Combined Method of Complex Coacervation and Electrospray for Encapsulate Preparation

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ABSTRACT: Encapsulate beads composed of alginate and chitosan as shell and bovine serum albumin (BSA) as core were prepared by combined method of complex coacervation and electrospray. The main objective of this work was to produce mono-sized and spherical capsule of chitosan-alginate with controlled sizes of capsules and shell. However, the effects of applied voltage, flow rate, and molecular weight of chitosan were investigated on the size, size distribution, membrane thickness of the prepared capsules, as well as the release rate of BSA. The results revealed that by the method developed in this study, it was possible to produce spherical capsules with controlled size and narrow size distribution. Increasing the voltage and decreasing the flow rate reduced the radius of capsule and its shell thickness from 2.09 mm to 750 μm and from 1.31 mm to 490 μm, respectively. Furthermore, the molecular weight of chitosan had no significant effect on the capsules' size and the release rate of BSA, whereas the rate of BSA release was increased with increase of the voltage. The later effect would be due to the increase of shell porosity at the higher voltages. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 322–328, 2010

Key words: alginate; chitosan; core-shell beads; particle size distributions; release properties

INTRODUCTION

Encapsulation of active agents into polymeric membranes has broad fields of applications, such as encapsulation of flavors in food industries,[1] drugs and proteins in drug delivery systems,[2,3] and enzymes in biosensors.[4] Chitosan and alginate are two natural macromolecules that have been used widely in the above-mentioned areas. Chitosan is a biomacromolecule and can be used for wall material capsule in food and drug industries,[5,6] protein and DNA encapsulation,[7,8] and chemical and biochemical areas.[9] Alginate is a random, linear, and anionic polysaccharide macromolecule. This macromolecule can be used in biomedical applications, including drug delivery systems[10] and cell encapsulation etc.[11] Chitosan-alginate polyionic complexes are formed through the ionic gelation via interaction between the carboxyl groups of alginate and the amine groups of chitosan.[6] Chitosan-alginate microspheres or beads have been prepared by different methods, including emulsion crosslinking,[12] coacervation/precipitation,[6] spray drying,[9] emulsion-droplet coalescence,[13] and sieving methods.[14]

A relatively simple method for preparing chitosan-alginate capsule is complex coacervation.[15] Coacervation is the separation of an aqueous polymeric solution into two distinct liquid phases: a dense coacervate phase, and a dilute equilibrium phase.[16] The coacervation process may be divided into two types: simple and complex, depending on the number of polymeric ingredients.[17] Complex coacervation refers to the separation of an oppositely charged polyion mixture into two distinct phases. Neutralization of the positive charge of one of the polymers by the negative charge of the other causes phase separation of the polymer-rich phase.[18] This technique is widely used for microencapsulation. In surfactant-free complex coacervation, shape, size, and size distribution of the produced capsules are mainly controlled by the method used in droplet formation. Simple dripping and other methods of droplet formation, such as mechanical vibrating nozzle, atomizing air jet, or simply a laminar jet may not work satisfactorily for viscous liquids such as alginate solutions. The viscosity of these liquids varies in the range of 1000-5000 mPa s even sometimes up to 25,000 mPa s, depending on its concentration.[19] Electrospray is a technique in which small droplets (some times smaller than 100μm) may be produced from the high-viscous liquids depending on their conductivity. The idea of using an electrostatic field between a capillary tip such as a nozzle and a flat counter electrode was initially used to reduce the diameter of droplets by applying an additional force

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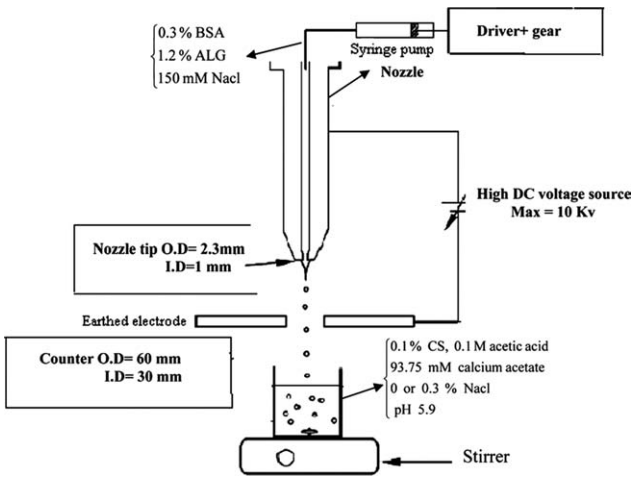

Figure 1 Schematic diagram of experimental apparatus.

