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ORIGINAL ARTICLE

Influence of the preparation procedure and chitosan type on physicochemical properties and release behavior of alginate–chitosan microparticles

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Abstract

Background: The potential for use of chitosan-treated alginate microparticles as a vehicle for oral phenytoin delivery has not been thoroughly exploited. *Aim*: We studied the influence of preparation procedure and chitosan type on physicochemical properties and release behavior of alginate-chitosan microparticles. *Method*: The total number of 24 microparticles formulations prepared by varying contents of calcium gelling ions and varying contents and type of chitosan was examined. As an additional variable, two different hardening times (1 and 24 hours) were employed. Possible interactions of components, surface morphology of microparticles as well as release profile of phenytoin were studied. *Results*: Both series of formulations with regard to hardening times, irrespective of the chitosan type and/or concentration employed appeared to be highly loaded with the model drug (above 90%). The drug release studies showed that the kinetics of phenytoin cannot be straightforwardly predicted based on the molecular weight of chitosan alone. On the other hand, prolonging the hardening time from 1 to 24 hours had significantly improved phenytoin kinetics, and gave rise to a formulation with the liberation half-time of about 2.5 hours. *Conclusion*: This study showed that the latter formulation is eligible for further modifications aimed at improving the regularity of phenytoin absorption.

Key words: *Alginate–chitosan microparticles; phenytoin; physicochemical characterization; preparation; release behavior*

Introduction

Among various drug delivery systems investigated with the aim to obtain efficient and selective delivery of drugs to the site of action, the multiparticulate systems have received a considerable interest[1,2]. Additionally, there has been increased interest in the use of multiparticulate systems as a way to largely avoid the negative influence of erratic gastric emptying and intestinal motility on intra- and inter-subject variability in the rate and the extent of drug availability[3–7]. Namely, it is accepted that the size of most such systems enables them to pass through the constricted pyloric sphincter so that they are able to distribute themselves along the entire gastrointestinal tract[6–8].

In recent years, attention has been focused on microparticles prepared with natural polymers such as alginate, a high molecular weight hyaluronic acid-like biodegradable polymer, which in the presence of divalent (calcium, strontium, barium, except for magnesium) and multivalent cations (such as aluminum) forms gels or precipitates in a process designated as ionotropic gelation[2,9].

Alginate is a family of polysaccharides composed of 1,4-linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues extracted from brown seaweeds

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(Phaeophyceae, mainly *Laminaria*). These residues are arranged in three types of blocks: homopolymeric M blocks (MM), homopolymeric G blocks (GG), and heteropolymeric sequentially alternating blocks (MG). It has been reported that alginate gels with a high G content exhibit high porosity, low shrinkage during gel formation, and lack of swelling after drying, while those with increasing M content are characterized as less porous, softer, and more elastic[10–13]. The process of ionotropic gelation takes place by forming egg-box junctions to associate divalent, for example, calcium ions with the GG blocks of the alginate chain. The alginates rich in guluronic acid form the more rigid gels, which are less prone to erosion, and the drug release would be slower[2,10,14]. It seems that alginate microparticles have the advantages of being nontoxic and having high biocompatibility[15]. Another advantageous property would be their inability to reswell and to substantially release encapsulated drug by the mechanism of erosion, as well as to limit the release of the drug by diffusion in acidic environment. As a consequence, the loaded drug may evade the gastric environment and the direct contact with the gastric mucosa[6,13,16,17], which would be especially advantageous for the acid-sensitive drugs[18].

Alginate microparticles are prone to erosion and subsequent destabilization in the presence of chelators such as phosphate, lactate, citrate, or nongelling cations like sodium or magnesium imposes a significant constraint because of accelerated drug release. In biological systems such as the human body, there is need for a stabilizing agent, which can be a membrane forming polycation bound to the alginate gel by electrostatic interactions in a process known as polyelectrolyte complexation. This membrane imposes the mechanical contractile forces resisting to an osmotic swelling pressure built up by network chains toward infinite dilution, and also reduces the permeability of these microsystems; both effects are desirable in order to control the disintegration and to extend the drug release. Chitosan and polylysine are the most commonly used polycations for this purpose[9,19,20].

Because of its low toxicity, good biocompatibility, biodegradability, and mucoadhesive properties, chitosan, a natural linear biopolyaminosaccharide, obtained by alkaline deacetylation of chitin[19] is preferred today. Chitosan comprises a series of copolymers of glucosamine and *N*-acetyl-glucosamine[21–23]. It possesses one primary amino and two free hydroxyl groups for each C6 building unit. Because of the easy availability of free amino groups in chitosan, it carries a positive charge, and thus in turn reacts with different negatively charged surfaces and polymers[24].

Usually, when considering the process of ionotropic gelation with the external calcium source, alginate–chitosan microparticles are obtained by two principally different procedures, first of them being one-step and the other being two-step procedure. In the one-step procedure, a complex coacervate membrane is formed at the interface between the alginate and chitosan solutions when the alginate solution is dropped directly into the solution of chitosan. This yields particles with a complex alginate–chitosan membrane surrounding a liquid alginate core. Gelling of the core is achieved either by having calcium chloride in chitosan solution during the formation of the particles[25–29], or by treating the liquid core particles with the salt after the membrane has been formed[26]. The two-step procedure comprises the formation of calcium alginate beads, followed by a subsequent coating with chitosan[30].

