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Preparation and *In Vitro* Evaluation of Budesonide Spray Dried Microparticles for Pulmonary Delivery

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Abstract

The present study describes development and *in vitro* evaluation of budesonide microparticles prepared by spray drying for delivering drug directly to lungs via dry powder inhaler. This paper introduces new formulations for pharmaceutical applications which includes conventional formulations and novel spray dried microparticles *viz.*, pulmosols, microspheres and porous particles. Optimized spray drying parameters for generation of microparticles were: inlet temperature, 130 °C; outlet temperature, 80 °C; aspirator rate, 240 mWc (60%); solution feed rate, 2 ml/min; spraying air flow pressure, 2 bar. Microparticles appeared to be spherical, low-density particles characterized by smooth surface. MMAD and GSD ranged from 2.5–4.6 µm and 1.5–2.7 respectively. Effective index of microspheres (54.48) and porous particle formulations (64.22) was higher than the conventional formulation (49.21) indicating more effective deposition of microparticles to the lungs. Carr's Index (20–30%) and Hausner ratio (1.2–1.7) for all formulations indicated good powder flow properties. Formulations emitted a fine particle fraction of 25–47%. Microparticles showed extended *in vitro* drug release upto 4 hours with high respirable fractions, thus use of microparticles potentially offers sustained release profile along with improved delivery of drug to the pulmonary tract.

Keywords

Spray drying • Budesonide • Microparticles • Dry powder inhaler • Drug deposition

Introduction

Pulmonary route presents several advantages in treatment of respiratory diseases. Drug inhalation enables a rapid and predictable onset of action and induces fewer side effects than administration by other routes [1, 2]. Dry powder inhalers (DPIs) are easier to use, more stable and efficient systems with better lung delivery than nebulizers/ MDIs and are typically formulated as one-phase, solid particle blends [3]. As a result, preparation of dry powder formulations for inhalation is an interesting and appreciated proposition [4]. When preparing a formulation suitable for a DPI, micronization is usually employed to reduce particle size of the drug powder to less than 5 μm [5], however powders in this size range exhibit strong interparticulate cohesion leading to poor powder flow properties. Furthermore, factors known to influence aerosolization properties of dry powders (e.g. particle morphology, density and surface composition) cannot be controlled effectively during the micronization process [6]. Researchers have investigated a number of approaches to improve powder aerosolization, such as mixing the micronized drug with inert carrier particles or modification of particle morphology, particle surface roughness, particle porosity or powder density [7-9]. An alternative approach in generation of dry powders for pulmonary drug delivery is offered by spray drying technology. Spray drying is one-step constructive process that provides greater control over particle size, particle morphology and powder density whereas micronization is a destructive technique [1, 3, 10].

Spray dried powders that exhibit sustained drug release properties may be generated through inclusion of drug release modifiers such as chitosan [11]. Chitosan, a polysaccharide derived from deacetylation of naturally occurring polymer chitin, is a promising excipient that can be employed in a wide range of applications, including sustained release preparations [12]. There are many advantages for developing sustained release formulations for pulmonary drug delivery which includes reduced dosing frequency, improved patient compliance and reduction in side effects [11, 13, 14]. Chitosan not only acts as a drug release modifier but also has mucoadhesive properties thus it appear to be a useful excipient while preparing sustained release formulations for pulmonary drug delivery [10, 11]. Chitosan has shown to be both biocompatible and biodegradable [15, 16]. The oral LD_{50} for mice, 16 g/kg, indicates a very low toxicity potential for this product [17, 18]. It is an approved food additive that has been considered for pharmaceutical formulation and drug delivery applications, in which attention has been focused on its absorption-enhancing, controlled release and bioadhesive properties. Recently, chitosan-based delivery systems have been proposed to increase the bioavailability of drugs both at the nasal mucosa and in the lungs [19, 20]; also these systems have been reported as efficient vehicles for pulmonary gene delivery [21–23]. Nevertheless chitosan is not included in the FDA Inactive Ingredient Guide and very sparse data on its pulmonary toxicity are available.

It is now widely accepted that inhaled corticosteroids (ICSs) are effective in controlling inflammation, improving lung function and reducing asthma symptoms. As a result, ICSs are recommended as first-line therapy for all patients with persistent asthma. There is considerable evidence that treatment with anti-inflammatory ICSs reduces morbidity and mortality in asthma [24]. ICSs appear to have a place in management of severe COPD, perhaps by decreasing the frequency of exacerbations and improve quality of life in patients with COPD [25]. Budesonide (BUD) has high glucocorticoid receptor affinity and prolonged tissue retention and it inhibits inflammatory symptoms such as edema and

vascular hyperpermeability [25]. Budesonide has low molecular weight of 430.53 Da having oral bioavailability of 6–11% with half life of 2–3 hr [26]. Budesonide forms esters in all tissues, PK modeling has shown that ester formation occurs primarily in the large airways and lungs, sustaining local anti-inflammatory activity. High doses of ICSs are recommended in the treatment of moderate to severe persistent asthma. It is well accepted that ICSs have fewer systemic side effects than oral or parenteral glucocorticosteroids [27, 28], but there is still some concern about the long-term safety of high doses of ICSs [29, 30]. Long-term systemic side effects of high doses of ICSs include thinning of the skin and easy bruising. The aim of inhaled administration of corticosteroids in respiratory disease is to achieve high local concentrations of active drug in the lungs while limiting systemic exposure. A dose-response relationship has been demonstrated for budesonide [31, 32] and an increase in the dose and frequency of budesonide administration has been shown to be beneficial in quickly reducing inflammation and broncho-constriction in patients with unstable asthma [27]. Thus, inhaled corticosteroids should preferentially combine a high fraction of the dose that reaches the airways with a low swallowed fraction [33, 34]. Respirable fraction of currently available DPI formulations is not more than 30% [35–37] which means that only 30% of total dose reaches at the site of action thus increasing frequency and dose of drug administration. This also increases the systemic side effects mentioned above. Conventional DPI formulations of this drug are available in the market but not as controlled release formulations. This necessitates the development of novel formulations. Pulmonary targeting can be achieved by prolongation of pulmonary residence time either by reducing dissolution rate of drug particle (drug lipophilicity or crystal structure), reducing release from drug delivery system (liposomes or microparticles) or by initiation of biological interaction resulting in prolonged pulmonary residence time (ester formation or capturing in membrane structures). Thus, development of useful controlled-release formulations for use in the respiratory tract presents additional challenges because apart from controlling drug release in the lung environment, drug particles need to avoid removal by the lung clearance mechanisms for the period of drug delivery.

No report is available comparing effect of various carriers on respirable fraction of drug and particle engineering technology in order to generate sustained release microparticles using chitosan as polymer with improved deposition profiles of BUD. The objective of current work was to develop and characterize conventional formulations (using various grades of inhalable lactose) and novel spray dried microparticles *viz.*, pulmosols (prepared with various sugars), microspheres and porous particles (generated using natural polymers *viz.* gelatin and chitosan) in order to achieve sustained release profile and to improve respirable fraction of formulation to the lungs. Microparticles could be used in DPI after being blended with standard excipient such as lactose.

