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Effect of formulation and process variables on the formation of chitosan–gelatin coacervates

Carmen Remuñán-López*, Roland Bodmeier[1]

College of Pharmacy, The University of Texas at Austin, Austin, TX 78712-1074, USA

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Abstract

The formation of complex coacervates between the oppositely charged polyions, chitosan and type B gelatin, was investigated. The complex formation was rapid and only observed at very diluted chitosan concentrations over a narrow pH range. The optimum chitosan–gelatin ratio was found to be 1:10 to 1:20, above or below which the coacervate yield decreased significantly. The coacervate yield decreased at higher temperatures and increased ionic strength. Complex coacervation was found to be dependent upon the molecular weight and bloom strength of the polymers. Several model compounds (clofibrate, piroxicam, sulfamethoxazole) were successfully encapsulated within the chitosan–gelatin coacervates.

Keywords: Chitosan; Complex coacervation; Gelatin type B; Microencapsulation

1. Introduction

The use of new natural hydrophilic polymers as drug carriers has received considerable attention in the last few years, especially from the viewpoint of cost, environmental concerns and safety. Chitosan [β-(1→4)-2-amino-2-deoxy-D-glucose] is prepared by *N*-deacetylation of chitin, one of the polysaccharides widely distributed in nature as a principal component of crustaceans shells and insects (Muzarelli, 1977). Chitosan and chitosan derivatives have been used as flocculants for the treatment of waste water and in personal care products (Sandford, 1989). In addition, chitosan has attracted attention in biomedical and pharmaceutical fields because of its reactive groups and favorable properties of biodegradability, low toxicity and biocompatibility (Knapczyk et al., 1989; Hirano et al., 1990). Biomedically, chitosan is useful for hypocholesterolemic effects, antacid and antiulcer activities, wound and burn healing, in soft and hard contact lenses, artificial organ membranes and in the immobilization of enzymes or living cells (Desai et al., 1986; Kim and Rha, 1989). In pharmaceutical applications, chitosan

* Corresponding author. Present address: Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Campus Sur, 15706-Santiago de Compostela, Spain. Tel. +81 563100, extn. 5009; Fax +81 594595; E-mail ffcarelo@usc.es.

[1] Present address: Institut für Pharmazie, Freie Universität Berlin, Berlin, Germany.

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has been used as a direct-compression diluent (Nagai et al., 1984), as a vehicle for sustained release of drugs (Hou et al., 1985; Kawashima et al., 1985; Inouye et al., 1988), as well as for the enhancement of the dissolution rate and bioavailability of water-insoluble drugs (Nagai et al., 1984).

Few investigations on the preparation of chitosan microcapsules and microspheres have been published. Several drugs or drug-containing microparticles were entrapped within beads prepared by the ionotropic gelation of chitosan with tripolyphosphate (Bodmeier et al., 1989a,b); albumin was microencapsulated within a chitosan–alginate complex membrane in the presence of calcium chloride (Polk et al., 1994). Several studies have been focused on the utilization of an emulsion–solvent evaporation method to obtain microcapsules of theophylline (Lin and Lin, 1992; Thanoo et al., 1992), of 5-fluouracil (Ohya et al., 1993) or magnetic microspheres of oxantrazole (Hassan et al., 1992; Hassan and Gallo, 1993) and of cisplatin (Nishioka et al., 1990). Li et al. (1991) used a dry-in-oil procedure to prepare ordinary and reacetylated chitosan microspheres containing 5-fluouracil. In order to sustain the drug release from these microcapsules, glutaraldehyde (Hassan et al., 1992; Nishioka et al., 1990; Thanoo et al., 1992; Ohya et al., 1993) and sodium tripolyphosphate (Lin and Lin, 1992) were added to the microencapsulation process at different stages.

