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DEVELOPMENT OF CAPSULE BASED DRY POWDER FOR INHALATION CONTAINING GLYCOPYRRONIUM BROMIDE

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ABSTRACT

A capsule based unit dose dry powder for inhalation of long acting muscarinic antagonist, Glycopyrronium bromide with the device Turbospin® #3 dry powder inhaler was prepared to obtain increased drug deposition in the alveolar absorptive region with a view to treat bronchospasm associated with chronic obstructive pulmonary diseases. The influence of different inhalation grade α-lactose monohydrate as carrier on the drug-to-carrier interaction and the performance of lactose-based dry powder inhaler formulations were evaluated. The Glycopyrronium bromide (< 5 μm) was incorporated with lactose of different grades and their combinations as carriers to deliver dose using an inhaler device. Solid-state characteristics of the carrier as well as the drug powder were assessed by particle size and distribution measurement. The aerosol behavior of the powder was studied by dispersion using Turbospin® connected to a twin-stage liquid impinger (TSLI) operating at flow rates of 60 Lit/min. Significant differences in aerosol performance of Glycopyrronium bromide measured as fine particle fraction (FPF) from the carriers was observed with different grade inhalation lactose. Specifically, as carrier size increased, FPF decreased. Different weight ratios and different size ranges for the fines with coarse lactose showed significant change for in vitro deposition of the drug from formulation. Primary packaging material like hard gelatin capsule and HPMC capsule influenced the DPI performance during the storage period. Formulation containing 70:30 mixture of respitose SV003 and sorbolac 400 as carrier imparts well disaggregation of drug and higher fine particle fraction was achieved at 60 Lit/min. Thus the object of the present invention is to provide DPI comprise LAMAs, allows highly efficient and sufficient amount of dosing when inhaled by the patients even in a low pressure.

KEYWORDS: Long acting muscarinic antagonist, dry powder inhaler, fine particle fraction, twin-stage liquid impinger, drug-to-carrier interaction.

INTRODUCTION

The respiratory tract is established as an attractive route for drug delivery. The potential advantages of delivering a drug to the lung by inhalation have been well known to scientists, physicians and drug abusers for many years. For drugs that exert their biological effect in the lung, these include rapid onset of action, reduced dose and minimized side effects compared to the same drug delivered by mouth.[1]

Development of pharmaceuticals for inhalation is a particular challenge, as it involves the preparation of a formulation and the selection of a device for aerosol dispersion. The lungs have lower buffering capacity than other delivery sites (eg, the gastrointestinal tract or the blood), which limits the range of excipients that could enhance delivery outcomes. An additional variable, unique to pulmonary delivery, is the patient, both in terms of inhalation mode and respiratory-tract anatomy and physiology.[2] There are many more ways to administer an inhaled aerosol than there are to swallow a tablet. Variability in delivered dose to an individual or a population of patients can be substantial.[3,4] Consequently, reproducible therapeutic effect is difficult to assure.

Treating respiratory diseases with inhalers requires delivering sufficient drug to the lungs to bring about a therapeutic response. For optimal efficacy, drug administration must be reliable, reproducible, and convenient. This goal can be achieved by a combination of formulation, metering, and inhaler design strategies.[5] The performance of the DPI system depends not only on the powder formulation but also the inhaler device. However, devices are much less explored than the powder formulations.[6] There is a wide range of passive (breathe driven) and active (power driven) single or multiple dose DPI devices in the market. The market is currently driven by passive devices which rely on the

inspiratory airflow of the patient for powder dispersion into individual particles.

Since DPIs are typically formulated as one-phase, solid particle blends, they are also preferred from a stability and processing standpoint.[7] Dry powders are at a lower energy state, which reduces the rate of chemical degradation and the likelihood of reaction with contact surfaces. By contrast, pMDI formulations, which include propellant and co solvents, may extract organic compounds from the device components.[8] There is a wide range of passive (breathe driven) and active (power driven) single or multiple dose DPI devices in the market. The market is currently driven by passive devices which rely on the inspiratory airflow of the patient for powder dispersion into individual particles. Even with active research on development of newer DPI devices, the concept of powder interaction with device is not well understood. The relative effect of air turbulence and mechanical impaction (particle-particle and particle-device) for controlling powder dispersion in the device as well as role of capsule and influence of airflow is still unclear.[9]

Two classes of long-acting bronchodilators are now available: long-acting beta$_2$ agonists (LABAs) and long-acting muscarinic antagonists (LAMAs). At present, Glycopyrronium bromide have been approved for the treatment of COPD in different countries.[10] Glycopyrronium bromide is an inhaled long-acting muscarinic receptor antagonist (anticholinergic) for once daily maintenance bronchodilator treatment of COPD. This was approved by the European Medicines Agency in late 2013 for the maintenance treatment of patients with moderate to severe COPD.[11] It significantly prolonged time to first moderate or severe COPD exacerbation and reduced the rate of moderate or severe COPD exacerbations. Schroeckenstein et al discloses the use of glycopyrrolate in an aerosol formulation for treating asthma where a single administration of a metered dose achieved bronchodilation for up to 12 hours.[12]