Results and Discussion

Optimization of spray drying process parameters

Effect of aspirator rate, spraying air flow pressure and inlet temperature on moisture content of product, % yield and % drug entrapment was studied (Table 1) [38] by plotting surface response curves (Figure 1) and was interpretated in terms of % contribution of each factor and from equations obtained from Stat-Ease Design-Expert v.7 software. Effect of aspirator rate and spraying air flow pressure on % yield was graphically shown in

Figure 1a and their individual and combined effects on yield of product is found to be 98.32%, 1.05% and 0.63% respectively; which was calculated from Eq. 6. This indicated that aspirator rate was the major parameter affecting product yield.

Eq. 6. Log_{10}(% yield) = 1.0803 + 6.3538 ×Aspirator rate − 0.0246 × Spraying air flow pressure + 6.6669 Aspirator rate × Spraying air flow pressure

Effect of aspirator rate and spraying air flow pressure on % drug entrapment was graphically shown in Figure 1b and their individual and combined effects on drug entrapment is found to be 51.44%, 1.62% and 46.94% respectively; which was calculated from Eq. 7. This proved that aspirator rate alone and aspirator rate- spraying air flow pressure together were major parameters affecting the drug entrapment.

Eq. 7. Log_{10}(% Drug entrapment)= −0.6103 + 0.0445 × Aspirator rate + 0.5706 × Spraying air flow pressure − 0.011 × Aspirator rate × Spraying air flow pressure

Effect of aspirator rate and inlet temperature on % moisture content was graphically shown in Figure 1c. Their individual (7.39% and 15.55%) and combined (77.05%) effects on moisture content were calculated from Eq. 8. This indicated that aspirator rate- inlet temperature together was major parameter affecting moisture content.

Eq. 8. Log_{10}(Moisture content) = −4.5851 + 0.0874 × Aspirator rate + 0.0332 × Inlet temp. −7.3062 × Aspirator rate × Inlet temp.

From all this data, it was concluded that aspirator rate and inlet temperature were the major parameters affecting yield, drug entrapment and moisture content of product.

Tab. 1. Combinations of factors and their effect on various responses in optimization of spray drying

Sr. No.	**Combination of factors**	**Response noted*** **F1 (LL)**	**F2 (HL)**	F3 (LH)	F4 (HH)
1.	Aspirator rate and feed spray pressure	**Effect on % yield**			
		21.8 ± 3.7	22 ± 6.7	31.06 ± 2.4	33.33 ± 5.7
2.	Aspirator rate and feed spray pressure	**Effect on % drug entrapment**			
		27.0392 ± 0.07	49.3174 ± 0.09	76.2938 ± 0.32	50.5038 ± 0.18
3.	Aspirator rate and inlet temperature	**Effect on % moisture content**			
		0.206 ± 0.14	0.267 ± 0.28	0.272 ± 0.38	0.186 ± 0.17

* Mean ± SD; n = 3; LL, Both factors at low limit; HH, Both factors at high limit; HL, First factor at high limit and another at low limit; LH, First factor at low limit and another at high limit

