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Modifiable chemically crosslinked poli(κ-carrageenan) particles

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ABSTRACT

Micron size κ-carrageenan hydrogel particles, p(CRN) from linear κ-carrageenan, were prepared via microemulsion polymerization using divinyl sulfone (DVS) as chemical crosslinker in a sodium bis(2-ethylhexyl) sulfosuccinate (AOT) reverse miceller system. Magnetic field responsive (m-p(CRN)) composite particles were also synthesized by encapsulating magnetic ferrite (Fe_3O_4) nanoparticles together with linear κ-carrageenan within the AOT reverse micelle before the crosslinking reaction. The synthesized bare p(CRN) particles were further modified to produce positive charges on the particles (q-p(CRN)) by a quaternization reaction with an 3-chloro-2-hydroxypropyl trimethyl ammonium chloride aqueous solution. Scanning electron microscopy (SEM), dynamic light scattering (DLS), zeta potential measurements and FT-IR analysis confirmed that particle sizes and charges were altered by chemical modification. Furthermore, a model drug, phenylephrine HCl was used for in vitro drug delivery studies to compare the effectiveness of modification of p(CRN) microgels by comparing bare p(CRN), m-p(CRN) and q-p(CRN) particles drug release capabilities in phosphate buffer solution (PBS) at pH 7.4.

1. Introduction

κ-Carrageenan (p(CRN)) is a linear sulfated water soluble polysaccharide extracted from different species of red seaweed (Pomin, 2009). This natural polymer is composed of repeating disaccharide units of 3-linked β-D-galactose 4-sulfate and 4-linked 3,6-anhydro-α-D-galactose. According to the number and position of the ester sulfate groups, there are three main types of carrageenans which are lambda (λ), kappa (κ), and iota (ι) (Belyaeva, Valle, & Poncelet, 2004; Daniel-da-Silva, Ferreira, Gil, & Trindade, 2010; Grenha et al., 2010; Leong et al., 2011; Thrimawithanaa, Younga, Dunstanb, & Alanya, 2010). Amongst them κ-carrageenan has been reported to be nontoxic, mucoadhesive, biodegradable, and biocompatible. A useful material, the potential applications of κ-carrageenan, in numerous industries including food, cosmetics, pharmaceuticals, and biomedical devices, as drug delivery systems and cell encapsulation, have been widely investigated (Daniel-da-Silva et al., 2010; Leong et al., 2011).

Over the past few decades, many successful research breakthroughs were made in designing pharmaceutical drugs for various diseases. A widely pursued strategy is to design a target-specific drug delivery system that can transport an effective dosage of drug molecules to the targeted cells and tissues (Chertok, David, & Yang, 2011; Pradhan et al., 2010). Therefore, there is continuously growing interest in the pharmaceutical industry in drug delivery systems that provide a controlled and sustained release of therapeutic agents (Liu et al., 2007). There is also a great necessity for dosage formulations allowing high loading, particularly for drugs requiring high therapeutic concentrations in circulation and/or target specific organs. For this purpose, various polymeric systems have been investigated for pharmaceutical applications as microparticles, nanoparticles, capsules and so on (Daniel-da-Silva et al., 2010; Grenha et al., 2010; Satarkar & Hilt, 2008) with high loading capacity. It has been reported that many parameters influence the characteristics of drug release behavior such as the loading path, the types of polymer and/or its functional groups. Therefore, in some cases new functional groups are required. These functional groups can be attained by chemical modification e.g., introduction of new functional groups, and even a guiding ability can be obtained i.e., magnetic responsiveness and so on (Daniel-da-Silva et al., 2010). Modified magnetic particles for the delivery of therapeutic drugs to specific locations in the body have attracted great attention for targeted and triggered delivery as self regulatory delivery devices (Mejías et al., 2008). The main goal is to prepare particles capable of carrying the desired amount of active agent to the required target site and to release on demand in a controlled rate and/or fashion with or without a stimulus. Magnetic particles, embedded in biodegradable polymers can release previously absorbed/conjugated drugs in a controllable fashion. Magnetic-carrageenan composite particles could take advantage