(i.e., electric force) in the direction of gravitational force to overcome the upward capillary force of liquid on the tip of nozzle. Application of an electrostatic field in liquid spraying provides an external force that can be manipulated to control the size and shape of droplets.[20] Thus, using the method of combined complex coacervation and electrospray may lead to spherical beads with uniform size distributions, where the size of droplets may be controlled easily by electric field intensity (i.e., voltage).

The main aim of this work was to produce spherical beads of chitosan-alginate with controlled size and uniform size distributions, using combined method of complex coacervation and electrospray. However, the effects of applied voltage, flow rate, and molecular weight of chitosan were investigated on the size, size distribution, and membrane thickness of the prepared beads, as well as the release rate of encapsulated drug. To study the release properties of the prepared beads, bovine serum albumin (BSA) was encapsulated in the chitosan-alginate shell.

EXPERIMENTAL

Materials

Alginic acid sodium alginate (medium viscosity, 3500 cps, 2% w/v aqueous solution at 25°C, from Sigma-Aldrich, USA) and chitosan with different molecular weights (1.5, 4, and 6×10^6 Da, from Fluka, USA) were used as coating macromolecules in the preparation of beads. Acetic acid (98%), sodium chloride, and calcium acetate were obtained from Merck Chemical Co., Germany. BSA from Merck Chemical Co. was used as a model protein for the encapsulation. All reagents were at least of analytical grade.

Preparation of capsules

Figure 1 shows the schematic diagram for generation of beads. The solution of 1.2% w/v alginate, 0.3% w/v sodium chloride, and 0.3% w/v BSA flowed through a stainless steel nozzle using a syringe pump at certain flow rate adjusted in 75 or 100 mL/h. Electric field strength was set using a high voltage, D.C. power supply. The positive and negative potential polarities of the power supply were connected to a nozzle and a stainless steel ground electrode, respectively. Distance between the nozzle and the ground electrode was adjusted by two polyethylene insulator plates. The liquid meniscus at the tip of nozzle was affected by the electric field where droplets were formed and drown down. Prevailing of the electric field and gravitational forces on the upward surface tension of liquid capillary leads to the separation of droplet. The droplet was dripped into a container where the complex coacervation process of droplet was occurred. The solution in the container was made of chitosan (0.1% w/v), acetic acid (0.1 *M*), and calcium acetate (93.75 m*M*) where the pH was adjusted at 5.9. The solution was gently stirred for 5 min while the droplets were cured. The cured capsules were then collected and washed for further size analysis and release test.

Capsules size and membrane thickness measurement

The size of wet capsules and the thickness of their membranes were measured by analyzing the digital images captured by a CCD camera installed on a microscope, using Motic Images Advanced software. The average diameter (or radius) and shell thickness of these capsules were reported as the mean particle size and the membrane thickness, respectively.

Evaluation of protein release

The protein release profiles of the capsules were determined using distilled water. About 5 g of the sample was placed in 100 mL of distilled water container where the system was agitated at 90 rpm in a shaker bath (Memmert GmbH, Germany) at 37°C ± 0.2. Aliquots were piped out at various time intervals. The Bio-Rad protein assay method was used to measure the protein content by a spectrophotometer (Shimadzu UV-160). The percentage of BSA release was calculated from the change of protein concentration during the time course.

