Release of Prednisolone and Inulin from a New Calcium-Alginate Chitosan-Coated Matrix System for Colonic Delivery

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ABSTRACT: Putative colonic release formulations of calcium (Ca)-alginate coated with chitosan containing two different actives, prednisolone and inulin, were prepared in three different sizes, beads ($D_{50} = 2104\ \mu m$) and microparticles ($D_{50} = 354$ and $136\ \mu m$). The formulations were tested in standard phosphate buffer and biorelevant Krebs bicarbonate buffer at pH 7.4, and were further evaluated in the presence of the bacterium *E. coli*. Product yield and encapsulation were higher with prednisolone than with inulin. In Krebs bicarbonate buffer, a clear relationship between particle size and prednisolone release was observed. In contrast, release of inulin was independent of the particle size. In phosphate buffer, the particles eroded quickly, whereas in Krebs buffer, the particles swelled slowly. The difference in behavior can be attributed to the formation of calcium phosphate in the phosphate buffer medium, which in turn weakens the Ca-alginate matrix core. In the presence of *E. coli*, the formulations were fermented and the release of prednisolone was accelerated. In conclusion, the buffer media affects formulation behavior and drug release, with the bicarbonate media providing a better simulation of *in vivo* behavior. Moreover, the susceptibility of the formulations to bacterial action indicates their suitability as carriers for colonic drug delivery. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2748–2759, 2013

Keywords: biodegradable polymers; coacervation; hydrogels; chitosan; inulin; alginate; calcium alginate; polysaccharides colonic drug delivery

INTRODUCTION

It has been shown that the use of bacteria as a trigger mechanism for colonic drug release improves specificity compared with a pH-responsive approach. These bacteria produce enzymes that are capable of breaking down undigested polysaccharides in the colonic contents.[1] Polysaccharides such as alginate and chitosan are preferentially degraded by bacteria in the colon[2–4]; these findings have led to the development of chitosan–alginate drug delivery systems for site-specific drug delivery in the colon.[2–7]

Procedures for the manufacture of particles of calcium (Ca)-alginate core coated with chitosan have been extensively studied. The main formulation factors studied have been the effect of composition of the alginates and the cross-linking ions,[8] the molecular weight and degree of acetylation of chitosan on the formation of the polyelectrolyte complex,[9,10] and coagulation time and the pH of the coagulation medium.[11] One of the most effective procedures is the two-step method, where Ca-alginate microparticles are recovered and subsequently coated with chitosan.[12] The reaction occurs mainly on the surface of Ca-alginate core to form a membrane. It has been reported that a thicker membrane with better antiswelling ability is obtained with low molecular weight chitosan compared with high molecular weight chitosan.[10] Thus, in this work, it has been used to optimize the conditions described in the literature for the two-step method. In this system, we have encapsulated active ingredients (AIs) with high

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molecular weight as proteins and conventional low molecular weight drugs. According to our knowledge, to date, there have been no reports on encapsulation and drug delivery behavior of a combination of high-/low-molecular-weight AIs in this system. In particular, it has been reported in the literature that alginate/chitosan microparticles containing prednisolone[13] and Ca-alginate beads containing inulin[14] but not the combination of both in chitosan–alginate system intended for colon-specific delivery. Some recent studies suggest that prebiotics that produce quite selective changes in the composition of the microbiota may have benefits in irritable bowel syndrome. It has been suggested that inulin should receive more attention for colon-specific delivery of bioactive food components as it is cheap, has many health benefits by itself, and can be applied in combination with almost all encapsulation techniques.[15–17]

The aim of this work was to develop novel formulations of Ca alginate coated with chitosan containing prednisolone and inulin as the AIs with the intention of evaluating drug release in simulated intestinal and colonic conditions in the presence of the bacterium *E. Coli*.

In particular, in this work, we prepare three different sizes of particles from beads to microparticles by dropping or spraying alginate solution into $CaCl_2$ solution, which was then coated by chitosan. The effect of the particle size on the load of the drug was studied, and the drug-dissolution behavior was evaluated based on the swelling/erosion behavior in both phosphate and Krebs buffers. The swelling/erosion data were analyzed according to the obstruction-scaling model proposed by Amsden[18] to estimate the intragel diffusion of prednisolone and inulin in the core of the Ca alginate. Additionally, the formulations were tested against bacterial-strains-cultured *E. coli* ATCC 25922.

MATERIALS AND METHODS

Chitosan (low molecular weight) was obtained from Sigma–Aldrich Inc. (St. Louis, Missouri), which had the following properties: intrinsic viscosity ($\eta_{sp/c}$) = 203 mL/g, viscometric molecular weight (M_v) = 269 kDa, and degree of acetylation (DA) (%) = 21.7. These properties were determined using conditions described elsewhere.[19]

Alginic acid sodium salt of medium viscosity from *Macrocystis pyrifera* (AS) was obtained from Sigma–Aldrich Inc. . The viscosity of the 2% solution at 25°C was 3500 mPas.

1. Prednisolone was obtained from Ferring Pharmaceuticals (København S, Denmark).
2. Inulin–FITC was obtained from Sigma–Aldrich Inc., St. Louis, MO
3. Calcium chloride was supplied from Scharlau Chemie (Barcelona, Spain).
4. All other chemicals used were of analytical grade.
5. *E. coli* ATCC25022 (Biomedical Laboratory Reference National Public Health Institute, Santiago, Chile).

Preparation of Beads and Microparticles Loaded with Prednisolone and Inulin–FITC

The beads and microparticles were made by dispersing 100 mg of prednisolone, inulin–FITC, and Pluronic F-127 (block copolymer of ethylene oxide and propylene oxide, Sigma–Aldrich Inc., St. Louis, MO) in 20 mL of an aqueous solution of sodium alginate (medium viscosity 3500 mPas) at 1% (w/v) with a magnetic stirrer. This dispersion was pumped using a peristaltic pump (Masterflex 7523-35; L/S tubing 14; Masterflex, Barrington, Illinois) at different rates (1–3–5 mL/min) into an automatic spray gun (Walther Pilot mod WA-XV, Wupertal, Germany) fitted with a 0.5- or 1.5-mm diameter nozzle. The dispersion was dropped (beads) or sprayed (microparticles, varying air flow and pressure) into 500 mL of a solution of calcium chloride at 0.5% (w/v) in water. The mixture was stirred with a magnetic stirrer until all of the suspension was added. Then, the particles obtained were separated by using a sieve and were washed three times with water. Next, the particles were immersed in 10 mL of chitosan solution at 1% (w/v) in acetic acid (Aldrich; low molecular weight) for 30 min. Then, the particles were separated by using a sieve and dried in a tray dryer (Labtech mod LDO-080F, Namyangu, Korea) for 24 h at 30°C and sieved through 16 mesh for the beads, 20 mesh for micro-M, and 100 mesh for micro-S. Particles with a moisture content of 10% were obtained.

