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Chitosan/agarose hydrogels: Cooperative properties and microfluidic preparation

Vanessa Zamora-Mora [a,b], Diego Velasco [b], Rebeca Hernández [b], Carmen Mijangos [a,b], Eugenia Kumacheva [a,c,d,*]

[a] Institute of Polymer Science and Technology, The Spanish National Research Council (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain
[b] Department of Chemistry, University of Toronto, 80 Saint George Street, Toronto, Ontario M5S 3H6, Canada
[c] Institute of Biomaterials & Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario M5S 3G9, Canada
[d] Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario M5S 3E5, Canada

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ABSTRACT

The preparation of composite biopolymer hydrogels offers the capability to produce biocompatible and biodegradable materials with cooperative properties. In this paper, two natural polymers, namely, chitosan and agarose were employed to prepare composite hydrogels with dual pH and temperature properties. The elastic modulus of the composite hydrogels increased with agarose concentration reaching the value of 1 kPa for the chitosan/agarose gel with a 2% (w/v) concentration of agarose. In addition, composite gels exhibited a higher stability in acidic aqueous solutions, in comparison with agarose gels. The drug release properties of the composite hydrogels were tested by loading a model anticancer drug, 5-Fluorouracil, in the hydrogel interior. At pH = 7.4, the cumulative release of 5-FU was ~50% within 96 h and decreased to ~33% at pH = 5.2, which was attributed to the different solubility of 5-FU as a function of pH. The preparation of composite microgels with controllable dimensions in the range from 42 to 18 μm and with narrow size distribution (polidispersity not exceeding 1.5%) was achieved by the microfluidic emulsification of an aqueous mixture of chitosan and agarose and subsequent gelation of the precursor droplets by cooling.

1. Introduction

Hydrogels obtained from natural polymers are currently the focus of considerable scientific research for the development of biomedical applications due to their inherent biocompatibility and biodegradability (Li, Rodrigues, & Tomas, 2012), (Dang & Leong, 2006). In particular, agarose, an alternating copolymer found in some seaweeds consisting of 1,4-linked 3,6-anhydro-α-L-galactose and 1,3-linked β-D-galactose derivatives is a neutral polysaccharide that forms thermorreversible gels upon cooling agarose aqueous solutions. The mechanical and thermal properties of agarose hydrogels depend on polymer concentration, pH and solvent type (Fernández et al., 2008; Guenet, 1992; Ramzi, Rochas, & Guenet, 1998). The biocompatibility of agarose and the mild conditions of its gelation make agarose suitable for applications in tissue engineering (Kong & Dumitriu, 2004), however, agarose hydrogels exhibit low cell adhesiveness (Lin et al., 2005) and slow degradation rate (Zhang et al., 2012). To overcome these disadvantages, agarose can be chemically functionalized (Luo & Shoichet, 2004) or blended with others biopolymers, *e.g.*, with gelatin (Imani, Emami, Moshtagh, Baheiraei, & Sharifi, 2012; Tripathi, Kathuria, & Kumar, 2009). In particular, the combination of chitosan and agarose in composite gels has improved the mechanical and cell-adhesive properties of agarose (Cao, Gilbert, & He, 2009; Gómez-Mascaraque, Méndez, Fernández-Gutiérrez, Vázquez, & San Román, 2014). Chitosan, the linear cationic (1-4)-2-amino-2-deoxy-β-D-glucan with typical degree of acetylation *ca.* 0.25, is soluble in certain acidic aqueous solutions, owing to the protonation of the primary amine groups. Due to the pH-responsiveness and inherent biocompatibility, chitosan gels are attractive for biomedical applications (Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Muzzarelli & Muzzarelli, 2005; Muzzarelli et al., 2012) and especially, as materials for controlled drug delivery (Park, Saravanakumar, Kim, & Kwon, 2010; Puga, Lima, Mano, Concheiro, & Alvarez-Lorenzo, 2013; Wei, Cai, Lin, Wang, & Zhang, 2011; Zhang, Mardyani, Chan, & Kumacheva, 2006).

* Corresponding author at: Department of Chemistry, University of Toronto, 80 Saint George Street, Toronto, Ontario M5S3H6 Canada. Tel.: +416 978 3576.
E-mail address: ekumache@chem.utoronto.ca (E. Kumacheva).

