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RESEARCH PAPER

Aqueous Preparation and Evaluation of Albumin-Chitosan Microspheres Containing Indomethacin

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ABSTRACT

Controlled-release egg albumin-chitosan microspheres containing indomethacin as a model drug were successfully prepared by coacervation method. The proposed method can offer a simple method for microsphere preparation in an aqueous system with the elimination of the use of organic solvents that are usually needed in conventional techniques of microencapsulation. The interaction between negatively charged egg albumin molecules in phosphate buffer, pH 7.2, or sodium hydroxide solution and positively charged chitosan molecules dissolved in diluted acetic acid to form an insoluble precipitate was the principle for the formation of the microspheres. The effects of many process variables, such as amount of formaldehyde as a cross-linking agent, stirring time, final pH of encapsulation medium, initial drug loading, and albumin concentration or albumin-to-chitosan weight ratio, on the properties of the prepared microspheres were investigated. Incorporation efficiencies of the microspheres to the drug were high in most cases and ranged between 63.3±3.6% and 92.39±3.2%, while particle sizes were 435.2±12.6 up to 693.9±34.6 μm for the different tested batches. On the other hand, the values of angles of repose and compressibility indices were in the range of 23.5±0.4 to 32.0±0.7 degrees and 11.1±0.7% to 23.6±0.7% respectively, which indicate overall good free flowing nature of the microspheres of all batches. The maximum required amount of the cross-linking agent was determined to avoid excessive unnecessary chemicals. It was also noticed that excessive time of stirring and excessive initial drug loading are not recommended as it may lead to microspheres of low properties. The pH of the encapsulation media (pH 3.77 up to pH 4.91) significantly affected the properties of the microspheres. As the pH of the encapsulation media was increased, the incorporation efficiency, particle size, and flowability decreased, along with increase of drug release rate, which could be related to incomplete cross

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linking of the microspheres matrix. It was also observed that high concentration of albumin solution and accordingly the increase of albumin-to-chitosan weight ratio were accompanied with increases in incorporation efficiency and particle size with improved microsphere flowability and slow indomethacin release. Thus, the proposed microspheres showed the ability to control the release of indomethacin, and their properties were highly affected by many process variables that could be controlled to obtain an optimized system.

Key Words: Albumin-chitosan microspheres; Indomethacin; Coacervation; Microencapsulation; Process variables.

INTRODUCTION

Chitosan, a natural biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin that is found widely in nature. Chitosan has attracted significant interest in recent years. This is largely due to the proposed novel application of the polymer in biomedical, food, and various industrial and biotechnological fields. These applications are possible because of the polymer reactive groups and their biodegradability, low toxicity, and biocompatibility.[1] The amine groups of chitosan are mostly protonated in acidic solutions and the resultant soluble polysaccharide is positively charged. Oppositely charged polyelectrolytes will interact rapidly with chitosan in solution to form an insoluble precipitate. This principle has been used for the production of chitosan microcapsules and microspheres to control drug release.[2] Controlled-release microspheres formulated with bovine serum albumin or egg albumin as a naturally occurring polymer have been also extensively studied using different methods of preparation.[3–8] Albumin is suitable for producing nonantigenic microspheres whose physicochemical properties can be widely moderated according to the cross-linking method employed for their production and the nature of the drug. Albumin microspheres are chemically and physically stable and nonimmunogenic.

Microspheres designed to control drug delivery using a single polymeric material are sometimes unable to fulfill all the required physical properties, encapsulation efficiency, or rate and mechanism of drug release. Thus, a combination of two or more polymers can offer an acceptable means of avoiding such problems in order to achieve optimum control of drug release. Combination of chitosan with other polymers for the production of a sustained release oral dosage form was the interest of many investigators. Prolonged-release microspheres prepared from the combination of chitosan with other naturally occurring polymers such as alginate, pectin,[9] and casein[10] were recently evaluated. On the other hand, combination of albumin with other polymers for the production of microspheres was also investigated. Nishioka et al.[11] prepared cisplatin microspheres by the coacervation technique using albumin and ethylcellulose. Furthermore, chitin was incorporated during preparation of albumin microspheres while chitosan was used to treat the preformed albumin microspheres to control drug release with no immunogenicity in vivo.[12]

The aim of this study was to formulate and prepare prolonged-release indomethacin microspheres made from the combination of egg albumin and chitosan, with the advantage of using an aqueous system. Indomethacin was chosen as a model of poorly soluble acidic drugs. The effects of different process variables on physical properties of the prepared microspheres, including particle size and flowability, were investigated in addition to incorporation efficiency and drug release profile in order to formulate prolonged indomethacin microspheres with acceptably high quality.