Determination of the Size of the Beads and Microparticles

Dried bead or microparticles (40 mg) were dispersed in 25 mL of distilled water and observed under a microscope (ZEISS model AXIOSTAR PLUS; magnification 5×; Oberkochen, Germany) equipped with a digital camera (NIKON mod E450; Tokyo, Japan). For each experiment, at least 50 particles were measured. The diameter of the particles was measured using Axiovision 4.8 software (ZEISS, Oberkochen, Germany). The particle size distribution and span were characterized by the ratio of $(D_{90}–D_{10})/D_{50}$, in which D_{90}, D_{10}, and D_{50} represent the diameter below which 90%, 10%, and 50% by diameter of the particles are found, respectively.

Drug Release Studies

Beads or microparticles (10 mg) were placed on a polyvinylidene fluoride membrane filter (0.45 μm) in a stainless steel basket normally used in the USP apparatus 1. The basket was properly fitted to a 100-mL beaker and immersed in 100 mL of buffer phosphate (0.02 M KH_2PO_4, adjusted with 3 M NaOH solution to pH 7.4) or Krebs buffer (1.18 mM KH_2PO_4, 24 mM $NaHCO_3$, 118.07 mM NaCl, 4.69 mM KCl, 2.52 mM $CaCl_2$, 1.18 mM $MgSO_4 \cdot 7H_2O$, pH 7.4). The test was performed in an orbital shaker at 37 ± 0.1°C and 50 ± 5 rpm. Each assay was performed in triplicate. Aliquots of 5 mL were taken at different times between 5 min and 7 h. These aliquots were replaced with an equal volume of the medium. For the determination of the loading capacity (LC) of prednisolone and inulin–FITC, samples were taken at 24 h in both dissolution media. LC was calculated using the following equation:

$$\mathrm{LC}\,(\%) = \frac{\mathrm{MAI}_{24\,\mathrm{h}}}{\mathrm{MP}} \times 100$$

where $\mathrm{MAI}_{24\,\mathrm{h}}$ is the amount of AI released at 24 h and MP is the total amount of particles.

The quantitative determination of prednisolone and inulin–FITC was performed by HPLC. For prednisolone, the following conditions were used: symmetry C18 column (150 × 3.9 mm^2, 100 Å pore size, 5 μm); mobile phase = acetonitrile:0.1% acetic acid (v/v in water) (40:60); flow rate = 1.0 mL/min; UV photodiode array detector model 996 set to 245 nm (Waters, Milford, Massachusetts); injection volume = 20 μL; and room temperature. For inulin–FITC, the following conditions were used: symmetry 300 C18 column (250 × 4.6 mm^2, 300 Å pore size, 5 μm); mobile phase = acetonitrile:0.1% acetic acid (v/v in water) (50:50); flow rate = 1.0 mL/min; fluorescence detector model 2475 set to 485 nm excitation and 530 nm emission (Waters); injection volume = 10 μL; and room temperature.

The dissolution data were evaluated according to multifactorial ANOVA analysis using the software STATGRAPHICS Centurion XV.II (Statpoint Technologies, Inc., Warrenton, Virginia) for each dissolution medium. The response used was the percentage of AIs released in relation to the following factors: AI, particle size, and time.

Determination of Chitosan Content in the Particles, Yield of Encapsulation, and Yield of Particles

The quantitative determination of chitosan in the particles was performed by SEC (size exclusion chromatography) according to the method described by Yu et al.[20] The following conditions were used: Protein Pak 300 SW column (Waters, Milford, Massachusetts) (300 ± 7.8 mm^2); mobile phase = 0.2 M CH_3COOH/0.1 M CH_3COONa; flow rate = 0.6 mL/min; and refractive index detector model 2414 (Waters, Milford, Massachusetts). The system (column and detector) was set at 30°C. The method was lineal ($r^2 = 0.9993$) in the range from 1.0 to 10 mg/mL. Briefly, the particles (beads–micro-M–micro-S) were immersed in the chitosan solution at 1% (w/v) in acetic acid for 30 min (w/s). The relation particles–chitosan was 1:2. Then, the particles were separated by using a sieve, and the remaining solution was used for the quantization. The control was a chitosan solution a 10-mg/mL in acetic acid and all samples were filtrated by membrane de 0.45 mm (Millipore, Billerica, Massachusetts).

Chitosan content (CC) in the particles was calculated using the following equation:

$$\mathrm{CC}\,(\%) = \frac{\mathrm{TC} - \mathrm{RC}}{\mathrm{MP}} \times 100$$

where TC is the total amount of chitosan in the solution, RC is the remaining amount of chitosan in the solution after the coating of Ca-alginate core, and MP is the total amount of particles.

Yield of encapsulation (YE) was calculated using the following equation:

$$\mathrm{YE}\,(\%) = \frac{\mathrm{LC} \times \mathrm{MP}}{\mathrm{MAI}}$$

where LC is the LC of AI, MP is the total amount of particles, and MAI is the total amount of AI (mg).

Yield of particles (YP) was calculated using the following equation:

$$\mathrm{YP}\,(\%) = \frac{\mathrm{MP}}{\mathrm{MP}_0} \times 100$$

where MP is the total amount of particles (mg) and MP_0 is the sum of the initial dry weight of alginate and AIs; the amount of chitosan was estimated from CC (%) and the amount of calcium chloride was estimated as 10% of alginate as described by Gamboa.[21]

Evaluation of Swelling Behavior

The same equipment and conditions used in the drug release studies were used in these experiments. At each sampling time, the basket containing the beads or microparticles was removed, the dissolution medium was eliminated, and the basket was weighed. Each assay was performed in triplicate. The swelling data were analyzed according to the obstruction-scaling model proposed by Amsden[18] to estimate the prednisolone and inulin intra-Ca-alginate diffusivity

(D_g) according to the following equation:

$$\frac{D_g}{D_o} = \exp\left[-\pi\left(\frac{r_s + r_f}{k_s(\phi a)^{-0.5}(\alpha C)^{-0.25} + 2r_f}\right)^2\right] \quad (1)$$

where D_g is the AI (prednisolone or inulin) intra-Ca-alginate diffusivity, D_o is the AI diffusivity in the aqueous medium, r_s is the AI hydrodynamics radius, r_f is the radius of the polymer chain, k_s is a scaling constant, ϕ is the volume fraction of the polymer, a is the monomer length, α is the degree of ionization of the polymer chain, and C is the polymer characteristic ratio.

