buffer (pH = 7.4), during 48 h at room temperature. Then, the discs were removed from the supernatant, and the amount of remainder drug was evaluated using an UV−visible (Thermo Evolution 300) spectrophotometer at 287 nm with an appropriate calibration curve (λ = 287 nm; ε = 26454 M^{-1} cm^{-1}) in phosphate buffer (pH = 7.4). The amount of drug loaded in milligrams (mg) by g of hydrogel was calculated as the difference between the initial amount and that remaining in the supernatant.

For the preparation of disc formulations, each dry disc (50−60 mg) of NIPA:AAc (70:30) was immersed and swelled in 5 mL of aqueous solution of OFL (7×10^{-3} M) for 48 h at 25 °C. The loaded disc was dried at room temperature for 2 days and then at vacuum for 6 h at 35 °C in order to hold the drug. The amount of OFL incorporated into the disc was quantified by difference from the initial and the remainder amount of OFL present in the solution after 48 h of contact. Lightly yellow OFL-containing disc of approximately 1.5 mm thickness and 1 cm of diameter was obtained.

To study the swelling behavior of NIPA:AAc (70:30)-OFL disc form, it was swollen in PBS (pH = 6.8) or SGF (pH = 1.2) at 25 and 37 °C. Samples were superficially dried with tissue paper, weighted by an electronic balance, and dried. Swelling index (q_w) was calculated according to eq 2, in triplicate. Then, q_w was plotted versus time.

$$q_w = W_e/W_d \quad (2)$$

2.9.2. Preparation in Dispersion form. NIPA:AAc (70:30) was finely pulverized using an automatic mortar. Then, 50% of the acid groups of NIPA:AAc (70:30) were neutralized by acid−base reaction with OFL in ethanolic medium. Suitable amounts (720 mg) of finely granular polymer and 400 mg of OFL were placed in a mortar and mixed slowly in the presence of 35 mL of ethanol for a period of 15 min to ensure complete wetting of the solid. The mixture was allowed to react in ethanolic medium by 24 h. The ionic complex was dried at room temperature (23−25 °C) for 2 days and then in an oven at 30 °C. NIPA:AAc (70:30)-OFL ionic complex was obtained as a yellowish powder. Then, 280 mg of the ionic complex was finely dispersed in 10 mL of Milli-Q water to yield OFL 1% (w/v) on the final hydrogel dispersion.

2.10. *In Vitro* Drug Delivery Studies. *2.10.1. Disc form.* The release profiles of OFL from the discs were studied in a dissolute SOTAX smart AT7 equipment according to Apparatus 2 (Padles) of US Pharmacopeia[34] fixing the stirring at 50 rpm and the temperature at 37 °C. Approximately 50 mg of NIPA:AAc (70:30)-OFL dry disc was placed in a recipient containing 500 mL of PBS (pH = 6.8) or SGF (pH = 1.2) in order to study the release profiles from the disc form. At different time intervals, 4 mL aliquots were removed from the receptor solution, and 4 mL of fresh buffer was replaced into the receptor solution. The amount of OFL released was quantified spectrophotometrically using the corresponding calibration curve for each medium (SGF, pH = 1.2; λ = 294 nm; ε = 30346 M^{-1} cm^{-1}; PBS, pH = 6.8; λ = 288 nm; ε = 20049 M^{-1} cm^{-1}). Each analysis was performed in triplicate.

2.10.2. Dispersion form. Samples of approximately 1 mL of NIPA:AAc (70:30)-OFL in gel form were collected using polypropylene syringes and placed into a donor compartment

of Franz cells. Receptor compartment was filled with 15 mL of three different media, Milli-Q water (deionized), saline solution (0.9% NaCl), and PBS pH = 6.8, at 37 °C. Aliquots of 1 mL of receptor solution were taken at different time intervals for 8 h using a syringe of 1 mL, in order to quantify the OFL released by UV−visible spectroscopy. Each withdrawn aliquot was immediately replaced by the same volume (1 mL) of the corresponding fresh medium, maintained at 37 °C. For quantification of the withdrawn aliquots from PBS, a calibration curve of OFL (at pH = 6.8; λ = 288 nm; ε = 20049 M^{-1} cm^{-1}) was used. In addition, for the quantification of the withdrawn aliquots from assays in Milli-Q water and saline, a calibration curve at pH = 1.2 (λ = 294 nm; ε = 30346 M^{-1} cm^{-1}) was used. For this, each aliquot previously withdrawn was acidified with a drop of 1.0 N HCl solution. Each analysis was performed in triplicate.

2.10.3. OFL Release Analysis. Three common mathematical models, zero-order, Higuchi, and Korsmeyer−Peppas, were used in order to determinate the release mechanism of each formulation. The zero-order, Higuchi,[35] and Korsmeyer−Peppas[36] models are represented by eqs 3, 4, and 5, respectively.

$$M_t/M_\infty = kt \quad (3)$$

$$M_t/M_\infty = kt^{1/2} \quad (4)$$

$$M_t/M_\infty = kt^n \quad (5)$$

where M_t/M_∞ is the fraction of drug released at time t for the models. k is the zero order release constant expressed in units of concentration/time, for the zero-order model (eq 3); the Higuchi dissolution constant, in Higuchi model (eq 4); and the release rate constant, for the Korsmeyer−Peppas model (eq 5). In this last model, the value of n characterizes the release mechanism of the drug. For the case of cylindrical tablets or disc, $n \geq 0.45$ corresponds to a Fickian diffusion mechanism; $0.45 < n < 0.89$ to non-Fickian transport; $n = 0.89$ to Case II (relaxation) transport; and $n > 0.89$ to Super Case II transport.[37] Equation 5 is applied to the initial swelling stages (60%). Plots of ln F versus ln t were drawn using the swelling kinetic data, and n and k values were calculated from the slopes and intercepts of the lines, respectively.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Hydrogels. Figure 2 shows the scheme of reaction to yield the polymers by free radical polymerization in aqueous medium. The polymers were prepared in solution using NIPA and AAc as the principal monomers, DAT as cross-linking agent, the redox couple APS/TEMED as generator and activator for the formation of free radicals, respectively, and water at 25 °C. Five polymers were prepared with different mol monomer compositions using the same amount of DAT. So, NIPA 100; NIPA:AAc (70:30); NIPA:AAc (50:50); NIPA:AAc (30:70); and AAc 100 were synthesized using the experimental conditions summarized in Table 1. All polymers were obtained in rod form and able to maintain the macroscopic structure after extraction from the reactors. However, NIPA:AAc (30:70) and AAc 100 products were less easy to manipulate. Of these two products, only AAc 100 was subjected to some studies for comparative purposes with the rest of NIPA-containing products.

