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Synthesis and Characterization of Polyelectrolyte Complex Microparticles for Drug Release

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ABSTRACT: Free radical copolymerization of acryloxyethyl–trimethylammonium chloride with 2–hydroxyethyl methacrylate using sodium persulfate as initiator was carried out at 60°C and of its electrostatic interaction with sodium alginate (NaAlg) allowed obtaining polymeric microparticles by complex coacervation. The solute transport in this swellable matrix was investigated to check the effect of cross-linking and simulated physiological condition over release process. Cefazolin Sodium loaded microparticles were prepared by incorporation of drug directly in the reaction mixture and their ability of releasing resulted in more extensive in enzyme-free simulated intestinal medium. The polymeric microparticles prepared in this study were characterized by scanning electron microscopy showing irregular spherical form and rough structure. Particles showed unimodal distribution and the size distribution ranged from 1.1 to 1.8 mm ($n = 100$). © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: polyelectrolytes; drug delivery systems; microgels

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INTRODUCTION

Development of polymeric systems based on a diffusion release mechanism continue increasing their importance for the delivery of a variety of drugs at controlled rates due to their number advantages over the conventional dosage forms.[1–5] A variety of natural and synthetic polymers with hydrophilic groups, such as hydroxyl, carboxyl, sulfonate, and quaternary ammonium have been used in the development of controlled release systems. According to the characteristic of polymeric matrix, drug and technologic method it can be obtained innumerable systems like films, membrane, microparticles, and nanoparticles.[6–8]

Microencapsulation using polyelectrolytes complexes promises results in terms of stability and controlled release, but the use of acrylic polymer matrices in this field requires careful control of several parameters as viscosity solution, polymer concentration, stirring rate, drug solubility, among others.[9–12] In addition, this method has additional advantages such as a short time reaction and a well–controlled simple production process.[13] Nevertheless, in the last decades the preparation of microparticles as drug delivery system has been majority focus on microparticles prepared with natural polymers alone or their combinations because of their nontoxic, biodegradability and biocompatibility. The most commonly used natural polymers are polysaccharides as chitosan, alginate, and cellulose, etc.[14,15] For example, Simonoska et al. reported delivery system for the inflamed colonic mucosa using Chitosan-coated Ca-alginate microparticles.[16] An interpolymer complex of pectin and chitosan were proposed for colon-specific drug delivery by Fernández–Hervás et al.[17] Jay et al. developed a new controlled and sustainable method to release VEGF from small alginate microparticles.[18] Thus, a wide variety of oppositely charged natural polyelectrolytes has been reported to use as carrier for the drugs.[19,20] However, based on the combination of synthetic and natural polymer to form microparticles by ionic crosslinking interaction offer stable and desired architecture in mild conditions, we wish to examine the possibility of preparing a new pH-dependent drug release system using a synthetic polymer prepared for the first time by our research group with sodium alginate. The possibility to mix their attractive properties (hydrophilicity, biodegradability, and pH-sensitive response to environmental stimuli) will allow entrapping and release ionic hydrophilic drug as well as drug needs to be protected from absorption and/or environment of the gastric fluid. Alginate polymers have been widely used in numerous biomedical applications and their delivery system is formed when water soluble

Table I. Composition of poly(Q–*co*–H)/NaAlg Microparticles, Encapsulation Yield, Encapsulation Efficiency, and % Cefazolin Released in Distilled Water ($\alpha = 0.05$)

Samples	m($CaCl_2$) (g)	EY ± Δ_{EY}(%)	EE ± Δ_{EE} (%)	DR ± Δ_{DR} (%)
M_1	0.08	62.8 ± 0.4	63.8 ± 0.6	76.5 ± 0.7
M_2	0.12	67.5 ± 0.4	73.7 ± 0.9	60.9 ± 0.4
M_3	0.16	70.5 ± 0.7	81.9 ± 0.8	50.7 ± 0.6
M_4	0.20	72.3 ± 0.5	84.6 ± 0.5	36.4 ± 0.5

alginate salts undergo an aqueous sol–gel transformation due to the addition of divalent ions such as calcium forming "egg–box" shaped structure.[21–23] Alginate is one of the natural polysaccharides remain intact in the physiological environment of stomach and small intestine. From this point of view and taking into account their properties it provides biodegradability and biocompatibility of studied system and the possibility of using it as oral colon specific drug. There is relativity few reports of acryloxyethyl–trimethylammonium chloride polymers despite the presence of quaternary ammonium group increase hydrophilic ability proportioning multipoint interaction with anionic matrix. This hydrophilicity confers permeability of molecules and improve soft consistency which makes the matrix similar to the physical characteristic of living tissues. In previous manuscript we report growth goat mammary epithelial cells culture over microcapsules of Q.[24,25] The combination of sodium alginate with this acrylic polymers could be a promote scaffold in biomedical application.[12,24,25,26]

