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Formulation and Evaluation of β -Cyclodextrin Microbeads as A Carrier for DPI

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ABSTRACT

Conventional forms of DPI's are prepared by micronization methods which are often blends of fine drug particles and lactose as a carrier where drug particles are expected to adhere to the carrier surface. This leads to only 30% of drug deposition in the lung region from the conventional form of DPI. The micronization process mainly affects the mean median aerodynamic diameter (MMAD) and fine particle fraction (FPF) of the DPI resulting in less lung deposition. This leads to an increased frequency of administration. To optimize the delivered dose, conventional formulations can be substituted by engineered powders containing the active ingredient (API) alone or in combination with excipients displaying various properties such as liposomes, poly (lactic-co-glycolic acid) (PLGA), or leucine. Several strategies have already been developed including promising nanoparticles, while some safety concerns still need to be addressed. Increased lung persistence is of particular interest in various pulmonary pathologies. Among them, asthma is characterized by chronic airway inflammation. Innate and adaptive immunity effectors induce mucus overproduction, bronchial hyper-reactivity, and airway wall re-modeling leading to variable levels of airflow obstruction. Inhaled corticosteroids such as budesonide are used as first-line therapy agents to reach control airway inflammation and therefore to improve patient pulmonary functions. Several major parameters influence the activity of corticosteroids including the lung residence time which is highly influenced by the release rate locally obtained. Indeed, a decrease in absorption out of the lungs will extend the contact time between corticosteroids and their receptors, thus enhancing their anti-inflammatory effect. By considering all these limitations, there is a need for carrier-free budesonide loaded microbeads DPI formulation with sustained drug release which could be useful to enhance the deposition of drugs in the lungs.

INTRODUCTION

Treating respiratory diseases with inhalers requires delivering a sufficient concentration of drugs to the lungs to bring about a required therapeutic response. For optimum efficacy, drug administration must be reliable, reproducible, and convenient. The development of an inhalation therapy that is efficacious and safe depends not only on a pharmacologically active molecule but also on a well-designed formulation. The lung has served as a route of drug administration for thousands of years. The origin of inhaled therapy can be traced back 4000 years ago to India where people smoked the leaves of the Atropa belladonna plant to suppress the cough. In the 19th and early 20th centuries, asthmatics smoked asthma cigarettes that contain stramonium powder mixed with tobacco to treat the symptoms of their disease. The development of modern inhalation devices can be divided into three different categories:-

1. Metered-dose inhalers (MDI)

2. Dry powder inhalers (DPI)

3. Nebulizers

The DPI formulation aims at pulmonary drug delivery having uniform distribution, small dose variation, good flowability, and adequate physical stability in the device. The DPI performance also can be enhanced by the preparation of ternary mixture using fine carriers which reduce the bulk density. This helps to reduce particle contacts lowering the inter-particulate force. Further particle interaction can be lowered by modifying surface composition.

Based on claims for improving aerosol performance, the DPI's may be classified into the four broad categories.

1. Blend and ternary system

2. Reducing aerodynamic diameter through porous/low-density particles.

3. Preparing less cohesive and adhesive particles.

4. Novel DPI.

MATERIAL AND METHOD:

Table No. 1: List of materials and chemicals

S. N.	Solvent / Miscellaneous	Source
1.	Methanol, GR grade	Merck Ltd, Mumbai, India.
2.	Potassium Dihydrogen phosphate, GR grade	Merck Ltd, Mumbai, India
3.	KBr-IR grade	Merck Ltd, Mumbai, India
4.	Micropipette	Bio Era Medical Systems, Pune, India

Table No. 2: List of Chemicals and sources

S.N.	Excipients	Source
1.	Budesonide	Cipla (Kurkumbh, Dist. Pune)
2.	B-Cyclodextrin	Sigma Aldrich, Mumbai
3.	Diphenyl carbonate	Otto Pharma.