Our previous work has showed the potential of chitosan reinforced calcium-induced alginate network to control the release of a model drug, but with a rather complex influence of chitosan coating on this parameter, thereby necessitating further modifications[31]. In order to elucidate this issue, and in an attempt to further improve the drug release profile, the objective of this study was to prepare and evaluate the microparticles with varying contents of calcium gelling ion using different types of chitosan (regarding the molecular weight and viscosity), and with hardening times of 1 and 24 hours. The microparticles were loaded with the model substance phenytoin, a standard antiepileptic agent, in its acidic form. The extended release formulations of antiepileptics decrease daily fluctuations and simplify the treatment of this chronic condition. Such commercial formulations with phenytoin sodium appear to be well designed, but with one possible shortcoming. That concern deals with the potential for irregular absorption that appears to occur particularly in the case of elderly patients[32]. Taking into account the ability of multiparticulate systems to overcome, to a large extent, the issue of erratic gastric emptying time and intestinal motility, an additional benefit of the present investigation might be the improvement of the regularity of phenytoin absorption.

Materials and methods

Manugel GHB, the medium viscosity sodium alginate with high guluronic acid ('high G') content was kindly donated by International Specialty Products (ISP, Wayne, NJ, USA). Phenytoin was provided by Fluka (Neu-Ulm, Germany). High (310–375 kDa) and low (50–190 kDa) molecular weight chitosans (degree of deacetylation, >75% and 75%–85%, respectively) were purchased from Aldrich (Milwaukee, WI, USA), whereas the combination of high and low molecular weight chitosans (50–2000 kDa, degree of deacetylation, 75%–85%) was purchased from Cognis (Monheim, Germany). Calcium chloride was obtained from Merck KGaA (Darmstadt, Germany).

Microparticles preparation

Microparticles were prepared according to the one-step procedure using a custom-made air-jet device. The volume of approximately 7 mL of 2% phenytoin suspension (bubble free) in 2% aqueous solution of alginate (drug:polymer ratio = 1:1) was extruded through a flat-cut 20 gauge needle at a rate of 120 mL/h into 70 mL of a gelling medium consisting of aqueous solution of different concentrations of calcium chloride mixed with the defined concentration of chitosan. The pH value of gelling medium was adjusted to 5.5 with acetate buffer. The laminar air flow in coaxial cylinder, pointed to the tip of the needle producing small droplets, was constant (1200 L/h). The prepared microparticles were allowed to harden for 1 or 24 hours depending on the procedure variation. Afterward, they were collected, washed, and dried at room temperature for 72 hours. The 24 different formulations of inhomogeneous microparticles were made according to this procedure (Table 1).

Microparticles size determination

The particle size of 50 microparticles was measured with Motic digital microscope DMB3-223ASC and Motic Images Plus v.2.0 software (Motic GmbH, Wetzlar, Germany) for each formulation and the mean particle size was determined.

Scanning electron microscopy

The surface structure and morphology of microparticles was examined by scanning electron microscopy (SEM) (DSM 940 A, Zeiss, Oberkochen, Germany) using gold sputter technique.

Table 1. Composition of model formulations of inhomogeneous microparticles prepared according to the one-step procedure.

Formulation		$CaCl_2$ concentration (% w/w)	Chitosan concentration (% w/w)
Hardening time			
1 hour	24 hours		
			Mixture of high and low MW chitosans
F4	F4′	0.5	0.1
F6	F6′	2.0	0.1
F7	F7′	0.5	0.2
F9	F9′	2.0	0.2
			Low MW chitosan
F4L	F4L′	0.5	0.1
F6L	F6L′	2.0	0.1
F7L	F7L′	0.5	0.2
F9L	F9L′	2.0	0.2
			High MW chitosan
F4H	F4H′	0.5	0.1
F6H	F6H′	2.0	0.1
F7H	F7H′	0.5	0.2
F9H	F9H′	2.0	0.2

Thermal analysis

Differential scanning calorimetry (DSC) analysis was used to characterize the thermal behavior of the particles components. DSC thermograms were obtained using a differential scanning calorimeter STA 409 PG (Netsch, Selb, Germany). Samples were crimped in a standard aluminum pan and run at a scanning rate of 20°C/min from 25°C to 330°C.

Drug assay

Determination of phenytoin in microparticles was performed using RP-HPLC method after microparticles were broken with the aid of ultrasonication in 0.1 M NaOH for 15 minutes, with subsequent centrifugation at 4000 rpm for 5 minutes. Phenobarbital was used as an internal standard. The column used was Zorbax SB-C18, 250 × 4.6 mm, 5 μm. The composition of the mobile phase was ethanol:water:0.5% triethanolamine in ratio 40:59.5:0.5 (v/v/v), respectively. The pH of the mobile phase was adjusted to 3.0 with 85% ortho-phosphoric acid. Chromatography was conducted at ambient temperature at a flow rate of 1.0 mL/min. The absorbance of each sample was determined spectrophotometrically at 228 nm using Agilent 1100 Series variable wavelength UV detector (Agilent Technologies, Waldbronn, Germany). All determination tests were performed in triplicate.