The delivery of the active substances, such as long acting muscarinic antagonists (LAMAs), which may show high efficiency even at low doses to the lungs in efficient and sufficient amounts so as to obtain the desired effects, is of great importance. It is considerably difficult to deliver sufficient amounts of these active substances including LAMA group to the lungs as they are very small in amount per dose required for the treatment. Therefore, said active substances have to be diluted with pharmaceutically acceptable carriers.[13] pharmaceutically acceptable carriers are used as a flow aid and facilitate the dose of the active substances into the lungs. Therefore the properties of the particles of the carrier play an important role in the formulation of dry powder inhaler (DPI). Thus, carriers should be carefully selected, designed and controlled for the use in a dry powder inhalation formulation.[14]

MATERIALS AND METHODS

Materials

Glycopyrronium bromide was kindly supplied by Khandelwal lab. Pvt. Ltd, India. Inhalac 70 and Sorbolac 400 were gifted by Meggle excipients and technology, Germany. Pharmatose DCL 11 was provided by DMV International, Netherlands. Respitose SV 003, Lactohale 201, Pharmatose 450M were generously given by DFE pharma, Europe. All other chemicals were of analytical grade.

Drug excipient compatibility study

The compatibility of drug and the formulation component is important prerequisite before formulation. Drug and excipients were taken in the ratio of 1:1000. The mixture blend was then filled in size 3 hard gelatin capsules as well as HPMC capsules. Finally the filled capsules were packed in HDPE bottle and exposed to different storage conditions. Blend of Glycopyrronium bromide and different grade of α– lactose monohydrate were subjected to the physical observation at initial 1,2,3 and 4 weeks and for DSC study.

Method of Preparation of Dry Powder

Glycopyrronium bromide 50 microgram formulation were prepared with various grade of coarse lactose and fine lactose with 25 mg fill weight as per composition given in table -1.

An accurately weighed amount of Glycopyrronium bromide was mixed separately in each case with coarse and fine lactose stated in table in geometric progress and passed through 60# mesh and blended in double polythene bag and filled in to size "3" hard gelatin capsules with partial filling manual capsule filling machine with fill weight of 25 mg per capsules. Quantity of API per capsule was calculated from below formula,

$$API : Capsule = \frac{dose(\mu g) \times 100 \times 100}{\%assay\ of\ API\ on\ dried\ bases \times (100 - LOD\ of\ API) \times 1000}$$

Glycopyrronium bromide 50 microgram formulations were prepared with 10% different grade of fine lactose (Lactohale 201, pharmatose 450M, sorbolac 400) and 90% respitose SV003 as a coarse lactose with 25 mg fill weight as per composition given in table -1 to select the fine lactose grade by evaluating the DPI performance. Furthermore, Glycopyrronium bromide 50 microgram formulation were prepared with 20%, 30%, 40% fine lactose (sorbolac 400) with 25 mg fill weight and respitose SV003 as coarse lactose to optimize the ratio of coarse lactose: fine lactose by evaluating the DPI performance.

Evaluation of Dry Powder Inhaler

Physical appearance

The capsules were visually observed.

Averages fill weight per capsule
Open the 20 capsule without losing any part of the shell and remove the contents as completely as possible. Weighed the 20 capsules content and determine the average of fill.

Moisture content
Transfer 35 to 40 ml of a mixture of methanol to the titration vessel and titrate with Karl Fischer reagent to detect any moisture that may be present. Quickly add about 200 mg of powder, mix and again titrate with the Karl Fischer reagent. Calculate the water content of the specimen, in mg, taken by the formula:
SF x 100/W

Where W = Weight of the Sample, in mg. S = Volume of the KF reagent, in ml. F = the water equivalence factor of KF reagent, in mg.

Assay
Transferred 10 capsules into a 100 ml volumetric flask and added 10ml of water sonicate to dissolve the capsule and added the suitable volume of diluent. Sonicate to dissolve for about 10 minutes with intermittent shaking (for complete dispersion) made the volume with diluent. Filtered through a 0.45µ membrane and estimated the drug content with suitable analytical method.

Content uniformity
This test was performed on 10 capsules. Each capsule was transferred separately into a volumetric flask both the shell and the contents of the capsule were treated with 5 mL of water followed by a solvent mixture of 25 mL of methanol: water (70:30) mixture, sonicated for 10 minutes with intermittent shaking (for complete dispersion) and the volume was made up to 50 ml with diluent. It was then filtered through a 0.45 µ membrane filter and the samples were analyzed by using HPLC technique for drug content. The same procedure was repeated for the remaining 19 capsules.

Uniformity of the delivered dose and emitted dose
Uniformity of the delivered and emitted dose was determined according to the official procedure specified in Indian Pharmacopoeia (2007).[15] The samples were analyzed with HPLC technique. These tests were performed repeatedly with 10 capsules in each case.