Table No. 3: List of Instruments and model

S.N.	Instrument	Model
1.	UV/visible Spectrophotometer	V-530, JASCO, Japan
2.	FT-IR spectrometer	FTIR-8400, Jasco corporation, Japan.
3.	Differential Scanning Calorimeter	Mettler-Toledo DSC 821e instrument equipped with an intercooler (Mettler-Toledo, Switzerland).
4.	Centrifuge	Beckman Coulter, Allegra 64R centrifuge
5.	pH meter	Toshniwal Instruments Ltd. India
6.	Hot plate	Metro Lab
7.	Magnetic stirrer	REMI
8.	Electronic balance	Contech Electronic balance, India

Methods

1. Calibration curve of budesonide

2. Experimental work

2.1 Synthesis of β-cyclodextrin microbeads

3 Characterization of microbeads

3.1 Particle size analysis

3.2 Drug content and entrapment efficiency

3.3 Flow properties of formulated

3.4 Transmission electron microscopy (TEM)

3.5 Scanning electron microscopy (SEM)

3.6 Fourier Transform-Infrared Spectroscopy (FTIR)

3.7 Differential scanning calorimetry (DSC)

3.8 X-ray Diffraction (XRD)

3.9 Release profile

3.10 In vitro deposition study using ACI

4. Acute toxicity study

1 Calibration curve of budesonide

1.1 Calibration curve in phosphate buffer pH 7.4.

The complete scan of a solution of budesonide in phosphate buffer pH 7.4 revealed the λmax at 243 nm. Serial dilution of budesonide was prepared in phosphate buffer pH 7.4 concentration ranging from 1µg/ml to 10µg/ml and the UV absorbance was measured at 243 nm.

2 Experimental works

2.1 Synthesis of β-cyclodextrin microbeads anhydrous beta-cyclodextrin (567.49 mg) was put to react in melted diphenyl carbonate (856.84 mg) at 90°C for 5 h. The reaction was carried out using an excess cross-linker at different molar ratios, e.g. 1:2, 1:4, 1:8 (b-CD: cross-linker). Then the obtained solid was grounded in a mortar and Soxhlet extracted with ethanol to remove either impurities or unreacted diphenyl carbonate. After purification, microbeads were stored at 25°C until further use [18]. The prepared microbeads and budesonide powder were mixed geometrically in a ratio (1:1 w/w). The resultant powder (1g) is suspended in 200 ml distilled water under constant stirring. The suspension was kept stirring for 5 hours and then filtered to remove any undissolved impurities. The filtrate was then lyophilized to get budesonide–loaded microbeads [19].

3 Characterization of microbeads

3.1 Particle size analysis: The mean particle size was determined by laser diffraction techniques using Malvern 2000 SM (Malvern Instruments, Malvern, UK) which allows sample measurement in the range of 0.05-20,000 µm. The analysis was carried out at room temperature keeping the angle of detection 90°. The mean particle size was expressed in terms of D (0.9) i.e. size of 90% of the particle. The data presented are the mean value of three independent samples produced under identical production conditions.

3.2 Drug content and entrapment efficiency: The solid powder (10mg) was dissolved in a suitable quantity of phosphate buffer pH 7.4. The drug content was determined at 243 nm using a spectrophotometer (V-530; JASCO, Japan) after suitable dilution, and the % drug content was calculated by the following formula [20].

$$\text{Drug content} = \text{Drug in microbeads} / \text{Weight of microbeads analyzed} \times 100$$

The amount of drug entrapped in the formulation was calculated by estimating the amount of unentrapped drug by dissolving 10 mg powder in phosphate buffer pH 7.4. The obtained solution was assayed spectrophotometrically at 243 nm for free drug content. In this process, the percent entrapment efficiency (%EE) was calculated as the percent of drug entrapped in the final dosage form to its initial concentration.

The %EE was calculated using the following formula:

%EE= Total Drug concentration - drug concentration in supernatant / Initial drug concentration ×100

3.3 Flow properties of formulated DPI

The fixed height cone method was used to check the flow property of the formulation. A glass funnel with a 5mm internal diameter was fixed at 2.5cm height over the flat surface. The gentle flowing of the powder through the funnel was carried out. The diameter of the powder cone-formed was measured. [21]

The angle of repose was calculated by the following equation

$$\text{Tan } \Theta = \text{Height/Radius}$$

The tapped and untapped densities were evaluated using a small graduated tube with a defined volume size into which the known weight of the powder was added. Tapped volume is calculated by using a tap density tester (Electrolab, Tap density tester, USP) following 100 taps. Tapped density is determined by dividing the mass of polymer by volume. Bulk density is determined by dividing the mass of the powder by the volume. Clark's index (Ci) is calculated using the value of bulk and tapped density.