Drug release studies

The release of phenytoin from microparticles was measured quantitatively on an automatic sampling and analysis system Erweka DT 800 (Erweka GmbH, Heusenstamm, Germany) using the rotating basket method at 100 rpm with the temperature maintained at 37°C in 900 mL phosphate buffer (pH 7.4 USP23). The obtained samples were mixed with solution of internal standard, and phenytoin content was determined as explained under drug loading determination. All release tests were performed in triplicate.

Results and discussion

It was shown that the polyionic complexation between chitosan and anionic polymers depends on the pH value of the media, the ionic strength, and temperature[33]. Polk et al.[34] investigated the effect of the pH of gelling medium containing chitosan on microparticle strength and flexibility and reported that the strength and flexibility of the

microparticle membrane were maximal as the pH of the chitosan solution increases to 5.5. Furthermore, it was shown for drugs of poor water solubility encapsulated into alginate–chitosan microparticles that molecular weight and viscosity of alginates should not affect the drug release[12,13]. Regarding the time allowed for hardening in gelling medium, there are considerable number of strategies, starting with the hardening time as short as 1 minute through 10 minutes, 45 minutes[34], 1 hour[35], 12 hours[12], 24 hours[6,17] up to 3 and 5 days[14]. Taking into consideration these reports and findings, the 24 microparticle formulations were prepared using different concentrations of calcium chloride and alginate of medium viscosity characterized with high G content, and with different types and concentrations of chitosans (Table 1). Twelve of them were allowed to harden in the gelling medium for 1 hour, and the other series of 12 microparticle formulations was allowed to harden for 24 hours. In addition, the pH of all gelling solutions was adjusted to 5.5.

Microparticles size determination

The recorded sizes of microparticle formulations incubated for 1 hour in gelling medium were in range of 460.2 ± 14.8 to 635.5 ± 6.2 μm (Figure 1). Although statistical analysis (not shown) pointed to some inhomogeneity in mean diameters of microparticles, the range of obtained values did not indicate any clear influence of composition, especially of chitosan type, on microparticle size, which is in good accordance with the results described previously[12].

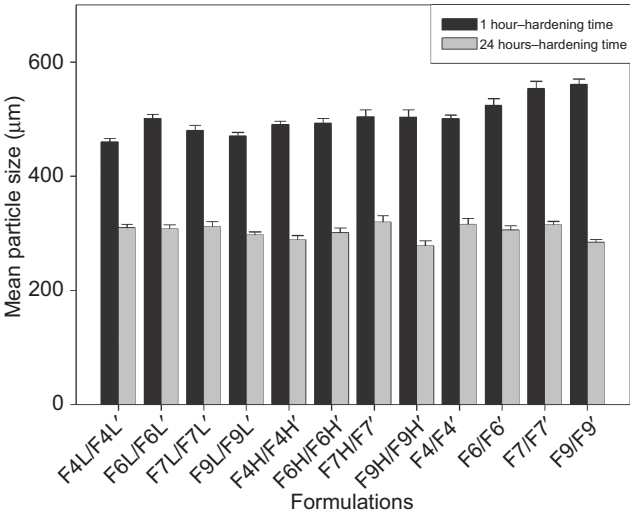

Figure 1. Particle size of formulations made with different chitosan types hardened for 1 hour (black bars) and of corresponding formulations hardened for 24 hours (gray bars).

The sizes of formulations hardened for 24 hours in the gelling medium were in range of 278.3 ± 18.2 to 320.4 ± 16.3 μm (Figure 1), and for these samples too statistical analysis (not shown) did not indicate any clear influence of composition variation on microparticle size. Smaller particle sizes of formulations incubated for 24 hours in the gelling medium compared to formulations incubated for 1 hour were probably the result of further contraction of alginate network in conditions of prolonged exposure to the source of gelling cation, calcium chloride.

Scanning electron microscopy

SEM observation revealed that higher concentration of either grade of chitosan used tended to distort the acceptable spherical shape of microparticles during the manufacturing and the drying phase (Figure 2) leading to the appearance of the tail-like structures in cases of high chitosan concentration. Viscosity values of the gelling mediums at 20°C with 0.1% of mixture of chitosans, low, and high molecular weight chitosans were 27, 16, and 57 mPas, respectively, while of those with 0.2% of the corresponding chitosan type present were 51, 30, and 109 mPas, respectively.

In the one-step procedure using external source of calcium, with chitosan present in the gelling medium, it has been observed that droplets of alginate are not able to break easily through the surface because of increased viscosity of solution[9]. The high velocity of alginate suspension of phenytoin droplets leaving the nozzle of device, alongside with instantaneous reactions of ionotropic gelation and polyionic complexation, may contribute to the nonspherical shape and tail-like structures of microparticle formulations with higher chitosan concentration. Moreover, the spherical shape of alginate–chitosan microparticles in the wet state was usually lost after drying, indicating that the integrity of alginate–chitosan membrane may be compromised during the drying process[35].

The samples produced with 0.2% of either grade of chitosan tended to form agglomerates on the surface of gelling medium during production process, which, partially, caused the subsequent presence of agglomerates after drying (Figure 3a). This tendency may be attributed to the adhesive properties of chitosan. In a preliminary study, increasing the percentage of high molecular weight chitosan to values close to 0.25% provoked permanent microparticles agglomeration, and an effective production process was impossible. Similar results were reported earlier[6,36]. Namely, these authors have observed that higher concentrations of chitosan made the dropping process difficult and microparticles could not have been readily produced. This was the reason why concentrations of either grade of chitosan used in this study did not exceed 0.20%.