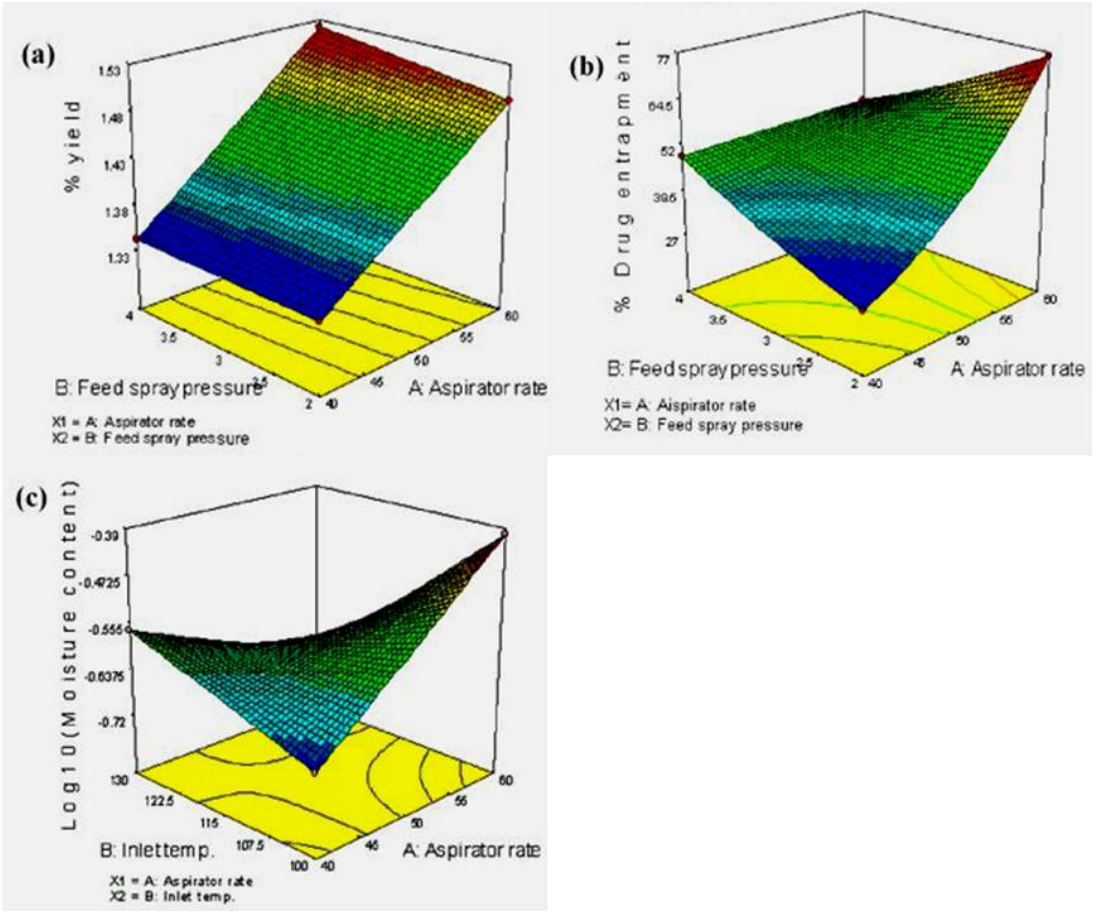


Fig. 1. Factorial design for optimization of process parameters

Development of HPLC method for estimation of Budesonide

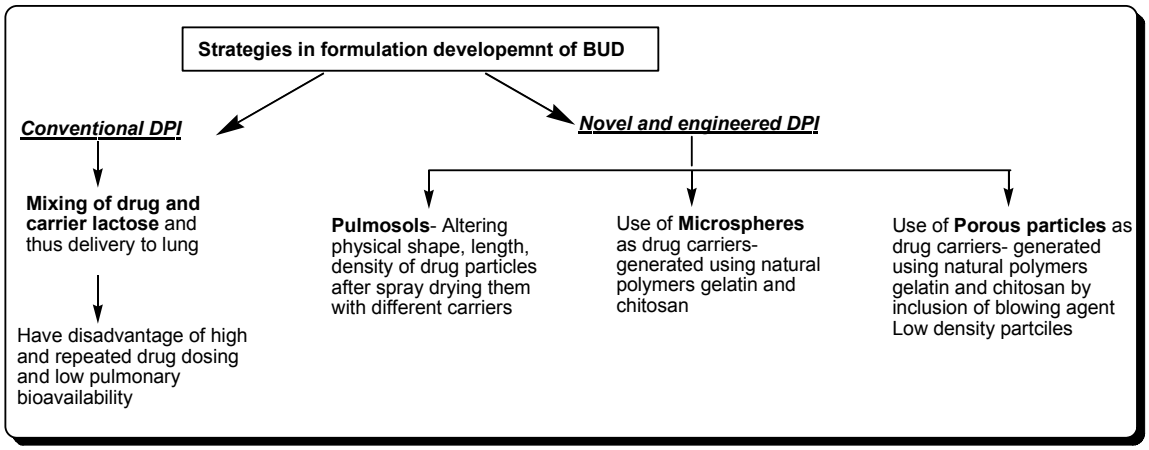
A review of literature revealed that all HPLC methods published so far for budesonide are based on separation of two epimers even though both epimers are similar in their potency with respect to their anti-inflammatory activity [39–41]. Since our work involved design and development of pulmonary drug delivery systems for budesonide, our objective was to develop a simple, rapid and stability indicating HPLC method of analysis. Thus, we developed a method, which elutes drug quickly and also separates all degradation products and other impurities from the main peak. This method was successfully used to analyze the developed budesonide formulations (polymeric microspheres and porous particles prepared by spray drying technology).

Proportions of the organic and aqueous phases were adjusted to obtain a rapid and simple assay method for budesonide with a reasonable run time, suitable retention time and sharpness of the peak. Under experimental conditions, the chromatogram of budesonide showed a single peak around 4 min. Our method has several advantages over earlier reported methods *viz*. [1] Cheaper mobile phase (methanol and water) in comparison with earlier methods involving acetonitrile and phosphate buffers; [2] Routinely used C18 column; [3] Short run time (5 min). The mobile phase was easily prepared and gave reproducible results. With the use of non-buffered mobile phases, problems associated with buffers *viz*. time required in its preparation, pH adjustments, chocking of tubings, and

proper washing of the system after its use has been avoided. The method was linear over a concentration range 10 ng-100 µg/mL (R2 = 0.9990). LOD of budesonide was 25 ng/mL and LOQ was 100 ng/mL, values which are lower than other HPLC methods. The method has been proven to be stability indicating. Peaks of degradation products, as well as those of excipients in the budesonide loaded microparticles did not interfere with the analysis. Recovery of budesonide from the developed dry powder inhaler formulations was essentially quantitative. The structure of major degradation product formed under basic aqueous conditions was also identified. Furthermore, degradation products formed under alkaline condition are very well resolved using newly developed HPLC method [42].

Preparation of microparticles

There are no literature reports which compare effects of various carriers on respirable fraction of budesonide and particle engineering technology in order to generate sustained release microparticles using chitosan as polymer with improved deposition profiles of BUD. Therefore we developed and characterized conventional formulations (using various grades of inhalable lactose) and novel spray dried microparticles *viz.*, pulmosols (prepared with various sugars), microspheres and porous particles (generated using natural polymers *viz.* gelatin and chitosan) in order to achieve sustained release profile and to improve drug-targeting to lungs. Microparticles could be used in DPI after being blended with standard excipient such as lactose. Formulation of BUD dry powder inhalers was designed based on two different approaches-



All sixteen conventional batches (Table 2) developed using inhalable lactose in combination of fine lactose: coarse lactose, 60: 40 and 70: 30 were evaluated for various parameters but the optimized batch was selected based on the % FPF. Batch L2 (Lactohale 300M: Pharmatose 150M, 60: 40) was selected as it gave maximum FPF (34.50%) compared to other batches.

Tab. 2. Development of BUD DPI formulations

Formulation type	Batch code	Excip./ drug: polymer ratio	Excip. (w/w)	% drug content/ loading	% FPF	EI	% CI	Hausner ratio
Conventional	L1	60: 40	A + B	105.6875	31.7606	46.3303	28.57	1.40
Fine Lactose:	L2		C + B	109.6011	34.5082	49.2185	25.0	1.33
Coarse	L3		D + B	115.6885	26.1204	44.8396	42.85	1.75
Lactose	L4		F + B	113.0335	22.1722	43.9040	28.57	1.40
	L5		A + E	101.0742	12.8226	31.8021	22.22	1.28
	L6		C + E	103.4362	27.2027	36.4843	20.0	1.25
	L7		D + E	107.0000	24.5655	43.0648	18.18	1.22
	L8		F + E	103.5188	31.6768	51.7964	37.50	1.60
Conventional	K1	70: 30	A + B	109.3114	31.2728	49.6251	22.22	1.28
Fine Lactose:	K2		C + B	112.5635	30.9298	43.6226	18.18	1.22
Coarse	K3		D + B	109.9085	30.7926	48.2394	25.0	1.33
Lactose	K4		F + B	106.0086	31.8673	48.8284	33.33	1.50
	K5		A + E	101.7149	18.4171	37.7805	25.0	1.33
	K6		C + E	104.6747	33.3384	45.5773	22.22	1.28
	K7		D + E	99.1107	31.4507	48.5436	25.0	1.33
	K8		F + E	108.5848	30.4080	48.3153	27.27	1.37
Pulmosols	PS1	1: 50	10%	31.2680	–	–	–	–
Drug: mannitol	PS2	1: 200	20%	10.0160	–	–	–	–
	PS3	1: 100	20%	49.6030	–	–	–	–
	PS4	1: 50	20%	87.7118	29.3275	48.5685	12.50	1.14
Microsph. D.	G1	1: 1	1%	56.8719	–	–	–	–
gelatin	G2	1: 2		88.5608	18.2420	38.2699	30.0	1.42
	G3	1 : 5		38.8170	–	–	–	–
	G4	1 : 7		24.1402	–	–	–	–
Microsph. D. gelatin: HPβ-CD	G5	1: 0.5: 1.5		92.4756	–	–	–	–
Microsph. D.	CH1	1: 2	1%	7.3474	–	–	–	–
chit.	CH2	1: 5		106.7436	–	–	–	–
	CH3	1: 2	0.5%	85.9734	35.6785	54.4813	30.76	1.44
	CH4	1: 4		90.6250	–	–	–	–
	CH5	1: 7		101.3991	–	–	–	–
Microsph. D.	CH6	1: 1: 1	0.5%	101.4084	–	–	–	–
chit.: HPβ-CD	CH7	1: 0.5: 1.5		102.8516	–	–	–	–
Microsph. D.	GC1	1: 2: 2	1%	83.0364	–	–	–	–
gelatin: chit.	GC2	1: 2: 5		109.8440	–	–	–	–
	GC3	1: 1: 1		55.7134	–	–	–	–
Microsph. D.	CM1	1: 1	1%	44.7347	–	–	–	–
compritol 888	CM2	1: 2		49.2864	–	–	–	–
ATO	CM3	1: 5		91.1380	–	–	–	–
Porous. Part. Drug: gelatin	P1	1: 2	1%	98.3962	12.1883	31.7858	25.0	1.33
Porous. Part. Drug: chit.	PC1	1: 2	0.5%	95.9390	46.8199	64.2257	26.66	1.36

A, Pharmatose 125M; B, Pharmatose 150M; C, Lactohale 300; D, Lactohale 200; E, Lactohale 100; F, Inhalac; FPF, fine particle fraction; EI, Effective index; Chit., Chitosan; CI, Carr's index; Excip., Excipients; Microsph. D., Microspheres Drug; Porous Part., Porous Particles.

