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DESIGN AND DEVELOPMENT OF A MICROPARTICLE BASED INHALATION FORMULATION OF ANTIASTHMATIC DRUG BY QbD APPROACH

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➢ **Abstract:**

The method of solvent evaporation was chosen as it is a well-established technique for producing inhalable microparticles with controlled drug release properties. By using this method, we aimed to encapsulate Salbutamol Sulphate within biocompatible polymers, ensuring its stability and enhancing its therapeutic efficacy.

S.S. microparticles are created by employing the biodegradable polymer PEG (poly ethylene glycol).The ability to effectively aerosolize different inhalable Salbutamol Sulphate formulations with appropriate (MMAD) mass median aerodynamic diameter 3.32& fraction of fine particles (FPF) ranging up to 62.40%.

According to ICH requirements, stability tests on the improved formulations were conducted done with a relative humidity of 75% at a temperature of 40 °C. The % CDR at 12 hours was shown by the optimised batch F9 (85.32%). The optimised batch F9 had an average particle size of 3.06µm and kinetic models displays Fickian diffusion kinetics. The stability investigation on the improved batch F9, conducted in accordance with ICH criteria, revealed no appreciable change in the drug composition. These findings effectively showed that respirable microparticles made from salbutamol sulphate could be created, making them a promising delivery method for the quick and efficient treatment of COPD.

Keywords: Salbutamol Sulphate, PEG, Meg, Solvent evaporation method.

➢ INTRODUCTION:

Mast cells, eosinophils, and lymphocytes in particular, as well as other cells, play a function in the chronic inflammatory condition of the airways known as asthma.[1] There are two types of asthma: extrinsic (allergic) and intrinsic (chronic).[2] Asthma sufferers' oxygen levels dropped because of inadequate ventilation, which induce hypoxemia (alveolar hypoventilation).[3]

Salbutamol stimulates 2 receptors, which has a strong bronchodilator action. It is given orally, inhaled, and intravenously. Salbutamol sulphate was typically administered orally in doses of 2 to 4 mg every 4 hours and intramuscularly in doses of 0.6 mg every 4 hours for inhalation. Another non-invasive systemic medication delivery strategy was the respiratory pathway using administration of salbutamol sulphate by oral inhaler As an alternative to oral delivery.[4] It is a short-acting, 4-6-hour plasma half-life beta-2 adrenoreceptor stimulant that must be dosed frequently to effectively treat asthma on a daily basis. The delivery of medications via aerosol was a development of the 20th century.

Given that polyethylene glycol (PEG) conjugation lengthens the protein's retention in intact form in the body and boosts its stability. PEGylation seems to be a potential strategy for ensuring the therapeutic efficacy of proteins in this context.[5] It entails joining a molecule with

one or more polymer strands of PEG made up of ethylene glycol repeating units.[6]

In order to increase flow, lessen agglomeration, and promote dispersion, big carrier particles (excipients) are typically combined with micronized medicinal particles, which typically have a diameter between 1-5µm.[7]

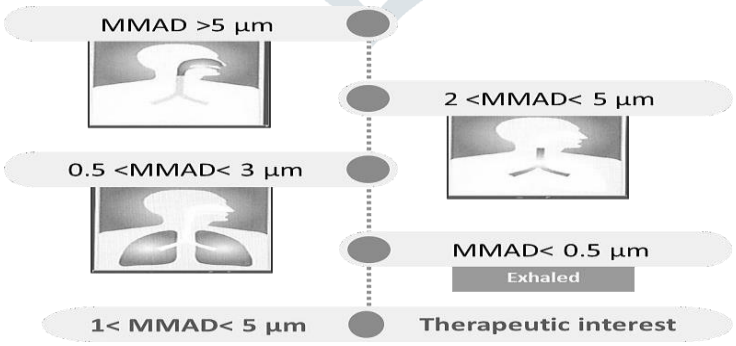

Fig.No.1.Depending on their size, particles are deposited in the respiratory tract

Evaluation of these innovative carrier systems' *in -vitro* and *in- vivo* performance is required for pulmonary delivery..[8]

A number of variables, including aerosol turboelectric charge, particle shape, size, size distribution, and moisture content, and inter-particulate forces, affect how much of the medicine is deposited in the airways.[9]