RESULTS AND DISCUSSION

In this work, the ability of the developed method was investigated to prepare spherical capsules with

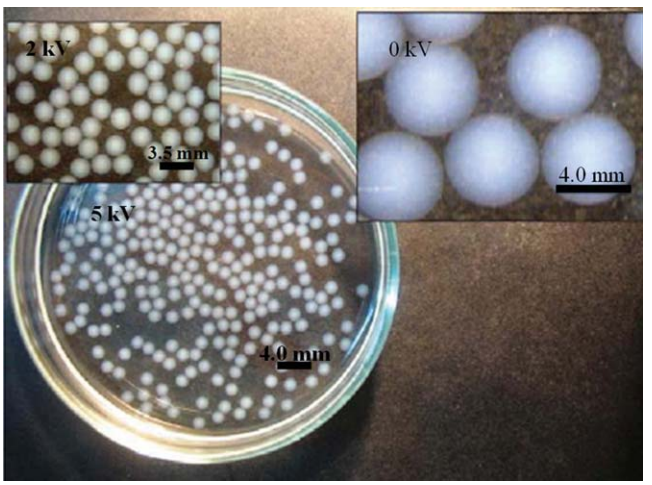

Figure 2 Image of the prepared capsules by combined electrospray and complex coacervation method at 100 mL/h and different applied voltages. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

controlled size and narrow size distribution. Figure 2 represents the prepared capsules by combined method of complex coacervation and electrospray in different operational conditions. This figure indicates that in all conditions spherical capsules with uniform size distribution could be prepared. Figure 3 shows the image of two distinct polymeric layers consisting of chitosan and alginate as shell and a core of active agent (BSA). Several parameters may affect properties of the prepared core-shell beads (i.e., molecular weight of chitosan, electric field intensity, volumetric flow rate of alginate-BSA solution, concentration of solution, nozzle diameter, and diameter of counter electrode). However, in this study, the effects of molecular weight of chitosan, intensity of electric field, and volumetric flow rate of alginate-BSA solution were investigated on the size, size distribution, membrane thickness, and release properties of the prepared capsules.

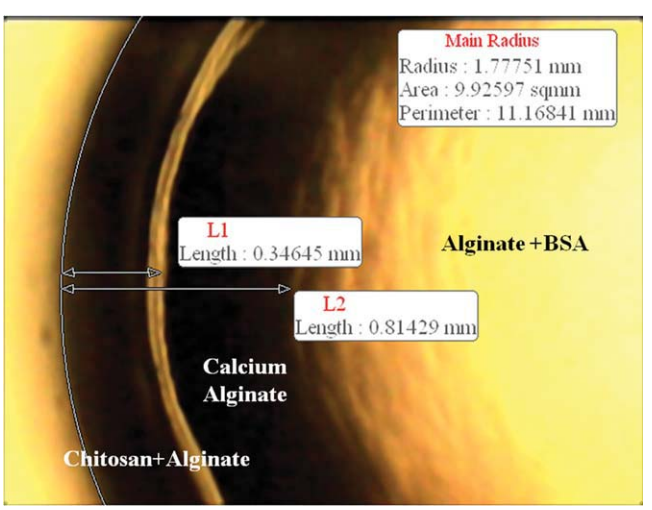

Figure 3 Structure of core-shell beads prepared by combined electrospray and complex coacervation method. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Effect of chitosan molecular weight on size of capsules

The effect of chitosan molecular weights on the radius of prepared beads is presented in Table I. The capsules were prepared on the following operational conditions: applied voltage (0, 4, and 7 kV), volumetric flow rate of alginate-BSA solution (100 mL/h), and nozzle diameter (1 mm). Chitosan with three different molecular weights (1.5, 4.0, and 6.0 $\times$ 10^5 Da) were used in this set of experiments. The results presented in Table I show that chitosan molecular weights have no significant effects on the size of the prepared capsules. Chen and Tsiah[15] have also reported the same results.

Effect of electrical field intensity on size of capsules

Intensity of electrical field is the most important parameter affecting the properties of prepared capsules. Electrical field intensity is obtained from eq. (1):

$$E_0 = \frac{V}{AR' \ln\left(\frac{4D}{R}\right)} \quad (1)$$

where V, R, and D are voltage, inside diameter of counter electrode, and distance between the nozzle and counter electrode, respectively. In eq. (1), the constant A equals to $\frac{1}{\sqrt{2}}$.[19] In this article, applied voltage was selected as a variable parameter and R, and D were fixed to 10 mm and 30 mm, respectively.