Ci = Tapped density - bulk density / Tapped density ×100

Hausner's ratio defines the flowability of the powder mixture. The value indicates the ratio of bulk and tapped density.

Hausner's ratio = Bulk density/ Tapped density

3.4 Transmission electron microscopy (TEM):

It was employed to evaluate the particle shape and size. A Philips CM 10 transmission electron microscope was used, and the particle size was measured using the NIH image software. The microsponge suspensions were sprayed on a Firmware-coated copper grid and air-dried before observation.

3.5 Scanning electron microscopy (SEM):

The external morphology of the formulated DPI was studied by SEM (Stereo scan S120; Cambridge, UK). The sample was mounted on double-faced adhesive tape and coated with a

thin gold-palladium layer by sputter-coated unit (VG-Microtech, Uckfield, UK) and surface topography was analyzed.

3.6 Fourier Transform Infrared Spectroscopy (FTIR):

To investigate the possible interaction between the drug and polymer, FTIR spectra were taken on the JASCO V5300 FTIR (Tokyo, Japan). Samples were crushed with KBr to produce pellets by applying a pressure of 150 kg/cm2. FTIR spectra of formulated DPI, pure drug, and polymer were scanned in the 400-400 cm-1 range.

3.7 Differential Scanning Calorimetry (DSC):

The DSC measurements were performed using the METLER Toledo DSC 821e module controlled by STARe software (METLER Toledo GmbH, Switzerland). Each sample (5-10mg drug, polymer, and formulated DPI) was sealed separately in a standard aluminum pan and the samples were purged in DSC with pure dry nitrogen set at a flow rate of 10 mL/min. The temperature speed was set at 10°C/min and the heat flow was recorded from 0 to 350°C. An empty aluminum pan was used as a reference.

3.8 X-ray Diffraction (XRD):

The powder X-ray diffraction patterns of drug, polymer and formulated DPI were recorded using an X-ray Diffractometer (PW 1729. Philips, Netherland) with Cu as anode material and crystal graphite monochromator operated at a voltage of 30 kV and a current of 30 mA. The samples were exposed to Cu-Kα radiation over a range of 2θ angles from 2 to 50º. The range and the chart speed were 5 × 103 CPS and 10 mm/º2θ, respectively.

3.9 Release profile:

The in vitro release for formulated DPI and pure drug were carried out in PBS (pH 7.4) using the dialysis bag diffusion technique. Formulation equivalent to 200 µg of Budesonide was added into the dialysis bag (Membrane MD 34-14, cutoff 14KD). This membrane is placed in a beaker containing 100 ml of phosphate buffer solution. The entire system was kept at 37±0.5°C with continuous magnetic stirring at 100 rpm/min. At the selected time interval, the sample was removed and replaced with a fresh medium to maintain sink conditions. The sample was analyzed by UV spectrophotometry at 243 nm.

3.10 In vitro deposition study using ACI:

Hard gelatine capsule (size 3) filled with microbead powder equivalent to 200 µg of the drug was dispersed through a Rotahaler® (Cipla Limited, Mumbai, India) into an ACI (Model no. WP-ACISS-0289, westech, UK) at a flow rate of 60 L/min for 5 sec. The drug deposited in the capsule, inhaler, mouthpiece, induction port, pre-separator, and ACI stages were collected by rinsing methanol in a Petri dish and kept for maximum extraction of the drug. [23]

Drug content was assessed with UV absorption spectroscopy at 243 nm. Each stage of the Impactor was rinsed individually for adequate time and the solution was made up to fixed volume. Different parameters were employed to characterize the deposition profile. The recovered dose (RD) was the sum of the drug collected from the capsule, inhaler device, induction port, pre-separator, and all stages of the Impactor. The emitted dose (ED) was the amount of drug released from the inhaler device i.e. the sum of drug collected at pre-separator, induction port, and all stages of the Impactor. Fine particle dose (FPD) was defined as the amount of drug deposit on stage 2 and below of the Impactor. The fine particle fraction (FPF) was calculated as the ratio of FPD to RD. The total recovery (%recovery) of the drug was assessed by the ratio of RD to the total drug.