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The combination of chitosan and agarose in microgel particles, cross-linked particles in the colloidal size range, has been achieved by employing suspension cross-linking methods (Hu, Li, & Yang, 2012; Xue, Yang, Wang, Niu, & Cao, 2007). However, these methods yielded particles with high polydispersity. It is currently well-established that continuous microfluidic (MF) synthesis and assembly of polymer particles offers a facile approach to the generation of microgels with dimensions in the range from tens to hundreds of micrometers (Tumarkin & Kumacheva, 2009). The distinct features of these particles include exquisite control in their size distribution, morphologies, shapes and internal structures (Dendukuri, Tsoi, Hatton, & Doyle, 2005; Kumacheva & Garstecki, 2011; Gokmen, De Geest, Hennink, & Du Prez, 2009; Nie, Xu, Seo, Lewis, & Kumacheva, 2005; Tumarkin & Kumacheva, 2009; Utada et al., 2005). Microfluidic preparation of microgels has been explored for a broad range of biopolymers including alginate (Fang & Cathala, 2011), gelatin (Huang et al., 2009), chitosan (Xu, Zhao, Lan, & Luo, 2012; Yang, Huang, Lin, & Lin, 2007), agarose (Chung, Lee, Khademhosseini, & Lee, 2012; Kumacheva & Garstecki, 2011; Kumachev, Tumarkin, Walker, & Kumacheva, 2013; Velasco, Tumarkin, & Kumacheva, 2012) and κ-carrageenan, and carboxymethylcellulose (Zhang, Tumarkin, et al., 2006). Currently, research in this field is developing toward applications of microgel particles prepared by the MF means, which may be challenging when a microgel is formed by a single polymer. Combining several polymers in microgel particles enables the realization of their collective properties.

The aim of this research is the development of a simple and straightforward method for the preparation of composite chitosan/agarose gels, without chemical crosslinking, for applications in controlled drug delivery. To this end, we report the structure, the mechanical properties and the stability of the composite chitosan/agarose macrogels as a necessary step for the evaluation of their feasibility in the proposed application. We also demonstrate the generation of composite CS/Aga microgels with controlled dimensions and narrow polydispersity by using microfluidic emulsification of mixed aqueous chitosan and agarose solutions and subsequent gelation of the precursor droplets.

2. Materials and methods

2.1. Materials

Ultra-low gelling temperature agarose (gelling temperature in the range of 8–17 °C and melting point in the range of 40–50 °C) was purchased from SeaPrep (Lonza, Switzerland, www.lonza.com). Chitosan with a degree of deacetylation of 65% and molecular weight determined by the viscosity method (ASTM D 2857) of 362 kDa was supplied by Polymers Laboratory, National University, Costa Rica. This chitosan sample was isolated from shrimp's shell (*Heterocarpus vicarious*).

Phosphate buffer saline (PBS, pH = 7.4) (Gibco-BRL, Rockville, MD), a non-ionic surfactant Span-80, acetic acid and mineral oil (viscosity 30 cPs) were supplied by Sigma–Aldrich (Canada) and used as received.

Photoresist resin (SU-8 50, 3050) was purchased from Microchem Co. (MA, USA). Sylgard 184 Silicon Elastomer kit was received from Dow Corning Corp. (Midland, MI). The deionized water was obtained from the Millipore Milli-Q water purification system. 5-Fluorouracil (5-FU), buffer pH 5.2 and pH 7.4 were purchased from Sigma Company (Sigma Company, St. Louis, MO, USA).

Table 1
Concentrations of agarose and chitosan in precursor solutions.

Sample	Agarose (%, w/v)	Chitosan (%, w/v)
Agarose-1.5	1.5	0
Chitosan/agarose-1	1	0.5
Chitosan/agarose-1.5	1.5	0.5
Chitosan/agarose-2	2	0.5

2.2. Preparation of chitosan–agarose solutions

Composite chitosan/agarose microgels were prepared with varying agarose concentration, which was controlled by changing agarose content in the precursor chitosan/agarose solution. Different amounts of agarose were dissolved at 60 °C in 0.5% (w/v) aqueous chitosan solutions containing 1% (v/v) of acetic acid. Solutions with a higher chitosan concentration were not used, because they had high viscosity not suitable for microfluidic emulsion. The concentration of agarose in the mixed solution was 1.0, 1.5 and 2.0% (w/v). The samples were designated as chitosan/agarose-1, chitosan/agarose-1.5 and chitosan/agarose-2 (Table 1). The chitosan-free sample was used as a control and was labeled as agarose-1.5.

2.3. Preparation of chitosan/agarose macrogels

Mixtures of chitosan and agarose with agarose content of 1.0, 1.5 and 2.0% (w/v) were prepared by dissolving the corresponding amount of agarose in 0.5% (w/v) aqueous chitosan solution. Mixed solutions were poured in Teflon molds (~8 mm in diameter and ~5 mm in height) and maintained overnight at 4 °C to allow the mixtures to gel.

Composite chitosan/agarose-1.5 macrogels and agarose-1.5 macrogels were loaded with a model anticancer drug 5-Fluorouracil (5-FU), which was dissolved directly in chitosan/agarose solution to the concentration 5 mg/mL and stirred at 60 °C. The procedure of the preparation of 5-FU-loaded chitosan/agarose macrogel was identical to that used for the preparation of 5-FU-free composite macrogels.

2.4. Characterization of macrogels

Attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR) experiments were carried out on freeze-dried samples using a Perkin Elmer spectrometer. The spectra were acquired in the wave number range of 600–4000 cm^{-1} at a 4 cm^{-1} resolution.