The following values were used to estimate D_g/D_o for inulin and prednisolone: $r_{sinulin} = 160\,A$,[22] $r_{sprednisolone} = 12.2\,A$,[23] $r_f = 8\,A$ (effective polymer radius for Ca alginate),[24] $k_s = 76.5\,A$ (scaling constant for Ca alginate),[18] $a = 5.14\,A$ (monomer length for alginate),[18] $\alpha = 1.0$ (degree of ionization of the alginate chain), and $C = 26$ (alginate characteristic ratio).[17]

The volume fraction of alginate (ϕ) was considered to be equivalent to the molar fraction of alginate ($x_{alginate}$). The weight composition of the Ca–alginate coated with chitosan for each size of particle was estimated based on LC of both actives, CC and MP, described in the above section; the amount of Ca chloride was estimated as 10% of alginate as described by Gamboa.[21] The weight composition estimated of the particles is described in Table 1. The monomer molecular weight of chitosan (anhidroglucosamine) is 161 and the monomer molecular weight of alginate (anhidromannuronic/guluronic) is 176, and the molecular weight of calcium chloride is 111. Thus, $X_{alginate0}$ before swelling was estimated using the following equation:

$$X_{alginate0} = \frac{n_{alginate}}{n_{alginate} + n_{chitosan} + n_{CaCl_2}} \quad (2)$$

Thus, $X_{alginatet}$ during the swelling experiment was estimated as follows:

$$X_{alginatet} = \frac{n_{alginate}}{n_{alginate} + n_{chitosan} + n_{CaCl_2} + n_{water_t}} \quad (3)$$

where n_{water_t} was estimated as follows:

$$n_{water_t} = \frac{m_t - m_0}{18} \quad (4)$$

where m_t is the mass in time t, and m_0 is the mass in time 0

Table 1. Composition of Particles of Calcium Alginate Coated with Chitosan

Components	Beads (%)	Micro-M (%)	Micro-S (%)
Prednisolone[a]	13.0	10.6	4.4
Inulin–FITC[a]	6.7	3.2	3.0
Chitosan[b]	9.7	4.3	7.2
Alginate[c]	64.2	74.5	77.6
Calcium chloride[d]	6.4	7.4	7.8
Total	100.0	100.0	100.0
Matrix without actives			
Alginate	79.9	86.4	83.8
Calcium chloride	8.0	8.6	8.4
Chitosan	12.1	5.0	7.8
Total	100.0	100.0	100.0
Monomer Mw alginate	176		
Mw calcium chloride	161		
Monomer Mw chitosan	111		
$X_{alginate_0}$	0.755	0.819	0.793

[a] Obtained from loading capacity (LC).
[b] Obtained from chitosan content in the particles (CC).
[c] Estimated from LC, CC, and mass of particles.
[d] Estimated as 10% of alginate as described on Gamboa.[21]

Characterization of the Microstructure

Scanning Electron Microscopy

The microstructure characterization of the beads and microparticles was carried out in both the dry and swollen states using scanning electron microscopy (SEM). The microparticles (100 mg) were submerged in a flask with 100 mL of phosphate buffer or Krebs buffer. The flask was shaken at 37°C for 2 h in an orbital shaker (Shaking Water Bath, PolyScience, Niles, Illinois). Then, the swollen microparticles were separated from the dissolution media. The SEM of the swollen particles was obtained by using the critical point drying method. In this method, the particles are desiccated with acetone and then saturated with CO_2.[25]

Fourier Transform Middle Infrared Region Spectroscopy

Fourier transform middle infrared region spectroscopy measurement were made in a Spectrum 400 (PerkinElmer, Beaconsfield, United Kingdom) with a range of reading in the middle infrared region (MIR) between 450 and 4000 cm^{-1}.

MIR Sample Preparation

Approximately 10 mg of the sample was ground thoroughly with 500 mg KBr, and a pellet was formed using a manual press (Pike technologies, Madison Wisconsin). The characteristic absorption bands for prednisolone, inulin–FITC, and blank microparticles in three different sizes were designated as beads, micro-M, and micro-S, respectively; and the same particles loaded with prednisolone and inulin–FITC were measured. The spectra were obtained by averaging 10 scans in the spectral range of 4000–450 cm^{-1}.

Table 2. Manufacture Conditions and Particle Size Distribution of Beads and Microparticles Loaded with Prednisolone and Inulin

	Spraying Conditions						Particle Size (μm)			
Type	Nozzle Diameter (μm)	Air Pressure (Mpa)	Air-Flow Rate (Lpm)	Alginate-Flow Rate (mL/min)	Microparticles Sieved by Mesh #	Lot #	D_{10}	D_{50}	D_{90}	Amplitude
Beads	500	0	0	1	16	1	1705	1994	2357	0.3
						2	1759	2150	2568	0.4
						3	1779	2169	2654	0.4
						Mean	1748	2104	2526	0.4
						SD[a]	38	96	153	0.1
						CI[b]	43	109	173	0.1
Micro-M	500	0.3	50	5	20	1	227	356	555	0.9
						2	247	364	462	0.6
						3	232	354	565	0.9
						Mean	235	358	527	0.8
						SD	10	5	57	0.2
						CI	12	6	64	0.2
Micro-S	500	0.5	60	3	100	1	81	120	165	0.7
						2	112	151	198	0.6
						3	98	137	178	0.6
						Mean	97	136	180	0.6
						SD	16	16	17	0.1
						CI	18	18	19	0.1[a]

[a]standard deviation
[b]confidence interval (p = 0.05)

Microbiological Assay and Growth Conditions

Formulations were tested against bacterial-strains-cultured *E. coli* ATCC 25922. Bacteria were grown at 37°C in Luria–Bertani Broth (LB)[26] with shaking until OD_{600} 0.8 (6.0×108 cfu/mL) and 0.7 (5.5×108 cfu/mL), respectively. For the strain, 40 mL of culture (initial pH 6.5) were taken and 10 mg of microparticles tested were added. The cultures were incubated at 37°C with constant stirring (50 rpm) and a volume 1-mL sample was harvested at 30, 60, 120, and 180 min (final pH 5.5). To determine the release profile of prednisolone by HPLC, samples were previously centrifuged at 3000 rpm for 15 min. Sterile LB culture was used as control. Each experiment was performed in triplicate.

Statistical Analysis

The experimental data were analyzed by analysis of variance and significance of differences between means by Tukey's multiple range tests (STATGRAPHICS Centurion XV.II; Statpoint Technologies, Inc.). A p level of 0.05 was used to determine significance.