The polymer mass recovery for the products was determined by the weight of the dry polymer after an exhaustive

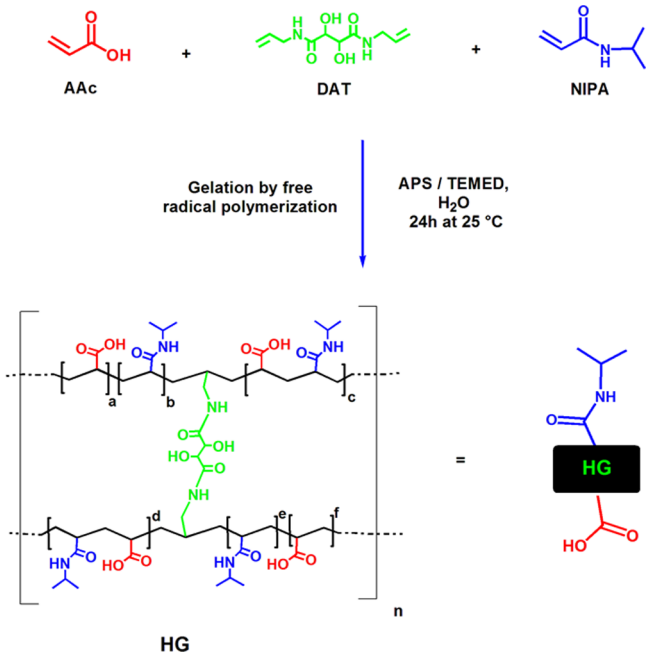


Figure 2. Schematic reaction of polymerization to yield hydrogels (HG).

purification process with distilled water. For NIPA-containing products: NIPA 100; NIPA:AAc (70:30); NIPA:AAc (50:50); and NIPA:AAc (30:70), the polymer masses recovery were almost quantitative (97−99%, see Table 1), while for the hydrogel prepared with 100% of AAc, it was also acceptable (85%) but not quantitative, probably due to lower reactivity ratio of acrylic monomer (AAc) than acrylamide derivatives (as NIPA) in water. However, after purification and a cycle of drying/swelling/drying, no mass loss was detected for all the hydrogels as a strong evidence of the cross-linking nature of the networks. For biomedical applications, it is very important that the polymeric material do not leach from the polymeric network toward any biological environment.

The chemical characterization of the hydrogels was carried out by ^{1}H NMR. Figure 3 shows ^{1}H NMR spectra of NIPA 100; NIPA:AAc (50:50); and AAc 100.

The ^{1}H NMR spectrum of NIPA 100 shows the characteristic bands of poly(NIPA) at δ (ppm) = 1.13 [6H, CONCH-(CH_3)$_2$]; 1.47 [2H, polymer backbone]; 2.02 [1H, polymer backbone]; and 3.86 [1H, CONC*H*-(CH_3)$_2$]. Similarly, the ^{1}H NMR spectrum of AAc 100 shows the characteristic bands of the polymer backbone of poly(AAc) to δ (ppm) = 1.75 [2H, polymer backbone] and 2.40 [1H, polymer backbone]. Confirmation of the copolymerization reaction between NIPA and AAc is shown in the spectrum of NIPA:AAc (50:50). Both, the characteristic bands of poly(NIPA) [δ (ppm) = 1.13; 1.47; 2.03; and 3.86)] and poly(AAc) bands [δ (ppm) = 1.75 and 2.40] are present in the spectrum giving clear evidence that both monomers are forming part of the hydrogel network NIPA:AAc (50:50).

3.2. Swelling Studies. Because of the thermoresponsive swelling behavior presented by NIPA-containing polymers and the pH-sensitive swelling of the AAc based hydrogels (pK_a AAc = 4.35), the influence of the pH on the swelling properties at 37 and 25 °C [above (37 °C) and below (25 °C) poly(NIPA) LCST (32 °C)] was studied. Figure 4 shows the swelling (DS_e) of NIPA 100, NIPA:AAc (70:30), NIPA:AAc (50:50), and AAc

dx.doi.org/10.1021/mp400615n | *Mol. Pharmaceutics* 2014, 11, 2239−2249

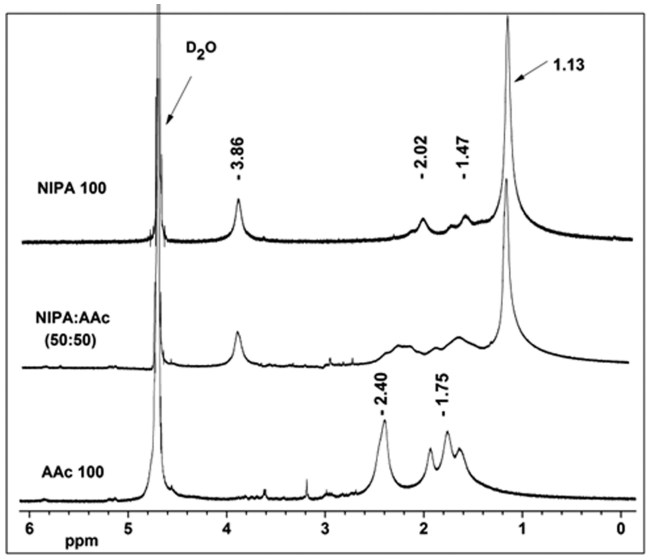


Figure 3. ^{1}H NMR spectra of NIPA 100, NIPA:AAc (50:50), and AAc 100 in D_2O at 25 °C.

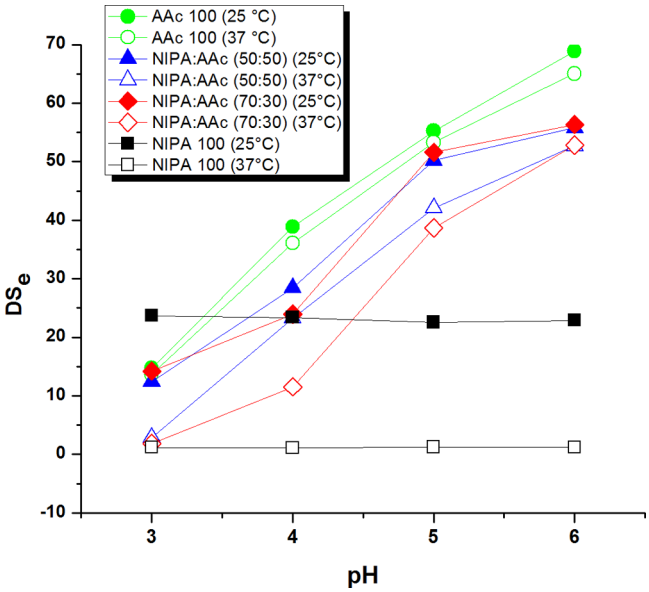


Figure 4. Degree of swelling (DS_e) of hydrogels in Britton–Robinson buffers (pH = 3.0; 4.0; 5.0; and 6.0) at 25 and 37 °C.

