Properties of pH-Dependent Tertiary Amine-Based Gels as Potential Drug Delivery Matrices

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ABSTRACT: The rheological and morphological properties and *in vitro* theophylline release of tertiary amine-based microgels were evaluated. The testing of such a formulation through *in vitro* diffusion experiments revealed that the release of theophylline from the microgels was pH-dependent and differs significantly with respect to a nonresponsive gel like scleroglucan (Scl). The microgels were obtained from 2-(diethyl amino) ethylmethacrylate (DEA) in the presence of a bifunctional crosslinker at pH 8–9. As the resulting microgels are pH-responsive and an increase in viscosity from high to low pH range is exhibited, the *in vitro* release of theophylline as model drug was studied at different pHs of both the matrix and the receptor medium. The release behaviors of PDEA-based microgels were compared to nonresponsive natural gel Scl, studied previously. For microgels, diverse release patterns were found at different acidity conditions. This observation seems to be related to complex diffusion phenomena and the different gel structure obtained for samples prepared at dissimilar pH. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 4035–4040, 2007

Key words: hydrogels; microgels; drug delivery; diffusion; theophylline; dynamic rheology

INTRODUCTION

Increasing attention is being paid to the applications of stimulus-responsive microgels and the poly(*N*-isopropylacrylamide) is perhaps the most studied example as pH-responsive system.[1–5] These systems are network-like that contain pH dependent ionizable groups. A slight pH variation can modify the network electrical charge, controlling the interaction between chains and, hence, the polymer mesh dimensions. Scleroglucan (Scl) is a hydrogel-forming nonionic polysaccharide, produced by fungi of the genus *Sclerotium*.[6] The primary structure of Scl has been characterized as a linear chain of β-1,3-linked D-glucose units with single D-glucose side chains linked β-1,6 to every third unit of the main chain.[7] In aqueous solution, the polysaccharide adopts a stable triple-stranded helical conformation held together by hydrogen bonds.[8] In previous work, we studied Scl hydrogels as matrices for controlled release.[9–11]

Controlled release of theophylline includes microencapsulated ion-exchange resins,[12,13] pellets,[14] and hydrogels.[10,15,16] For instance Ward and Peppas[15] prepared the hydrogels by *in situ* UV-polymerization of poly(ethylene glycol) methacrylate. Shaheen and Yamaura[16] had prepared the drug delivery system using poly(vinyl alcohol) as polymeric matrix, showing a Fickian type drug release (Higuchi Model).

A previous study of 2-(diethyl amino) ethylmethacrylate-based microgels[17] shows that unlike high T_g microgels they are soft and film forming. In the same study the latex-to-microgel transition was observed at around neutral pH (human applications) and that swelling was reversible.

In view of these distinctive physical characteristics, we felt that a detailed investigation of such theophylline-containing microgels as drug delivery matrices was warranted.

EXPERIMENTAL

Materials

2-(Diethylamino) ethyl methacrylate (DEA, Aldrich) and poly(propylene glycol) diacrylate (PPGDA,

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Aldrich) were treated with basic alumina to remove inhibitor. Sodium bicarbonate, sodium dodecyl sulfate (SDS), and ammonium persulfate (APS) were used as received. PEGMA macromonomer (M_n = 2000; M_w/M_n = 1.10, steric stabilizer in the microgel synthesis) was supplied by Cognis Performance Chemicals (Hythe, UK) as a 50% w/w aqueous solution. Doubly distilled de-ionized water was used in every polymerization.

Scl of molecular weight 4.5×10^5 was provided by CarboMer (USA) and used at 2% w/w in all preparations. The model molecule for release studies, theophylline (Th) ($C_7H_8N_4O_2$, M_w = 180.17), was obtained from Phoenix Laboratories (Argentina), and was incorporated in all matrices at 0.2% w/w. Its pKa was determined as 8.8 by potentiometric titration with NaOH aqueous solution. This value is in agreement with that reported by Spiller.[18] This molecule was chosen for release studies owing to its stability in water solutions, practical applications, and its easy detection by UV absorption. For pH adjustment HCl aqueous solutions of analytical grade were used.

