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STUDY THE AMINO ACID BEHAVIOUR IN CHITOSAN CONTAINING SPRAY DRIED POWDER FOR PULMONARY DRUG DELIVERY

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Abstract: For a dry powder carrier platform to be suitable for pulmonary delivery of potent biomacromolecules, it has to be aerosolisable and capable of reach to the deep part of the lung such as alveoli. In the present study, strategies aiming to produce a multi-component spray-dried powder formulation with a stable amorphous glassy matrix containing mannitol as a deaggregating agent, while using different amino acid such as acids arginine, leucine, phenylalanine and threonine as a particle formation and aerosolisation enhancing agent were investigated. Parameters such as spray-dried yield, tapped density, and Carr's Index were performed. In addition, whilst the majority of amino acid-modified powders displayed suitable particle size distribution for pulmonary administration and potentially favourable high fine particle fractions (FPF) and formulations in vitro particle deposition was only enhanced for the leucine-modified powder. Scanning electronic micrographs (SEM) revealed distinct morphological features of these. In conclusion, the results suggest that with suitable particle size, good dispersibility leucine can be used to enhance the dispersibility and aerosolisation properties of spray-dried powders for pulmonary drug delivery. And appear to have good potential for development into a universal carrier platform for pulmonary delivery.

Keywords: Pulmonary Drug Delivery, Amino acid, Leucine

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INTRODUCTION

Pulmonary drug delivery systems (nebulisers, dry powder inhalers and pressurised metered dose inhalers), dry powder systems have many advantages including ease of drug administration, convenient portability, relatively simple formulation, low cost and inherently improved stability[1–4].Dry powders for the administration of therapeutics via inhalation require specific characteristics to optimize their delivery. Spray-drying, a single-step particle processing technique, has been shown to be an effective way to prepare dry particles within the respirable size range. For instance, for efficient particle delivery into the alveolar region of the lungs, the aerodynamic particle size should be less than five micrometers, preferably 1–3 μm In this size range, capillary, van der Waals, electric double layer and Coulomb forces between powders induce particle agglomerates. Unfortunately, spray dried particles commonly aggregate into larger species which consequently decreases the air-flow properties of the powders and their subsequent deposition into the deep lung [5].To overcome such problems the powder characteristics such as size, size distribution, shape, hardness and morphology should be manipulated [3]. Several attempts have been made to enhance the dispersibility of spray-dried powders for pulmonary delivery, including the use of carrier-based formulations [6,7], altering particle density [8] and incorporating modified components [9, 10].The use of excipient materials with specific properties is a way to improve the characteristics of powders [8–11]. Materials, such as polymers and surface-active compounds have been used to stabilize drug formulations [12–14], to modify the particle morphology and density [15] and to reduce adhesion between particles [16]. The use of hydrophobic α-amino acids, particularly leucine derivatives, has been shown to improve powder dispersion when delivered from dry powder inhalators (DPI).significantly improve the in vitro deposition of these bioactive macromolecules with significant quantities of powder deposition in regions equating to the lower respiratory tract [10,11].

In this study, the amino acids arginine (ARG),leucine (LEU), phenylalanine (PHE) and threonine(THR) are investigated as potential 'dispersibility enhancers' in spray-dried powders containing Mannitol as a primary excipient and Fluticasone Propionate as a model drug.The influence of these amino acids on the physical properties,including particle diameter, dose emission and in vitro deposition pattern of the spray-dried particles was investigated, with the aim of determining the utility of amino acids in enhancing the deposition drugs delivered via inhaler and reach to the deep part of the lung.[17]

MATERIALS AND METHODOLOGY

Materials

FP was gifted by Sun Pharmaceutical Company, Halol, Chitosan (grade M, M.W 100,000 – 2,000,000 da, degree of deacetylation: 80%) was gifted by Cognis Gmbh Pvt. Ltd.,Germany, L-

arginine (ARG), L-leucine (LEU), L-phenylalanine (PHE) and L-threonine gifted by Devson Implex Pvt Ltd, Mumbai, Mannitol of AR grade was gifted by Astron Chemicals Pvt Ltd,