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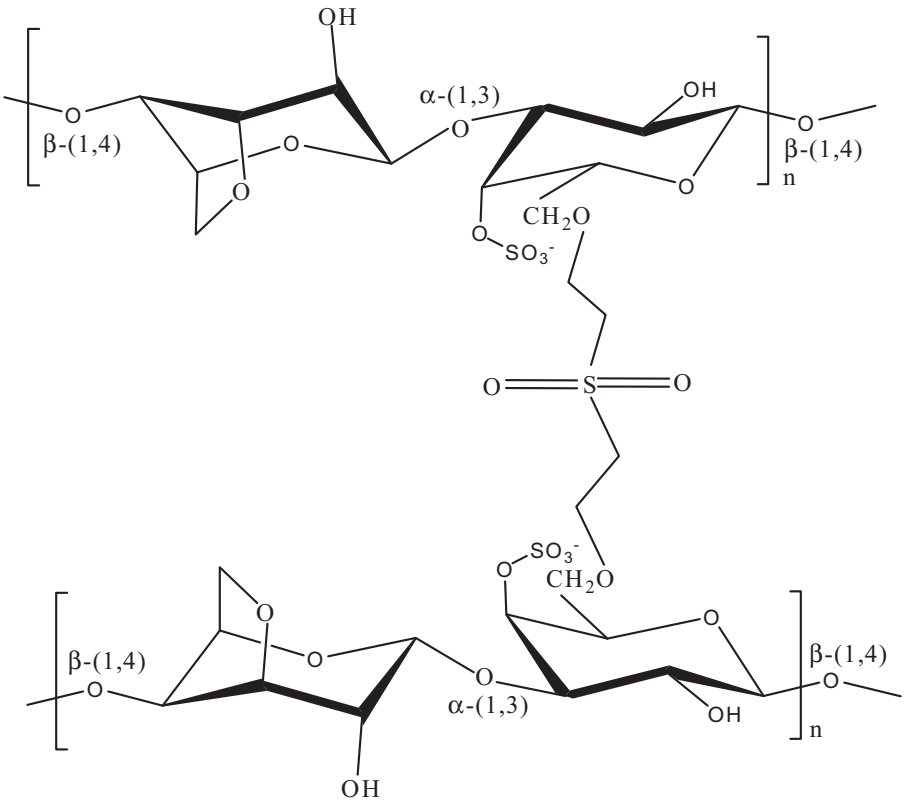

Fig. 1. Schematic illustration of crosslinking linear CRN polymers with DVS.

of the properties of these two components. The magnetic responsive behavior could make it possible to guide the particles to the specific site by means of an externally applied magnetic field. The polymeric p(CRN) particles could have the role of carrying the drug and releasing it upon reaching a target site.

κ-Carrageenan shows characteristic ion binding for monovalent cations and this form is the basis for their gelling properties (Daniel-da-Silva et al., 2010). Previously, the soluble carrageenan particles were obtained by physical crosslinking of linear carrageenan with potassium chloride (Thrimawithanaa et al., 2010). In this study, κ-carrageenan hydrogel particles were prepared by chemical crosslinking using divinyl sulfone (DVS) in a water-in-oil AOT microemulsion system. Furthermore, p(CRN) particles embedding ferromagnetic materials were also prepared to investigate their potential for guidable biomedical applications. The main purpose of this work is to develop a simple procedure for rapid preparation of natural p(CRN) and m-p(CRN) microparticles for biomedical applications. Furthermore, the synthesized p(CRN) particles were quaternized using 3-chloro-2-hydroxypropyl trimethyl ammonium chloride as a modifying agent in an aqueous solution to increase the positive charge on the particle by the procedure reported by Stepnova et al. (2007). The obtained bare, magnetic and quaternized particles were used for encapsulation and in vitro release of a water-soluble model drug, phenylephrine HCl.

2. Experimental

2.1. Materials

κ-Carrageenan (Fluka), divinyl sulfone (DVS, 98%, Merck) as a chemical crosslinker, sodium bis(2-ethylhexyl) sulfosuccinate (AOT, 98%, Sigma–Aldrich) as a surfactant, 2,2,4-trimethylpentane (isooctane) as a solvent, and 3-chloro-2-hydroxypropyl trimethyl ammonium chloride solution (65%, Fluka) as a quaternization agent (QA) were used as received. Sodium hydroxide (NaOH, Sigma–Aldrich), hydrochloric acid (HCl, 37%, Riedel-de haen), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Acros), and iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, Fluka) were used for magnetic particle preparation. All the solvents, acetone and ethanol, were highest purity available. All aqueous solutions were freshly prepared using ultra pure distilled water (DI) (18.2 MΩ cm from Millipore-Direct Q UV3). Phenylephrine HCl was used as a model drug and obtained from a local vendor.

2.2. Synthesis of p(CRN) particles

Earlier, our group reported micro hydrogel particle preparation from different natural polymers employing a water-in-oil microemulsion system (Ekici, Ilgin, Butun, & Sahiner, 2011; Ilgin et al., 2010; Sahiner & Jia, 2008). Here a similar technique was employed, e.g., 0.50 mL of κ-carrageenan solution (0.03 g/mL carrageenan in 0.2 M NaOH at 50 °C) was dispersed in 15 mL of 0.2 M AOT solution in isooctane. Then, the mixture was vortexed until a clear solution was obtained. DVS (50 mol% relative to p(CRN) repeating unit) was subsequently added as a crosslinking agent to the this mixture, and it was stirred for 1 h at 1200 rpm. The reaction mixture was filtered and the obtained p(CRN) particles were precipitated into acetone and kept overnight. Then the p(CRN) carrageenan particles were purified in a water–acetone mixture and collected by centrifugation at 10,000 rpm for 15 min at 20 °C (at least 3 times). The particles were dried at 40 °C and stored in a closed container for modification, drug loading and release studies.