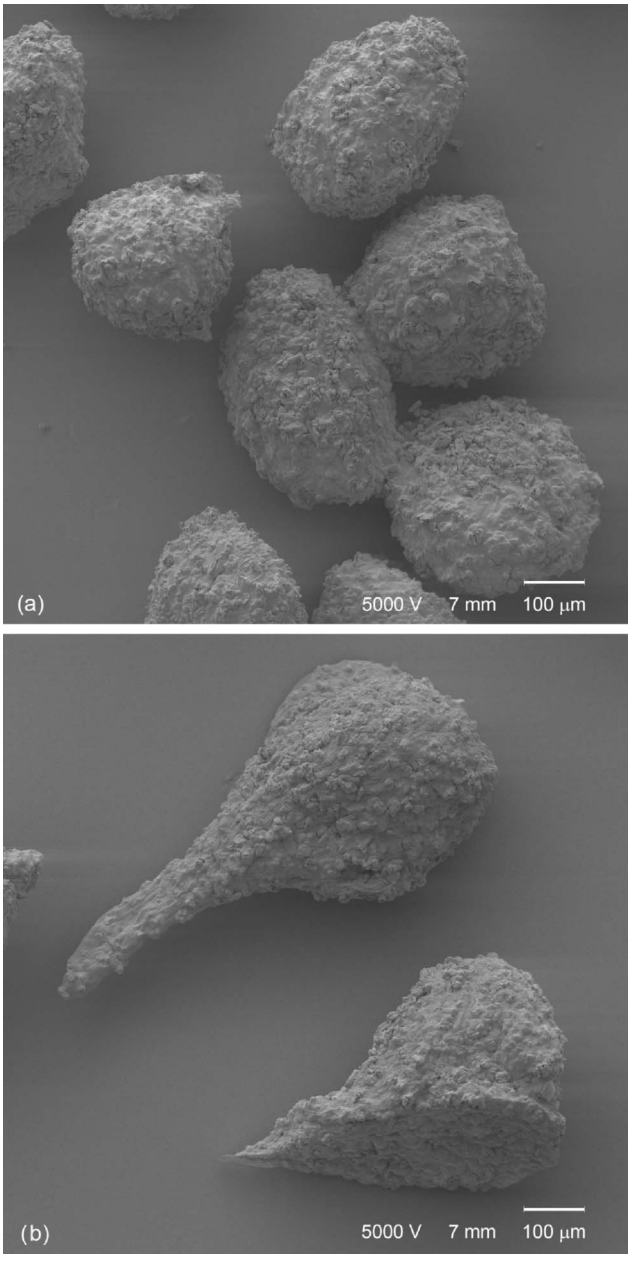

Figure 2. SEM micrographs of formulations F6′, made with 0.1% of chitosan (up), and F9′, made with 0.2% of chitosan (down).

A notable property of the dried microparticles' surface is its higher porosity and more extensive fracturing, which made the phenytoin particles more discernible when chitosan was not present[31] in hardening solution (Figure 3b), compared to the case where it was present (Figure 3c). In order to demonstrate the presence of visible drug particles on surface of chitosan nontreated microparticles, the inset of Figure 3b shows, comparatively, the pure drug before encapsulation procedure. When increasing the concentrations of chitosans employed, the magnitude of fracturing was lowered. These pores and fractures are the points through which the dissolution medium enters first. Further, a close scrutiny of microparticles prepared with either grade of chitosans used reveals the presence of a very fine mesh-like structure because of a polyelectrolyte reaction (Figure 3d). This structure is quite comparable with the other micrographs where alginate–chitosan polyelectrolyte complex is likely to be present[37]. The surface of all observed model drug-loaded microparticles appeared rough, with invaginations originating from the encapsulated phenytoin particles which can be anticipated underneath the surface.

Thermal analysis

The DSC thermograms of phenytoin, sodium alginate, blank alginate–chitosan microparticles, phenytoin-loaded alginate–chitosan microparticles, and chitosan are shown in Figure 4. The thermogram of sodium alginate is characterized by an endothermic peak around 105°C and a sharp exothermic peak at 250°C. The first peak corresponds to a dehydration process, while the second one denotes a degradation exotherm[18,29]. This degradation exotherm of sodium alginate was missing in blank microparticles, and a broad exothermic peak appeared at a range 215°C–315°C, which can be explained by the 'egg-box' conformation in alginate–chitosan microparticles. An additional broad endothermic peak at about 210°C was observed, which was attributed to an alginate–calcium interaction.

The thermogram of phenytoin is characterized by a sharp endothermic peak at 296°C pointing to its melting point. Considering that this peak is present in phenytoin-loaded alginate–chitosan microparticles, it can be concluded that phenytoin in the dried loaded microparticles is incorporated in its crystalline form. This was not unexpected since phenytoin has very low water solubility.