Dynamic oscillatory experiments were carried out in an AR-G2 rheometer (TA Instruments, USA) using the 20 mm-diameter steel parallel plates. Temperature sweeps were performed from 5 to 80 °C at 10 °C/min and at 1 Hz frequency. All the experiments were carried out at a fixed torque (10 μN m) in the linear viscoelastic regime.

To examine temporal stability (mass loss) of the composite macrogels, the samples were immersed at 37 °C in buffer solutions at pH 7.4 or 5.2 for various time intervals. Mass loss was determined as $(m_t - m_o)/m_o$, where m_t is the mass of the sample after a given time interval and m_o the mass of the 'as prepared' gel. Before mass measurements, the gel samples were quickly dried with a filter paper to remove excess water from the surface.

The surface of macroscopic gel samples was examined using an SEM (XL30 ESEM, Philips). All the gels under study were freeze-dried.

To study *in vitro* release of 5-FU loaded in chitosan/agarose-1.5 gels at different pH values, the following procedure was used. Macroscopic gel samples were placed in vials containing 10 mL

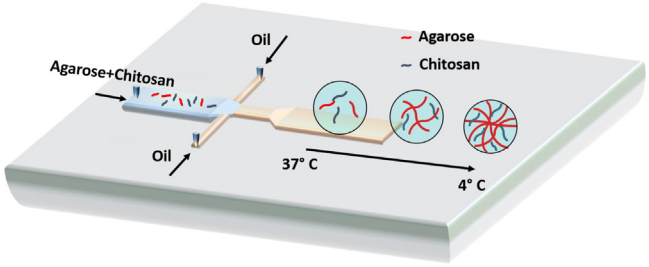

Fig. 1. Schematic of the MF preparation of microgels from chitosan/agarose solutions. Emulsification of the chitosan/agarose solution at 37 °C in a flow-focusing droplet generator is followed by crosslinking of the precursor droplets upon cooling to 4 °C. The height of the channel was 50 μm. The width of the horizontal channel supplying the mineral oil and the chitosan/agarose solution were 70 μm. The length of the mixing channel was 1.02 cm.

of buffer at pH of 5.0 (acetate buffer) or 7.4 (PBS buffer), both at 37 °C, and stirred. An aliquot of 1 mL of the solution was periodically withdrawn to determine the concentration of 5-FU using UV/vis spectrometry (Perkin Elmer Instrument Lambda 35 UV/VIS spectrometer, wavelength of 265 nm). The solution medium was replaced with the same volume of fresh medium.

2.5. Fabrication of microfluidic devices

Photolithographic masters were prepared from SU-8 25 photoresist (Micro-Chem) in bas-relief on silicon wafers. The MF devices were fabricated in poly(dimethylsiloxane) using a soft lithography method (Xia & Whitesides, 1998). Prior to experiments, a MF device was maintained for 48 h in an oven at 140 °C.

2.6. Microfluidic emulsification and generation of the chitosan/agarose microgels

An aqueous chitosan/agarose solution and mineral oil containing 3% (w/v) of the non-ionic surfactant Span 80 heated to 37 °C were supplied to the flow-focusing MF droplet generator (Fig. 1) using two independent syringe pumps (Harvard Apparatus 33 Dual Syringe Pump, USA). Following MF emulsification, the droplets traveled through the downstream channel and exited the device through the outlet tubing (HPFA tubing, Upchurch Scientific, USA).

The outlet tubing was enclosed within a hose connected to a water circulator cooled to 4 °C by a 1:4 (v/v) mixture glycerol and water. The droplets moved through the cooled tubing, where they gelled, and were collected in a 15 mL vial containing PBS solution (pH 7.4). The vial was placed on an ice bath for 45 min to ensure complete gelation of the composite microgels. The resulting suspension was centrifuged at 14,000 rpm for 30 min (Eppendorf Microcentrifuge, Model 5430R), washed with a PBS buffer, and transferred into a PBS buffer (pH 7.4).

2.7. Characterization of droplets and chitosan/agarose microgels

The diameters of the precursor droplets and the corresponding microgels were determined by analysing optical microscopy images of 100 droplets or microgels using Image Pro 5.0 (media Cybernetics, USA) software.

3. Results and discussion

3.1. Composite chitosan/agarose macrogels

The chitosan/agarose gels retained a transparency in the hydrated state, which suggested uniform distribution of chitosan in the host agarose matrix, without noticeable phase separation, as shown in Fig. 2a. The microstructure of the agarose-1.5 and chitosan/agarose-2 macrogels was examined using scanning electron microscopy (SEM) after lyophilization of the samples. The structure of the surface and cross-section of the agarose-1.5 and chitosan/agarose-2 macrogels are shown in Fig. 2b and c, respectively. Both types of hydrogels had a similar sponge-like structure and porosity. The size of pores of the agarose-1.5 and chitosan/agarose-2 gels (determined from SEM images of the sample cross-section; Fig. 2c) was 73 ± 16 μm and 102 ± 39 μm, respectively. The individual agarose and chitosan domains were not distinguishable in the composite macrogels.

