ELSEVIER

International Journal of Pharmaceutics 151 (1997) 175–182

international journal of pharmaceutics

In vitro and in vivo respirable fractions of isopropanol treated PLGA microspheres using a dry powder inhaler

Vinod A. Philip [1], Rahul C. Mehta [2], Patrick P. DeLuca *

Department of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Kentucky, 907 Rose St., Lexington, KY 40536-0082, USA

Received 6 September 1996; accepted 23 January 1997

Abstract

The purpose of this study was to evaluate the effect of surface treatment with isopropanol on the in vitro and in vivo distribution of poly (D,L-lactide-co-glycolide) (PLGA) microspheres from a dry powder inhaler (DPI). ^{111}In-labeled PLGA microspheres were prepared by solvent extraction/evaporation in order to determine the in vitro/in vivo respirable fraction (RF) and deposition pattern in the respiratory tract (RT) of an animal model. The in vitro RF, determined by cascade impaction, was significantly larger than that of untreated microspheres while the in vivo deposition in the RT, via intranasal administration using a breathing chamber, was two-fold larger for the isopropanol treated microspheres. About 20–30% of the radioactivity recovered from the RT was deposited beyond the trachea. Formulation strategies for powders in DPIs which maximize turbulence and minimize pressure drop should provide increased RFs, thereby providing more useful targeting to the pulmonary area. © 1997 Elsevier Science B.V.

Keywords: Dry powder inhalers; Isopropanol; PLGA microspheres; Respirable fractions

1. Introduction

The respirable fraction (RF), or the fraction of an aerosol dose that deposits in the respiratory tract, is commonly regarded as an index of the efficacy of an aerosol product. The RF of a metered dose inhaler (MDI) is roughly twice as large as that obtained with a dry powder inhaler (DPI), but usually below 20% for any type of aerosol product (Byron, 1986, 1990). The residence time of the small fraction of drug that does manage to reach the desired sites in the respiratory tract (RT) is very short due to rapid absorption and clearance of the deposited drug (Clark

* Corresponding author.

[1] Present address: Storz Ophthalmics Division, American Home Products, Pearl River, NY 10965, USA

[2] Present address: ISIS Pharmaceuticals, 2292 Faraday Ave., Carlsbad, CA 92008, USA

PII S03 8-5173(97)04908-9

and Byron, 1985). Most DPI formulations presently use drug in micronized form along with diluents such as lactose. Microspheres offer an alternative form that can be prepared with different morphological characteristics such as size, shape, and porosity by modifying the processing parameters (Philip et al., 1997).

The delivery of respiratory drugs via biodegradable microspheres have particular appeal since sustained release of drug can improve patient compliance and provide prolonged therapeutic drug levels. Delivery of small amounts of drug in a microsphere-encapsulated isoproterenol system provided prolonged and enhanced protection from bronchoconstriction in the airway smooth muscles of rats, when compared with non-encapsulated drug (Lai et al., 1993). The success of such a dosage form would depend on the delivery of the carrier particles to targeted areas within the RT, uniform release of the encapsulated drug, biodegradation of the polymer matrix in a reasonable time, and freedom from tissue damage (Philip et al., 1992).

Microspheres prepared from hydrophobic poly (D,L-lactide-co-glycolide) (PLGA) are especially suitable for respiratory drug delivery because they are less susceptible to the effects of hygroscopic growth and biodegrade at predictable rates (DeLuca et al., 1993). The critical aspect of a DPI formulation of microspheres would be the ability of the particles to remain deaggregated and free-flowing, since aggregation of the powder affects the RF of the delivered dose. The molecular, electric, coulombic and capillary forces responsible for powder aggregation are influenced by particle size, surface morphology/charge, and moisture content (Zimon, 1982; Newman, 1967; Atkins et al., 1992).

Isopropanol (IPA) treatment of PLGA microspheres was determined to be beneficial in improving the RF during studies of the effect of surface charge and moisture content on aggregation of the powder (Philip et al., 1997). The high relative unipolarity of the IPA formulation resulted in its deaggregation, a consequence of interaction among particles of similar charge being much lower than that with particles of opposite/no charge (Zimon, 1982). The IPA treatment of the microspheres may have resulted in a cleaning of the surface of the particles, which in turn may have caused modifications of the powder properties that resulted in a more deaggregated form of the powder in the dry state.

