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Preparation and characterization of theophylline loaded chitosan/β-cyclodextrin microspheres

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Abstract The purpose of this project was to develop sustained release chitosan/β-cyclodextrin microspheres of theophylline (TH) prepared by spray drying method. The effect of several formulation variables on the characteristics of microspheres was studied. The B microspheres had a narrower particle size distribution with the diameter between 1 and 10 μm. SEM showed spherical microspheres with smooth or slightly wrinkled surfaces. FT-IR spectroscopy revealed that hydrogen bonds were formed between TH and chitosan or β-cyclodextrin. The drug entrapments significantly increased from 13.33 to 35.70% with an increase of the ratio of drug/polymer. The encapsulation efficiencies were from 85.16 to 91.40%. The in vitro release of TH from microspheres was related to the pH of the medium, swelling ability, especially in the ratio of drug/polymer. The B microspheres had a prolonged release pattern with the release rate of 60.20% (pH 6.8) within 8 h.

Introduction

Theophylline (TH) is one of the most important drugs for the treatment of asthma and recent studies indicate that it has anti-inflammatory effects [1, 2]. Thus, great attentions have been shown due to its new effect on the treatment of asthma. Unfortunately, since its short half-life (6 h), conventional dosage forms have to be administered 3–4 times a day in order to avoid large fluctuations in plasma concentrations, which lead to poor patient compliance [3]. The large fluctuations of plasma TH level lead to gastrointestinal and cardiovascular adverse effects. Moreover, its therapeutic index is narrow (10–20 μg/mL). The therapeutic effects of TH require a plasma TH concentration at least 5–10 μg/mL and toxic effects are frequent above 20 μg/mL. Sustained release dosage forms can overcome these drawbacks, but long-term oral therapy can't avoid the side effects of gastrointestinal, cardiovascular and central nervous system's disturbances because its elimination half-life varies widely between patients. Hence a long-term TH therapy also leads to serious management problems in asthmatics. Despite the occurrence of numerous new sustained release products of TH, an optimal therapeutic use continues to evolve. Currently, pulmonary delivery has become an increasingly attractive route for drug delivery owing to its enormous surface area for absorption (140 m^2), highly permeable epithelium compared with the gastrointestinal tract and avoiding the first pass hepatic metabolism. Inhalation aerosols of TH can be absorbed but caused local irritant [4, 5], while dry powder inhalation is another potential pharmaceutical form for the direct administration of compounds to the lung. It has been proposed to enhance absorption when drugs were incorporated with colloidal polymer microspheres made of biocompatible materials such as starch, gelatin, albumin, chitosan (CTS) and dextrin [6]. However, there have been few studies on dry powder inhalation of TH from such microspheres as sustained release preparations.

CTS is a cationic natural copolymer of glucosamine, obtained from the deacetylation of chitin which is the second

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most abundant polysaccharide after cellulose in the world. It has been widely used in several pharmaceutical formulations as sustained release carrier systems such as beads [7], gels [8], films [9], sponges [10] and microspheres [11, 12] for its many unique properties such as low toxicity, biocompatibility, biodegradability and mucoadhesive property. Furthermore, CTS has been used as a drug carrier to attain the desirable drug release profile and enhance dissolution rate of low water soluble drugs [13–15]. Recently, CTS has become a good candidate for using in pulmonary delivery [16]. It can bind with mucosal surfaces due to its cationic nature, which leads to bioadhesion and a reduced mucociliary clearance [17]. In addition, CTS has another dramatic effect in terms of improving drug absorption by opening the intercellular tight junctions of the lung epithelium [18].

In this paper, CTS/β-cyclodextrin (β-CD) microspheres loaded TH were prepared as sustained release delivery system administrated to the lung by spray drying method, while β-CD was used as an excipient to improve pharmaceutical and biopharmaceutical properties of drugs [19]. The microspheres were characterized by a series of pharmaceutical properties such as yield, drug entrapment, encapsulation efficiency, morphology, size, swelling, humidity and drug release.