100 at two different temperatures (25 and 37 °C), in four different Britton–Robinson buffers (pHs = 3.0; 4.0; 5.0; and 6.0). The pH values of the buffers were chosen below and above the pK_a of AAc to study the effect of ionization of the acid groups of AAc-containing polymers. The information regarding the influence of the pH and temperature over the swelling behavior of each hydrogel and accordingly with their monomer composition can be obtained from Figure 4.

It is clearly observable that those polymers containing easy ionizable groups in their network structures, AAc 100, NIPA:AAc (70:30), and NIPA:AAc (50:50), present a remarkable increase in the swelling capacity after increment of pH (from 3.0 to 6.0) of the swelling solution. This increment is also independent of the temperature at which the hydrogels were submitted to swell since the increment occurs at 25 °C but also at 37 °C. An increment of pH above the pK_a of AAc produces the ionization of acid groups (COO^-) in the polymer network and consequently repulsion forces of negative charges, generating great network expansion and increase in buffer absorption capacity. As expected, nonmodification on the swelling behavior of NIPA 100 with changes in the pH was observed at both temperatures since this polymer has no easily ionizable groups.

Then, analyzing each polymer at any similar pH (3.0; 4.0; 5.0; or 6.0), there is more noticeable difference between the swelling values at different temperatures (25 or 37 °C), while major is the amount of NIPA into the structures. For example, there is minimal influence of the temperature over the DS_e values for AAc 100 at pH = 4 (DS_e = 38.9 at 25 °C and 36.1 at 37 °C). Then, a more noticeable influence of the temperature on the DS_e values was observable for NIPA:AAc (70:30) (DS_e at 25°C = 23.5; DS_e at 37°C = 10.1) and in a lesser extent for NIPA:AAc (50:50) (DS_e at 25 °C = 28.6; DS_e at 37 °C = 23.4) in the swelling behavior between 25 and 37 °C. At pH = 4, NIPA 100 showed the biggest influence of the temperature on the DS_e values presenting a difference of approximately 22 points on their swelling values (DS_e at 25 °C = 23.6; DS_e at 37 °C = 1.0). Deswelling properties are due to the collapse produced by poly(NIPA) [LCST at 32 °C] with the increment of temperature from 25 to 37 °C.

The combination of the properties of AAc and NIPA produces networks with dual pH–thermo responsive behavior. Polymer networks with more or less influence of pH and/or temperature can be prepared modifying the initial monomer feed composition. NIPA:AAc-containing products presented both pH and temperature responsive swelling behavior. However, thermosensitivity property of NIPA:AAc (70:30) resulted better than NIPA:AAc (50:50), so that has been selected to be used in drug delivery application.

3.3. Rheological Studies. Rheological studies are important to investigate the material response to any applied force. Information about the microstructure of the polymeric materials at any swelling state can be obtained using oscillatory rheological measurements. Because of the possible application of the yielded materials in oral and topical formulations, the investigation of any change on the viscoelastic properties of NIPA:AAc (70:30) and NIPA 100 swelled in simulated physiological fluids was performed. So, SGF (pH = 1.2) and PBS (pH = 6.8) at 37 °C were used to determine the hydrogel behaviors in these experimental conditions under small deformations imposed on different frequencies into the linear viscoelastic range (LVR). To determine the storage modulus G' and loss modulus G'' of each swollen hydrogel, frequency sweep assays at 37 °C were performed.[12]

Figure 5 shows the storage modules of NIPA 100 and NIPA:AAc (70:30). The viscoelastic behavior of the polymer swelled in SGF or PBS at 37 °C is typical of slightly swollen cross-linked gel materials, which present a storage module G' superior to the loss module G'' in the whole range of frequencies analyzed[13] (G'' is not shown in Figure 5). So, both polymeric materials show behavior more similar at an elastic solid than a Newtonian fluid in SFG and PBS at 37 °C.[14]

NIPA 100 does not present significant differences in their storage modulus G' and consequently in their network microstructure into the fluids of swelling assayed SGF or PBS since the effect of the collapse by the temperature is strong. However, as can be clearly seen in Figure 5, NIPA:AAc (70:30) presents widely different values of G' depending on the pH of the fluid in which the polymer has been previously swelled. The G' value is approximately 570 Pa at pH = 6.8 and close to 7800

dx.doi.org/10.1021/mp400615n | Mol. Pharmaceutics 2014, 11, 2239–2249

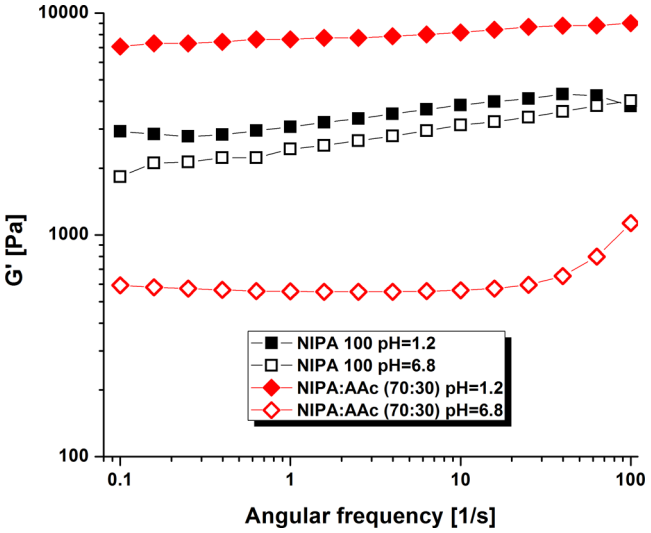


Figure 5. Storage modules G' for NIPA 100 and NIPA:AAc (70:30) swollen in SGF (pH = 1.2) and PBS (pH = 6.8) determined at 37 °C.

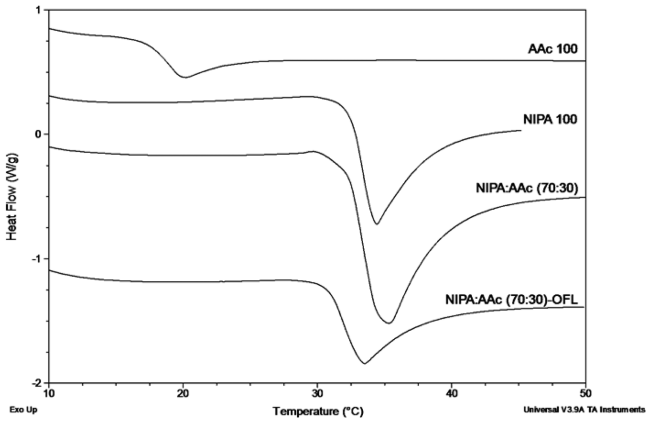


Figure 6. DSC studies of AAc 100, NIPA 100, NIPA:AAc (70:30), and NIPA:AAc (70:30)-OFL.