Complex coacervation is the spontaneous liquid/liquid phase separation that occurs when oppositely charged colloids are mixed as a result of electrostatic attractions. Two distinct phases are formed: a dense coacervate phase, which is rich in colloids, and a diluted equilibrium phase or supernatant, which is poor in colloids (Bungenberg de Jong, 1949). The coacervation process occurs under mild conditions, consequently has great potential for the microencapsulation of living cells and labile molecules, which are unable to withstand harsh conditions (heat, organic solvents) involved in other microencapsulation processes (Ecanow, 1989; Singh, 1992). As coacervation can be induced in systems containing both cationic and anionic hydrophilic colloids, complex coacervation is likely to occur between chitosan, a water-soluble cationic polysaccharide, and type B gelatin, a protein which is negatively charged at pH values above its isoelectric point.

The objective of this study was to investigate the formation of complex coacervates between chitosan and type B gelatin as a function of several variables. The optimal complex coacervation conditions were established. Finally, some model compounds were microencapsulated within the chitosan–gelatin systems.

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial suppliers and used as received: clofibrate, piroxicam, sulfamethoxazole (Sigma Chemical Co., St. Louis, MO); chitosan glutamate (supplier's specification: the viscosities of 1% (w/w) aqueous solutions at 25°C for Sea cure +110 or +210 were <20 or 20–200 cps; Protan, Drammer, Norway); chitosan (supplier's specification: the viscosities of 1% (w/w) aqueous solutions in 1% (v/v) acetic acid at 25°C for Sea cure 123, 223 or 320 were <20, 20–200 or >200 cps and the degrees of deacetylation were >80, >80 or >70%; Protan, Raymond, WA); type B gelatin, bloom strength 100 (Fisher Scientific Co., Fair Lawn, NJ), bloom strength 75 and 225 (Sigma Chemical Co., St. Louis, MO); and glycerin (Fisher Scientific Co., Fair Lawn, NJ); sodium chloride, sodium hydroxide, acetic acid and hydrochloric acid were of analytical grade quality; water was double-distilled.

2.2. Methods

Aqueous solutions of chitosan and gelatin were combined in 15 ml glass tubes and agitated with a vortex mixer after heating the solutions to 40°C. The pH of the polymer solutions was previously adjusted with diluted hydrochloric acid or sodium hydroxide. The mixtures were observed with an optical microscope (Micromaster™, Fisher Scientific, Model E, magnification ×100) in order to assess if coacervation occurred. The coacervate

