ELSEVIER

International Journal of Pharmaceutics 242 (2002) 239–242

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Note

Formulation factors in preparing BTM–chitosan microspheres by spray drying method

Y.C. Huang [a], M.K. Yeh [b], C.H. Chiang [c,*]

[a] *Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan, ROC*
[b] *Clinical Pharmacy, Tri-Service General Hospital, Taipei, Taiwan, ROC*
[c] *School of Pharmacy, National Defense Medical Center, Taipei, Taiwan, ROC*

Received 11 December 2001; received in revised form 8 January 2002; accepted 9 January 2002

Abstract

Chitosan (CTS) microspheres were prepared by a spray drying method using type-A gelatin and ethylene oxide–propylene oxide block copolymer as modifiers. Surface morphological characteristics and surface charges of prepared microspheres were investigated by using scanning electron microscopy (SEM) and microelectrophoresis. The particle shape, size and surface morphology of microspheres were significantly affected by the concentration of gelatin. Betamethasone disodium phosphate (BTM)-loaded microspheres demonstrated good drug stability (less 1% hydrolysis product), high entrapped efficiency (95%) and positive surface charge (37.5 mV). The in vitro drug release from the microspheres was related to gelatin content. Microspheres containing gelatin/CTS 0.4 ~ 0.6(w/w) had a prolong release pattern for 12 h. These formulation factors were correlated to particulate characteristics for optimizing BTM microspheres in pulmonary delivery.

Keywords: Betamethasone; Chitosan; Pluronic F68; Gelatin; Spray drying

Betamethasone, one of the most important glucocorticosteroids, is an effective anti-inflammatory agent for the treatment of respiratory diseases (Smith and Bernstein, 1996), which might be developed as a prolonged pulmonary delivery system for minimizing side effects. Particulate characteristics including density, particle size, morphology, surface charge and drug release crucially influenced pulmonary delivery. Porous microspheres with suitable diameter (5–10 μm) and density (0.01–0.4 g/cm^3) could be inspired into the lung and less deposited in trachea, to achieve better pulmonary bioavailability (Ben-Jebria et al., 1999; Ewards et al., 1997). Chitosan (CTS) has been applied in delivery system for offering mucoadhesive characteristics (Leffler and Muller, 2000). In acidic conditions, CTS carries positive charges binding with the mucous surface of the respiratory tract by electrostatic interaction (Mooren et al., 1998). Recently, Carlvo et al. (1997) investigated the physicochemical properties, surface composition, and mechanism of

* Corresponding author. Present address: 161, Min-Chuan East Rd., Sec. 6, Neihu, Taipei 114, Taiwan, ROC. Tel.: + 886-2-879-23100x18894
E-mail address: cch@ndmctsgh.edu.tw (C.H. Chiang).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00164-3

protein association to CTS/Pluronic (F68) nanoparticles. They demonstrated that the hydrophilic surface of CTS microspheres is formed by F68; it has excellent capacity for the association of protein or peptide. In addition, Thacharodi and Rao (1995) investigated CTS membranes containing varied ratios of collagen; the collagen plays an important role in regulating the swelling properties and permeability properties of the CTS membranes. Their studies may implicate F68 and collagen to be important modifiers in CTS preparation. In this study, we used betamethasone disodium phosphate (BTM) as a model drug combining CTS with varied ratios of F68 and gelatin in preparing bioadhesive microspheres. Formulation factors were investigated to correlate particulate characteristics including particle size, surface morphology, and surface charge in the preparation of CTS microspheres.

CTS (the viscosity of 1% CTS in 1% acetic acid ~ 100 mPa/S, 87% hydrolysis) was provided from Fluka BioChemika (Buchs, Switzerland). BTM, Gelatin (type-A), F68, betamethasone free base and other reagents were also used. BTM microspheres were prepared by spray drying technology using a spray dryer (Büchi-190, Switzerland). The inlet temperature was controlled at 170 °C. The zeta potential of prepared microspheres was measured by a zeta potential analyzer (Brookhaven Instruments Corp., Holtsville, NY) after dispersing in KCl solution (10^{-3} M) with concentration 0.3% (W/V). Additionally, microspheres were coated with gold/palladium and examined by a scanning electron microscope (SEM) (Jeol-5400, Japan).