Pa at pH = 1.2. Although NIPA:AAc (70:30) contains NIPA, its combination with a little amount of AAc changes drastically the properties of the network. The observed behavior is consequence of ionization of acid groups and the subsequent expansion of the polymer network at pH higher than pK_a of AAc. Network expansion produces major absorption capacity of fluids producing an increasing on the swelling. Thus, the network became softer, and consequently, a decreasing on the storage modulus G' of the swollen polymer was reached. The rheological behavior of NIPA:AAc (70:30) is very interesting for a potential application in oral drug delivery formulations. Changes in the microstructure of the gels from SGF to PBS (chains separation by chains repulsion) could trigger the release of drugs in the intestine after passing the stomach without or with minimal release in an oral formulation.

NIPA:AAc (70:30) presents major storage G' modulus at 37 °C at both fluidic pH of SGF and PBS with respect to polymers containing 100% of AAc with similar cross-linking density (as Carbomer), which could be positively reflected in a better mechanical performance after oral administration in this environment.[30]

3.4. DSC Studies. The LCST can be assigned as an endothermic transition peak, related to the breaking of hydrogen bonds between water molecules and the hydrophilic polymer segments.[38] Taking into account the peak of the thermograms as the phase transition temperature for the NIPA-containing products, it can be noticed (Figure 6) that an increment from 34.71 °C (for NIPA 100) to 35.22 °C [for NIPA:AAc (70:30)] was observed. Incorporation of AAc hydrophilic monomer increases the hydrophilicity of the system, and the polymer−water interactions occur rather than polymer−polymer interactions, reducing the driving force of the phase transition with increase of the LCST.[39,40] For NIPA:AAc (70:30)-OFL, the interaction between polymer and drug is basically ionic. The acid−base neutralization of AAc carboxylic groups with OFL produces decreases in the hydrophilic segment of the hydrogel with consequent diminution in the phase transition (33.50 °C; Figure 6).

3.5. SEM Study. Figure 7 shows the micrographs of AAc 100, NIPA 100, and NIPA:AAc (70:30) obtained at 150× with the purpose to analyze the hydrogel morphology. Although the products were expandable and swellable in water, they showed very different superficial morphology. It is important to notice that NIPA 100 and AAc 100 resulted in rough and smooth morphology, respectively, while NIPA:AAc (70:30) presented an interesting porous structure.

3.6. Cytotoxicity Studies. Considering a potential pharmaceutical application for the new hydrogels, the quantitative *in vitro* cytotoxicity was performed by MTT assays by direct-contact of swollen hydrogels in culture medium with fibroblast cells line. Cytotoxicity effect of the hydrogel NIPA:AAc (70:30) was compared with that of a hydrogel prepared from the commercial Carbomer 974P recognized as nontoxic and nonirritant material (Generally Recognized As Safe, GRAS FDA Classification). Figure 8 presents the cell viability of the fibroblast cells in contact with medium alone (blank control), 2% NaCl solution, hydrogel from Carbomer 974P, and hydrogel NIPA:AAc (70:30) in different amounts (5 and 10 μL).

The new hydrogel NIPA:AAc (70:30) showed cell viabilities above 70% for the different gel amount assayed (Figure 8), which can be considered as a potential nontoxic material according to ISO 10993-5 standards.[32] In addition, the hydrogel of NIPA:AAc (70:30) at 2.3% w/v showed cell viability greater than Carbomer 974P hydrogel prepared at the same concentration. Furthermore, no change in the morphology of the fibroblasts nor their adhesions could be observed microscopically after 24 h of contact at 37 °C with swollen NIPA:AAc (70:30). This result suggests that NIPA:AAc (70:30) copolymer could be considered as a useful drug-carrier in pharmaceutical applications.

3.7. OFL Loading. The maximum amount of OFL that can be incorporated by discs was evaluated. Dry discs showed OFL loading efficiencies of 12.5 and 312 mg/g of polymer for NIPA 100 and NIPA:AAc (70:30)-OFL, respectively (Figure 9). Both the increased swelling and the capacity of ionic interaction between amine and acid groups of OFL and NIPA-AAc, respectively, could be related with the important increase of OFL loading efficiency (close to 25-fold) for AAc-containing discs.

In general, the discs incorporated a minor amount of OFL (12.5 and 312 mg/g of polymer for NIPA 100-OFL and NIPA:AAc (70:30)-OFL, respectively), with respect to the usual doses (500 mg drug/g polymer). The highest amount of OFL loaded by NIPA:AAc (70:30) observed in Figure 9 can be

dx.doi.org/10.1021/mp400615n | *Mol. Pharmaceutics* 2014, 11, 2239−2249

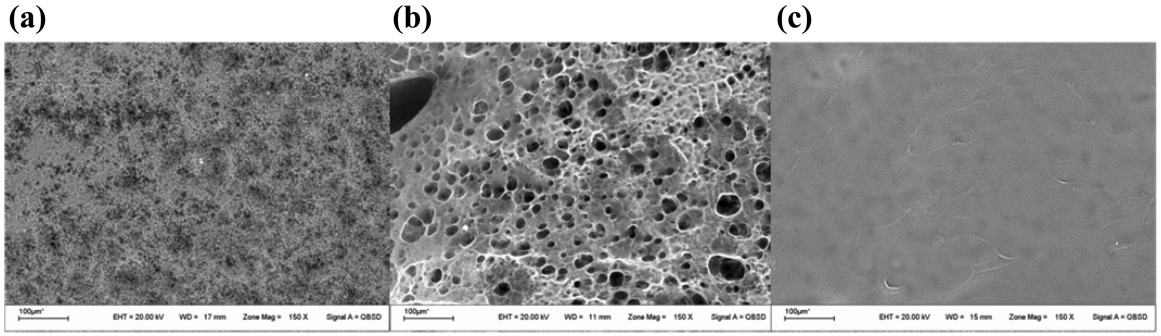


Figure 7. SEM of (a) NIPA 100, (b) NIPA:AAc (70:30), and (c) AAc 100.

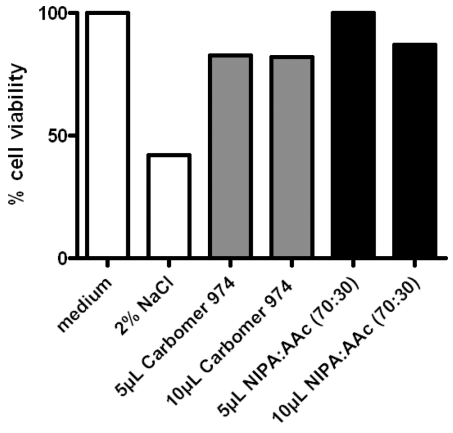


Figure 8. Cell viability of L-929 cells incubated with culture medium alone (blank control), 2% NaCl solution (positive control), Carbomer 974P, and NIPA:AAc (70:30) in different amounts.

