Society For Biomaterials

New hydrogel obtained from a novel dendritic monomer as a promising candidate for biomedical applications

Julio César Cuggino,[1] Germán Charles,[1] Gerardo Gatti,[2,3] Miriam Cristina Strumia,[1] Cecilia Inés Alvarez Igarzabal[1]

[1]Departamento de Química Orgánica, IMBIV-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba 5000, Argentina
[2]Departamento de Bioquímica Clínica, CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba 5000, Argentina
[3]Fundación para el Progreso de la Medicina—Laboratorio de Alta Complejidad, 9 de Julio 941, Córdoba 5000, Argentina

Received 14 August 2012; revised 8 January 2013; accepted 9 January 2013
Published online 2 April 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34640

Abstract: Acid functional hydrogels are a type of materials with many advantages. Over the last years, increasing attention for the synthesis of dendronized polymers has been drawn due to their unique properties of high multivalence in the same surface as compared with conventional polymers. In this study, we report the preparation of novel acid dendronized hydrogels using a dendritic monomer obtained from Behera's amine. The swelling and rheological performance, the non-toxicity over fibroblast cells and the drug encapsulation capacity of the novel hydrogels suggests that the new materials can achieve great potential as carrier for drug delivery and other potential biomedical applications. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 101A: 3372–3381, 2013.

Key Words: biomaterials, dendronized polymers, drug carriers, hydrogels, soft materials

How to cite this article: Cuggino JC, Charles G, Gatti G, Strumia MC, Alvarez Igarzabal CI. 2013. New hydrogel obtained from a novel dendritic monomer as a promising candidate for biomedical applications. J Biomed Mater Res Part A 2013:101A:3372–3381.

INTRODUCTION

Hydrogels are three-dimensional structures containing highly polar groups with the ability to retain large amounts of fluid. Normally, hydrogel networks are insoluble due to the presence of physical and chemical crosslinks of elastic nature. These properties grant the hydrogel the ability to swell in water or biological fluids without dissolution. Hydrogels are extremely suitable for a variety of applications in the pharmaceutical and medical industry since they are capable of retaining large amounts of water and because their soft and rubbery consistence closely resembles that of living tissues. Moreover, the high water content also contributes to their excellent biocompatibility. Thus, hydrogels have proved to be particularly useful for drug delivery of active agents,[1-4] cell support in tissue engineering,[5,6] among other biomedical applications. Novel non-toxic and biocompatible hydrogels with improved properties are always extensively required for drug delivery applications. Hydrogels containing acid functional groups are a type of materials with many additional advantages for drug delivery. They have the "smart" property of changing the macroscopic volume of their network structure with a change in pH.[7,8] In addition, the acid groups offer functionality in order to incorporate active ingredients such as drugs, dyes and peptides into the hydrogel structures through ionic or chemical interaction. The acid groups also provide materials with both good water swelling and mucous adhesive properties which are important from the view point of drug delivery applications. The most simple and industrially used monomer for the synthesis of anionic based hydrogels is the acrylic acid. This monomer is used to prepare Carbomer® products, which are slightly crosslinked polymeric materials widely accepted in the pharmaceutical industry to prepare gel formulations for drug vehiculization. However, over the last years, special attention has been directed to the synthesis of new high functionalized or dendronized polymers due their unique properties of high multivalence in the same surface, as compared with conventional polymers.[9,10] The high functionality and architecture of dendronized hydrogels could bring advantages in drug delivery applications with respect to the normal hydrogels. So, the high functionality of dendronized hydrogels could exhibit the capacity to load the same amount of drug using less amount of vehicle polymer. Further, these gel formulations can present the same viscosity and mechanical properties using lower polymer amounts as those prepared with conventional polymers. Physical or chemical crosslinking reactions of

Correspondence to: C. I. Alvarez Igarzabal; e-mail: cia@fcq.unc.edu.ar
Contract grant sponsors: MECyT; CONICET; SECyT (Universidad Nacional de Córdoba); FONCyT

TABLE I. Experimental Reaction Conditions and Parameters Determined for Hydrogels

Hydrogel[a]	Polymer Mass Recovered[b] (%)	v_{2s}	%ESR[c]
HG 0.60/4	65	[d]	[d]
HG 0.80/4	68	0.010	9805
HG 1.00/2	75	0.011	8546
HG 1.00/4	73	0.012	7456
HG 1.00/6	77	0.027	2300
HG 1.20/4	75	0.013	5201
HG 1.40/4	75	0.020	3748
HG 1.60/4	74	0.024	2592
HG 1.80/4	72	0.026	1952

[a] In the HG 0.60/4 nomenclature, HG means hydrogel, 0.60 and 4 indicate that the hydrogel was prepared using 0.6*M* of DM and 4% mol ratio of DAT with respect to the total DM moles. A 2% mol ratio of APS with respect to DM was used for the preparation of all hydrogels.

[b] Determined from the dry weight of the final product after purification.

[c] Determined in water from hydrogel discs at 25°C.

[d] Not determined since HG did not maintain the solid consistence.

functionalized dendritic or dendrimer monomers,[11,12] in addition to the dendronization of existing polymers,[13] are convenient strategies for the synthesis of this new kind of materials.

Behera's amine (BA) is a commercially available dendritic amine developed by Raijani Behera in Newkome's group.[14] This material was recently used for the synthesis of multifunctional dendrimers for drug delivery applications[15,16] and theranostics.[17] In a previous work, we also reported the preparation of linear dendronized polymers derived from BA and their efficient interaction with a cationic surfactant was shown by rheology.[18] However, the preparation of hydrogel materials using BA as a precursor is yet unexplored. The preparation of this kind of material could exhibit interesting applications in biomedical fields such as drug delivery or cell support. In this report we present, for the first time, the synthesis of new dendronized hydrogels with a high acid functionality using the traditional vinyl free radical polymerization of a special vinyl monomer prepared from BA. The chemical characterization, swelling behavior, rheology, fibroblast cytotoxicity and ionic drug load-release capacity of the new materials were studied in order to analyze the properties and the possibilities of biomedical application in drug delivery formulations.

MATERIALS AND METHODS

Materials

The following chemicals were used as purchased: Behera's amine (BA, Frontier Scientific); dichloromethane (CH_2Cl_2, Anedra); triethylamine (TEA, Aldrich); formic acid (FA, 85% Cicarelli); ammonium persulphate (APS, Aldrich); tetramethylenethylendiamine (TEMED, Aldrich); *N,N′*-diallyltartardiamide (DAT, Aldrich); azithromycin (AZI, Parafarm); Carbomer 934 (Parafarm, $M_w = 3 \times 10^6$ g mol^{-1}); glacial acetic acid (CH_3COOH, Cicarelli); phosphoric acid (H_3PO_4, Cicarelli); boric acid (H_3BO_3, Cicarelli); sodium hydroxide (NaOH, Cicarelli); and 3-(4,5-dimethylthiazol-2yl)-2,5, diphenyl tetrazolium bromide (MTT, Sigma). The dendritic monomer (DM) 4-acryloylamine-4-(carboxyethyl) heptanedioic acid was synthesized according to a previous work.[18] Britton Robinson (BR) buffers were prepared dissolving 2.3 mL of glacial CH_3COOH, 2.7 mL of H_3PO_4, and 2.4720 g of H_3BO_3 in 1000 mL of distilled water. Aliquots of 100 mL were taken and the pH of each solution was adjusted at pH 3.02, pH 5.01, and pH 7.00 with 2*M* NaOH solution.