For optimization of pulmosols, initially blank batches of lactose, sucrose, mannitol, fructose, dextrose, albumin and PEG 4000 were prepared. Except mannitol, all other carriers *viz.* lactose, sucrose, fructose, dextrose, albumin and PEG 4000 yielded cohesive product which could not be removed from the cyclone, so mannitol was chosen for drug loading as it gave free flowing powder. Different drug: carrier ratios, 1:50, 1:100 and 1:200 at concentrations of 10% and 20% w/w solution were tried (Table 2). Batch PS4 was selected to determine % FPF (10 mg of formulation ≈ 200 µg of BUD) as drug: carrier ratio was minimum (1: 50) with optimum drug loading of about 88.0% w/w as compared with other batches of pulmosols.

Microspheres of gelatin, chitosan (alone and in combination) and compritol 888 ATO were generated with drug: polymer ratios as shown in Table 1. Batch G2 (1% w/w solution, 1:2 drug: gelatin) and CH3 (0.5% w/w solution, 1:2 drug: chitosan) were selected for which % drug entrapment was about 89.0 and 86.0% w/w, respectively. During preparation of chitosan microspheres, initially 1% w/w solution was used for batches CH1 and CH2 for spray drying. Although batch CH2 showed maximum entrapment efficiency, this batch was not further continued because blocking of feed pipe was noticed sometimes during spray drying process due to viscosity of the solution. Further batches of chitosan microspheres were prepared with 0.5% w/w solution and drug: chitosan ratio of 1:2, 1:4 and 1:7; batch CH3 (1:2, drug: chitosan) was selected as the amount of chitosan was less with 86.0% w/w drug content as compared with other batches. Effect of HPβ-CD on entrapment efficiency and drug release was studied and its addition increased entrapment efficiency of BUD without changing the release profile. Combinations of gelatin-chitosan were evaluated but not continued as the quantity of polymer required for these batches was larger than batches prepared with gelatin and chitosan alone. Similar observations were noted in case of batches prepared using compritol 888 ATO.

Porous particles of gelatin and chitosan were prepared (Table 2) with % drug entrapment of about 98% and 96% w/w, respectively. Microspheres and porous particles were formulated with inhalable lactose (lactohale 300M: pharmatose 150M, 60: 40) based on the entrapment efficiency such that 25 mg of formulation ≈ 200 µg of BUD.

In vitro assessment of developed aerosol formulations

Selected formulations were characterized for *in vitro* deposition by twin stage impinger and Anderson cascade impactor [43]. Pulmosols, formulations of microspheres and porous particles prepared using gelatin showed FPF of 29.32%, 18.24% and 12.18%, so these were not selected for further characterization. FPF for conventional (L2), microspheres (CH3) and porous particles formulation (PC1) was about 34%, 36% and 47% respectively with SD of ≤ 0.5 was very promising. Values were compared using normality ($p= 0.159>0.05$) and paired t test ($p= 0.727>0.05$). The difference is considered to be statistically significant ($p<0.001$) (One Way Analysis of Variance Test). MMAD, for conventional, microspheres and porous particles formulation were 2.75, 4.60, and 4.30 respectively. GSD for above batches was 2.56, 1.75 and 2.54.

Drug content, content uniformity and in vitro release studies

Drug content and content uniformity for all conventional formulations was in the range of 90-110% w/w. Drug entrapment for developed novel formulations varied from 10-110% w/w as given in Table 2. *In vitro* release profile is shown in Figure 2a. Mechanism of drug

release was determined using various kinetic models. Coefficient of correlation were calculated from plots of Q *vs* t (cumulative % drug release *vs* time), log Q *vs* log t and Q *vs* square root of t [44]. Regression coefficients (near to 1) for zero order, matrix and korsmeyer-peppas kinetic equations confirmed the release of drug by slow zero order kinetics through diffusion matrix (Table 3, Figure 2b and 2c). Korsmeyer-peppas plot indicated good linearity (r^2 = 0.9877).

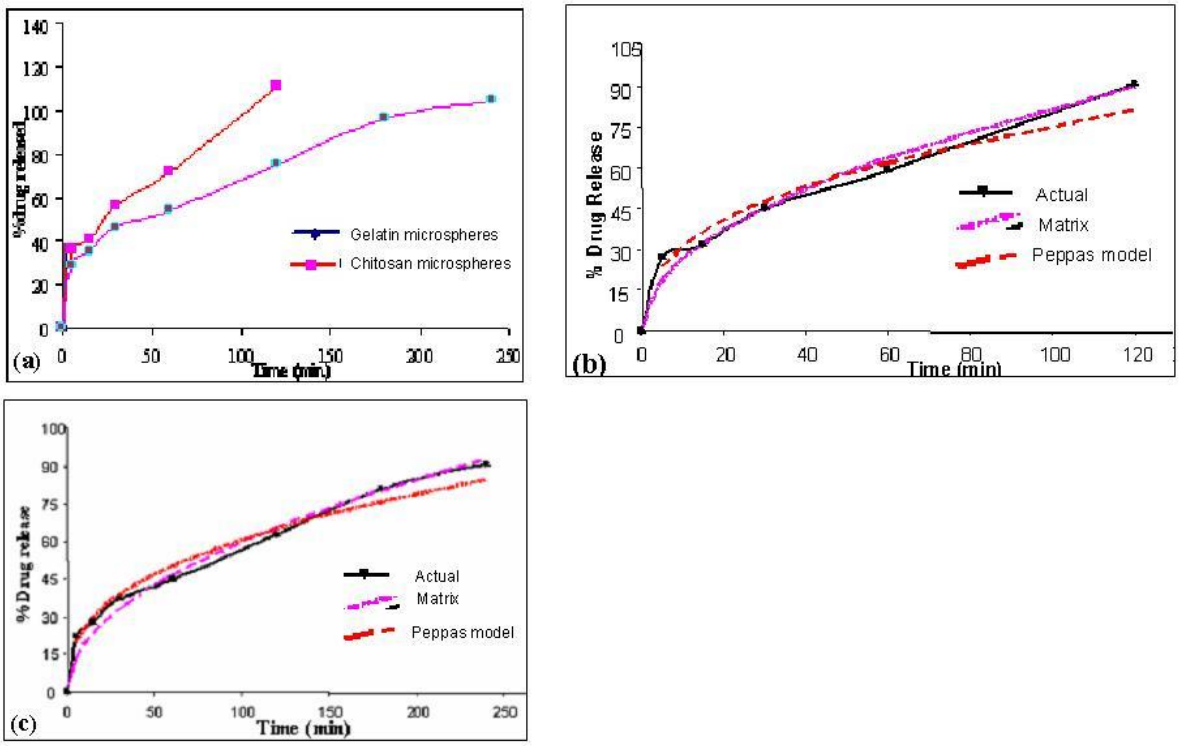


Fig. 2. *In vitro* release profile and release kinetics of formulations (a) *In vitro* release profile of microspheres and porous particles prepared by spray drying method; (b) Release kinetics of chitosan microspheres; (c) Release kinetics of porous particles of chitosan