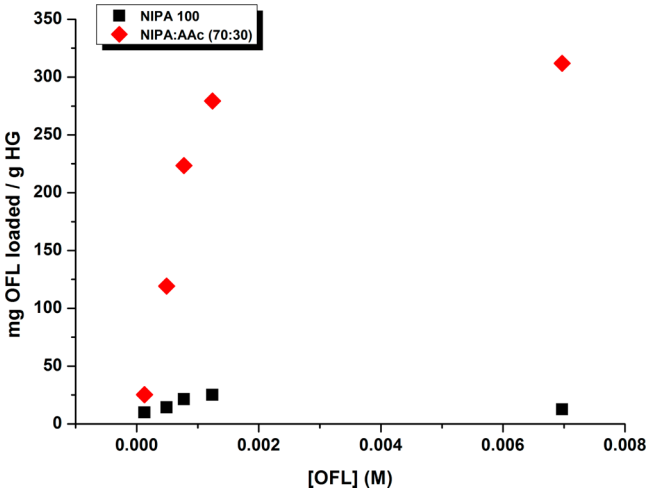


Figure 9. Evaluation of OFL loading efficiency in discs.

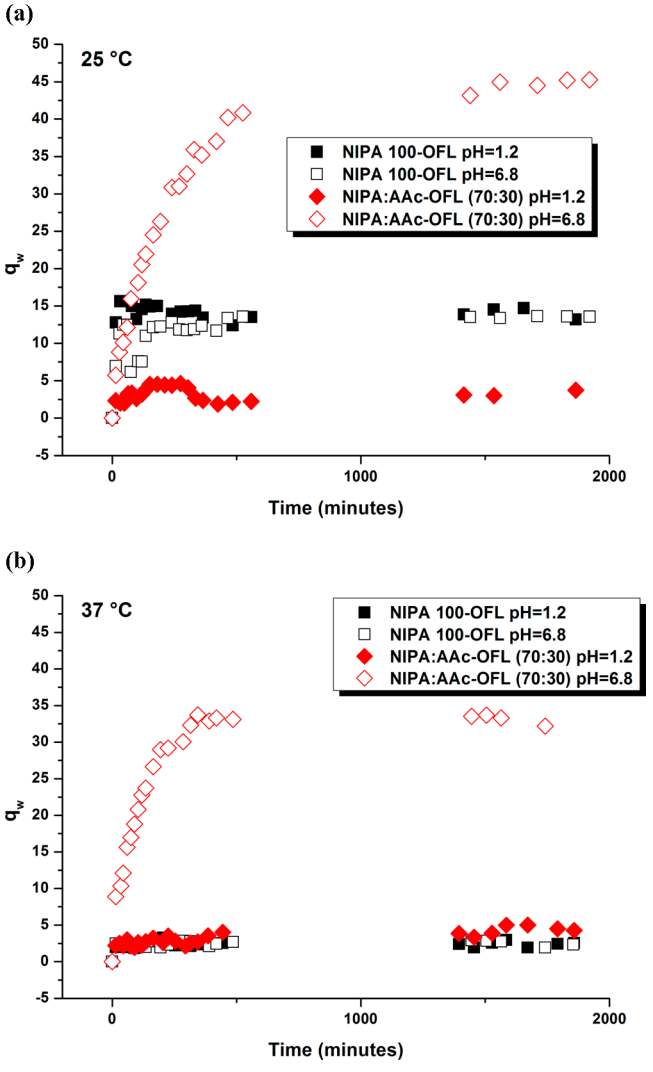


Figure 10. Swelling indexes (q_w) at (a) 25 °C and (b) 37 °C, from discs NIPA 100-OFL and NIPA:AAc (70:30)-OFL at different simulated physiological fluids pHs, SGF (pH = 1.2) and PBS (pH = 6.8).

attributed to the interesting porous structure (Figure 7), the high swelling capacity, and strong polymer−drug interactions.

Swelling studies were performed on both NIPA:AAc (70:30) and NIPA 100-containing OFL discs (Figure 10). In the case of NIPA 100-OFL discs, the increase in temperature (from 25 to 37 °C) produces an important diminution in the q_w at both pHs due to the collapse in the network, as it was previously mentioned. On the other hand, the effect of the increase in temperature for NIPA:AAc (70:30)-OFL discs at both pHs produce diminution in the swelling values. However, such diminution at pH = 6.8 was slight compared with that produced at pH = 1.2. At pH = 6.8, the swelling remains high, possibly due to electrostatic repulsions generated between the chains of the polymer network (whose acid groups are ionized) and the drug (as zwitterion).

In addition, for loading of OFL into the dispersions through salt formation, NIPA:AAc (70:30) and OFL, both in solid state, were immersed in ethanolic medium in an appropriated

dx.doi.org/10.1021/mp400615n | *Mol. Pharmaceutics* 2014, 11, 2239−2249

proportion to neutralize until 50% of the total acid carboxylic groups were present in the polymer. Through DSC and hot-stage microscopy analysis performed on dried NIPA:AAc (70:30)-OFL complex, the endothermic signal corresponding to free-OFL melting temperature was not observed (data not showed).

3.8. *In Vitro* Drug Delivery Studies. *3.8.1. Dissolution Test of Dry Discs.* In order to know the release profiles of HG loaded with OFL, dissolution tests of dry discs were performed. Figure 11 shows the average of two measurements of OFL

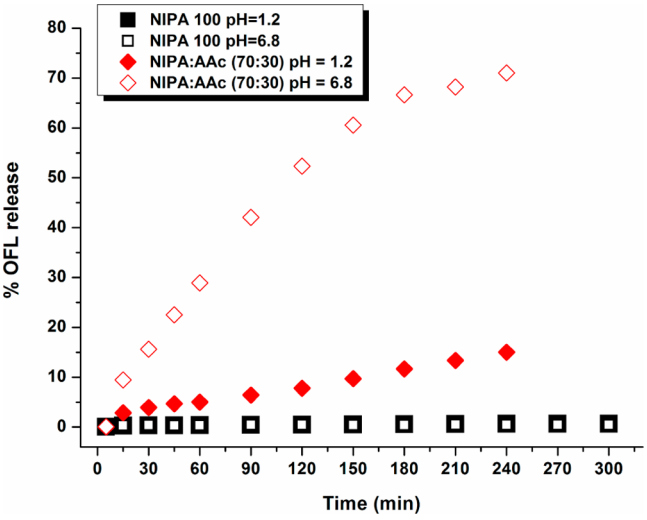


Figure 11. Release kinetics at 37 °C from loaded discs NIPA 100-OFL and NIPA:AAc (70:30)-OFL at different pHs.

released from polymeric discs loaded with the maximum amount of OFL (12.5 and 312 mg/g of polymer for NIPA 100 and NIPA:AAc (70:30)-OFL, respectively) at different simulated physiological fluid pHs, SGF (pH = 1.2) and PBS (pH = 6.8), at 37 °C. The release of OFL was very low for NIPA 100 at 37 °C, with total percentage less than 1% in both simulated media, SGF and PBS. This behavior is probably associated with the strong collapse that NIPA 100 suffers at 37 °C (above the LCST) as was previously observed in the swelling values at different temperatures (Figure 4) and corroborated with the swelling studies of NIPA 100-OFL discs (Figure 10). The great collapse of the network does not permit water incorporation into the structure; consequently, the processes of drug dissolution and posterior diffusion into the outside environment were practically impossible.