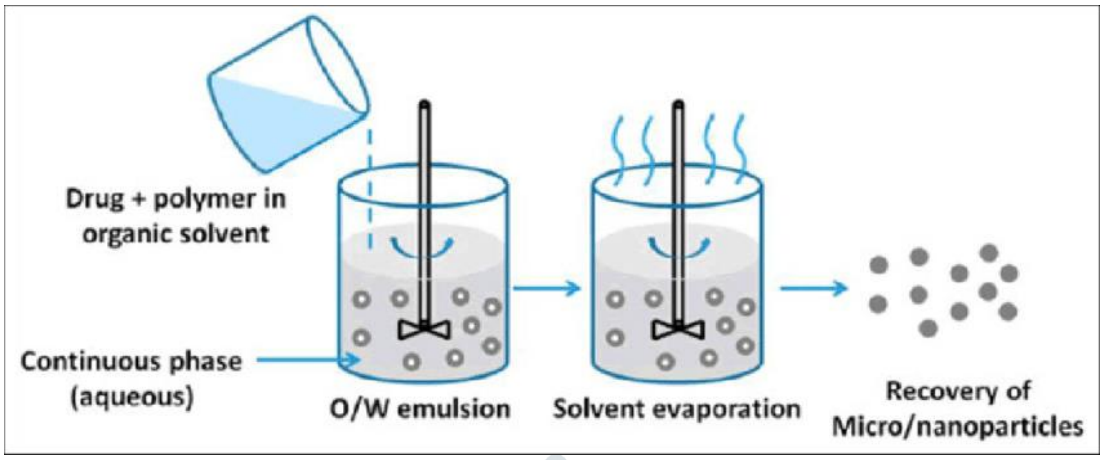

Fig.no.2. Process of Solvent Evaporation schematic diagram.

➢ Materials and Methods:

Salbutamol Sulphate, manufactured by Yarrow Chem in Mumbai, India. S.D. Fine Chem. Ltd., Mumbai, India, PEG 4000. S. Puram, Bangalore, India, Magnesium Stearate. Mumbai, India's HPMC- S.D. Fine Chem. Ltd. SDFCL Lower Parel, Mumbai, India, liquid paraffin. Acetone LR is produced in N-hexane is produced by S.D. Fine Chem. Ltd., a company situated in Mumbai, India.

➢ Inhalable microparticle preparation:[10]

Inhalable microparticles were produced by the solvent evaporation procedure. In order to spread the various HPMC and PEG 4000 ratios, Each was dissolved separately in 8.5 ml of acetone and stirred with a magnetic stirrer. The major component was mixed completely into the polymer solution for 15 minutes before adding 100 mg of magnesium stearate.The resulting dispersion was applied in a thin stream to a mixture of 250 mL beaker containing 90 mL light liquid paraffin and 10 mL n-hexane. It took 3 hours of continuous stirring to thoroughly evaporate the acetone. Filter paper No. 1 by Whatman was used to filter the microparticles that were produced. Using 50 ml volumes with n-hexane There were four or five washings of the residue. A 24-hour period of room temperature drying followed, drying the product.

Central Composite Design: Table.1. Codes and compositions of the S.S formulations

		Factor 1	Factor 2	Response 1	Response 2
Std	Run	A: PEG 4000 mg	B:mg stearate mg	Particle size µm	Entrapment efficiency %
8	1	75	104	4.04	86.02
12	2	75	90	2.03	55.01
4	3	100	100	4.03	85.01
3	4	50	100	2.04	54.01
11	5	75	90	2.1	52.9
7	6	75	75	4.01	65.02
6	7	110	90	3.02	60.07
5	8	39	90	2.01	74.09
2	**9**	**100**	**80**	**3.06**	**88.03**
1	10	50	80	3.07	68.06
13	11	75	90	2.6	56.05
9	12	75	90	2.5	57.01
10	13	75	90	2.7	59.01

Table.2: Regression terms for responses

	R^2	Predicted R^2	Adjusted R^2
Y1	0.9302	0.7919	0.8804
Y2	0.7205	-0.8924	0.5208

Analysis of variance (ANOVA) is used to determine whether any level of the tested factor's response differs noticeably from the other levels. The model terms must affect the response if the Prob>F value is very low (less than 0.05 is significant).ANOVA is used to assess if any level of the tested factor's response varies significantly from the other levels.

- **The signal-to-noise ratio is measured by Adeq Precision.**

$Y1=+2.38+0.4251*A+0.0345*B+0.5000*AB+0.0240*A^2+0.7353*B^2$

$Y2=+56.00+1.22*A+4.73*B+8.26*AB+4.97*A^2+8.63*B^2$

Equation (1) revealed elements A and B have a favourable effect on the PS. Consequently, rising the amount of all these factors PEG-4000 & Mg. Stearate would increase in PS.

Equation (2) uncovered variables A and B. Positive impact on the EE. As a result,increasing the amount of all these factors PEG-4000 & Mg Stearate would result increase in E.E.

- **Evaluation of Inhalable Microparticles:**

1.Particle size determination: The laser diffraction method was used to determine the size of the microparticles. Particle size was determined using Sympatec Gmbh's particle size analyzer Helos and the Vibro/Rodos drug dispersion equipment. To obtain the requisite obscuration of 5%, about 100 mg of the powder were employed. The data on particle size were expressed as D0.5.