The objective of this work was to evaluate the effect of surface treatment with isopropanol on the in vitro and in vivo distribution of PLGA microspheres using a DPI. The microspheres were tagged with an ^{111}In radiolabel, treated with isopropanol, and characterized for particle size and surface morphology. In vitro RF was determined with an Anderson cascade impactor while in vivo distribution was measured in the RT of rats following intranasal administration. ^{111}In was employed in these studies since it was easily incorporated with high specific activity into the PLGA microsphere system and had a conveniently short half life of 2.8 days. The intranasal route of administration was preferred over the intratracheal route, since the latter would not permit the evaluation of redispersability of powder caused by breathing of the animal. Although intranasal administration can be performed more efficiently with nasal delivery devices, a DPI was used since the influence of surface charge on the redispersion of the microspheres would only be truly represented by using the actual delivery system.

2. Experimental

2.1. Preparation of PLGA microspheres

2.1.1. ^{111}In-labeling

Microspheres were prepared using a previously reported method (Philip et al., 1992). The ^{111}In chloride stock solution ($t_{1/2}$: 2.8 days, Medi-Physics, Arlington Hts, IL) was diluted with 4 ml 0.1 M acetate buffer (pH 5.0) and converted to ^{111}In oxine by reacting with 200 μl of a 1 mg/ml 8-hydroxy quinoline solution in 95% ethanol (Sigma, St. Louis, MO). The complex was extracted using 2 ml chloroform (Fisher Scientific, Fairlawn, NJ) and subsequently evaporated to dryness under in a 50°C water bath. The complexation/extraction steps were performed

two more times to ensure maximum conversion of the chloride to oxine.

The dispersed phase (DP) consisted of the dry ^{111}In oxine complex with 0.5 g PLGA (Resomer® RG 503, 50:50, 34 000 Da, Boehringer Ingelheim, Ingelheim, Germany) and 5 mg of indium oxine (Sigma), in 10 ml of chloroform/methylene chloride (1:1 v/v). The indium oxine was used to saturate the continuous phase (CP) to prevent loss of the ^{111}In label from the ^{111}In oxine complex by solubilization. The CP was a 0.04% w/v solution of sodium oleate (EM Science, Cherry Hill, NJ) in distilled water. The DP was added to the CP, using a Silverson dispersator (Model L4R, Silverson Machines, Chesam, England) under controlled processing conditions. The mixing speed was 7000 rpm and the temperature of the continuous phase was increased from 6 to 25°C during the run. The emulsion was transferred to a magnetic stirrer/hot plate, and briskly stirred while warming at about 35°C to evaporate the final traces of methylene chloride. The resulting microspheres were recovered by filtration, washed with distilled water and dried under vacuum.

2.1.2. Isopropanol treatment

A suspension of 400 mg ^{111}In-labeled PLGA microspheres was prepared in 100 ml IPA by sonication and subsequently agitated for 12 h at room temperature using a previously described method (Philip et al., 1997). The suspension was filtered, rinsed with IPA and vacuum dried for 2 h. The microspheres were triturated lightly and dried again for 2 h.

2.2. Characterization of microspheres

2.2.1. Particle size analysis

The particle size and size distribution were analyzed by a Malvern® laser light scattering device (Malvern Instruments, Worcestershire, England). Samples were prepared by suspending microspheres in Milli-Q® water (Millipore) containing 0.01% w/v Tween-80 (Sigma).

2.2.2. Scanning electron microscopy (SEM)

Samples for surface morphology and particle size analysis were prepared in the dry state using DPIs since the surface of the powder would be altered by suspension in a liquid medium. The powder was loaded in a Pfeiffer DPI (Pfeiffer, Princeton, NJ) and sprayed directly on a polycarbonate membrane filter (Poretics, Livermore, CA) that was secured on an aluminum sample mount (Ted Pella, Redding, CA). A conductive gold/palladium coating was applied to the surface prior to examination using a Hitachi Model S-800 SEM instrument (Hitachi, Mountain View, CA) at an energy level of 5 to 10 kV and magnifications of 1–8K.

2.2.3. Specific activity/in vitro release

The specific activity of the microspheres was determined using a Capintec CRC-12 Radioisotope Calibrator (Capintec Instruments, Ramsey, NJ). The in vitro release of the ^{111}In oxine from the PLGA microspheres was determined in saline containing Tween-80 (0.1% w/v). The ^{111}In-labeled microspheres were quantitatively transferred to centrifuge tubes, and the release medium was quantitatively added to the tubes. The tubes were placed on a rotating wheel for pre-determined time intervals, centrifuged and the supernatant sampled and analyzed for radioactivity using a Packard Autogamma Scintillation Counter. An equivalent amount of fresh release medium was added at each sampling point.

