

Poly(N-Isopropylacrylamide-Co-Acrylic Acid) Smart Nanocarriers for Drug Release: A Study of Theophylline Delivery

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Abstract

Copolymeric nanoparticles of poly(N-isopropylacrylamide-co-acrylic acid) have been synthesized by inverse microemulsion polymerization. The obtained nanoparticles have been characterized by FTIR and DSC, to detect their molecular structure and glass transition temperatures respectively, by transmission electron microscopy (TEM) and quasielastic light scattering (QLS) to determine the sizes and size distribution of gels synthesized. The swelling of these nanogels has been studied in order to know their response at different pH and crosslinking agent concentration. These nanoparticles have subsequently been charged with a vasodilator drug (theophylline) to study the release-kinetics of the drug release at different pH. These nanogels have shown a controlled release at basic environment. After further biological viability studies this system could be used as smart carriers in nanomedicine.

Keywords: Hydrogel; Drug release; Microemulsion polymerization; Nanoparticles; Theophylline

Introduction

In recent years the use of hydrogels in biomedicine has increased as they have very good biocompatibility with the human body, mainly due to its physical properties, which make them similar to living tissue, especially for its high content in water, soft and elastic consistency and low interfacial tension which does not allow proteins absorb body fluids [1-6]. Taking into account the fast evolution of nanotechnology smaller carriers are demanded and in this sense nanogels appear as ideal candidates. Those particles consist in a crosslinked polymeric network which is able to swell up, hosting large amounts of solvent inside; as a result, those structures allow the drug delivery in a very specific way, targeting the tissues where the drug is needed without damaging the healthy ones. The most efficient way to obtain nanogels is through inverse microemulsion polymerization [7,8].

One of the most studied thermosensitive monomers is N-isopropylacrylamide [9,10], being the resulting polymers capable of suffering a conformational transition at body temperature [11]. Taking into account this exceptional feature and the pH-sensitivity of acrylic acid [12-15], the main objective of the present work is the synthesis and characterization of NIPA-co-acrylic acid copolymeric nanoparticles obtained by inverse microemulsion polymerization. Besides, a study of the release [16] of Theophylline at different pH values has been carried out in the nanoparticles. Theophylline, also known as dimethyl xanthine, is a drug which is used in therapy for respiratory diseases such as COPD and asthma under a variety of brand names.

Being a member of the xanthine family, it shares structural and pharmacological similarities with caffeine. It is naturally found in tea,

although in trace amounts (~1 mg/L), significantly less than the required therapeutic doses [17]. It is also present in cocoa beans, 3.7 mg/g have been extracted from Criollo cocoa beans.

The main actions of theophylline involve: relaxing bronchial smooth muscle, increasing heart muscle contractility and efficiency; as a positive inotropic. Increasing heart rate: positive chronotropic. Increasing blood pressure. Increasing renal blood flow. Some anti-inflammatory effects and central nervous system stimulatory effect mainly on the medullary respiratory center.

The objective of the present research is to synthesize polymeric nanogels which are able to respond to various external stimuli releasing the active substance. This system could eventually be used in controlled and targeted drug delivery especially for diseases which treatment has been carried out in a basic medium such as ophthalmic mucosa or colon. This medical approach would reduce the amount of drug employed, decreasing the side effects of the treatment.

Experimental

Materials

N-Isopropylacrylamide (NIPA) (Across Organics, 97%), sorbitan sesquiolate (ARLACEL-83, Sigma Aldrich), polyoxyethylene sorbitol hexaoleate (ATLAS G-1086, Sigma Aldrich), isoparaffinic oil (Isopar M, Esso Chemie), chloroform (Riedel de Haën, > 99.8%), MilliQ water and diethyl ether (Panreac, 99.7%) were used without further purification. Acrylic acid (AA, Sigma Aldrich, 99%) was purified through an ion exchange column and the radical initiator sodium metabisulfite (DSNa, Merck, > 98%) was recrystallized from methanol prior to use. The crosslinking agent, N,N'-methylene-bis-acrylamide (NMBA, Aldrich Chemical, > 98%) and the drug used for the delivery

study, theophylline (Sigma Aldrich, 99%), were used without further purification.

Methodology

Synthesis of the nanogels: The monomer is solved an adequate volume of water (in all cases the aqueous phase has presented the maximum proportion of monomer that its solubility allows, 20%) together with NMBA. Separately the compounds of the organic phase are mixed Isopar M, Atlas G-1086 and Arlacel-83). Then the aqueous phase is added dropwise to the organic one, stirring the mixture vigorously over 30 minutes.