The thermogram of chitosan showed a broad endotherm around 120°C, corresponding to the water evaporation, and a degradation exotherm at 300°C[29]. These peaks of the single component are not visible when combined into a microparticle. Loss of degradation exotherms of chitosan and alginate from final formulations may be explained by the chemical reaction occurring in solution, which modified chitosan, and also by strong stabilizing interaction in the microparticle, since degradation exotherms of single polymeric components are not present in the final formulation.

Drug assay

Both series of formulations, one hardened for 1 hour and the other hardened for 24 hours, independently of the type of chitosan used, appeared to be highly loaded with the model drug (Table 2).

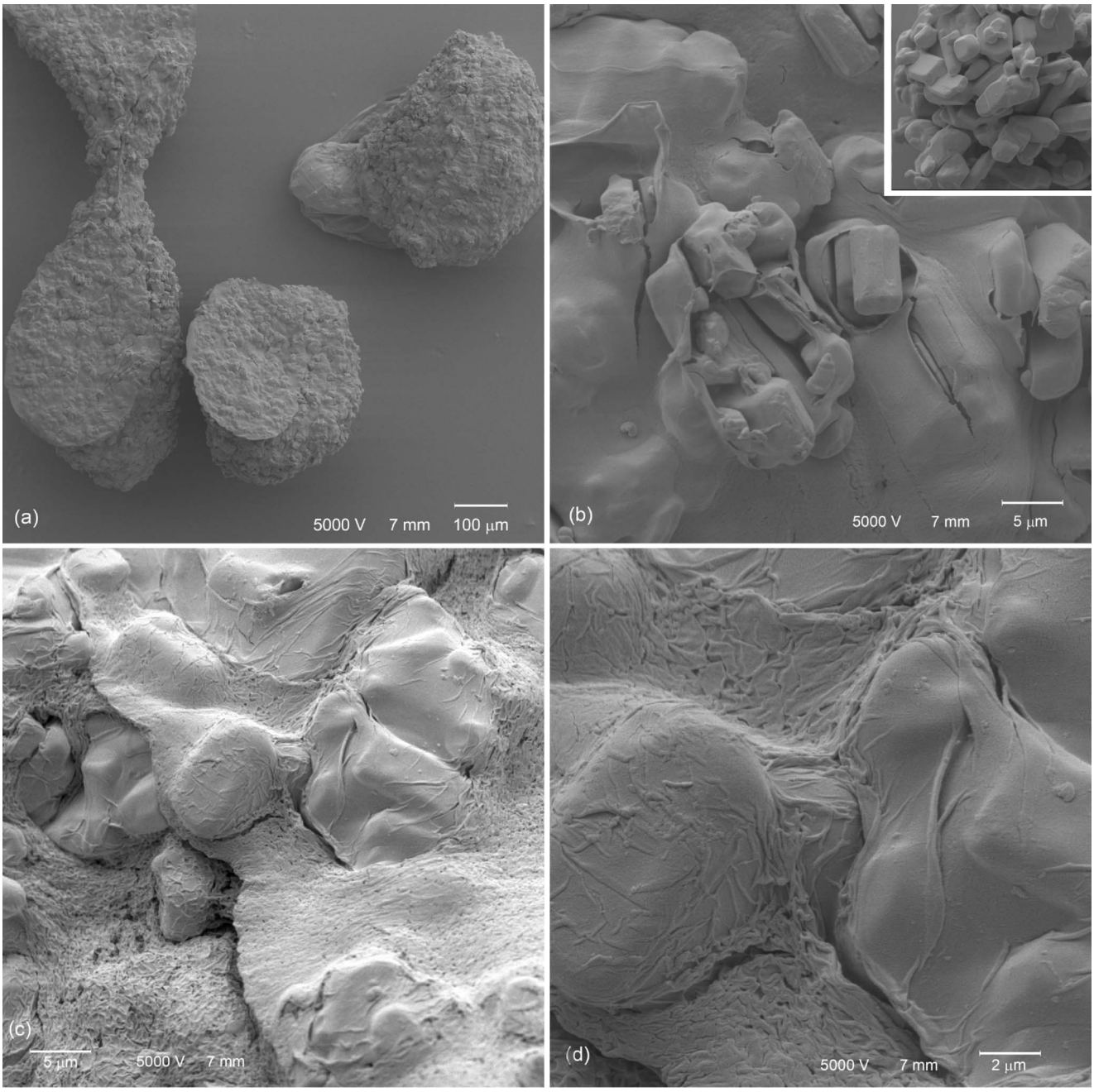

Figure 3. (a) SEM micrograph of formulation F9H′ made with 0.2% of high MW chitosan; (b) micrograph of formulation F1 made without chitosan; in the inset is the micrograph of phenytoin alone before the encapsulation procedure; (c) micrograph of formulation F7′ made with 0.2% of combination high and low MW chitosans; and (d) mesh-like structure of formulation F7′ at high magnification (5000×).

The drug contents of the dried microparticles were determined to be more than 90% in all the cases studied. No clear relationship between the drug loading and concentration of the crosslinking ion and chitosan was found, neither any influence of mere chitosan presence of any type used was detected. Further, it appeared that the observed morphological changes did not affect the encapsulation efficiency. Measuring the phenytoin particles in alginate suspension has shown the average particle perimeter of 44 μm[31]. Along with its poor solubility in aqueous media, such a high perimeter possibly prevented the exit of phenytoin particles through the pores of instantaneously formed microparticles, which may add to the high loading outcome. Therefore, the present method is useful to encapsulate small molecular weight drugs with low water solubility.