RESULTS AND DISCUSSION

RESULT:

1 Calibration Curve:

1.1 Calibration curve of budesonide in phosphate buffer pH 7.4

The calibration curve of budesonide was found to be linear over a concentration range of 1 to 10 µg/ml at 243 nm by using UV visible spectroscopy with an equation of y = 0.059x + 0.107 and linearity constant of R2 = 0.994.

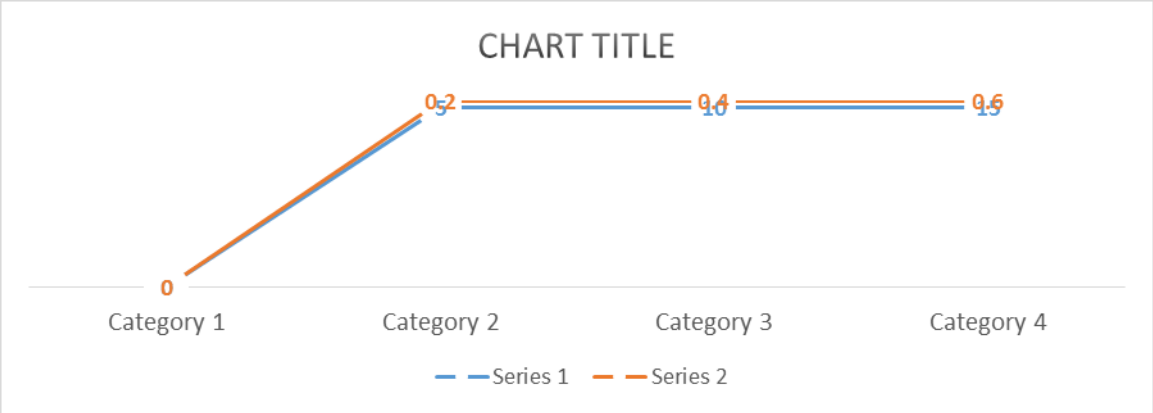


Figure No. 1: Calibration curve

1.2 Optimization of formulation

The concentration of β-cyclodextrin and diphenyl carbonate gives a prominent effect on the particle size and drug loading. Cross-linking is the process of chemically joining two or more molecules by a covalent bond. Cross-linking reagents (or crosslinkers) are molecules that contain two or more reactive ends capable of chemically attaching to specific functional groups. The reaction was carried out using an excess cross-linker at different molar ratios, e.g. 1:2, 1:4, 1:8 (b-CD: cross-linker). In the study formulation of microbeads using various concentrations of DPC with a fixed concentration of β-CD was carried out. At a high concentration of DPC, particle size was increased due to aggregation whereas drug content was low at a low concentration of DPC. Uniform size with spherical shape and high drug content was achieved at 1:8 (β-CD: DPC, molar ratio) interference of Vander-wall's force between the small particle and formation of pores on the particle by DPC. The inhalation particle properties of formulation were further carried out and further characterized and compared to marketed formulation.

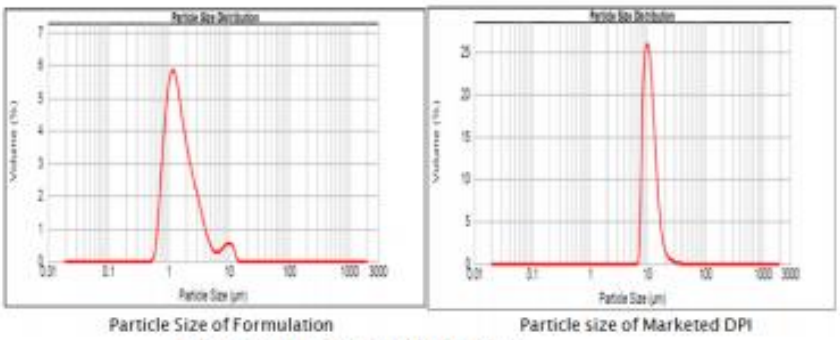
Table No. 4: Trial batches

B-CD: DPC	% Entrapment	Particle size(um)
1:2	68.3	15.46
1:4	71.4	11.73
1:6	76.7	8.6
1:8	96.02	2.43
1:10	81.5	13.15
1:12	87.3	17.43

