

Electrospray-assisted encapsulation of caffeine in alginate microhydrogels

Alireza Mehregan Nikoo^a, Rassoul Kadkhodaei^{b,*}, Behrouz Ghorani^b, Hussam Razzaq^c, Nick Tucker^d

^a Department of Food Chemistry, Research Institute of Food Science and Technology (RIFST), PO Box 91895/157/356, Mashhad, Iran

^b Department of Food Nanotechnology, Research Institute of Food Science and Technology (RIFST), PO Box 91895/157/356, Mashhad, Iran

^c Scientist in Department of food innovation, The New Zealand Institute for Plant and Food Research, New Zealand

^d School of Engineering, Brayford Pool, Lincoln LN6 7TS, United Kingdom

ARTICLE INFO

Article history:

Received 15 February 2018

Received in revised form 27 March 2018

Accepted 29 April 2018

Available online 2 May 2018

Keywords:

Electrospray

Microhydrogel

Electrostatic interactions

Caffeine delivery

Encapsulation

ABSTRACT

One of the major challenges with microencapsulation and delivery of low molecular weight bioactive compounds is their diffusional loss during storage and process conditions as well as under gastric conditions. In an attempt to slow down the release rate of core material, electrospray fabricated calcium alginate microhydrogels were coated with low molecular weight and high molecular weight chitosans. Caffeine as a hydrophilic model compound was used due to its several advantages on human behavior especially increasing consciousness. Mathematical modeling of the caffeine release by fitting the data with Korsmeyer–Peppas model showed that Fick's diffusion law could be the prevalent mechanism of the release. Electrostatic interaction between alginate and chitosan (particularly in the presence of 1% low molecular weight chitosan) provided an effective barrier against caffeine release and significantly reduced swelling of particles compared to control samples. The results of this study demonstrated that calcium alginate microhydrogels coated by chitosan could be used for encapsulation of low molecular compounds. However, more complementary research must be done in this field. In addition, electrospray, by producing monodisperse particles, would be as an alternative method for fabrication of microparticles based on natural polymers.

© 2018 Published by Elsevier B.V.

1. Introduction

Alginate is an anionic natural polysaccharide mainly extracted from brown seaweeds. It is often the first choice for food, pharmaceutical and biomedical applications owing to its biocompatibility and low toxicity [1]. Considering the facile sol-gel transition of alginate, it is by far one of the most extensively used biopolymers for microencapsulation of functional components such as vitamins and probiotics, immobilization and tissue engineering [2,3]. Alginate gelation occurs through binding of calcium ions to the carboxylic groups existing in its polymer chain resulting in egg box structures [4]. Direct mixing of alginate and Ca^{2+} ions solutions does not produce a homogenous gel due to the very rapid and irreversible formation of interchain junctions. The only exception is when alginate solution is mixed with small amounts of crosslinking ions at high shear rates [5]. To control the gel structure two different methods have been proposed: diffusion and internal setting. In the diffusion method, the crosslinking ions (Ca^{2+}) diffuse from an outer reservoir into the alginate solution, leading to the formation of more ordered gel structure. In the internal setting method, Ca^{2+} ions are released in a controlled manner from an inert calcium source

in response to changes in physicochemical conditions of the surrounding environment, for instance the release of Ca^{2+} from CaCO_3 by decreasing the pH [6].

In general, the component to be encapsulated is mixed with alginate solution. Then, the mixture is injected into a solution containing Ca^{2+} ions. Despite the simplicity and quickness of such encapsulating system, the surface tension of the polymeric solution is a limiting factor for the production of small homogeneous droplets with low polydispersity index (PDI) which should be overcome by carefully adjusting the process conditions.

Electrospraying is a method of atomizing a liquid or polymeric solution by applying electrostatic forces stronger than its surface tension [7,8]. In this process a liquid droplet at the tip of a capillary nozzle once exposed to a high electric field, undergoes a shape deformation to a cone, due to internal electrostatic repulsions and external attractive Coulombic forces, from which a jet is emitted that subsequently breaks up into fine droplets [7] as a result of varicose instability [9].

Calcium alginate hydrogel owing to its interconnected open pore network does not effectively retain small molecular size compounds and thus often leads to an initial burst release in aqueous environments [10–13]. Therefore, for controlled delivery purposes it is necessary to retard the diffusion rate of such compounds by coating the surface of hydrogel particles or reinforce their structure with a secondary polymer.

* Corresponding author.

E-mail address: r.kadkhodaei@rifst.ac.ir (R. Kadkhodaei).

Chitosan is a natural polysaccharide which is composed of glucosamine and *N*-acetylglucosamine units displaying remarkable polycation capabilities at a pH lower than 4.0 [14]. This characteristic of chitosan ensures its strong interaction with the alginate of negative charge [15]. It has been demonstrated that the zeta potential of calcium alginate particles is negative in weak acidic and neutral pHs [16]. Therefore, once calcium alginate microhydrogels are introduced into a chitosan solution, a membrane is created around the particles as a result of the electrostatic interactions between alginate carboxylic groups and chitosan amine groups [17]. However, the efficiency of coating as well as the crosslink density greatly varies with chitosan molecular weight. This is assumed to considerably affect the network strength and thus the ability of particles to retain the encapsulated compound within the gel matrix. Alginate-chitosan micro/nanoparticles have been employed for the encapsulation and controlled release of a number of compounds such as ampicillin [18], sodium diclofenac [19], triamcinolone [4], genipin [20] 5-aminosalicylic acid [21], brilliant blue [17], 5-fluorouracil and tegafur [10], as well as high molecular weight components like bovine serum albumin [22], glucose oxidase [23] and enoxaparin [24].

Caffeine is the most widely consumed psychoactive or central nervous system (CNS) stimulant in the world [25]. Once ingested it is rapidly absorbed into the blood stream and immediately affects CNS leading to increased alertness, reduced drowsiness and boosted energy level. Owing to these beneficial effects caffeine has received an increasing attention by the food and pharmaceutical industries. However, its bitter taste and the gastrointestinal problems it may cause in sensitive individuals are the main challenges for the direct use of this bioactive compound. Also, the body is not able to store it and thus is quickly excreted through renal system [26,27].

The present study was designed to entrap caffeine as a model bioactive ingredient of small molecular size in electrosprayed calcium alginate microparticles. To block the diffusion paths of caffeine and reinforce the hydrogel structure against swelling the particles were coated by chitosan of low (CHI) and high (CHh) molecular weight. The capability of the developed microcarriers for controlled delivery applications were assessed by investigating their resistance to gastrointestinal conditions and the release rate of caffeine.

2. Materials and methods

2.1. Materials

Food grade high quality commercial sodium alginate (MW ~ 120 k Da, guluronic to mannuronic ratio of 1.7:1) was kindly gifted by Fibrisol (Ladenburg, Germany). CHI [viscosity average molecular weight MW_v ~ 50–190 kDa, degree of deacetylation (DD): 75–85%], CHh (MW_v ~ 310–375 kDa, DD < 75%), caffeine (MW = 194.19 g/mol) and calcium chloride were purchased from Sigma Aldrich (Steinheim am Albuch, Germany). Glacial acetic acid, hydrochloric acid, sodium phosphate monobasic dehydrate and sodium phosphate dibasic heptahydrate were supplied by Merck (Darmstadt, Germany).

2.2. Preparation of solutions

Feed solution (3% w/w) was prepared by progressively dispersing appropriate amount of dry sodium alginate powder to distilled water at 40 °C. Then, caffeine was added to it to make a final concentration of 1% (w/w). The solution was kept in refrigerator overnight for complete hydration of polysaccharide.

Collector solution containing 2% (w/v) CaCl₂ was prepared in distilled water. In order to minimize the concentration gradient of caffeine across the alginate particles during gelation 1% caffeine was added to the collector solution.