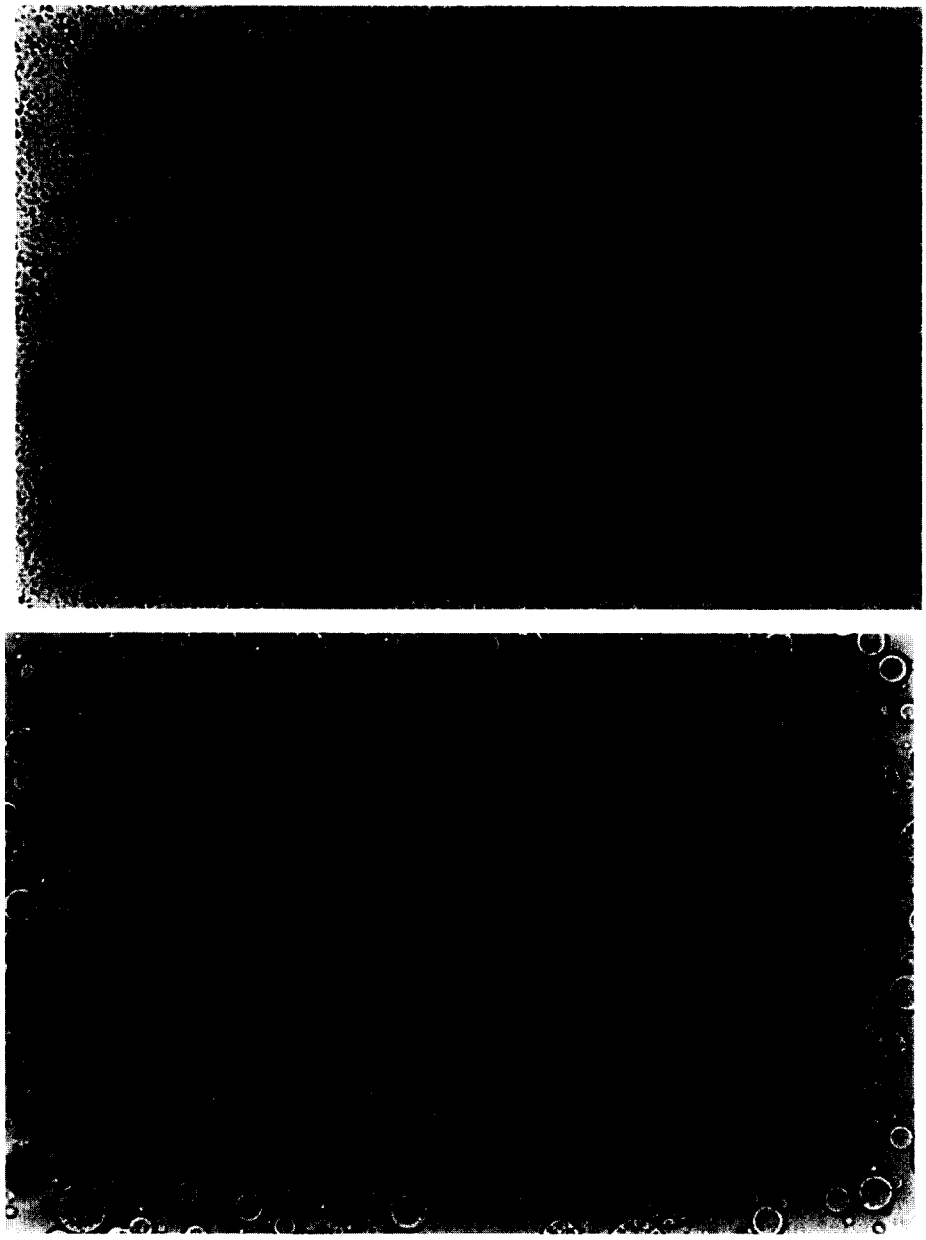

Fig. 1. Photographs of the chitosan glutamate–gelatin coacervate droplets after the addition of gelatin to chitosan: (a) small coacervation nuclei; (b) larger spherical coacervate droplets (× 400).

droplets separated at the bottom of the glass tube and formed the coacervate phase.

The extent of coacervation was evaluated by measuring the volume of the coacervate phase (height of coacervate phase divided by the total sample height, expressed in percent) and the solids content of the coacervate phase (dry coacervate yield, in percent of total polymer concentration in the system). The coacervate volume was measured with a ruler directly on the tube. The dry coacer-

vate yield was obtained by decanting the supernatant and drying the coacervate phase at 60°C until constant weight.

The standard formulation for the coacervation studies was as follows: Sea cure + 110; gelatin 100 bloom; pH 5.5; chitosan:gelatin ratio 1:20; total polymer concentration 3.15% w/w; temperature 40°C; incubation time 4 h).

The following variables affecting the coacervation process were investigated ($n \geq 4$): coacervation time (0.25, 0.5, 1, 2, 4, 8, 16 and 24 h), temperature (25, 30, 40, 50, 60, 70°C), pH (pH 4.5–6.5, intervals of 0.25), chitosan:gelatin ratio (1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:40 and 1:50), total polymer concentration (0.26, 0.52, 1.05, 1.57, 2.10, 3.15% w/w), NaCl concentration (0, 1, 5, 10, 20, 30, 40, 50, 70, 90, 110, 130 mM), type of chitosan (chitosan glutamate: Sea cure + 110 and + 210; chitosan: Sea cure 123, 223 and 320) and gelatin bloom strength (75, 100, 225).