Synthesis of the dendritic monomer and the hydrogels

The dendritic monomer (DM) was synthesized by reaction of amidation between BA and acryloyl chloride. *Tert*-butyl ester groups were then hydrolyzed with formic acid for 12 h at 45°C to yield the tri-acidic dendritic monomer DM.[18] The general procedure for the synthesis of the hydrogels was as follows: the DM, the crosslinking agent, DAT and APS as initiator were dissolved in 2 mL of distilled water in a tube with a septum. The solution was then bubbled using N_2 for 2 min in order to eliminate the oxygen of the system; the corresponding volume of 0.32*M* aqueous solution of TEMED was added. Immediately, the reaction mixture was transferred to a 5 mL disposable hypodermic syringe of poly(propylene) which served as reactor. The syringe was placed in a bath at 25°C and allowed to react for 24 h. After reaction, the product was extracted from the syringe and the polymer was cut in regular tablets of 1 cm diameter and 3 mm thick. The discs were exhaustively washed with distilled water for 48 h for removal of unreacted monomers, dried at room temperature for 3 days and in an oven at 30°C until constant weight. Table I describes the experimental reaction conditions.

NMR characterization

A nuclear magnetic resonance spectrometer BRUKER 400 MHz NMR was used for the acquisitions. For the ^{1}H-NMR measurements, approximately 5 mg of fine powder polymer was swelled in 0.8 mL of D_2O for 24 h before each measurement in order to allow water incorporation.

Network parameter

The Archimedes' principle was used to measure the volume of the dry polymer (V_p). The mass of the disc was determined in air and in heptane used as non-solvent; Eq. (1) was used for V_p determination:[19]

$$V_p = \frac{W_a - W_h}{\delta_{heptane}} \tag{1}$$

where W_a and W_h are the weights of dry polymer in air and heptane, respectively, and $\delta_{heptane}$ is the density of heptane (0.684 g mL^{-1}) at 25°C. The density of each disc, $\delta_{polymer}$, was determined using the mass and V_p of the dry polymer.

The volume fraction of polymer after swelling equilibrium (v_{2s}) was calculated from Eq. (2):

$$v_{2s} = \left[1 + \left(\frac{\left(\frac{m_s}{m_d} - 1\right)\delta_{polymer}}{\delta_{solvent}}\right)\right]^{-1} \tag{2}$$

where m_s is the mass of the disc swollen in the swelling solvent, m_d is the mass of the dry disc and $\delta_{solvent}$ is the density of the solvent used.

Dynamic and equilibrium swelling studies

Dry discs were placed in 50 mL of milli-Q water or buffer (Britton-Robinson) at 25°C. The gels were removed from the solution at different time intervals, weighted (m_t), and then returned back into the corresponding solution. This procedure was repeated until constant swelling weight was achieved. Then, the percentage of swelling ratio [*% SR*, Eq. (3)] *versus* time was plotted as an average of the determinations by triplicate.

$$\%SR = \left(\frac{m_t - m_d}{m_d}\right) \times 100 \quad (3)$$

The equilibrium swelling ratio (*%ESR*) is the *%SR* measured at equilibrium.

To determine the process of diffusion of water into the matrices at 25°C, the Fick's law model was used as shown in Eq. (4):[20–22]

$$F = M_t/M_\infty = k\ t^n \quad (4)$$

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 water diffusion. Equation (4) 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.

Rheological characterization

The rheological characterization of the hydrogels was carried out using an Anton Paar Physica MCR 301 controlled-strain rheometer. A 25 mm plate-plate (PP25) geometry and 1.5 to 1.8 mm gap were used for all the experiments. Amplitude sweep studies (strain 0.01%–100% at constant frequency of 10 Hz) on each hydrogel disc swollen in milli-Q water at 25°C were performed to establish the linear viscoelastic region (LVR) profiles and the critical strain (CS) region of each material. Then, frequency sweep from 0.1 to 100 Hz at a fixed strain within the LVR (0.3–0.7% depending on each hydrogel) was also performed to determine the storage moduli (G') and the loss moduli (G'') in the LVR. All the determinations were made by triplicate.

Cytotoxicity studies

The cytotoxicity evaluation of the hydrogels was carried out by direct contact assay with a monolayer of skin fibroblast cells according to ISO standards.[23] Approximately 90 mg of polymer in powder form was swelled in 4 mL of fresh culture medium for 24 h at 37°C. Briefly, fibroblast cells were subcultured from stock culture by trypsinization and seeded onto multi-well tissue culture plates. Cells were fed with Dulbecco's minimum essential medium supplemented with bovine serum and incubated at 37°C in 5% CO_2 atmosphere. When the cells attained a monolayer, 2.5; 5; and 10 μL of the culture swollen hydrogel (previously sterilized for 30 min by UV exposition) were carefully kept in contact with the cells, by triplicate, for direct contact assay seeking to cover only one tenth of the cell surface. After incubation of the gel in contact with fibroblast cells for 24 h at 37°C, the cytotoxicity of gels was quantitatively assessed by MTT assay which measures the metabolic reduction of 3-(4,5-dimethylthiazol-2yl)-2,5, diphenyl tetrazolium bromide to a colored formazan by viable cell.[24]

Ionic polymer-drug interaction

Dried discs were powdered into small particles. The powder with particle size between 100 and 120 mesh (140–117 μm) was selected through the standard testing sieves. The polymer-drug ionic complex was prepared by addition of an appropriate amount of AZI to neutralize 50% of carboxylic groups. The drug-polymer complex was prepared by mixing of the corresponding amount of the polymer and the drug in a mortar; 1 mL of ethanol was then added. The mixture was gently stirred for 10 min and left to react for 24 h. The complex was dried at room temperature and then in an oven at 40°C. To corroborate the polymer-drug ionic interaction, the effect of the addition of 3*M* NaCl solution to the ionic equilibrium of complex dispersions (100 mg hydrogel in 8 mL of water) was evaluated. Thus, the pH of the dispersion *versus* the volume of NaCl added[25] were plotted as average of the determinations by triplicate.