Materials and methods

Materials

TH was kindly supplied by Minsheng Pharmaceutical Factory (Hangzhou, China). CTS (molecular weight 1 300 KDa, deacetylation degree 80%) was prepared in our laboratory. All other chemicals and solvents such as β-CD, acetic acid were provided by Shanghai Chemical Reagent Company (Sigma Co. ST. Louis, USA).

Preparation of microspheres

A predetermined amount of TH, CTS and β-CD was dissolved in 200 mL of 1% acetic acid aqueous solution according to various formulations (Table 1). The solutions were spray-dried, using a spray-dryer (Büchi® Mini Spray Dryer, B-191, Switzerland). Blank microspheres were made as a comparison. The spray-dried microspheres were collected and stored in a desiccator (with anhydrous $CaCl_2$) at room temperature.

Size and morphology

The sizes of microspheres were measured by laser diffraction (Malvern MasterSizer, model MS 2000, Malvern Instruments Ltd, Malvern, UK), determining the volume mean diameter.

Table 1 Composition of formulations used for aqueous solution to be spray dried

Microspheres	TH (%) (w/v)	CTS (%) (w/v)	β-CD (%) (w/v)	TH/CS/β-CD
A	1	1	1/3	1:1:0.33
B	1/3	1	1/3	1:3:1
C	1/5	1	1/3	1:5:1.67

The morphology of microspheres was evaluated by scanning electron microscopy (KYKY2800B, KYKY Technology Development LTD., China). The microspheres were sputter-coated with a thin layer of Au/Pd and photographed.

FT-IR

Infrared (IR) spectra were carried out using an Avater-360 Fourier Transform Infrared Spectroscope (Nicolet). The samples were prepared by grinding the dry blend microspheres (2 mg) with KBr powders (100 mg) and then compressing the mixtures to form disks.

Swelling ability

Swelling behavior was established by measuring water uptake. Dried microspheres of known weight (Wd) were incubated in phosphate buffer solution (PBS, pH 6.8) or HCl (pH 1.2) for 12 h to make microspheres reach swelling equilibrium, centrifuged, removed water on the surface with filter paper and weighed immediately on an electronic balance. The weight of the swollen microspheres (Ww) was recorded. Swelling ability was determined by calculating the water content: (Ww–Wd)/Ww. All experiments were carried out in triplicate.

Moisture uptake

Humidity was evaluated by the moisture uptake. About 100 mg of microspheres (Wd) which were dried to constant weight under vacuum before used, was packed into each polystyrene tubes which were stored in the chamber at 40 °C/RH 75%. At the end of different predetermined intervals (4, 8, 12, 24 and 48 h), the weight of microspheres (Wh) was recorded. The increase in weight represented the weight of moisture taken by the microspheres. The moisture uptake was calculated as a ratio of the weight of absorbed moisture to the weight of the dry microspheres at each period of time as followed: [(Wh–Wd)/Wh] × 100%. All samples were analyzed in triplicate.

Determination of drug entrapments

The drug contents of microspheres were determined using an UV spectrophotometer (Shimadzu UV-Vis Spectrophotometer

UV-1700, Japan) at the wavelength of 275 nm. An equivalent of 30 mg microspheres was dissolved in 100 mL of PBS (pH 6.8), sonicated for 3 min, extracted after vigorous shaking for 3 h, which was sufficient to ensure TH release completely, and calculated according to the standard curve equation $C = 18.228H + 0.1287$ ($n = 3$, $r = 0.9997$) (C: the concentration of TH; H: the value of absorbance at 275 nm). All tests were carried out in triplicate.

In vitro drug release

In vitro release of TH from the microspheres was studied using a dialysis system comprising a dialysis bag and receptor chamber. Specifically, microspheres (30 mg) were put into a dialysis bag and 1 mL of the release medium (PBS, pH 6.8 or HCl, pH 1.2) was added. Then, the dialysis bag was put into a 250 mL flask containing 200 mL of the same medium. The whole apparatus was placed in a water bath shaker with horizontal shaking at 100 rpm, and thermostated at 37 ± 0.5 °C. At set time intervals, 4 mL of samples were withdrawn from the flask, and the same volume of blank medium with the same temperature as that of the tested medium was added immediately. The drug was spectrophotometrically determined in the receiving phase (Shimadzu UV-Vis Spectrophotometer UV-1700, Japan) at 275 nm. All experiments were carried out in five samples and average values were plotted.