Oral administration is one of the preferred routes for drug because of its noninvasive nature, convenience, safety and patient complacence. Moreover in case of oral drug delivery, the use of microparticles loaded with antibiotic would be beneficial for various diseases such as gastric diseases,[27] intestinal infections,[28] ulcerative colitis, and carcinomas.[29] Recommended treatments of the local diseases of the colon include the administration of anti-inflammatory drugs, chemotherapy drugs, and/or antibiotic. Anti-microbial agents such as amocixillin, metronidazole, and tetraciclyne HCL, from the polymeric matrix have been carried out by various coworkers.[30–32] In our work Cefazolin was selected as a model drug because of their ionic character allow their ease microencapsulation. Furthermore, for patient with penicillin allergies Cefazolin is alternative antibiotic. Cefazolin is a first–generation cephalosporin antibiotic and is usually administrated as sodium salt. It is mainly used to treat bacterial infections of the skin. It can also be used to treat moderately severe bacterial infections involving the lung, bone, joint, stomach, blood, heart valve, and urinary tract. It is clinically effective against infections caused by staphylococci and streptococci of Gram (+) bacteria.

With all these considerations in mind, the purpose of this research work was to: (i) prepare poly(acryloxyethyl–trimethylammonium chloride)–*co*–2–hydroxyethyl methacrylate)/Alginate (poly(Q–*co*–H)/NaAlg) polymeric microparticles; (ii) investigate the effect of calcium chloride content on drug release from polymeric microparticles prepared in i); (iii) The study of Cefazolin Sodium release in two simulated medium from prepared polymeric microparticles.

MATERIALS AND METHODS

Materials

2–Hydroxyethyl methacrylate (H, Merck), acryloxyethyl–trimethylammonium chloride (Q, AQUATECH), potassium persulfate ($K_2S_2O_8$, Fluka), acetonitrile (CH_3CN, Merck) and calcium chloride anhydrous ($CaCl_2$, BDH) were used as received. Sodium alginate was purchased from SIGMA Chemical Co. It was carefully purified prior to use. Enzyme-free Simulated Gastric Fluid (SGF, pH = 1.2) and enzyme-free Simulated Intestinal Fluid (SIF, pH = 6.8) were prepared according procedure described USP 24. Cefazolin Sodium was purchased from Pharmaceutical Laboratory of Cuba (IMEFA).

Copolymerization Reaction

The poly(acryloxyethyl-trimethylammonium chloride–*co*–2–hydroxyethyl methacrylate) copolymer (poly(Q–*co*–H)) was prepared by free radical polymerization of the mixture of corresponding monomers (i.e., Q and H) in aqueous solution without the use of any crosslinking agent. The polymerization was carried out for 8 h at 60°C that permitted to achieve high (>90%) conversions. Since a cationic polyelectrolyte was required for this study, the copolymers with high content of Q units were synthesized. For this purpose the feed monomer composition of Q/H = 95/5 wt %, that corresponds to 92/8 mol %, was used. Copolymerization of these two monomers was detailed investigated previously and it was shown that this ratio between Q and H was optimum for synthesis of the copolymer with the highest concentration of quaternary ammonium (cationic) units in the polymer chain.[24,25]

Preparation of Alginate/poly(acryloxyethyl–trimethylammonium chloride)–*co*–2–hydroxyethyl methacrylate), poly(Q–*co*–H)/NaAlg and Incorporation of Cefazolin Sodium

For the preparation of the poly(Q–*co*–H)/NaAlg polymeric microparticles, 2.0% (w/v) aqueous sodium alginate solution was dropped