Fine particle distribution
Fine particle distribution (FPD) was determined for aerodynamic assessment of fine particles by twin stage liquid impinger. Place the device in position at the end of the throat so that the mouthpiece end of the actuator, when inserted to a depth of about 10 mm, lines up along the horizontal axis of the throat and the open end of the device, which accepts the capsules, is upper most and in the same vertical place as the rest of the apparatus. Prepare the capsules for use and locate the mouthpiece-of the apparatus in the apparatus by means of a suitable adapter. Switch on the pump for 3 seconds. Switch off the pump and remove the device. Perform the test using further 9 capsules.

Stability studies
The optimized Glycopyrronium bromide DPI formulations were stored at 40°C/75% RH and 25°C/60% RH for six months as per IGH guidelines. Later, the formulations were evaluated for assay of number of doses delivered, deposition of emitted dose and net fill weight. The observed data were compared with the initial results.

RESULTS AND DISCUSSION

Physical properties such as bulk density, tapped density and particle size of drug and various lactose grades were evaluated and showed in table.-2. Results of bulk Characterization of API showed poor flow property of API. Based on flow properties, lactose grades can be as sorbolac 400 < pharmatose450M < Lactohale 201 < respitose SV003 < DCL 11 < Inhalac 70. Fine lactose (Sorbolac 400, Pharmatose450M, Lactohale 20) alone are not suitable as carrier for dry powder inhaler, hence they are blended with coarse lactose to improve the flow properties and performance of dry powder inhaler.

Water content for lactose monohydrate
Water content plays an important role in formulation and performance of dry powder for inhalation. Water content should be within the limit for better performance of formulation. Water content of different grade of **α**-lactose monohydrate used in present investigation was carried out using Karl Fischer apparatus. Results obtained for different grade of lactose monohydrate are shown within 4.6% to 5.4%. Results for water content of different grade of lactose were within specific limit.

Compatibility study
(A) Physical compatibility study
Results for hard gelatin capsules containing Glycopyrronium bromide with different lactose grade blends shows that lump formation was observed at 60°C open storage condition while there was lump formation as well as sticking in case of 40°C/75% RH open storage condition. There was no change observed in capsule content at 40°C/75% RH closed storage condition. Results for HPMC capsule containing different lactose grade shows that lump formation was observed at 60°C open, 40°C/75% RH open storage condition. There was no change observed in capsule content at 40°C / 75% RH closed storage condition.

No significant change was noted in all cases of formulation containing Glycopyrronium bromide with different grade of α-lactose monohydrate filled in hard gelatin as well as HPMC capsules at 40°C/75% RH closed indicating compatibility of Glycopyrronium bromide with α-lactose monohydrate whether packed in hard gelatin capsule or HPMC capsule.

(B) DSC study

As shown in figure-1 to 4, DSC thermo gram of API, alpha lactose monohydrate and blend filled in hard gelatin capsule and HPMC capsule were obtained and compared. Figure -1 shows peak at 195.39°C with onset 194.56°C and recovery at 196.41°C for Glycopyrronium bromide. While figure -2 shows peak at 219.62°C with onset 209.91°C and recovery at 228.48°C for alpha lactose monohydrate.

Thermogram for Glycopyrronium bromide and alpha lactose monohydrate with hard gelatin capsule (figure -3) shows peak for Glycopyrronium bromide onset 194.24°C, peak 195.73°C, and recovery 196.81°C. Thermogram of Glycopyrronium bromide alone shows sharp peak at 195.39°C with onset 194.56°C and recovery at 196.41°C, which indicate that there was no change in peak maxima and hence no interaction between drug and alpha lactose monohydrate in Hard gelatin capsule. Thermogram for Glycopyrronium bromide and alpha lactose monohydrate with HPMC capsule (figure -4) shows peak for Glycopyrronium bromide onset 194.45°C, peak 195.61°C, and recovery 196.78°C. No significant change in peak onset, peak maxima and recovery were noted in all these cases indicating compatibility of Glycopyrronium bromide with alpha lactose monohydrate.

Blend uniformity and assay

Results for in process evaluation of blend with different grade coarse lactose are shown in table -3. Different formulation batches F1, F2, F3 were evaluated for blend uniformity and assay, which comply with given standard specifications. Average weight of filled capsule was within the limit that is ± 3% of targeted fill weight. Formulations with different grade coarse lactose were subjected to the desired in process and finished product tests. Uniformity of delivered dose was tested by using dosage unit sampling apparatus at 60 L/m flow. All the formulation compiled content uniformity, uniformity of delivered dose, assay and water content.

However significant differences in fine particle fraction were observed with respect to lactose grades (figure-5). Respitose SV003 showed better fine particle fraction (31%) than other coarse lactose grade (Inhalac 70, DCL11-22%, 26% respectively). Average weight of filled capsule was within the limit that is ± 3% of targeted fill. All the formulations complied content uniformity, uniformity of delivered dose, assay and water content. However significant differences in fine particle fraction were observed with respect to lactose grades (figure-6). Sorbolac 400 showed better fine particle fraction (45%) than other coarse lactose grade (36%, 41% for Lactohale 201, Pharmatose 450M respectively).