2.Entrapment efficiency[11]: The microparticles were used to estimate the medication content. Following their dissolution in acetonitrile, the microparticles were treated using 0.1 M sodium hydroxide to get rid of the SS. The SS concentration at 278 nm was measured using a UV/Vis spectrophotometer (Jasco) after the necessary dilutions. The formula used to determine entrapment efficiency was: Entrapment effectiveness is calculated as follows: (estimated% drug content)/(% drug content (theoretical))100.

3. zeta potential[12]: Using Zetasizer Nano ZS (HORIBA SZ 100 Scientific Instruments, UK), the 12zeta potential was calculated. A dynamic light scattering particle size analyser with a 635 nm wavelength and a fixed scattering angle of 90° was used in the device.The z-average diameter values were employed. Samples were measured at 25°C and diluted with water to 2% prior to analysis.

4.Poly dispersity index[13]: The PDI value is calculated using cumulants analysis and a two-parameter fit to the correlation data. Because of the dimension lessness and scale of this measure, values lower than 0.05 are often observed only with extremely monodisperse standards. When the particle distribution index (PDI) is more than 0.7, the sample likely cannot be studied using DLS, or dynamic light scattering (more on this in the section below). Data between these two PDI extremes (between 0.05 and 0.7) can be used with various size distribution techniques. The formulas employed to calculate size and PDI The ISO specification publications ISO 22412:2008 and 13321:1996 E establish parameters. Essentially, The PDI measures the distribution of population sizes within a given sample.The numerical value of the PDI varies from 0.0 (for a sample) to 1.0 (for totally uniform particle size) (for a sample with numerous particle size populations that is extremely polydisperse).The numerical value of the PDI varies from 0.0 (for a sample) to 1.0 (for totally uniform particle size) (for a sample with numerous particle size populations that is extremely polydisperse). In practise, values of 0.2 and below are commonly

recognised as acceptable for polymer-based nanoparticle materials.A PDI of 0.3 or less is regarded appropriate for drug delivery applications using lipid-based carriers, such as liposome and nanoliposome formulations. a population of phospholipid vesicles that is homogeneous. Despite the fact that it has been a while since it was updated, the FDA's "Guidance for Industry" relating to liposome medicinal products still emphasises size and size distribution as "critical quality attributes (CQAs).. and crucial elements of stability studies of these goods, although the standards for an appropriate PDI are left out. The regulatory authorities must establish more precise rules recommendations for the approval of the product PDI range for various bioactive delivery channels and applications (such as food, cosmetics, and pharmaceuticals).

5.Scanning electron microscopy[14]: SEM (VEGA3 LMU, TESCAN) was used to look at the surface morphology and shape of the created microparticles, with a maximum magnification of 10,00,000*X and a resolution of 3 nm at a maximum applied voltage of 30 kV.A greater magnification digital recording was made of the electrical images. The DPI microparticles' structural parameters, particle size makeup, as well as surface morphology were all observed using scanning electron microscopy. The powders were stored on Radius 6.25 mm aluminium stubs with an adhesive coating, and the excess powder was expelled by blowing a compressed gas jet and tapping the stubs devoid of particles.The samples were then inspected in a scanning electron microscope, which ran at its highest vacuum and had a working distance of 12 minutes for each specimen while using an accelerating voltage of 5 to 15 KV.

6. IN VITRO DRUG RELEASE STUDIES [15] **:** Using a dialysis bag diffusion approach, the in vitro salbutamol sulphate dissolution from the microparticles was examined. buffer with phosphate Saline (PBS), pH 7.4, was employed as the diffusion medium for the in vitro release.. and a a membrane for dialysis having a molecular weight of 14 kDa (Himedia, Mumbai, India) was employed. The dialysis bag contained an aquatic dispersion containing Salbutamol sulphate 10 mg microspheres that was sealed at both ends. 250 ml of diffusion media were added to the dialysis bag, which was then swirled at 100 rpm. At regular intervals, samples were taken out and after each sample was removed, A volume of blank was given to the receptor phase. pH 7.4 was used to dilute the samples. PBS after being filtered through a 0.46 m filter. Using pH 7.4 PBS as a blank, UV/Vis spectrophotometry was used to calculate the samples' absorbance at 278 nm. Calculations and a time plot were made for the cumulative percent of the drug at different points in time. Three copies of each experiment were performed.

7. Aerosol performance of salbutamol sulphate MP's: [16] The removal of the MPs has other connections besides Dae. Their density also has a significant impact. Inversely correlated with A lower respiratory system's MP density is the possibility of MP accumulation. MPs should have a bulk density of around 0.4 g/cm3. The uneven shape and porosity may be a desirable quality to attain. These MPs are less dense than others, and as a result, their Dae is less than their actual size. Additionally, agglomerates cannot develop on an uneven surface. The other three aerodynamic parameters associated with Dae are the geometric standard deviation (GSD), the mass median aerodynamic diameter (MMAD), and the fine particle fraction (FPF).MPs are split in half by MMAD: 50% of MPs have Dae values that are lower than MMAD and 50% of MPs have values that are higher than MMAD.