RESULTS AND DISCUSSION

Synthesis of hydrogels

Figure 1 shows the synthesis of the high functionalized acid hydrogels (HG) by free radical solution polymerization. The polymerization was carried out using DM as monomer, DAT as crosslinker, water as solvent, and the redox couple APS/TEMED as generator and activator of the formation of free radicals, respectively.

With the aim of investigating the influence of the initial monomer and crosslinker concentrations over the properties of the hydrogel, a systematic analysis was performed by modifying the DM initial concentration and the DM/DAT feed ratio on the synthesis. DAT was chosen as hydrophilic crosslinking agent instead of the normally used *N′N*-methylenebisacrylamide (BIS). The choice was based on the structural similarity that present the dendritic monomer prepared from BA and the monomer *N*-acryloyl-*tris*-(hydroxymethyl) aminomethane (NAT) which was copolymerized and crosslinked with DAT.[26] Table I describes the experimental details for the preparation of the hydrogels. In the HG 0.60/4 nomenclature, HG means hydrogels, 0.60/4 indicates that the hydrogel was prepared using 0.6*M* concentration of DM and 4% mol ratio of DAT with respect to the total DM moles**.** The polymers were obtained in rod shaped with good polymer mass recovery after purification (65–77%). The low conversion could be related with the low reactivity of the allyl group of DAT respect to the

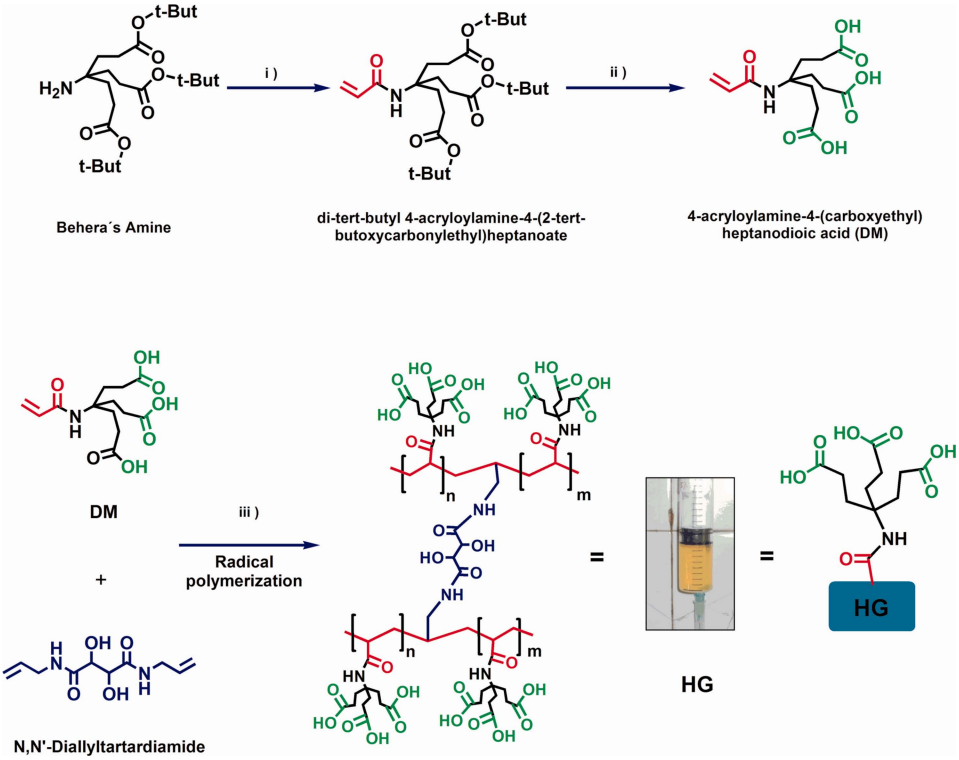

FIGURE 1. Hydrogel synthesis by free radical polymerization. Reagents and conditions: (i) acryloyl chloride, triethylamine (TEA), CH_2Cl_2, 0 to 25°C, 4 h (92% yield); (ii) formic acid 85 %, 45°C, 16 h (99% yield); (iii) APS/TEMED, distilled water, 25°C, 24 h (65–77% yield). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

acrylamide group of monomers like DM in the radical copolymerization. For the reactions in which DAT was used as crosslinker and despite the fact that the conversion of the allyl group may be low, the studies performed on the yielded products showed homogeneity in the structures. The products resulted mechanically stable in general and not showed collapse as easily as softer gelatinous materials.

As a test to corroborate the existence of chemical crosslinked continues networks, all the products were able to swell but not dissolve in excess of distillated water after 48 h of contact. In addition, not polymer mass loss was noticeable by gravimetry after a cycle of dry/maximum swelling/dry assayed on the products. This behavior is highly important for the possible applications of these products in biomedical fields since it is desirable that the polymeric material do not leach from the network over sustained periods.

^{1}H-NMR characterization was carried out to investigate the chemical structure of DM and hydrogels. Figure 2 displays the ^{1}H-NMR chemical characterization of HG 1.00/4 compared with the precursor monomer DM. The polymerization reaction of the DM precursor was corroborated through the disappearance of the characteristic vinyl bands in the HG 1.00/4 spectrum (HG 1.00/4 in Fig. 2) between δ: 5.70–6.29 ppm which was present in the monomer spectrum (DM in Fig. 2). In addition, the signals of -C***H***-C***H***$_2$- polymer backbone could be observed as broad signals at approximately δ: 2.40 and 2.06 ppm, respectively. The polymer backbone signals appear superposed with the signals of the monomer -C***H***$_2$-COOH and -C-(C***H***$_2$-CH$_2$-COOH)$_3$. A signal corresponding to the incorporation of DAT in the network structure -CH-C***H***$_2$-NHCO- (see Figs. 1 and 2) is shown at δ: 2.97 ppm.

Network parameters

Since the favorable properties of hydrogels stem from their hydrophilicity, the characterization of their water-sorption capabilities is fundamental to understand the nanoscopic structure of hydrogel networks.[27] In order to evaluate the potential application of the hydrogels in biomedicine, it is important to know their network parameters. One of the most important parameters is v_{2s}, which provides an idea of the fraction in volume that the polymer occupies in the total hydrogel (polymer + fluid). This parameter can be easily determined using swelling theory measurements [Eq. (2)].[19] As shown in Table I, the values of v_{2s} ranged between 0.010 and 0.027 depending on each hydrogel. In the analysis of the experimental values of v_{2s}, an increase in the initial monomer concentration for the polymerization (for HG 0.80/4; HG 1.00/4; HG 1.20/4; HG 1.40/4; HG 1.60/4 and HG 1.80/4) resulted in a decrease in the swelling capacity of the dense polymer networks formed. Consequently, the fraction of polymer in the total swelled hydrogel (v_{2s}) increases. The polymers with low v_{2s} values showed high water swelling capacity. The dendronized polymers occupy only between 1.0 and 2.6% of the total volume of the hydrogel network, suggesting that the major component of the material is water. This important water fraction (from 97.4 to 99.0%) in the gels (polymer + water) enhances the