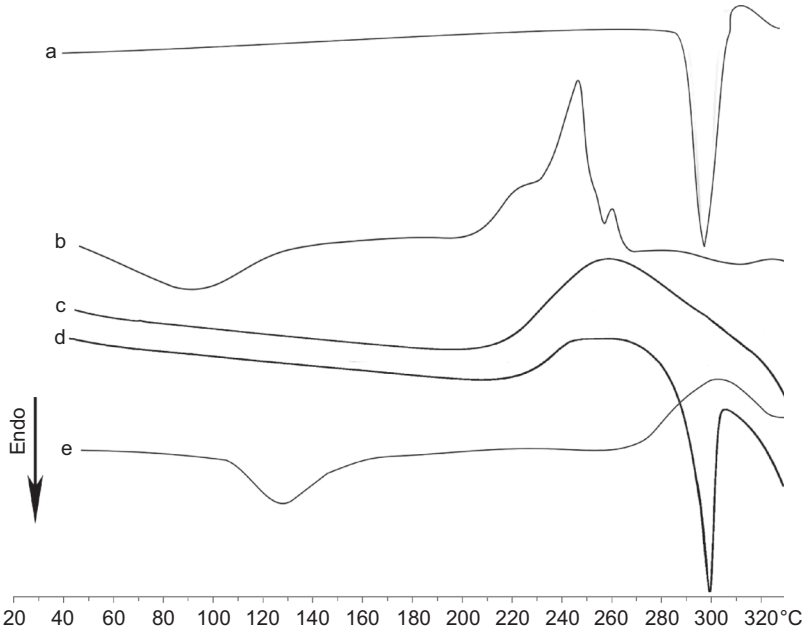

Figure 4. DSC of: (a) phenytoin; (b) sodium alginate; (c) blank alginate–calcium–chitosan microparticles; (d) phenytoin-loaded alginate–calcium–chitosan microparticles; and (e) chitosan.

Table 2. Drug loading of 24 studied model formulations.

Formulation	Drug loading (% w/w)	Formulation	Drug loading (% w/w)
F4	96.02 ± 4.10	F4′	92.31 ± 1.97
F6	96.34 ± 1.99	F6′	93.35 ± 2.07
F7	90.63 ± 3.87	F7′	95.32 ± 2.56
F9	95.88 ± 2.73	F9′	94.23 ± 3.24
F4L	96.11 ± 2.07	F4L′	93.47 ± 2.52
F6L	95.27 ± 2.89	F6L′	91.54 ± 2.85
F7L	94.42 ± 2.82	F7L′	96.04 ± 3.45
F9L	96.37 ± 3.01	F9L′	93.47 ± 3.80
F4H	94.83 ± 3.08	F4H′	93.43 ± 2.90
F6H	93.89 ± 2.14	F6H′	94.92 ± 2.87
F7H	98.77 ± 3.37	F7H′	95.22 ± 3.48
F9H	92.61 ± 4.06	F9H′	92.72 ± 3.96

Drug release studies

Figure 5 shows the phenytoin release profiles from alginate–chitosan microparticle formulations manufactured in one-step procedure using external source of the crosslinking ion, with three types of chitosan studied, and hardened for 1 or 24 hours. The times for 50% and 90% of the drug to be released ($t_{50\%}$ and $t_{90\%}$, respectively), as the parameters suitable for comparing the different formulations[12,18], are shown in Table 3.

The influence of chitosan coating on phenytoin release cannot be clearly deduced from the release profiles we found. On the other hand, the increased hardening time brought forward further improvement of phenytoin release profile, in that it tended to decrease the release of phenytoin in the release medium (Figure 5a–c). Such improvement of the phenytoin release profile was particularly apparent when comparing the $t_{50\%}$ and $t_{90\%}$ values of F9 formulation, which amounted 18 and 130 minutes, respectively, with formulation F9′, where these parameters were 140 and 293 minutes, respectively (Table 3, Figure 5a). Polk et al.[34] reported that no difference existed when hardening time was increased, presumably because of the fact that reaction time required for ionotropic gelation and polyionic complexation is very short. On the contrary, Sankalia et al.[18] reported increased $t_{50\%}$ and $t_{90\%}$ values with increased hardening time. However, in the latter study, chitosan coating was not employed, and the difference in hardening time of about 10 minutes was much lower compared to this study. Probably, this can be attributed to further decreasing the permeability (decreasing magnitude and size of fractures) of the microparticle surface with chitosan coating employed, and subsequent suppression of erosion in the release medium (Figure 3b and c). The exceptions were formulations F4 and F6, incubated with the mixture of low and high molecular weight chitosan chains, where increased hardening time increased the release of the model drug (Table 3, Figure 5a). This anomaly could not be easily explained with the phenomenon of destroying the integrity of chitosan–alginate complex membrane during the drying process[6,34,35], since with higher concentration of the same chitosan type this was not true, nor SEM examination (micrographs not