2.3. Synthesis of Fe_3O_4 coated p(CRN) particles

The magnetic Fe_3O_4 particles were prepared according to the method reported in the literature (Liu, Ma, Xing, & Liu, 2004;

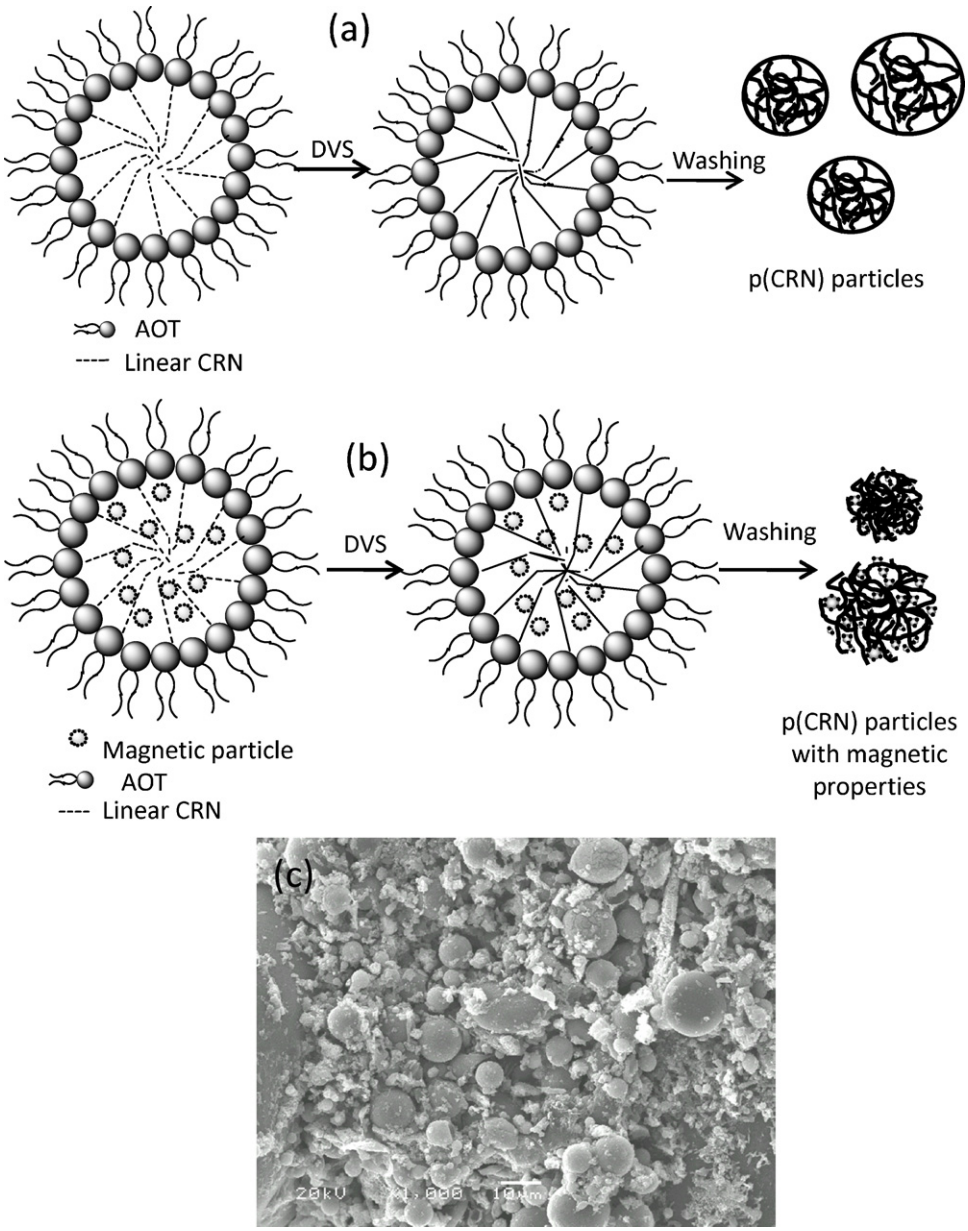

Fig. 2. (a) Schematic illustration of the synthesis of p(CRN) and m-p(CRN) particles in AOT reverse micelles. (c) The SEM images of p(CRN) particles.