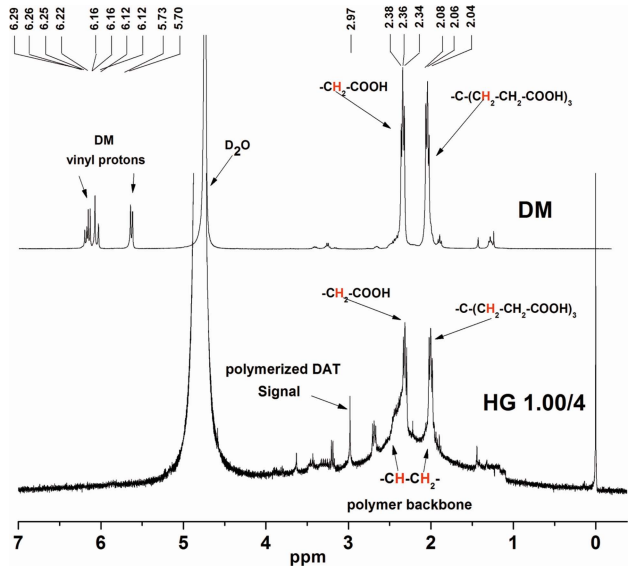

FIGURE 2. ^{1}H-NMR (400 MHz) spectra of dendritic monomer (DM) compared with that of the hydrogel (HG 1.00/4) in D_2O at 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

potential use of the new dendronized hydrogels as biomaterials.

Dynamic and equilibrium swelling studies

Effect of initial monomer concentration. Table I and Figure 3(a) show, respectively, the *%ESR* and water absorption rate studies in milli-Q water for the HGs prepared with different DM concentration. The dendronized hydrogels absorbed a large amount of water (between 18 and 95 g water per g of polymer). Thus, in a strict classification relative to water sorption, the novel hydrogels are superabsorbent polymers.[28] The water absorption of the new hydrogels could be tuned depending on the initial monomer concentration, from 18 to 95 g of water per gram of polymer, which is significant from the perspective in drug delivery applications. The possibility of modifying the fluid absorption capacity of the hydrogels will allow the regulation of two important parameters such as mechanical properties and drug release rate from the gel formulations.

As Figure 3(a) shows, the increase in the initial monomer concentration resulted in a decrease in both: rate of water absorption and swelling equilibrium (*%ESR*, Table I). Clearly, when the initial monomer concentration was major, a decrease in the *%ESR* was produced. There is minor space between the chains in the networks formed after polymerization, possibly due to the presence of a significant amount of crosslinks by secondary interactions of hydrogen-bridged type. Consequently, in those hydrogels prepared with high DM concentration, the chain mobility is reduced, resulting in a more rigid and stable structure.

In order to know the type of mechanism regulating the entrance of water into the HGs, the Fick's law [Eq. (4)] was applied until 60% of total water absorption. For cylindrical shape hydrogels, the law predicts that if $0 < n < 0.5$, it corresponds to a Fickian diffusion type mechanism (only diffusion is present); if $0.5 < n < 1$, it represents non-Fickian diffusion (diffusion and relaxation polymer chains are present); if $n = 1$, it corresponds to type II mechanism (only relaxation polymer chain is present).[22]

As observed in Figure 3(b), for samples prepared with initial monomer concentration lower than 1.20 M (HG 1.00/4 and HG 0.80/4), the values of n indicate that relaxation chain processes govern the rate of water absorption, being independent of time. For these slightly rigid structures (HG 1.00/4 and HG 0.80/4), the diffusion rate is higher than that of relaxation, probably arising from the major amount of pre-existent spaces in the network, being the relaxation of the chains the rate-determining step. However, the rate of water diffusion inside HGs with initial monomer concentration above 1.20 M (HG 1.20/4 to HG 1.80/4) has non-Fickian control. Possibly, the more rigid structures formed with high initial monomer concentrations produced minor rate of water diffusion into the hydrogels, and both, diffusion and relaxation rates occur at the same time.

Effect of DAT amount and pH. The effects on HGs swelling properties by the variation in the amount of crosslinking agent (DAT) were studied. The *%ESR* and swelling kinetic curves for HG 1.00/2, HG 1.00/4 and HG 1.00/6 in water are shown in Table I and Figure 4(a), respectively. As

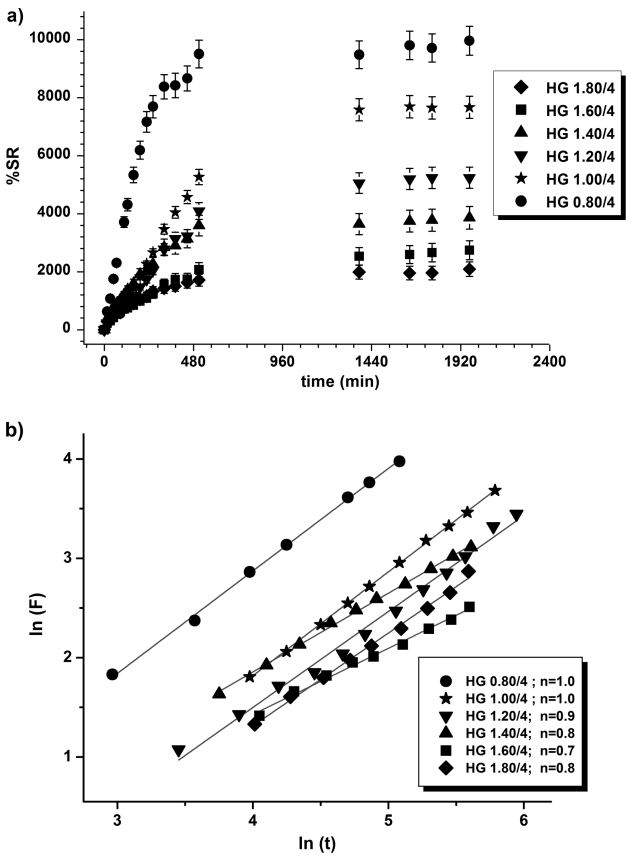

FIGURE 3. (a) Swelling kinetics for HG 0.80/4 to HG 1.80/4 in milli-Q water. (b) Fick's law applied to the first stage of the swelling process.