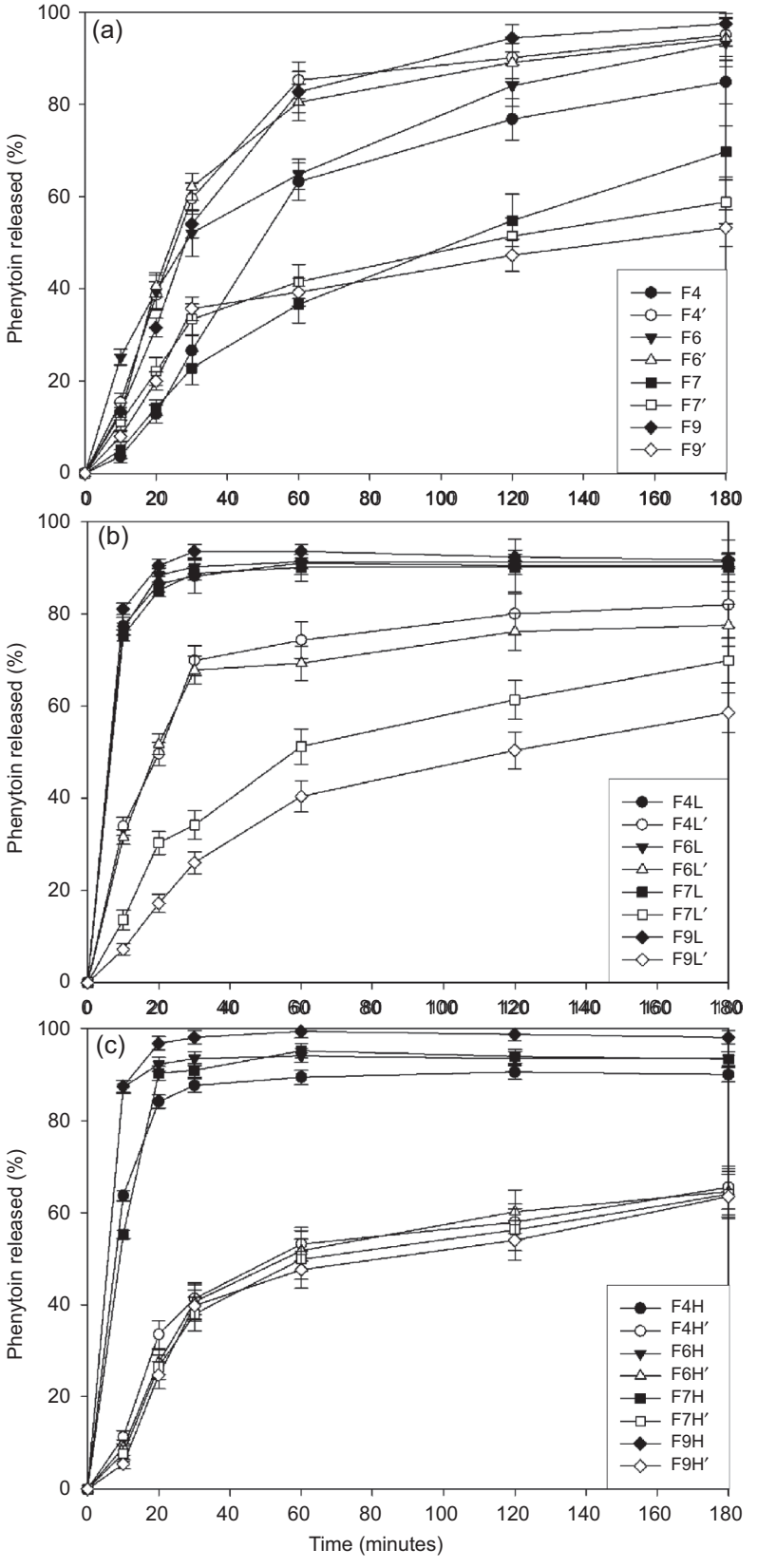

Figure 5. Effect of hardening time on release characteristics of alginate–chitosan microparticles made with (a) combination of high and low MW chitosans; (b) low MW chitosan; and (c) high MW chitosan.

Table 3. Times for 50% and 90% of the drug to be released, $t_{50\%}$ and $t_{90\%}$, respectively.

Formulation	F4L	F6L	F7L	F9L
$t_{50\%\,(\text{minutes})}$	9.94	9.71	10.12	9.26
$t_{90\%\,(\text{minutes})}$	62.44	59.91	64.50	54.99
Formulation	F4H	F6H	F7H	F9H
$t_{50\%\,(\text{minutes})}$	10.55	8.93	9.75	8.08
$t_{90\%\,(\text{minutes})}$	66.54	51.44	60.35	42.99
Formulation	F4	F6	F7	F9
$t_{50\%\,(\text{minutes})}$	46.40	21.53	114.79	17.79
$t_{90\%\,(\text{minutes})}$	163.45	147.01	219.50	130.55
Formulation	F4L′	F6L′	F7L′	F9L′
$t_{50\%\,(\text{minutes})}$	18.76	21.45	97.12	129.34
$t_{90\%\,(\text{minutes})}$	163.02	178.73	213.37	258.70
Formulation	F4H′	F6H′	F7H′	F9H′
$t_{50\%\,(\text{minutes})}$	100.55	102.73	108.26	111.96
$t_{90\%\,(\text{minutes})}$	230.63	231.68	233.85	238.50
Formulation	F4′	F6′	F7′	F9′
$t_{50\%\,(\text{minutes})}$	17.10	17.73	124.80	140.11
$t_{90\%\,(\text{minutes})}$	133.41	135.94	262.78	293.19