However, the presence of a small amount of AAc had marked influence on the release properties of these systems. As shown in Figure 11, NIPA:AAc (70:30) released an appreciable and different amount of OFL, depending on the tested release medium (SGF or PBS). After 4 h of dissolution experiment, 71 and 15% cumulative release for OFL in PBS and SGF, respectively, was produced. This resulted in accordance with the behavior observed from swelling studies of NIPA:AAc (70:30)-OFL discs at different simulated physiological fluids pHs (Figure 10), which promotes the drug dissolution processes and posterior diffusion-release of the drug into the outside environment.

From the analysis in SGF (pH = 1.2) (Table 2), the slow release rate showed a marked tendency toward zero order kinetic (R^2 = 0.997) and additionally high diffusional exponent (n > 0.80) using Peppas equation, proving a high control of drug release. However, in PBS (pH = 6.8), a sharp increase of release rate was observed, close to 7.4 times higher than acidic medium, without significant changes in kinetic control (see R^2, Table 2), whereby the high and controlled drug release toward PBS could be attributed at the predominant network hydrogel relaxation mechanism. The hydrogel becomes ionized, negatively charged at pH = 6.8, increasing the electrostatic repulsion between the polymer network and resulting in an expansion that causes a faster dissolution and diffusion of OFL to the surrounding medium.

All release studies were performed under *sink* conditions (volume of dissolution medium of 5 to 10 times greater than the volume needed to prepare a saturated solution with the amount of drug within the gel). In this case, the volume of dissolution medium was 500 mL and the amount of OFL that was incorporated into the gels were about 12 and 5 mg for NIPA:AAc (70:30) and NIPA 100, respectively. The OFL solubility is about 3 and 36 mg/mL at pH = 6.8 and pH = 1.2, respectively.[41] Definitively, the copolymerization of NIPA with small amounts of AAc were very interesting since a new material that can control the drug release, according to the medium pH, was yielded, acquiring a potential application for administration of oral matrix systems. As is known, it is sometimes necessary to protect the gastric pH (pH = 1.2) to very labile drugs, allowing their release once passed the stomach and reached the gastro intestinal system, in which the pH increases drastically. Accordingly, the material NIPA:AAc (70:30) has promising biopharmaceutical properties since it is capable to load a high proportion of drug and to modify the release in SGF or PBS for design of oral delayed drug delivery systems.

3.8.2. Release Studies from NIPA:AAc (70:30)-OFL Viscous Dispersions. The OFL release property of the finely granulated NIPA:AAc (70:30) was studied to evaluate the properties of the polymer−drug system in potential topical application. The choice of this system was based on the thermo and pH-sensitive properties of the material. Subsequently, the release from the 1% dispersion of OFL into the complex NIPA:AAc (70:30)-OFL was studied in three different media, Milli Q water, saline, and PBS (pH = 6.8) in diffusion Franz cells at 37 °C. As shown in Figure 12, the release rates and the percentage of total drug released after 7 h were totally different for each analyzed medium. The cumulative percentage of drug was about 60, 30, and 10% after 7 h when the receiver solutions were PBS (pH = 6.8), saline, and Milli Q water, respectively. Furthermore, adjustment to three different release kinetic models (zero-order, Higuchi, and Peppas) are summarized in Table 3. In

Table 2. Adjusted Release Kinetics from Discs of NIPA:AAc (70:30)-OFL to Different Models

	zero-order		Peppas		Higuchi	
receptor medium	k (%·min^{-1})	R^2	n	R^2	k (%·min$^{-0.5}$)	R^2
SGF	0.056	0.997	0.809	0.989	1.32	0.980
PBS	0.386	0.993	0.852	0.997	6.42	0.995

dx.doi.org/10.1021/mp400615n | *Mol. Pharmaceutics* 2014, 11, 2239−2249

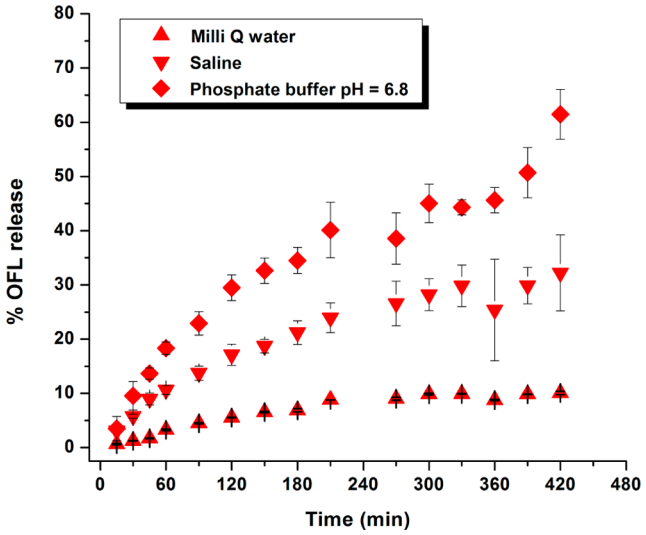


Figure 12. Release profiles in Franz's cells at 37 °C from dispersions of ionic complex NIPA:AAc (70:30)-OFL (50% neutralized acid groups) prepared to yield dispersion of OFL 1% (w/v).

Table 3. Adjusted Release Kinetics Data in Franz's Cells at 37 °C from Dispersions of Ionic Complex NIPA:AAc (70:30)-OFL (50% Neutralized Acid Groups) Prepared so until 1% Dispersion of OFL

	zero-order		Peppas		Higuchi	
receptor medium	k (%·min^{-1})	R^2	n	R^2	k (%·min$^{-0.5}$)	R^2
Milli-Q water	0.015	0.957	0.634	0.989	0.351	0.985
NaCl 0.9% soln.	0.076	0.969	0.653	0.989	1.866	0.997
PBS (pH = 6.8)	0.132	0.936	0.675	0.981	3.078	0.987

general, the release profiles had a better fit to both Peppas and Higuchi models (see R^2 Table 3) and not for the release model of zero-order. Thus, when the acceptor medium was PBS (pH = 6.8), release rate (k, from Higuchi model, Table 3) was 1.7 and 15.3 times larger than in saline and Milli Q water, respectively. The differences in release rate and cumulative amount of drug released between the different release media can be explained by ion exchange phenomenon. As it was explained, the type of interaction between polymer and drug is basically ionic, forming ionic pairs of polymer−$COO^{-}{}^{+}$OFL, which act as a reservoir for OFL, releasing it slowly. The presence and concentration of ions in the receptor medium have a very important role, promoting the ionic exchange and consequent increase of OFL release. The release of OFL from NIPA:AAc (70:30)-OFL was higher in receptor media containing ions: phosphate buffer and saline, than Milli Q water (without ions). In all media, the *sink* conditions were assured. The volume of the acceptor medium was 15 mL, and the amount of total drug loaded into NIPA:AAc (70:30) was between 8 and 9 mg. OFL solubility in water and in PBS (pH = 6.8) is about 1.4 and 3.2 mg/mL, respectively. The release temperature plays an important role in the release of active molecules, when NIPA-containing polymers are used. Collapsing property of this NIPA-containing polymer at 37 °C allowed more delay and control in the release of OFL compared with a homologous polyelectrolyte such as Carbomer, which has no such property, from which both the amount of OFL and release rate were 2-fold higher under similar experimental conditions.[42]