Effect of applied voltage was investigated using two different volumetric flow rates as shown in Figure 4. Increasing applied voltage from 0 to 7 kV generally resulted in decreasing beads radius from 2.03 mm to 1.1 mm and from 1.89 mm to 921 μm for 100 and 75 mL/h, respectively. Furthermore, increasing the volumetric flow rate of alginate-BSA solution increased the size of the capsules. As shown

TABLE I
Effect of Chitosan Molecular Weight on the Radius of Prepared Capsules

	Capsules radius (mm)		
Voltage (kV)	Low molecular weight chitosan	Medium molecular weight chitosan	High molecular weight chitosan
0	2.09 ± 0.0096	2.075 ± 0.0091	2.07 ± 0.008
4	1.682 ± 0.088	1.672 ± 0.089	1.669 ± 0.084
7	0.845 ± 0.036	0.844 ± 0.034	0.850 ± 0.031

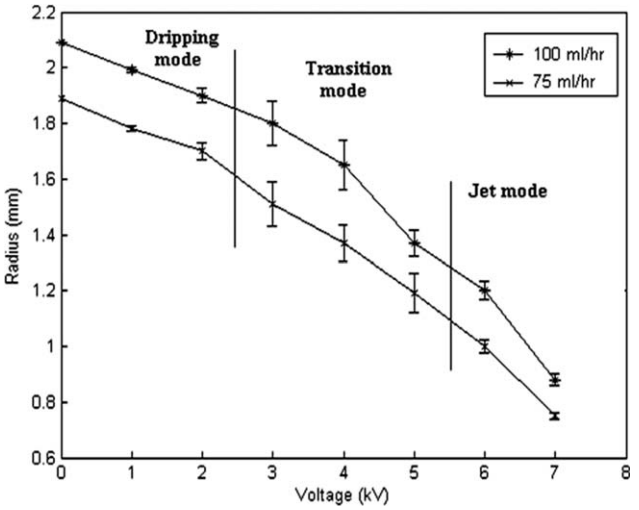

Figure 4 Effect of applied voltage on radius of the prepared capsules.

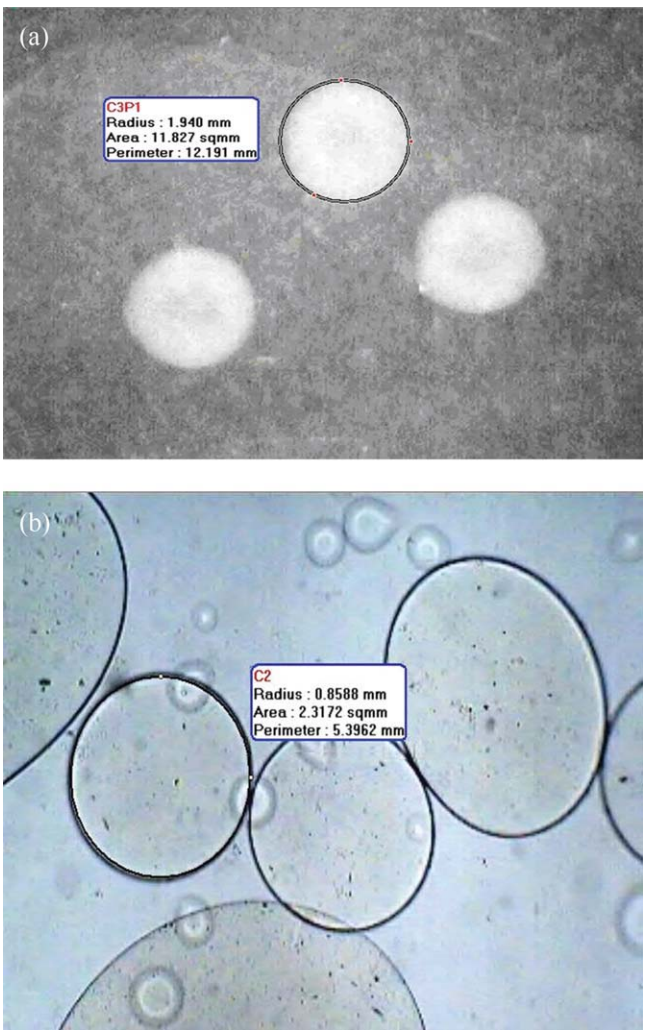

Figure 5 Produced capsules at (a) dripping mode (2 kV, mono-size), and (b) jet mode (7 kV). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