Coating solutions of 0.5 and 1% (w/w) CHI and CHh were prepared by dispersing appropriate amount of each chitosan fraction in 1.5% (v/v)

acetic acid solution containing 2% CaCl₂ (w/v) and 1% caffeine (w/w) followed by holding at 4 °C overnight for full hydration.

2.3. Preparation of hydrogel particles

Alginate microhydrogels were prepared by electrospraying of the feed solution into the collector solution in dripping mode using an electrospinning machine (Electrospin Ltd., New Zealand) under the optimum operational conditions achieved in our previous study [28]. They were as follows: operating voltage: 8 kV, inner diameter of the nozzle: 500 μm, distance between the nozzle and the collecting bath (D): 8 cm and header tank height (H): 20 cm. Fig. 1 illustrates the schematic diagram of the experimental setup used for electrospraying of alginate solution. To ensure the completion of gelation process the particles were kept immersed in the collector solution for 20 min and then separated using an 80 mesh laboratory sieve (180 μm). Larger particles were prepared in the gravity-induced dripping mode at the same operational conditions without applying any voltages. In order to reinforce the hydrogel particles they were transferred to chitosan solution and gently stirred at 100 rpm overnight [29].

2.4. Particle size analysis and microscopic observations

The size and morphology of alginate microhydrogels were examined using a light microscope (Leica DM 5000-D, Leica Microsystems Inc., USA) and image analysis was performed via Leica application suite software, version 4.0.0 (Leica Microsystems Inc., USA). The microstructure of gel particles was observed by a scanning electron microscope (JEOL NeoScope JCM-5000 bench top SEM, USA). Samples were freeze dried (Labconco, FreeZone 12 L, UK) at –55 °C and 0.1 mBar for 48 h, coated with a gold layer by a sputter coater (Q150R Rotary-Pumped Sputter Coater, Quorum Technologies Ltd., UK) under an argon atmosphere and their surface image, was captured at an accelerating voltage of 10–15 kV under high vacuum.

A Malvern laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK) was used to determine the size distribution of specimens. Drops of micro-hydrogels dispersed in distilled water were progressively added into the sample holding tank of the

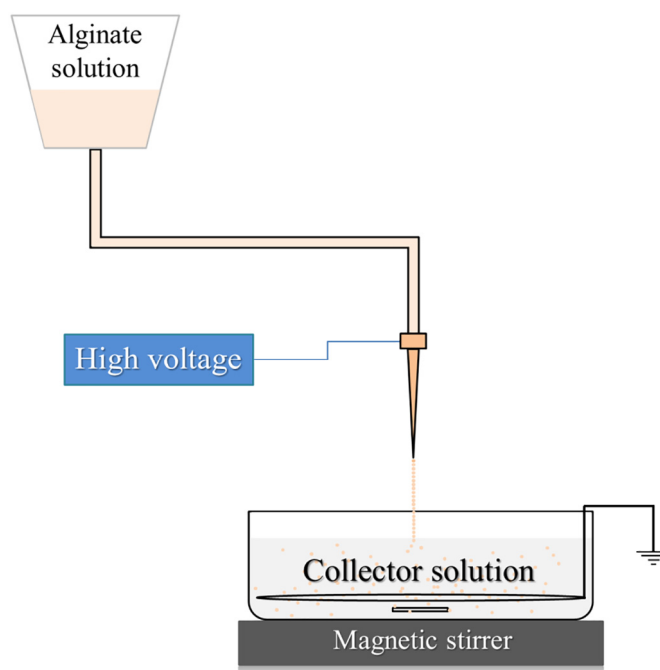


Fig. 1. Schematic of the experimental setup for electrospray assisted fabrication of calcium alginate particles in dripping mode.

instrument until the desired obscuration level of the laser light was obtained as instructed by the manufacturer. This was found to be at about 0.4 wt%. To homogeneously disperse the particles, the speed of agitator was set at 500 rpm. A refractive index of 1.53 was taken for alginate hydrogel. The volume mean diameter, $D[4,3]$, of particles was calculated by the following equation.

$$\overline{D}[4,3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

Where n_i is the number and d_i is the diameter of particles.

2.5. Determination of encapsulation efficiency (EE)

To determine the caffeine content of particles they were mixed with 2% (w/v) sodium citrate solution followed by being vigorously shaken in a shaking incubator (Hanyang Scientific Equipment Co., Ltd. Korea) for 1 h at 37 °C. This led to the dissolution of alginate microhydrogels and complete release of caffeine which was spectrophotometrically (Cromtech, CT-5700, India) measured at 273 nm. The caffeine content of particles was estimated in mg caffeine/l using the calibration curve (Absorbance = $0.0474 \times \text{concentration} + 9 \times 10^{-5}$, $R^2 = 0.99$) and the EE was calculated by Eq. (2).

$$EE (\%) = \frac{\text{Mass of loaded caffeine}}{\text{Mass of initial caffeine used}} \times 100$$

2.6. Fourier transform infrared spectroscopy (FTIR)

The IR spectra of freeze-dried calcium alginate microparticles were obtained using an FT-IR spectrometer (Bruker Alpha FTIR, US). Samples were powdered by a mortar and pestle, mixed with KBr at a ratio of 1:100 and pressed into pellets. IR spectra were recorded in the wave number range 4000–400 cm^{-1} with a resolution of 1 cm^{-1} .

2.7. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (TA Instruments, Q2000, Wilmington, USA) was used to investigate the thermal behavior of samples. Freeze-dried particles (2.00 ± 0.10 mg) were sealed in a standard aluminum pan (resistant to high pressures) after weighing on an analytical balance (Sartorius®, CP225D, Germany) and heated under a stream of nitrogen (20 ml min^{-1}) from 20 °C to 300 °C at a heating rate of 10 °C/min.

2.8. Measurement of particles swelling

The swelling property of freeze-dried calcium alginate particles was studied by stirring their suspension in hydrochloric acid (pH = 1.2) and phosphate buffer (pH = 7.4) solutions at 100 rpm and 37 °C in a shaking incubator (Hanyang Scientific Equipment Co., Ltd. Korea) for 2 and 6 h, respectively. The swelling ratio (%) was calculated by the following Equation:

$$\text{Swelling} (\%) = \frac{(D_{\text{Current}} - D_{\text{Initial}})}{D_{\text{Initial}}} \times 100$$

Where D_{current} and D_{initial} are the average diameters of 10 particles measured through image analysis at hourly intervals and the onset of experiment, respectively [30].

2.9. Determination of in vitro caffeine release

The release of caffeine from particles was studied by placing about 2 mg of freeze-dried sample in 100 ml agitated (100 rpm) hydrochloric

acid (pH = 1.2) and phosphate buffer (pH = 7.4) solutions in a shaking incubator at 37 °C for 2 and 6 h, respectively. An aliquot of 3 ml was taken at predetermined time intervals; its absorbance was recorded at 273 nm against distilled water as blank and immediately returned to the release medium. The percent release of caffeine was calculated by dividing the amount released at time t by the total amount existing in particles at $t_0 = 0$.

2.10. Studying the kinetics of caffeine release

To describe the caffeine release profile from particles, the release data were fitted to first order and Korsmeyer-Peppas models. The validity of models was judged according to their R^2 , RMSE and χ^2 statistics.

In the first order model, there is a straight relationship between the compound concentration and its release [31]. The equation can be written in decimal logarithm as:

$$\log Q_t = \log Q_0 + \frac{K_1 t}{2.303}$$

Where Q_t is the amount of drug released at time t , Q_0 is the amount of drug in the solution and K_1 is the first order release constant.