Statistical analysis

All the data are the arithmetic mean ± SD, statistical data were analyzed using SPSS13.0 and differences were considered to be significant at a level of $p < 0.05$, using a two tailed paired *t*-test.

Results and discussion

The drug entrapment and encapsulation efficiency are important factors of the microspheres. Table 2 shows the drug entrapments, encapsulation efficiencies and yields with various formulations were different. The drug entrapments of A, B and C microspheres were from 13.33% to 21.09% and 35.70%, which increased with an increase of the ratio of drug/polymer. It can be changed by different ratios to meet the clinical needs. The yields of A, B and C microspheres were 52.98%, 46.08% and 45.81%, respectively, which were higher than that of Alpar reported [20]. This could be probably explained that the viscosity of the solution decreased when β-CD was added which made little product deposit in the chamber during the process of spray drying. The encapsulation efficiencies of A, B and C microspheres ranged from 85.16% to 88.7% and 91.40%, which had no significant differences ($p > 0.05$). These results show that three kinds of microspheres have been successfully developed by spray drying method with considerable yields, encapsulation efficiencies and high drug entrapments.

Table 2 Production yield, entrapment percent and encapsulation efficiency of spray dried microspheres ($n = 3$, mean ± SD, %)

Microspheres	Production yield	Drug entrapment	Encapsulation efficiency
A	52.98 ± 1.05	35.70 ± 0.09	88.79 ± 0.23
B	46.08 ± 0.13	21.09 ± 0.62	91.40 ± 2.71
C	45.81 ± 0.04	13.33 ± 0.33	85.16 ± 2.15

The most important factor of the aerosol that deposits in the lung is the diameters. The aerodynamic diameters of particles for optimal lung administration should be approximately 1–5 μm [21], larger than 6 μm are generally deposited in the upper respiratory tract and less than 1 μm are exhaled without deposition. Therefore, the particle size must be 1–5.0 μm so that the drug may deeply penetrate the lungs. The diameter distribution curves of microspheres are shown in Fig. 1. The particle size distribution analysis of A, B, and C microspheres showed that the volume distributions of 50% [d (0.5)] of the particles were below 4.97, 4.90 and 6.43 μm, while the blank microspheres were below 3.76 μm. The distributions of B and blank microspheres were narrower than that of A and C microspheres, which were more suitable for inhalation. SEM photomicrographs of A, B, C and blank microspheres are shown in Fig. 2. The A, B and C microspheres showed a regular spherical shape and a smooth or slightly wrinkled surface. A similar morphology was observed for blank microspheres. This suggests TH has little effect on the morphology of the spray dried microspheres. The numerous invaginations of the microspheres were probably due to the rapid drying process of spray dried. This similar wrinkled surface morphology was observed in previous studies [11, 12]. The A Microspheres presented few needle-like structures which suggested that some whickers of TH existed on the surfaces of the microspheres.

Figure 3 shows the FT-IR spectra of TH, CTS, β-CD, blank and B microspheres. In the B microspheres (e), the carbonyl stretching vibration near 1,716 cm^{-1} for TH (a) was shifted to 1,706 cm^{-1} with a lower frequency about 10 cm^{-1}. On the other hand, the spectrum of CTS (c) showed the stretching vibration of the amide group appeared at 3,414 cm^{-1}, which shifted to a lower frequency side in the blank microspheres (d) and B microspheres. These results indicated that an intermolecular hydrogen bond had formed between the carbonyl group of TH and the amide group of CTS. The peaks corresponding to the stretching of amide group with strong overlapping hydroxyl group were at 3,000–3,600 cm^{-1}. The spectrum of β-CD (b) showed the hydroxyl group vibration appeared