This slow addition is crucial for the final aspect of the microemulsion (lower viscosity, transparent look and higher micelle formation) because of the rise of the bluish tone of the microemulsion. The mixture is placed in a reaction flask, under nitrogen atmosphere and with constant magnetic stirring at 25 °C for an hour before the polymerization reaction is carried out. The reaction flask is provided with a thermopar to indicate any temperature change. The adequate solvent to extract the nanohydrogels from the microemulsion and to purify them from the possible residues of monomers, oils and surfactant agents, resulted to be diethyl ether. As the objective is to extract crosslinked hydrogels, the fact that the solvent can dissolve the linear polymers or monomers results beneficial because as it would clean the hydrogel from the starting materials.

The microemulsion is poured onto the diethyl ether vortex and a white, thin powder begins to form at the bottom of the pot. Afterwards the solvent is eliminated by decantation and dried under vacuum. In order to eliminate possible residues, the solid is precipitated again in diethyl ether and a thin white powder is obtained. As it is shown in Table 1, several compositions of NIPA-co-AA have been synthesized, with different degrees of crosslinking agent (NMBA).

% NIPA	% AA	% NMBA		
100	0	1	3	5
90	10	1	3	5
80	20	1	3	5
70	30	1	3	5
60	40	1	3	5

Table 1: Synthesized copolymeric composition.

Drug load of theophylline: The nanogels were loaded by immersion in a solution of 2.5 mg/mL of theophylline in MilliQ water for 72 hours. During that period the nanogels are vigorously stirred and light-isolated due to the high photosensitivity of the drug. When the UV-Vis signal of theophylline in the supernatant decreases and it remains constant the load is considered completed.

The nanogels are centrifuged and washed through nanometric membranes in order to eliminate the possible drug residue stuck to the external surface. After the filtration the nanogels are dehydrated with dry-air.

Characterization

Fourier transform infrared studies (FTIR): FTIR spectrophotometer (Nicolet 6700) was employed to obtain spectra of copolymeric

nanohydrogels. The spectra were collected using Attenuated Total Reflectance (ATR) Smart Orbit accessory. All the spectra were recorded with the average of 100 scans with a resolution of 4 cm⁻¹.

Differential scanning calorimetry studies (DSC): Glass transition temperatures were measured using a TA Instruments calorimeter (DSC 2920). Standard indium (156.68 °C) and zinc (419.58 °C) were used for calibration. The thermal analysis for poly(NIPA-co-AA) copolymers was performed from 0 to 180 °C at a heating rate of 10 °C/minute under nitrogen flow (100 mL/min⁻¹). Glass transition temperature was assigned by the mid-point criterion.

Quasielastic light scattering (QLS) and transmission electron microscopy (TEM) measurements: QLS was employed to determine particle size and distribution of the nanohydrogels. An AMTEC light scattering spectrophotometer was used at an angle of 90°. Intensity correlation function measurements were carried out using a Brookhaven BI-9000AT 522-channel digital correlator, equipped with a water-cooled argon-ion laser operated, at 514.5 nm, as a light source. Samples were prepared by dispersing the purified powder in water and acetone for 24 hours under stirring. All measurements were recorded at 25 °C. Size distribution was obtained by CONTIN analysis at pH 7.0.

TEM micrographies were collected using a JEOL JSM7000F electron microscopy at 10.0 kV, equipped with a Field Emission Gun (FEG). An ultrathin coating of electrically-conducting material is deposited by high vacuum evaporation or by low vacuum sputter coating of the sample. For these experiments a gold film was deposited over the sample. Sphericity of the synthesized nanohydrogels was confirmed by this technique.

Swelling measurements: The swelling measurements were carried out using a nanometric-pore membrane. The liquid passed by diffusion through the membrane, swelling the polymer up, allowing the determination of the swelling values by analytical weights. All the measurements were done to every sample and at different pH.

Nanogels releasing-kinetics (UV-Vis): The releasing-kinetics measurements were followed by UV-Vis Cintra 303 spectrophotometer, using a black quartz tray of continuous flow coupled to a peristaltic pump. The nanogels were introduced in a buffer solutions of pH 5.0, 7.0 and in bare MilliQ water to evaluate the releasing of the drug. After passing through a nanometric filter (to avoid the entrance of the nanogels to the tray) the solution flown through the tray and values of the absorption wavelength of the drug (250 nm) were measured every second.