EXPERIMENTAL

Materials

High molecular weight chitosan (2,000,000) with viscosity of 2000 mPa.s for 1% w/v solution in 1% v/v acetic acid at 20° C, was purchased from Fluka Chemie AG (Buchs, Switzerland). Egg albumin was obtained from Riedel-deHaen AG (Seelze, Germany). Indomethacin (Winlab Laboratory Chemicals, Leicestershire, United Kingdom) and formaldehyde 40% m/v (Farmitalia Carlo Erba, Milano, Italy) were used as received. Other reagents and solvents were of analytical grade.

Methods

Preparation of Albumin-Chitosan Microspheres

A total of 25 mL of egg albumin solution (5%, 10%, 15%, or 20% w/v) in phosphate buffer pH 7.2 (0.05 M) or in a sodium hydroxide solution of concentration 0.1, 0.2, 0.3, or 0.5 M was freshly prepared to give albumin

dispersions of different pHs (7.09, 12.15, 12.36, 12.56, and 12.78, respectively). The calculated amount of indomethacin was dispersed in 25 mL chitosan solution of concentration 1.5% w/v and pH 3.44 in 5% w/v acetic acid (0.88 M). Chitosan solution containing the drug was transferred into a 200-ml beaker and stirred at the rate of 500 rpm for 5 minutes (Eurostar digital, IKA Labortechnik, Staufen, Germany). Egg albumin solution was slowly added dropwise into the beaker containing the chitosan-drug dispersion with continuous stirring for 15 minutes. Formaldehyde solution (40% m/v) was then added and the reaction was allowed to take place with stirring for 1, 3, 5, or 7 hours. The formed microspheres were filtered using a Buchner funnel and then washed two times each with 20 mL distilled water and then allowed to dry at ambient temperature for 24 hours.

The effect of different concentrations of egg albumin (5%, 10%, 15%, and 20% w/v), initial drug loading (5%, 10%, 20%, 30%, and 40% w/w), amount of added formaldehyde (3%, 6%, 10% v/v), stirring time for 1, 3, 5, or 7 hours, and different final pH of encapsulation media (3.77, 4.18, 4.33, 4.52, and 4.91) on the characteristics of the prepared microspheres were investigated in order to obtain microspheres with acceptable good flowability, high drug content, and slow drug release profile. It was taken in consideration that when a variable was investigated, the others were kept constant.

Particle Size Analysis

Albumin-chitosan microspheres containing indomethacin were placed separately on a set of standard sieves (British Standard) of size range 250–1000 μm and shaken for 15 minutes. The resulting fractions remaining on the sieves were weighed and the mean microspheres diameter was calculated after sieving using the following equation:[13]

$$d_{ave} = \Sigma nd/\Sigma n \qquad (\Sigma n = 100)$$

where d_{ave}=arithmetic mean diameter of microspheres, n=percent weight fraction retained on smaller sieve, and d=arithmetic mean size of openings.

Standard deviation of mean particle size was calculated from particle size determination of three similar batches.

Flow Properties

Flow properties of the microspheres were evaluated by determining the angle of repose and the compressibility index. Static angle of repose was measured according to the fixed funnel and free standing cone method of Banker and Anderson.[14] A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height (1 cm), H, above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel. Thus, with R being the radius of the base of the microspheres conical pile:

$$\tan\theta = H/R \text{ or}$$
$$\theta = \arctan(H/R)$$

where θ=the angle of repose.

Compressibility index (I) values of the microspheres were determined by measuring the initial volume (V_o) and final volume (V) after subjecting to 100 tappings in a graduated measuring cylinder using the equation:

$$I = [1 - V/V_o)] \times 100$$

Each of the reported value of angle of repose and the compressibility index was the average of three determinations.

Determination of Drug Loading and Incorporation Efficiency

An aliquot of 200 mg of each batch of the microspheres was triturated and triplicate samples of 50 mg of the triturate were sonicated each with 10 mL of methanol for 40 min. An aliquot of 100 mL of phosphate buffer, pH 7.2, was then added to the solution and shaken for another 40 minutes. The solution was completed with phosphate buffer (pH 7.2) to 250 mL. An aliquot of 10 mL of each sample solution was filtered through 0.45-μm filter (Millipore, Bedford, MA) and assayed spectrophotometrically for its content of indomethacin at 267 nm. The incorporation efficiency was determined using the relation:

$$(D_e/D_t) \times 100$$

where D_e=experimental drug load, D_t=theoretical drug load, and drug load is defined as the weight of encapsulated drug per weight of microspheres.

The yield of production for each batch was also determined by using the relation:

$$(Y_e/Y_t) \times 100$$

where Y_e=experimental yield in grams and Y_t=theoretical batch size in grams. All reported values were the average of three determinations.