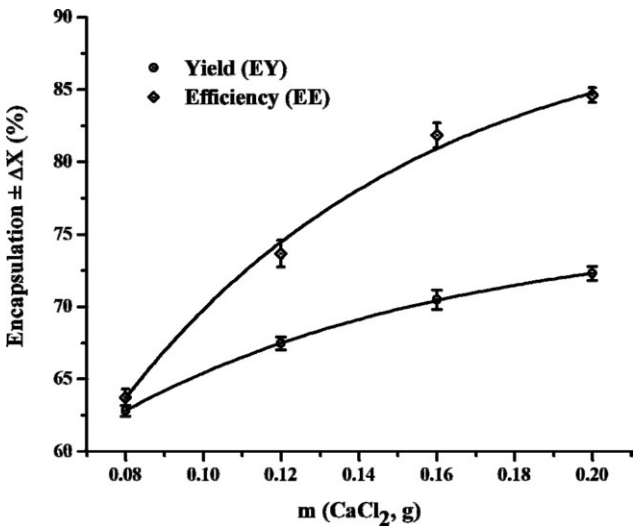

Figure 1. Encapsulation yield and efficiency as function of calcium chloride mass.

Table II. Parameters of the Fit Model for EY and EE ($\alpha = 0.05$)

Parameter	(EY) ± se(%)	(EE) ± se (%)
y_0	75.6 ± 0.2	90 ± 4
A	−31.6 ± 0.4	−75 ± 20
T	0.088 ± 0.002	0.08 ± 0.03

into 1.5% (w/v) aqueous poly(Q–*co*–H) solution containing $CaCl_2$ (0.06–0.20 g), through a microsyringe of 0.70 mm external diameter employing an air-driven droplet generator (KdScientific, Switzerland). These experiments were carried out at room temperature under magnetic stirring and droplets formed were lyophilized. The compositions of samples are listed in Table I.

Cefazolin-loaded microparticles were prepared by the same process described above. Totally, 20 mg of Cefazolin was added in sodium alginate aqueous solution under magnetic stirring at room temperature. Following aqueous sodium alginate solution with drug incorporated was dropped into aqueous (poly(Q–*co*–H) solution employing an air–driven droplet generator. The resulting loaded microparticles were lyophilized.

Microscopic Observations

The shape and morphology of the unloaded and loaded freeze–dried microparticles were observed by scanning electron microscopy (SEM). The microparticles were coated with gold–palladium and their external surface was examined with JEOL–FX 2000, Japan; using a 15 kV accelerating voltage. For the observation of inner structure of microparticles, samples were cryofractured by immersing them in liquid nitrogen and cutting them in half with a sharp scalpel, sputter coated with gold, and examined with a JEOL JSM 1000 F, Japan, at 10 kV. The size of poly(Q–*co*–H)/NaAlg microparticles was determinate employing 100 polymeric microparticles in each composition by using a Nikon Eclipse H550S Optical microscope, Japan.

Encapsulation Yield

For determination of encapsulation yield (EY) six batches of poly(Q–*co*–H)/NaAlg polymeric microparticles were recollected and were evaluated in terms of:

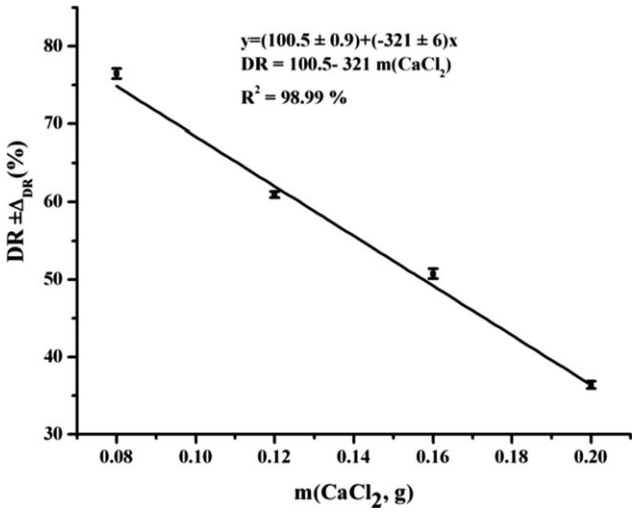

Figure 3. Maximum DR vs. calcium chloride mass.

$$EY(\%) = \frac{m_{mp}}{m_D + m_P} \times 100$$

where m_{mp} is the mass of microparticles obtained in the process, m_D and m_P were the mass of drug and polymer initially dissolved in the solution.