Korsmeyer-Peppas model is based on the Fick's Law. It is used to describe the release profile of a compound from a polymeric system when the dominant mechanism is a combination of Fickian-diffusion- and non-Fickian transport, which is controlled by the relaxation of polymer chains. Korsmeyer-Peppas is a suitable model to predict the release mechanism in the first 10 h being described by the following equation [32]:

$$\frac{M_t}{M_\infty} = K t^n$$

Where, M_t/M_∞ is the fraction of compound released at time t (M_t and M_∞ are the amount of compound released at time t and the total amount initially present in the particles, respectively). K is the release rate constant and n is the release exponent. The numerical value of n identifies the release mechanism. For spherical shaped particles, $n \leq 0.43$ corresponds to Fickian diffusion mechanism, partially through a swollen matrix and water filled pores, $0.43 < n < 0.85$ to non-Fickian transport and $n \geq 0.85$ to case-II (translational) transport [33].

2.11. Statistical analysis

One-way ANOVA was performed for the analysis of the experimental data based on completely randomized design ($\alpha < 0.05$). Duncan's multiple range test was used to compare the means at a significance level of 0.05 using SPSS version 16.0.

3. Results and discussion

3.1. Particles size and morphology

The size of microhydrogel particles prepared by electrospray process was much smaller than those obtained by gravity-induced dripping method. As can be seen in Fig. 2 the mean volume diameter, $D[4,3]$ of electrosprayed particles was estimated to be $765.29 \pm 14.53 \mu\text{m}$ with a PDI of 0.209, while for gravity-induced particles (without applying voltage) it was determined to be $2740 \pm 115 \mu\text{m}$. The size distribution curve in Fig. 2 is monomodal and almost sharp and narrow indicating that the electrosprayed calcium alginate microhydrogels were nearly monodisperse and reasonably uniform. This confirms the ability of process for producing homogeneous fine particles which are of importance for reproducible release purposes [34]. Scanning electron micrographs showed that a rough membrane formed on the surface of microspheres (Fig. 3) due to attractive electrostatic forces between negatively charged

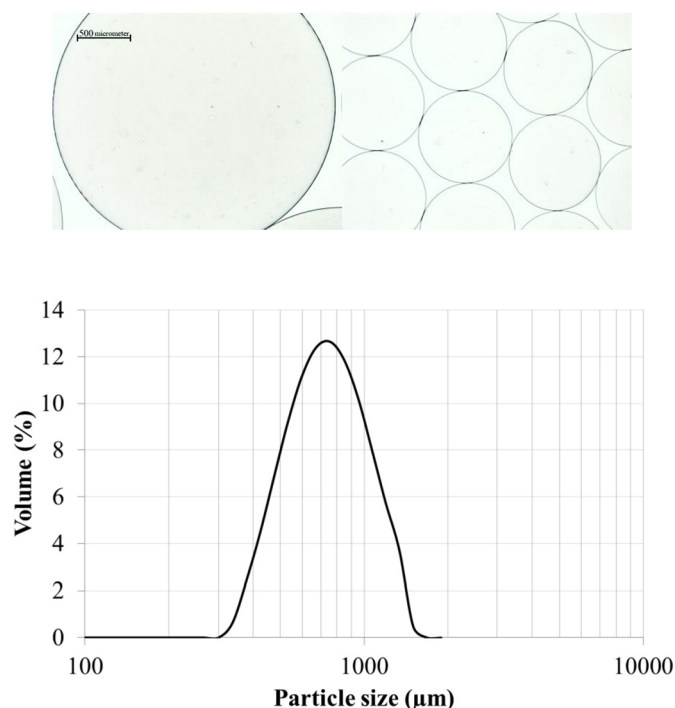


Fig. 2. Top: Calcium alginate hydrogels a) gravity-induced, b) electrospayed. Bottom: Size distribution of electrospayed calcium alginate microhydrogels.

carboxylic groups of alginate and positively charged amino groups of chitosan. Increasing the concentration of chitosan led to a thicker and denser membrane with a prominent surface roughness. This can be related to the enhanced bonding of chitosan molecules owing to the availability of more positively charged groups that somehow dominates the steric effects. It is also obvious from the SEM images in Fig. 3 that the particles coated with CHh had a rougher surface with distinct fibrous morphology indicating that chitosan molecules because of their large

size did not diffuse into the alginate network and thus mostly adhered to the surface. On the other hand, CHI due to having shorter chain length and less acetyl groups and hence smaller size probably diffused into the core and left the particles surface smoother but slightly granular because of the typical pattern of bonding in which loop-like regions incorporates uncoupled units of the two polyelectrolytes.

3.2. Encapsulation efficiency

For uncoated calcium alginate particles EE was measured to be 37.51% which means that a considerable amount of caffeine leached out during gelation and hardening due to the porous matrix of the gel network. Chitosan coating significantly improved the entrapment of caffeine in alginate microspheres. It was shown that 0.5% CHh and CHI increased EE to 42.06 and 59.81%, respectively. Raising the concentration of chitosan to 1% increased this parameter further leading to 46.19 and 64.28% in the case of CHh and CHI, respectively. These results clearly demonstrate that the electrostatic interactions between alginate carboxylate groups and the protonated amine groups of chitosan considerably changes the gel microstructure and blocked the diffusion paths of caffeine [35,36]. The lower EE of CHh-coated particles can be related to the inability of this fraction to diffuse deeply into the gel core and mainly remained at the surface. In contrary, CHI owing to its smaller molecular size and less steric effect moved into the depth of the gel network and enhanced the retention of caffeine by creating a thick and dense membrane as seen in SEM micrographs (Fig. 3). It seems that increasing the concentration of chitosan especially CHI resulted in the diffusion of a higher number of molecules into the alginate microhydrogels; in other words the crosslink density and thus the thickness and strength of chitosan membrane greatly increased at higher concentration.

3.3. FTIR

Fig. 4 shows the FTIR spectra of alginate, chitosan, caffeine and chitosan coated particles containing caffeine. As can be seen there two distinct bands at 1600 and 1405 cm^{-1} in FTIR spectrum of sodium