In Vitro Release Studies

Release of indomethacin from albumin-chitosan microspheres of particle size range 355–800 μm was carried out using microspheres equivalent to 50 mg of the drug. The dissolution apparatus [United States Pharmacopeia (USP) I] with 75-rpm basket rotational speed was utilized in the studies. Phosphate buffer pH 6.8 of volume 750 mL was used as a dissolution medium at 37±1° C. The drug concentration and the percentage released were determined with respect to time for 8 hours. The drug release was monitored at 267 nm using a Philips PU 8620 spectrophotometer connected with an IBM computer model P530 equipped with a dissolution system PU 9605/60 software (Pye Unicom Ltd., Cambridge, England). The in vitro release studies were performed in triplicate for each of the samples.

Statistical Analysis

Data of microspheres properties and indomethacin release were compared statistically using unpaired t-test and analysis of variance (ANOVA) at a significant level $P \leq 0.05$.

RESULTS AND DISCUSSION

Chitosan being insoluble at neutral and alkaline pH was dissolved in 5% v/v acetic acid with the formation of acetate salt.[15] Upon dissolution (pH 3.44), the amine groups of the polymer are mostly protonated and the resultant soluble polysaccharide is positively charged. On the other hand, a colloidal dispersion of egg albumin was prepared in a phosphate buffer or alkaline sodium hydroxide solution to get pH in a range of 7.09–12.78. The protein molecules are amphoteric in nature and expected to attain mostly negative charges at solution pH above its isoelectric point. The isoelectric point of egg albumin was reported to be at pH 4.5.[16] Thus, the two oppositely charged polymers (chitosan in acidic pH and albumin at pH higher than its isoelectric point) are expected to interact in the aqueous solution to form an insoluble precipitate, which could be the principles for the formation of albumin-chitosan microspheres. The interaction between the two oppositely charged polymers appeared to be rapid by observing the immediate formation of a precipitate. The fast interaction of chitosan with oppositely charged molecules was also previously observed and described to be rapid or even instantaneous by other investigators.[17,18] One of the important advantages of the proposed method is that organic solvents were avoided during microsphere preparation to eliminate safety hazards, toxicity, and high cost of organic solvents usually used in the conventional technique of microencapsulation. This advantage could be suitable for simplifying the process for obtaining prolonged release microspheres with low cost and high safety, which are priorities in the pharmaceutical industry. Generally, the proposed microencapsulation method showed to give high yield production of the microspheres for all batches and ranged between 96.31 ±1.02% and 89.43±1.83%.

Tables 1–5 show the effect of some process variables on important properties of the prepared albumin-chitosan microspheres, including incorporation efficiency, particle size, angle of repose, and compressibility index. It was clear that the variables differently affected the properties of the microspheres. Incorporation efficiencies were high in most cases and ranged between 63.3±3.6% and 92.39±3.2%, while particles sizes were 435.2±12.6 μm up to 693.9±34.6 μm for the different tested batches. On the other hand, the values of angles of repose and compressibility indices were in the range of 23.5±0.4 degrees to 33.1±0.7 degrees and 11.1±0.7% to 23.6±0.7% respectively, which indicate an overall good free flowing nature for microspheres of all batches. Values of angle of repose $\leq 30°$ usually

Table 1. Effect of concentration of formaldehyde as a cross-acting agent (% v/v) on the characteristics of albumin-chitosan microspheres containing indomethacin (mean±SD).

Formaldehyde concentration (% v/v)	Incorporation efficiency (%)	Particle size (diameter) (μm)	Angle of response (θ) (o)	Compressibility index (I) (%)
3.0	75.5±3.4	435.1±21.9	32.0±0.7	23.6±0.7
6.0	84.3±2.6	579.3±24.9	25.5±0.3	13.7±0.9
10	90.1±3.8	631.3±31.4	23.5±0.4	14.0±0.7

Experimental condition: egg albumin solution, 10% w/v; chitosan solution concentration, 1.5% w/v; initial drug loading, 20% w/w; pH of encapsulation medium, 3.77; stirring time, 3 hours.

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Table 2. Effect of stirring time (hours) on the characteristics of albumin-chitosan microspheres containing indomethacin (mean ± SD).

Stirring time (hours)	Incorporation efficiency (%)	Particle size (diameter) (μm)	Angle of repose (θ) (o)	Compressibility index (I) (%)
1	76.7 ± 4.7	512.2 ± 22.9	29.1 ± 0.6	17.1 ± 0.7
3	84.3 ± 2.6	579.3 ± 24.9	25.5 ± 0.3	13.7 ± 1.1
5	77.7 ± 3.1	565.2 ± 22.8	24.4 ± 0.4	14.3 ± 0.9
7	70.2 ± 2.4	550.9 ± 25.7	26.8 ± 0.5	14.3 ± 0.8

Experimental condition: egg albumin solution concentration, 10% w/v; chitosan solution concentration, 1.5% w/v; initial drug loading, 20% w/w; pH of encapsulation medium, 3.77; using 6.0% v/v formaldehyde as a cross-linking agent.