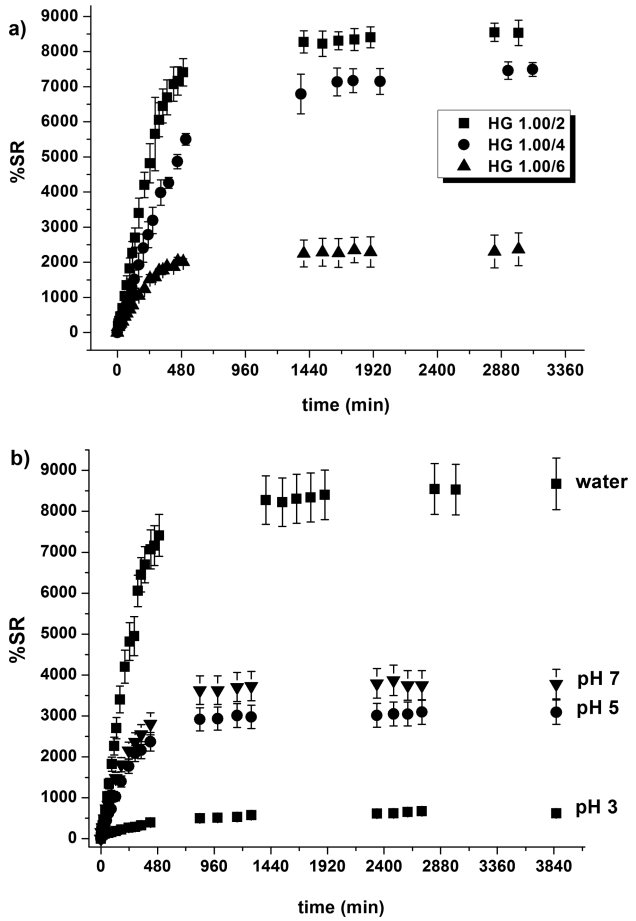

FIGURE 4. (a) Swelling kinetic curves of HG 1.00/2, HG 1.00/4, and HG 1.00/6 in water at 25°C. (b) Swelling kinetic curves of HG 1.00/2 at pH 3.02, 5.01, 7.00, and water at 25°C.

expected, an increase in the amount of crosslinking agent to form the polymeric networks results in a decrease in water swelling capacity. With 2% of crosslinker, the water absorption capacity is around 80 g/g polymer; however, it is reduced to 20 g/g polymer when 6% of crosslinker is incorporated in the reaction. Thus, the water absorption capacity of the hydrogels prepared can be also modified by changing the crosslinking density according to the application requirements. Moreover, dendronized hydrogel dispersion with tunable consistence could be prepared by changing the crosslinking density of the polymer.

The influence of the pH environment on the swelling properties of the dendronized hydrogels was also studied. Table II exhibits the %ESR values for the HG 1.00/2, HG 1.00/4, and HG 1.00/6. Figure 4(b) shows the representative swelling curve of HG 1.00/2 studied in four different swelling media: water and three different BR buffer solutions of pH 3.02, 5.01, and 7.00 at 25°C. Clearly, the pH of the medium has considerable importance in the swelling properties of these ionic gels since *%ESR* is different depending on pHs. In the case of HG 1.00/2, the swelling capacity increases 6.3 times between pH 3 and 7. The pK_a estimated by potentiometric titration from a solution of DM with a NaOH solution proved to be between 5 and 6. At pH 3, lower than that of monomer pK_a, the carboxylic groups of the material are protonated. However, at pH 5 or 7, the acid groups are partially or totally deprotonated; consequently, the swelling is greater than at pH 3 because the electrostatic repulsion between the chains increases the water absorption capacity. The use of BR buffer allowed studying the swelling properties at different pH by maintaining the identical chemical environment and ionic strength ($I = 0.1M$). The equilibrium swelling of HG 1.00/2 in milli-Q water (pH 6.13, $\%SR = 8546$) was even greater than at pH 7 [$\%SR = 3800$, Table II and Fig. 4(b)]. Comparing the results of swelling in water and those in buffer solutions, evidently, the ionic gels decreased the swelling capacity with an increase in the ionic strength of the medium. One of the driving forces of the swelling process in ionic networks is the osmotic diffusion of ions from the solution to the gel network, in order to maintain electroneutrality. Great differences between the concentration of ions inside and outside of the polymeric network will produce greater swelling of the gel due to osmotic forces. As was previously descript for a similar ionic network,[29] in the case of the water, the few free ions remain inside the gel to neutralize the fixed charges on the network chains. When salt is added to the system, the ions diffuse from the solution into the network. The overall concentration of mobile ions in the gel is higher than in water, but the difference between ion concentrations inside and outside is reduced and therefore the osmotic forces decrease. Consequently, swelling decreases gradually with increasing salt concentration because the osmotic forces are lower. Similar swelling behaviors to HG 1.00/2 were observed for HG 1.00/4 and HG 1.00/6 at different pHs and milli-Q water, but presenting less noticeable changes in the swelling by the major crosslinking density. Minor influence of the crosslinker amount over the %ESR values was observed for all polymers at pH 3.02. As was explaining above, the carboxylic groups of the materials are protonated at pH 3.02 and consequently there is less swelling and expansion of the networks than at pH 5.02 and 7.00 where the carboxylic groups are negatively charged. So, at pH 3.02, the crosslinking density has not considerable influence on the swelling behavior of HG 1.00/2, HG 1.00/4, and 1.00/6 because the polymers present low swelling and the amount of crosslinking point into the network does not play an important role for counter the small swelling forces. On the other hand, at pH 5.02 or 7.00, where the acid groups are partial or totally negatively charged the

TABLE II. %ESR Values at 25°C for HG 1.00/2, HG 1.00/4, and HG 1.00/6 in Different Swelling Media

	%ESR			
Hydrogel	pH 3.02[a]	pH 5.01[a]	pH 7.00[a]	Milli-Q Water pH 6.13
HG 1.00/2	600	3100	3800	8546
HG 1.00/4	430	1800	2500	7456
HG 1.00/6	400	1600	1800	2300

[a] BR buffers allowed to study the swelling properties at different pH maintaining the identical chemical environment and ionic strength ($I = 0.1M$).