2.3. Determination of respirable fractions

The selection of a DPI that would enable easy characterization of the dry powder formulations in vitro and in vivo was limited to a few devices (Philip et al., 1994). The Pfeiffer DPI is convenient since it delivers the dose by actuation of a spring/air displacement mechanism, unlike most DPIs which require special mechanisms to dispense a dose without the breathing force of a patient.

2.3.1. Determination and validation of the DPI output dose

The Capintec radioisotope calibrator was used to determine the dose of ^{111}In-labeled microspheres for the in vitro and vivo studies. The radioactive dose delivered from the DPI (in μCi)

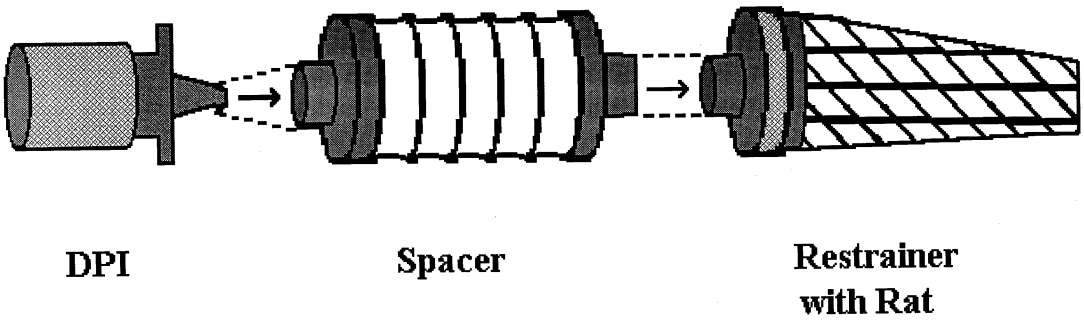

Fig. 1. Schematic of apparatus for administration of powder via DPI to rat.

was recorded following each actuation of the device, and an equivalent amount of radioactivity (in the form of ^{111}In-labeled microspheres) was taken in a sample tube and used to determine the radioactivity in counts per minute (cpm) with a Packard Gamma counter (Packard Instrument, Downers Grove, IL). The radioactivity obtained in μCi from the radioisotope calibrator and cpm from the gamma counter showed adequate correlation (r^2, 0.982) and could be used to estimate the radioactive dose delivered from the DPI.

2.3.2. Determination of in vitro RFs

The aerodynamic size distribution of a powder determined with a cascade impactor can be used to evaluate its degree of aggregation. The Andersen 1.0 CFM Cascade Impactor (Andersen Samplers, Atlanta, GA) was used along with its preseparator and a glass adapter/throat (University of Kentucky glass shop) for the determination of RF of the powder samples.

A previously reported method was used for the aerodynamic sizing of the microsphere formulations (Philip et al., 1992). The desired amount of powder was delivered to the cascade impactor, and the glass fiber substrates placed on the individual stages of the impactor were measured for radioactivity. The RF of the microspheres was determined by dividing the total activity in the stages corresponding to the respirable sizes (i.e., below 5.8 μm) by the total activity delivered to the cascade impactor (Philip et al., 1994).

2.3.3. Determination of in vivo RF/distribution in the respiratory tract

In vivo deposition of the ^{111}In-labeled PLGA microspheres was studied by intranasal delivery using the Pfeiffer DPI in Long-Evans rats. The set up used in these studies for the administration of the powder to the rat is shown in Fig. 1. The unanesthetized rats were positioned in a restrainer in such a manner that only their nostrils and a small portion of the mouth would be exposed to the spray of the DPI. A prefabricated spacer device (Tobacco and Health Research Dept., University of Kentucky) was used to deliver a finer particle size fraction to the rats.

The DPI was filled with approximately 30 μCi of ^{111}In-labeled microspheres, the powder sprayed into the spacer for the animal to breathe, and the delivered amount of activity measured. The rat was immediately sacrificed with a lethal injection of 3 ml of a 12 mg/ml sodium pentobarbital solution (Sigma, St. Louis, MO). The rat was dissected to recover the trachea, left and right lobes of the lung, and the weights of the individual tissues were recorded. The sampled tissues were counted for radioactivity and the RF was calculated by dividing the total activity recovered in the trachea and the lungs by that delivered to the rat. Statistical analyses of differences between treatment were performed using analysis of variance and the Student's t-test.