Microgel syntheses via emulsion polymerization

Polymerizations were carried out in a 100-mL round-bottomed flask, fitted with a nitrogen gas inlet, water condenser, and an overhead mechanical stirrer operating at 250 rpm. Charge-stabilized microgel was prepared using SDS as temporary stabilizer and removed later by ultrafiltration. For batch reactions using APS initiator, the required amount of water (typically 85 g) and a mixture (typically 10.5 g) of DEA and PPGDA crosslinker was added to the flask and the solution was stirred for 30 min under nitrogen flow at 70°C. The polymerization commenced on addition of a previously degassed aqueous solution (typically 5.0 g) of the initiator (1.0 wt % based on monomer). When a reactive macromonomer stabilizer was used, the required amount (10.0 wt % based on monomer) was added to the aqueous solution prior to the addition of the monomer and crosslinker. In all syntheses the reaction solution turned milky-white within 5–10 min and was stirred for 16–20 h at 70°C under a nitrogen atmosphere. For more experimental details on microgel synthesis and characterization see Ref. 17.

Purification of microgels

Serum replacement (ultrafiltration) was used to eliminate excess stabilizer or SDS, as well as traces of monomer and initiator, to purify the PDEA latex particles. Ultrafiltration was performed using a homemade stirred cell (500 mL) by replacing the serum with water in continuous mode; this serum was periodically collected to assess the extent of purification. Purification was continued until the serum surface tension was close to that of pure water (68–72 mN/m) or the conductivity of incoming water was close to the out-coming one.

Preparation of hydrogels loaded with theophylline

Well-defined stirring conditions and temperature (25°C) were kept constant for every gel preparation.

Scl-based hydrogels

Scl concentration was kept constant in all experiments (2% w/w) by using 0.20 g Scl/10 g of total system (Th, water, and Scl). The necessary amount of polymer powder was dispersed in water containing the dissolved Th. These dispersions were kept under magnetic stirring for 96 h to obtain proper polymer swelling and homogeneous gel formation.

Tertiary amine-based gels

Polymer concentration was 5.5% or 10% solids w/w. The necessary amount of polymer was added to water containing the dissolved Th. These dispersions were kept stirred at constant temperature for 4 h. To form the gel, HCl was added drop wise to achieve the desired pH.

Microgel characterization

Environmental scanning electron microscopy

Compared to traditional scanning electron microscopy (SEM), the most prominent advantage of environmental scanning electron microscopy (ESEM) is that the imaging of a sample is performed under the protective atmosphere of water vapor. Hydrophilic samples such as hydrogels remain intact and the observed topography represents the realistic surface structure of the material. ESEM was used as a direct technique for measuring the pore size, as the network mesh or pore size is one of the important parameters in controlling the rate release.

Micrographs of PEGMA-containing microgels were obtained at 20°C using a FEI Company ESEM 2010. This technique allows the imaging of uncoated and untreated wet systems with no prior sample preparation. The sample chamber was kept at a constant pressure of 10–20 Torr (1 Torr $\cong$ 133 N/m^2) that is displayed at the bottom of each micrograph. The electron beam used had a voltage of 20 kV.

Dynamic rheology

A weighted sample of each gel was used to completely fulfill the 1 mm gap space under a 50 mm in

diameter of rough flat plate device of a Paar Physica controlled stress rotational shear rheometer (MCR 300, Stuttgart, Germany). To keep the sample temperature constant at 25°C, a Peltier system (Viscotherm VT2, Paar Physica) was used. Drying out of the samples was prevented by creating a water-saturated atmosphere around them using a small container with distilled water. After the loading of each sample, a resting time of 10 min was set to reach thermal and mechanical equilibrium. The linear viscoelastic region (LVR) was found performing an oscillatory stress sweep from 0.1 to 10 Pa at 1 Hz. After the LVR was found, a constant strain of 1% was selected to perform the frequency sweep. For every sample the elastic (G') and viscous (G'') moduli were determined from the frequency sweeps from 0.05 to 3 Hz.

Drug release behavior

Measurements

Before beginning release experiments, the gel was centrifuged (10 min at 3000 rpm, at room temperature) to remove entrapped air.