Encapsulation Efficiency

Drug loading was determined by dispersing accurately weighed amounts of microparticles (10 mg) in 10 mL of distilled water. The supernatant was filtrated through 0.45-μm membrane (Millipore) and the drug loading, expressed as weight of polymeric microparticles was determined in triplicate for each composition using UV/Vis spectrophotometer (Cintra 10e, Australia) at a wavelength of 272 nm. The percentage encapsulation efficiency (EE) was calculated as follows:

$$EE(\%) = \frac{C_{mp}}{C_0} \times 100$$

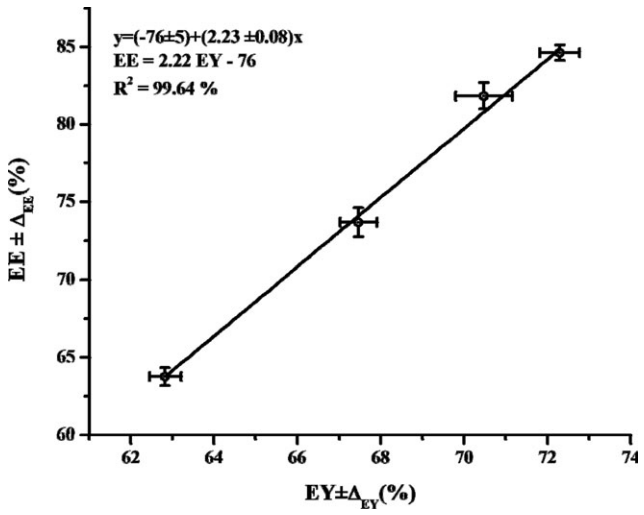

Figure 2. Relationship between encapsulation yield and encapsulation efficiency.

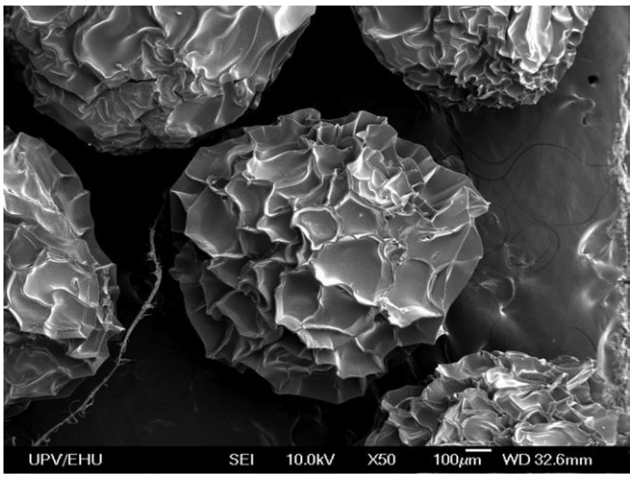

Figure 4. Scanning electron micrograph of external surface of poly(Q–*co*–H)/NaAlg with 0.08 g $CaCl_2$.