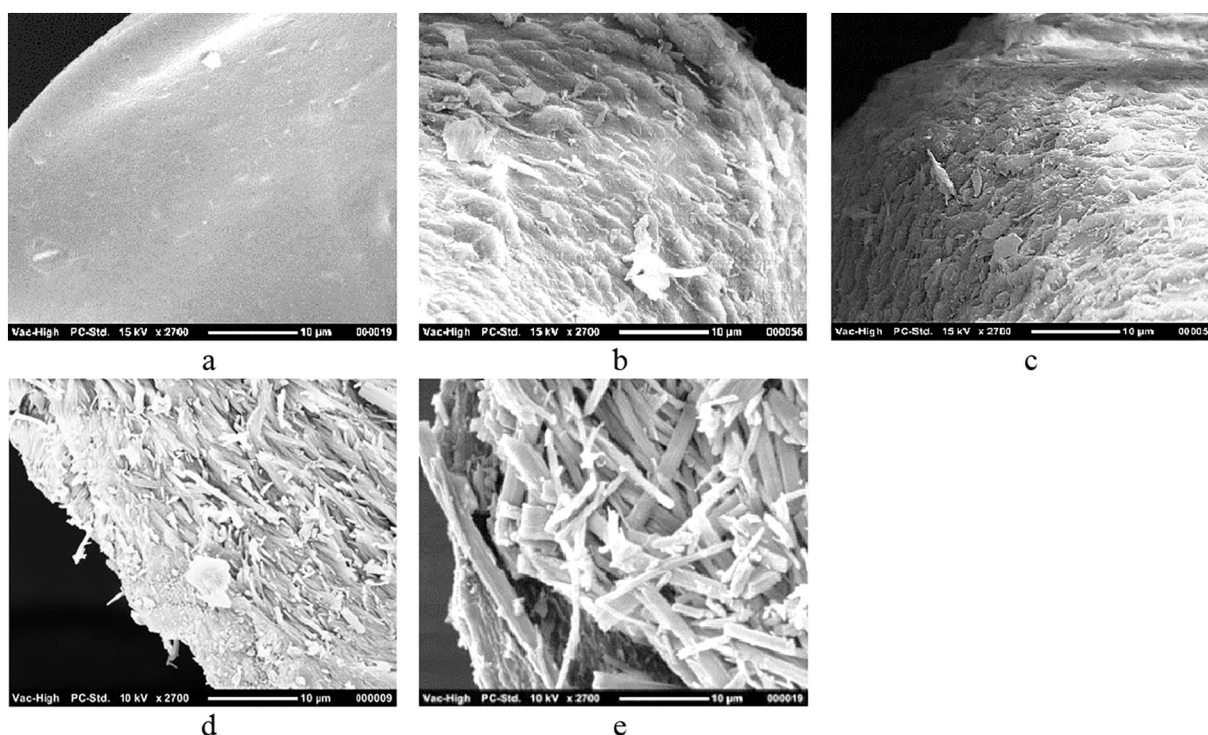


Fig. 3. Scanning electronic micrographs of calcium alginate microparticles a) uncoated b) coated with CHI 0.5% c) coated with CHI 1% d) coated with CHh 0.5% e) coated with CHh 1%.

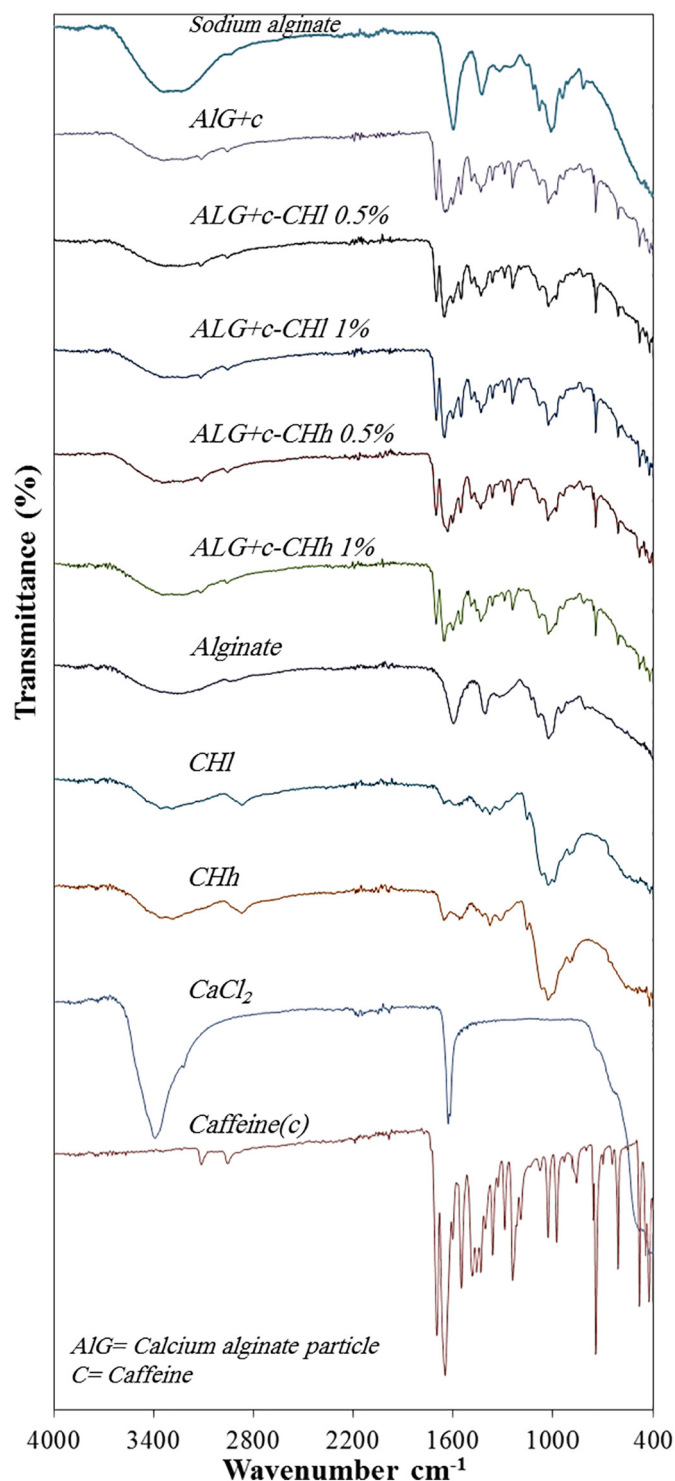


Fig. 4. FTIR spectra of sodium alginate, chitosan, CaCl_2 , caffeine and different formulations of encapsulated calcium alginate particles.

alginate which are assigned to asymmetric and symmetric stretching vibration of carboxylate groups, respectively. They were shifted to 1596 and 1429 cm^{-1} upon interaction with calcium chloride indicating the replacement of Na^+ with Ca^{+2} . This band displacement caused the separation between asymmetric and symmetric stretching vibrations to decrease from 189 cm^{-1} to 169 cm^{-1} after spraying sodium alginate into calcium chloride solution that can be interpreted as ionic interaction between Ca^{+2} and carboxyl groups of alginate that act as chelating or bridging ligands. It should be noted that the degree of shift in the

position of bands may differ depending on the ratio of M/G as well as the length of G blocks. The characteristic bands of caffeine at 1696 and 1647 cm^{-1} (amide I), 1547 cm^{-1} (amide II) and 3119 cm^{-1} (C—H stretching in heterocyclic compounds) were noticeable in FTIR spectra of chitosan coated alginate particles but shifted to 1700 , 1653 , 1555 and 3130 cm^{-1} , respectively, denoting to the physical entrapment of this compound in biopolymer network. The typical bands of chitosan at 1657 (amide I) and 1564 cm^{-1} (amide II and bending vibration of N—H) are also distinguishable in the infrared spectrum of the particles, although they showed some shifts in their positions owing to the interactions with alginate and also the overlap resulted from caffeine contribution. The shoulders at 1539 and 1637 cm^{-1} can be assigned to partial protonation of chitosan (vibration of $-\text{NH}_3^+$) which confirm presence of this polymer in the particles. The broaden band in the region $3200\text{--}3500\text{ cm}^{-1}$ is due to the stretching vibration of N—H and —OH of chitosan that overlaps with that of hydroxyl groups in alginate [29,37,38].

3.4. DSC

DSC can be used to characterize the thermal behavior of polymers and correlate it to their structure, hydrophilic properties and association states. Shifts of exothermic and endothermic peaks are usually associated with interactions between drugs and polymers [39]. The DSC thermogram of caffeine showed an endothermic peak at 235.56°C (Fig. 5) which corresponds to the melting point of this compound. It also appeared in the thermograms of pure and chitosan coated alginate particles which implies caffeine entrapment in the gel network. As can be seen this peak is much more intense for the particles coated with CHI that can be attributed to their higher caffeine content. The early endothermic peaks in the thermograms of individual polymers or particles which fall in the range $50\text{--}90^\circ\text{C}$ are associated with the evaporation of the absorbed water. This is related to the hydrophilic nature of alginate and chitosan [40,41]. The first exothermic peak at 216°C in the thermogram of sodium alginate may be attributed to partial crystallization of this polymer following dehydration [42]. The second exothermic peak that revealed at 251°C is probably due to the degradation (partial decarboxylation) and oxidation of this polymer. The exothermic peak at 281°C in chitosan thermogram also indicates the onset of thermal decomposition [43]. The thermograms of particles, whether coated or uncoated, show that the first exothermic peak was shifted, the second one disappeared and a new endothermic peak appeared at 242°C compared to the thermograms of alginate and chitosan. These changes especially the appearance of the new endothermic peak can be attributed to the formation of ionic pair between $-\text{COO}^-$ in alginate and $-\text{NH}_3^+$ in chitosan. It should also be noted that the shift of the second exothermic peak to higher temperatures in calcium alginate or chitosan coated particles demonstrate that their thermal stability was improved [44].