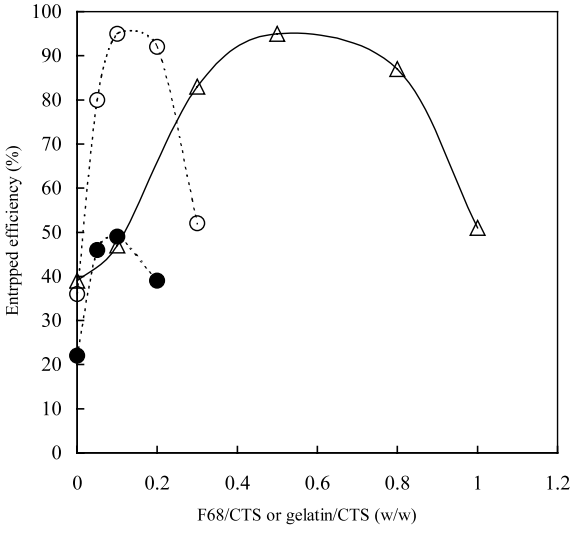

Fig. 1. The entrapped efficiency of BTM-loaded microspheres in different formulations. Key: (△) varied ratios of gelatin/CTS with a constant ratio of F68/CTS: 0.1 (w/w); (●) varied ratio of F68/CTS, without gelatin; and (○) varied ratio of F68/CTS, with a constant ratio of gelatin/CTS: 0.5 (w/w).

The content of BTM in prepared microspheres was determined by HPLC method with UV detector (at 254 nm). Microspheres were dispersed in methanol by vortex for 12×10 s; the supernatant was obtained after centrifugation and analyzed by HPLC for measuring surface drug content. Following, the residual of microspheres was dissolved in 0.1% (w/v) acetic acid solution and neutralized by 0.1 M Na_2CO_3 for measuring inner core drug content. Samples were eluted with a mobile phase (CH_3OH:0.07 M $K_2HPO_4 = 6{:}4$) through a RP18 column at a flow rate of 1 ml/min. Drug release from microspheres was studied by paddle method at 50 rpm in water at 37 °C. Released BTM was directly determined by a spectrophotometer at 254 nm using an automatic dissolution tester (Vankel VK7000, Cary, NC).

Incorporating gelatin in microspheres substantially increased the zeta potential, especially in the ratio of gelatin/CTS at 0.4–0.6 (w/w) with a value about 56 mV. But combining F68, the zeta potentials of microspheres were decreased, as the ratio of F68/CTS larger than 0.3 (w/w), the zeta potential decreased to a constant value (20 mV). Additionally, combined gelatin and F68, (gelatin/CTS: 0.4–0.6, w/w; F68/CTS: 0.1, w/w) in preparing CTS-microspheres, the microspheres were significantly improved with a yield rate $> 50\%$, but microspheres prepared without gelatin and F68 only with a 5% yield rate.

BTM-loaded microspheres containing BTM from 10 to 50% were prepared. The inner core of microspheres contained about 35% BTM of total entrapped drug in microspheres. No significant degraded BTM was noticed in the core portion of prepared microspheres. However, a minor hydrolysis product (free base) of BTM was detected ($< 2.5\%$) in the surface portion of prepared mi-