Tab. 3. Regression coefficients for formulations

Formulations	r^2					
	Zero order	**Korsmeyer-peppas**	**Matrix**	**$T_{50\%}$ (min)**	**Log Q *vs* Log t**	**Q *vs* SQRT**
Gelatin microspheres	0.4272	0.9920	0.9166	4	0.6749	0.7637
Chitosan micropsheres	0.8338	0.9722	0.9898	27	0.8132	0.9680
Gelatin porous particles	1.0000	1.0000	1.0000	2	0.6684	0.7953
Chitosan porous particles	0.8315	0.9877	0.9906	56	0.8595	0.9619

Fourier transform infrared spectroscopy

IR spectrum of BUD showed peaks at 3378 cm^{-1} (O-H stretch), 2935 (C-H stretch) and 1720, 1659 cm^{-1} (C=O stretch). Chitosan showed typical peaks at 3446 and 1633 cm^{-1} for N-H stretch and C=O stretch for free amine and amide carbonyl functionalities respectively. In the IR spectrum of BUD chitosan microparticles, typical peaks for the N-H stretch of free NH_2 of chitosan disappeared due to possible crosslinking of chitosan with drug via amine and hydroxyl functionalities while the broad peak ranging from 2800–2600 cm^{-1} was observed as well as intensity of peaks in the range of 1650–1720 cm^{-1} was dramatically reduced which confirmed entrapment of BUD in chitosan.

Characterization of particle shape by scanning electron microscopy

Morphology of microparticles was investigated and SEM micrographs are illustrated in Figure 3. Figure 3a of pulmosols showed spherical particles with wide particle size distribution (1–20 µm) but uniform spherical microspheres and porous particles (Figure 3b and 3c) were obtained with diameter ranging from 1 to 4 µm, with similar particle morphology and size.

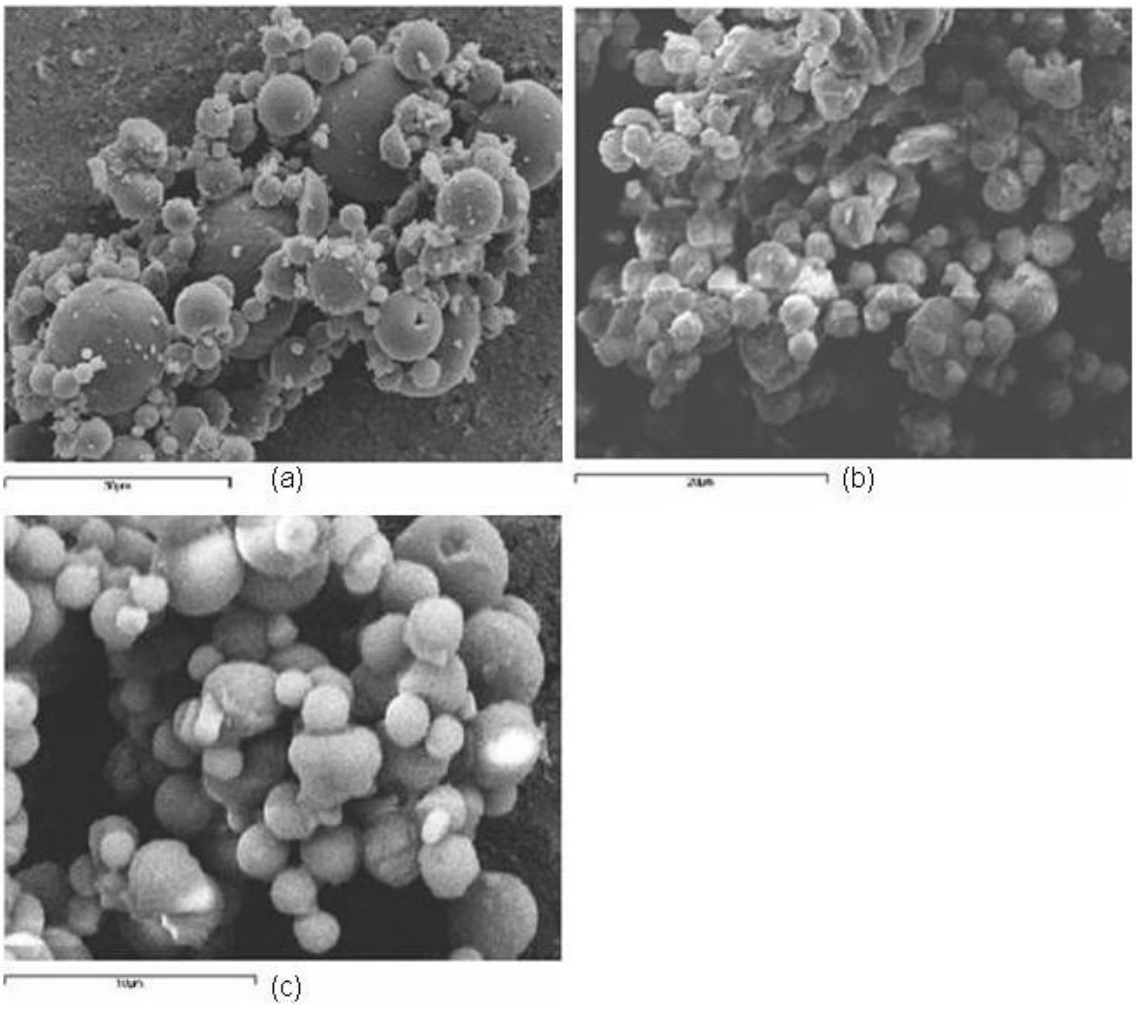


Fig. 3. SEM micrographs (a) Pulmosols; (b) Chitosan microspheres; (c) Chitosan porous particles

Characterization of microparticles by differential scanning calorimetry and crystalline state by X-ray powder diffraction

DSC scans are shown in Figure 4. Chitosan showed broad endotherm (Figure 4d) at 109.13 °C while BUD showed sharp endotherm (Figure 4c) at 264.14 °C. DSC spectra of microspheres revealed 2 endotherms (Figure 4b) for chitosan and BUD at 105.18 °C and 235.38 °C respectively. In case of porous particles 2 endotherms (Figure 4a) were observed for chitosan and BUD at 106.50 °C and 222.98 °C respectively. BUD conventional formulation showed 2 sharp endotherms (Figure 4e) for inhalable lactose and BUD at 148.44 °C and 221.06 °C respectively. This confirmed no interaction between BUD and excipients occurred after spray drying process [45].