indicate a free-flowing material, while values of compressibility index reading below 25% usually give rise to good flow characteristics.[14]

Effect of Cross-Linking Agent

Cross linking of the prepared microspheres was necessary to harden the coacervated albumin-chitosan matrix in order to ensure good control of drug release. Microspheres prepared without the addition of formaldehyde were found to be fragile and did not sufficiently control indomethacin release. Cross linking of coacervated chitosan with other polymers was previously shown to be necessary for preparation of controlled-release microspheres.[10,17,19] The cross-linking reaction of aldehydes with these polymers is highly initiated in acidic medium where cross linkers have been selected for reactivity with the available amino groups.[20] Formaldehyde was reported to be used as a cross-linking agent for the production of both chitosan and albumin microspheres[3,4,7,10,17] and was successfully tested in this study. This cross-linking agent is water soluble and can be added and used in aqueous systems.

Table 1 shows the effect of added % v/v formaldehyde solution (40% m/v) on the characteristics of the prepared microspheres. It was clear that the increase of added formaldehyde from 3% into 6% v/v significantly increased the incorporation efficiency of the drug and the particle size of the microspheres, in addition to improving the flowability of the microspheres as indicated from the obtained values of angles of repose and compressibility indices ($P<0.05$). The improvement of the above characteristics could be due to more efficient hardening effect of the matrix of the microspheres at the higher formaldehyde concentration, and these results are in good agreement with previous studies.[21,22] The increase of added formaldehyde from 6% up to 10% v/v showed a small but nonsignificant increase in the above characteristics ($P>0.05$), which

Table 3. Effect of pH of polymer solution on the characteristics of albumin-chitosan microspheres containing indomethacin (mean ± SD).

pH of chitosan solution[a]	pH of albumin solution	Final pH of polymer solution	Incorporation efficiency (%)	Particle size (diameter) (μm)	Angle of repose (θ) (o)	Compressibility index (I) (%)
3.44	7.09[b]	3.77	84.3 ± 2.6	579.3 ± 24.9	25.5 ± 0.3	13.7 ± 1.1
3.44	12.15[c]	4.18	80.3 ± 4.0	531.5 ± 23.6	24.4 ± 0.5	16.1 ± 0.9
3.44	12.36[d]	4.33	74.4 ± 2.9	508.6 ± 21.5	25.5 ± 0.6	18.4 ± 1.2
3.44	12.56[e]	4.52	66.1 ± 3.4	468.9 ± 23.4	27.9 ± 0.6	21.0 ± 1.4
3.44	12.78[f]	4.91	63.3 ± 3.6	445.3 ± 21.9	27.5 ± 0.5	21.7 ± 1.2

Experimental condition: egg albumin solution concentration, 10% w/v; chitosan solution concentration, 1.5% w/v; initial drug loading, 20% w/w; stirring time, 3 hours using 6.0% v/v formaldehyde as a cross-linking agent.
[a]Chitosan dissolved in 5% v/v acetic acid.
[b]Egg albumin dissolved in phosphate buffer pH 7.2.
[c]Egg albumin dissolved in 0.1 M sodium hydroxide solution.
[d]Egg albumin dissolved in 0.2 M sodium hydroxide solution.
[e]Egg albumin dissolved in 0.3 M sodium hydroxide solution.
[f]Egg albumin dissolved in 0.5 M sodium hydroxide solution.

Table 4. Effect of initial drug loading (% w/w) on the characteristics of albumin-chitosan microspheres containing indomethacin (mean ± SD).

Initial drug loading (% w/w)	Incorporation efficiency (%)	Particle size (diameter) (μm)	Angle of repose (θ) (o)	Compressibility index (I) (%)
5	84.7 ± 4.0	489.2 ± 24.6	28.8 ± 0.6	16.3 ± 1.3
10	87.5 ± 2.1	534.5 ± 17.0	26.6 ± 0.4	11.1 ± 0.8
20	84.3 ± 2.6	579.3 ± 24.9	25.5 ± 0.3	13.7 ± 1.0
30	88.1 ± 3.4	635.5 ± 27.5	25.8 ± 0.6	14.8 ± 0.8
40	87.7 ± 5.8	501.4 ± 27.0	27.8 ± 0.5	15.6 ± 1.1

Experimental condition: egg albumin solution concentration, 10% w/v; chitosan solution concentration, 1.5% w/v; pH of media is 3.77; stirring time, 3 hours using 6.0% v/v formaldehyde as a cross-linking agent.

could indicate that no further hardening of the microspheres was indicated after the addition of 6% v/v or more formaldehyde. On the other hand, the release profile of indomethacin from the microspheres (Fig. 1) shows that the low amount of cross-linking agent is accompanied by fast drug release, and as the volume of added formaldehyde was increased from 3% to 6% v/v, the release of indomethacin slowed down. However, the increase in formaldehyde volume up to 10% v/v showed to have a nonsignificant effect on drug release ($P > 0.05$) as tested at 2, 4, 6, and 8 hours of drug release. This could indicate that 6% v/v of added formaldehyde solution (40% m/v) is sufficient to give the required hardening for the microspheres to control drug release where excessive cross linking agent is not preferred.