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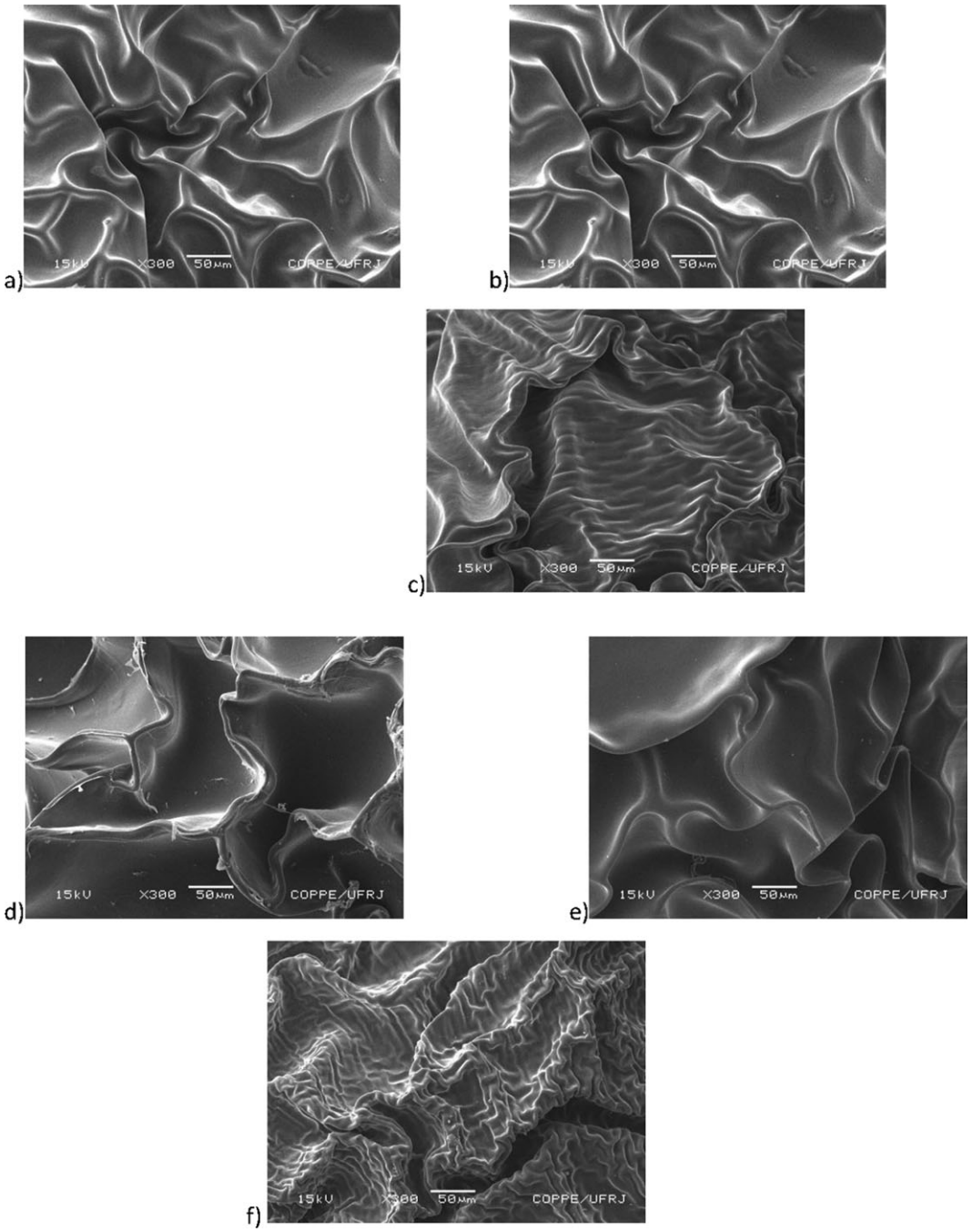

Figure 5. Scanning electron micrograph of poly(Q–*co*–H)/NaAlg microparticles: unloaded microparticles: (a) 0.08 g $CaCl_2$, (b) 0.12 g $CaCl_2$, and (c) 0.16 g $CaCl_2$; loaded microparticles: (d) 0.08 g CaCl2, (e) 0.12 g $CaCl_2$, and (f) 0.16 g $CaCl_2$.

where C_{mp} is the drug concentration in the microparticles, C_0 is the drug concentration in the initial solution which the microparticles were obtained.

In Vitro Release Studies

In order to evaluate their potential as drug delivery system poly(Q–*co*–H)/NaAlg polymeric microparticles were conjugated with 20 mg of Cefazolin Sodium. Lyophilized-loaded microparticles (25 mg) were placed into a flask containing 10 mL of release medium (distilled water, Simulated Gastric Fluid, Simulated Intestinal Fluid) at 37°C for 5 h (Bioblock Scientific, Switzerland).

Simulated fluids conditions were achieved by using different dissolution media. Simulated gastric fluid (SGF) pH 1.2 consisted of NaCl (2.0 g), HCl (7 mL); pH was adjusted to 1.2 ± 0.5. Simulated intestinal fluid (SIF) pH 7.4 consisted of KH_2PO_4 (6.8 g), 0.2 *N* NaOH (190 mL); pH was adjusted to 7.4 ± 0.1.

At predetermined intervals all volume was withdraw and replaced with fresh release medium. The drug concentration release (DR)

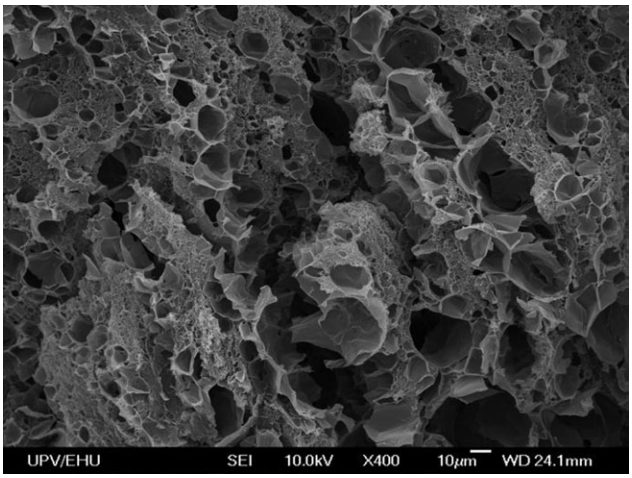

Figure 6. Scanning electron micrograph of the inner surface of loaded poly(Q–*co*–H)/NaAlg microparticles.

into the different mediums as a function of time was monitored by UV-spectrophotometry at 272 nm and expressed as shown below:

$$DR(\%) = \frac{M_t}{M_\infty}\ x\ 100$$

where M_t is the drug amount released at time "t" and M_∞ is the total drug amount in the microparticles.