The result is used to compute the geometric standard deviation (GSD) by dividing the diameter at the 16th centile by the square root of the diameter at the 84th centile.According to this, 68 percent of the particles between MMAD/GSD and MMAD/GSD should fit. based on the characteristic of the log-normally distributed particle size distribution.

$$GSD = \frac{84.1\%}{50\%} * 100 \quad or \quad GSD = \frac{84.1\%}{50\%} * 100$$

MPs are considered monodisperse if their GSD value is 1, and hetero disperse if it is greater than 1.2. The term "FPF" refers to the "The proportion of particles with an aerodynamic diameter of less than 5 mm.".

The aerosol's normalised mass per stage (H') is analysed as a histogram to visually confirm that it follows a unimodal and log-normal distributionThe difference between logDp+1 and logDp, where Dp is the lower cut-size limit and Dp+1 is the higher cut-size limit of the associated impactor stage, is equal to logDp.Calculate the histogram fH' by equation:

$$\text{fH}' = Nf \times \frac{\text{mass /stage D}}{\Delta \text{logDp}}$$

Using the following equation, determine the log-normal mass distribution y'(Dae) = Nf x y(Dae) as a function of the aerodynamic diameter (Dae):

$$\text{y}'(\text{Dae}) = exp[-\frac{(\log \text{Dae} - \log MMAD)2}{2 \times \log 2 \ GSD}]$$

likewise apply the normalising factor (Nf):(18)

$$\text{Nf} = \left(\frac{\Sigma mass}{\log GSD \times \sqrt{2\pi}}\right)^{-}1$$

Fine particle dose:

Formula: FPD (mg) = drug mass deposited ≤4.46m

The FPF is the proportion of particles with an aerodynamic diameter of 5µm. The FPF is derived using an equation.

$$FPF = \frac{Fine\ Particle\ dose(Dae < 1\mu m)}{Delivered\ Dose} * 100$$

where the mass of particles with a size less than 2.5 m is the fine particulate dose.D_{ae} <5µm and The given dose is the total mass of the drug provided to the device through the inhaler's mouthpiece.Two further factors characterise the MPs for inhalation that are less frequently mentioned but can be found in the literature.

These are the size distribution's median diameter (DV50) and emitted dosage (ED).DV50 implies that the particle size in micrometres is half of the total amount of dry powder delivered by the device during inhalation. The amount of drug that escapes the device, stated as a percentage, is known as ED.

$$\text{Respirable Fraction(RF\%)} = \frac{mass\ of\ Powder < 4.46\mu m(stages\ 2\ though\ 7)}{Total\ particle\ mass\ on\ all\ stages} * 100$$

$$\text{Emitted Dose(ED\%)} = \frac{Initial\ mass\ in\ Capsule - Final\ mass\ remaining\ in\ Capsules}{Initial\ mass\ in\ Capsules} * 100$$

The shape factor is calculated with the respect to the shape of a perfect sphere. For non spherical particles, the value of the shape factor value varies between 1.1 to 1.75 mm.

It is insufficient to convey particle characteristics solely through estimated average diameter.Particle characteristics are heavily influenced by the particle size distribution law.

Particle and Size Distribution: The dispersity of particles, which is the number or mass percentage of particles of different sizes within the group of particle clusters, is synonymous with the distribution of particle size.

where the given dosage is dose refers to the entire amount of medication delivered to the apparatus through the inhaler's mouthpiece. The mass of particles with a diameter of less than 5µm (Dae) is the small particulate dose.

Equation used for calculation of the particle density

MMAD = D50 x√ Particle Density

8.Studies on stability.

Stability testing is done to show how different environmental elements, including light, humidity, and temperature, , affect how well a medicine formulation performs over time. This study included information on suggested storage conditions, retest intervals, and the drug's self-life.

According to ICH requirements, a stability test of the chosen Optimised formulations was conducted for one

month at temperatures of 400°C/75°RH and 300°C/65°RH. The formulations' physical characteristics, pH, drug content, and percentage of drug released by the prior processes were examined.