Massart, 1981; Yan, Li, Zhang, Liu, & Yang, 2009) with some modifications and then enclosed within p(CRN). Briefly, both $FeCl_2{\cdot}4H_2O$ (0.22 g) and $FeCl_3{\cdot}6H_2O$ (0.606 g) were dissolved in 30 mL DI water in an ultrasonic bath. The mixture was vigorous stirred under nitrogen gas at 50 °C, and 2.5 mL of aqueous ammonia (26%) was added slowly into this mixture under vigorous stirring to obtain good dispersion of the metallic particles. As an indication of ferrite particle formation, the color of the bulk solution turned from orange to black immediately. Then, stirring was stopped, and a strong magnet was used to settle the black precipitate. The magnetite precipitates were centrifuged at 5000 rpm for 5 min and washed three times with DI water. The prepared Fe_3O_4 particles average size was reported as 6–15 nm (Yan et al., 2009). Magnetite nanoparticles were dispersed in 30 mL deionized water, and 1 mL oleic acid (OA) was added to this mixture as a stabilizing agent. Then 200 μL of magnetic nanoparticles were added to the κ-carrageenan precursor solution before the addition of DVS. The same procedure was followed as for p(CRN) particle preparation.

2.4. Quaternization of p(CRN) particles

The quaternization of p(CRN) particles was carried out according to the previously reported procedure with some modifications (Butun, Ince, Erdugan, & Sahiner, 2011). A particle solution consisting of 0.2 g of p(κ-carrageenan) particles was prepared in 40 mL 0.2 M NaOH solution. Another mixture containing 1.5 mL of quaternization agent (QA) was prepared in 20 mL 0.2 M NaOH solution. Then the second mixture was added into the first particle solution and stirred at 800 rpm for 12 h. The resultant mixture was neutralized with equal molar HCl solution. The quaternized p(κ-carrageenan) (q-p(CRN)) particles were precipitated in ethanol. The precipitate was washed with ethanol and centrifuged at 10,000 rpm for 10 min (at least three times) and the q-p(CRN) particles were dried at 40 °C and stored for further use.

2.5. In vitro drug loading and drug release studies

Phenylephrine HCl was used as a model drug to investigate loading and release behavior of p(CRN)-based particles. A weighed

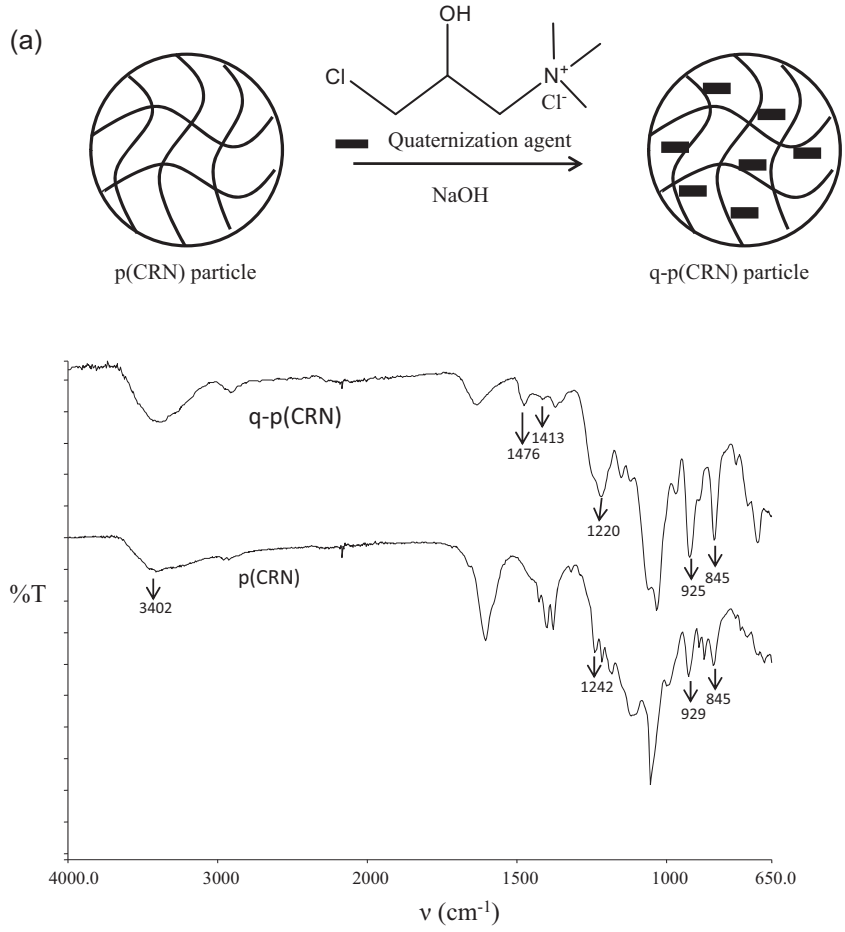

Fig. 3. (a) Schematic illustration of the quarternization reaction mechanism, and (b) FT-IR spectra of bare CRN particles, and q-CRN particles.