Effect of Stirring Time

Preliminary studies showed that the stirring rate of 500 rpm is suitable to obtain clearly distinct microspheres with acceptable appearance. A slower stirring rate (300 rpm) gave aggregated nonspherical particles, while a faster rate (700 rpm) gave much smaller but cohered particles. However, it was necessary to determine the required stirring time and how it may affect the properties of the microspheres. Stirring time is expected to be mainly the time for cross-linking reaction, while coacervation reaction is expected to be instantaneous at the experimental conditions.

Table 2 shows the effect of stirring time on the characteristics of the microspheres. Stirring for 3 hours gave a higher drug incorporation efficiency as well as larger particle size with improved flowability compared with stirring for 1 hour. However, it was clear that 5 and 7 hours of stirring gave lower drug incorporation efficiency than 3 hours of stirring. This may indicate that 3 hours of stirring is sufficient for producing the required microspheres, while long stirring allows for some more indomethacin to be lost in the aqueous system. This conclusion was in agreement with Bodmeier and Wang[23] and Bayomi et al.[10] On the other hand, change in particle sizes and flowability was not significant ($P > 0.05$) on long stirring. Figure 2a shows the spherical morphology of the microspheres that were prepared by stirring at 500 rpm for 3 hours in presence of 6% v/v formaldehyde at a standard condition (chitosan solution concentration 1.5% w/v; albumin solution concentration 10% w/v; initial drug loading 20% w/w; pH of encapsulation medium 3.77).

Table 5. Effect of egg albumin solution concentration on the characteristics of albumin-chitosan microspheres containing indomethacin (mean ± SD).

Albumin solution conc. (% w/v)	Albumin-to-chitosan ratio (w:w)	Incorporation efficiency (%)	Particle size (diameter) (μm)	Angle of repose (θ) (o)	Compressibility index (I) (%)
5	3.3:1	68.3 ± 2.8	435.2 ± 12.6	27.8 ± 0.5	16.6 ± 0.9
10	6.7:1	84.3 ± 2.6	579.3 ± 24.9	25.5 ± 0.3	13.7 ± 0.8
15	10.0:1	88.5 ± 3.1	611.9 ± 25.9	24.4 ± 0.3	14.4 ± 1.1
20	13.3:1	92.4 ± 3.2	693.9 ± 34.6	24.4 ± 0.4	11.1 ± 0.7

Experimental conditions: chitosan concentration, 1.5% w/v; initial drug loading, 20% w/w; pH of encapsulation medium adjusted to 3.77; formaldehyde solution, 6.0% v/v as a cross-linking agent, and stirring time for 3 hours.

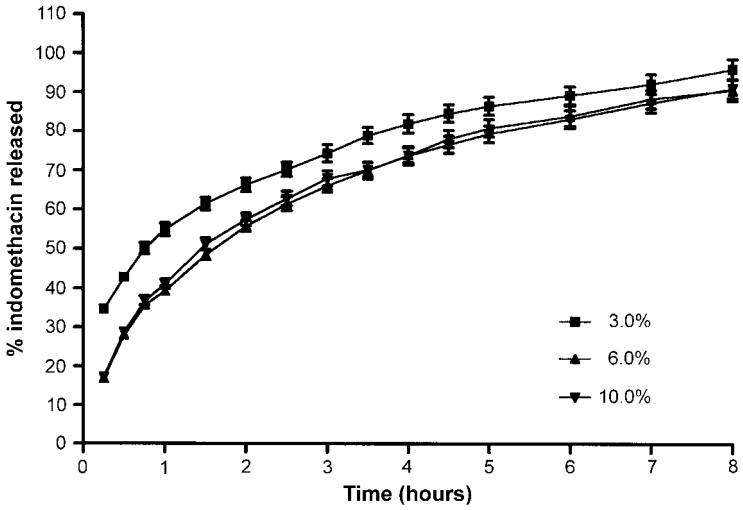

Figure 1. Effect of amount (% v/v) of formaldehyde solution 40% m/v on the release of indomethacin from albumin-chitosan microspheres (n=3).

Figure 3 shows also that 1 hour of stirring is accompanied by significant fast indomethacin release ($P<0.05$) compared with stirring at 3, 5, and 7 hours, which may indicate that 1 hour of stirring led to incomplete cross linking. Stirring for 3 or more hours, however, gave slower and comparable drug release, which indicates maximum microspheres hardening within 3 hours. Thus, 6% v/v formaldehyde and 3 hours stirring time are suggested to be suitable for the production of the albumin-chitosan microspheres.