Studies were carried out to evaluate the effect of fine lactose sorbolac 400 percentage (10%-40%) and coarse lactose respitose sv003 on performance of DPIs containing Glycopyrronium bromide. Results for content uniformity, UODD, Assay, water content are shown in table 5.16. All the formulations showed good content uniformity and UODD as per specification given in pharmacopoeia. Fine particle fraction was determined with TSLI apparatus and samples were analyzed by HPLC.

A significant difference was noticed in the emitted dose and fine particle deposition with respect to composition of the formulation. The emitted dose was found to be decreased with increasing concentration of fine lactose and the fine particle deposition was increased with the incorporation of fine lactose. Thus the ratio of fine and coarse lactose influences the performance of dry powder inhalers. Ratio 30:70 for fine lactose: coarse lactose showed better fine particle fraction (54%) as compare to 20:80 for fine: coarse lactose. in case of 40:60 ratio (sorbolac400: respitose sv003) did not show any significant improvement in fine particle fraction and have the chances of more actuator retention of drug if we use more percentage of fine lactose carriers.

The results revealed that formulation F8 containing 30% fine grade lactose (Sorbolac 400) and 70% coarse grade lactose (Respitose SV003) can be considered as optimum formula as it showed better DPI performance in relation to delivering higher fine particle fraction (FPF) compared to other formula.

Microbial limit

All the formulation F1 to F9 prepared in present investigation complied with the microbial limit test specification as per IP. Total bacterial & fungal count and yeast and mould count were found within the limit less than 10cfu/gm. Content of capsule for all the formulation batches confirmed the absent of Escherichia coli, salmonellae SP, Staphylococcus aureus, Pseudomonas aeruginosa. Bacterial endotoxins were also found less 5EU/g for all the formulations.

Stability study of optimized formula

It can be seen from the stability study table-4, that all of the formulations dropped in T2 (fine particle fraction) performance during the stability period when stored 40°C/75% RH. However, at 40°C/75% RH, formulations with HPMC capsules had relatively small drop in T2 compared to the formulations with hard gelatin capsules, which dropped more sharply. The data showed that formulation manufactured with hard gelatin capsule exhibited approximately 25% reduction in T2 than the respective formulation with HPMC capsule which show reduction in T2 approximately 14%.

Table 1: Preparation of dry powder for inhalation with different grade of coarse and fine lactose.									
Batch	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Inhalac 70									
Pharmatose 450M	24.94								
Respitose SV003		24.94					5	7.5	10
Respitose Sv003-fine			24.94	22.44					
Lactohale 201--fine				2.5					
Pharmatose 450M--fine					2.5				
Sorbolac 400--fine						2.5	19.94	17.44	14.94

* API-0.05045mg/cap.

Table 2: Results for Physicochemical properties of API and excipients.					
Material	**Bulk density (g/ml)**	**Tapped density (g/ml**	**Carr's index (%)**	**Hausner's ratio**	**Angle of repose (°)**
API	0.32	0.48	33.33	1.50	59.45
Inhalac 70	0.68	0.87	21.83	1.28	42.12
Pharmatose DCL11	0.63	0.85	25.88	1.34	44.69
Respitose sv003	0.56	0.81	30.86	1.44	54.23
Lactohale 201	0.44	0.75	41.33	1.70	68.31
Pharmatose 450M	0.41	0.73	43.83	1.78	71.37
Sorbolac 400	0.38	0.71	46.47	1.86	74.13

Table 3: Effect of different grade of coarse and fine lactose on DPI performance.										
Batch No.		**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Content Uniformity (%)	Mean	100.5	103.1	102.6	95.4	103.6	102.1	101.8	100.6	100.1
	Max	105	106.4	109.6	99.5	107.6	110.9	104.1	107.6	111.3
	Min	96.2	106.4	95.3	90.2	99.8	93.5	97.8	92.8	88.9
Uniformity of Delivered Dose (%)	Mean	90.3	98	100	100.8	101	100	100	99.1	97.1
	Max	99.8	101	102	117.6	125.3	108.1	107.3	102.1	99.7
	Min	78.2	94.3	97.4	93.4	79.6	95.4	93.2	96.8	95.2
Fine Particle distribution (%)	AR	5	5	6	6	6	5	5	7	8
	T1	72	70	61	55	54	49	46	37	32
	T2	22	26	31	36	41	45	48	54	55
	Mass balance	99	101	98	97	101	99	99	98	95
Assay (%)		102	102.1	99.8	101.4	100.4	99.6	101.5	99.7	99.5
Water content (%)		5.22	5.35	5.47	5.29	4.78	5.47	4.49	4.59	5.13