Methodology

Preparation of spray-dried powders

Aqueous solutions containing fluticasone propionate as a model drug, Chitosan as a polymer and mannitol as a deaggregating agent in the presence of selected amino acids (ARG, LEU, PHE or THR) as dispersibility enhancers were prepared, with a total powder mass of 2% w/v. The prepared formulations (100 mL) were subsequently spray-dried using a spray-dryer equipped with a mini nozzle, using the following standard operating conditions: inlet temperature, 120 °C; spray flow rate, 5 ml/min these conditions resulted in an outlet temperature of 85–91 °C. A total of 12 powders were prepared, each containing 0.5% w/w fluticasone propionate, 5–20% w/w amino acid and 70-90%w/w mannitol. In addition, a powder containing 0.5% w/w fluticasone propionate and 90% w/w mannitol (i.e. no amino acid) was produced as a control.

EVALUATION PARAMETERS

Powder characterisation

- **Spray-drying yield**

The production yield was calculated as the weight percentage of the final product after drying, with respect to the initial total amount of ingredients used for the preparations. Production yield was calculated by using equation (1) [18].

% Production yield"="W1" /"W2" ×"100" ---------- **(Eq.1)**

W1= weight of dried nanoparticle

W2= dry weight of starting materials

- **Drug content**

The FP content of each prepared powder was measured in triplicate, with analysis by HPLC, and expressed as the percentage of nominal load.

- **Scanning electron microscopy**

10 mg nanoparticles fastened onto a brass stub with double-sided adhesive tape. The stub was fixed into a sample holder and placed in the vacuum chamber of (JEOL, Tokyo, Japan) scanning

electron microscope and observed under low vacuum (1023 mm HG). The nanoparticles were observed for surface characteristics.

- **Particle size analysis**

The size analysis of nanoparticles was performed by zetatrac particle size analyzer. Prepare nanoparticulate solution containing 10 mg nanoparticles in 20 ml solvent. The aqueous nanoparticulate dispersion was added to the sample dispersion unit containing probe. Coated-window optical probes are paired with their opposite electrodes in an insulating sample cell. An electric field is applied between the optical probes and their corresponding electrodes. Particle motion is analyzed while under the influence of the field. Particle size distribution is determined from the velocity distribution of particles suspended in a dispersing medium, using the principles of dynamic light scattering. Particle size distribution graph was obtained by using flex software. For the study of particle size distribution polydispersibility index was calculated by using equation (2).

Polydispersibility index = "D0.9 - D0.1" /"D0.5" ---------- (Eq.2)

Where D0.9, D0.1 and D0.5 particle diameters determined at 90th, 50th and 10th percentile of particles respectively [18].

- **Powder density**

The poured density of the spray-dried powder was determined by pouring a known mass of powder under gravity into a calibrated measuring cylinder and recording the volume occupied by the powder. The tapped density of the same samples was subsequently determined using a tamping volumeter (Tapped Density Assessor: Copley Scientific Ltd., Nottingham, UK) to displace powder until no further change in the powder volume was observed. Measurements were performed in triplicate. Carr's Index values for each spraydried powder were derived according to Equation 3.

Carr's Index(%) =100(Tapped Density – Poured Density)/ Tapped Density----------(Eq.3)

- **Aerodynamic properties evaluation**

The aerodynamic properties of the nanoparticle were investigated using cascade impactor 20mg of each sample was loaded into a hard gelatin capsule manually. The experiment is carried out at an air flow rate of 60 l/min. A coating of 1% (w/v) solution of silicon oil in hexane is used on the impaction plates to prevent particle bounce and re-entrainment. A capsule filled with particles is loaded into a Rotahaler® (Glaxo) which is used as the inhaler to aerosolize the particles. An actuation time of 20 s is allowed for each capsule to completely disperse all the

particles. Particles remaining in the capsule, inhaler, throat, pre-separator, individual impaction plates, and stages are extracted using methanol. Amount of drug deposited on capsule, inhaler, throat, pre-separator, individual impaction plates, and stages were assayed using a UV spectrophotometrically at 237.5nm calculate % respirable fraction (RF) by using equation (4). The respirable fraction (RF) was the ratio of RD to total loaded dose [19, 20].