3. Results and discussion

In this study, the effect of various formulation and process variables on the formation of chitosan type B gelatin coacervates was investigated. The first step was to identify the experimental conditions at which chitosan–gelatin coacervates formed. Experiments were performed by mixing aqueous solutions of both colloids at 40°C in various concentrations and at a pH where both carried opposite charges. The pH was therefore not changed from a pH where the polymers originally carry the same charge to a pH at which they carry opposite charges; this is commonly done in complex coacervation, for example with gelatin–acacia systems. Mixtures, which became turbid, were examined under an optical microscope for evidence of coacervation. A preliminary screening of different chitosan–gelatin combinations (chitosan glutamate: 0.05 to 1% w/w, type B gelatin 100 bloom: 0.5 to 10% w/w) showed that both polymer ratio and total polymer concentration were decisive for the complex coacervation. The coacervate formation was confined to very dilute solutions of chitosan (0.05–0.5% w/w). Fig. 1 shows typical coacervate droplets formed from chitosan glutamate and gelatin. The addition of the gelatin solution to the chitosan initially resulted in small coacervation nuclei (Fig. 1a), which rapidly coalesced into larger spherical coacervate droplets (Fig. 1b).

The objective of this paper was to examine the effect of various variables including pH, polymer ratio, total polymer concentration, coacervation temperature, incubation time and ionic strength on the formation of the coacervates as expressed by the solid content of coacervate formed (dry coacervate yield).

A preliminary study was initially performed to investigate the effect of agitation on the formation of the coacervate phase. The method of agitation (vortex-mixing, magnetic stirrer, no agitation) affected the size of the coacervate droplets, however not the coacervate yields; the differences between the procedures was $< 5\%$.

Studies were carried out to determine the effect of the time allowed for coacervation to occur on the dry coacervate yield. As can be seen in Fig. 2, coacervation is a spontaneous, rapid process; the coacervate yield increased up to 1 h before reaching a plateau. A coacervation time of 4 h was used for the following studies.

The effect of equilibration temperature on the coacervate formation is shown in Fig. 3. Increasing the temperature of the polymer solutions decreased the coacervate yield. This result is in accordance with other investigated coacervation systems (Veis and Aranyi, 1960; Dhruv et al., 1975; Burgess and Carless, 1986). The decrease in the coacervate yield could be explained with the increased solubilities of the polymers and probably also of the complex formed. The coacervate yield produced at each temperature remained the same after several cooling/heating cycles.

Complex coacervation is a pH-sensitive process since the charge and charge density of the polymers vary with pH. Chitosan is always positively charged in solution while the charge of the gelatin molecules depends on the pH. Coacervation between chitosan and gelatin should be restricted to a narrow pH range, where both molecules carry opposite charges. The investigated pH range was 4.5 to 6.5, which is above the PI of gelatin and below the pH of precipitation for chitosan (Fig. 4). The optimum pH for maximum coacervate

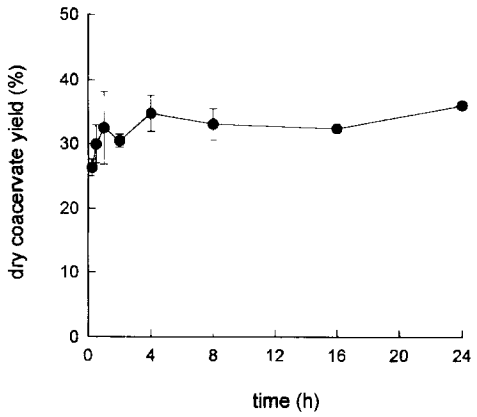

Fig. 2. Effect of the time allowed for chitosan–gelatin coacervation to occur on the dry coacervate yield ($T = 40°C$; pH 5.5; polymer ratio 1:20; total polymer conc. 3.15% w/w).