In general, the results reached with OFL-containing NIPA:AAc (70:30) in dispersion of ionic complex form are very promising for the design of smart systems useful in topical and mucosal drug controlled release. In addition to advantageous properties of swelling, releasing, and being rheological, the hydrogels have proven to be easily extrudable through a syringe (Figure 13), which would facilitate its application in body specific sites through the formation of the complex when placed in contact with mucous membranes at approximately 37 °C.

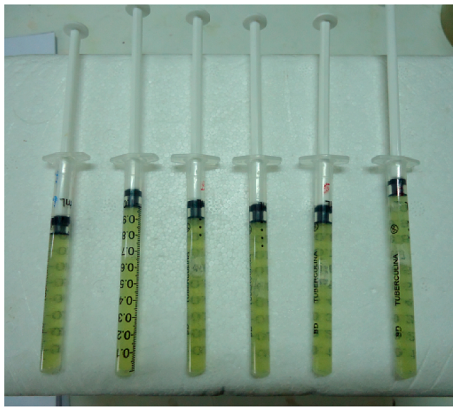


Figure 13. Photography of syringes containing dispersions of ionic complex NIPA:AAc (70:30)-OFL (50% neutralized acid groups) prepared so until 1% dispersion of OFL.

4. CONCLUSIONS

The development of new drug carrier materials with any improved properties is a permanent topic of academic and industrial research. In view of this context, novel thermo- and pH-sensitive hydrogels slightly cross-linked with DAT were prepared exploiting the temperature-sensitive properties of NIPA and the ionic properties that depend of the pH, originating from AAc. The addition of small proportion of AAc to NIPA-derivative material caused increasing in the swelling properties and control in the swelling according to the pH, which was confirmed by rheological measurements.

NIPA:AAc (70:30) that was chosen by its pH/thermosensibility properties showed low cytotoxicity over fibroblast cells. Besides, the capacity of loading of OFL significantly increased, which was demonstrated for NIPA:AAc (70:30) discs. These showed promising properties for developing systems of modified-delayed drug release for oral administration. Furthermore, NIPA:AAc (70:30) in dispersion form may be easily loaded by acid−base reaction maintaining the drug (OFL) ionically bonded. Interestingly, the complex formed NIPA:AAc (70:30)-OFL was easily dispersed in water to yield a gel formulation of OFL 1% (w/v) that presented different release rates of the drug at 37 °C, depending on the environment in which release was performed. The releases in physiological medium and in buffer phosphate (pH = 6.8) were maintained for 7 h when the test was performed. The results reached with OFL-containing NIPA:AAc (70:30) were very promising for the design of intelligent systems for drug controlled release of local topical or mucosal uses.

dx.doi.org/10.1021/mp400615n | *Mol. Pharmaceutics* 2014, 11, 2239−2249

AUTHOR INFORMATION

Corresponding Author

*(C.I.A.I.) Tel/Fax: 54-0351-5353867/69. E-mail: cia@fcq.unc.edu.ar.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank SECyT (Universidad Nacional de Córdoba), FONCyT, and CONICET for financial assistance. J.C.C. and C.B.C. also acknowledge receipt of fellowships from CONICET and SECyT, respectively.

REFERENCES

(1) Wichterle, O.; Lim, D. Hydrophilic gels for biological use. *Nature* **1960**, *185* (4706), 117−118.

(2) Andrade, J. D., Ed. *Hydrogels for Medical and Related Applications*; ACS Symposium Series; American Chemical Society: Washington DC, 1976; Vol. *31*, p 380.

(3) Graham, N. B.; McNeill, M. E. Hydrogels for controlled drug delivery. *Biomaterials* **1984**, *5* (1), 27−36.

(4) Peppas, N. A. Hydrogels and drug delivery. *Curr. Opin. Colloid Interface Sci.* **1997**, *2* (5), 531−537.

(5) Qiu, Y.; Park, K. Environment-sensitive hydrogels for drug delivery. *Adv. Drug Delivery Rev.* **2012**, *64* (Supplement,(0)), 49−60.

(6) Langer, R.; Peppas, N. Chemical and physical structure of polymers as carriers for controlled release of bioactive agents: A review. *J. Macromol. Sci., Part C* **1983**, *23* (1), 61−126.

(7) Chaturvedi, K.; Ganguly, K.; Nadagouda, M. N.; Aminabhavi, T. M. Polymeric hydrogels for oral insulin delivery. *J. Controlled Release* **2013**, *165* (2), 129−138.

(8) Ichikawa, H.; Peppas, N. A. Novel complexation hydrogels for oral peptide delivery: In vitro evaluation of their cytocompatibility and insulin-transport enhancing effects using Caco-2 cell monolayers. *J. Biomed. Mater. Res., Part A* **2003**, *67A* (2), 609−617.

(9) Onuki, Y.; Hoshi, M.; Okabe, H.; Fujikawa, M.; Morishita, M.; Takayama, K. Formulation optimization of photocrosslinked polyacrylic acid modified with 2-hydroxyethyl methacrylate hydrogel as an adhesive for a dermatological patch. *J. Controlled Release* **2005**, *108* (2−3), 331−340.

(10) Navath, R. S.; Menjoge, A. R.; Dai, H.; Romero, R.; Kannan, S.; Kannan, R. M. Injectable PAMAM dendrimer−PEG hydrogels for the treatment of genital infections: Formulation and in vitro and in vivo evaluation. *Mol. Pharmaceutics* **2011**, *8* (4), 1209−1223.

(11) Nanjawade, B. K.; Manvi, F. V.; Manjappa, A. S. Retracted: In situ-forming hydrogels for sustained ophthalmic drug delivery. *J. Controlled Release* **2007**, *122* (2), 119−134.

(12) Ricci, E. J.; Lunardi, L. O.; Nanclares, D. M. A.; Marchetti, J. M. Sustained release of lidocaine from Poloxamer 407 gels. *Int. J. Pharm.* **2005**, *288* (2), 235−244.

(13) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* **2000**, *50* (1), 27−46.