% RF=Amount of drug deposited in lower stage /Total loaded dose ×100---- (Eq.4)

RESULTS AND DISCUSSION

Spray-dried powder characteristics

A total of 13 spray-dried powders along with control were investigated. Physical characteristic was depicted in below table 1.The yield of the control powder was in range 36-51%The addition of the amino acid dispersibility enhancers caused considerable variation in the yield of powder produced (Table 1). The addition of phenylalanine to the spray-drying solution appeared to cause the greatest deviation in spray-drying yield from that of the control powder with a maximum yield of 81% at 15% amino acid concentration. amino acidmodified spray-dried powders reporting the greatest spray-dried yield from phenylalanine containing powders [18]. Only those formulations modified with leucine showed an increase in powder yield at all the amino acid concentrations tested, i.e. 5%, 10%, 15% It has previously been suggested that low spray-drying yields are indicative of cohesive powders that demonstrate poor aerosolisation properties, and that the addition of formulation excipients may change the physicochemical characteristics of the resultant spray-dried powders, resulting in an improved spray-drying yield and potentially enhanced aerosolisation properties [21]. Alternatively, low spray-drying yields may be a reflection of highly adhesive particles resulting in adhesion of the powder to the walls of the spray-drier.

Table 1: Characteristic of spray dried powders

Batch No.	Amino Acid	Conc.	% Yield	Particle Size (µm: Mean±Sd, N=3)	PDI	Tapped Density (G Cm−3: Mean±Sd, N=3)	Carr's Index (%)	Flowability
1	BLANK	---	39	4.81±0.51	0.299	0.36±0.02	25.6	Poor, Cohesive
2	ARG	5	36	2.42±0.17	0.172	0.39±0.03	14.7	Good
3		10	39	3.08±0.11	0.189	0.40±0.02	24.1	Poor
4		15	45	1.95±0.21	0.190	0.28±0.10	22.1	Poor
5	PHE	5	21	3.28±0.14	0.182	0.33±0.01	24.4	Poor

6		10	42	6.91±0.31	0.178	0.41±0.04	25.4	Poor, Cohesive
7		15	69	2.08±0.44	0.181	0.34±0.11	26.1	Poor, Cohesive
8	LEU	5	49	4.18±0.32	0.171	0.31±0.08	21.1	Poor
9		10	44	1.18±0.18	0.173	0.37±0.13	15.2	Good
10		15	52	1.08 ±0.21	0.168	0.35±0.01	13.1	Good
11	THR	5	29	2.36±0.11	0.211	0.29±0.07	18.2	Fair
12		10	33	1.77±0.34	0.174	0.32±0.15	16.2	Good
13		15	39	1.81 ±0.21	0.169	0.39±0.05	29.3	Poor, Cohesive

Carr's Index flowability: 5–12%, excellent; 12–18%, good; 18–21%, fair; 21–25%, poor, fluid; 25–32%, poor, cohesive; 32–38%, very poor; N40%, extremely

Particle size was getting in range 1.18 to 6.91 µm. Particle size getting less in formulation containing leucine as compare to another amino acid that are suitable for pulmonary drug delivery system. PDI Was found in range 0.168 to 0.299 indicates good uniformity in the formulations. Tapped density was found in range 0.29 to 0.41 along with leucine containing formulation have good flow property as compare to another. These all formulations pass all characteristic as compare to the formulations having without any aminoacid.

Only those formulations modified with leucine showed an increase in powder yield at all the amino acid concentrations tested, i.e. 5%, 10% and 15%. It has previously been suggested that low spray-drying yields are indicative of cohesive powders that demonstrate poor aerosolisation properties, and that the addition of formulation excipients may change the physicochemical characteristics of the resultant spray-dried powders, resulting in an improved spray-drying yield and potentially enhanced aerosolisation properties [21]. Alternatively, low spray-drying yields may be a reflection of highly adhesive particles resulting in adhesion of the powder to the walls of the spray-drier.

Analysis of the salbutamol content of the spray-dried powders indicated that drug loading varied from 47% to 96% of nominal load. The 5% THR and 5% PHE spray dried powders contained significantly lower drug content than the control powder.

SCANNING ELECTRON MCROSCOPY

The morphology of the optimized batch was examined by SEM. The SEM photographs of the FP loaded nanoparticles are shown in figure 1.It can be seen that spray-dried nanoparticles are spherical shape and smooth surface.