Effect of pH of Polymers Solution

Chitosan solution at concentration of 1.5% w/v in 5% acetic acid had a pH of 3.44. At this acidic pH, chitosan molecules are expected to be mostly protonated and can interact with negatively charged sites of egg albumin molecules prepared in solution at pH higher than its isoelectric point. Initial mixing of the two solutions resulted in the formation of coacervated product. The mixing of the two-polymer solution should end up with encapsulation media bearing a final pH value depending on pH of egg albumin dispersion. No interaction is expected between albumin and chitosan molecules at pHs below or even equal to the isoelectric point of egg albumin. Thus, the final pH of encapsulation media can only affect the cross linking reaction with formaldehyde as long as the coacervation process was initially formed on mixing of the two polymers. The pH of the albumin dispersion media was carefully chosen to end up with final encapsulation pH that covers a pH range below, at, and above the isoelectric point of the protein. Table 3 shows the effect of pH of the encapsulation media on the physical properties and drug incorporation efficiency of the produced microspheres. It is notable that microspheres were successfully prepared at all tested pHs although the final pHs were in some cases less than the isoelectric point of egg albumin (pH 3.77, 4.18, and 4.33), at pH close to isoelectric point (pH 4.52) and at higher pH (pH 4.91). This could indicate that initially when the two oppositely charged polymer molecules were gradually mixed, immediate interaction occurred with the formation of the insoluble coacervate. However, the effect of final pH on the properties of the prepared microspheres could be related mostly to cross-linking reaction. The highest incorporation efficiency, largest particle size, and best flow properties of the microspheres were obtained at the final pH 3.77, while the lowest incorporation efficiency, smallest particle size, and least flowability were obtained at pH 4.91 with nonspherical appearance of the microspheres, as shown in Fig. 2b. The cross-linking interaction was initiated at acidic pH[20] in the presence of amino groups, and as the pH of solution was increased, the efficiency of cross-linking reaction was decreased and the microspheres became less rigid, resulting in irregular particles with a decrease in particle size and corresponding flowability of the particles. This trend of changes was fast at pH 4.52, which is close to the isoelectric point of egg albumin where the molecules are nearly overall electroneutral and incomplete coacervation (if any) and/or incomplete cross linking of the microspheres is possible. The

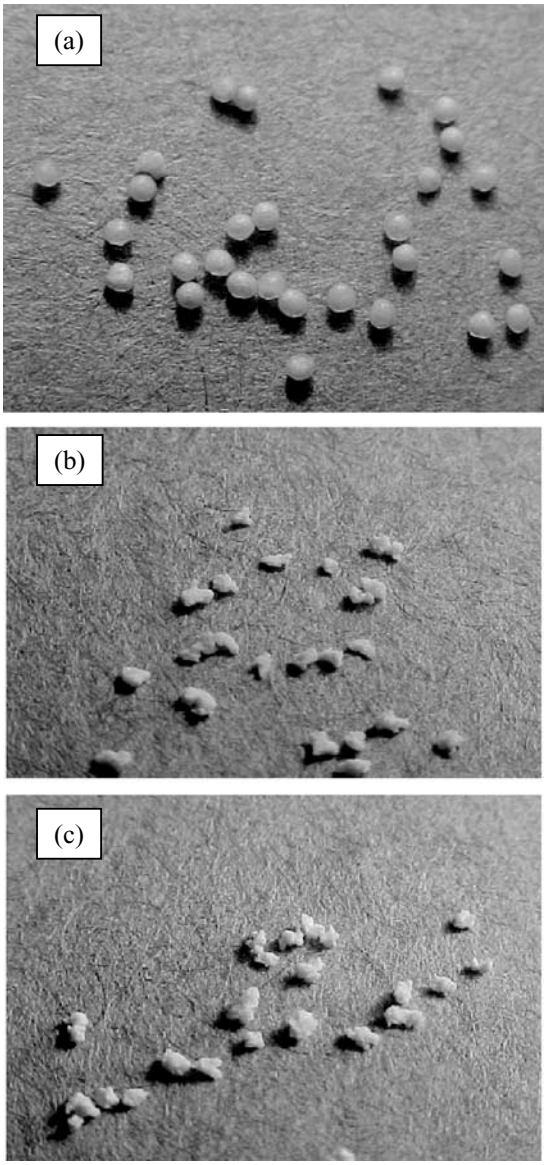

Figure 2. Morphology of albumin-chitosan microspheres prepared at different conditions: (a) Experimental condition: egg albumin solution concentration, 10% w/v; chitosan solution concentration, 1.5% w/v; initial drug loading, 20% w/w; pH of encapsulation medium, 3.77; using 6.0% v/v formaldehyde as a cross-linking agent and stirred for 3 hours (particle size = 579.3 ± 24.9 μm). (b) Experimental condition: egg albumin solution concentration, 10% w/v; chitosan solution concentration, 1.5% w/v; initial drug loading, 20% w/w; stirring time, 3 hours using 6.0% v/v formaldehyde as a cross-linking agent; pH of encapsulation medium, 4.91 (particle size = 445.3 ± 21.9 μm). (c) Experimental condition: egg albumin solution concentration, 10% w/v; chitosan solution concentration, 1.5% w/v; pH of media is 3.77; stirring time, 3 hours using 6.0% v/v formaldehyde as a cross-linking agent, and initial drug loading is 40% w/w (particle size = 501.4 ± 27.0 μm). (*View this art in color at www.dekker.com.*)