2 Characterization of budesonide loaded microbeads

2.1 Particle size

Significant particle size variation was observed with different concentrations of DPC and β-CD. The particle size of the final formulation is 2.43 µm as compared to the 10.2 µm of the commercial form of DPI. Depending on their particle size, inhaled drug particles will deposit in different regions of the lung. Particles 5 µm will predominately deposit in the oropharynx



Particle Size of Formulation Particle size of Marketed DPI

Figure no. 3: Particle size

2.2 Drug content and Entrapment efficiency (DC, %EE)

The percent entrapment efficiency of the formulation was 96.02% and the drug content was 76.4%. The entrapment efficiency of the formulation is one of the important parameters in the microbeads DPI. The polymer and cross-linker have a positive impact on entrapment efficiency.

2.3 Flow Properties:

The aerosolization efficiency of the microbead- DPI was governed by the flow properties. The angle of repose, carr's index, and Hausner's ratio for the final formulation were in the range of 24.43°, 10%, and 1.11 for formulated DPI as compared to 28.4°, 23.3%, and 1.30 of the marketed DPI. A better angle of repose and Carr's index was observed for formulated DPI. The uneven surface of the formulated DPI helps to reduce the adhesion force and improve flow property. The particles have large geometric diameters because of their low density; they exhibit aerodynamic diameters comparable to smaller particles having higher densities. They may be ideal for pulmonary drug delivery because of their low density and large surface area which causes excellent dispersibility Furthermore, their large geometric size may reduce clearance by macrophage action, thereby improving the bioavailability of

inhaled pharmaceuticals. Whereas the ideal Hausner's ratio for DPI is 1.0-1.11 as excellent and 1.26-1.34 as passable flow property. The ideal Carr's index for DPI is ≤10 as excellent and 21-25 as passable flow property.

Table No. 5:

	Bulk Density (g/cm3)	Tapped density (g/cm3)	Angle of repose	Carr's index	Hausner Ratio
Marked DPI	0.15	0.19	28.4	23.3	1.30
Formulated DPI	0.081	0.090	24.43	10	1.11

2.4 Scanning electron microscopy (SEM)

The surface nature and morphology of the formulated DPI were verified by the SEM technique. Optimized microbead DPI evident from the photograph represents the spherical shape with a porous surface. The SEM image also indicates significant uniformity of size and the least amount of fines in the formulated DPI.

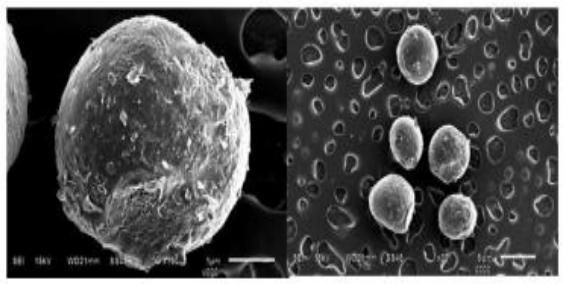


Figure no. 4: SEM images

2.5 Transmission electron microscopy (TEM)

To investigate the microbeads morphology further, microscopy studies were carried out using TEM analysis. TEM photomicrographs confirm that budesonide-loaded microbeads were spherical with a rather uniform distribution.

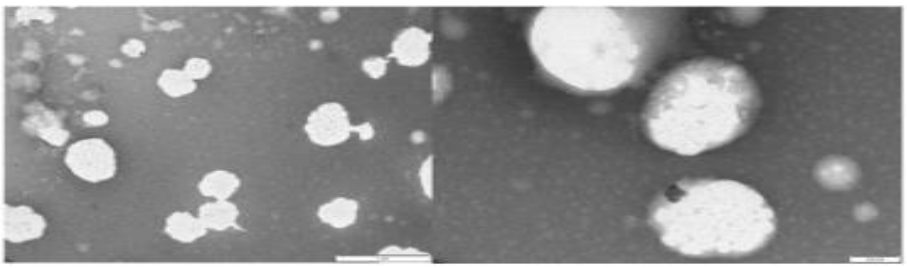


Figure no. 5: TEM images

2.6 Fourier transform-infrared spectroscopy (FTIR)

Potential intermolecular interactions between the polymer and drug were analyzed by the FTIR spectra. Budesonide showed peaks at 3499 cm-1,2956 cm-1,1722 cm-1 and 1690 cm-1 due to O-H stretching, C-H stretching and C=C stretching. The final spectrum of formulation budesonide showed minor shifting of peaks. This shifting of functional groups was attributed due to the formation of hydrogen bonding and conversion to an amorphous form.