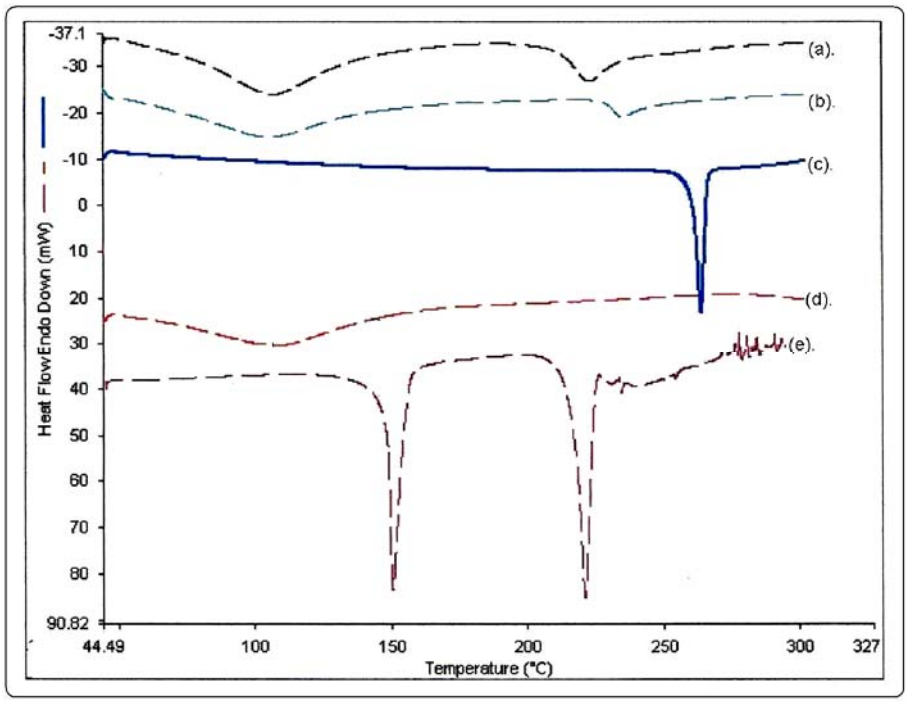


Fig. 4. DSC spectra of developed formulations (a) BUD porous particles; (b) BUD microspheres; (c) BUD pure; (d) Chitosan pure; (e) BUD conventional formulation

On the other hand, X-ray powder diffraction patterns (Figure 5) showed that spray-drying process did not completely affect the crystalline form of BUD (Figure 5a). Peaks that represent the spray dried samples (both microspheres and porous particles) (Figure 5b and 5c) correspond to those of chitosan (Figure 5d) but differ in intensity, indicating that the major component (in formulations) is partly amorphous.

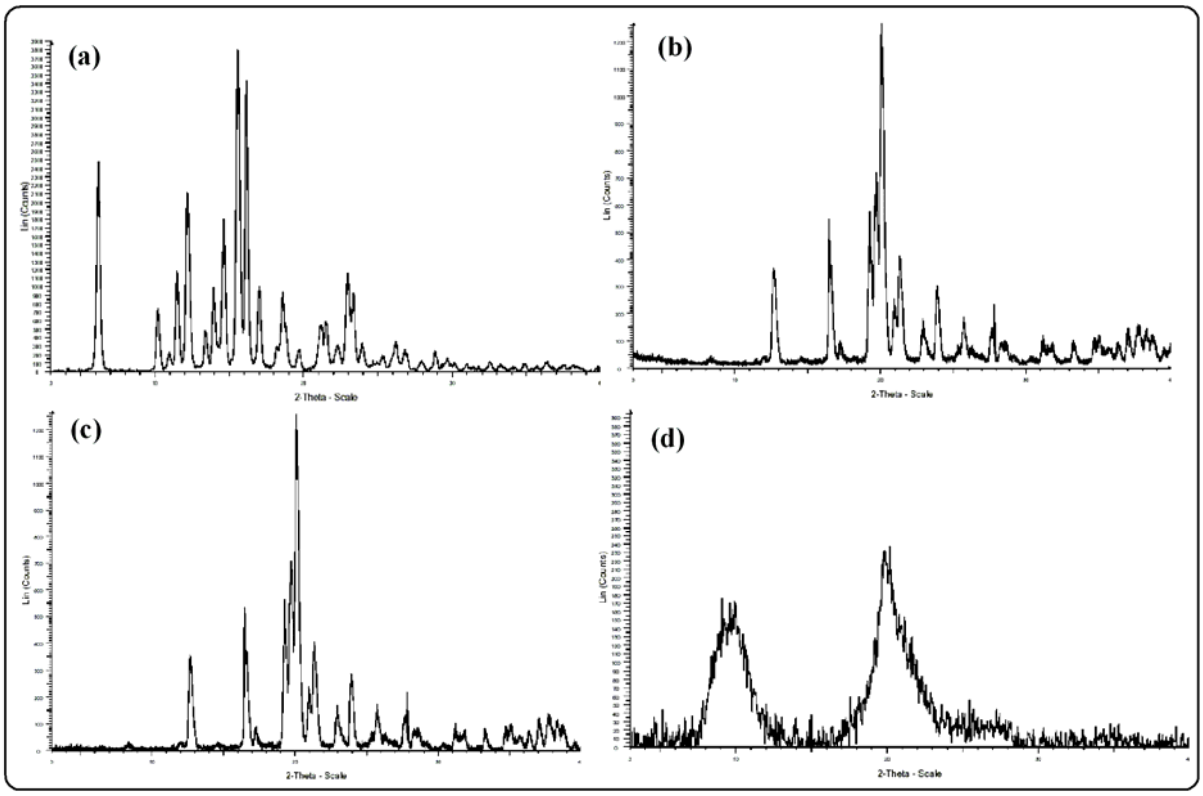


Fig. 5. X-ray powder diffraction patterns of (a) BUD; (b) Microspheres; (c) Porous particles; (d) Chitosan

Evaluation of other physicochemical characteristics

Effective index (EI) of microspheres (54.48, Table 2) and porous particle formulations (64.22) was higher than the conventional formulation (49.21) suggesting more effective microparticles drug deposition to the lungs might be possible [37]. Carr's Index and Hausner ratio, which are considered as appropriate methods of evaluating flow properties of solids, were also determined from tapped and bulk density values [38]. Carr's Index values of less than 25 are usually taken to indicate good flow characteristics; values beyond 40 indicate poor powder flowability. Carr's Index values (Table 2) for all formulations were found to be in the range of 20-30% which indicated good powder flow properties. Hausner ratio is measure of flowability of powder. A low Hausner ratio means that the powder has a high flowability (but it should be >2.0). For all formulations this ratio was in the range of 1.2–1.7 (Table 2) indicating good flowability. Extent of porosity for chitosan microspheres and porous particles formulation was 30.76 and 26.66 respectively which was good as compared to developed conventional formulations (6–20%). Moisture content for all formulations was <1% w/w.

Discussion

Chitosan, a polysaccharide derived from deacetylation of naturally occurring polymer chitin is a promising excipient that can be employed in a wide range of applications, including sustained release preparations. Deacetylation value of chitosan was determined using IR spectroscopy in order to know quality of chitosan employed in formulation development.