3.5. Swelling of particles

Almost no water uptake and hence insignificant swelling was observed when particles were incubated in hydrochloric acid solution ($\text{pH} = 1.2$). This is attributed to ion exchange phenomenon in which the interchain calcium ions are replaced by H^+ resulting in the formation of insoluble alginic acid that may limit water penetration into the particles [30,45]. There are, however, contradictory reports on the swelling behaviour of calcium alginate microhydrogels in acidic pH. Some researchers have observed a partial shrinkage for particles in these conditions [46]. Ouwerx et al. [47] explained that at pH values lower than 4 carboxyl groups of alginate become protonated and the network may undergo a volume shrinkage as a result of decreased electrostatic repulsions between undissociated groups. In contrast, others have demonstrated partial swelling of particles when placed in acidic media. Lucinda-Silva et al. (2010) calculated a maximum swelling rate of 12% for the particles prepared with 1.5% calcium chloride. Apart

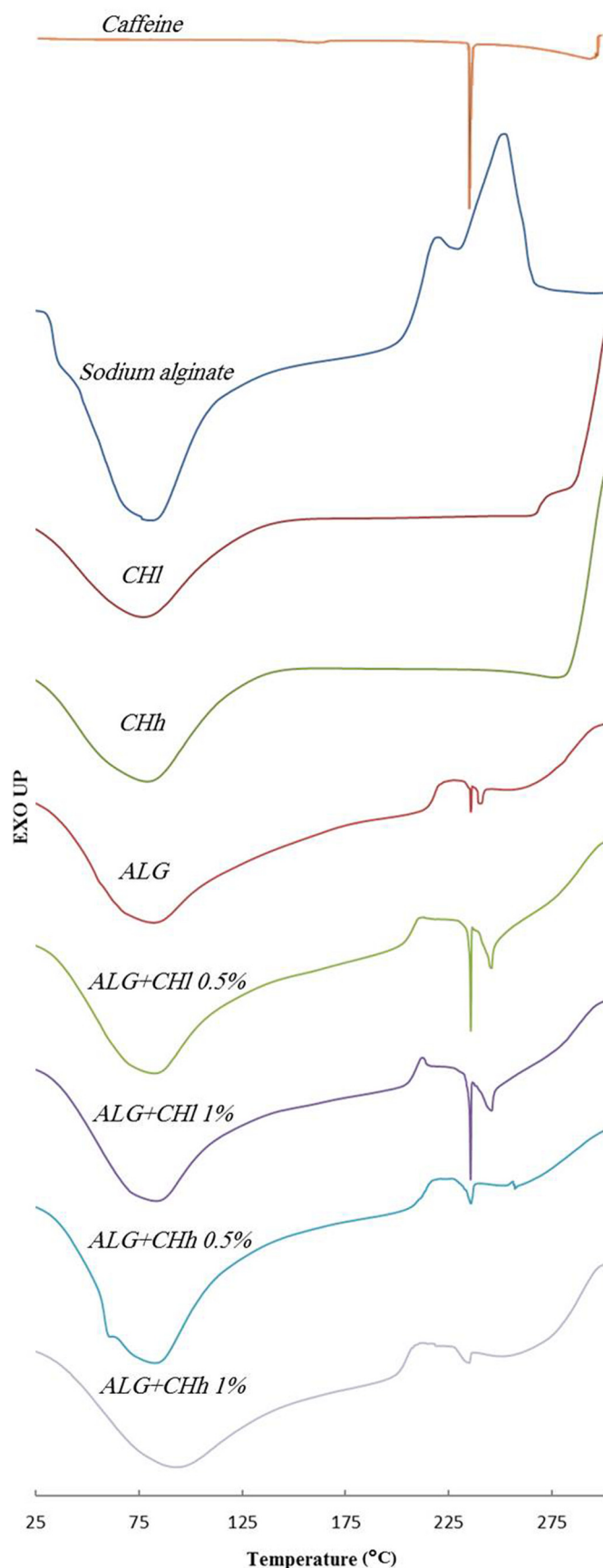


Fig. 5. DSC thermograms of caffeine, sodium alginate, chitosan, and different formulations of caffeine encapsulated calcium alginate particles.

from the Ca^{2+} – H^+ exchange effect, acidic pH may cause dissolution of chitosan membrane by converting free amine groups to highly soluble NH_3^+ leaving the swelling to be mainly dominated by alginate matrix [35]. This may be the reason why different observations have been reported on the swelling behaviour of alginate–chitosan particles. In this study negligible changes in the surface morphology of chitosan coated particles were observed during 2 h of incubation in acidic medium.

The swelling behavior of calcium alginate particles in phosphate buffer solution is controlled by the ion exchange between Na^+ in buffer solution and Ca^{2+} within the particles. Sodium ions do not have the ability to bridge between carboxylic acid groups and thus result in a lower crosslink density and consequently more water absorption [48].

Fig. 6a shows the swelling profile of particles in phosphate buffer solution. As can be seen it is a time dependent process exhibiting a sudden increase after a time lag of 3–4 h. Swelling behavior could be well explained by the fact that soaked particles tend to absorb water until they reach an equilibrium in order to fill the void regions in the gel structure. This phenomenon is driven by osmotic pressure leading to the relaxation of polymer network. Swelling of the particles continues until osmotic pressure becomes equivalent to the force of cross-linked bonds maintaining the structure of network. When these two forces are equal, no more water absorption will occur [35]. The curves in Fig. 6a indicate that chitosan coating of particles decreases their swelling rate. This is due to complex formation between the carboxyl groups of alginate and the amino groups of chitosan. Electrostatic interaction between those polymers leads to a compact structure posing a great resistance against swelling in aqueous media [35]. Swelling rates and the percentage of unbroken particles after 8 h incubation in the swelling media are reported in Table 1. Coating the particles with 0.5% chitosan, especially CHh, could not retain the integrity and spherical shape of particles until the final hours of incubation and only 40% of them preserved their sphericity after 8 h. This may be due to the low crosslink density of CHh as a result of being unable to diffuse into alginate microhydrogels and form a thick and dense membrane. The swelling rate for the particles coated with 0.5% CHI was lower, and >80% of them retained their spherical shape. Although uncoated particles (ALG) exhibited a higher swelling rate, they greatly preserved their integration and spherical shape (>90%). Raising the concentration of chitosan to 1% significantly strengthened the gel network as suggested by the low swelling rate data and increased percentage of unbroken particles (Table 1). However, CHI coated revealed again a more enhanced solidity due to their more compacted and thicker membrane. Similar results have been reported by Abreu et al. [49]. Anal & Stevens (2005) had recorded a swelling rate of 222% for the calcium alginate particles coated by chitosan at the end of incubation time.