RESULTS AND DISCUSSION

Polymeric Microparticles Obtained by Complex Coacervation, Encapsulation Yield, and Efficiency Encapsulation

Polyelectrolyte complex microparticles were formed immediately from a pair of oppositely charged polymers. This formation of microparticles was strongly influenced by the association of calcium ions preferentially with guluronic blocks. The higher encapsulation yield values was appreciated for the samples with a higher $CaCl_2$ content due to the increasing cross-linking offers better interaction between macromolecules and therefore more quantity of microparticles.[33–36] Table I lists the $CaCl_2$ content in

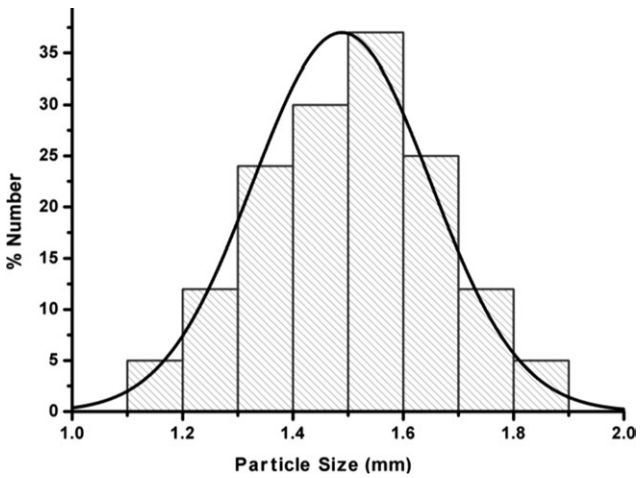

Figure 7. Size distribution of poly(Q–*co*–H)/NaAlg microparticles M1.

Table III. Mean Diameter and Size Distribution of Poly(Q–*co*–H)/NaAlg Microparticles

Microparticles	$m_{(CaCl2)}$ (g)	Size particle mm	Average size ± SD
M_1	0.08	1.1–1.9	1.5 ± 0.2
M_2	0.12	1.1–1.9	1.5 ± 0.2
M_3	0.16	1.1–1.7	1.5 ± 0.1
$^*M_5$	0.08	1.1–1.8	1.5 ± 0.2
$^*M_6$	0.12	1.1–1.8	1.4 ± 0.1
$^*M_7$	0.16	1.1–1.7	1.4 ± 0.1

polymeric microparticles, encapsulation efficiency and efficiency yield. Values encapsulation efficiency increased with increasing $CaCl_2$ content too and more than 60% of Cefazolin could be loaded into the matrix, so this method is useful to encapsulate ionic drugs like sodium cefazolin.

Both relationship EY vs. m($CaCl_2$) and EE vs. m($CaCl_2$) fit to exponential decay with formulae: $y = y_0 + Ae^{-x/t}$ (Figure 1) with R^2 of 99.99 and 99.24%, respectively with which the model explains a highest percent of the variability in the dependent variables, EY and EE. In the mathematical model the obtained parameters for both models showed at Table II.

As it can see the difference between "t" parameter was not significant ($P > 0.05$) and the model establishes two behaviors from the values of "y_0" and "A" parameters (Table II). The microparticles manufacturing lead to the drug encapsulation as a process is more efficient than the microparticles formation itself. It is a fact because the drug encapsulation was made in the same solution to microparticles formation. When it occurs almost all the microparticles formed have the possibility of drug caption from the original solution. In this sense, it could be said that microparticles formation (efficiency yield) is the lead process for the obtained of drug-charged microparticles as showed in Figure 1.

On the other hand, the drug released had a strong dependence of calcium chloride mass which was responsible of cross–linking in the microparticles formation (Figure 2). It can be observed that the samples which 0.20 mg de $CaCl_2$ to cross–linking reach only the 30% of drug released in the scheduled time. This fact could be led to think in an approximation to the lower limit in the range of calcium quantities admissible by the copolymer in order to control de drug released (Figure 3).