The variation in the elastic modulus of the composite macrogels as a function of temperature was also studied (Fig. 3). No variation in the elastic modulus G'_e was observed below the melting temperature of the gel, T_m. Above T_m, the value of G'_e dramatically decreased and ultimately reached the second plateau at ~70 °C, above which the agarose network completely melted down. The value of T_m increased with agarose concentration from 57 ± 1 °C (chitosan/agarose-1) to 62 ± 1 °C (chitosan/agarose-2). The elastic modulus of the composite gels increased with agarose concentration, reaching the value of 1 kPa at 20 °C for chitosan/agarose-2. In addition, composite gels had a higher elastic modulus than agarose gels ($G' = 824 \pm 10$ Pa for the sample chitosan/agarose-1.5 and $G' = 540 \pm 44$ Pa for agarose-1.5).

The dependence of the elastic modulus on agarose concentration in the chitosan/agarose hydrogels was analyzed using the scaling approach as (de Gennes, 1979)

$$G' \sim C^n, \quad (1)$$

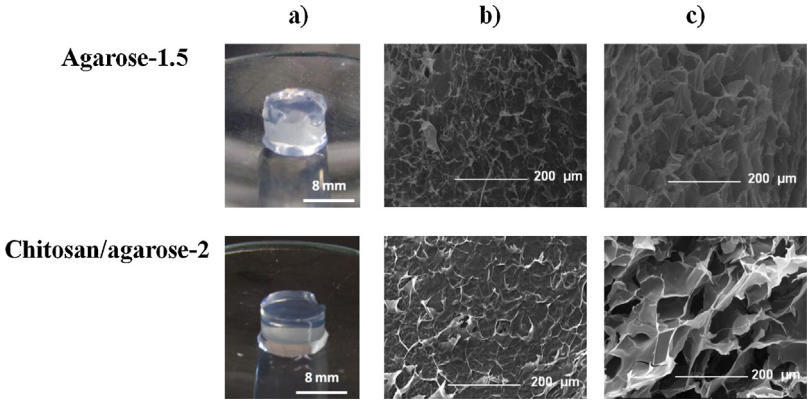

Fig. 2. (a) Photographs of Aga-1.5 and chitosan/agarose-2 macroscopic gels; (b) SEM images corresponding to the surface; and (c) cross sections of the lyophilized samples.

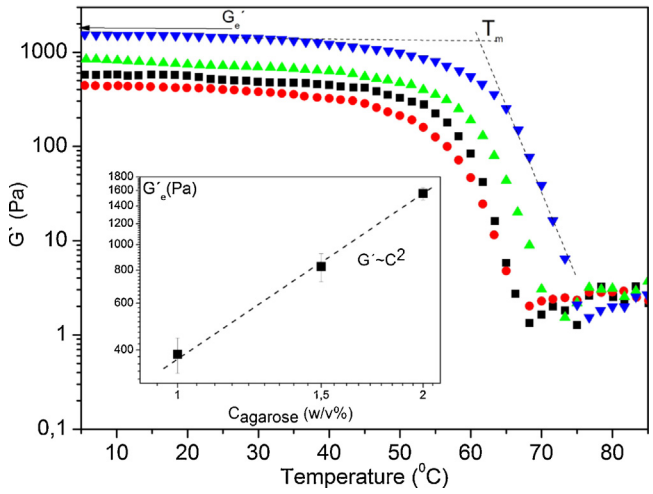

Fig. 3. Variation in elastic modulus, G', plotted as a function of temperature, for agarose-1.5 (■), chitosan/agarose-1 (●), chitosan/agarose-1.5 (▲) macrogel and chitosan/agarose-2 (▼) macrogels. Dashed lines show the determination of the melting point, T_m, of the gel. Insert shows the variation in G' as a function of agarose concentration. The black dashed line corresponds to the linear fit of the data.

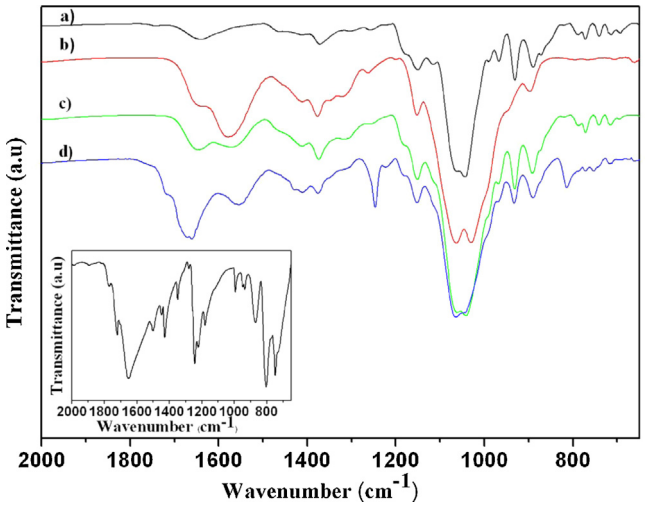

Fig. 4. ATR-FTIR spectra of (a) agarose-1.5, (b) chitosan, (c) chitosan/agarose-1.5 and (d) chitosan/agarose-1.5 loaded with 5-FU. The insert image correspond to the ATR-FTIR spectra of 5-FU.

where C is the agarose concentration and n is an exponent related to fractal dimensions of the hydrogel. Individual agarose chains are intrinsically rigid and, on cooling, they form bundles (nanofibrils), which assemble in a gel (Guenet & Rochas, 2006). Jones and Marques theory (Jones & Marques, 1990) relates the exponent n to the longitudinal fractal dimensions of the fibrils as (Guenet, 2008)

$$G' \sim C^{(3\upsilon+1)/(3\upsilon-1)} \quad (2)$$

where the constant υ is the inverse of the fractal dimensions.