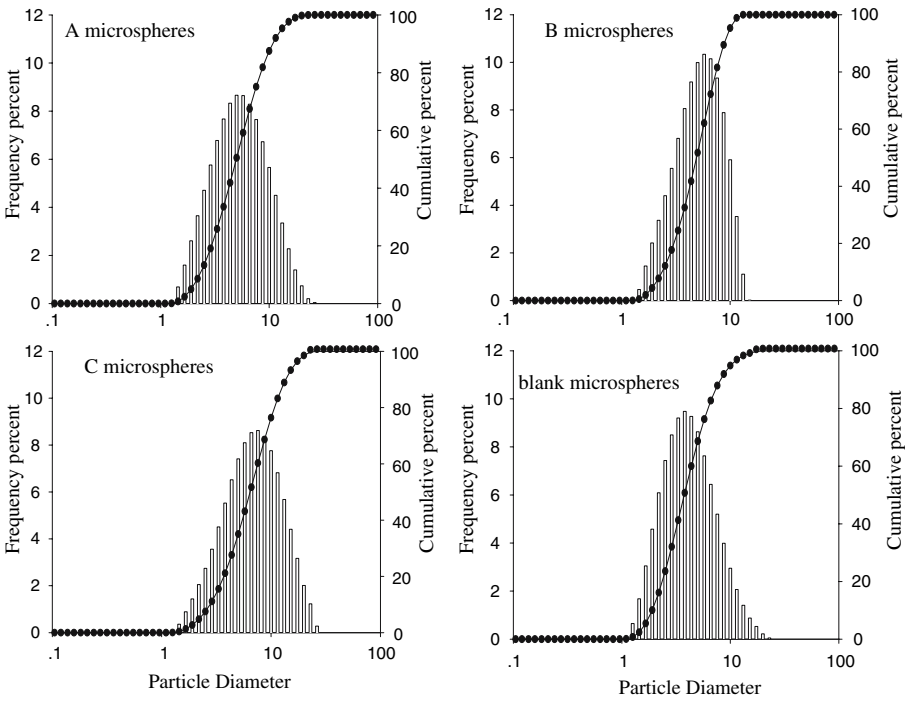

Fig. 1 Particle size distributions of microspheres systems

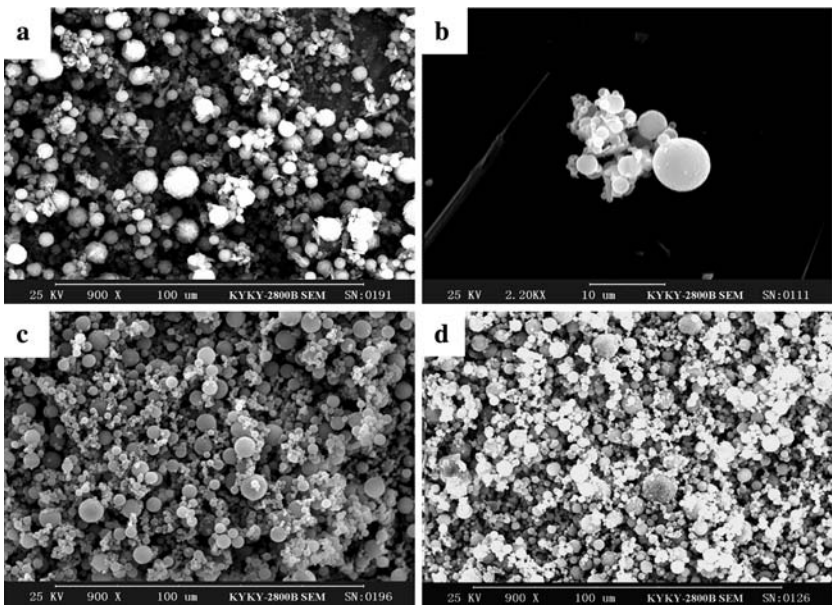

Fig. 2 SEM of spray-dried microsphere. (**a**) A microspheres; (**b**) B microspheres; (**c**) C microspheres; (**d**) blank microspheres

at 3,381 cm^{-1}. Compared with B microspheres, hydroxyl band was less intense with a shift to a lower frequency. It was considered that an intermolecular hydrogen bond had formed between the carbonyl group of TH and the hydroxyl group of β-CD. In short, carbonyl group of TH formed intermolecular hydrogen bonds with amide group of CTS or hydroxyl group of β-CD.