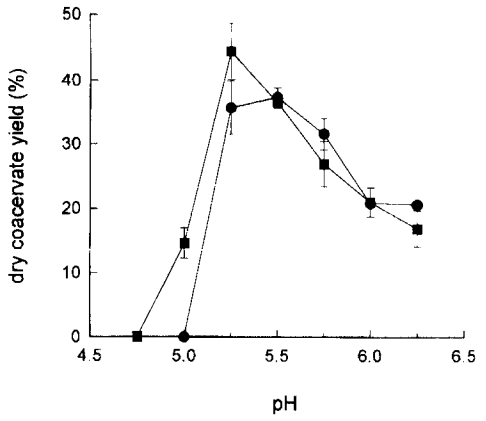

Fig. 4. Effect of the pH on the dry coacervate yield: (■) 1.57% w/w and (●) 3.15% w/w total polymer conc. ($t = 4$ h; $T = 40°C$; polymer ratio 1:20).

formation was between 5.25 and 5.50. The coacervate yield dropped at pH values below and above this pH. Above pH 6.25 chitosan precipitated from solution. The coacervation between chitosan and gelatin was always accompanied by a slight pH increase of the mixture when compared to the pH of the individual polymer solutions.

The pH of maximum coacervate yield is believed to correspond to the electrical equivalence pH (EEP), where both polymers carry equal but opposite charges (Burgess and Carless, 1984; Peters et al., 1992). At the EEP, attracting forces between the charged components neutralize each other leading to a strong binding and the highest coacervate yield. At pH values where the charges are no longer balanced, a reduction in the interaction between the polymers causes a reduction in the coacervate yield.

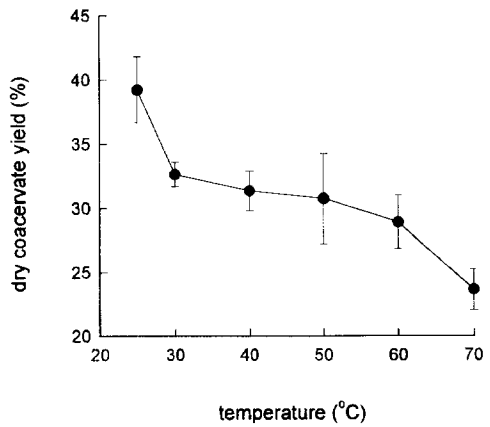

Fig. 3. Effect of the equilibration temperature on the dry coacervate yield ($t = 4$ h; pH 5.5; polymer ratio 1:20; total polymer conc. 3.15% w/w).

Preliminary results revealed that the mixing ratio of the polymers and the total polymer concentration greatly affect the coacervation process. The effect of the gelatin:chitosan ratio at a total polymer concentration of 3.15% w/w was investigated. The amount of coacervate formed was at an optimum at a ratio of 10 (Fig. 5). This ratio might correspond to equal but oppositely charged groups under the experimental conditions chosen. Above or below this ratio, the uncomplexed negatively or positively charged groups of either gelatin or chitosan are in excess, thus keeping the complex in solution. Coacervation was completely suppressed at a ratio of 50:1; the colloidal mixture remained clear. The lower yield at high gelatin concentrations may be a consequence of a combination of factors, such as salt effects and possibly an increased viscosity of the system (Dhruv et al., 1975). In addition, a large excess of one polymer may only lead to partial neutralization and therefore to soluble complexes. A ratio of chitosan:gelatin of 1:20 was selected in this study; the

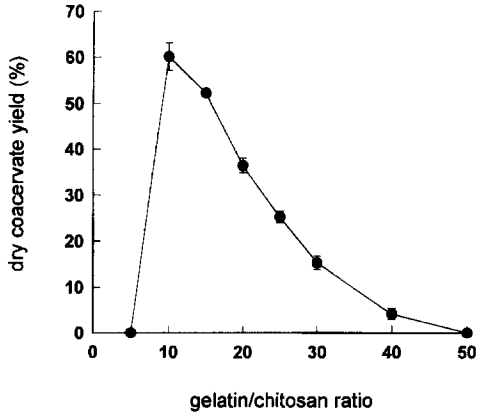

Fig. 5. Effect of the gelatin:chitosan ratio on the dry coacervate yield ($t = 4$ h; $T = 40$°C; pH 5.5; total polymer conc. 3.15% w/w).