(14) Chakraborti, C. C.; Sahoo, S.; Behera, P. K. Characterization of an Ofloxacin/Carbopol 940 mucoadhesive polymeric suspension. *Int. J. Curr. Pharm. Res.* **2012**, *4* (2), 92−100.

(15) Babu, J. A.; Ayyappan, T.; Vetrichelvan, T. Novel site specific delivery system containing ofloxacin for the treatment of periodontal infection. *Res. J. Pharm., Biol. Chem. Sci.* **2011**, *2* (1), 590−602.

(16) Hoffman, A. S. Stimuli-responsive polymers: Biomedical applications and challenges for clinical translation. *Adv. Drug Delivery Rev.* **2013**, *65* (1), 10−16.

(17) Don, T.-M.; Huang, M.-L.; Chiu, A.-C.; Kuo, K.-H.; Chiu, W.-Y.; Chiu, L.-H. Preparation of thermo-responsive acrylic hydrogels useful for the application in transdermal drug delivery systems. *Mater. Chem. Phys.* **2008**, *107* (2−3), 266−273.

(18) Ilić-Stojanović, S. S.; Nikolić, L. B.; Nikolić, V. D.; Milić, J. R.; Petrović, S. D.; Nikolić, G. M.; Kapor, A. J. Potential application of thermo-sensitive hydrogels for controlled release of phenacetin. *Hem. Ind.* **2012**, *66* (6), 831−839.

(19) Sadeghi, M.; Ghasemi, N.; Kazemi, M.; Soleimani, F. Synthesis, swelling behavior, salt- and pH- sensitivity of cross linked carrageenan-graft-poly (acrylamide-co-itaconic acid) superabsorbent hydrogel. *Middle East J. Sci. Res.* **2012**, *11* (3), 311−317.

(20) Mohd Amin, M. C. I.; Ahmad, N.; Halib, N.; Ahmad, I. Synthesis and characterization of thermo- and pH-responsive bacterial cellulose/acrylic acid hydrogels for drug delivery. *Carbohydr. Polym.* **2012**, *88* (2), 465−473.

(21) Masteiková, R.; Chalupová, Z.; Šklubalová, Z. Stimuli-sensitive hydrogels in controlled and sustained drug delivery. *Medicina* **2003**, *39* (2), 19−24.

(22) Milašinović, N.; Kalagasidis Krušić, M.; Knežević-Jugović, Z.; Filipović, J. Hydrogels of *N*-isopropylacrylamide copolymers with controlled release of a model protein. *Int. J. Pharm.* **2010**, *383* (1−2), 53−61.

(23) McNeilla, M. E.; Grahamb, N. B. Properties controlling the difusion and release of water-soluble solutes from poly(ethylene oxide) hydrogels: 3. Device geometry. *J. Biomater. Sci., Polym. Ed.* **1996**, *7* (11), 937−951.

(24) http://clinicalpharmacology.com/Forms/Monograph/monograph.aspx?cpnum=450&sec=monindi&t=0.

(25) Chavanpatil, M. D.; Jain, P.; Chaudhari, S.; Shear, R.; Vavia, P. R. Novel sustained release, swellable and bioadhesive gastroretentive drug delivery system for ofloxacin. *Int. J. Pharm.* **2006**, *316* (1−2), 86−92.

(26) Sahoo, S.; Chakraborti, C. K.; Mishra, S. C.; Naik, S. Qualitative analysis of controlled release ofloxacin/HPMC mucoadhesive suspension. *Int. J. Drug Dev. Res.* **2011**, *3* (2), 217−232.

(27) Saigeethika, S.; Gautam, S. Ramanjaneyulu. Formulation and evaluation of floating tablets of ofloxacin. *Int. J. Pharm. Sci. Res.* **2012**, *3* (11), 4291−4296.

(28) Ali, M. S.; Ali, J.; Ahuja, A.; Alam, M. S. Formulation and characterization of dental film containing ofloxacin. *J. Appl. Pharm. Sci.* **2012**, *2* (11), 114−119.

(29) Chaitral, P. P.; Sachin, S. S. Formulation and in-vitro evaluation of ofloxacin microspheres for controlled release. *Int. J. Adv. Pharm. Res.* **2013**, *4* (5), 1803−1809.

(30) Romero, V. L.; Pons, P.; Bocco, J. L.; Manzo, R. H.; Alovero, F. L. Eudragit E100® potentiates the bactericidal action of ofloxacin against fluoroquinolone-resistant *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* **2012**, *334* (2), 102−110.

(31) Çaykara, T.; İnam, R. Determination of average molecular weight between crosslinks and polymer−solvent interaction parameters of poly(acrylamide-*g*-ethylene diamine tetraacetic acid) polyelectrolyte hydrogels. *J. Appl. Polym. Sci.* **2004**, *91* (4), 2168−2175.

(32) International standars ISO 10993-5. Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity; 2009.

(33) Ciapetti, G.; Cenni, E.; Pratelli, L.; Pizzoferrato, A. In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials* **1993**, *14* (5), 359−364.

(34) *The United States Pharmacopeia-The National Formulary*; United States Pharmacopeial Convention, Inc.: Rockville, MD, 2009.

(35) Higuchi, T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J. Pharm. Sci.* **1963**, *52* (12), 1145−1149.

(36) Korsmeyer, R. W.; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N. A. Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.* **1983**, *15* (1), 25−35.

(37) Korsmeyer, R. W.; Peppas, N. A. Solute and penetrant diffusion in swellable polymers. III. Drug release from glassy poly(HEMA-co-NVP) copolymers. *J. Controlled Release* **1984**, *1* (2), 89−98.

(38) Geever, L. M.; Cooney, C. C.; Lyons, J. G.; Kennedy, J. E.; Nugent, M. J. D.; Devery, S.; Higginbotham, C. L. Characterization and controlled drug release from novel drug-loaded hydrogels. *Eur. J. Pharm. Biopharm.* **2008**, *69* (3), 1147−59.

(39) Chearúil, F. N.; Corrigan, O. I. Thermosensitivity and release from poly *N*-isopropylacrylamide−polylactide copolymers. *Int. J. Pharm.* **2009**, *366* (1−2), 21−30.
(40) Caykara, T.; Kiper, S.; Demirel, G. Thermosensitive poly (*N*-isopropylacrylamide-co-acrylamide) hydrogels: Synthesis, swelling and interaction with ionic surfactants. *Eur. Polym. J.* **2006**, *42* (2), 348−355.
(41) Ross, D. L.; Riley, C. M. Aqueous solubilities of some variously substituted quinolones antimicrobials. *Int. J. Pharm.* **1990**, *63*, 237−250.
(42) Romero, V. L. *Sistemas portadores de antimicrobianos: evaluación de los efectos de polímeros aniónicos y catiónicos en el desempeño y la eficacia de agentes antimicrobianos*. Ph.D. thesis, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina, 2012. Available at http://fcq.biblio.unc.edu.ar/cgi-bin/koha/opac-detail.pl?biblionumber=7817.