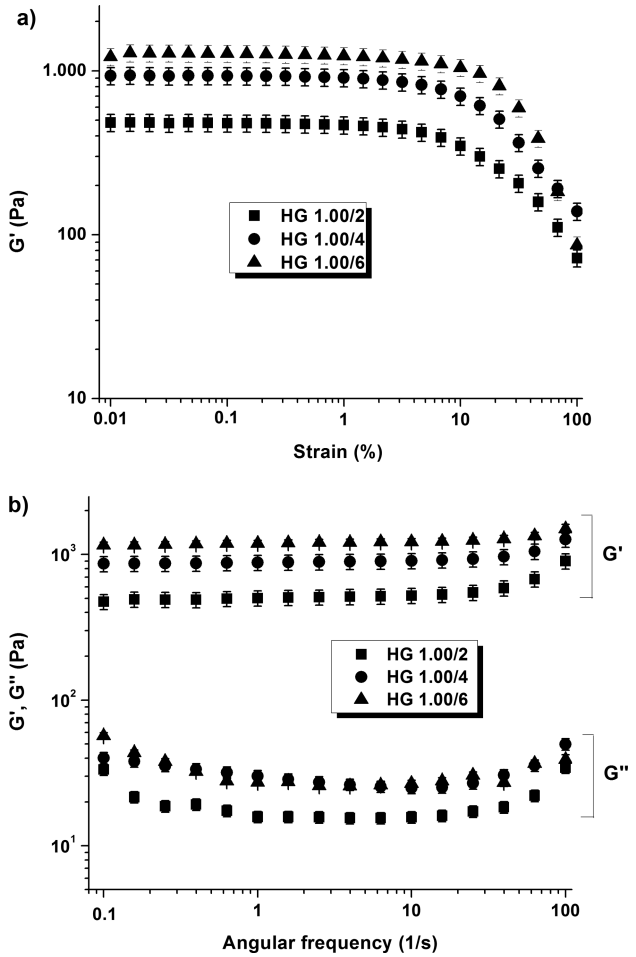

FIGURE 5. (a) Amplitude sweep assay for HG 1.00/2, HG 1.00/4, and HG 1.00/6 in milli-Q water at 25°C. (b) Frequency sweep for HG 1.00/2, HG 1.00/4, and HG 1.00/6 swelling in milli-Q water at 25°C.

networks present major swelling and expansion than at pH 3.02. In this case, the amount of crosslinking points plays an important role to counter the big swelling forces.

The pH of the skin is around 5.5, which conditions the pH for topical formulations between pHs 5.5 and 6 to avoid irritation. In this case, the new dendronized hydrogel HG 1.00/2 presents its maximum swelling capacity (31 g water for g of polymer) at pH 5, which could allow efficient topical gel formulations.

Rheological properties

The rheological characterization of hydrogels allow investigating the response of these materials *versus* an applied strain. Thus, oscillatory rheology was used to study the mechanical response of the new hydrogels under different conditions. First, strain sweeps were performed to determine the linear viscoelastic region (LVR) for the polymers. As shown in Figure 5(a), the LVR for the hydrogels studied, HG 1.00/2, HG 1.00/4, and HG 1.00/6, showed strains of 0.01 to 4.4, 0.01 to 6.8, and 0.01 to 9.9%, respectively. Thus, the G' values of all materials rapidly decrease above the critical strain (CS) region, which proved slightly different depending on the amount of crosslinker used (HG 1.00/2, CS = 4.6%; HG 1.00/4, CS = 6.8 %; HG 1.00/6, CS = 9.9%). After the determination of LVR for the hydrogels, their responses over a range of frequencies were examined. In Figure 5(b), the storage moduli, G' and loss moduli G'' are shown for HG 1.00/2, HG 1.00/4, and HG 1.00/6 as a function of angular frequency at a fixed strain (0.3–0.7%, depending on each hydrogel). All samples displayed a single plateau region in their dynamic modulus. In addition, the G' values had good elastic response and were, in all cases, larger than the G'' values over the whole range of frequencies. The elastic behavior of the samples predominates over the viscous behavior, and the swollen samples exhibited mechanical rigidity. In addition, at increasing frequencies, i.e. at low relaxation time, the sample flexibility of the samples decreased and the swollen samples became increasingly rigids. This behavior is typically found in slightly crosslinked polymers. The G' values were 490, 870, and 1150 Pa for HG 1.00/2, HG 1.00/4, and HG 1.00/6, respectively.

Interestingly, the value of G' for HG 1.00/2 (490 Pa, 1.2% w/v polymer in water) proved close to a formulation prepared using a major amount of Carbopol 974 polymer (360 Pa, 4% w/v of Carbopol 974 in water) as reported in literature.[30] So, this new dendronized polymer could permit to obtain gel formulations with similar rheological properties than those from Carbopol 974 but using less mass amount of polymer. The equilibrium swelling performance of both HG 1.00/2 and Carbopol 974 in milli Q-water was experimentally compared. The equilibrium volume swelling ratio q_v (q_v = swelling volume of sample/ dry volume of sample) of Carbopol 974 ($q_v = 28$) was slightly major than HG 1.00/2 ($q_v = 21$) due possibly at minor degree of crosslinking density in the network. Carbopol 974 (a Carbomer family polymer) is one of the most common thickening agents for water-based topic bioadhesive formulations. As shown in Figure 5(b), G' values depend on the amount of crosslinking agent. Among the three crosslinking percentages studied, 6% (mol ratio) of DAT yielded the hydrogel with greatest G' value because the major amount of crosslinking points in the material increased the solid component of the structure. This result clearly opens the possibility of changing the mechanical properties of the new materials in a widely range of storage moduli. In addition, these behaviors are in line with the swelling behavior of the hydrogels where those with a high crosslinking density (HG 1.00/6) presented a lower swelling degree or, in rheological terms, a more elastic structure. As a complement to the mechanical properties, all materials prepared were able to flow through the syringes used as reactor. This thixotropic behavior demonstrated that the novel hydrogels present a desired property in the development of materials with applications in topic drug delivery formulations.

Cytotoxicity studies

Considering a potential application for the new hydrogels as carrier in topic drug delivery systems, the quantitative *in vitro* cytotoxicity was performed by MTT assays by direct contact of swollen hydrogels in culture medium with

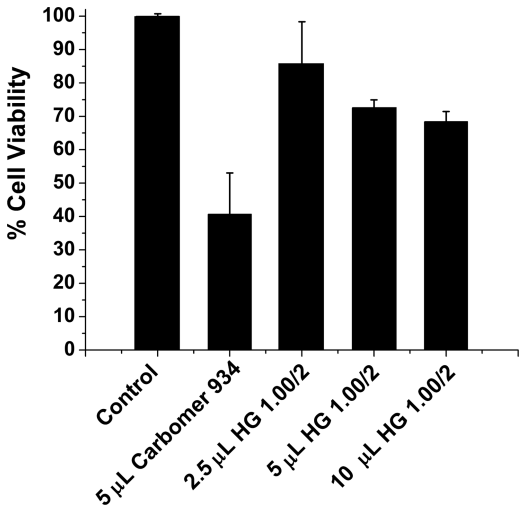

FIGURE 6. Cell viability of fibroblast incubated with culture medium alone (control), Carbomer 934 hydrogel, and different amounts of HG 1.00/2 high functionalized hydrogel.

fibroblast cells. The cytotoxicity effect of the new hydrogel HG 1.00/2 was compared with those of both the culture medium alone (control) and the hydrogel prepared from the commercial polymer Carbomer 934 (commercial polymer widely used in the pharmaceutical industry). Figure 6 presents the cell viability of the fibroblast cells in contact with: medium alone (control), hydrogel from Carbomer 934 (2.3% w/v), and HG 1.00/2 (2.3% w/v) in different amounts (2.5, 5, and 10 μL).