Results and Discussion

FTIR characterization

Figure 1 shows the FTIR spectrum for the studied samples. All the spectra (except 100% poly(NIPA)) confirm the presence of acrylic acid into the copolymeric structure. The band at 1.700 cm⁻¹ confirms the presence of this monomer due to the stretching absorption of carbonyl group. The rest of the bands belong to NIPA and AA. It is also observed that as the composition is enriched with the acid the characteristic band of the carbonyl group is clearly predominant. The sample containing 40% of AA shows the most intense carbonyl band.

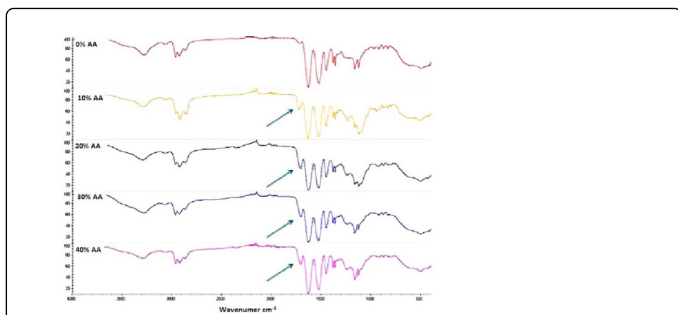


Figure 1: FTIR spectrum of the studied compositions.

DSC characterization

Calorimetric curves were obtained for all samples. The curves show an increase of stiffness of the nanoparticles when the composition of AA rises. As a consequence of the hydroxyl group present in the monomer, the glass transition temperature increases. The hydroxyl groups form hydrogen bonds into the hydrogel reducing the movement of polymeric chains. Figure 2 exemplifies this behavior when the glass transition temperature is measured in samples of different proportion of AA. This figure also shows that the glass transition temperature depends on the crosslinking agent percentage. For instance, a nanogel of 90% NIPA and 5% of NMBA presents 80 °C glass transition temperature. Besides, a polymer of the same concentration of NIPA but containing just a 1% of crosslinking agent, suffers a decrease in the glass transition temperature, reaching a value of 65 °C. All the samples analyzed present the same tendency.

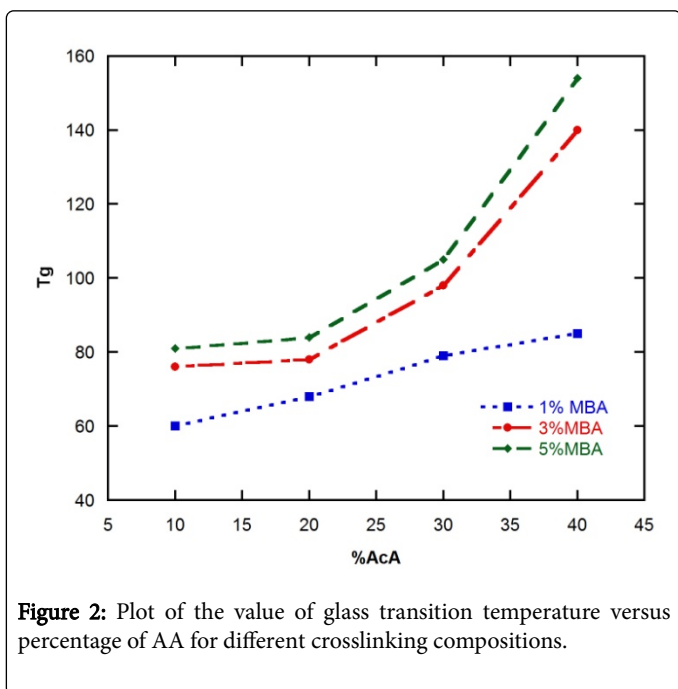


Figure 2: Plot of the value of glass transition temperature versus percentage of AA for different crosslinking compositions.

Analyzing the behavior based on AA concentration in the hydrogel, we find that for the same percentage of crosslinking agent the glass transition temperature value increases. For example, when the concentration of crosslinking agent is maintained constant (5%) and

the AA percentage is raised from 10 to 40% the glass transition temperature changes from 80 to 163 °C. This occurs in every case studied and is due to the fact mentioned above.

QLS and TEM characterization

The particle size measurements obtained by QLS indicate a predominant molar mass distribution of 63 nm for all samples. This is confirmed in Figure 3 with TEM imaging. The obtained micrographs show that a particle size of 40-70 nm, observing spherical morphology for all the studied nanoparticles.