The double logarithmic dependence of G'_e *vs.* agarose concentration for the composite gels is shown in the inset of Fig. 3. The elastic modulus of the composite gel increased with agarose concentration, in agreement with the reported changes for agarose gels (Guenet, 1992; te Nijenhuis, 1997). The linear fitting of the data plotted in the inset of Fig. 3 yielded a slope of ~2. A relation of $G' \sim C^2$ and the fractal dimension $1/\upsilon \sim 1$ obtained for the chitosan/agarose hydrogels indicated that agarose gels with embedded chitosan chains were composed of rigid nanofibrils identical to those in pure agarose gels (Fernández, Hernández, Teresa Cuberes, Mijangos, & López, 2010; Guenet, 2008). This result was in agreement with the earlier measurements of the elastic modulus of agarose gels, which gave a power law dependence of G' corresponding to a longitudinal fractal dimension $1/\upsilon \approx 1.1$ (Ramzi et al., 1998; Rochas, Brulet, & Guenet, 1994). From these results, we infer a close similarity between the structural organization of agarose fibrils in the chitosan-free and chitosan/agarose hydrogels.

The increase in the elastic modulus of the composite chitosan/agarose gels with respect to agarose gels could be caused by the hydrogen bonding between the chitosan chains and the agarose network, as it was reported for agarose mixed with collagen (Lake, Hald, & Barocas, 2011; Ulrich, Jain, Tanner, MacKay, & Kumar, 2010), κ-carrageenan, (Amici, Clark, Normand, & Johnson, 2002) or xylans (Meena, Lehnen, Schmitt, & Saake, 2013). The nature of interactions between the chitosan and agarose components in the composite gels was examined in ATR-FTIR spectroscopy experiments. The ATR-FTIR spectrum of agarose-1.5 (Fig. 4a) shows the peak at 1046 cm^{-1} for C—O (characteristic of axial deformation), 1371 cm^{-1} ascribed to C—C bending, 890 cm^{-1} attributed to C—H angular deformation of anomeric carbon, and 931 cm^{-1} assigned to 3,6-anhydrogalactose (Zhang et al., 2012). The chitosan spectrum exhibited the band at 1579 cm^{-1} corresponding to the N—H bending vibration overlapping the amide II vibration and the band at 1649 cm^{-1} that corresponded to the amide I vibration (Fig. 4b). Absorption bands at 1153 cm^{-1} (antisymmetric stretching C—O—C bridge), 1089 cm^{-1} and 1033 cm^{-1} (skeletal vibrations involving the C—O stretching) were characteristic of the CS saccharide structure (Zamora-Mora et al., 2014).

The ATR-FTIR spectrum corresponding to the sample chitosan/agarose-1.5 (Fig. 4c) showed the characteristic bands of each polymer, however, the band located at 1579 cm^{-1} in chitosan (Fig. 4b) shifted to 1564 cm^{-1} (Fig. 4c), suggesting intermolecular hydrogen bonding between the chitosan amino groups and agarose hydroxyl groups. In addition, band broadening indicated interaction between the polymers.

In vitro stability of the composite chitosan/agarose gels, important for their drug delivery and release applications, was measured as their temporal mass loss at physiological temperature and pH 7.4. The temporal mass loss for the composite and agarose gels was also studied (Fig. 5). The study was extended to pH = 5.2, in order to simulate acidic tumor environment (Kim et al., 2013). At physiological temperature of 37 °C and pH 7.4, the chitosan/agarose-1.5 macrogel had a significantly weaker mass loss than agarose-1.5 gels (Fig. 5a).

We note that agarose gels are known to be stable at physiological temperature and pH. Generally, they are prepared by dissolving agarose in water or PBS buffer and subsequently gelling the solution under reduced temperatures. In our work, agarose-1.5 gel was prepared by dissolving agarose in an aqueous 1% (v/v) acetic acid solution (pH = 3.5), which could promote the light hydrolysis of 1–4 glucosidic links and decrease the stability of agarose gels. At pH = 5.2 the increase in stability in chitosan/agarose gels, in comparison with agarose samples was evident (Fig. 5b). Thus hindering the hydrolysis of agarose polymer in the presence of chitosan led to an enhanced stability of the composite gels.