RESULTS AND DISCUSSION

As described in Table 2, three sizes of particles were obtained with a span less than 0.9. The order of the size of the particles, expressed by the value of D_{50}, was beads, micro-M, and micro-S with sizes of 2104, 354, and 136 μm, respectively, which in relation to the smallest particle size gave an approximate ratio of beads–micro-M–micro-S of 15:3:1. Figure 1 shows the YE of prednisolone and inulin for each particle size in phosphate buffer at pH 7.4 (Fig. 1a), the load of drug (LC) for each particle size in both phosphate and Krebs buffers at pH 7.4 (Fig. 1b), and the YP (Fig. 1c). In the case of prednisolone, the YE increased when the particle size increased ($p < 0.05$), but there is no difference in the YE of inulin for the smaller particles (136 and 358 μm). The YE of prednisolone was higher compared with inulin, except for the smallest size (136 μm). The load of prednisolone increased with the size of the particle. There are no differences as a result of the buffer, except for the 358-μm particle, where the load was higher in Krebs buffer. In the case of inulin, the load was independent of the buffer, and there is no difference between the 135- and 358-μm particles. The load of prednisolone was higher compared with that of inulin. The YP increased significantly when the size of particles increased (see Fig. 1c).

Inulin and prednisolone are very different in their water solubility and molecular weight; the water solubility for inulin–FITC is 10 mg/mL and its molecular weight is 3900 Da (from dahlia tuber, Sigma–Aldrich Inc., St. Louis, MO).[14] In the case of prednisolone, its water solubility is 0.223 mg/mL at 37°C and its molecular weight is 360.5 Da.[27] Thus, the water solubility of inulin–FITC is 45 times higher than prednisolone, and its molecular weight is 11 times higher than prednisolone. Thus, the lower YE and load of inulin in the Ca-alginate chitosan-coated (CACC) matrix system can be explained by its higher molecular weight and water solubility compared with prednisolone.

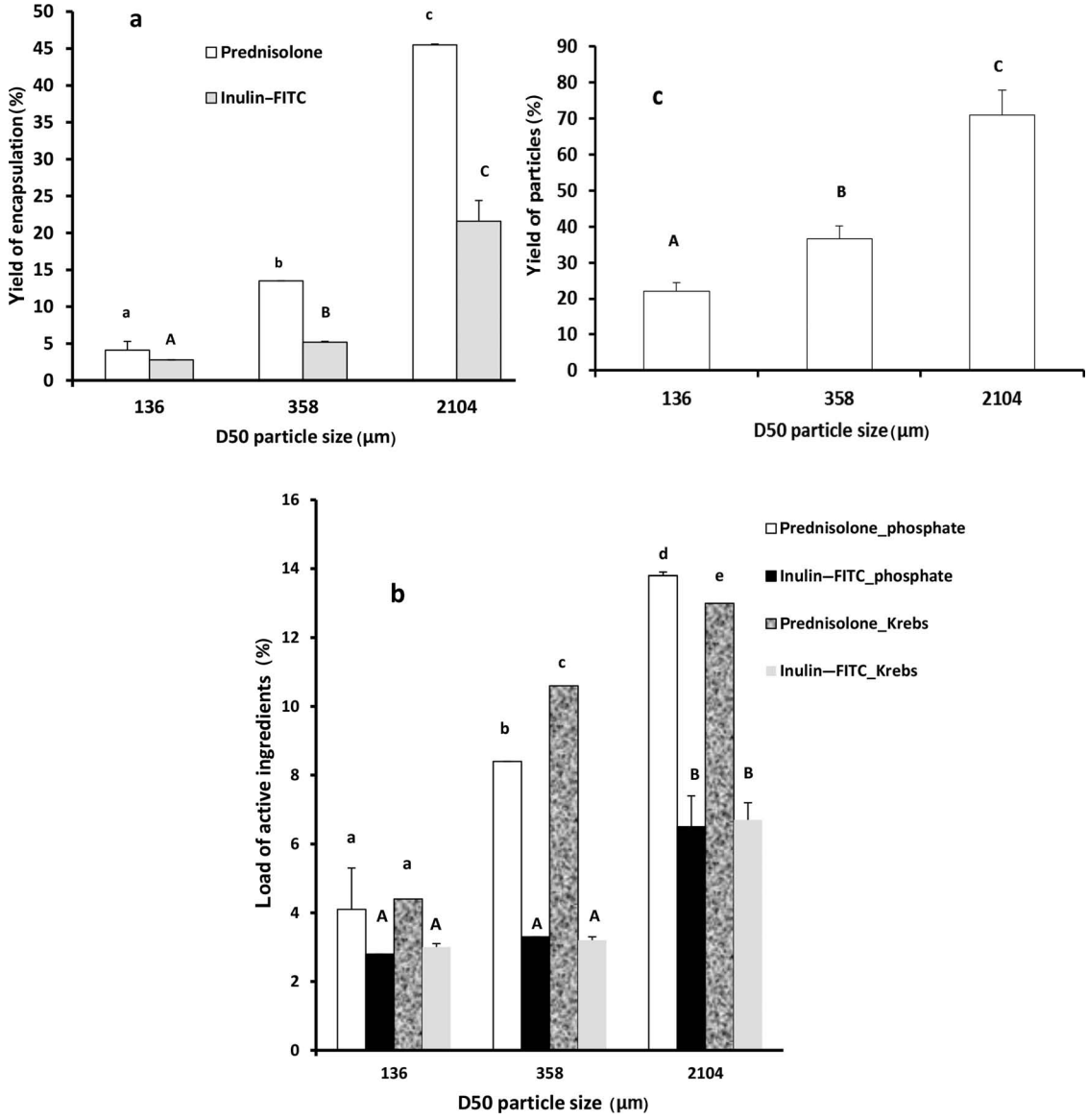

Figure 1. Yield of encapsulation and particles, and load of active ingredients (a) Yield of encapsulation (%). (b) Load of active ingredients (%). (c) Yield of particles against D_{50} particle size (μm).