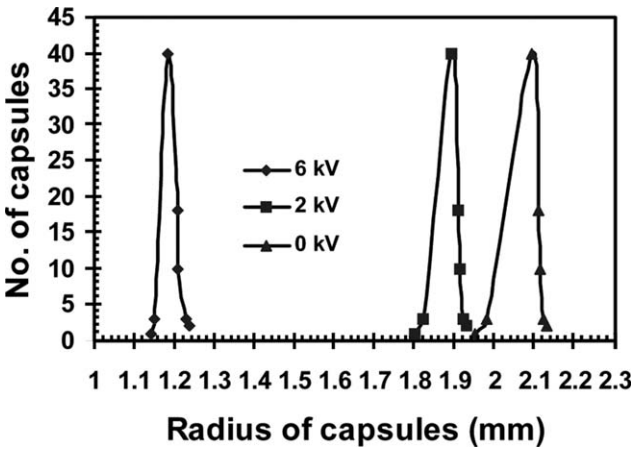

Figure 6 Size distribution of prepared capsules in three applied voltages (0, 2, and 6 kV).

in Figure 4, three distinct modes of bead formation were observed. The first region was dripping mode at the voltage range from 0 to about 3 kV. In this mode, there was a slight change in the size of beads. The main feature of electrospray in dripping mode was mono-size distributions of the beads. In voltage range about 3 to 5 kV, an unstable transition of spraying was observed from dripping mode to jet mode while the sizes of beads were reduced. In this range, a wide size distribution of beads was observed as a result of fluctuation between dripping and jet modes. At the voltages higher than 5 kV, where jet mode was dominated, the size of beads was decreased further. However, at the voltages over 7 kV, no capsules were readily produced as the droplets were drown toward the lateral flat surface of counter electrode and therefore the droplets could not pass through the counter hole. The deflection of droplets was due to high electric field strength as

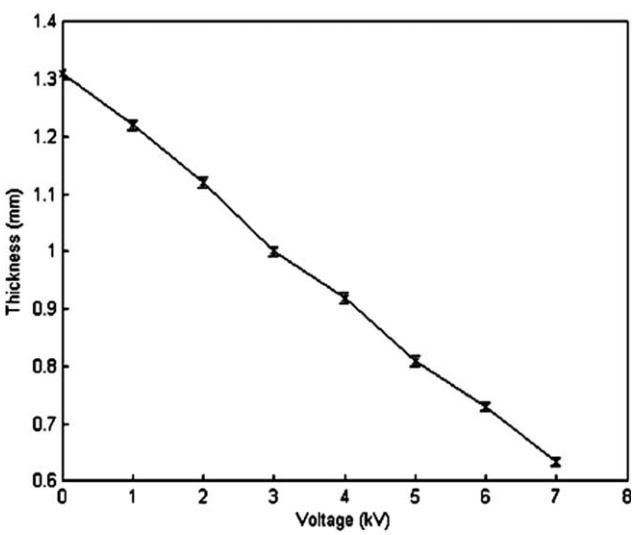

Figure 7 Effect of applied voltage on shell thickness of the prepared capsules (100 mL/h).

TABLE II
Effect of Chitosan Molecular Weight on Release Percent of BSA from Prepared Capsules

		Percentage of release BSA (%)		
Voltage (kV)	Time (h)	Low molecular weight chitosan	Medium molecular weight chitosan	High molecular weight chitosan
0	1	17.9	17.2	16.6
0	5	28.4	28.8	28.0
0	10	41.0	40.2	40.0
4	1	21.0	20.6	20.2
4	5	39.3	39.0	38.0
4	10	47.0	47.0	46.6
7	1	34.0	34.9	34.4
7	5	46.0	45.4	45.0
7	10	60.6	60.1	59.6

well as small size of droplets. Figure 5 shows the beads formed in dripping and jet modes. Comparing the images of beads [i.e., Figure 5(a,b)] in two modes reveals a mono-size distribution beads in dripping mode, despite jet mode in which bimodal size distribution is main feature. The bimodal size distribution of beads is a result of separation of small satellites from the droplets at the nozzle tip.