We recall that the agarose gels below 17 °C and melts in the range of 40–50 °C. These transitions depend on the degree of hydroxyethylation of agarose component of the hydrogel. In order to determine the effect of chitosan on stability of the composite gels above agarose melting temperature, we determined mass loss at 45 °C at pH 7.4 and at pH 5.2. After 24 h, at pH 7.4 the mass loss was ~28% and ~41% for the chitosan/agarose-1.5 gel and agarose-1.5 gel, respectively (Fig. 5c). A similar trend was observed for agarose and chitosan/agarose hydrogels at pH 5.2 (Fig. 5d). We conclude that at T = 45 °C, the stability of the gels was lower than at T = 37 °C

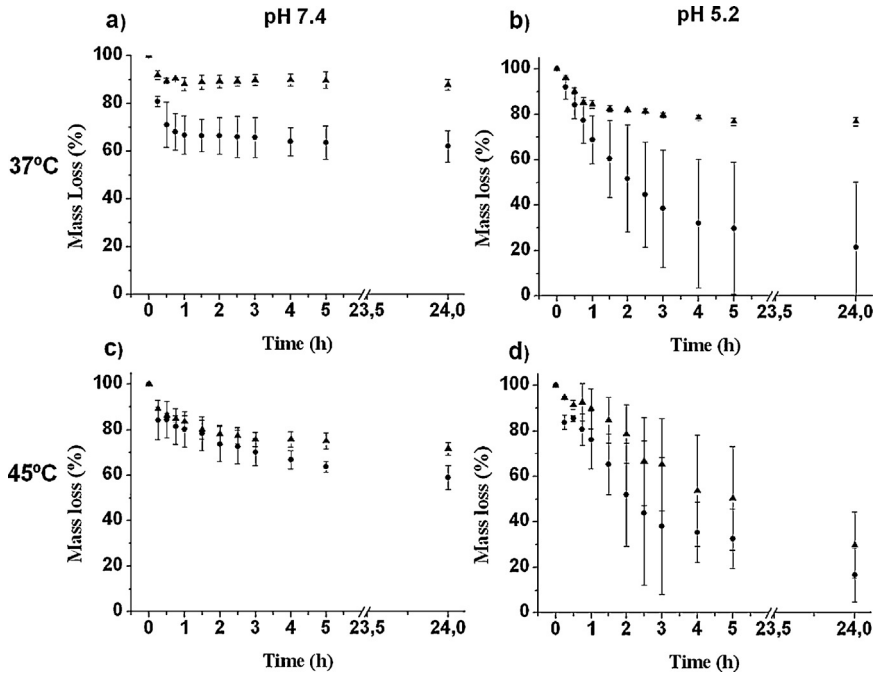

Fig. 5. Kinetics of stability of chitosan/agarose-1.5 macrogels (▲) and agarose-1.5 macrogel (●) at $T = 37\,^{\circ}C$ and pH 7.4 (a) and at pH 5.2(b) and at $T = 45\,^{\circ}C$ at pH 7.4 (c) and pH 5.2 (d).

as a consequence of partial melting of the agarose gel, yet, it was higher in the presence of chitosan.

Finally, we selected 5-Fluorouracil as a model drug to evaluate its release from the composite chitosan/agarose hydrogel at 37 °C and at pH of 7.4 and 5.2. The loading of 5-Fluorouracil in the chitosan/agarose macrogels was confirmed in ATR-FTIR experiments. In Fig. 4, the characteristic absorption bands of 5-Fluorouracil were located at 1651 cm^{-1}, corresponding to C=O stretching of the N-acetyl group (amide I band), 1501 cm^{-1} assigned to N—H amide II band, N—H stretching of amide at 1429 cm^{-1}, 1244 cm^{-1} for C—F and 804 cm^{-1} for C—H out of plane deformation. After 5-Fluorouracil loading in the gel, the absorption peak located at 1569 cm^{-1} in the composite gel (Fig. 4c) shifted to 1555 cm^{-1} (Fig. 4d), which suggested hydrogen bonding between the polar functional groups in 5-Fluorouracil and NH_2 groups of chitosan. Furthermore, the band located at 804 cm^{-1} in 5-Fluorouracil shifted to 814 cm^{-1}, when the drug was encapsulated in the composite gel chitosan/agarose (Fig. 4d) (Ganguly, Aminabhavi, & Kulkarni, 2011; Zhu, Ma, Jia, Zhao, & Shen, 2009).