Assays for Th release from all types of hydrogels were made in a Flat Ground Joint type Franz Cell (PermeGear, USA). A Franz Cell is a device with two vertical compartments. The upper donor chamber is filled with the gel sample containing the drug and the lower one is the receptor compartment, initially filled with distilled water or an aqueous solution. In this compartment the theophylline concentration increases as drug permeation occurs and from this chamber, samples are taken at different times. A membrane to sustain the gel from which drug release is being studied covers the area at the top of the receptor chamber. A membrane of cellulose (Arthur Thomas, USA) was placed between the upper section of the Franz cell and the lower receptor compartment initially containing distilled water (pH 5.6) or an HCl aqueous solution. The pore size of the hydrophilic cellulose membrane used (48 Å) provided a M_w cut-off of 12,000. Taking into account the radius of gyration of Th (3.8 Å),[19] the membrane pores does not introduce a limiting step in the release process. The kinetic experiments were run under thermostatic control at 25°C.

For experiments with the receptor aqueous liquid at pH 3 or 4, HCl solutions were used to attain the pH.

Measurements of drug concentration were performed by taking samples (0.50 mL) with a precision syringe at fixed times from the sampling port of the receptor compartment. The Th absorbance was measured at 271 nm in a Shimadzu UV-2401 spectrophotometer (Shimadzu, Kyoto, Japan). After each aliquot was taken, the volume (20 mL) in the receptor chamber was made up with distilled water, assuring a constant volume in the lower compartment and a full contact between polymer matrix supported by the membrane and the receptor liquid.

Kinetic data treatment

Because the only area available for drug release is the surface of the membrane at the bottom of the gel, in contact with the liquid of the receptor compartment of the Franz diffusion cell, we assumed a one-dimensional release process.

For each experiment, the cumulative concentration of drug released was calculated and the curves of Th concentration ([Th]) as a function of time (t) were plotted. The molar absorption coefficient of Th at 271 nm in water, ε, was determined as 1.0×10^4 M^{-1} cm^{-1}. For each set of experimental conditions, data from replicated experiments were plotted as a group. The temporal behavior of Th concentration during the release process was adjusted to a power-law type relationship:[20,21]

$$M_t/M_\infty = kt^n \quad (1)$$

wherein M is the mass or concentration of Th in the receptor compartment at time t or at time approaching infinity, and hence M_t/M_∞ is the fractional drug release up to time t, k is a constant depending on kinetic features, and n is the exponent that depends on the release mechanism. M_∞ and k were included in k', and eq. (2) was used to fit the data:

$$[\mathrm{Th}] = k't^n \quad (2)$$

where [Th] is the molar concentration of Th in the receptor compartment at time t.

A global analysis of the replicate experiments using nonlinear least-squares fitting by the method of Levenberg-Marquardt[22] was used to fit eq. (2). The 95% confidence interval of the nonlinear least square estimation was reported for all parameters (Table II).

RESULTS AND DISCUSSION

Critical swelling pHs of microgels prepared using PDEA are summarized in Table I. Entry 1 corresponds

TABLE I
Summary of the Physicochemical Data for the Tertiary Amine Methacrylate-Based Microgels and Scleroglucan Gel Tested in this Study

Entry number	Gel type	Critical swelling (pH)
1[a]	Charge-stabilized PDEA	7.0–7.4
2[a]	PEGMA/PDEA	6.3–6.6
3	Scleroglucan	N.A.

[a] PPGDA was used as crosslinker at 1.0% w/w.

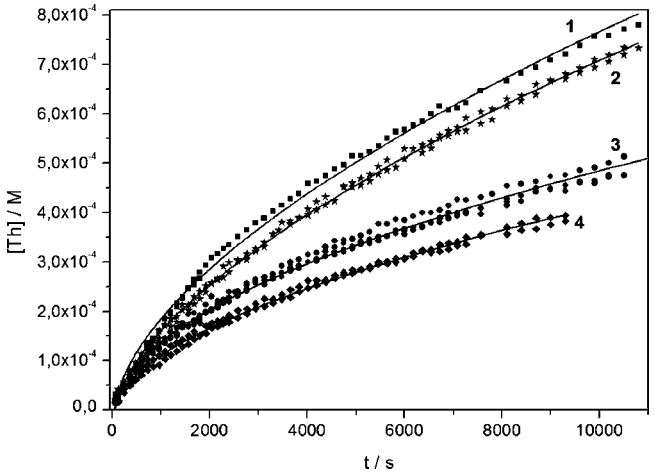

Figure 1 Cumulative Th concentration released as a function of time. Experimental conditions for each curve: see Table II.

to a simple charge-stabilized latex by the sulfate groups coming from the initiator and entry 2 to a PEGMA stabilized microgel.

Figure 1 shows the release patterns from the microgel and Scl matrices, at different pH conditions, reported in Table II.