Interestingly, the new dendronized hydrogel HG 1.00/2 showed cell viabilities above 70 % for different gel amounts assayed, which demonstrates that can be considered as a potential non-toxic material according to ISO 10993-5 standards.[23] In addition, the gel formulation (2.3% w/v) prepared with the new material HG 1.00/2 showed cell viability greater than that of the gel formulation (2.3% w/v) prepared with the same amount of the commercially available polymer Carbomer 934. This result could indicate high potential for biomedical application since this new material showed less toxicity compared with Carbomer 934 in the experimental conditions used. Furthermore, no change in the morphology of the living fibroblasts neither their extensions/focal adhesions could be observed microscopically after 24 h of contact at 37°C with swollen 1.00 HG/2.

Ionic polymer-drug interaction

To determine the potential of these new materials in their possible application as carriers for topic drug delivery formulations, the loading capacity through ionic interaction between a voluminous model basic drug as azithromycin (AZI) and the new polymers was studied. Azithromycin is an anti-bacterial drug approved by the food and drug administration (FDA), prescribed for the treatment of patients with mild to moderate infections caused by susceptible strains of the designated microorganisms. It was chosen as an anti-bacterial basic model drug. As known, the acid-base interaction between acid polymers and basic drugs yields a high degree of counter ion interaction through ion pair according to the equilibrium shown in Figure 7.

Particularly, both basic groups of AZI, the amino sugar (pK_a 8.6) and the lactone ring (pK_a 9.5), have enough strength to combine with the numerous amount of carboxylic groups of the hydrogels, showing a large number of possible interactions. The ionic complex HG-AZI was easily prepared by mixing both components in ethanol and neutralization of 50% of the total carboxylic groups of the polymer with AZI (365 mg of AZI/g of polymer). In addition, HG-AZI formulations were also prepared efficiently by dispersion of the ionic complex HG-AZI in distilled water. The effective ionic interaction between the dendronized hydrogel and AZI and their potential release in physiological medium was demonstrated by titration of an aqueous dispersion of the ionic complex HG-AZI by ionic exchange using a neutral salt (NaCl) according to previous works.[25,31] As clear evidence, the protogenic effect occurring from the displacement of AZI-NHR_3^+ according to Equations 5 and 6 was easily measured by pH determination as shown in Figure 8.

$$[R{-}COO^{-+}AZI\text{-}NHR_3] + NaCl \leftrightarrows [R\text{-}COO^{-+}Na] + Cl^- + AZI\text{-}NHR_3^+ \quad (5)$$

$$AZI\text{-}NHR_3^+ \leftrightarrows AZI\text{-}NR_3 + H^+ \quad (6)$$

Two differentiated steps were observed along the titration. At the beginning, a marked decrease in pH stopping at about 0.6 mEq of NaCl with respect to 1 mEq of AZI was observed. After that, a slower decrease in the pH was found. Such pH decrease remained even after addition of 2.4 mEq of NaCl. This behavior reveals that both basic groups of AZI are involved in the interaction with the carboxylic groups of

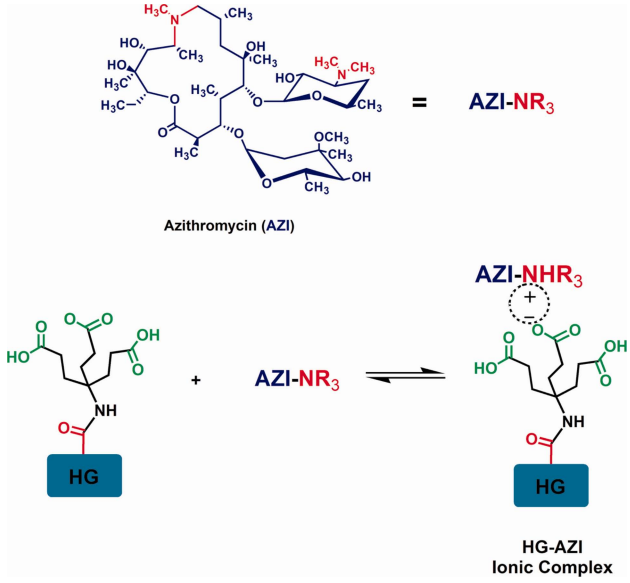

FIGURE 7. Schematic representation of the formation of the ionic complex between the hydrogel and AZI. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

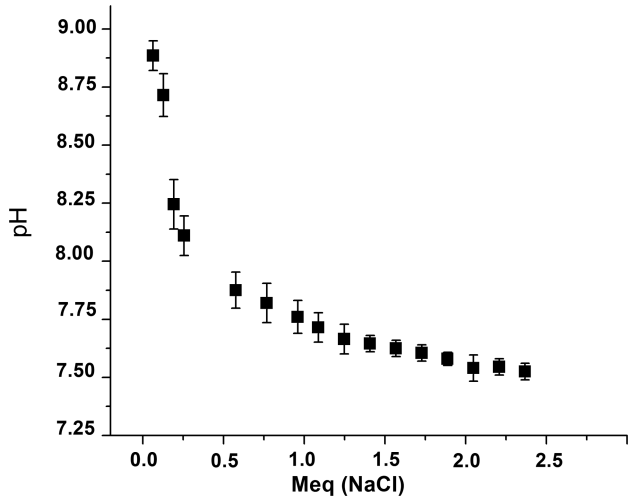

FIGURE 8. Protogenic effect occurring from the displacement of AZI-NHR_3^+ from an aqueous dispersion of HG 1.00/6-AZI with a solution of NaCl (3*M*).

the high functionalized acid hydrogel. The first step would be associated with the titration of weaker ionic interaction relative to the second one, exhibiting higher affinity between AZI and dendronized hydrogel. In addition, the ionic interaction between the polymer and AZI was reversible and the drug could be release by ionic exchange, which is an important property for the potential applications of these materials in release formulations. In this sense, the ionic interaction between polymer and AZI could prevent the rapid diffusion of AZI from the hydrogel, and consequently, this material could have potential uses as carrier in sustained drug delivery.

CONCLUSIONS

There is always much interest in the development of new and innovative functional materials for biomedical applications. In this sense, the development of dendronized polymers for a wide range of applications is an area which has gained growing attention over the last years because of their multivalence properties. In addition, the development of new drug carrier materials with improved properties is a permanent topic of academic and industrial research. In view of this context, the synthesis and network characterization of structurally novel dendronized hydrogels with interesting properties for biomedical applications is presented. The new materials developed in this report were easily obtained and showed required properties for their application as biomaterials in topic drug delivery formulations: (1) superabsorbent and tunable swelling properties; (2) accessible regulation of mechanical properties; (3) thixotropic behavior; (4) low cytotoxicity over fibroblast, even lower than a Carbomer 934 gel; (5) efficient load drug capacity; and (6) drug ionic exchange of a model basic drug. Moreover, in view of these relevant properties, the preparation, release profiles and antibacterial activity of topic formulations between antibacterial drugs and these new dendronized hydrogels are currently under development. Besides their suggested applications in drug delivery, these new biocompatible materials may have potential applications in other biomedical fields, such as cellular and tissue regeneration since they showed low toxicity over fibroblast cells. Finally, the utilization of the dendritic monomer precursor DM to prepare new polymeric biocompatible structures should be strongly considered.