shown) or DSC analysis revealed such an effect. In the series of microparticle formulations with corresponding calcium chloride concentration hardened for 1 hour, it was unexpectedly observed that the increase of either type of chitosan coating over 0.1% markedly accelerated phenytoin release. The exceptions were formulation F7, prepared with 0.5% calcium chloride and 0.2% chitosan and, to much lesser extent, formulation F7L (Table 3). For example, $t_{90\%}$ values for formulations F9H and F9 prepared with 0.2% of chitosan were 43 and 130 minutes, respectively, whereas for formulations F6H and F6 prepared with 0.1% of chitosan the $t_{90\%}$ values were 51 and 147 minutes, respectively. The similar phenomena were reported earlier[6,34,35]; some authors attributed them to destroying of the integrity of chitosan–alginate complex membrane during the drying process[35]. Notably, a distinct delay in release of phenytoin from microparticles was observed for formulation F7, compared to all other formulations in this series (Table 3, Figure 5a).

When examining the influence of the type of chitosan present in the gelling medium, the greatest effect was noticed when the microparticles were prepared with a combination of high and low molecular weight chitosans (formulations F4–F9 and F7′–F9′). Polk et al.[34] reported similar data and attributed this significant decrease in microparticles permeability to a combination of increased chitosan–alginate ionic interaction and to an enhanced interpenetration of polymer network by mixture of chitosans and crosslinking ions. This greater penetration would have produced stronger enhancement of alginate–chitosan membrane, and thus created a greater barrier to erosion and, subsequently, barrier to liberation of the drug.

The influence of calcium chloride concentration on phenytoin release in both series of microparticle formulations was also examined. The $t_{50\%}$ and $t_{90\%}$ values of formulations incubated for 1 hour, with the corresponding type and concentration of chitosan used (F4L-F9L, F4H-F9H, F4-F9), indicated that the incremental addition of calcium chloride increases drug release. Similar data were reported earlier[38], with an explanation that increased concentration of crosslinking ions yields increased porosity of the formed network gel. In our study, this effect was much more pronounced for the mixture of chitosans. For example, $t_{90\%}$ value of formulation F4 prepared with 0.5% of calcium chloride was 163 minutes, whereas $t_{90\%}$ value of formulation F6 prepared with 2.0% of the source of crosslinking ions was 147 minutes. Likewise, $t_{90\%}$ values for formulations F7 and F9 were 219 and 130 minutes, respectively. Conversely, in the series of microparticle formulations hardened for 24 hours, the $t_{50\%}$ and $t_{90\%}$ values indicated that increasing calcium chloride concentration results in decreased drug release rates for each group of particular concentration and type of chitosan used. Similar data were published by Sankalia et al.[18]. This phenomenon was more pronounced with low molecular weight chitosan, and when 0.2% of mixture of chitosans was employed. Namely, the $t_{90\%}$ values for formulations F4L′ and F6L′ were 163 and 178 minutes, respectively, while the same parameters for formulations F7L′ and F9L′ were 213 and 259 minutes, respectively. Similarly, the $t_{90\%}$ values for formulations F7′ and F9′amounted 263 and 293 minutes, respectively. It appears that combination of the prolonged hardening time and increased calcium chloride concentration, resulting in the increased porosity of the formed alginate gel, could create conditions for increased crosslinking and more thorough interpenetration of polymer network in the case of low molecular chains of chitosan present in the hardening medium.

It is known that alginate–chitosan polyelectrolyte complex erodes slowly in environments at pH values higher than 6.5, thus leading to the suppression of the initial drug release in the stomach and controlling the release in the lower segments of gastrointestinal tract[6,13,17]. Taking into consideration the possible shortcoming of commercial formulations with phenytoin, which deals with the potential for irregular absorption[32], as well as the ability of multiparticulate systems to exclude to a large extent the influence of physiological changes in gastric emptying time and intestinal motility, an additional benefit of the formulations with longer liberation half-time (e.g., formulation F9′, where $t_{50\%}$ was about 2.5 hours) might be the improvement of the regularity of phenytoin absorption. Further investigations are needed in order to clarify this issue.

Conclusion

The air-jet method used, in the case of small molecular weight drugs of low water solubility, confirms itself as an effective method of producing microparticles of very high model drug loading, the acceptable sphericity and size, with preparation procedure having great influence on the latter parameter, that is, formulations hardened for a longer period of time yielded smaller particles. The results suggest that alginate–chitosan microparticles may be used as a delivery system for delayed release of the kind of drugs used in our study. The key factor found to affect the release of phenytoin was the combination of hardening time and chitosan molecular weight. Namely, in the series of microparticle formulations hardened for 24 hours, as a rule, chitosan coating tended to further decrease the release of model drug compared to particles with 1 hour hardening time, thus offering further retardation and better control of phenytoin release. The delay was marked in the cases where the mixture of high and low molecular weight chitosans was employed. One such formulation, with the liberation half-life of about 2.5 hours, is eligible for further modifications and studies aimed to achieve better control and regularity of phenytoin absorption.

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