Table 1
Distribution of radioactivity from ^{111}In-labeled microspheres in the cascade impactor assembly

Microsphere formulation	n	(%) Distribution in		In vitro respirable fraction
		Glass throat + preseparator[a]	Cascade impactor[b]	
Untreated PLGA (± S.D.)	3	89.67 ± 2.7*	10.33 ± 2.7	4.92 ± 0.34
IPA treated PLGA (± S.D.)	3	80.70 ± 1.1	19.30* ± 1.1	11.69 ± 1.27

[a] Difference in radioactivity obtained as: (DPI—Cascade impactor), expressed as percentage of total radioactivity delivered from the DPI following cascade impaction.
[b] Radioactivity penetrating beyond first impaction stage of cascade impactor, expressed as percentage of total radioactivity delivered from the DPI following cascade impaction.
* Significantly greater ($p = 0.01$).

3. Results

3.1. Characterization of microspheres

3.1.1. Particle size and surface morphology

The microspheres had an average particle size of 2.8 μm, and 100% of the particle volume was below 5.0 μm. The process of preparation of the sample for Malvern analysis by ultrasonication essentially separates most of the aggregates and therefore provides an estimate of the best state of deaggregation of the microspheres.

The SEM analysis of untreated and IPA treated ^{111}In-labeled microspheres indicated that both formulations were aggregated, with many particle clumps of 10–30 μm size. There were also a number of separate particles and smaller aggregates of microspheres that were in the respirable size range. Scanning electron microscopy did not reveal any noticeable differences in surface morphology between the treated and untreated microspheres. This agreed with previous findings with unlabeled PLGA microspheres (Philip et al., 1997).

3.1.2. Specific activity/in vitro release

The specific activity (corrected to zero time) of the ^{111}In-labeled microspheres was 1.69 and 1.83 μCi/mg for the two batches. The IPA treated microspheres had a lower specific activity of 1.24 μCi/mg, due to the loss of some activity to the isopropanol wash during the coating process.

The ^{111}In-labeled microspheres released 11.7% of the radiolabel in 15 min and then the release leveled off with a total of 14.5% released after 10 h, and no further release up to 22 h. The low figure for total release of the ^{111}In label from the microspheres indicates that the label is adequately stable and acts as a suitable marker for the presence of the microspheres. Since the microspheres are in contact with the fluids in the RT for a very short time, the measured radioactivity can be assumed to reflect intact ^{111}In label on the microspheres.

3.1.3. Aerodynamic size distribution

The distribution of radioactivity in the cascade impactor assembly following sampling of ^{111}In-labeled microspheres is shown in Table 1. The IPA treated microspheres were more easily redispersed as compared to the untreated microspheres, indicated by a significantly smaller fraction in the preseparator/throat and a larger fraction entering the cascade impactor.

The distribution of radioactivity in the various stages of the cascade impactor is shown in Fig. 2. The untreated microspheres had a higher fraction of radioactivity deposited in the first stage as compared to the IPA treated microspheres (39.7 and 25.1%, respectively). Moreover, the IPA treated microspheres had a much larger fraction in the aerodynamically useful size ranges (1.1 to 5.8 μm) as compared to the untreated spheres (53.8 and 39.7%, respectively). Thus, the IPA treated microspheres were easily redispersible unlike the untreated microspheres.

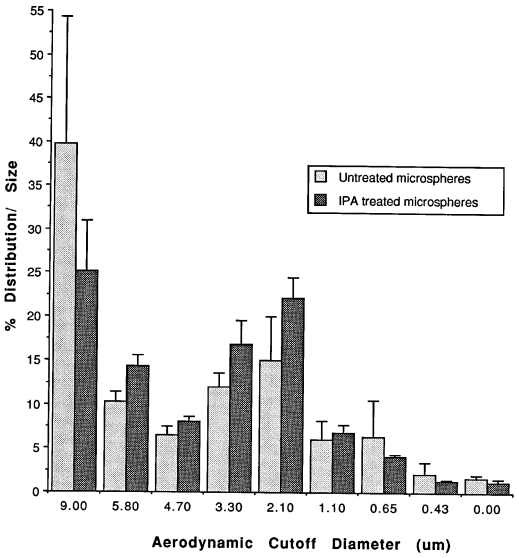

Fig. 2. Radioactivity distribution of ^{111}In-labeled PLGA microspheres entering the cascade impactor following sampling with a Pfeiffer DPI.