We will discuss the release patterns of microgels (prepared with PEGMA) taking into account the pH of the matrix and the receptor aqueous liquid, specified in Table II. In the case of matrix at pH 3 coincident with the receptor pH, the release is described by curve 4 on Figure 1. Comparing this curve with curve 3, an enhancement of the drug release is observed when the receptor liquid is water. This fact can be understood taking into account the existence of a flux of HCl in the same direction of Th transport that favors drug delivery. This hypothesis has been validated in a separate experiment by the observation of a slight but detectable acidification of the water in the receptor compartment as drug release occurs.

Curve 3 shows the same release behavior for matrices at pH 3, 4, or 7, when the receptor compartment contains water (pH reported approximately as 6 in Table II). For matrices at pH 3 or 4, the flux of HCl out of the polymeric matrix (as described in the last paragraph) can account for the observed release pattern, compared to curve 4. In the case of the microgel at pH 7, the release enhancement respect to curve 4 can be understood taking into account that the structure of the matrix at pH near 7 is more open than the observed for a microgel at acid pH. This fact is supported by Figures 2 and 3. These ESEM micrographs correspond to 5.5% w/w microgels of pH 2.1 and 6.3, respectively. It can be observed the huge differences between both structures. At acidic pH, the completely swollen particles are impossible to be distinguished from each other and the pore diameter is approximately of 2 μm, but at pH 6.3 the rounded particles are partially swollen and are separate by 6–25 μm.

Release experiments performed using microgels synthesized with or without PEGMA at 5.5% w/w solids lead to the same release pattern as curve 3 in Figure 1. These results indicate that the type of stabilization (charge or steric) and the variation of the microgel concentration in the tested range (from 5.5 to 10% w/w solids) do not affect the release behavior. At both concentrations the mesh size of the gel is larger than the hydrodynamic diameter of the diffusing drug. Microgel at 5.5% w/w and pH 2.1 shows a pore diameter of approximately 2 μm (Fig. 2) and the radius of gyration of Th is reported as 3.8 Å.[19] With this huge difference is expectable that an increase in gel concentration does not modify the release of Th.

Drug release data results are correctly described and fitted by eq. (2), as can be seen in Figure 1. In Table II, calculated n-values are between 0.60 and 0.63 for the Scl and close to 0.5 for the DEA-based microgels, indicating a predominant difusional process responsible for theophylline delivery.

Scl is a nonionic polymer that forms hydrogels with a rod-like triple helical structure insensible to pH lower than 12.5.[23] In all preparations, Scl matrix was at pH 6. As Figure 1 shows, when the receptor compartment is filled with water (pH *ca*. 6, curve 1),

TABLE II
Experimental Conditions and Th-Release Kinetic Parameters[a] Obtained from Data Treatment

Curve on figure 1	Polymer	Matrix pH	Receptor pH	n[b]	k'[b]($(10^{-6}$ M/s^n)
1	Scleroglucan	6	6	0.60 ± 0.01	2.9 ± 0.3
2	Scleroglucan	6	3	0.63 ± 0.01	2.2 ± 0.1
3	Microgel[c]	3, 4, or 7	6	0.53 ± 0.01	3.6 ± 0.3
4	Microgel[c]	3	3	0.56 ± 0.01	2.4 ± 0.2

[a] Mean and confidence intervals are informed. Statistical treatment of results is mentioned in kinetic data treatment section. All parameters are within the 95% confidence interval of the nonlinear least square estimate.
[b] Kinetic parameters were obtained by fitting the data through eq. (2).
[c] Polymer synthesized with PEGMA. Microgel prepared at 10% w/w solids.