➢ Result and Discussions:

Response surface plots for optimization of Salbutamol Sulphate inhalable MP's

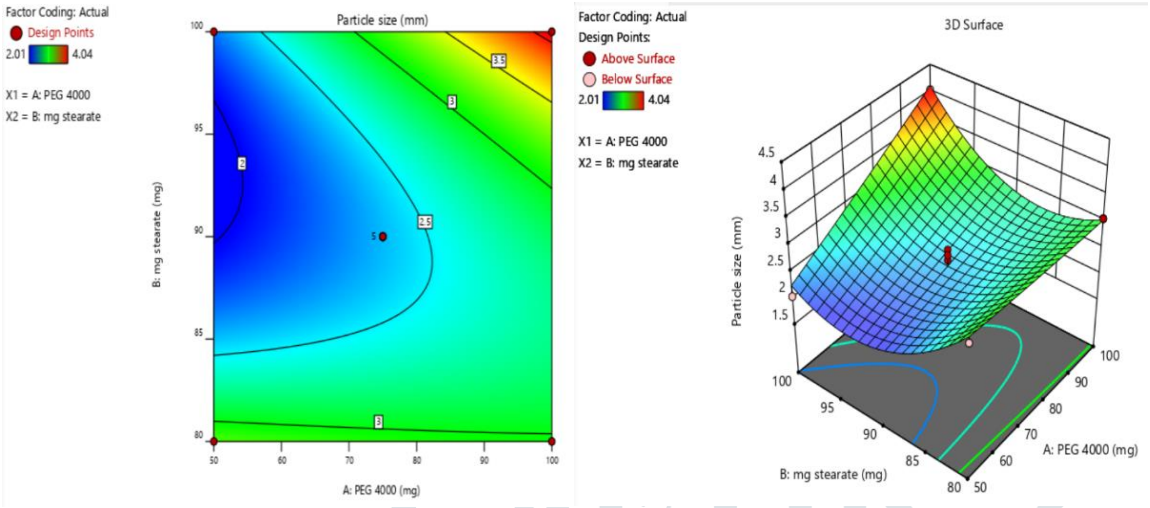

Fig.no.2. Effects of variables on P.S.

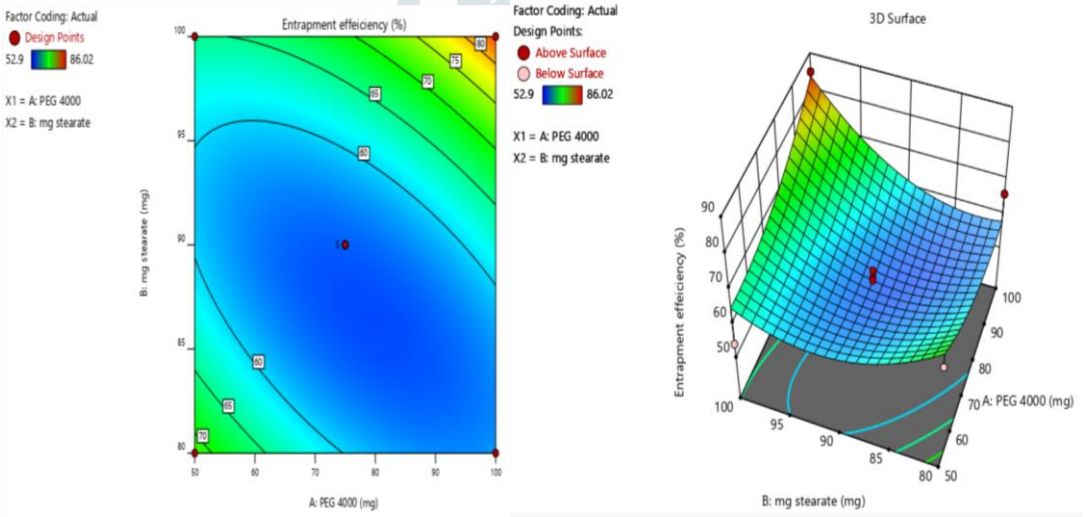

Fig.no.3.Effects of variables on E.E

1.Particle size determination:

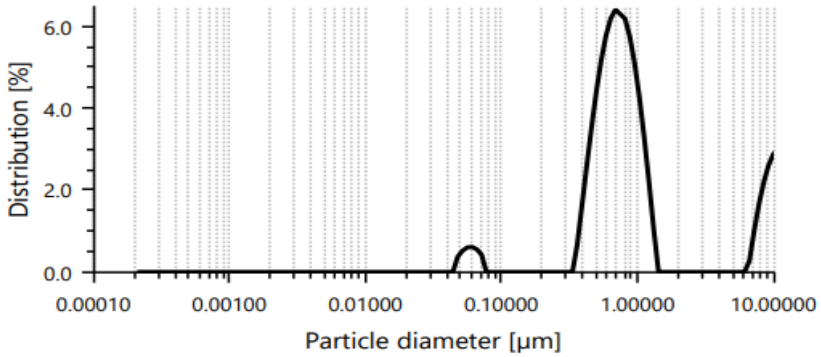

Fig.no.4: Particle size analysis of Salbutamol Sulphate

2.Entrapment Efficiency of S.S MP's: All of the polymers, excipients, and surfactants utilised were found to have an increasing relationship with entrapment efficiency. To obtain the desired Entrapment efficiency of Microparticles, PEG-4000, a powerful encapsulating agent, is especially important. The greatest EE was 88.03(%) in the optimised formulation, which contained PEG-4000 and mg stearate.