In Figure 6, the size distribution of beads are presented in three different applied voltages (0, 2, and 6 kV) where at 6 kV the size of satellite is excluded. As it can be seen, using electrospray technique at 2 and 6 kV results in narrower size distributions, as compared to 0 kV.

Effect of electrical field intensity on shell thickness of capsules

Effect of applied voltage on the shell thickness of beads was studied as shown in Figure 7. As it is clear from the figure, increasing the applied voltage leads to decreasing of shell thickness. This is as a result of reducing the size of bead with voltage.

Effect of molecular weight of chitosan on BSA release

Effect of molecular weight of chitosan on BSA release from capsules is presented in Table II. Capsules were prepared using the following operational conditions: applied voltage (0, 4, and 7 kV), volumetric flow rate of alginate-BSA solution (100 mL/h), and nozzle diameter (1 mm). According to the data presented in Table II, there is no significant difference in percentage of BSA released from the capsules with different molecular weight of chitosan but at the same time interval and voltage. However, as it is seen in Table II, with increasing the voltage the release rate of BSA increases.

Effect of electrical field intensity on BSA loading in capsules

Table III shows the percentage of BSA loading in the prepared capsules at different voltages and 100 mL/h. The percentage of loading was calculated using the following equation:

$$\text{BSA Loading Percent} = \left[\frac{\text{Amount of encapsulated BSA}}{\text{Total amount of BSA in pumped solution}}\right] \times 100 \quad (2)$$

As it is seen in Table III, the loading percent of BSA decreases with increase of applied voltage. As shown in previous sections, increasing applied voltage results in decreasing radius and shell thickness of the prepared capsules. On the other hand, decreasing the radius of capsules increases the surface to volume ratio of the beads and therefore, mass-transfer area. Furthermore, decreasing the shell

TABLE III
Effect of Chitosan Molecular Weight and Applied Voltage on BSA Loading in Prepared Capsules

Molecular weight of chitosan	Loading percentage (%) ± SD		
	0 kV	4 kV	7 kV
Low molecular weight	99.5 ± 0.18	98.6 ± 0.21	71.1 ± 0.3
Medium molecular weight	99.7 ± 0.16	98.7 ± 0.23	66 ± 0.32
High molecular weight	99.1 ± 0.17	97.4 ± 0.20	75.3 ± 0.31

thickness of capsules decreases resistance against mass transfer. Considering all the effects mentioned above, the rate of mass transfer is promoted. Therefore, BSA release during capsules curing in chitosan solution increases, whereas the loading percent of BSA decreases.

Effect of electrical field intensity on BSA release

In this work, BSA release from the prepared capsules at three different voltages (0, 4, and 7 kV) was studied in distilled water at 37°C. The results are presented in Figure 8. As it can be seen, the BSA release rate increases with increasing of the applied voltage. Furthermore, the amount of BSA released during 20 h increases with increase of the applied voltage. As noted in previous section due to decreasing the particle size and shell thickness, increasing of voltage results in increasing of mass transfer surface area and decreasing of mass transfer resistance, whereby the BSA release rate and the amount of released BSA increase. The release profiles in Figure 8 show that the release of BSA never reaches close to 100%. BSA shows a negative charge at distilled water (Its pI in water at 25°C is 4.7), whereas chitosan is a positively charged polymer. The presence of permanent positively charged may be responsible for a stronger electrostatic interaction with BSA, preventing its complete release (Fig. 8).