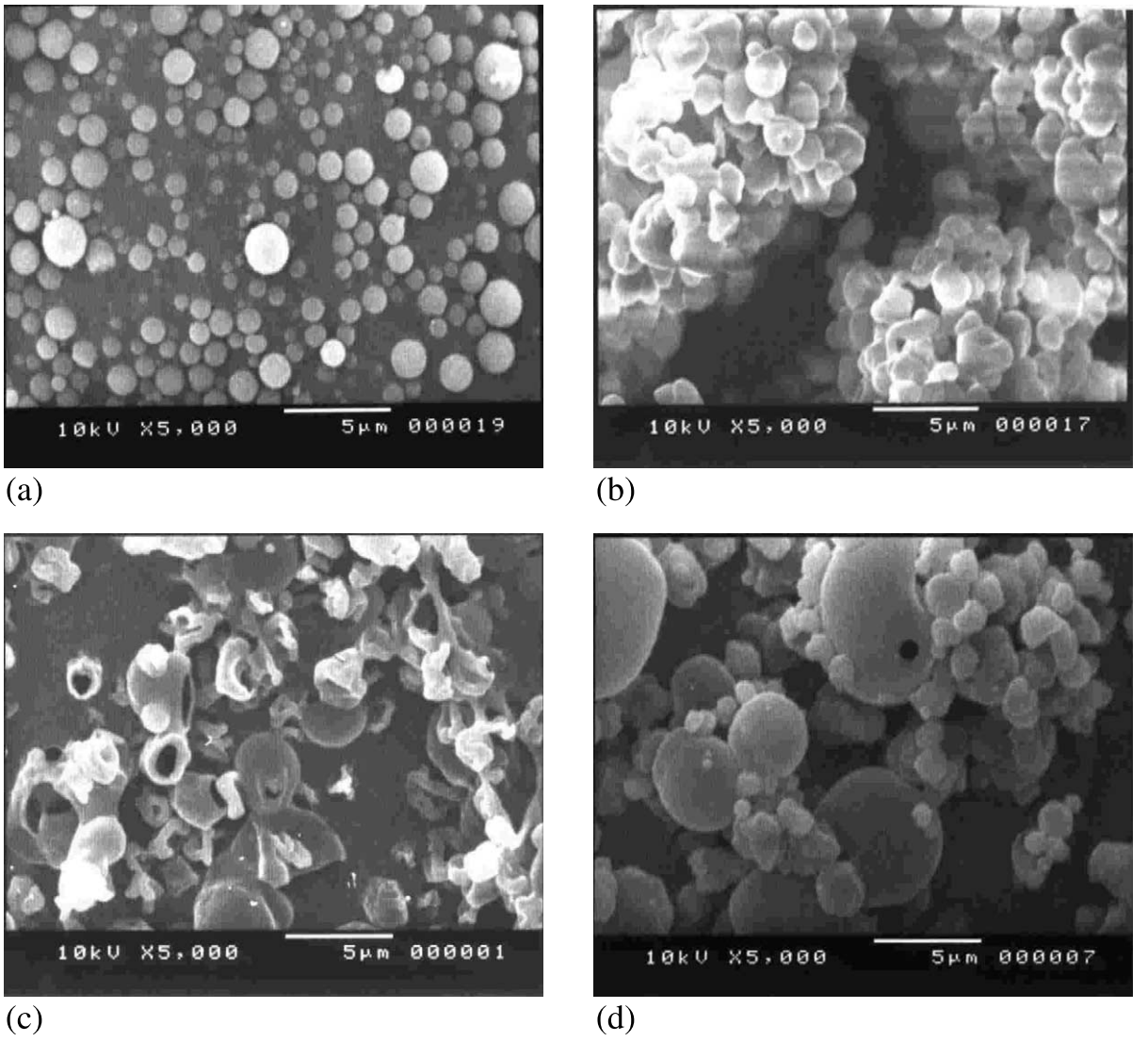

Fig. 2. Electroscanning microphotograms of CTS microspheres with different components: (A) blank microspheres; (B) BTM-loaded microspheres; (C) BTM-loaded microspheres combined gelatin; and (D) BTM-loaded microspheres combined F68 and gelatin.

crospheres, which was counted about 1% of total entrapped BTM drug. Surface BTM has a larger exposure area to heat and air which might have more chance to be decomposed during spray drying process. Fig. 1 shows the entrapped efficiencies of BTM-loaded microspheres, formulations with gelatin/CTS: 0.4–0.6 (w/w) and F68/CTS: 0.1 (w/w) obtained better results. An optimal formulation was achieved to combine CTS/gelatin/F68 = 1:0.4:0.1 in microspheres, the formulation has excellent entrapped efficiency (95%), higher yield rate ($\sim$50%) and positive surface charge (37.5 mV).