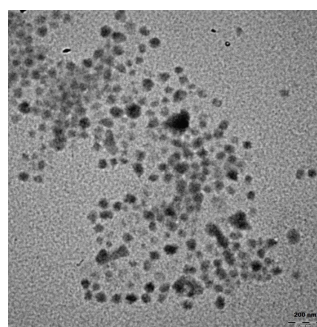


Figure 3: TEM Microographies of PNIPAA-co-acrylic acid nanoparticles (40% AA).

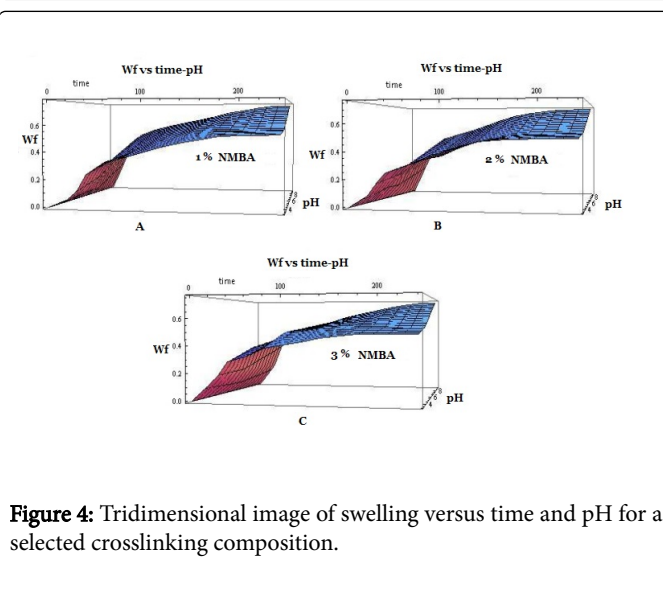


Figure 4: Tridimensional image of swelling versus time and pH for a selected crosslinking composition.

Swelling properties

The study of swelling behavior of the specific composition poly(NIPAA-co-AA) 90:10 at different pH values and crosslinking percentages were carried out. Figure 4 shows the tridimensional image of swelling values as a function of time and pH for a selected crosslinking percentage. It can be observed that for each curve swelling degree increases as time and pH increase. On the contrary swelling increases inversely proportional to the crosslinking agent percentage. The swelling degree is affected by the pH due to pKa of the AA, if the environment is acid, the acrylic acid will present its acid form (-COOH) but at high pH values, the deprotonated form will be shown (-

COO⁻). The presence of negative charges causes the appearance of repulsive forces between them, thus leading to a tendency of the chains to separate the macromolecular network and expand, thereby allowing greater amount solvent inside.

Figure 5 represents the study of swelling at infinite time for a poly(NIPA-co-AA) 90:10 sample, varying the crosslinking agent (1, 3, and 5%) into 3 different buffer solutions. This figure shows that a sample with a defined composition and same pH presents a small increase in W_{∞} when the percentage of NMB acrosslinking agent increases.

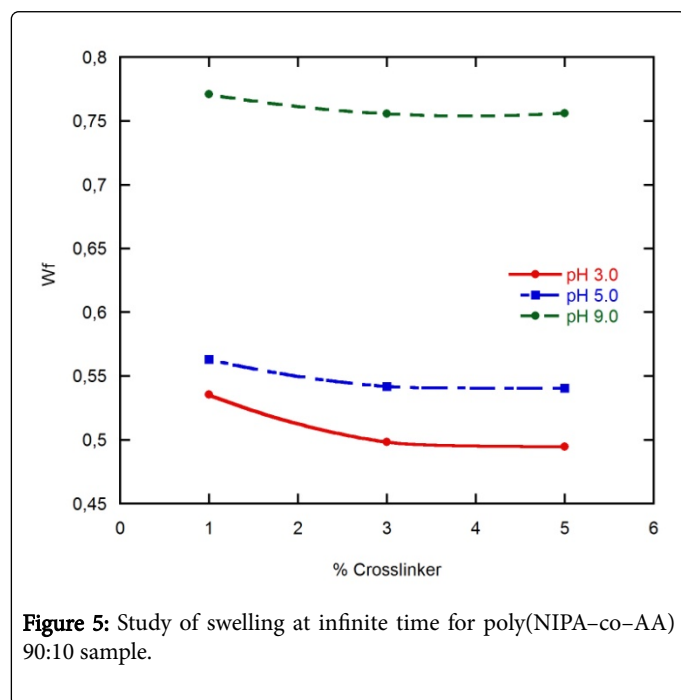


Figure 5: Study of swelling at infinite time for poly(NIPA-co-AA) 90:10 sample.