Table 4: Stability study of optimized formula with different primary packaging material at 40 ±2°C/75 ± 5%RH.									
Parameter		**Hard gelatin capsule**				**HPMC capsule**			
		Initial	**1M**	**2M**	**3M**	**Initial**	**1M**	**2M**	**3M**
Content Uniformity (%)	Mean	102.6	99.7	100.5	100.2	98.9	103.2	99.2	99.9
	Max	109.6	108.4	105.1	107.6	109.3	106.9	105.8	104.7
	Min	89.2	91	96.2	90.12	94.3	92.3	91.7	91.1
Uniformity of Delivered Dose (%)	Mean	92.6	88.4	85.5	82.9	99.8	97.8	96.2	94.9
	Max	95.4	98.2	95.6	94.1	103	102.7	101.9	102.1
	Min	86.9	76.8	78.6	76.7	96.6	95.6	92.0	91.7
Fine Particle distribution (%)	AR	5	5	6	7	5	5	5	6
	T1	39	48	51	55	39	42	44	48
	T2	53	42	36	28	55	51	48	41
	Mass balance	97	95	93	90	99	98	97	95
Assay (%)		101.1	101.5	99.9	101.2	100.5	99.8	101.9	101.7
Water content (%)		4.49	5.13	5.58	6.01	4.41	4.48	4.51	4.94

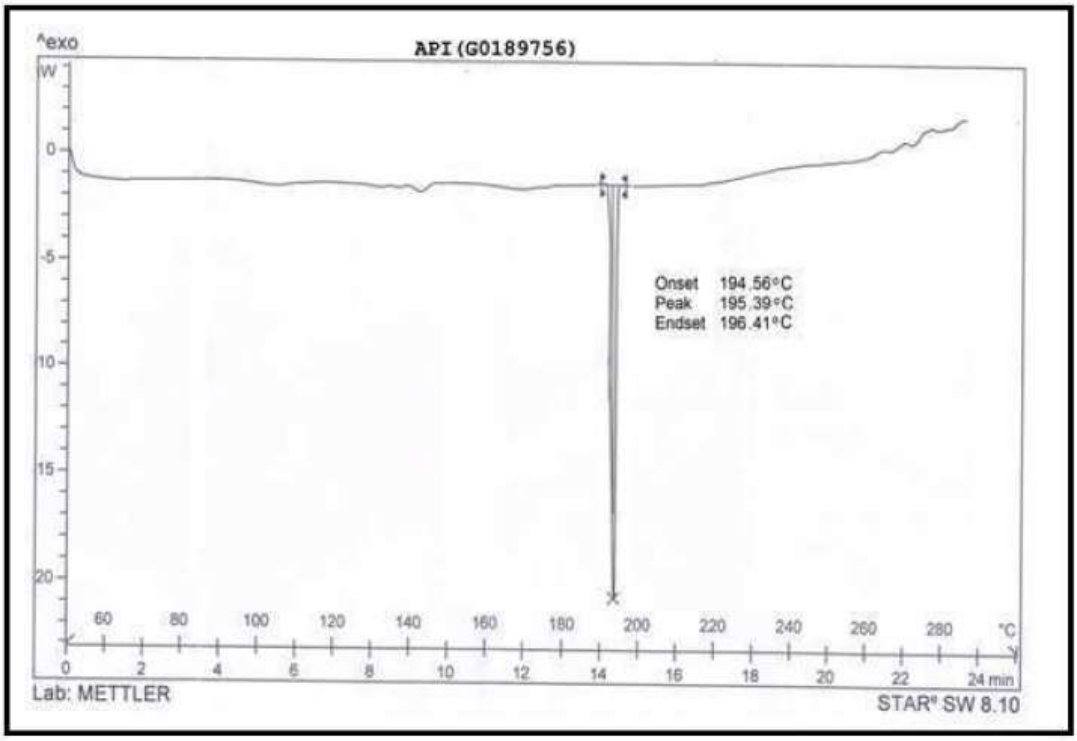

Figure 1: DSC curve of API.