The swelling ratio of three kinds of microspheres at different pH is shown in Table 3. The microspheres absorbed water very fast and dissolved after 12 h at pH 1.2. However, at pH 6.8, the microspheres attained equilibrium within 12 h. The swelling ability of the microspheres was more than three times of their weight. The significant differences of swelling ability were not observed between B and C microspheres, but both less than that of A microspheres.

The humidity rates of microspheres are shown in Table 4. The humidity rates of A, B and C microspheres were from

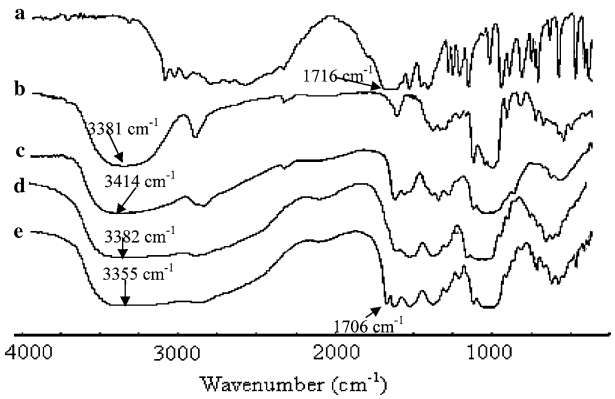

Fig. 3 IR spectra of TH/CTS/β-CD systems. (**a**) TH; (**b**) β-CD; (**c**) CTS; (**d**) blank microspheres; (**e**) B microspheres

1.40% to 4.10% and 3.75% within 4 h. With time prolonged, the humidity rates increased significantly with an increase of the ratio of drug/polymer. The equilibrium was attained in 24 h and the humidity rates of A, B and C microspheres increased little which were 9.00%, 13.5% and 13.98% up to 48 h. These results suggest that the microspheres can absorb an amount of moisture at 40 °C/RH 75%, however it wouldn't be the case when they are stored inside inhalers. Since the humidity rates of microspheres can affect the lung deposition, these results indicate that microspheres should be avoided humidity within storage.

The results of drug release from microspheres in different conditions are shown in Figs. 4 and 5. The drug release from A, B and C microspheres depended on the pH of the release media. For example, the drug release from B microspheres was more than 61.00% in the first hour and attained 74.09% within 8 h at pH 1.2, while at pH 6.8 the drug release was only 39.00% and 60.21%, respectively. Therefore, the release rate of B microspheres was faster at pH 1.2 than that at pH 6.8. In the case of A and C microspheres, the drug release was faster at pH 6.8 than that at pH 1.2 in the first hour, and had no differences within 8 h. This could be explained that CTS had a higher solubility in acidic solution and formed a gel, which was in accordance with the results of swelling ratio. While in the medium of pH 6.8, the microspheres were insoluble and easy to swell, and TH diffusion became slow through the more hydrophilic CTS/β-CD matrix layer. In addition, the solubility of the drug is another factor that has a marked effect on the drug release. Drugs with low solubility in water are the most slowly released [22]. TH, insoluble in water, also decreased the release rate from CTS microspheres. Furthermore, due to its basic nature (pK_a 8.8), the release rate is expected to be higher under acidic conditions, while under the alkaline conditions, TH solubility becomes minimal, which might be reflected in the reduce dissolution of the dispersed drug particles and consequently decreased the release rate [7].