A two-step method has been described for the preparation of alginate/chitosan microparticles containing prednisolone by complex coacervation using Ca alginate as the gel core, followed by coating with chitosan. For particles between 1 and 2 mm, drug content between 30% and 80% was obtained using concentrations of prednisolone between 2% and 15% (w/v) in alginate, which shows the versatility of the CACC matrix system in the encapsulation of prednisolone.[28] In preliminary tests with the micro-M particles (D_{50} = 358 μm, results not shown), 0.5% (w/v) prednisolone in alginate was encapsulated with a yield of over 10% in the CACC. Although inulin–FITC has a higher solubility than prednisolone, it was necessary to include 0.5% (w/v) Pluronic F-127, to obtain an encapsulation yield of approximately 5%. Pluronic F-127 is a well-known nonionic surfactant with high solubilizing capacity. At the concentration used in this work (0.5%, w/v), the molecules form multimolecular aggregates consisting of a hydrophobic central core with their hydrophilic polyoxyethylene chains facing the external medium.[29] Although the encapsulation of inulin—FITC in Ca alginate beads of approximately 0.7 mm in diameter without Pluronic F-127 has been described, there is no information about the YE and the load of the AIs in the beads.[14]

Characterization of Particles by MIR

Figure 2a shows MIR spectra for inulin–FITC, the beads, and microparticles M and S. Blank microparticles are characterized mainly by absorptions of

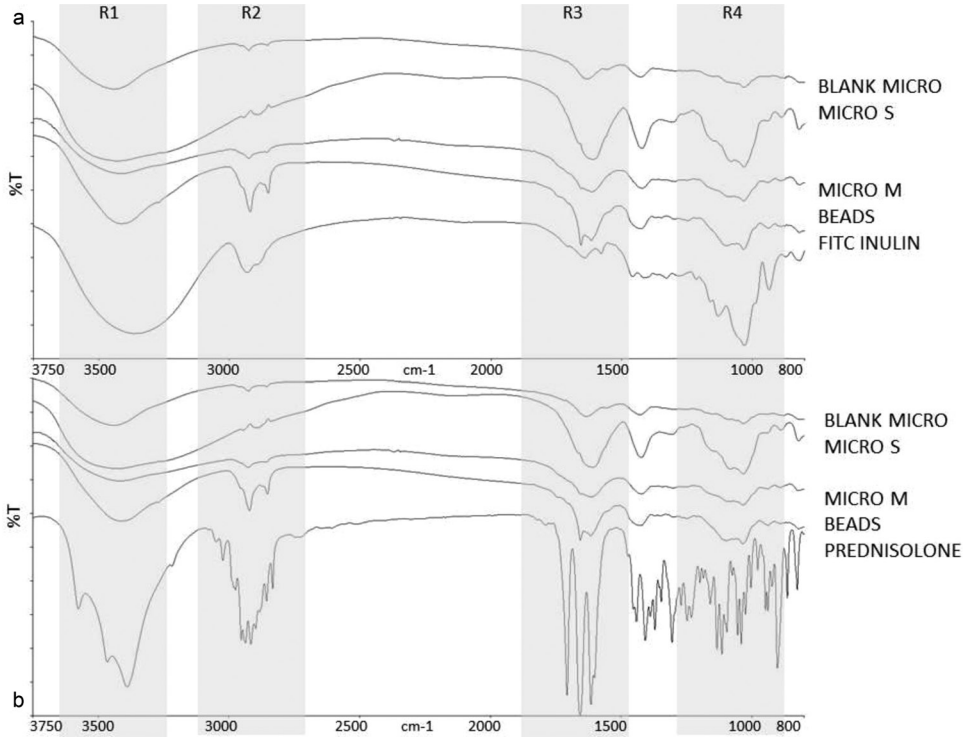

Figure 2. MIR spectra of inulin-FITC and prednisolone in the particles (a) MIR spectra of inulin–FITC, beads, and microparticles. (B) MIR spectra of prednisolone, beads, and microparticles.

alginate at 1636–1434 cm^{-1}, which are attributed to carboxylate symmetric stretching signals and water associated with the polymer. Chitosan displays a peak at 1555 cm^{-1}, which is attributed to N—H bending. Inulin–FITC exhibits characteristic absorptions at 1135 and 1033 cm^{-1} associated with the C—OH stretching of the alcohol moiety and asymmetric stretching of C—O—C, as well as a peak at 935 cm^{-1}, which is associated with a CH_2 out-of-plane deformation. These bands overlap with the absorbance of the saccharide residues of alginate and chitosan, increasing the signals observed in the blank microparticles. The main signal of inulin is because of a CH_2 out-of-plane deformation. In Figure 2b, the MIR spectrum of prednisolone showed characteristic bands at 1708, 1655, and 1613 cm^{-1}, associated with C=O-unconjugated ketone stretching at C-20, C=O-conjugated ketone stretching at C-3, and C=C-conjugated stretching, respectively. Of these bands, the band at 1655 cm^{-1} can be observed in the beads and, at lesser intensity, in the micro-M and micro-S. The absorptions are masked by other stronger signals from the components of the microparticles.

Drug Release Studies

Figure 3 shows the dissolution profiles of prednisolone, and Figure 4 shows the dissolution profiles of inulin in both phosphate and Krebs buffers at pH 7.4 for each particle size. A multifactorial ANOVA analysis was performed for each dissolution medium using the percentage of AI released as the response in relation to the AI, particle size, and time. The three main factors ($p < 0.0000$) and also the interactions AI × time ($p < 0.0000$), AI × particle size ($p < 0.0000$), and particle size × time ($p < 0.0000$) were significant. The formulations were compared using the mean fraction of AI released in the range of time used in dissolution tests. The results indicate that in phosphate buffer (pH 7.4), the mean fraction release of inulin (0.42 ± 0.01) was significantly higher than that of prednisolone (0.23 ± 0.01) ($p < 0.000$). The interaction of AI × particle size was evaluated in detail (see Fig. 5a). It was observed that for both AIs the smallest percentage released was from the larger particles (beads, 2104 μm). For the other particles, the percent of AIs released depends on the AI. In the case of prednisolone, there is no significant difference between the 136- and 358-μm particles. For inulin, release percentage from the 358-μm particles was higher compared with the 136-μm particles. The same analysis was applied to the dissolution data in Krebs buffer (pH 7.4). The mean fraction release of inulin (0.53 ± 0.01) was significantly greater than that of prednisolone (0.39 ± 0.01). There is a higher mean fraction release of prednisolone and inulin in Krebs buffer as compared with that in phosphate buffer. Additionally, there is a significant interaction between the AIs and size ($p < 0.000$). However, in this case

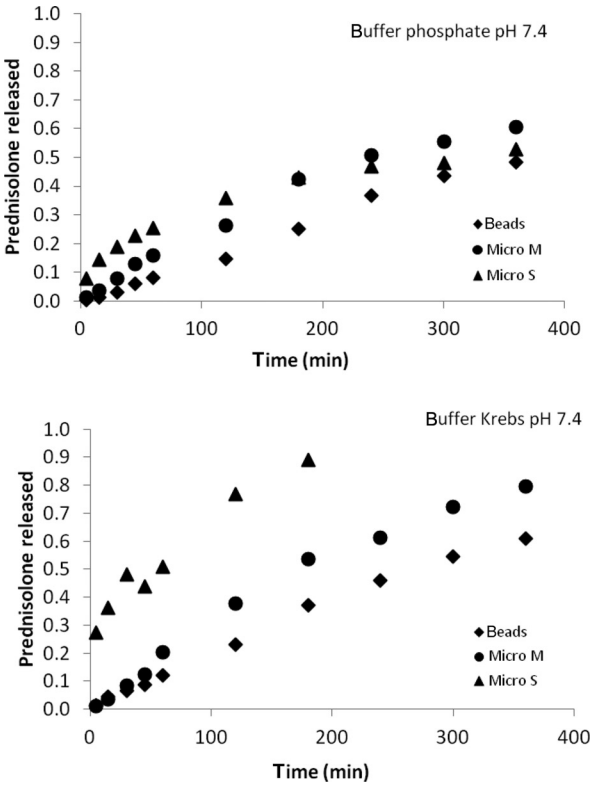

Figure 3. Dissolution profiles of prednisolone in buffer phosphate pH 7.4 and buffer Krebs pH 7.4.