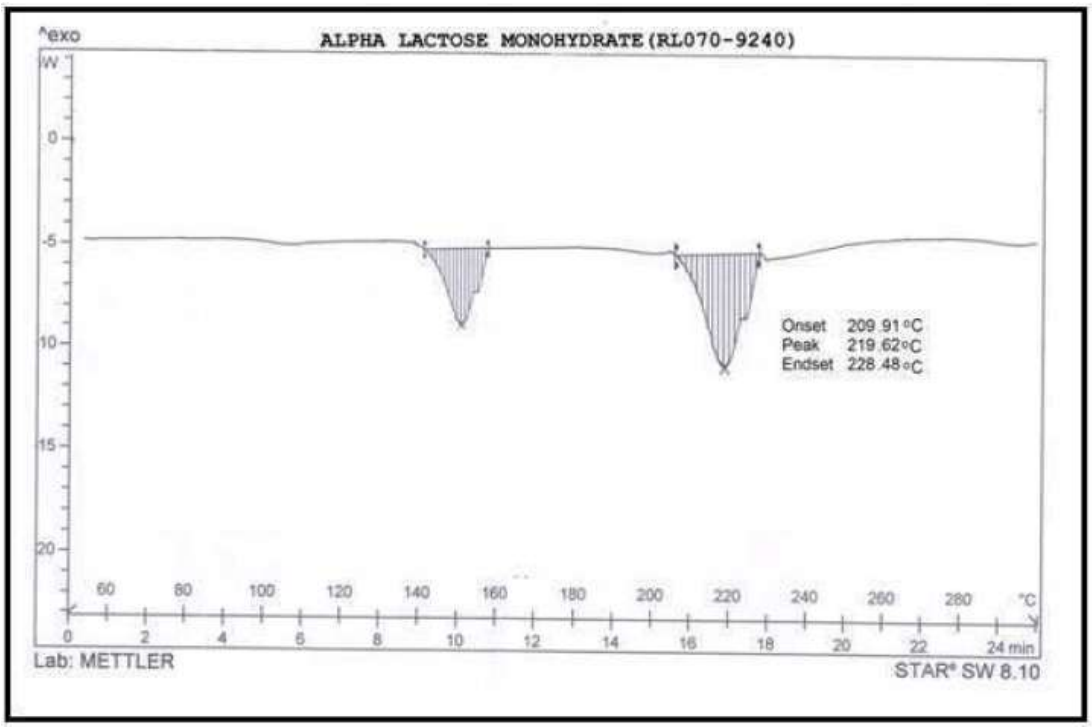

Figure 2: DSC curve of alpha lactose monohydrate.

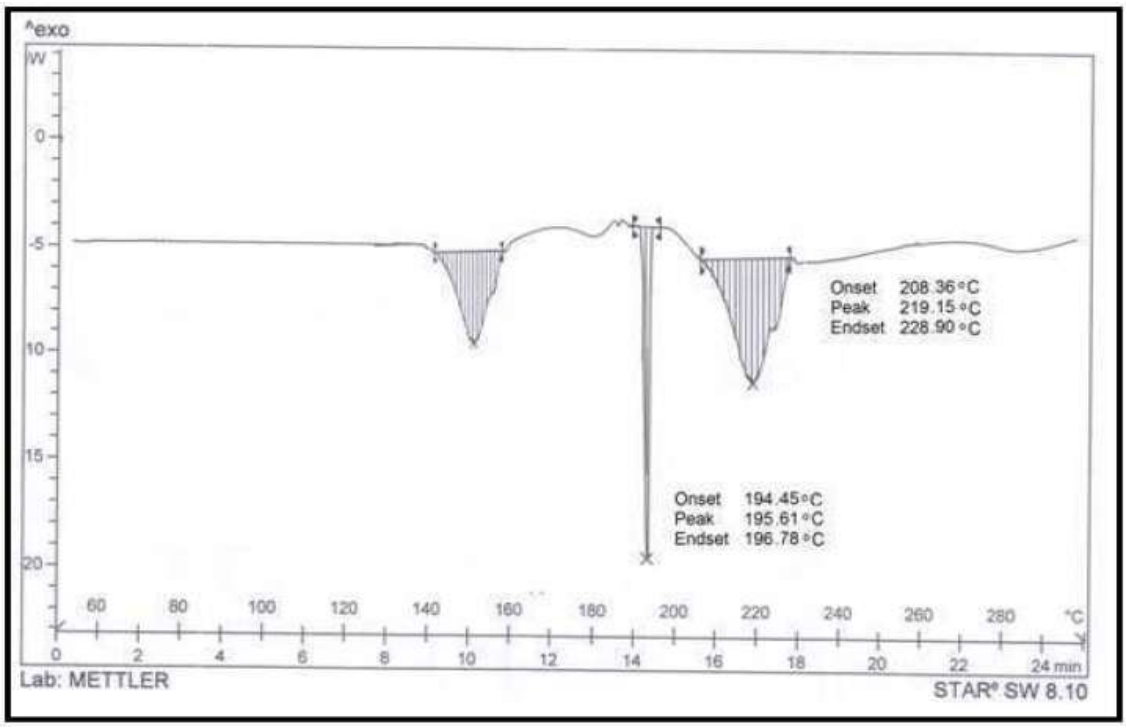

Figure 3: DSC curve for API + Lactose filled in hard gelatin capsule.