The drug release was evaluated for the chitosan/agarose -1.5 hydrogel in buffered solutions at pH 7.4 and 5.2 and at 37° C (Fig. 6). It can be observed that at pH = 7.4, the cumulative release of 5-Fluorouracil was ~50% within 96 h, whereas at pH = 5.2 it was ~33%. In the initial period (~3 h) high release rates of 5-Fluorouracil were measured, due to the localization of the drug at or near the gel surface and a stronger relative mass loss of the gels after a short time interval, as shown in Fig. 5. After 5 h, the release rate did not change with time. At pH of 7.4 and 5.2, part of the 5-Fluorouracil remained trapped in the hydrogel as a result of drug–polymer interactions, as demonstrated by ATR-FTIR spectra. In principle, due to the ionic nature of 5-Fluorouracil, its electrostatic interactions with chitosan in chitosan/agarose composite gels could affect drug release, however the enolic hydroxyl groups of 5-Fluorouracil ionize at pK_a of 8.1 (Ojugo et al., 1998), and at pH values studied, 5-Fluorouracil remained uncharged. The main reason for the difference in release profiles at different pH values could result from the varying solubility of 5-Fluorouracil: in alkaline media the solubility of 5-Fluorouracil is higher than that in acidic conditions (Rao, Chung, Reddy, & Ha, 2009). According to the release profiles shown in Fig. 6, chitosan/agarose gels enable drug delivery of 5-FU within a short period of time, which is appropriate because 5-FU has a half-life of 10–20 min. On the other hand, for a local application as a cream or subcutaneous injection, chitosan/agarose gels would allow controlled 5-FU delivery in a single dose, thus avoiding cycles of treatment and consequently, side effects.

3.2. Microfluidic preparation of chitosan/agarose microgels

Chitosan/agarose microgel particles were prepared using MF under identical conditions (time and temperature of gel formation) and with the same composition as chitosan/agarose macrogels. A mixed chitosan/agarose aqueous solution and mineral oil containing 3% (w/v) of Span 80, were forced into the orifice of the MF device, as shown in Fig. 1. The stream of the chitosan/agarose

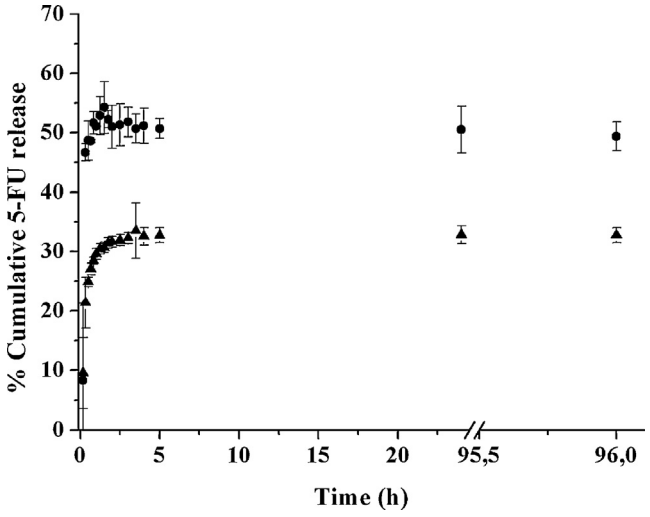

Fig. 6. *In vitro* release of 5-FU from the composite gel chitosan/agarose-1.5 at pH = 7.4 (●) and pH = 5.2 (▲) at 37 °C.

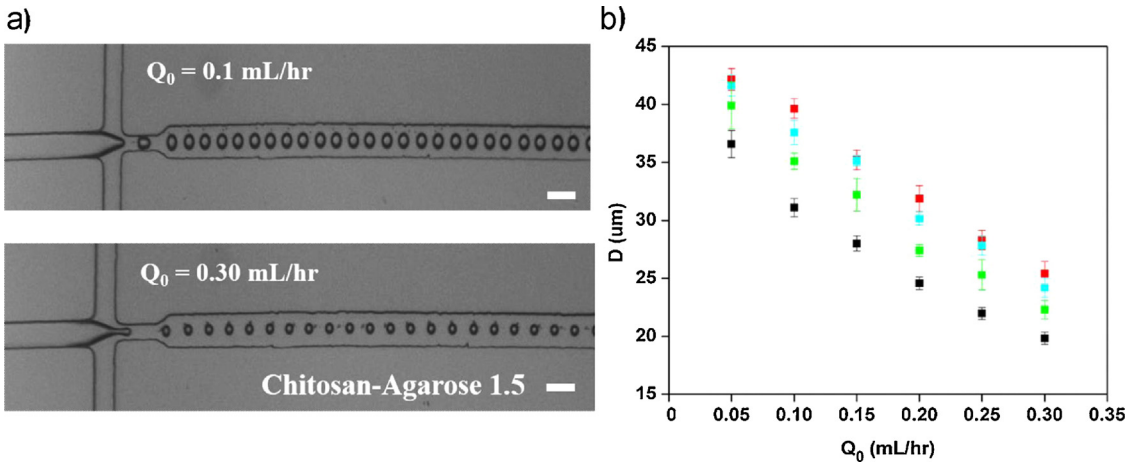

Fig. 7. (a) Optical microscopy images of the chitosan/agarose-1.5 droplets obtained at Q_o of 0.1 mL/h (top) and 0.35 mL/h (bottom) at Q_d = 0.05 mL/h. The scale bar is 70 μm. (b) Variation in the mean diameter, D, of droplets formed by MF emulsification of chitosan/agarose-1 (■), chitosan/agarose-1.5 (■), chitosan/agarose-2 (■) and agarose-1.5 (■) solutions, plotted as a function of the flow rate of the continuous oil phase. Q_d = 0.05 mL/h.