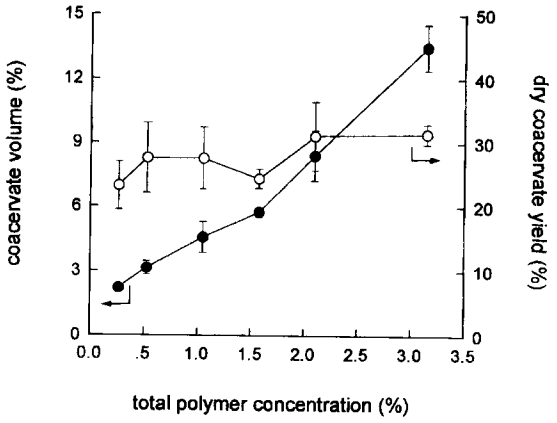

Fig. 6. Effect of the total polymer concentration on the coacervate volume and the dry coacervate yield ($t = 4$ h; $T = 40$°C; pH 5.5; polymer ratio 1:20).

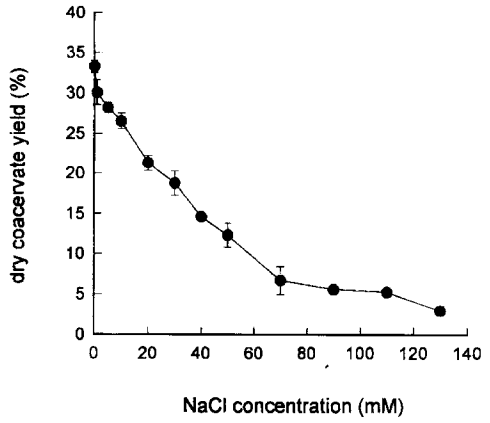

Fig. 7. Effect of NaCl concentration on the dry coacervate yield ($t = 4$ h; $T = 40$°C; pH 5.5; polymer ratio 1:20; total polymer conc. 3.15% w/w).

most spherical and isolated coacervate droplets were formed at this ratio.

After identifying the optimum polymer ratio, the effect of the total polymer concentration on the coacervate yield was investigated at a chitosan:gelatin ratio of 1:20. The coacervate volume formed linearly increased with increasing polymer concentration, while the coacervate yield remained almost constant (Fig. 6). More coacervate was formed with increasing total polymer concentration, however the polymer concentration of the coacervate phase reflected by the dry coacervate yield remained constant. Spherical coacervate droplets were formed up to a polymer concentration of 3.15% w/w, while a gel was formed at higher total concentrations.

Table 1
Effect of the chitosan type and gelatin bloom strength on the coacervate volume ($T = 40$°C; $t = 4$ h; polymer ratio 1:20; total polymer conc. 3.15% w/w; pH 5.5)

Chitosan type	Coacervate volume (%)		
	Gelatin 75 bloom	Gelatin 100 bloom	Gelatin 225 bloom[a]
Sea cure +110	10.71 ± 0.82	12.15 ± 0.40	CS
Sea cure +210	9.66 ± 0.80	10.86 ± 0.21	CS
Sea cure 123	4.48 ± 0.02	3.25 ± 0.16	CS
Sea cure 223	10.57 ± 0.41	8.88 ± 0.89	CS
Sea cure 320	23.06 ± 0.96	26.38 ± 0.30	ND

[a]CS = clear solution; ND = not determined.