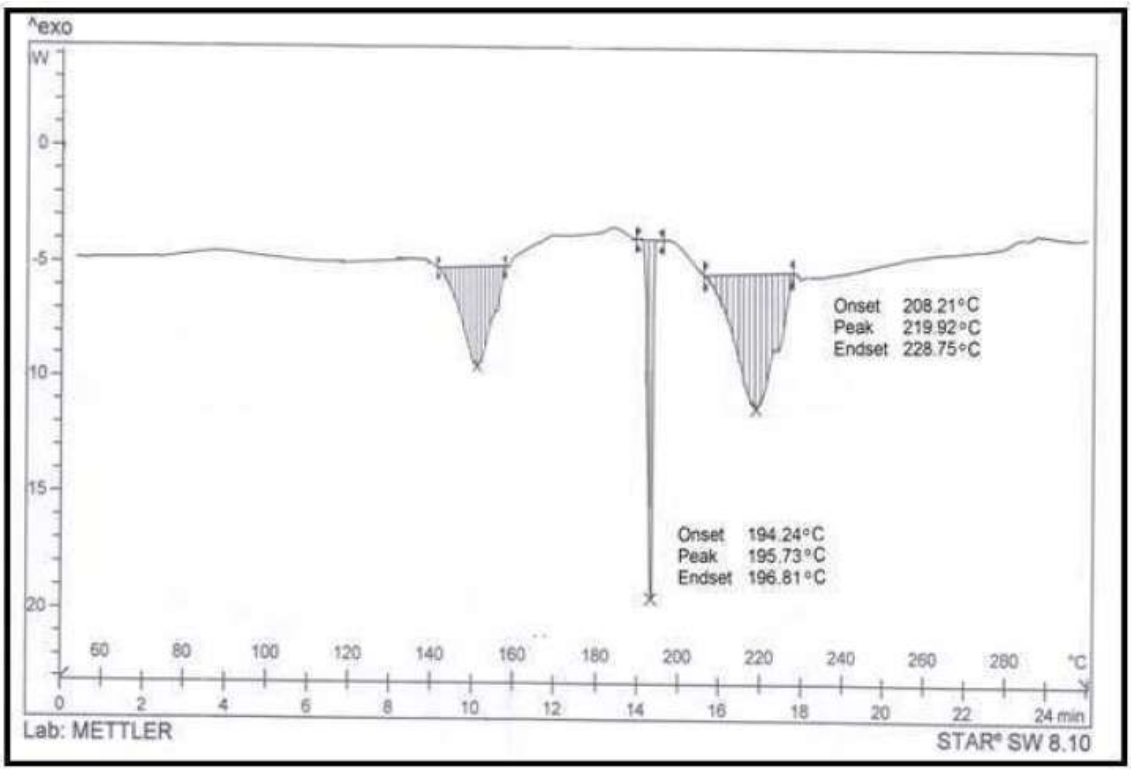

Figure 4: DSC curve for API + Lactose filled in HPMC capsule.

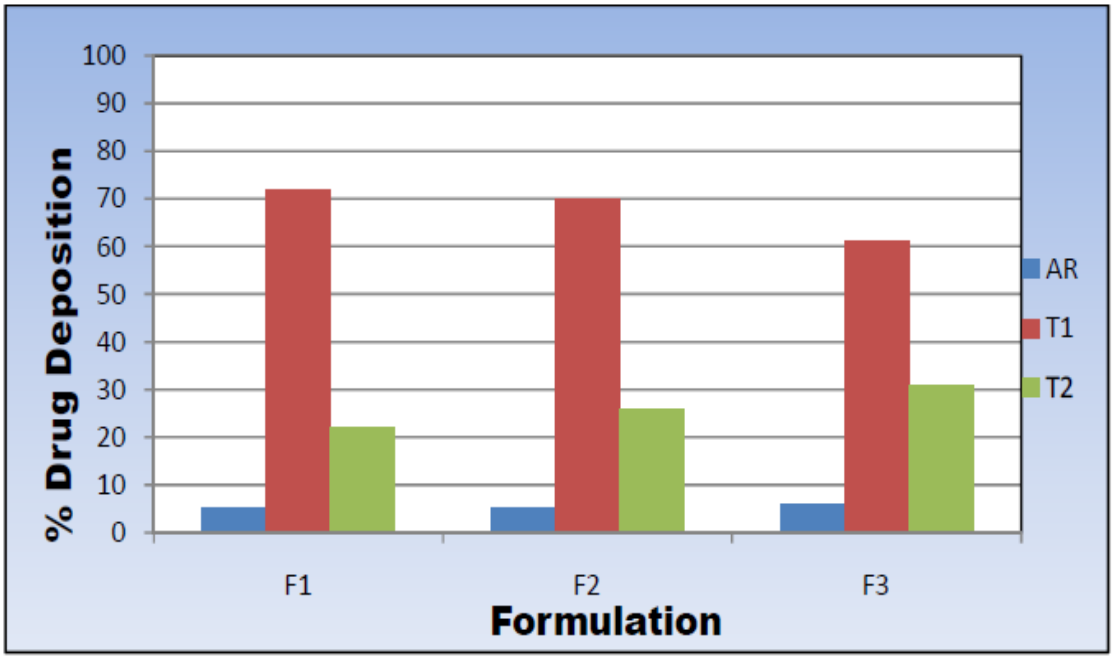

Figure 5: Histogram showing the % drug deposition in TSLI of formulation containing different grade of coarse lactose.