3.6. In vitro caffeine release studies

On transferring microparticles to hydrochloric acid solution, caffeine release started through diffusion due to its high solubility in the acidic media. The main portion of this burst release was related to the caffeine present on the surface of particles that easily released into the acidic solution. As is shown in Fig. 6b the highest rate of caffeine release was observed for uncoated calcium alginate particles. Chitosan coating, on the other hand, especially at 1% concentration significantly increased the retention of caffeine within the microhydrogels matrix. Although almost no changes were observed in the size of particles, it seems that Ca^{2+} – H^+ exchange and protonation of $-\text{COO}^-$ groups led to the relaxation of polymer chains which pushed out the entrapped caffeine due to space limitation. The positive effect of chitosan coating on the retention of caffeine can be related to the hindering effect it imposed on the diffusion of H^+ into the gel network through electrostatic repulsion from unbound $-\text{NH}_3^+$ groups as well as the increased diffusion length it caused following penetration into the particles or deposition on their surfaces. Similar observations have been reported by Zhang et al. (2016) and attributed to the internal shrinkage of microhydrogels due to the protonation of

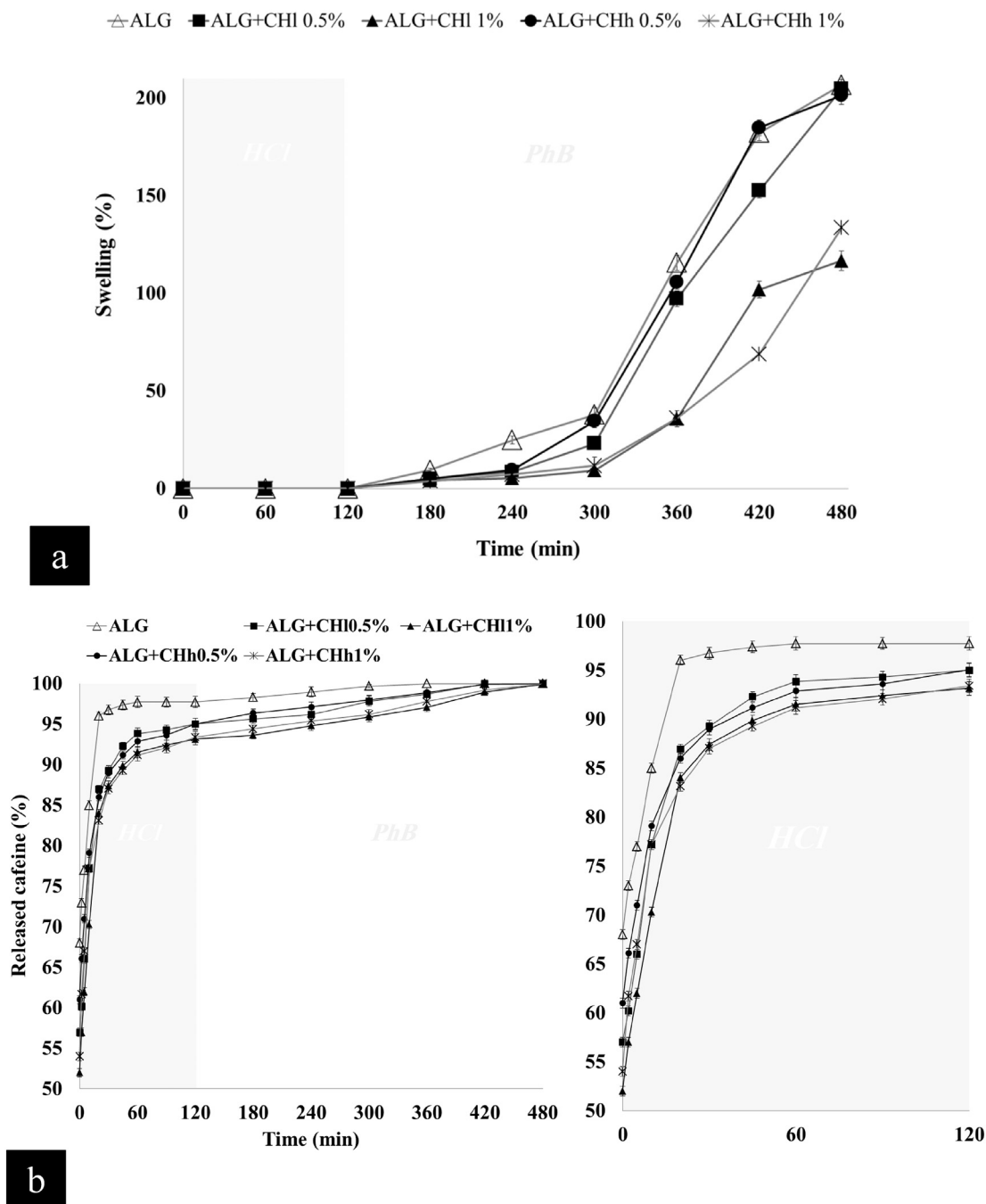


Fig. 6. a) Swelling profile of caffeine-encapsulated calcium alginate particles in in hydrochloric acid (pH = 2) and phosphate buffer (pH = 7.4) solutions at 37 °C; b) Cumulative caffeine release profile from calcium alginate particles in the same media.

carboxyl groups ($-\text{COO}^-$). Fig. 6b also shows that in phosphate buffer the release of caffeine from the particles coated with 0.5 and 1% chitosan occurred at a lower rate compared to the others. This is in agreement with the swelling trends depicted in Fig. 6a and the data given in Table 1 confirming the higher crosslink density of alginate-chitosan due to the availability of greater number of $-\text{NH}_3^+$ groups in CHI fraction as well as its lower molecular weight that permits deeper diffusion into the gel matrix. Previous studies on the release of other low molecular weight compounds have also proved that it could be sustained by coating the alginate particles with chitosan [4,10,18–20,38].

The release kinetics of caffeine was evaluated by Korsmeyer-Peppas and first order models. The former was found to best fit the release data as indicated by R^2 , RMSE and χ^2 statistics (Table 2). Accordingly, the release of caffeine from microparticles can be considered to be the

consequence three different processes of diffusion, swelling and dissolution of polymer matrix that simultaneously take place. The value of n in Table 2 is <0.43 for both coated and uncoated particles that denotes the release of caffeine can be described by the Fickian diffusion law.

Table 1

Swelling rate and percentage of unbroken particles after 8 h incubation in release media.

Particle type	Swelling rate (%)	Unbroken particles (%)
ALG	206.84 ± 4.49	>90
ALG + CHI 0.5%	204.80 ± 6.88	>80
ALG + CHI 1%	116.76 ± 3.12	100
ALG + CHh 0.5%	201.60 ± 4.01	>40
ALG + CHh 1%	133.60 ± 2.23	>95

Table 2

Kinetic parameters of caffeine release in hydrochloric acid (pH = 1.2) and phosphate buffer (pH = 7.4) solutions fitted to Korsmeyer-Peppas and first order models.

Formulation	R ²	RMSE	χ^2	n	K(h ⁻ⁿ)
ALG	0.7740	4.955	36.632	0.0702	68.56
ALG + CHI 0.5%	0.8656	5.232	40.499	0.1017	56.37
ALG + CHI 1%	0.8774	5.313	41.870	0.1109	52.78
ALG + CHh 0.5%	0.9198	3.513	18.054	0.0848	61.84
ALG + CHh 1%	0.9112	4.217	25.900	0.1006	55.99

4. Conclusions

The reinforcement of electrosprayed calcium alginate particles by chitosan coating and its effect on the release of caffeine was investigated in this study. It was shown that chitosan significantly enhanced the retention of caffeine within particles by controlling the swelling rate of hydrogel matrices. This was attributed to the electrostatic interactions between the two polymers which resulted in a more strengthened network being capable of maintaining its integrity in the release medium. Of the two types of chitosan, CHI was revealed to more efficiently control the swelling of particles and thus the release rate of caffeine than CHh. The molecular weight of chitosan seems to be a determining factor for crosslink density with alginate and network reinforcement. FTIR spectra and DSC thermograms both confirmed the ionic interaction of alginate-chitosan and the entrapment of caffeine within the gel network. Mathematical modeling indicated that Fickian diffusion was the prevalent mechanism of caffeine release from the particles. The findings of this study suggest chitosan coated electrosprayed alginate microhydrogels, owing to their tunable size and sustained release behaviour, as a potential carrier systems for encapsulation and delivery of a wide range of bioactive compounds.