retarded cross linking was the most probable cause for changes in microsphere properties; as the final pH of the medium was increased, fewer amino groups were available for the cross-linking reaction for both polymers. The above results were also supported from the release study. Figure 4 shows that microspheres prepared at pH 3.77 gave the slowest indomethacin release, as the cross linking was more efficient and produced harder microspheres compared with those prepared at pH 4.91, which showed the fastest release pattern. However, microspheres prepared at pH 4.17, 4.33, and 4.52 showed intermediate release. A higher pH range is not shown in this study, as higher concentration of sodium hydroxide solution (higher than 0.5 M) can dissolve egg albumin only with some difficulties, and the produced microspheres were bearing unacceptably low physical properties, low drug content, and fast drug release.

Effect of Initial Drug Loading

Table 4 shows that incorporation efficiency was not significantly changed ($P > 0.05$) with the increase in initial (theoretical) drug loading. This could indicate that the increase of initial drug loading was accompanied by a corresponding increase of experimental drug loading that resulted in unchanged incorporation efficiency. This was in agreement with previous studies on microencapsulation of drugs in aqueous systems.[10,23] The particle size of the microspheres was increased with the increase of drug loading from 5% up to 30% w/w. The increase of drug loading and corresponding increase of particle size were also accompanied by the improvement of microsphere flowability, as indicated from the decrease in the values of angles of repose and compressibility indices. However, it was also noticed that at 40% w/w initial drug loading, the particle became smaller than expected and less flowable. Figure 2c shows the morphology of the produced microspheres at 40% w/w drug loading. The microspheres were getting irregular and small, which can explain these changes in physical properties. The release profile of indomethacin from microspheres prepared at different initial (theoretical) drug loading is shown in Fig. 5. It was clear that 5% w/w initial drug loading was accompanied by fast drug release due to small particle size. Higher drug loadings up to 30% showed slower drug release as a result of increase of particle size. The above results could indicate that the presence of insoluble drug particles on using up to 30% w/w of initial drug loading did not interfere with the coacervation process and the coacervated phase within the

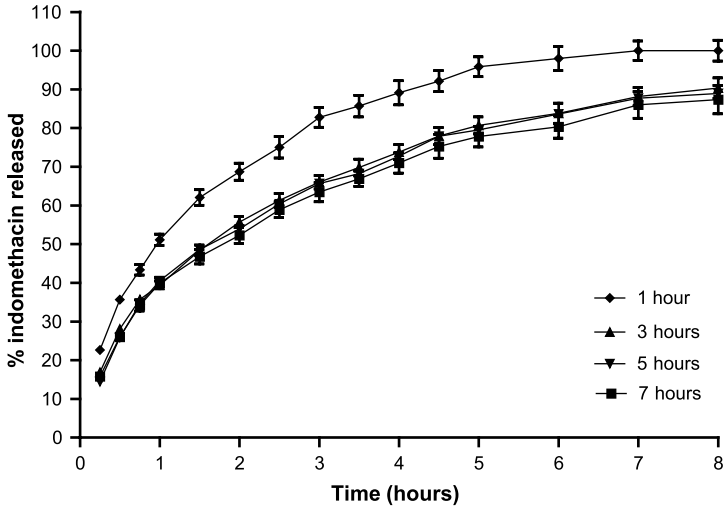

Figure 3. Effect of stirring time (hours) at 500 rpm on the release of indomethacin from albumin-chitosan microspheres (n=3).

microspheres. However, higher drug loading (40% w/w) started to interfere with the coacervation process and increased the rate of drug release. Similar results were obtained with coacervation of chitosan with gum karaya,[17] and it was concluded that this effect may be due to an increase in the number of drug particles, which reduced the amount of coacervated phase within the microspheres and might have interfered with cohesion of coacervate. In addition, there was no expected interaction between high molecular weight chitosan and the poorly water soluble indomethacin at the applied acidic condition,[24] which eliminates any possible effect of an interaction on the properties of the produced microspheres.