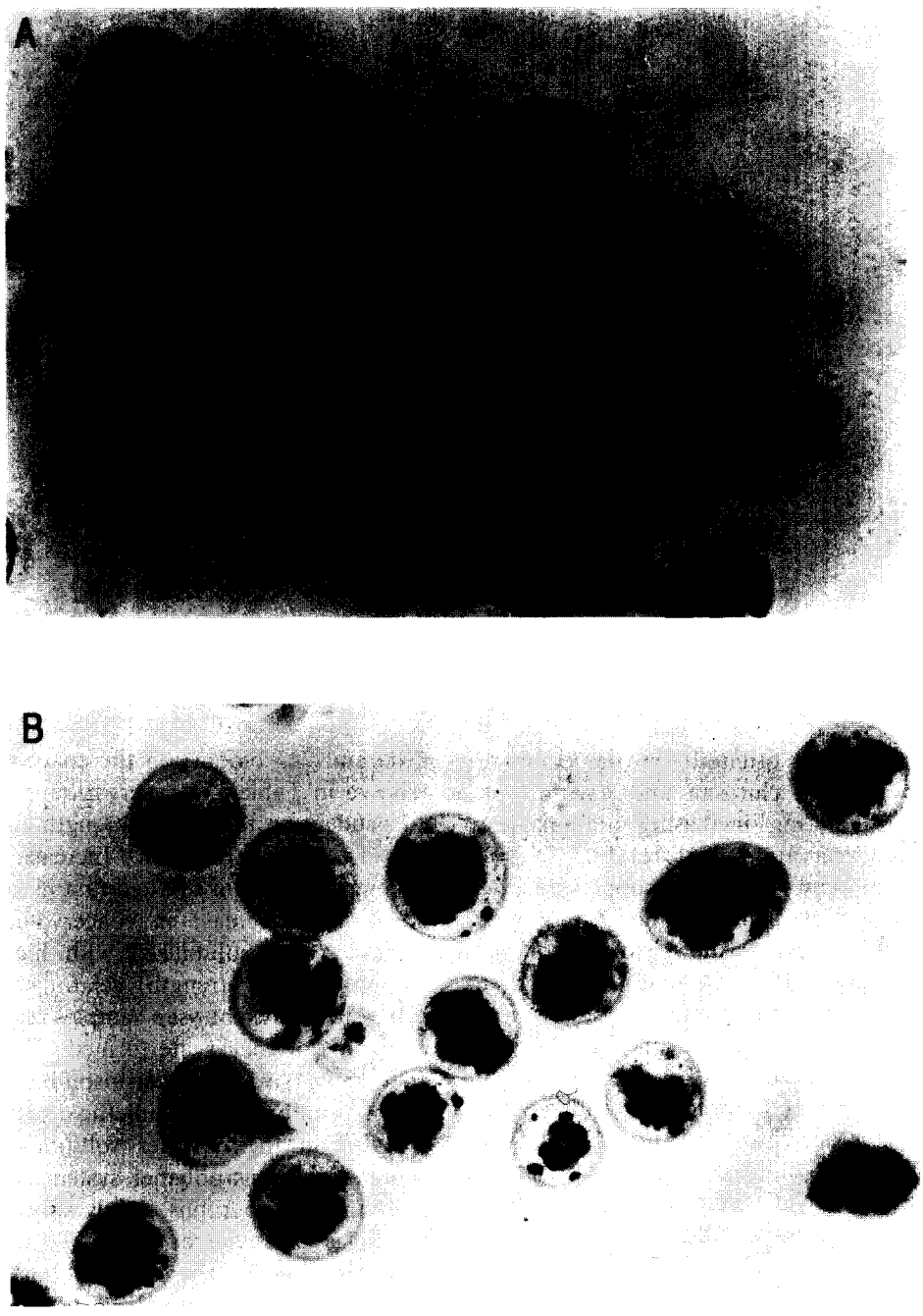

Fig. 8. Photographs of chitosan glutamate–gelatin coacervates containing (a) piroxicam (× 600), (b) sulfamethoxazole (× 600) and (c) clofibrate (× 160).