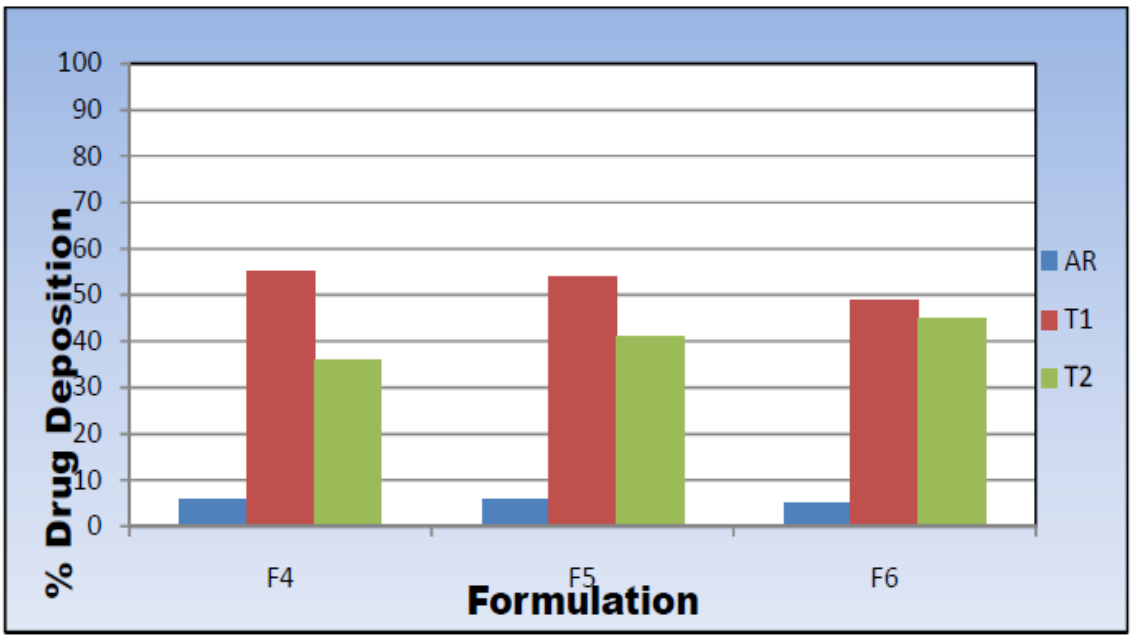

Figure 6: Histogram showing the % drug deposition in TSLI of formulation containing different grade of fine lactose.

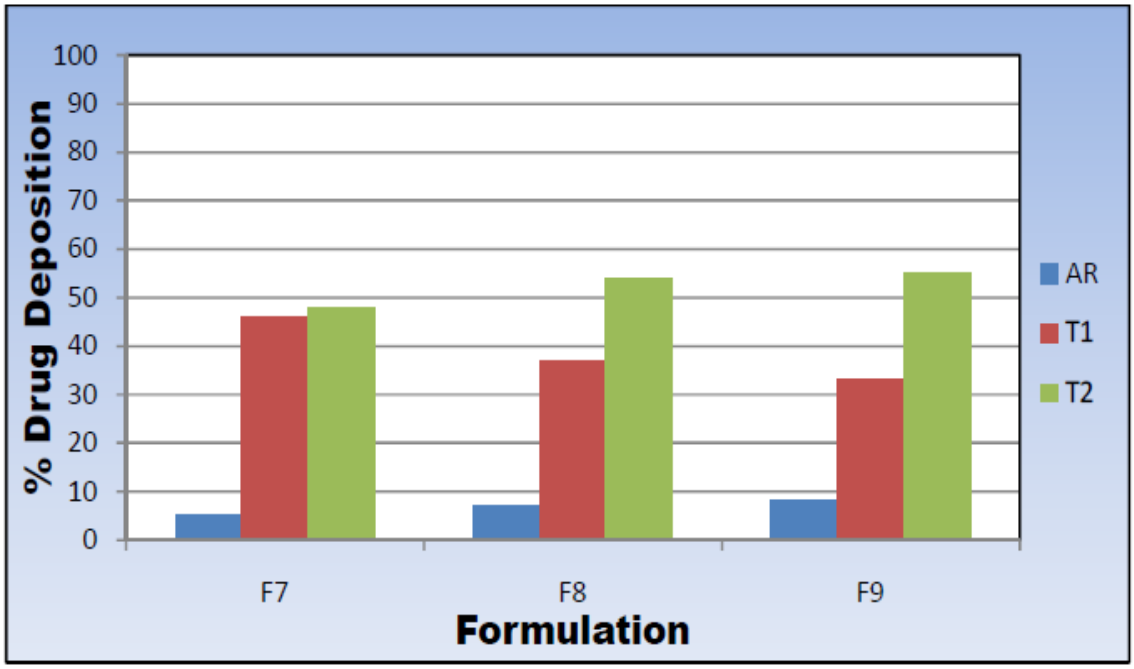

Figure 7: Histogram showing the % drug deposition in TSLI of formulation containing different percentage of fine lactose.

CONCLUSION

In carrier based mixtures for inhalation, a proper balance has to be obtained between the stability of the blend during storage and handling, and dispersibility during inhalation. The present study showed the effect of lactose particle size on inhalation behavior of Glycopyrronium bromide from dry powder inhaler. The performance of dry powder inhaler was found to be dependent on proportion of fine and coarse lactose grade employed in the preparation of dry powder inhaler. Formulation containing 70:30 mixtures of Respitose SV003 and Sorbolac 400 as carrier with HPMC capsule as primary packaging material imparts well deaggregation of Glycopyrronium bromide and higher fine particle fraction throughout stability period.

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