3.1.4. In vivo deposition of ^{111}In-labeled microspheres in the rat

The weight normalized distribution of the ^{111}In in the RT of the rats following administration of the ^{111}In-labeled PLGA microspheres by Pfeiffer DPI is shown in Table 2. The majority of the radioactivity was located in the trachea for both the treatment groups, and the distribution of radioactivity in the lungs was slightly greater in the left lung than the right lung, especially in case of the untreated microspheres.

The total radioactivity deposited in the RT was significantly larger for the IPA treated microspheres as compared to the untreated microspheres. There was a two-fold larger deposition for the IPA treated microspheres, even with a 19% smaller average dose.

3.1.5. In vitro/in vivo respirable fractions

The in vitro RFs of the ^{111}In-labeled PLGA microspheres are listed in Table 1, while the in vivo RFs are in Table 2. The RFs of the IPA treated ^{111}In-labeled microspheres were significantly higher than the untreated microspheres in both the in vitro and in vivo studies ($p < 0.01$).

4. Discussion

The cascade impaction results demonstrate that the IPA formulation was more easily redispersed as compared to untreated microspheres. Although the primary untreated particles were in the respirable sizes and separable following dispersion by suspension in Tween-80, they did not adequately redisperse following aerosolization. This also suggests that the Pfeiffer DPI by itself did not effect any significant deaggregation of the untreated microspheres, at least at the flow rate (28.3 l/min) used for the studies. This feature of the Pfeiffer DPI was desirable for the study of the effects of the formulation variables on aggregation, since it effectively limited the factor of redispersion by the DPI.

The radioactivity recovered in the RT was adequately distributed in the lower regions (about 20–30%), which compares well with recently reported data (Ganderton and Kassem, 1992). The slightly lower radioactivity in the right lung as compared to the left lobe may be expected due to the anatomy of the rat lungs. The left lung has a single large lobe (average weight, 0.53 g), while

Table 2
Weight normalized distribution of ^{111}In in the rat following intranasal administration of ^{111}In-labeled microspheres

PLGA microsphere formulation	Average dose (cpm; ± S.D.)	Radioactivity recovered (cpm)/g tissue (± S.D.)				In vivo respirable fraction
		Trachea	L. Lung	R. Lung	Total	
Untreated	859 255 ± 313 257	6328 ± 2488	1439 ± 288	1078 ± 244	8845 ± 2898	0.420 ± 0.17
IPA treated	696 587 ± 78 105	14 554 ± 5353	1926 ± 601	1575 ± 459	18 055* ± 5087	0.921 ± 0.25

* greater ($p < 0.01$).

the right lung has three smaller lobes (average weight, 0.92 g). The manner of branching of the right lung may be adversely affecting the penetration of the powder into the smaller subdivisions of the RT.

The in vitro RF values determined with the ^{111}In-labeled microspheres were similar to those obtained in studies with unlabeled microspheres: 3.84 and 12.9%, for untreated and IPA treated, respectively (Philip et al., 1997). This indicates that the addition of the ^{111}In label to the PLGA microspheres did not change the aerodynamic deposition characteristics of the microspheres.

The in vivo RFs are much lower when compared to the in vitro values. An important factor affecting the deposition of an aerosol is the physiology of respiration. In human subjects, high inhaled flow rates and breath holding immediately following dosing has been shown to significantly improve the deposition of the aerosol (Newman et al., 1994). The flow rate of the cascade impactor is about 28.3 l/min, while the peak inspiration flow rate of the anesthetized rats was determined to be 0.3 to 0.6 l/min. This difference in flow rate contributes significantly to the redispersion of the powder, and is reflected in the RF obtained from the two techniques.

Rapid, shallow breathing causes deposition in the large airways, whereas slow, deep breathing causes deposition in the alveolar regions (Thompson, 1992). It was difficult to synchronize the dose delivery with the breathing of the rat; moreover, the animals attempted to discontinue breathing the aerosol and even to exhale the powder after the initial doses. The RF values obtained by administration of powder intranasally are always lower than those obtained by oral administration, due to the more tortuous pathway involved in the transport of the powder to the interior regions of the RT. An alternative method available for administration of the powder is by intratracheal intubation, which was not attempted because the charge environment during dosing would not accurately reflect that present when using the DPI.