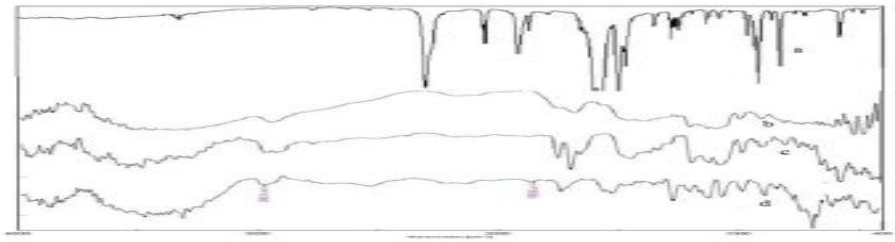


Figure no. 6: FTIR of (a) DPC (b) β-CD (c) Budesonide (d) formulated DPI

Table No.6: FTIR peaks value

Functional group	Budesonide value (cm-1)	Formulation value(cm-1)
CH3str.	2956	2997
C=O str. ketone	1722	1737
C-O str. ether	1225	1232

2.7 Differential Scanning Calorimetry (DSC)

DSC scan of budesonide showed a sharp endothermic peak at 230°C due to the melting transition point of the drug. The β-CD showed the endothermic peak at 280°C. The absence of endothermic peak of budesonide in the spectrum of formulation pointed out complete entrapment and reduction of drug crystallinity.

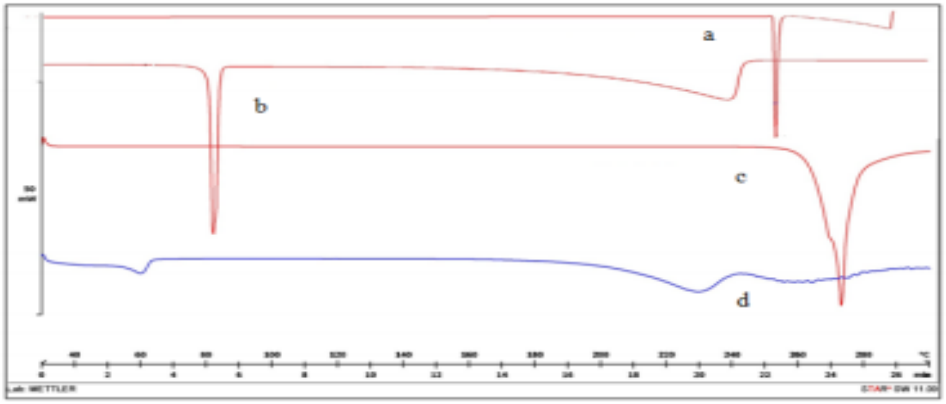


Figure no. 7: DSC plots of a) Budesonide, b) DPC, c) Cyclodextrin, d) formulated DPI

2.8 Powder X-ray diffraction (PXRD)

The PXRD diffraction data of pure drugs revealed characteristic peaks at high intensity representing high crystalline nature. PXRD data for microbeads showed reduced peak intensity as compared to pure drug and excipients thus it indicates the prepared formulated DPI was less crystalline. The intermolecular interaction between polymer and drug molecules results in the molecular complex which was responsible for fewer intensity peaks.

Table No.7: PXRD values

2..	BUD (intensity)	b-CD (intensity)	Formulation (intensity)
11.41	257	238	201
13.85	226	253	191
15.55	382	389	1017
16.1	698	347	234
22.91	259	379	201