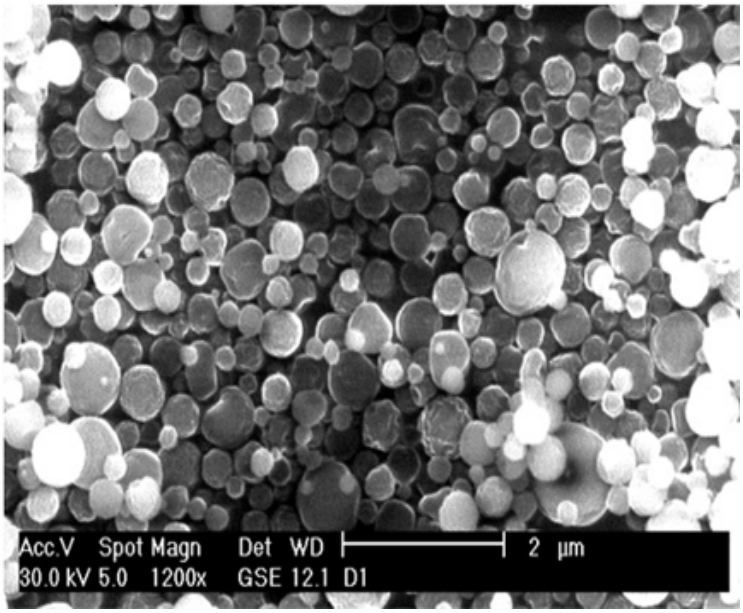

Figure 1: SEM images of optimized formulation

AERODYNAMIC BEHAVIOR

Table 2: Aerodynamic behaviour of nanoparticles formulations

Batch no.	Total amount remaining	% Respirable fraction
1	85.6	30.5
2	89.63	41.66
3	85.96	38.33
4	85.26	40.66
5	87.23	45
6	90.76	39.63
7	83.46	36.83
8	90.33	40
9	80.66	42.66
10	90.1	45
11	82.83	39.3
12	88.86	41.33
13	81.1	36.84

The deposition of total amount of remaining in capsule, device, throat, preseperator and stages 0-7 nanoparticles was found to be 82.83-90.76%. Percentage deposition at stages 6-7 of the cascade impactor indicates Percentage respirable fraction was used to evaluate the possibility of delivering the particles to deep parts of the lung such as alveoli [22]. Percentage respirable

fraction was vary from 36.83-42.66%. highest respirable fraction of 45%.Batch containing leucine having highest respirable fraction as compare to formulations containing another amino acid its indicates the high aerosol performance of nanoparticles. These kinds of nanoparticles possess high aerosolization efficiency, which improve the flowability of the particles of the inhaler and promotes the disaggregation into fine particles. Furthermore, by virtue of the low density, an effective lung delivery was achived, by minimizing oropharyngeal deposition of the particles [21]. Mannitol aid in disaggregation, as the formation of excipient layer around the nanoparticles and protect drug against thermal stress and denaturation during sprat drying. Implies stability of nanoparticles. Aerosol powders ranging from 1-5µm are considered the optimum size for deposition beyond the increasingly narrow airways into the alveoli.

CONCLUSIONS

Although spray-dried powders can be prepared to an appropriate particle diameter range for the pulmonary delivery of therapeutically active agents, the particles are prone to aggregation due to strong cohesive forces resulting in agglomerates with large aerodynamic diameters and reduced dispersibility during inhalation. Amino acids containing leucine, in spray-dried formulations can significantly reduce the interactions between the resulting particles, leading to enhanced dispersibility and functional in vitro pulmonary deposition. In this study, using Fluticasone propionate as the active drug, a significant enhancement in FPF was only observed in leucine-modified powders. Given that standard spray-drying operating conditions and validated assay procedures were employed in this study. It has been suggested that leucine displays surfactant like properties, and has the capacity to migrate to the droplet surface during the rapid drying phase in spray-drying, and hence influence the surface characteristics of the resultant particle. It has also been suggested that the surface activity of a solute is linked to the solute's hydrophobicity, and that leucine is a relatively hydrophobic amino acid. In addition to surface activity, the pitted surface of the leucine modified powders, facilitating dispersion of individual particles during aerosolisation and making leucine an effective co-excipient in spray-dried powders for pulmonary drug delivery. Finally it can be conclude that leucine used for improve aerosolization behaviour along with mannitol as a deaggregating agents.

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