amount (0.5 g) of p(CRN) particles was used in drug loading experiments by soaking the particles in 100 mL 300 ppm phenylephrine HCl aqueous solution for 24 h at ambient temperature at 500 rpm. After this loading period, the p(CRN) particles were purified by centrifugation and dried at 35 °C for 24 h. To investigate the release characteristics, 50 mg phenylephrine HCl loaded dry p(CRN) particles were suspended in 2 mL of phosphate buffered saline (PBS) at pH 7.4 and transferred to a dialysis membrane (molecular weight cut off <12,000 Da, Aldrich). The release sack was then placed into a closed beaker containing 25 mL of PBS under constant stirring at 150 rpm. The amount of drug released into the PBS buffer was evaluated by UV–vis spectrometer (T80 + UV/Vis Spectrometer, PG Ins. Ltd) at 273 nm as a function of time via a previously constructed calibration curve at the same wavelength. All experiments were conducted in triplicate, and the results are average values with standard errors. The loading capacity of bare p(CRN), m-p(CRN) and q-p(CRN) particles were also determined by the same method using a UV–vis spectrometer.

2.6. Particle characterization

The average hydrodynamic diameter of the synthesized p(CRN) particles were determined by a dynamic light scattering (DLS) instrument (Brookhaven Ins. and Cor. 90 plus particle size analyzer). The DLS experiment was carried out employing 90° angle detector, with 35 mW solid state laser detector operating at a wavelength of 658 nm. The results are average values of ten consecutive measurements with an integration time of 2 s. Zeta potential measurements were conducted with Zeta-Pals Zeta Potential Analyzer BIC (Brookhaven Inst. Corp.) in 0.01 M KNO_3 solution in water.

Scanning electron microscopy (SEM) images of p(CRN) hydrogel particles were obtained with an SEM (Joel JSM-5600). P(CRN) particles were placed onto carbon tape-attached aluminum SEM stubs at ambient temperature after coating with gold to a few nanometers thickness under vacuum, with an operating voltage of 20 kV.

The Fourier transform infrared spectroscopy (FT-IR) spectra of p(CRN)-based particles were recorded by using a Perkin-Elmer FT-IR spectroscope in the spectral range 4000–650 cm^{-1} using attenuated total reflectance (ATR) at 4 cm^{-1} resolution.

Thermal analyses of p(CRN)-based particles were carried out using a thermo gravimetric analyzer (SII TG/DTA 6300, Japan) to determine the magnetic ferrite content of m-p(CRN) particles. TGA was performed under nitrogen atmosphere with 100 mL/min flow rate with heating rate of 10 °C/min heating up to 800 °C.

Elemental analysis (LecoSL-144DR) was used to determine the presence of sulfur coming from DVS to confirm the crosslinkage of the polymer structure in the particle form.

Drug loading and releasing studies were done by a UV–vis spectrometer at 273 nm wavelength.

3. Results and discussion

P(CRN)-based hydrogel particles were synthesized in AOT reverse micelles using a water-in-oil micro emulsion system