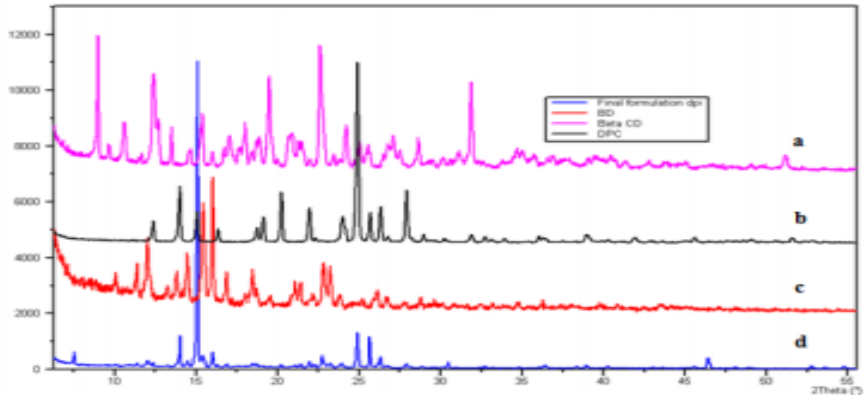


Figure No. 8: PXRD plots of a) β-CD, b) DPC, c) Budesonide, and d) formulated DPI

2.9 In vitro drug release

In vitro, drug release profiles of budesonide from DPI were carried out by dialysis technique using diffusion bag. The release studies were carried out in phosphate-buffered saline (pH 7.4) at 37°C. The rapid release of budesonide from commercial DPI was observed, nearly 100% in 8 h due to the rapid diffusion of budesonide in phosphate-buffered saline. The obtained DPI showed a biphasic release pattern with initial burst release (25%) within the first 2 h followed by controlled release up to 24 h. The initial burst release may be due to the presence of the free drug or adsorbed on the surface of the microbeads. The controlled release reflects the longer retention of the drug in the lung, which reduces the exhalation and improves the efficacy of budesonide.

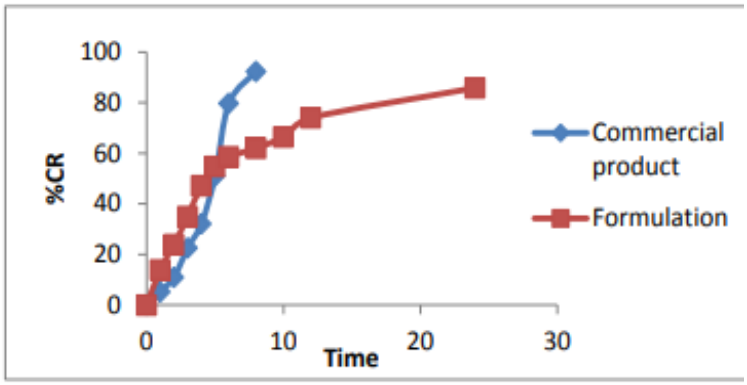


Figure No. 9: In vitro release profile of Commercial DPI and Formulated DPI

3 In vitro deposition study using Anderson Cascade Impactor

The aerodynamic diameter is the key factor for drug deposition in the lung. The key parameters such as FPF, MMAD, and GSD were prominently deciding the aerosolization efficiency and deposition of drugs in the lungs. GSD for a well-functioning stage should ideally be less than 1.2 (the GSD for an ideal size fractionator would be 1.0 and indicates a monodisperse aerosol) (MMAD) from close to 4.0 µm to 5.3 µm [4]. After the completion of dosing different plates were collected; they were washed with 10 ml of methanol. The deposition of formulated DPI on each stage of the Impactor was determined. MMAD and GSD were calculated from the deposition data using the MMAD calculator for the Anderson apparatus. The sample was analyzed by UV spectrometry at 243nm. To determine the drug deposition in various stages, Rotahaler was connected to the cascade Impactor at 60L/min and drug content was calculated on each stage. The formulated DPI showed the MMAD 2.05±0.01 and GSD2.9.