Morphology and Size Microparticle

The surface and internal texture of microparticles are shown in Figures 4–6. Examinations of the photographs indicate irregular spherical geometry and rough surface marked by large wrinkles (Figure 4). Change in morphology was revealed with increment of $CaCl_2$ concentration and the inclusion of drug in poly(Q–*co*–H)/NaAlg microparticles [Figure 5(a–f)]. The inner surface of loaded poly(Q–*co*–H)/NaAlg revealed an open porous microstructure similar to sponge appearance, appropriate characteristic for drug delivery system (Figure 4). Thus, when the microparticles are placed in simulated fluid medium their inner sponge structure

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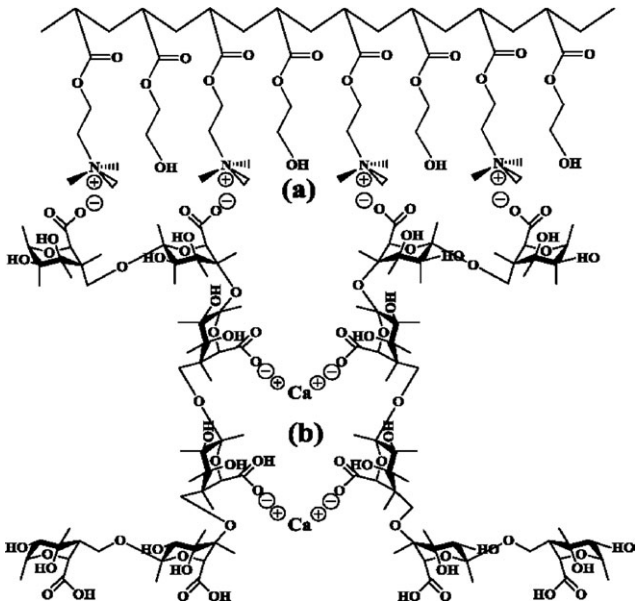

Figure 8. Schematic representation of poly(Q–*co*–H)/AlgNa: (a) electrostatic interactions and (b) ionotropic gelation.

rapidly absorb fluid medium, facilitating Cefazolin diffusion and release. A similar morphology for alginate–chitosan microparticles was observed by Acosta et al.[37] and system reported in previous publication using SEM.[24]

On the other hand, optical microscopic inspection of polymeric microparticles revealed physical integrity in each composition and a homogenous size distribution was observed (Figure 7).

The average size particle and size distribution of poly(Q–*co*–H)/NaAlg are given in Table III, the data revealed that increasing the concentration of $CaCl_2$ caused a slight decrease in the two parameters. However, the drug entrapment did not show any relationship with the variation of particle size of microparticles prepared.

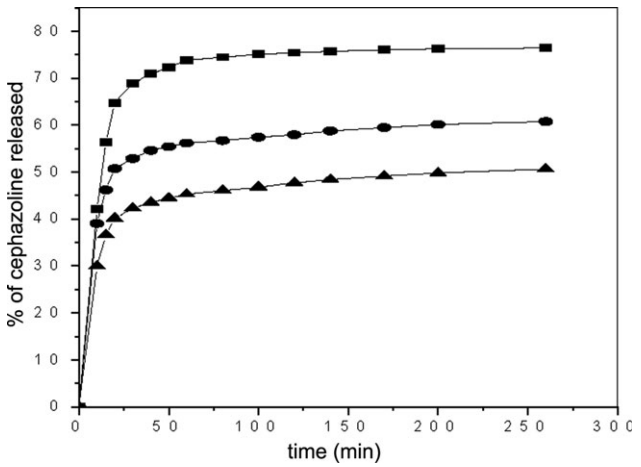

Figure 9. Sodium Cefazolin release from poly(Q–*co*–H)/NaAlg microparticles in water with different content of $CaCl_2$: (■) 0.08 g; (●) 0.12 g; (▲) 0.16 g.