reported earlier for other kinds of natural polymers (Butun et al., 2011; Sahiner & Jia, 2008). Fig. 1 illustrates the crosslinking of linear p(CRN) with DVS in the basic medium to produce hydrogel particles. According to the elemental analysis results, the sulfur content increased from 5.15 wt% to 7.013 wt% upon crosslinking of linear p(CRN) with DVS. The sulfur coming from DVS increased the sulfur content of the particles, confirming the crosslinking of linear CRN. In addition to the preparation of κ-carrageenan particles, we also prepared composite p(CRN) using magnetic Fe_3O_4 particles that were encapsulated within p(CRN) particles by inclusion into reverse micelles containing linear p(CRN) before crosslinking with DVS. This one step synthesis method for preparation of bare p(CRN) particles (Fig. 2a) and m-p(CRN) particles are demonstrated in Fig. 2b. The yield of particle formation from linear p(CRN) particles was estimated to be 70 ± 5.3%. Fig. 2c shows the SEM images of 50 mol% DVS crosslinked p(CRN) particles that are spherical in shape. The particle size as can be seen varies from 100 nm to 10 μm. Although the obtained p(CRN) particles have a broad size distribution, it is possible to separate them by simple filtration and/or gradient centrifugation as was reported earlier (Butun et al., 2011; Sahiner & Jia, 2008). To generate new charge (i.e., positive charge) and an amphiphilic character on p(CRN) particles, the particles were treated with QA. The quaternization (with 3-chloro-2-hydroxypropyl trimethyl ammonium chloride) reaction is depicted in Fig. 3a. Upon chemical modification of p(CRN) particles a trimethyl ammonium chloride group was generated on the particles that offers many possible biomedical applications, such as antimicrobial (Silan et al., 2012; Stepnova et al., 2007; Vallapa et al., 2011) and gene delivery (Dailey, Wittmar, & Kissel, 2005; Jintapattanakit, Mao, Kissel, & Junyaprasert, 2008), etc. To confirm that new functional groups were produced on the particles, the FT-IR spectra of p(CRN) particles and q-p(CRN) particles were taken and are shown in Fig. 3b. As can be clearly visualized from the FT-IR spectra of bare p(CRN) particles shown as (1), the specific bands of p(CRN) were identified as the wide peak at about 3400 cm^{-1} corresponding to –OH stretching, and a peak at 1242 cm^{-1} belonging to the sulfate groups, a peak at about 929 cm^{-1} for 3,6-anhydrogalactose, and a peak at 845 cm^{-1} corresponding to galactose 4-sulfate (Volery, Besson, & Schaffer-Lequart, 2004). After the quaternization reaction shown as (2) in Fig. 3b, the q-p(CRN) particles also have the same specific bands as p(CRN) particles but additionally the characteristic bands of QA also appear at about 1476 cm^{-1}, which corresponds to $^{+}N–CH_3$ stretching, and at 1413 cm^{-1} corresponding to C–N stretching. Furthermore, the broadened band between 3000 and 36,000 cm^{-1} due to new amino groups is clearly visualized.

Further confirmation of positive charge generation was done by zeta potential measurements. As shown in Fig. 4a, the zeta potential of the particles changed from −62.70 mV to −47.37 mV upon modification. This is a drastic change, allowing the particles to possess both negative and positive charges which can be used for the absorption of different species with different charges as will be shown later. The change in charge also affects the particle sizes, proven by the results of dynamic light scattering measurements and illustrated in Fig. 4b. As can be seen the bare p(CRN) and q-p(CRN) particle sizes increased from 845.2 to 963.9 nm. As the measurements were done in salt solution the interaction of charges were suppressed and a size increase upon quaternization of p(CRN) particles resulted.

To prepare composite p(CRN) particles responsive to a magnetic field, separately prepared magnetic ferrite particles (Fe_3O_4) were included in the AOT reverse micelle before crosslinking linear κ-carrageenan with DVS. As can be seen in the digital camera images in Fig. 5a, the bare p(CRN) particles are white in color (1), and the

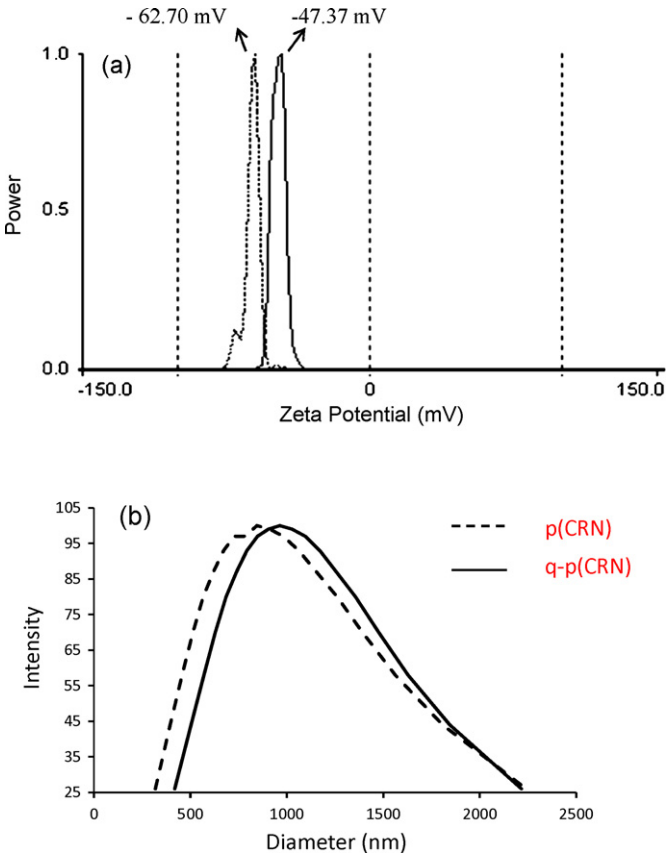

Fig. 4. (a) Zeta potencial measurement of p(CRN) particles (−62.70 mV), and q-p(CRN) particles (−47.37 mV), and (b) particle size disrubution of p(CRN) particles (845.2 nm), and q-p(CRN) particles (963.9 nm).