Table 4 The moisture absorption rate of microspheres (mean ± SD, n = 3, %)

Time (h)	Microspheres		
	A	B	C
4	1.40 ± 0.40	4.10 ± 0.75	3.75 ± 0.61
8	3.20 ± 0.30	6.57 ± 0.32	6.73 ± 0.41
12	4.06 ± 1.10	8.05 ± 0.23	8.26 ± 0.58
24	6.03 ± 0.29	13.20 ± 0.95	12.58 ± 0.99
48	9.00 ± 1.21	13.50 ± 0.80	13.98 ± 0.73

The ratio of drug/polymer also had effect on the release of the microspheres. As shown in Fig. 5, the initial drug release significantly increased with an increase in the ratio of drug/polymer. The drug released rapidly from A microspheres and reached 74.00% within 1 h, while B microspheres was 39.00%. This result is consistent with the morphology of the microspheres. For example, A microspheres showed that some TH was on the surfaces of the microspheres which resulted in higher initial drug release. In addition, owning to the high ratio of drug/polymer of A microspheres, the TH particles presented at or near the surface were also normally released during the initial burst effect. The release rate of TH from B microspheres were slower than that of A microspheres which showed more than 85.50% drug release within 8 h, while B microspheres were only 60.20% during the same period. C microspheres also showed a fast release behavior which indicated that the drug release didn't show a more sustained release with an increase of polymer. These results suggest that drug release depend on the ratio of drug/polymer. The release rate of TH decreased as the amount of polymer increased such as from 1:1:0.33 to 1:3:1 (from A microspheres to B microspheres), but the release rate increased with the amount of polymer increased to 1:5:1.67 (C microspheres). This result indicated that the prepared microspheres might

Table 3 Swelling ratio of spray dried microsphere (mean ± SD, n = 3)

Microspheres	2 h		12 h	
	1.2	6.8	1.2	6.8
A	7.22 ± 1.46	Incompletely swelling	Disintegrated	3.88 ± 0.07
B	7.39 ± 0.88	Incompletely swelling	Disintegrated	3.19 ± 0.21
C	8.26 ± 2.31	Incompletely swelling	Disintegrated	3.19 ± 0.77

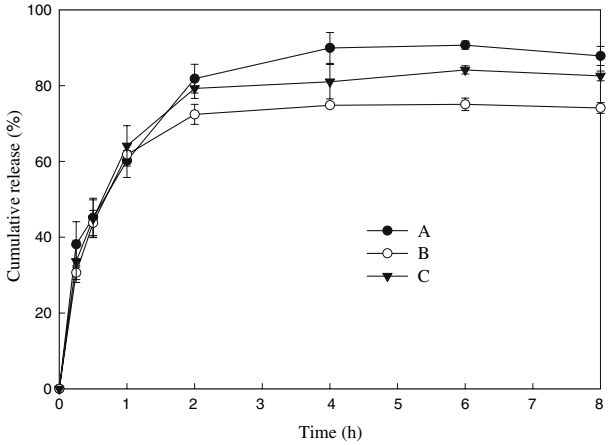

Fig. 4 The release profile of TH from the spray dried microspheres at pH 1.2 (mean ± SD, $n = 5$)

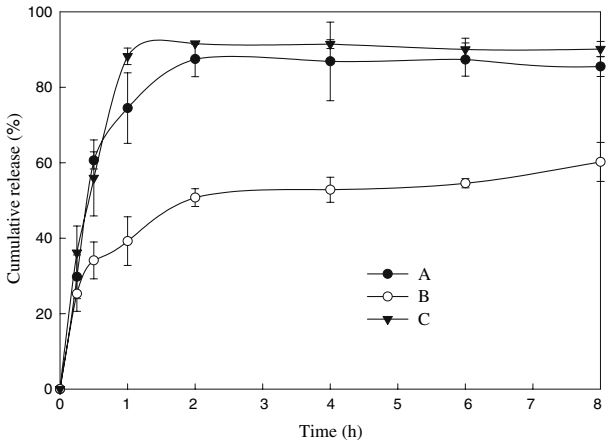

Fig. 5 The release profile of TH from the spray dried microspheres at pH 6.8 (mean ± SD, $n = 5$)

have the proper ratio of drug/polymer in order to achieve sustained release profile.