Acknowledgements

The authors would like to appreciate the financial support of the Research Institute of Food Science and Technology, Iran, (Grant No: 71293036-2014/08/23) and the scientific cooperation of the New Zealand Institute for Plant & Food Research.

References

- [1] W.R. Gombotz, S.F. Wee, Protein release from alginate matrices, *Adv. Drug Deliv. Rev.* 64 (2012) 194–205, <https://doi.org/10.1016/j.addr.2012.09.007>.
- [2] K.Y. Lee, D.J. Mooney, Alginate: properties and biomedical applications, *Prog. Polym. Sci.* 37 (2012) 106–126, <https://doi.org/10.1016/j.progpolymsci.2011.06.003>.
- [3] J.P. Paques, E. van der Linden, C.J.M.M. van Rijn, L.M.C.C. Sagis, Alginate submicron beads prepared through w/o emulsification and gelation with CaCl₂ nanoparticles, *Food Hydrocoll.* 31 (2013) 428–434, <https://doi.org/10.1016/j.foodhyd.2012.11.012>.
- [4] R.M. Lucinda-Silva, H.R.N. Salgado, R.C. Evangelista, Alginate-chitosan systems: in vitro controlled release of triamcinolone and in vivo gastrointestinal transit, *Carbohydr. Polym.* 81 (2010) 260–268, <https://doi.org/10.1016/j.carbpol.2010.02.016>.
- [5] E. Tavassoli-Kafrani, H. Shekarchizadeh, M. Masoudpour-Behabadi, Development of edible films and coatings from alginates and carrageenans, *Carbohydr. Polym.* 137 (2016) 360–374, <https://doi.org/10.1016/j.carbpol.2015.10.074>.
- [6] S. Al-Assaf, V. Amar, Handbook of hydrocolloids, *Handb. Hydrocoll.* (2009) 477–494, <https://doi.org/10.1533/9781845695873.477>.
- [7] A. Jaworek, A.T. Sobczyk, Electrospraying route to nanotechnology: an overview, *J. Electrostat.* 66 (2008) 197–219, <https://doi.org/10.1016/j.elstat.2007.10.001>.
- [8] A. Mehregan Nikoo, R. Kadkhodae, B. Ghorani, H. Razzaq, N. Tucker, Electrohydrodynamic atomization assisted encapsulation of bioactive compounds, *MOJ Food Process. Technol. Electrohydrodynamic.* 1 (2015) 10–11, <https://doi.org/10.15406/mojfpt.2015.01.00010>.
- [9] Lord Rayleigh, On the instability of jets, *Proc. Lond. Math. Soc.* 10 (1878) 4–13, <https://doi.org/10.1112/plms/s1-10.1.4>.
- [10] C.Y. Yu, X.C. Zhang, F.Z. Zhou, X.Z. Zhang, S.X. Cheng, R.X. Zhuo, Sustained release of antineoplastic drugs from chitosan-reinforced alginate microparticle drug delivery systems, *Int. J. Pharm.* 357 (2008) 15–21, <https://doi.org/10.1016/j.ijpharm.2008.01.030>.
- [11] A. Rajendran, S.K. Basu, Alginate-chitosan particulate system for sustained release of nimodipine, *Trop. J. Pharm. Res.* 8 (2009) 433–440, <https://doi.org/10.4314/tjpr.v8i5.48087>.
- [12] A.M.R. Al-Mayah, Simulation of enzyme catalysis in calcium alginate beads, *Enzyme Res.* 2012 (2012) <https://doi.org/10.1155/2012/459190>.
- [13] A. Lopez Cordoba, L. Deladino, M. Martino, A. López Córdoba, Effect of starch filler on calcium-alginate hydrogels loaded with yerba mate antioxidants, *Carbohydr. Polym.* 95 (2013) 315–323, <https://doi.org/10.1016/j.carbpol.2013.03.019>.
- [14] Y. Zhou, Y. Yang, H. Chuo, X. Liu, Preparation and rheological behaviors of 6-carboxy-chitosan, *J. Appl. Polym. Sci.* 94 (2004) 1126–1130, <https://doi.org/10.1002/app.21013>.
- [15] A. Rafiee, M.H. Alimohammadian, T. Gazori, F. Razi-rad, S.M.R. Fatemi, A. Parizadeh, I. Haririan, M. Havaskary, Comparison of chitosan, alginate and chitosan/alginate nanoparticles with respect to their size, stability, toxicity and transfection, *Asian Pacific J. Trop. Dis.* 4 (2014) 372–377, [https://doi.org/10.1016/S2222-1808\(14\)60590-9](https://doi.org/10.1016/S2222-1808(14)60590-9).
- [16] M. Simonoska Crcarevska, M. Glavas Dodov, K. Goracinova, Chitosan coated Ca-alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa, *Eur. J. Pharm. Biopharm.* 68 (2008) 565–578, <https://doi.org/10.1016/j.ejpb.2007.06.007>.
- [17] X.Z. Shu, K.J. Zhu, The release behavior of brilliant blue from calcium-alginate gel beads coated by chitosan: the preparation method effect, *Eur. J. Pharm. Biopharm.* 53 (2002) 193–201 http://ac.els-cdn.com/S0939641101002478/1-s2.0-S0939641101002478-main.pdf?_tid=44d7f9ce-9216-11e4-a4b5-0000aabb0f6c&acdnat=1420158687_52ace081e45ee9fc07d96b90e69d9c1.
- [18] A.K. Anal, W.F. Stevens, Chitosan-alginate multilayer beads for controlled release of ampicillin, *Int. J. Pharm.* 290 (2005) 45–54, <https://doi.org/10.1016/j.ijpharm.2004.11.015>.
- [19] M.L. González-Rodríguez, M.A. Holgado, C. Sánchez-Lafuente, A.M. Rabasco, A. Fini, Alginate/chitosan particulate systems for sodium diclofenac release, *Int. J. Pharm.* 232 (2002) 225–234, [https://doi.org/10.1016/S0378-5173\(01\)00915-2](https://doi.org/10.1016/S0378-5173(01)00915-2).
- [20] F.-L. Mi, H.-W. Sung, S.-S. Shyu, Drug release from chitosan-alginate complex beads reinforced by a naturally occurring cross-linking agent, *Carbohydr. Polym.* 48 (2002) 61–72, [https://doi.org/10.1016/S0144-8617\(01\)00212-0](https://doi.org/10.1016/S0144-8617(01)00212-0).
- [21] K. Mladenovska, O. Cruaud, P. Richomme, E. Belamie, R.S. Raicki, M.-C. Venier-Julienne, E. Popovski, J.P. Benoit, K. Goracinova, 5-ASA loaded chitosan-Ca-alginate microparticles: preparation and physicochemical characterization, *Int. J. Pharm.* 345 (2007) 59–69, <https://doi.org/10.1016/j.ijpharm.2007.05.059>.
- [22] O. Masalova, V. Kulikouskaya, T. Shutava, V. Agabekov, Alginate and chitosan gel nanoparticles for efficient protein entrapment, *Phys. Procedia* 40 (2013) 69–75, <https://doi.org/10.1016/j.phpro.2012.12.010>.
- [23] X. Wang, K. Zhu, H. Zhou, Immobilization of Glucose Oxidase in Alginate-chitosan Microcapsules, 2011 3042–3054, <https://doi.org/10.3390/jms12053042>.
- [24] A.P. Bagre, K. Jain, N.K. Jain, Alginate coated chitosan core shell nanoparticles for oral delivery of enoxaparin: in vitro and in vivo assessment, *Int. J. Pharm.* 456 (2013) 31–40, <https://doi.org/10.1016/j.ijpharm.2013.08.037>.
- [25] R. Pandey, G.K. Khuller, Chemotherapeutic potential of alginate-chitosan microspheres as anti-tubercular drug carriers, *J. Antimicrob. Chemother.* 53 (2004) 635–640, <https://doi.org/10.1093/jac/dkh139>.
- [26] M.J. Glade, Caffeine—not just a stimulant, *Nutrition* 26 (2010) 932–938, <https://doi.org/10.1016/j.nut.2010.08.004>.
- [27] D. Tan, B. Zhao, S. Mochhala, Y.-Y. Yang, Sustained-release of caffeine from a polymeric tablet matrix: an in vitro and pharmacokinetic study, *Mater. Sci. Eng. B* 132 (2006) 143–146, <https://doi.org/10.1016/j.mseb.2006.02.031>.
- [28] A. Mehregan Nikoo, R. Kadkhodae, B. Ghorani, H. Razzaq, N. Tucker, Controlling the morphology and material characteristics of electrospray generated calcium alginate microhydrogels, *J. Microencapsul.* (2016) 1–28, <https://doi.org/10.1080/02652048.2016.1228707>.
- [29] A. Belščak-Cvitanović, D. Komes, S. Karlović, S. Djaković, I. Špoljarić, G. Mršić, D. Ježek, I. Špoljarić, Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin, carrageenan, chitosan and psyllium, *Food Chem.* 167 (2015) 378–386, <https://doi.org/10.1016/j.foodchem.2014.07.011>.
- [30] K.M. Manjanna, K.S. Rajesh, B. Shivakumar, Formulation and optimization of natural polysaccharide hydrogel microbeads of aceclofenac sodium for oral controlled drug delivery, *Am. J. Med. Sci. Med.* 1 (2013) 5–17 <http://pubs.sciepub.com/ajmsm/1/1/2>.
- [31] D.W.A. Bourne, *Modern Pharmaceutics*, Marcel Dekker, New York, 2002.
- [32] S. Jose, J.F. Fanguero, J. Smitha, T.A. Cinu, A.J. Chacko, K. Premaletha, E.B. Souto, Predictive modeling of insulin release profile from cross-linked chitosan microspheres, *Eur. J. Med. Chem.* 60 (2013) 249–253, <https://doi.org/10.1016/j.ejmech.2012.12.011>.
- [33] S. Lee, M.S. Kim, J.S. Kim, H.J. Park, J.S. Woo, B.C. Lee, S.J. Hwang, Controlled delivery of a hydrophilic drug from a biodegradable microsphere system by supercritical anti-solvent precipitation technique, *J. Microencapsul.* 23 (2006) 741–749, <https://doi.org/10.1080/09687860600945552>.
- [34] N. Bock, M.A. Woodruff, D.W. Huttmacher, T.R. Dargaville, Electrospraying, a reproducible method for production of polymeric microspheres for biomedical applications, *Polymers (Basel)* 3 (2011) 131–149, <https://doi.org/10.3390/polym3010131>.
- [35] G. Pasparakis, N. Bouropoulos, Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads, *Int. J. Pharm.* 323 (2006) 34–42, <https://doi.org/10.1016/j.ijpharm.2006.05.054>.
- [36] J. Shi, N.M. Alves, J.F. Mano, Chitosan coated alginate beads containing poly(N-isopropylacrylamide) for dual-stimuli-responsive drug release, *J. Biomed. Mater. Res. Part B Appl. Biomater.* 84B (2008) 595–603, <https://doi.org/10.1002/jbm.b.30907>.
- [37] M. Bhavan, G. Nagar, CHITOSAN-SODIUM ALGINATE NANOCOMPOSITES BLENDED WITH CLOISITE 30B AS A NOVEL DRUG DELIVERY SYSTEM FOR ANTICANCER DRUG CURCUMIN Vijay Kumar Malesu, Debasish Sahoo and P. L. Nayak * ISSN 0976-4550 Materials Preparation of Chitosan-Alginate Nanocomposite, *Int. J. Appl. Biol. Pharm. Technol.* 2 (2011) 402–411.