3.Zeta potential of Salbutamol Sulphate: The magnitude of the zeta potential is assessed to assess the colloidal system's potential stability. The Microparticles will reject one another and exhibit dispersion stability if they all possess a strong zeta potential, either negative or positive. The optimised S.S formulation's zeta potential was discovered to be -7.5 mV, indicating that the produced S.S mp's formulation was stable and free of agglomeration.

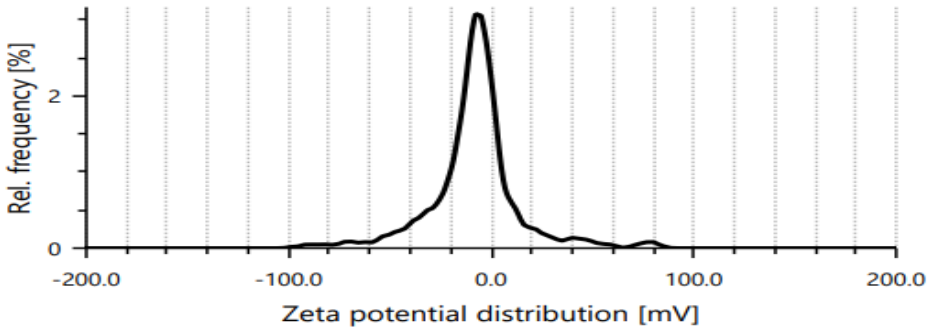

Figure.5: Zeta potential analysis of Salbutamol Sulphate mp's

4. Poly disperse index: Typical particle size distribution plots exhibit a mixture of particles with widely varying sizes (low PDI value).

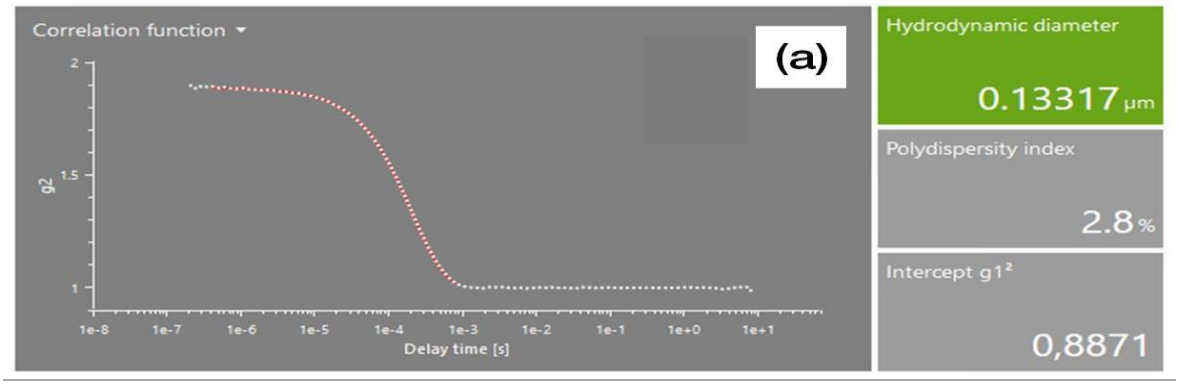

Fig No.6: PDI Values Of S.S Mp's

5. Scanning electron microscopy: SEM (Scanning Electron Microscope) was used to analyse surface morphology for final formulation of Salbutamol Sulphate microparticles. Microparticles shows particles in good shape.The surface morphology of Salbutamol Sulphate microparticles was studied using a scanning electron microscope (SEM) prior to their final formulation.

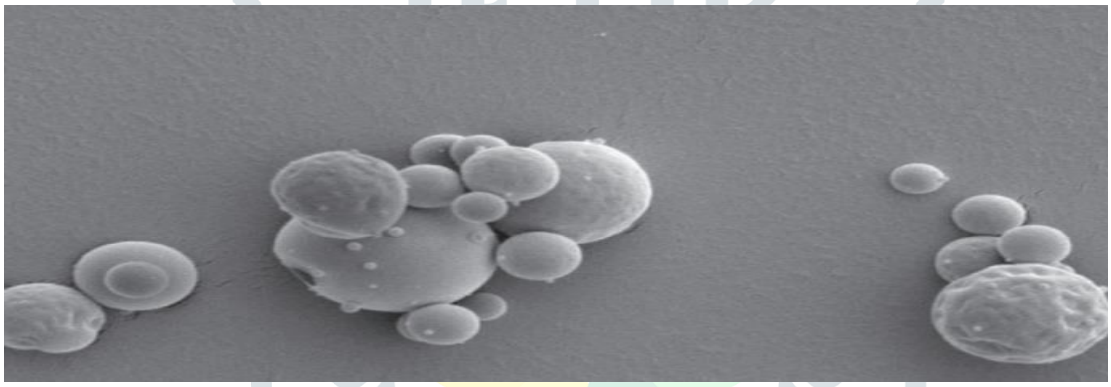

Fig. no.7: SEM of Salbutamol Sulphate

6.Aerosolization properties of S.S:

stages	%mass deposited at each stage
device	1.5213
capsules	1.7321
adapter	0.7854
induction port	2.7821
preseparator	4.341
stage -1	5.3742
stage -0	6.7852
stage 1 (5.8 um)	7.9102
stage 2 (4.7 um)	10.2563
stage 3 (3.3 um)	8.9113
stage 4 (2.1 um)	9.1376
stage 5 (1.1 um)	12.4781
stage 6 (0.7 um)	11.434
stage 7 (0.4 um)	10.185

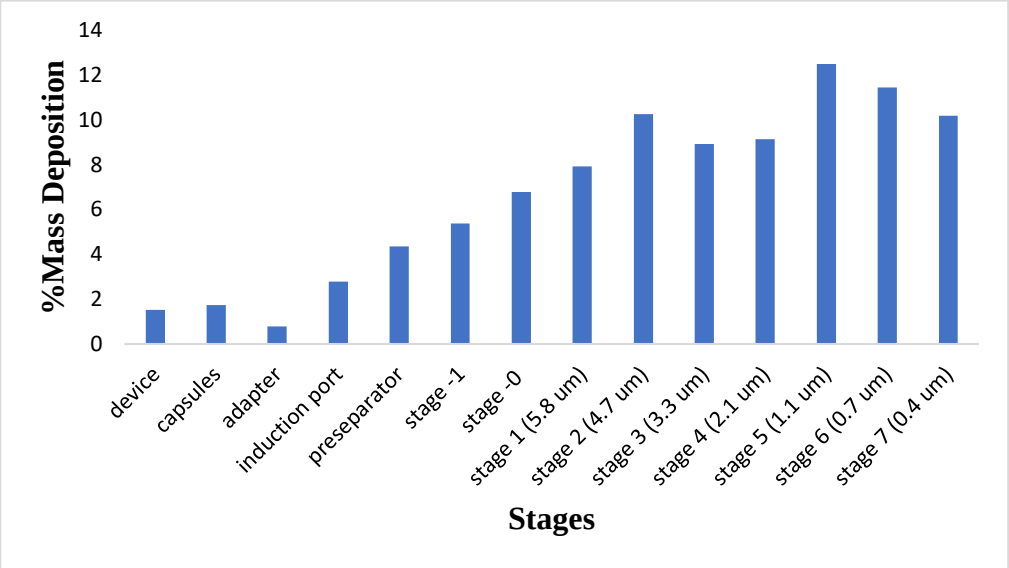

Fig.no.8.Percent deposition profiles in each stages for ACI.

➢ **Table.3. Fine particle properties of Salbutamol Sulphate Dpi's**

total (stage 2 - 7)	62.4023
total particle mass on all stages	93.6338
fine particle dose (mg)	62.4023
fine particle fraction (FPF %)	31.2
respirable fraction (RF %)	66.64
emitted dose (ED %)	0.9635

7. *IN-VITRO* DRUG RELEASE STUDY:

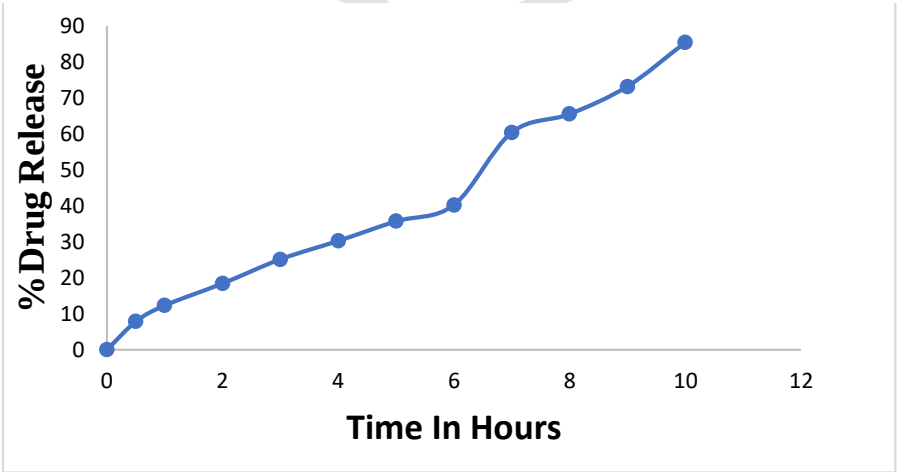

Fig.no.9: *In vitro* Cumulative % Drug Release.