Conclusions

The present study has demonstrated that CTS/β-CD microspheres loaded TH can be successfully produced by spray drying method with the yield about 50.00%, high drug entrapment and encapsulation efficiency. SEM showed the microspheres possessed spherical shape with smooth or wrinkled surfaces and the suitable diameters indicated a possibility for inhalation. FT-IR spectroscopy revealed that TH formed hydrogen bonds with CTS or β-CD. The in vitro release of TH from microspheres was slower at pH 6.8 than that at pH 1.2 and also related to the swelling ability, especially in the ratio of drug/polymer. B Microspheres had a prolonged release pattern, providing the release of 60.20% (pH 6.8) within 8 h. These results indicate that B microspheres are effective as sustained release drug delivery system for pulmonary delivery.

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References

1. J. P. FINNERTY, C. LEE, S. WILSON, J. MADDEN, R. DJUKANOVIC and S. T. HOLGATE, *Eur. Respir. J.* **9** (1996) 1672
2. Y. TOHDA, M. MURAKI, T. IWANAGA, H. KUBO, M. FUKUOKA and S. NAKAJIMA, *Int. J. Immunopharmacol.* **20** (1998) 173
3. T. MELLSTRAND, N. SVEDMYR and P. O. FAGERSTORM, *Eur. J. Respir. Dis. Suppl.* **109** (1980) 54
4. D. KANDT, I. SEHRT and H. IWAINSKY, *Z. Erkr. Atmungsorgane.* **157** (1981) 152
5. H. P. LI, G. J. HE and X. H. YI, *Shanghai Med. J.* **23** (2000) 465
6. C. R. BEHL, H. K. PIMPLASKAR, A. P. SILENO, J. De MEIRELES and V. D. ROMEO, *Adv. Drug. Deliv. Rev.* **29** (1998) 89
7. K. AIEDEH and M. O. TAHA, *Eur. J. Pharm. Sci.* **13** (2001) 159
8. V. R. PATEL and M. M. AMIJI, *Pharm. Res.* **13** (1996) 588
9. S. PUTTIPIPATKHACHORN, J. NUNTHANID, K. YAMAMOTO and G. E. PECK, *J. Control. Release* **75** (2001) 143
10. K. OUNGBHO and B. W. MÜLLER, *Int. J. Pharm.* **156** (1997) 229
11. P. HE, S. S. DAVIS and L. ILLUM, *Int. J. Pharm.* **187** (1999) 53
12. A. GANZA-GONZÁLEZ, S. ANGUIANO-IGEA, F. J. OTERO-ESPINAR and J. BLANCO MÉNDEZ, *Eur. J. Pharm. Biopharm.* **48** (1999) 149
13. M. ASADA, H. TAKAHASHI, H. OKAMOTO, H. TANINO and K. DANJO, *Int. J. Pharm.* **270** (2004) 167
14. Y. C. HUANG, M. K. YEH and C. H. CHIANG, *Int. J. Pharm.* **242** (2002) 239
15. F. MAESTRELLI, N. ZERROUK, C. CHEMTOB and P. MURA, *Int. J. Pharm.* **271** (2004) 257
16. H. OKAMOTO, S. NISHIDA, H. TODO, Y. SAKAKURA, K. IIDA and K. DANJO, *J. Pharm. Sci.* **92** (2003) 371
17. S. S. DAVIS, *PSTT* **2** (1999) 450
18. H. YAMAMOTO, Y. KUNO, S. SUGIMOTO, H. TAKEUCHI and Y. KAWASHIMA, *J. Control. Release* **102** (2005) 373
19. T. KINNARINEN, P. JARHO, K. JÄRVINEN and T. JÄRVINEN, *J. Control. Release* **90** (2003) 197
20. H. O. ALPAR, S. SOMAVARAPU, K. N. ATUAH and V. W. BRAMWELL, *Adv. Drug. Deliv. Rev.* **57** (2005) 411
21. C. BOSQUILLON, C. LOMBRY, V. PRÉAT and R. VANBEVER, *J. Control. Release* **70** (2001) 329
22. J. AKBUGA, *Int. J. Pharm.* **100** (1993) 257