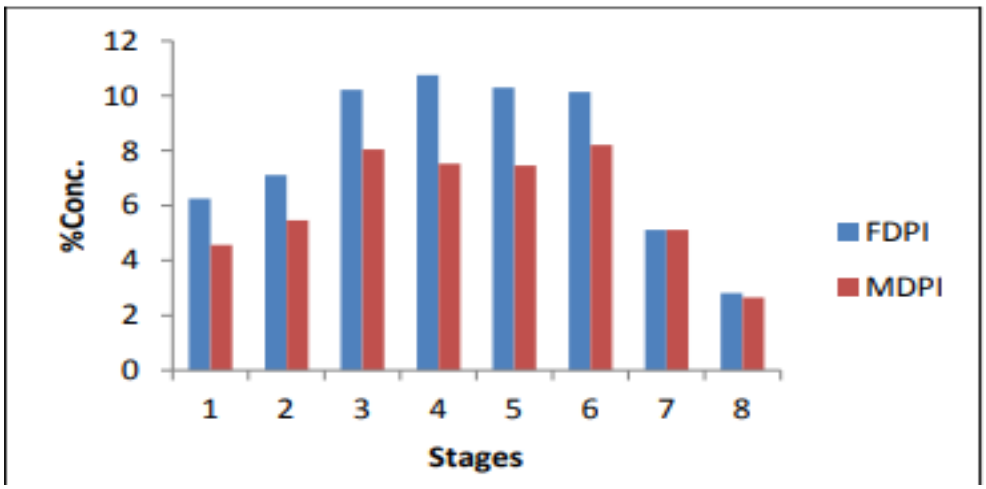


Figure No 10: In vitro deposition by Anderson Cascade Impactor

Table No.8: Lungs deposition profile of the drug in all stages

	%RD		%ED	%FPD	%FPF	MMAD	GSD
Marketed DPI	73.58		64.05	45.47	60.5	3.85	3.3
Formulated DPI	59.8	77.8	56.41	62.77		2.26	2.9

4 Acute toxicity study

The powder equivalent to 200µg of budesonide was administered. There was no death or hazardous sign-on tested animal were recorded. Formulated DPI has shown minimal congested alveolar capillaries as compared to the control group. Moreover, any abnormalities in cells or tissue were not seen in both formulated and marketed DPI. However, some inflammation was observed in the trachea was may be due to the installation procedure. From the result, it can be concluded that the given formulated DPI did not induce any major signs of toxicity in the final formulation and is safe for pulmonary administration.

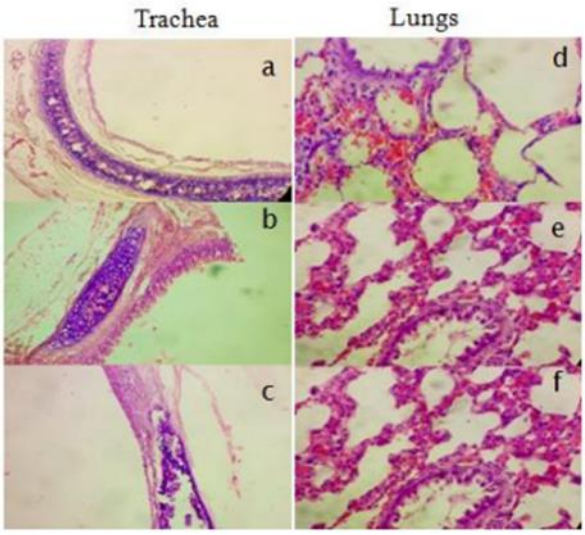


Figure No. 11: Histopathology of the trachea (a: control, b: marketed and c: formulation treated group) and lung (d: control group, e: commercial treated group, and f: formulation treated group)

DISCUSSION:

- To overcome the drawbacks associated with the commercial form of DPI, a novel dry powder formulation of β-CD microbeads was investigated for inhalation.

- For increasing deposition efficiency in the lungs and to achieve controlled release of the formulation microbeads were prepared.

- FTIR spectra suggested that there were no significant structural changes or complexation reactions between drug and polymer while PXRD spectra evidenced a reduction in the crystallinity of the formulation as compared to the drug which indicates the enhancement of solubility of the pure drug inside the formulation.

- SEM images showed the aggregates of microbeads having a spherical shape and uneven surface which helps to decrease the adhesion force and increase the aerosol performance.

- In vitro release study exhibited maximum drug release up to 24 hours which indicates the suitability of formulation for controlled release of the drug.

- In vitro aerosol performance study by using Anderson cascade Impact or Confirmed its feasibility for improved lung deposition.

- Acute cytotoxicity study proved its acceptability for enhanced lung deposition and therapeutic efficacy.

CONCLUSION:

Based on the experimental evidence, β-CD microbead ascertained a control drug release and improved lung deposition as compared to a commercial form of DPI which confirmed its feasibility for pulmonary administration. The toxicity study confirmed its safety and enhanced efficacy for pulmonary administration as compared to commercial DPI. These results concluded that β-CD microbeads formulation as a carrier for DPI is suitable for inhalation.

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