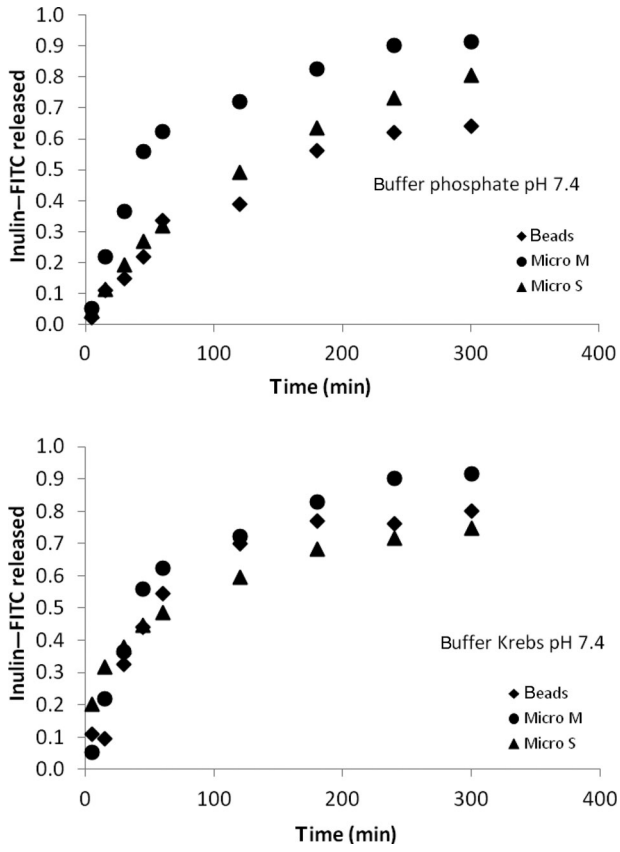

Figure 4. Dissolution profiles of inulin–FITC in buffer phosphate pH 7.4 and buffer Krebs pH 7.4.

(see Fig. 5b), the mean fraction release of inulin is independent of the particle size, but the mean fraction release of prednisolone decreases as particle size increases. Thus, a clear dependence of prednisolone release on the particle size in Krebs buffer (pH 7.4) is observed. In the case of using phosphate buffer (pH 7.4), this effect is only observed between the particles with large differences in particle size, the beads compared with micro-M and micro-S. It has been described that, irrespective of the type of drug, the relative release rate decreased with increasing system dimensions. This phenomenon was attributed to the decreased "surface area–volume" ratio with increasing microparticle size and to changes in the drug distribution patterns within the systems.[30] On the basis of the results, this effect of the decreased of surface area–volume ratio with increasing microparticle size is clear for the percentage of prednisolone released, particularly in Krebs buffer (pH 7.4), but not for inulin. In the case of inulin, it is observed (see Figs. 4 and 5) that the highest mean fraction release is for micro-M, which could be the explanation for the lowest CC in the particle compared with micro-S and beads (see Table 1).

Another important factor to consider is the polyelectrolyte behavior of the CCAS matrix, which includes the buffer capacity and ionic strength of the buffers. According to Fadda et al.,[31] Krebs buffer (pH 7.4) has a higher ionic strength (0.161) and lower buffer capacity (3.7 unstabilized and 5.45 stabilized mM/L per pH unit) compared with phosphate buffer (pH 7.4) (0.129 and 23 mM/L per pH unit, respectively). Thus, different ionic environments can influence the tortuosity and porosity of the matrix and, therefore, the drug diffusion. Additionally, physiological bicarbonate buffers have been shown to be more discriminative of the drug release for ileo-colonic delivery than compendial phosphate buffers and gave better reflections of *in vivo* disintegration times.[32]

Swelling/Erosion Studies

Figure 6 shows the swelling behavior in phosphate buffer (pH 7.4) (Fig. 6a) and Krebs buffer (pH 7.4) (Fig. 6b) for each particle size. Different swelling/erosion behaviors are observed in the two buffers. In phosphate buffer, the particles eroded quickly, whereas in Krebs buffer, the particles slowly swelled. The microstructure of the particles in the dry and swollen states in both buffers is shown in Figure 7. The SEM images clearly show the erosion effect of phosphate buffer over the particles, whereas the particles are swollen in Krebs buffer. The erosion effect of the particles of Ca alginate coated with chitosan observed in phosphate buffer can be explained by the

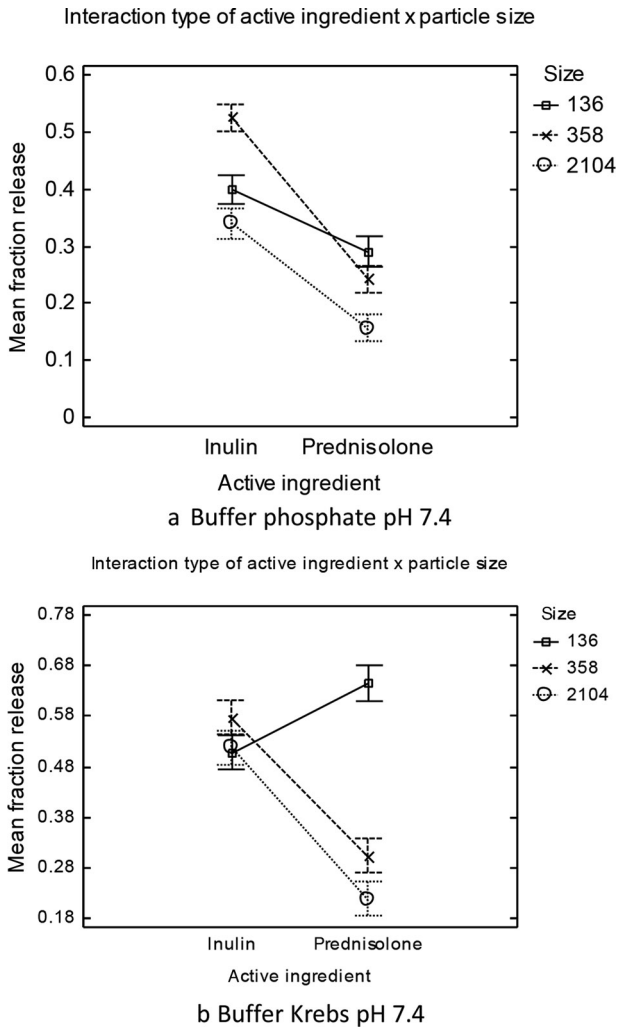

Figure 5. Interaction between AI and particle size in (a) buffer phosphate pH 7.4 and (b) buffer Krebs pH 7.4.