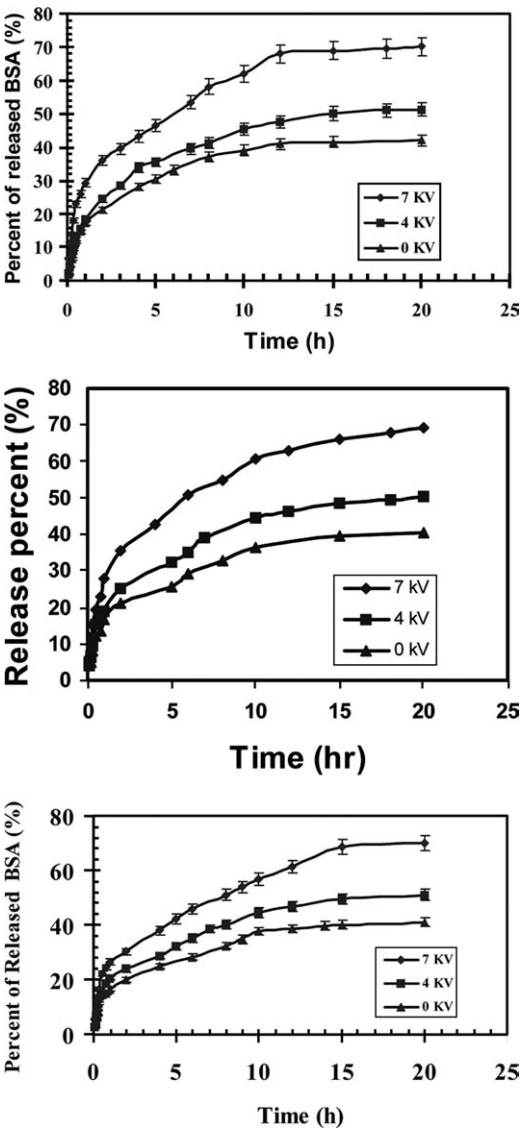

Figure 8 Effect of applied voltage on release percent of BSA from capsules prepared with (a) low molecular weight chitosan, (b) medium molecular weight chitosan, and (c) high molecular weight chitosan. Release percent was measured at 37 ± 0.2°C.

TABLE IV
Radius, Shell, Thickness and BSA Loading Percent of Two Samples Prepared at Different Operational Conditions

Measured Properties	Sample A[a]	Sample B[b]
Average radius (mm)	1.519	1.488
Shell thickness (mm)	1.001	0.991
BSA loading percent (%)	91.3	87.9

[a] Sample A was prepared at 3 kV, and 75 mL/h.
[b] Sample B was prepared at 5 kV, and 100 mL/h.

Effect of electrical field intensity on shell porosity of capsules

To investigate the effect of electrical field intensity on the shell porosity of capsules, two samples were prepared according to conditions presented in Table IV. As indicated in Tale 4, two samples had approximately same size and shell thickness. Figure 9 shows the release profile of BSA from these samples, where the rate of BSA release from Sample B (prepared in

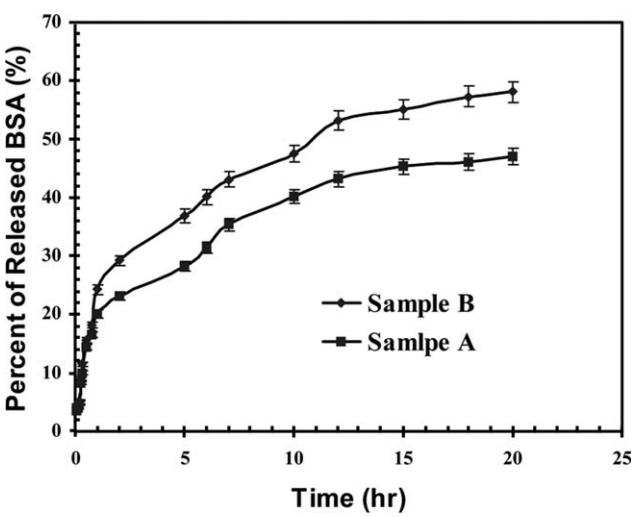

Figure 9 Release profile of BSA from capsules prepared at 3 kV, and 75 mL/h (Sample A), and capsules prepared at 5 kV and 100 mL/h (Sample B).

5 kV) is higher than Sample A (prepared in 3 kV). As two samples are in the same size and shell thickness, thus, it would be concluded that the higher rate of release for Sample B might be due to its higher shell porosity. Therefore, increasing applied voltage would increase the shell porosity of prepared capsules.

CONCLUSIONS

Considering the results presented in this study, it was possible to produce spherical capsules with controlled size and narrow size distribution. By enhancing the voltage and decreasing the volumetric flow rate, the radius of capsules was decreased from 2.09 mm to 750 μm, whereas the shell thickness was decreased from 1.31 mm to 490 μm, approximately. The results revealed that the molecular weight of chitosan had no significant effect on the capsules size and the BSA release rate from prepared capsules, whereas the release rate was increased with increase of voltage. Furthermore, the capsules prepared at the higher voltages would have higher shell porosity.

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