Spray drying process was optimized for aspirator rate, spraying air flow pressure and inlet temperature and its effect on moisture content of product, % yield and % drug entrapment was studied. Above mentioned optimized parameters were selected as it was observed that aspirator rate alone affected yield and drug entrapment, aspirator rate along with spraying air flow pressure was also affecting drug entrapment and aspirator rate- inlet temperature together was major parameter affecting moisture content of product. Initially conventional BUD formulations were developed using novel inhalable lactose and effect of those on respirable fraction of BUD was assessed. It was observed that for all formulations FPF was in the range of 12-34%. Microparticles *viz.* pulmosol, microspheres and porous particles were developed using spray drying technology at optimized process parameters in order to further improve FPF and thus delivery of BUD into deep lungs. These developed microparticles were also assessed for *in vitro* deposition studies using TSI and ACI. Data was statistically analysed. Drug content, content uniformity and *in vitro* release profile was monitored and it was found that developed microparticles showed drug release by slow zero order kinetics through diffusion matrix. From SEM micrographs, it was observed that spray drying process yielded hallow, porous and spherical micropsheres with uniform particle size distribution. To prove compatibility of drug with excipients, DSC studies confirmed that there was no interaction between drug and polymer as endotherms of drug and polymers were separate in formulation even after spray drying. From XRPD studies it was observed that spray drying did not affect crystalline form of BUD. From other physicochemical parameters like EI, Carr's index, Hausner ratio and % porosity it was clear that spray drying process generated particles for inhalation with uniform particle size distribution and good flow properties.

Experimental

Materials

Budesonide was obtained from Lupin Ltd., Mumbai; gelatin and chitosan were procured from S.D. Fine Chemicals, Mumbai and different grades of inhalable lactose were obtained as gift sample from DMV Int., The Netherlands. Methanol and chloroform were of analytical grade and were procured from S.D. Fine Chemicals, Mumbai.

Assay for degree of deacetylation of chitosan

Degree of deacetylation of chitosan affects overall charge density. An increasing presence of ammonium groups results in decrease in the crosslinking density related to hydrogen bonding and hydrophobic interactions [12, 46]. Increase in degree of deacetylation results in increased swelling due to an increase in number of ionic sites and their counter-ions. Degree of deacetylation dictates the reactivity, solubility and viscosity of chitosan solutions which was determined using FTIR spectroscopy (Nicolet, USA) by Eq. 1.

Eg. 1. $$\%\ Deacetylation = \frac{Absorbance\ of\ carbonyl\ stretch\ of\ amide\ (NHCOCH_3)}{Absorbance\ of\ NH\ stretch\ of\ free\ NH_2} \times 115$$

IR spectra of chitosan showed the characteristic peaks for N-H stretch (v_{max} 3346 cm^{-1}) for free amine and C=O stretch (v_{max} 1633 cm^{-1}) for amide carbonyl. From the Eq. 1, % deacetylation was calculated as 45% which was in the acceptable limit.

Optimization of spray drying process parameters

Microparticles were prepared at laboratory scale by spray drying using Labultima Mini Spray Dryer (Mumbai, India). Spray-drying is a one-step process that converts liquid feed (solution, coarse suspension, colloidal dispersion) to a dried particulate form. Principle advantages of spray drying with respect to pulmonary drug delivery are ability to manipulate and control particle size and size distribution, particle shape and density in addition to macroscopic powder properties such as bulk density, flowability and dispersibility [47]. Various process parameters were optimized by 2^2 factorial design. Surface response curves were plotted using Stat-Ease Design-Expert v.7 software. Effect of aspirator rate (varied form 40–60%), spraying air flow pressure (2-4 bar) and inlet temperature (varied from 100-130 °C) on moisture content of product, yield and entrapment efficiency of drug was studied (Table 1). Following were the optimal conditions of spray-drying: inlet temperature, 130 °C; outlet temperature, 80 °C; aspirator rate, 240 mWc (60%); solution feed rate, 2 ml/min; spraying air flow pressure, 2 bar [38, 48]. These process parameters were optimized for batch CH3 with concentration of chitosan solution as 0.5% w/v.

HPLC method development for estimation of Budesonide in formulations

HPLC apparatus

The HPLC analysis was carried out on Phenomenex C18 analytical column (5 µ, 250 mm × 4.6 mm) connected to a HPLC (model SPD-M2OA 230V) system (Shimadzu corporation, Japan) consisting a LC-8A pump, SPD- M2OA PDA detector, C3M-20A flow cell, a degasser unit and 20 µL injection loop. LC Solution software was used for data collection.

HPLC conditions

The mobile phase consisted of methanol: water (80:20 v/v) with the flow rate of 1.5 mL/min. The wavelength of detection was 244 nm (λ_{max} for BUD) and the injection volume was 20 µL [42].

Preparation of microparticles

Conventional DPI formulations

BUD DPI formulations were developed on laboratory scale (100 g) using various grades of inhalable lactose like pharmatose, lactohale, inhalac and mannitol in various combinations (fine lactose: coarse lactose, 60: 40 and fine lactose: coarse lactose, 70: 30). The development of DPI formulations of salbutamol sulphate using various carriers by sieving process was reported in literature [43, 49] therefore; we employed sieving process in development of BUD DPI formulations on laboratory scale. BUD was initially mixed with fine lactose using mesh #100 and this premix was blended with coarse lactose to homogeneity in geometric proportions using mesh #80. In all these 16 developed batches (Table 2), 25 mg of formulation was equivalent to 200 µg of BUD. Effect of particle size of excipients (fine or coarse) on fine particle fraction (FPF) of drug was assessed using TSI study [44].

Pulmosol formulations

Based on report of spray dried composites of drug bendroflumethiazide with polyethylene glycol by Corrogan *et al*. [50] we attempted development of composites of BUD with different sugars. Different sugars like lactose, sucrose, mannitol, fructose, dextrose, albumin and PEG 4000 were used as carriers in formulation development of pulmosols. Initially, blank batches were spray dried to find out optimum concentration of sugar as well as to determine powder flow properties. Mannitol was chosen as carrier in formulations as it yielded free flowing powder with maximum drug loading. Different ratios of drug: mannitol (Table 2) was spray dried at optimized process conditions.

Microspheres formulation

BUD and natural polymers *viz.* chitosan, gelatin was spray dried at optimized process parameters. Salbutamol chitosan co-spray dried multiparticulates were developed and reported by Corrigan et al. with varying concentration of 0.5–2% chitosan solution and there coworkers observed that 0.5% w/v was the optimum concentration of polymer based on entrapment efficiency and release profile from developed composites [44]. Hence gelatin (1% wt/ml solution in water), chitosan (0.5% wt/ml solution in 0.1M HCl) were spray dried and drug: polymer ratio was optimized based on the % drug entrapment and release profile (Table 2). Final concentration of solution to be spray dried was adjusted to 1% wt/ml and 0.5% wt/ml for gelatin and chitosan, respectively. BUD and polymer were dissolved in equal parts of methanol and water. Polymeric phase was mixed using Ultra-turrax at 13000 rpm to which methanolic phase was slowly added and solution was stirred to homogeneity. This solution was spray dried to get microspheres.