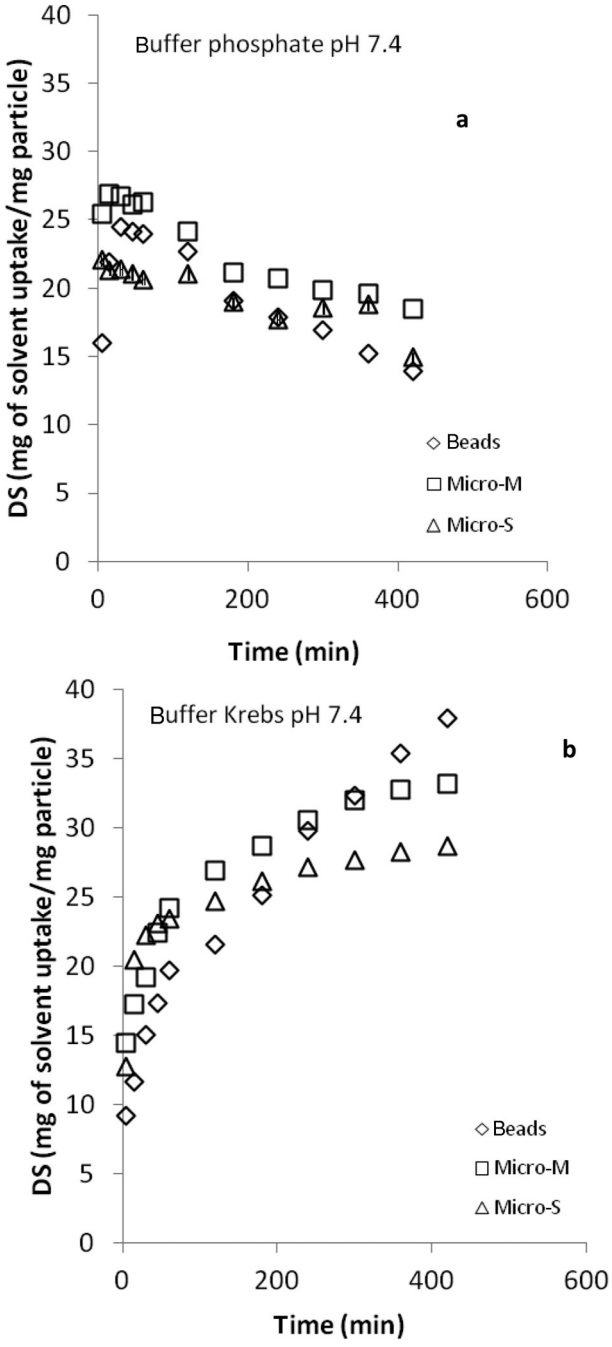

Figure 6. Degree of swelling (DS) against time in buffer phosphate pH 7.4 (a) and buffer Krebs pH 7.4 (b).

exchange of sodium from the phosphate buffer with calcium in the egg box structure of the Ca alginate core, thus leading to the formation of calcium phosphate and a weakening of the Ca-alginate core, which triggers the erosion process.[33,34] Thus, a similar process should occur between potassium from the phosphate buffer with the calcium in the egg box structure of the Ca-alginate core.

Because the CACC matrix formulations displayed an erosion process caused by the interaction between the phosphate buffer and Ca-alginate core, the swelling mechanism was only evaluated in Krebs buffer (pH 7.4). The swelling data in Krebs buffer (pH 7.4) were fitted to the potential equation $W = K_p \times t^n$ using nonlinear regression to estimate the kinetic constant of water penetration (K_p) in relation to the particle size.[14] The beads and micro-M showed a good fit to this model (R^2 adjusted to d.f. of 93.9% and 83%, respectively). The K_p of the beads (5.0 ± 0.5) was significantly lower compared with K_p of micro-M (10.3 ± 0.9).

To estimate the diffusion of prednisolone and inulin inside the Ca-alginate hydrogel, the obstruction-scaling model proposed by Amsden[18] was applied because this model has been applied to the diffusion of solutes within Ca-alginate hydrogels. The model was applied based on the kinetic data of swelling/erosion, according to the methodology described in the *Materials and Methods* section. Figure 8 shows the variation over time of the ratio D_g/D_o in Krebs buffer (pH 7.4).