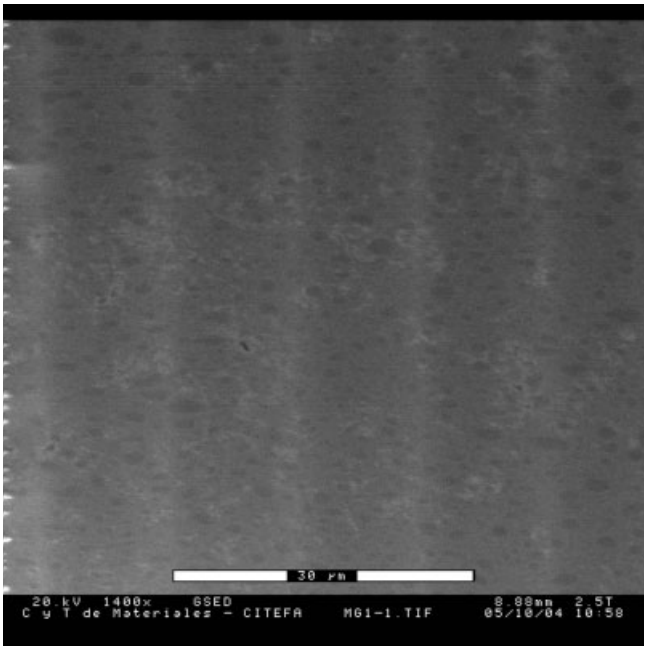

Figure 2 ESESM micrograph of 5.5% w/w microgel obtained at pH 2.1 (magnification: ×1400; water vapor pressure: 8.9 Torr).

we observed a drug release more significant than in the case of HCl solution at pH 3 as receptor liquid (curve 2). This fact can be rationalized proposing the existence of a transfer of HCl upwards that opposes to drug transport. This hypothesis has been supported by the observation of an acidification of the Scl matrix as drug release occurs.

For the same Th loading, Scl delivers a higher amount of drug than microgel matrices. This microgel behavior could be an advantage for some purposes, depending on expected drug effect. For example, a slower release will bring a wider delivery time. Comparing Figure 3 and Figure 4, this observation could be assigned to a more open structure of Scl, with a pore size larger than the interparticle distance observed for microgels at pH 6.3.

From dynamic rheological measurements we obtain the values of the storage or elastic modulus (G') and viscous or loss modulus (G''). The storage modulus G' is a measure of the energy stored and recovered per cycle of oscillatory deformation and represents the elastic behavior of the material. The loss modulus G'' is a measure of the energy dissipation per cycle of sinusoidal deformation and represents the viscous behavior of the system. Taking into account the results obtained in stress sweep experiments for microgels at pH 2.1 or 6.3, the frequency sweep at a constant strain of 1% was undertaken within the LVR. Figure 5(a,b), corresponding to frequency sweeps, shows that these systems behave like gels as G' values are always higher than G'' at both pHs. The frequency dependence of these moduli, particularly in the low frequency range, allows us to state that the system studied at different pH's is weakly structured.[24] In contrast, for well-structured systems (strong gels) G' and G'' are independent of the frequency.

At pH 6.3, the G' value at 1 Hz ($G' = 12.1$ Pa) and the LVR (0–0.50 Pa) are greater than the ones measured from the swollen microgel at pH 2.1 ($G' = 6.9$ Pa, LVR = 0–0.32 Pa). A bigger G' and a wider LVR are evidences of more rigidity.[24]

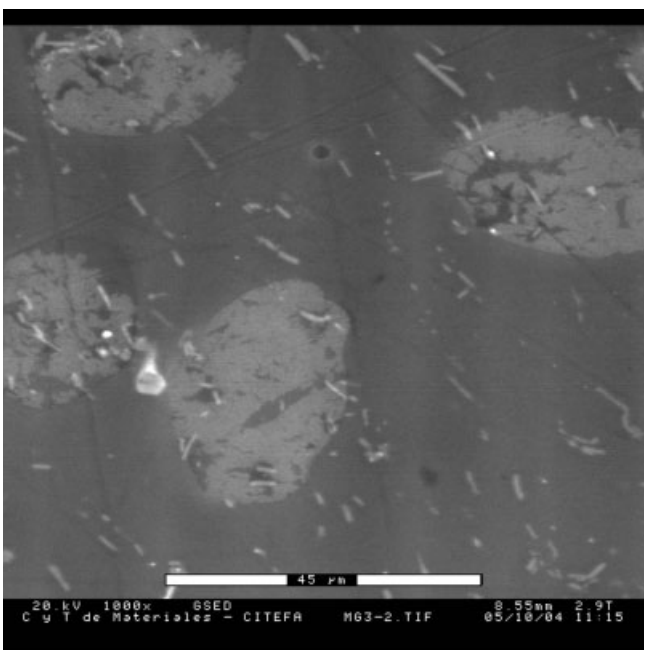

Figure 3 ESEM micrograph of 5.5% w/w microgel obtained at pH 6.3 (magnification: ×1000; water vapor pressure: 8.6 Torr).