Conventional DPIs available in the market present many difficulties in their use for testing powder aerosols in animals, since most of them require cumbersome procedures for filling and dose delivery. The Pfeiffer prototype DPI was suitable for the purposes of the experiments in this study, since it enabled easy in vitro and in vivo characterization of the dry powder formulations. The Pfeiffer prototype used in these studies did have some disadvantages such as non-reproducibility of the dose volume, and a limited ability to effect redispersion of the powder. The problem with the dose volume reproducibility was accounted for by dose calibration. DPIs such as the Spinhaler and the Turbohaler are designed to maximize turbulence and minimize the pressure drop, which could provide even higher estimates of RFs for the tested microsphere formulations.

5. Conclusions

The IPA treatment of PLGA microspheres resulted in significantly improved redispersibility of the particles following delivery by the Pfeiffer DPI. The in vivo distribution pattern of the IPA formulation was found to be suitable for pulmonary delivery. DPIs that improve the RF by maximizing turbulence and minimizing the pressure drop may provide even larger estimates for the RF of the microsphere formulations studied here. It has been suggested that sustained release systems (such as PLGA microspheres) could be useful in pulmonary therapy due to their potential to improve the therapeutic efficacy by utilizing the kinetic aspects of the drug in the RT (Niven, 1992). The residence time for a drug in microsphere-encapsulated form would be increased since it will not be removed completely from the RT by absorption/metabolism in the smaller airways and would be slowly released as the particles move up the mucociliary escalator.

Acknowledgements

This work was submitted as partial fulfillment of Dr. Vinod Philip's requirement for a Doctor of Pharmacy degree.

References

Atkins, P.J., Barker, N.P. and Mathisen, D., In: Hickey, A.J. (Ed.) *Pharmaceutical Inhalation Aerosol Technology*, Marcel Dekker, New York, 1992, pp. 155–185.

Byron, P.R., *Drug Dev. Ind. Pharm.*, 12 (1986) 993–1015.

Byron, P.R., In: Byron, P.R. (Ed.), *Respiratory Drug Delivery*, CRC Press, Florida, 1990, pp. 168–201.

Clark, A.R. and Byron P.R., *J. Pharm. Sci.*, 74 (1985) 939–942.

DeLuca, P.P., Mehta, R.C., Hausberger, A.G. and Thanoo, B.C., (Eds.), In: *Polymeric Delivery Systems*, Vol. 4, American Chemistry Society, 1993, pp. 53–79.

Ganderton, D. and Kassem, N.M., In: Ganderton, D. and Jones, T. (Eds.), *Advances in Pharmaceutical Sciences*, Vol. 6, Academic Press, London, 1992, pp. 165–191.

Lai, Y.L., Mehta, R.C., Thacker, A., Yoo, S.D., Mcnamara, P.J. and DeLuca, P.P., *Pharma. Res.*, 10 (1993) 119–125.

Newman, B.S., In: Bean, H.S., Beckett, A.H. and Carless, J.E. (Eds.), *Advances in Pharmarmaceutical Sciences*, Vol. 2, Academic Press, London, 1967, pp. 181–221.

Newman, S.P., Hollingworth, A. and Clark, A.R., *Int. J. Pharm.*, 102 (1994) 127–132.

Niven, R.W., In: Hickey, A.J. (Ed.), *Pharmaceutical Inhalation Aerosol Technology*, Marcel Dekker, New York, 1992, pp. 321–359.

Philip, V.A., Kulkarni, M.K., Doll, W.A., Mehta, R.C., Digenis, G.A., Lai, Y.L. and DeLuca, P.P., *Pharm. Res.*, 9(10) (1992) S251.

Philip, V.A., Mehta, R.C., Mazumder, M.K. and DeLuca P.P., *Pharm. Res.*, 11 (1994) S211.

Philip, V.A., Mehta, R.C., Mazumder, M.K. and DeLuca, P., *Int. J. Pharm.*, (1997) Accepted for publication.

Thompson, D.C., In: Hickey, A.J. (Ed.), *Pharmaceutical Inhalation Aerosol Technology*, Marcel Dekker, New York, 1992, pp. 155–185.

Zimon, A.D. (Ed.), *Adhesion of Dust and Powder*, Bureau, New York, 1982, pp. 1–24.