ACKNOWLEDGMENTS

The authors thank Dr. Marcelo Calderon and Carolina Mosconi for careful proofreading of this article.

REFERENCES

1. Garbern JC, Minami E, Stayton PS, Murry CE. Delivery of basic fibroblast growth factor with a pH-responsive, injectable hydrogel to improve angiogenesis in infarcted myocardium. Biomaterials 2011;32:2407–2416.
2. Kikuchi A, Okano T. Pulsatile drug release control using hydrogels. Adv Drug Deliv Rev 2002;54:53–77.
3. Jansen J, Mihov G, Feijen J, Grijpma DW. Photo-crosslinked biodegradable hydrogels prepared from fumaric acid monoethyl ester-functionalized oligomers for protein delivery. Macromol Biosci 2012;12:692–702.
4. Baldwin AD, Robinson KG, Militar JL, Derby CD, Kiick KL, Akins RE. In situ crosslinkable heparin-containing poly(ethylene glycol) hydrogels for sustained anticoagulant release. J Biomed Mater Res A 2012;100:2106–2118.
5. Van Vlierberghe S, Dubruel P, Schacht E. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: A review. Biomacromolecules 2011;12:1387–1408.
6. Ogawa M, Kitamura N, Kurokawa T, Arakaki K, Tanaka Y, Gong JP, Yasuda K. Poly(2-acrylamido-2-methylpropanesulfonic acid) gel induces articular cartilage regeneration in vivo: Comparisons of the induction ability between single- and double-network gels. J Biomed Mater Res A 2012;100:2244–2251.
7. Gupta P, Vermani K, Garg S. Hydrogels: From controlled release to pH-responsive drug delivery. Drug Discov Today 2002;7:569–579.
8. Pourjavadi A, Barzegar S, Zeidabadi F. Synthesis and properties of biodegradable hydrogels of κ-carrageenan grafted acrylic acid-co-2-acrylamido-2-methylpropanesulfonic acid as candidates for drug delivery systems. React Funct Polym 2007;67:644–654.
9. Grinstaff MW. Dendritic macromers for hydrogel formation: Tailored materials for ophthalmic, orthopedic, and biotech applications. J Polym Sci Part A: Polym Chem 2008;46:383–400.
10. Wang Q, Mynar JL, Yoshida M, Lee E, Lee M, Okuro K, Kinbara K, Aida T. High-water-content mouldable hydrogels by mixing clay and a dendritic molecular binder. Nature 2010;463:339–343.
11. Altin H, Kosif I, Sanyal R. Fabrication of "clickable" hydrogels via dendron-polymer conjugates. Macromolecules 2010;43:3801–3808.
12. Cuggino JC, Alvarez I CI, Strumia MC, Welker P, Licha K, Steinhilber D, Mutihac R-C, Calderon M. Thermosensitive nanogels based on dendritic polyglycerol and *N*-isopropylacrylamide for biomedical applications. Soft Matter 2011;7:11259–11266.
13. Deng J, Zhou Y, Xu B, Mai K, Deng Y, Zhang L-M. Dendronized chitosan derivative as a biocompatible gene delivery carrier. Biomacromolecules 2011;12:642–649.
14. Newkome GR, Weis CD. Di-tert-butyl 4-[(2-tert-butoxycarbonyl)ethyl]-4-aminoheptanedicarboxylate. Org Prep Proced Int 1996;28:495–498.
15. Ornelas C, Weck M. Construction of well-defined multifunctional dendrimers using a trifunctional core. Chem Commun 2009:5710–5712.
16. Ornelas Ct, Broichhagen J, Weck M. Strain-promoted alkyne azide cycloaddition for the functionalization of poly(amide)-based dendrons and dendrimers. J Am Chem Soc 2010;132:3923–3931.
17. Ornelas Ct, Pennell R, Liebes LF, Weck M. Construction of a well-defined multifunctional dendrimer for theranostics. Org Lett 2011;13:976–979.

18. Cuggino JC, Calderón M, Alvarez CI, Strumia MC, Silva KN, Penott-Chang EK, Müller AJ. New dendronized polymers from acrylate Behera amine and their ability to produce visco-elastic structured fluids when mixed with CTAT worm-like micelles. J Colloid Interface Sci 2011;357:147–156.
19. Peppas NA, Wright SL. Drug diffusion and binding in ionizable interpenetrating networks from poly(vinyl alcohol) and poly(acrylic acid). Eur J Pharmacol Biopharmacol 1998;46:15–29.
20. Sayil C, Okay O. Macroporous poly(*N*-isopropyl)acrylamide networks: formation conditions. Polymer 2001;42:7639–7652.
21. Raj Singh TR, McCarron PA, Woolfson AD, Donnelly RF. Investigation of swelling and network parameters of poly(ethylene glycol)-crosslinked poly(methyl vinyl ether-co-maleic acid) hydrogels. Eur Polym J 2009;45:1239–1249.
22. Peppas NA, Colombo P. Analysis of drug release behavior from swellable polymer carriers using the dimensionality index. J Controlled Release 1997;45:35–40.
23. International Standards ISO 10993-5. Biological Evaluation of Medical Devices. Part 5: Tests for In Vitro Cytotoxicity; International Organization for Standardization; 3rd. ed.; 2009.
24. Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. In vitro evaluation of cell/biomaterial interaction by MTT assay. Biomaterials 1993;14:359–364.
25. Esteban SL, Manzo RH, Alovero FL. Azithromycin loaded on hydrogels of carbomer: Chemical stability and delivery properties. Int J Pharm 2009;366:53–57.
26. Hermanson GT, Mallia A, Smith PK. Immobilized Affinity Ligand Techniques. California: Academic Press, Inc.; 1992. p 19.
27. Lin C-C, Metters AT. Hydrogels in controlled release formulations: Network design and mathematical modeling. Adv Drug Deliv Rev 2006;58:1379–1408.
28. Omidian H, Rocca JG, Park K. Advances in superporous hydrogels. J Controlled Release 2005;102:3–12.
29. Horkay F, Tasaki I, Basser PJ. Osmotic swelling of polyacrylate hydrogels in physiological salt solutions. Biomacromolecules 2000;1:84–90.
30. Bonacucina G, Martelli S, Palmieri GF. Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents. Int J Pharm 2004;282:115–130.
31. Jimenez-Kairuz AF, Allemandi DA, Manzo RH. Equilibrium properties and mechanism of kinetic release of metoclopramide from carbomer hydrogels. Int J Pharm 2003;250:129–136.