composite m-p(CRN) particles (2) are brown. The aqueous solution of dispersed p(CRN) particles is shown in (3) whereas the aqueous solution of dispersed p(CRN) particles, and their behavior under a magnetic field are illustrated in (4) and (5), respectively in the same figure. Therefore, it can be assumed that these magnetic composites can be guided by an externally applied magnetic field. In order to determine the amount of ferrite nanoparticles inside composite p(CRN) particles, thermogravimetric analysis was performed on bare p(CRN) and m-p(CRN) particles as shown in Fig. 5b. The weight loss of bare p(CRN) and m-p(CRN) particles were recorded by heating the samples up to 800 °C with a heating rate of 10 °C/min under nitrogen atmosphere. As can be clearly seen from the thermogram, both particles start to degrade about 200 °C and yielded almost similar degradation behavior. The difference between bare and m-p(CRN) particles can be assigned to the extent of the inorganic materials at about 800 °C. Therefore, the TG analysis revealed that the magnetic ferrite content of m-p(CRN) particles is about 12% by weight.

One of the possible applications of p(CRN)-based particles is their utilization as drug delivery systems. Therefore, we used phenylephrine HCl as a model drug for loading and release studies from p(CRN), q-p(CRN), and m-p(CRN) particles. Phenylephrine HCl is a decongestant, mydriatic drug in an acidic salt form and its chemical structure is shown in Fig. 6a. Phenylephrine HCl was loaded onto the p(CRN)-based particles for 24 h in aqueous solution and the loading capacities were measured by UV–vis spectrometer at 273 nm wavelength from a calibration curve. Drug loading capacities of p(CRN)-based particles varied greatly. For example, the amount m-p(CRN) particles can absorb at almost 5.54 mg/g is