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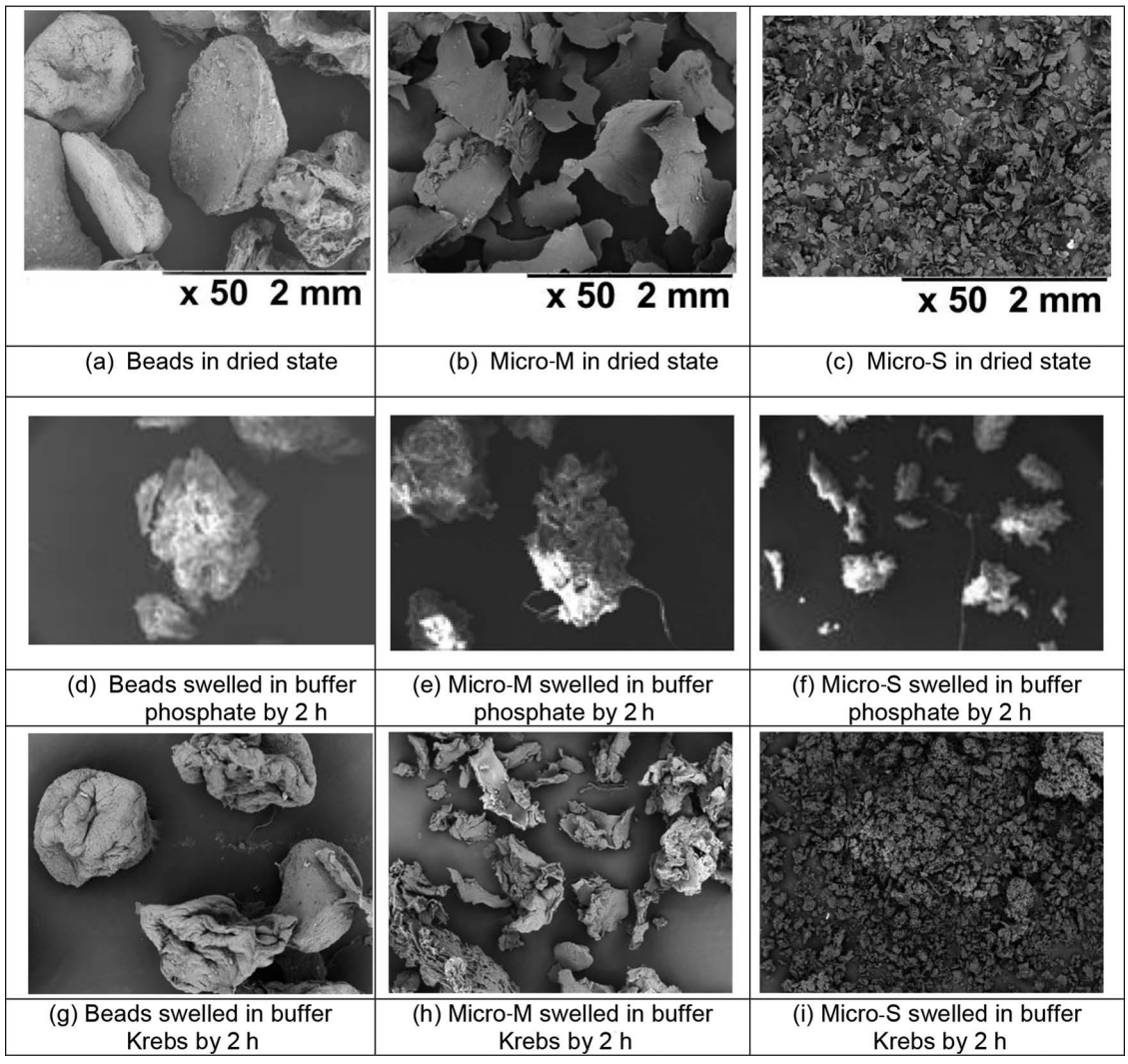

Figure 7. Microstructure measured by SEM, for beads. Micro-M and micro-S in (a–c) dried state, (d–f) swelled by 2 h in buffer phosphate pH 7.4, and (g–i) swelled by 2 h in buffer Krebs pH 7.4.

It is clear that for prednisolone, there is a low obstruction to the diffusion within the Ca-alginate hydrogel because the hydrodynamic radius of prednisolone (r_s) is close to the value of the monomer length (a). Therefore, intragel diffusion (D_g) is similar to the solvent diffusion (D_o). In contrast, with inulin, there is a clear effect of obstruction within the Ca-alginate hydrogel; thus, the ratio D_g/Do for inulin is significantly lower compared with prednisolone.

Microbiological Assay

The degradation of the CACC matrix formulations by bacteria and the subsequent release of prednisolone were studied using *E. coli* as a model microorganism. *E. coli* is an enterobacteria that is highly incident (~100%) in the autochthonous microbiota of the human colon; in addition, it is an aerobic, easily

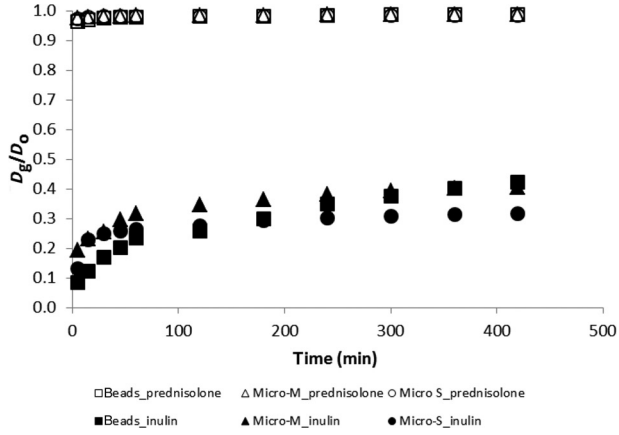

Figure 8. D_g (intra-Ca-alginate diffusivity)/D_o(diffusivity in the aqueous medium) ratio for prednisolone and inulin—FITC, against time. D_g–D_o ratio was estimated from the swelling data obtained in buffer Krebs pH 7.4.

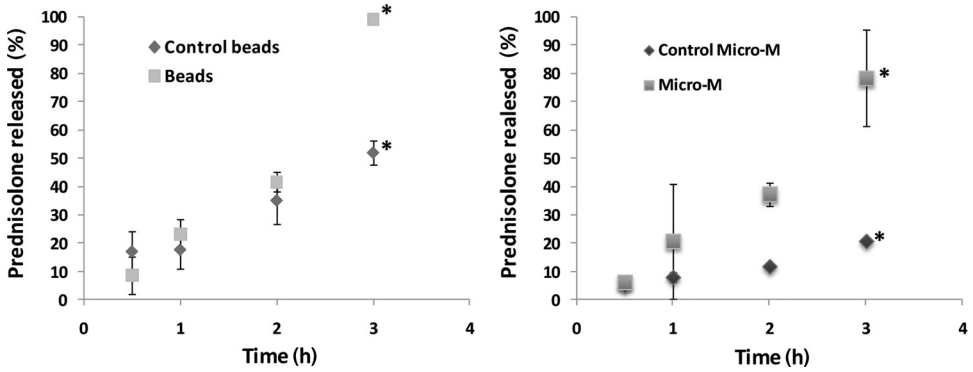

Figure 9. Prednisolone released (%) from beads and micro-M, during fermentation by *E. coli*.

cultivable microorganism. Figure 9 shows the prednisolone release profiles from beads and micro-M in cultures of *E. coli* and controls (sterile medium). It is clear that the matrices for both particle sizes (beads and micro-M) are degraded by *E. coli* because of the higher percentage of prednisolone released at 3 h of the assay compared with the control.

CONCLUSIONS

The appropriate buffer system to evaluate *in vitro* drug-dissolution behavior from a CCAS matrix intended for ileo-colonic drug delivery is Krebs buffer (pH 7.4), as compared with phosphate buffer, because there is a lower interaction with the Ca-alginate core and it is also a more biorelevant buffer compared with phosphate buffer. The release of prednisolone from CACC matrix system is dependent on the particle size, whereas the release of inulin is controlled by intragel diffusion of the Ca-alginate core. Prednisolone release is activated by *E. coli*, which indicates that this new platform has the potential to deliver drugs to the colon.

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