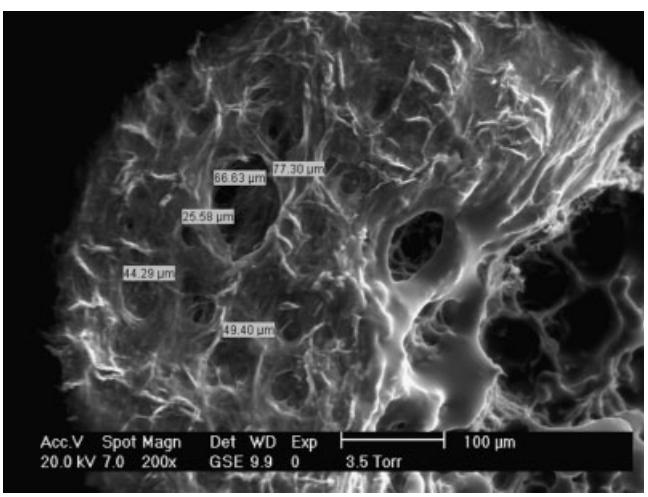

Figure 4 ESEM micrograph of 2% w/w Scl gel obtained at pH 6 (magnification: ×200; water vapor pressure: 3.5 Torr).

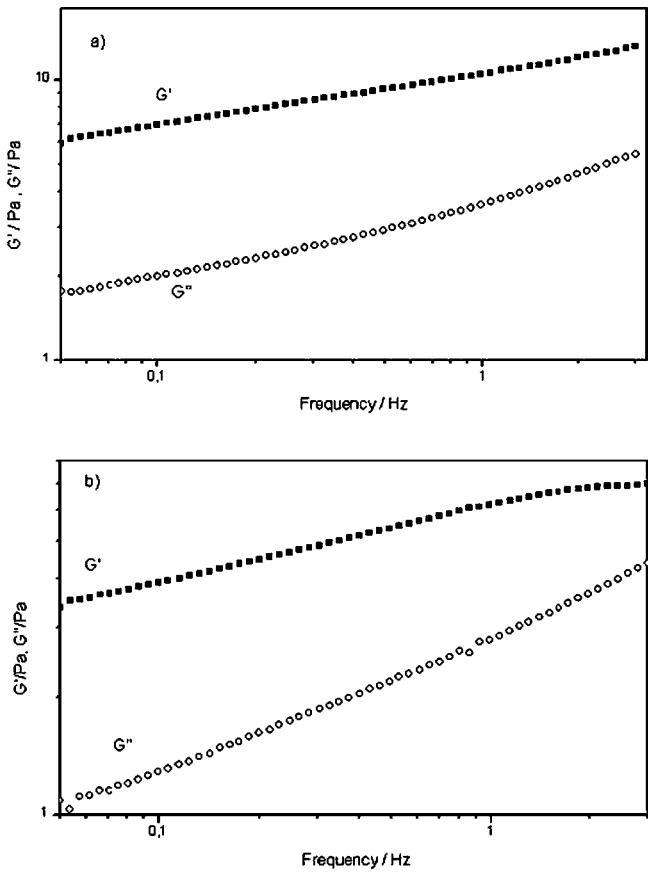

Figure 5 Storage (G') and loss (G'') moduli as a function of frequency. Sweep performed at 1% constant strain and 25°C for 5.5% w/w microgel loaded with Th. (a) at pH 6.3, (b) at pH 2.1.

The comparison between the stress (not shown) and frequency sweeps (Fig. 5) for samples at different pHs suggests that the swelling of the particles in acid medium enhances the softness of the structure. These observations are according to the previous results on particle morphology of microgels.[17] At high pH value the systems has a compact (collapsed) morphology, whereas at low pH the microgel structure is found less compact.

CONCLUSIONS

A new class of lightly crosslinked pH-responsive microgels based on 2-(diethylamino)ethyl methacrylate has been tested as drug delivery matrix and compared to a pH-independent gel. Microgels were also characterized by ESEM and dynamic rheology. These results show that the DEA-based microgel is pH sensitive with a hydrated state in acidic pHs; in contrast, it is quite rigid and dehydrated near neutral pHs.

Results on the characteristics of these new polymeric microgels can be considered important taking into account the possibility of application in the field of drug release.

The low T_g microgeles (16°C)[26] tested in this work are film-forming materials and new applications in this aggregation state as drug delivery systems are currently being evaluated.

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