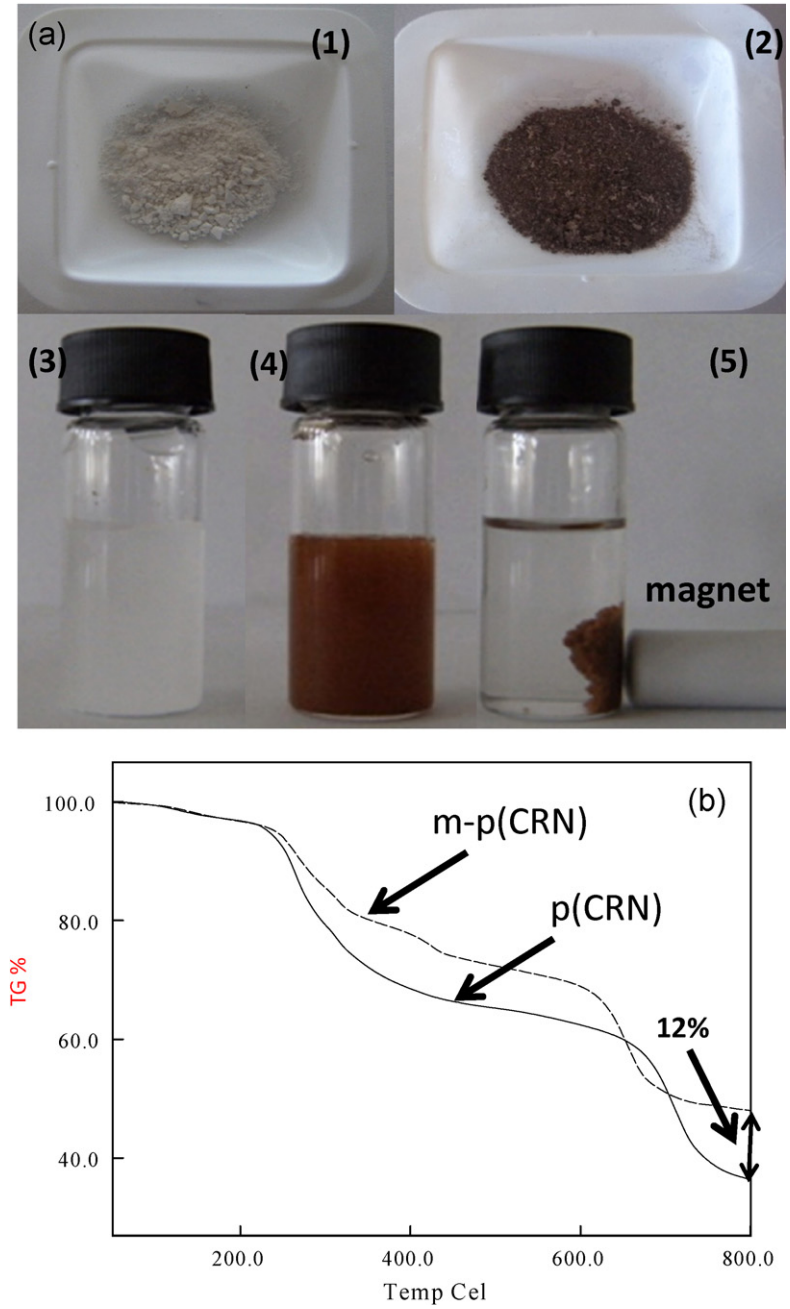

Fig. 5. (a) Digital camera images of (1) bare p(CRN) particles, (2) m-p(CRN) particles, and the aqueous suspensions of (3) p(CRN) and m-CRN particles (4). And (5) the behavior of m-p(CRN) particles and under a magnetic field. (b) Thermogram of bare p(CRN), and m-p(CRN) particles.

almost 2 times more than that of bare p(CRN) particles (3.67 mg/g). On the other hand, the amount loaded onto q-p(CRN) particles increased to 18.71 mg/g, more than fivefold loading than that of bare p(CRN). As illustrated in the release profile shown in Fig. 6b, the particles after quaternization (q-p(CRN)) contain much more phenylephrine HCl due to the new functional groups and released more drug than the other two particles. As can be seen from release profile, bare p(CRN) and q-p(CRN) particles released almost 100% of loaded drug whereas the m-p(CRN) particles released (about 90%) much less than the loaded amount. This can be attributed to the interactions of drug molecules with magnetic ferrite particles within m-p(CRN). All the particles showed similar release profiles but with quaternization the released amount increased almost 6 fold. Therefore, it was proven here that with chemical modification, the biocompatible particles loading and release capacity can be drastically increased and may have great potential for guided and higher dosage of active agents. Owing to the natural properties of p(CRN) such as biodegradability, biocompatibility, and nontoxic and modifiable characteristics, these types of delivery systems can also be used for other types of therapeutic delivery systems such as proteins, genes, DNA, peptides and other active agents (Daniel-da-Silva et al., 2010; Leong et al., 2011; Satarkar & Hilt, 2008). Moreover, the synthesis of p(CRN) particles are simple with the ability of desired functionality e.g., introduction of magnetic sensitivity by inclusion of magnetic ferrites within the p(CRN) network.

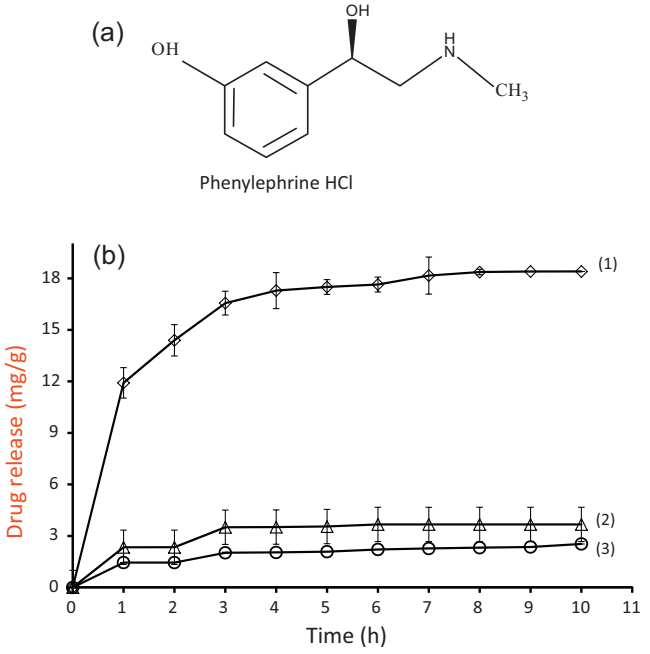

Fig. 6. (a) Chemical structures of the model drugs (phenylephrine HCl), and (b) phenylephrine HCl release profile from q-p(CRN) particles (1), m-p(CRN) particles (2), and bare p(CRN) particles (3) in PBS.

4. Conclusions

As demonstrated in this investigation, a simple water-in-oil micro emulsion polymerization can be employed to prepare biodegradable/biocompatible p(CRN)-based particles even with magnetic responsive composites such as m-p(CRN). The synthesized particles can also be readily modified using a quaternization agent, 3-chloro-2-hydroxypropyl trimethyl ammonium chloride, to generate positive charges on the particles. It was also further demonstrated that new functional groups (ammonium groups) originating from the quaternization reaction allowed increased amounts of drug absorption and release. These kinds of particles may have great potential as targeted drug delivery devices and allow increased amounts of drugs to be delivered.

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