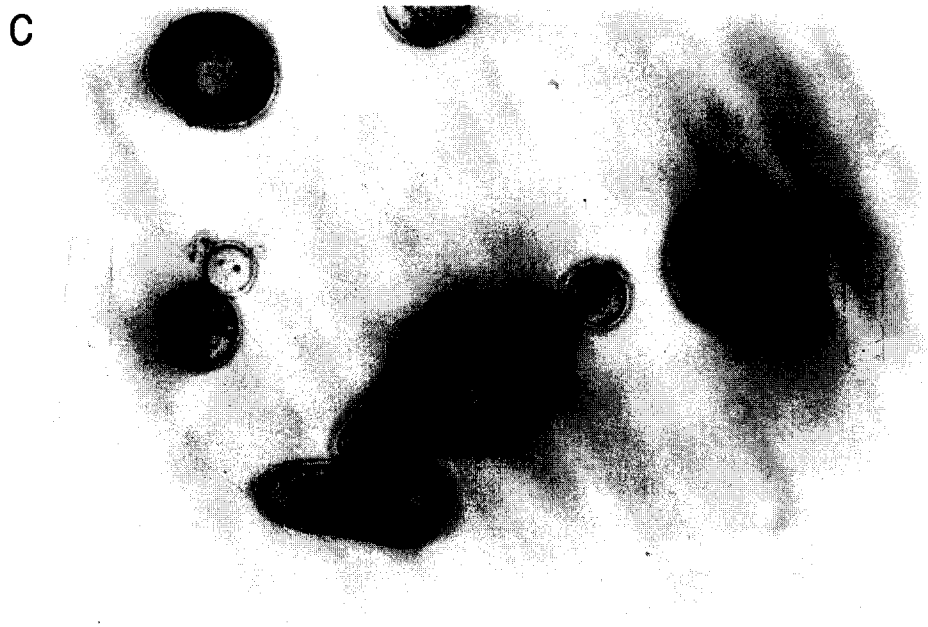

Fig. 8c

The effect of ionic strength on the complex coacervation between chitosan and gelatin was investigated by adding increasing amounts of NaCl to the coacervation mixture (Fig. 7). Increasing the amount of NaCl suppressed the coacervation between chitosan and gelatin and hence reduced the coacervate yield. It is well known (Vandegaer, 1974; Madan, 1978) that neutral salts influence the complex coacervation process due to the screening of the charged groups on the polyelectrolytes. The attraction between the polyions and hence the tendency to form coacervates decreases with increasing ionic strength. With the chitosan glutamate–gelatin system, 130 mM of NaCl were required for complete suppression; this was high in comparison with other coacervation systems such as the typical gelatin–acacia combination (Burgess and Carless, 1984). It could be an indication for a stronger interaction between gelatin and chitosan.

The effect of the molecular weight of chitosan (Sea cure +110 and +210, Sea cure 123, 223 and 320) and the bloom strength of gelatin (75, 100 and 225 bloom) on the coacervate volume is shown in Table 1. Coacervation was found to be dependent on the bloom strength and the molecular weight of chitosan. Increasing the bloom strength of gelatin resulted first in an increase and then in a decrease in coacervate volume. The complex was solubilized with higher molecular weight (bloom strength) gelatin samples. Higher viscosity grade chitosan samples resulted in higher amounts of coacervate formed.

Finally, various water-insoluble drugs (piroxicam, clofibrate, sulfamethoxazole) were suspended in the polymer solutions to show the potential of this polymer system for microencapsulation. Photographs clearly show that the drug particles are surrounded by the coacervate droplets (Fig. 8). The dispersed particles were well wetted by the coacervate droplets; no free particles were visible.

In conclusion, complex coacervation between chitosan and type B gelatin was achieved by carefully choosing the proper conditions. The optimized system will be used in the future for the encapsulation of various drugs.

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