SEM studies revealed that the amount of gelatin had an effect on the size and the surface morphology of blank CTS-microspheres. Fig. 2 shows SEMs of four typical formulations. Blank microspheres were spherical shape and size distri-

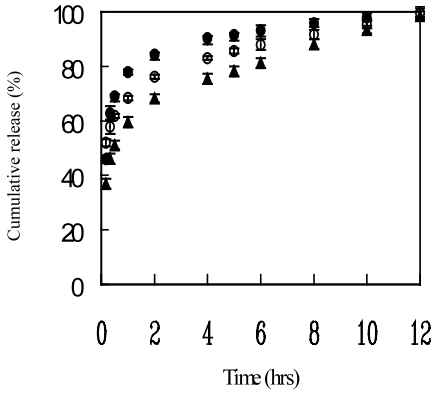

Fig. 3. BTM release profiles of BTM-loaded microspheres combined a constant ratio of F68/CTS: 0.1 (w/w) and different ratios of gelatin determined by USP paddle method at 50 rpm in water at 37 °C. Key: (●) gelatin/CTS, 0.1 (w/w); (▲) gelatin/CTS, 0.5 (w/w), (○) gelatin/CTS, 1.0 (w/w) (mean ± SD, $n = 3$).

bution from 1 to 4 μm (Fig. 2A). However, without gelatin and F68, blank microspheres could not be obtained, and irregular microparticles were produced, alternatively. The similar phenomenon occurred in preparing BTM microspheres without gelatin and F68. Irregular microparticles instead of spheres were produced (Fig. 2B) and also aggregated together. As combining gelatin but without F68 in formulation, most particles with a large hole, they might be formed by high heat (170 °C) to produce explosion during the spray drying process (Fig. 2C). Combined F68 and gelatin with CTS in preparing BTM microspheres, two different size distributions of microspheres, 1–4 μm and 5–10 μm were obtained (Fig. 2D). F68 might play an important role to offer more rigid matrix in preventing the explosion of microparticles. Fig. 3 shows the in vitro drug release of the prepared BTM microspheres. BTM microspheres combined gelatin/CTS: 0.5 (w/w) with the highest entrapped efficiency (95%) obtained the slowest release pattern. The preparation might provide an extended release more than 12 h.

Overall in the study, we successfully established a spray drying technique combining formulation factors in preparing high BTM-loaded microspheres. BTM was stable in CTS microsphere with modifiers of gelatin and F68 during spray drying process. The prepared microspheres have positive charge with suitable particle size, which might be potentially applicable as a pulmonary delivery system.

References

Ben-Jebria, A., Chen, D., Eskew, M.L., Vanbever, R., Langer, R., Edwards, D.A., 1999. Large porous particles for sustained protection from carbachol-induced bronchoconstriction in guinea pigs. Pharm. Res. 16, 551–561.

Carlvo, P., Remuñan-López, C., Vila-Jato, J.L., Alonso, M.J., 1997. Chitosan and chitosan/ethylene oxide–propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm. Res. 14, 1431–1436.

Ewards, D.A., Hanes, J., Caponetti, G., Hrkach, J., Ben-Jebria, A., Eskew, M.L., Mintzes, J., Deaver, D., Lotan, N., Langer, R., 1997. Large porous particles for pulmonary drug delivery. Science 276, 1868–1871.

Mooren, F.C., Berthold, A., Domschke, W., Kreuter, J., 1998. Influence of chitosan microspheres on the transport of prednisolone sodium phosphate across HT-29 cell monolayers. Pharm. Res. 15, 58–65.

Leffler, C.C., Muller, B.W., 2000. Influence of the acid type on physical and drug liberation properties of chitosan–gelatin sponge. Int. J. Pharm. 194, 229–237.

Smith, S.J., Bernstein, J.A., 1996. Therapeutic uses of lung aerosols. In: Hickey, A. (Ed.), Inhalation Aerosols. Marcel Dekker, New York, pp. 233–261.

Thacharodi, D., Rao, K.P., 1995. Collagen–chitosan composite membranes for controlled release of propranolol hydrochloride. Int. J. Pharm. 120, 115–118.