8.Stability Studies:

Table.4: Results of stability studies of Salbutamol Sulphate Mp's formulation

Sampling Interval (days)	Storage condition		
	40^0C±20C /75% RH±5%RH		
	pH	Drug content	% *In-vitro* Drug release (12 hrs)
0	7.4±0.34	96.23±0.86	85.32±0.32
30	7.41±0.26	95.69±0.70	85.34±0.31

CONCLUSION:

Salbutamol sulphate is dissolved in a solvent, which evaporates to form a fine powder with spherically shaped particles with a mean size suitable for pulmonary distribution. The medication was not chemically changed by solvent evaporation, but it did lose some of its crystallinity. In in vitro aerosol deposition experiments, the S.S. powder was found to perform similarly to the medication that had been micronized. Consequently, it offers a practical method of producing regulated particle sizes with a clearly defined physical character for the medicine.

Various methods are employed to overcome obstacles. In order to make them porous, modified emulsification is first used to create the majority of polymeric MPs. As a result, the aerodynamic diameter stays between 1 and 5 micrometres, while the physical diameter rises to as much as 20 millimetres, effectively preventing macrophage absorption. In addition, composite MPs are promptly redispersed after entering the lungs, allowing the mucus to enter the side of action with appropriate depth. The utilisation of materials with high drug loading capacities, appropriate release kinetics, and appropriate aerodynamic properties for MPs, respectively, makes it particularly intriguing. These methods have mostly been seen in recent publications, which suggests positive prospects for DPIs in the future.

➢ References:

1.McConnell W, Holgate ST. The definition of asthma: its relationship to other chronic obstructive lung diseases. In: Clark TJH, Godfrey S, Lee TH, Thomson NC, editors. Asthma. 4th ed. London: Arnold; 2002. p. 1-5.

2. The respiratory system. In Wilson KJW, Ross JS editors. Ross and Wilson's Anatomy and physiology health and illness. 7th ed. London: Churchill Livingstone; 1990. p. 138-40.

3. Pharmacotherapy of Bronchial Asthma and Rhinitis. In: Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotherapeutics. 14th ed. Mumbai: Popular Prakashan; 1994. p. 313-20.

4. Dandagi PM, Mastiholimath VS, Gadad AP, Iliger SR. Mucoadhesive microspheres of propanolol hydrochloride for nasal delivery. Indian J Pharm Sci 2009;69:402-7

5.S.A. Shoyele, S. Cawthorne, Particle engineering techniques for inhaled biopharmaceuticals, Adv Drug Del Rev, 58 (2006) 1009-1029.

6.M. Roberts, M. Bentley, J. Harris, Chemistry for peptide and protein PEGylation, Adv Drug Del Rev, 54 (2002) 459-476

7. P.C. Seville, H.-y. Li, T.P. Learoyd, Spray-Dried Powders for Pulmonary Drug Delivery, Critical Reviews™ in Therapeutic Drug Carrier Systems, 24 (2007) 307-360.

8.Todoroff J, Vanbever R. Fate of nanomedicines in the lungs. Curr Opin Colloid Interface Sci 2011;16:246-54.

9. Yang W, Peters JI, Williams RO. Inhaled nanoparticles - a current review. Int J Pharm 2008;356(1-2):239-47. 181.Kumaresan C, Sathishkumar K. Development of an inhaled sustained release dry powder formulation of salbutamol sulphate, an antiasthmatic drug. Indian journal of pharmaceutical sciences. 2016 Jan;78(1):136.

10.Nagendra R, Charan CS, K. Hanumanthachar Joshi, Jayanthi C. Preparation and evaluation of microparticles containing Oxybutynin Chloride for controlled release. Asian Journl of Biomedical and Pharmaceutical Sciences. 5(41).2015: 15-17.

11.Gupta S, Vyas SP. Carbopol carbopol p930/Chitosan based pH triggered *in-situ* gelling system for ocular delivery of Timolol maleate. Sci Pharm. 2010;78(1):959-76.

12.Worldwide, M.I. Dynamic Light Scattering, Common Terms Defined; Inform White Paper; Malvern Instruments Limited: Malvern, UK, 2011; pp. 1–6.pdi

13.Chen AZ, Yang YM, Wang SB, Wang GY, Liu YG, Sun QQ. Preparation of methotrexate-loaded, large, highly-porous PLLA microspheres by a high-voltage electrostatic antisolvent process. J Mater Sci Mater Med 2013;24:1917-25.Sem

14.Davies NM, Feddah MR. A novel method for assessing dissolution of aerosol inhaler products. Int J Pharm 2003;255:175-87.

15.Chew N. Y. K., and Chan H.-K. Use of solid corrugated particles to enhance aerosol performance. Pharm. Res. 2001; 18:1570-1577.

16.ICH Q1A (R2) Stability testing guidelines: Stability Testing of new drug substances and products.[Online]URL:http://www.tga.health.gov.au/docs/pdf/euguide/inch/273699r2en.pdf