However if the crosslinking agent percentage remains constant, W_{∞} value is deeply influenced by the pH. Effect of pH will be predominant in comparison to the effect of crosslinking agent amount. For example, when the crosslinker concentration is constant (1%) and pH is increased from 3 to 9, an increase of the 30% can be observed in the W_{∞} value, whereas when the percentage of crosslinker is changed, the W_{∞} difference does not exceed 4%. Therefore the most influential parameter on the swelling behavior of the synthesized nanoparticles is pH.

Drug delivery kinetics

The selected drug to incorporate into the polymer structure of nanogels was theophylline, specifically for poly(NIPA-co-AA) 80:20. Drug delivery system was carried out in 3 different pH buffer solutions and the drug delivery kinetic was measured by UV-Vis spectroscopy. The study was carried out measuring the absorption wavelength of theophylline (250 nm). The drug delivery kinetics depends on the pH, due to the possible hydrolyzation of the AA groups. The swelling will be faster in basic pH and the drug release will be more efficient, until equilibrium is reached (Figure 6).

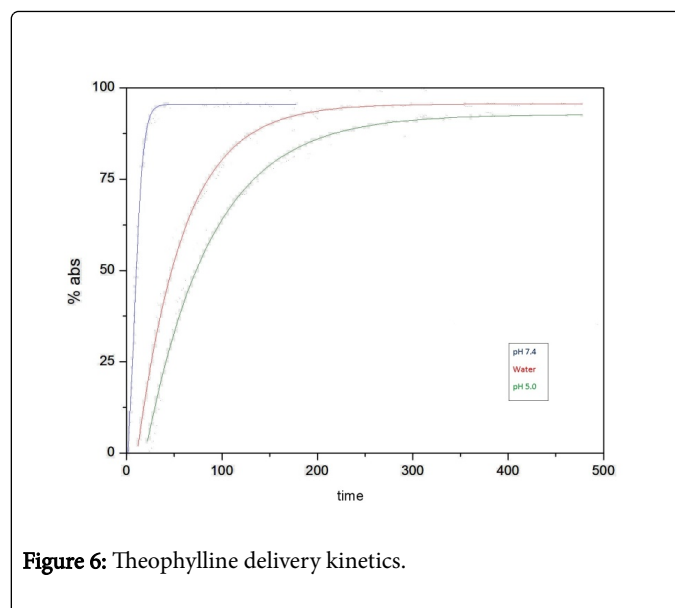


Figure 6: Theophylline delivery kinetics.

Conclusion

Nanoparticles of poly (NIPA-co-AA) have been synthesized by reverse microemulsion, varying NIPA/AA compositions. All copolymers were characterized by FTIR and DSC. It is observed in DSC thermograms that glass transition temperature increases when acrylic acid percentage in the polymer is higher, due to the ability of the hydroxyl groups to form H-bonds. The increment of the crosslinking agent causes also a rise in glass transition value, due to the restriction of the molecular chains, needing more temperature to allow free movement of the chains. The size of the particles was measured by quasielastic light scattering and electronic transmission spectroscopy; obtaining values of around 60 nm.

A study of the swelling of the nanogels has been carried out, finding that when the composition of the polymer is kept constant, lower crosslinking values present higher swelling values. Therefore, it is observed that for a set composition and constant crosslinking agent amount, the swelling values are higher in basic environment. This effect occurs as a consequence of the deprotonation of the -COOH group of the AA at high pH, causing a strong repulsion between the negative charges created, favoring the expansion of the polymeric network and allowing the hostage of greater amount of solution inside.

In this study the nanogels have been loaded with a vasodilator drug (theophylline) and the release-kinetics have been studied in three buffers of different acidity, measuring distinct release values were different in time and absorbance signal depending on the pH of the solution.

Given the data explained above, we have a promising drug delivery system. Due to its ability to be formulated with several monomers with different pKa, we can synthesize nanogels with different pH-sensitivity, achieving a drug delivery system selectively in the desired pH media. If these nanoparticles are functionalized with different target molecules, we would be building a promising drug delivery system, obtaining particles that release the exactly amount of drug in a precise time only where necessary.

Acknowledgement

This work was supported by MICINN (MAT2010-21509-CO2) and SAIOTEK2013.

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