- [38] P. Li, Y. Dai, J. Zhang, A. Wang, Q. Wei, Chitosan — alginate nanoparticles as a novel drug delivery system for nifedipine, *Int. J. Biomed. Sci.* 4 (2008) 221–228.
- [39] B. Sarmento, D. Ferreira, F. Veiga, A. Ribeiro, Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies, *Carbohydr. Polym.* 66 (2006) 1–7, <https://doi.org/10.1016/j.carbpol.2006.02.008>.
- [40] O. Borges, G. Borchard, J.C. Verhoef, A. de Sousa, H.E. Junginger, Preparation of coated nanoparticles for a new mucosal vaccine delivery system, *Int. J. Pharm.* 299 (2005) 155–166, <https://doi.org/10.1016/j.ijpharm.2005.04.037>.
- [41] A.J. Ribeiro, C. Silva, D. Ferreira, F. Veiga, Chitosan-reinforced alginate microspheres obtained through the emulsification/internal gelation technique, *Eur. J. Pharm. Sci.* 25 (2005) 31–40, <https://doi.org/10.1016/j.ejps.2005.01.016>.
- [42] M.D. Veiga, R. Cadorniga, R. Lozana, Thermal study of prednisolone polymorphs, *Thermochim. Acta* 96 (1985) 111–115, [https://doi.org/10.1016/0040-6031\(85\)80012-5](https://doi.org/10.1016/0040-6031(85)80012-5).
- [43] S.C. Barros, A.A. da Silva, D.B. Costa, C.M. Costa, S. Lanceros-Méndez, M.N.T. Maciavello, J.L.G. Ribelles, F. Sentanin, A. Pawlicka, M.M. Silva, Thermal-mechanical behaviour of chitosan-cellulose derivative thermoreversible hydrogel films, *Cellulose* 22 (2015) 1911–1929, <https://doi.org/10.1007/s10570-015-0603-5>.
- [44] X. Qi, X. Hu, W. Wei, H. Yu, J. Li, J. Zhang, W. Dong, Investigation of salectan/poly (vinyl alcohol) hydrogels prepared by freeze/thaw method, *Carbohydr. Polym.* 118 (2015) 60–69, <https://doi.org/10.1016/j.carbpol.2014.11.021>.
- [45] K.M. Manjanna, T.M. Pramod Kumar, B. Shivakumar, Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis, *Drug Discov. Ther.* 4 (2010) 109–122, <http://www.ncbi.nlm.nih.gov/pubmed/22491168>.
- [46] J.M.C. Puguán, X. Yu, H. Kim, Characterization of structure, physico-chemical properties and diffusion behavior of Ca-alginate gel beads prepared by different gelation methods, *J. Colloid Interface Sci.* 432 (2014) 109–116, <https://doi.org/10.1016/j.jcis.2014.06.048>.
- [47] C. Ouwerx, N. Velings, M. Mestdagh, M.a. Axelos, Physico-chemical properties and rheology of alginate gel beads formed with various divalent cations, *Polym. Gels Networks* 6 (1998) 393–408, [https://doi.org/10.1016/S0966-7822\(98\)00035-5](https://doi.org/10.1016/S0966-7822(98)00035-5).
- [48] S.K. Bajpai, S. Sharma, Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions, *React. Funct. Polym.* 59 (2004) 129–140, <https://doi.org/10.1016/j.reactfunctpolym.2004.01.002>.
- [49] F.O.M.S. Abreu, C. Bianchini, M.M.C. Forte, T.B.L. Kist, Influence of the composition and preparation method on the morphology and swelling behavior of alginate-chitosan hydrogels, *Carbohydr. Polym.* 74 (2008) 283–289, <https://doi.org/10.1016/j.carbpol.2008.02.017>.