Porous particle formulations

BUD and natural polymers *viz.* chitosan, gelatin was spray dried in water: methanol (1:1) as 1.0% and 0.5% w/v, respectively. Solution containing BUD, polymer and a blowing agent was atomized into the drying chamber and brought in contact with a hot air stream. Blowing agent which is trapped in droplets decomposes at higher temperatures creating a void in the center of particle [51, 52]. Since air stream temperature is greater than that of the droplet, the droplet temperature increases until the evaporation temperature of solvent is reached. Solvent at the surface (blowing agent) begins to evaporate causing solvent below the surface of droplet to diffuse to the surface. The droplet, as it passed through the spray chamber forms a hollow particle [45]. Hence porous particles were generated by adding chloroform (5% v/v) as a blowing agent. Drug: polymer ratio was optimized based on the % drug entrapment and release profile.

Effect of HPβ-CD (HPβ-cyclodextrin) on entrapment efficiency and drug release of microspheres and porous particles was assessed [53]. Microspheres and porous particles obtained by spray drying were formulated with inhalable lactoses.

In vitro assessment of developed aerosol formulations

In vitro deposition of dry powders for inhalation was determined using a twin impinger [Copley Instruments (Nottingham) Ltd]. A 25 mg formulation was weighed and loaded into size 3 hard gelatin HPMC capsules (Associated Capsules Pvt. Ltd., India), which were individually installed in a rotahaler device. The rotahaler was attached to the impinger which contained 7 and 30 ml of collecting solvent [acetonitrile: buffer (disodium hydrogen

orthophosphate pH 3.0) (650: 350)] in stages 1 and 2, respectively. Capsule contents were released by twisting the rotahaler and system was vacuumed to produce air streams of 60 l/min for 5 s. Liquid in stages 1 and 2 was collected, diluted to 100 mL and measured by UV spectrophotometry at 244 nm. Each deposition experiment involved aerosolization of ten capsules.

The fine particle fraction was calculated as the amount deposited in the lower stage as a percentage of the emitted dose (amount emitted into upper and lower stages excluding the amount remaining in the device). All formulations were analyzed in triplicate. Statistical analysis was carried out using Sigma Stat- 2.0, Jandel Scientific Software.

Formulations were also subjected to Andersen cascade impactor [Copley Instruments (Nottingham) Ltd] studies to determine mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) [43]. ACI utilizes eight jet stages enabling classification of aerosols from 9 µm and above to 0.4 µm (at 60 L/min) and allows airborne particulates to impact upon stainless steel impaction surfaces. A final filter collects all particles smaller than 0.4 µm. Rotahaler device was filled with a No. 3 HPMC stick free capsule (Associated Capsules Pvt. Ltd., India) loaded with 25 mg of powder (200 µg budesonide). Test was conducted at flow rate of 60 L/min for 4 s. Three fine particle determinations were performed on each test formulation and analyzed by UV spectrophotometry. Starting at the filter, a cumulative mass deposition (undersize in percentage) *vs.* cut-off diameter of respective stages was derived. Calculation by interpolation of mass of active ingredient with an aerodynamic diameter of less than 5 µm gave the fine particle fraction (FPF). It is considered to be directly proportional to the amount of drug able to reach the pulmonary tract *in vivo*: consequently, higher the percentage of FPF, deeper the estimated lung deposition will be. Data was statistically analyzed using Sigma Stat- 2.0, Jandel Scientific Software.

Drug content of BUD formulations recovered from twin impinger apparatus was determined by UV spectrophotometry. Absorbance was measured at λ_{max} of 244 nm for BUD analysis. Concentration was determined by reference to a calibration curve prepared from dilutions of stock solutions of BUD.

Drug content, content uniformity and in vitro release studies

Drug content for conventional formulations, drug entrapment of developed novel formulations and content uniformity was determined by method as described above. *In vitro* release profile of microparticles was performed using Flow Through Cell Apparatus-USP IV at 37 °C with flow rate of 16 mL/min (in 100 mL of phosphate buffer, pH 7.0). A 5 mL was taken, filtered through a 0.4 µm filter and replaced with 5 mL of fresh medium at 37 °C. Samples were analyzed by UV spectroscopy at 244 nm. Mechanism of drug release was determined using various kinetic models using PCP Disso V3 software.

Fourier transform infrared spectroscopy

Infrared (IR) spectra were recorded with FT-IR spectrometer (Nicolet, USA) to confirm drug entrapment in the polymer. Samples were prepared by processing compressed KBr disks [54].

Characterization of particle shape by scanning electron microscopy

Morphology of particles was evaluated by scanning electron microscopy, using JSM-840A-/WDS/EDS Sys- Jeol, Japan. Powders were scattered onto a thin film of a two-component epoxy resin and coated with a gold layer [45].

Characterization of microparticles by differential scanning calorimetry and crystalline state by X-ray powder diffraction

Thermal behavior of microparticles was investigated using a Perkin–Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with an Intracooler- 2 cooling system (Perkin-Elmer Instruments, U.S.A.) to prove drug- excipients compatibility in the formulations. Samples of about 3 mg were placed in 50 µL perforated aluminum pans and sealed. Heat runs for each sample were set from 50 to 300 °C, using nitrogen as the blanket gas. The apparatus was Indium–Cyclohexane calibrated.

X-ray powder diffraction (XRPD) is another powerful and widely-used tool for crystalline state evaluation. Diffraction patterns of BUD, excipients and microparticles were determined using a Siemens Diffractometer D5000 (Siemens, Germany), with a Cu line as the source of radiation (WL1Z1.5406 A, WL2Z1.54439 A).

Evaluation of other physicochemical characteristics

Effective index (EI) is the geometric mean of total emitted dose (ED) and fine particle fraction (FPF), represented by the Eq. 2 [37].

Eg. 2. $$EI = \sqrt{(100 - DF) \times FPF}$$

where DF is the device fraction (amount of drug retained in DPI device).

Bulk and tapped densities were measured using a tap density tester (Thermonik: Campbell Electronics). Apparent volume occupied by a mass of powder of about 10 mg, carefully placed into a 5 mL graduated cylinder, was determined before and after packing (tapped more than 500 times in order to obtain the closest packed densities). Bulk and tapped density values allow the determination of Carr's compressibility index by the Eq. 3 [38].

Eg. 3. $$Carr's\ Index\ (\%) = \frac{Tapped\ density - Bulk\ density}{Bulk\ density} \times 100$$

Hausner ratio is a measure of flowability of drug and is calculated using Eq. 4. A low Hausner ratio means that the drug has a high flowability [38].

Eg. 4. $$Hausner\ Ratio = \frac{Bulk\ density}{Tapped\ density}$$

Percent porosity (ε) is one of the method used to determine compressibility of powder that is the degree of volume reduction due to an applied pressure is measurement of porosity changes during compaction and is calculated using Eq. 5 [55].

Eg. 5. $$e = \left(1 - \frac{P_p}{P_t}\right) \times 100$$

where P_p and P_t are bulk density and tapped density, respectively.

Moisture content was determined by Karl Fischer method of analysis.

Authors' Statement

The authors declare no conflict of interest.

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