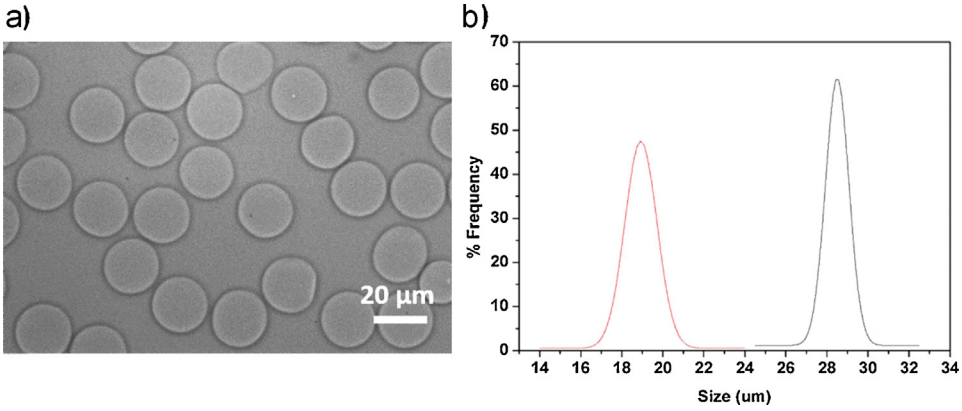

Fig. 8. (a) Optical microscopy image of chitosan/agarose-1 microgel in the PBS buffer at 25 °C. The droplets were generated at Q_o = 0.20 mL/h and Q_d = 0.05 mL/h, transferred in PBS solution and post-gelled at 4 °C. (b) Distribution of the diameters of droplets of chitosan/agarose-1 solution (right curve) and of the corresponding microgels (left curve). The experimental data points were fitted to Gaussian distribution.

solution periodically broke up to release droplets. The formation of droplets occurred in the flow-focusing regime (Anna, Bontoux, & Stone, 2003) at a frequency of 1200 s^{-1}. The formation of primary droplets was accompanied by the formation of small ~3 μm-diameter satellite droplets. With increasing volumetric flow rate, Q_o, of the oil phase from 0.1 to 0.30 mL/h, the diameter of primary droplets reduced (Fig. 7a), due to the increasing shear stress imposed by the oil phase on the aqueous stream. This was corroborated with the study of the variation in the mean diameter D, of the droplets formed from chitosan/agarose-1, chitosan/agarose-1.5, chitosan/agarose-2 and agarose-1.5 solutions, plotted as a function of the flow rate of the oil phase (Fig. 7b). The average diameter of droplets was tuned from 42 to 18 μm by varying Q_o from 0.05 to 0.3 mL/h, while maintaining the flow rate of the chitosan/agarose solution, Q_d, at 0.05 mL/h. For a particular Q_0, the diameter of droplets increased with increasing agarose concentration in the chitosan/agarose solutions, due to the increase in viscosity of the solution (Nie et al., 2008; Wang et al., 2013). A similar trend was observed for the droplets of chitosan/agarose-1.5 solution, compared to the droplets of agarose-1.5 solution. For all the chitosan/agarose and agarose solutions, MF emulsification produced primary droplets with polydispersity not exceeding 1.5%.

The precursor droplets exiting the MF device were cooled down in the outlet tubing to induce agarose gelation, while the chitosan molecules were distributed within the agarose network. Additional off-chip post-gelation yielded transparent microgels with a well-defined round shape and polydispersity below 1.5%. A representative image of chitosan/agarose-1 microgels generated at Q_o = 0.20 mL/h and Q_d = 0.05 mL/h, which were transferred in PBS solution, was shown in Fig. 8. The average diameter of the microgels was 18.4 ± 2.3 μm, which was ~30% smaller than the diameter of the corresponding precursor droplets, owing to microgel shrinkage upon gelation.

4. Conclusions

We report on the preparation of composite gels derived from chitosan and agarose having good transparency and mechanical stability. Chitosan/agarose gels had a higher elastic modulus than agarose gels due to the reinforcement of the composite chitosan/agarose gels, in comparison with agarose gels. The introduction of chitosan in agarose gels reduced mass loss of the chitosan/agarose gels, in comparison with agarose gels, at 37 °C and pH = 7.2. The increase in the stability of the chitosan/agarose gels was more evident at pH = 5.2. This result has an important implication for the utilization of these gels as drug delivery matrixes in areas with relatively low pH values such the acidic stomach or tumoral tissues. The release of 5-Fluorouracil from chitosan/agarose macrogels at pH 7.4 and 5.2 under physiological temperature demonstrated the feasibility of these hydrogel for

drug delivery applications. In addition, our work provides a useful strategy for the preparation of composite chitosan/agarose microgels by the MF method as a means to obtain colloidal materials with narrow particle size distribution and potential candidates for their employment in controlled drug release applications due to their dual pH and temperature responsive properties.

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