Effect of Polymer Concentration and Albumin-to-Chitosan Weight Ratio

It was observed that low and medium molecular weight chitosan failed to produce distinct microspheres when coacervated with egg albumin at the investigated conditions. At the limited concentration of chitosan, the effect of different concentrations of egg albumin on the properties of the microspheres was studied, as it can show the combined effect of total polymer content and albumin to chitosan weight ratio. Table 5 shows that the increase of albumin concentration or albumin to chitosan weight ratio was always accompanied by a significant increase in incorporation efficiencies and

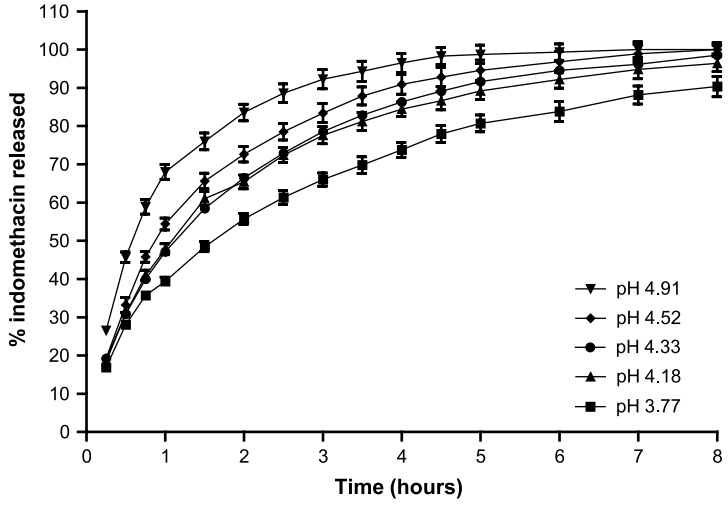

Figure 4. Effect of pH of encapsulation medium on the release of indomethacin from albumin-chitosan microspheres (n=3).

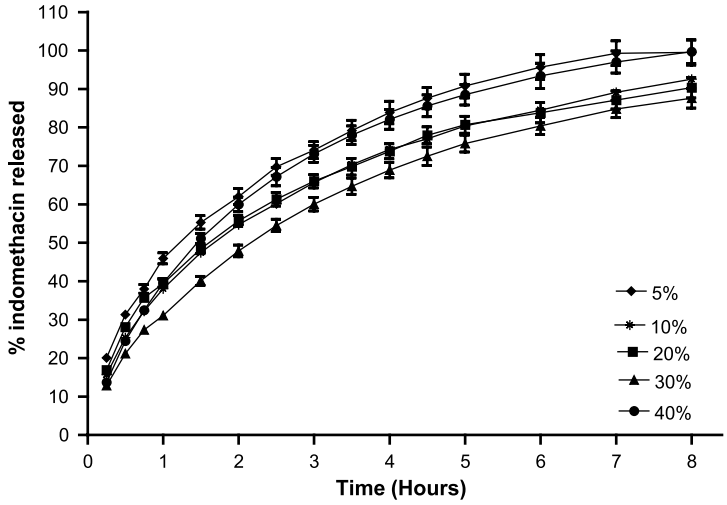

Figure 5. Effect of initial (theoretical) drug loading (% w/w) on the release of indomethacin from albumin-chitosan microspheres (n=3).

particle size with improvement of flow properties of the microspheres. This increase of the amount of albumin allowed the increase of the amount of incorporated drug, which may result in larger particle size of the microspheres. Thus, the increase of particle size can improve the flowability of the particles.[14] The increase in particle size with increase of concentration of albumin decreases the surface area of the microspheres and is accompanied by the slow release of indomethacin (Fig. 6). The above results indicate that high albumin-to-chitosan weight ratio is necessary to control drug release and to obtain microspheres of good properties.

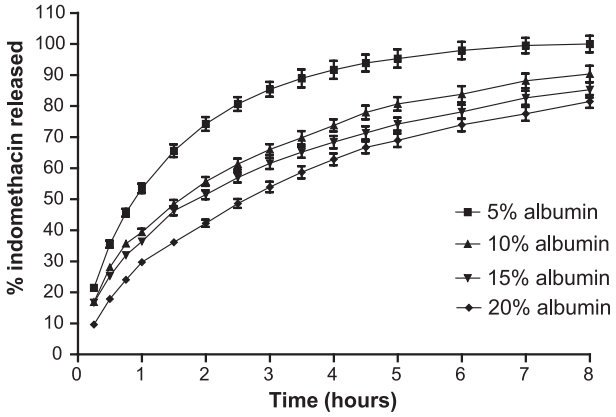

Figure 6. Effect of concentration of egg albumin dispersion (% w/v) on the release of indomethacin from albumin-chitosan microspheres (n=3).

In conclusion, albumin-chitosan microspheres were successfully prepared using a preferred aqueous system. The proposed system could have low cost with high safety compared with other conventional methods used in the preparation of microspheres. The proposed microspheres demonstrated the controlled release of indomethacin as an acidic model drug. The properties of the microspheres are highly affected by many process variables that could be controlled to obtain an optimized system.

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