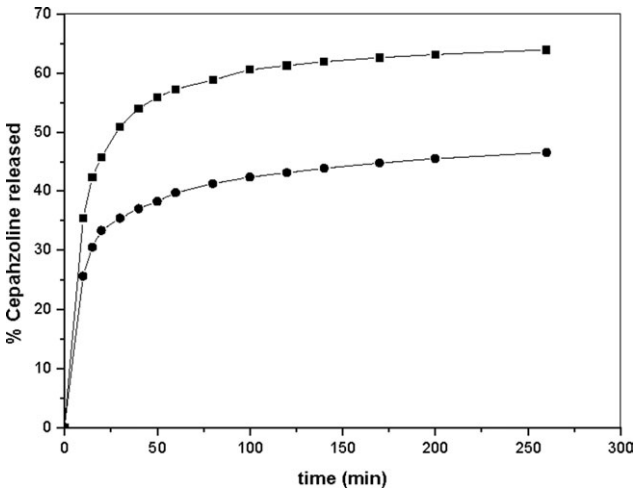

Figure 10. Cefazolin Sodium release from poly(Q–*co*–H)/NaAlg microparticles (M1) in different mediums: (■) Simulated Intestinal Fluid and, (●): Simulated Gastric Fluid.

In Vitro Release Studies of Cefazolin Sodium

Effect of $CaCl_2$ Content on the Release Process. There are two ionic interactions that contribute to the three-dimensional cross-linked networks of the polymeric microparticles: the interaction between opposite charges of the polymers and the junction formed by the calcium ion [Figure 8(b)]. The contributions of these factors will affect the structure of microparticles and consequently the release profile of drug incorporated into the matrix. That is why the aim emphasis was placed on the evaluation of the effect of cross-linking and medium over release process.

Figure 9 shows the *in vitro* release profile of different formulations in distilled water varying amounts of $CaCl_2$. All formulations present an initial burst effect may be attributed to the diffusion of the drug caused by rapid gel swelling and the release of drug adsorbed on the surface of the hydrophilic matrix. The results indicate that the release profile of Cefazolin was influenced by the concentration of the cross-linker, the higher concentration of $CaCl_2$ led to a lowest release due to the formation a closed matrix proportioning a reduction of drug diffusion through to release medium (Table I). In fact, microparticles with 0.20 g of cross-linking only release Cefazolin Sodium in the first 20 min (36.40%) because in this condition drug was greatly entrapped into the mesh space on the network. With more cross-linking agent, the higher crosslink density and consequently the lower release is obtained. This behavior are in contrast with the resulted obtained by external microstructural examinations of microparticles, samples with higher amount of $CaCl_2$ revealed a close network with an increment of roughness at the same magnification [Figure 5(a, c)].

Effect of Medium on the Release Process. The Sodium Cefazolin release profiles from poly(Q–*co*–H)/NaAlg microparticles containing 0.08 g of $CaCl_2$ in Simulated Intestinal Fluid and Simulated Gastric Fluid over a 5-h period is illustrated in Figure 10 showing a characteristic pattern of release in two mediums.

The percentage of Sodium Cefazolin released from polymeric microparticles in SIF (61.14%) was higher than in Simulated

Gastric Fluid (46.92%). This can be explained on the basis of the properties of polymers contain pendant acidic or basic groups change with pH of the environments (degree of ionization of the ionize sites of polymers chain change with pH). At low pH (acid pH), all species are in protonated form experimenting cross-linking by hydrogen bonds and proportioning more rigid network, that explains the lower aliquot of release in Simulated Gastric Fluid.

On the other hand, electrostatic interactions between polymers is clearly in this system and the addition of divalent ions produce a partial neutralization of carboxylate groups on the alginate chain. Thereby we considered a remarkable influence of quaternary ammonium group at high pH. In this sense, we analyzed two interactions: the interaction of Q with anionic charges present in release medium and the repulsion of positive charge of Q producing enlarge the distance of main polymer chain, resulted in the expansion of matrix. As the result of these factors the hydrophilic network to swell causing Sodium Cefazolin to be easily released.

CONCLUSIONS

The polymeric microparticles of poly(Q–*co*–H) and sodium alginate could be prepared by complex coacervation while synthetic copolymer has been synthesized by free radical polymerization using a conventional initiator. The Sodium Cefazolin has been successfully incorporated into microparticles and their release in distillated water was dependent on the content of cross-linker ($CaCl_2$). Moreover, *In vitro* release study of poly(Q–*co*–H)/NaAlg microparticles in gastric and intestinal simulated mediums indicate the markable influence of pH over release process. Images obtained by electronic microscopy of microparticles revealed rough out surface and sponge surface when Cefazolin Sodium is included, while their observation in a light microscope showed similar size distribution ranged from 1.1 to 1.8 mm. These results suggested that new polymeric scaffold were appropriate to orally delivery of drugs.

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