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Development and comparison of orally inhalable sustained release formulations for three respiratory drugs for asthma

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ABSTRACT

The present work was designed to develop and compare orally inhalable sustained release formulation for salbutamol sulphate (SS), ambroxol hydrochloride (AH) and montelukast sodium (MS). The emulsion solvent evaporation method was used to prepare microparticles with the polymers. The prepared polymer encapsulated microparticles were blended with carrier inhalable lactose and filled in size 3 hard empty gelatin capsule. Formulations T1-T9 were prepared with 1:1 ratio of PLGA (50:50), PLGA (75:25) and Eudragit RS100. The formulation T1 prepared with SS:PLGA (50:50) produces best result when compared with other formulations T2-T9. Formulation T1 gives in vitro release 91.23% at 12 h and having particle size of microparticles ($D_{0.5}$ µm) 1.94±0.6 and respiratory fraction 34.9 ± 2.59 %.

Key Words: Salbutamol, Ambroxol, Montelukast, Sustained release, Dry powder inhaler, Microparticles

Academic Discipline And Sub-Disciplines

Technical, Chemical sciences

SUBJECT CLASSIFICATION

Inhaler formulation and characters

1. INTRODUCTION

Today drugs were delivered through dry powder inhalers to treat respiratory diseases. The purpose of dry powder inhaler is to accumulate prescribed dose of powder aerosol into the air inhaled by patient in single large breath. During inhalation the powder particles were picked up and then broken up. Depending upon the size, particle will get deposited in different regions of respiratory tract during inhalation and releases drug to produce drug action. SS, AH, MS were very suitable for sustained release formulations [1-3]. Many research work were carried out with these drugs but orally inhalable sustained release formulation is new concept [4]. The drugs SS, AH, MS versus polymers PLGA (50:50), PLGA (75:25) and Eudragit RS100 in the ratio 1:1, which were kept constant to prepare microparticles for orally inhalable sustained release dry powder for effective treatment of respiratory diseases [5-7]. The emulsion solvent evaporation method is most influential for the production of microparticles [8-9].

2. EXPERIMENTAL SETUP

SS and AH was obtained as gift sample from Fourrts (India) Laboratories Ltd, Chennai. MS was obtained as gift sample from Orchid healthcare, Chennai. Poly (lactic-co-glycolic) acid (PLGA 75:25), Poly (lactic-co-glycolic) acid (PLGA 50:50) was obtained from Brimingham polymer Inc, USA. Eudragit RL100 and Eudragit RS 100 were obtained as gift from Evonik India Pvt. Ltd., Mumbai. Polyvinyl alcohol, HPMC, PVP K90 were obtained from Orchid Healthcare, Chennai. Lactohale LH100 was obtained as gift sample from DMV, Netherlands, Dichloromethane, methanol and acetonitrile was obtained from Qualigens, Mumbai and potassium dihydrogen orthophosphate, sodium chloride and sodium hydroxide were obtained from SD fine chemicals, Mumbai, India. MTT Reagent (Weigh 5mg of MTT in sterile condition and packed in 4ml amber bottle), Dulbecco's Modified Eagle's Medium (DMEM) media, Dimethyl sulphoxide (DMSO) Co_2 Incubator, ELISA Plate used in Orange Progene Pvt Ltd., Chennai.

2.1 Drug encapsulation:

The microparticles were prepared by emulsion solvent evaporation method with some modifications. This method involves preparation of o/w emulsion between organic phase (OP) consisting of SS, AH and MS and polymers in methanol and aqueous phase (AP) containing 2% w/v PVA [10-15]. Drugs and polymers were dissolve in methanol by sonication, using vibracel sonicator. For drug encapsulation, the organic phase was emulsified in aqueous phase by homogenization at 10000rpm for 10 minutes using a probe homogenizer. The emulsion obtained was stirred overnight (15 h) at 25±2ºC using magnetic stirrer to ensure complete evaporation of methanol. The encapsulated drug particles were recovered by centrifugation (15,000 rpm, 20 min,4^0C) using sorvall ultracentrifuge and the precipitate washed repeatedly three times with ice cold MilliQ water to ensure complete removal of polyvinyl alcohol. As a final point the product was dispersed in cold water and recovered by lyophilisation. Formulations T1-T9 were prepared keeping OP to AP ratio (1:5) and varying drug:polymer ratios given in Table 1. The unencapsulated drug presence were determined by measuring its absorbance at 278nm (SS), 257nm (AH), 351nm (MS).

Table 1. Formulation of microparticles

Trial Code	Drug:Polymer (1:1)	Weight of Drugs (mg)	Weight of polymer (mg)	Vol. of DCM (ml)- OP	AP Vol. (2% PVA) (ml)- AP	% yield
T1	SS:PLGA(50:50)	50	50	5	25	82.25±1.38
T2	SS:PLGA(75:25)	50	50	5	25	80.12±1.01
T3	SS:Eudragit RS100	50	50	5	25	75.21±1.72
T4	AH: PLGA(50:50)	50	50	5	25	77.42±1.82
T5	AH: PLGA(75:25)	50	50	5	25	75.92±1.01
T6	AH: Eudragit RS100	50	50	5	25	74.82±1.62
T7	MS: PLGA(50:50)	50	50	5	25	77.10±1.08
T8	MS: PLGA(75:25)	50	50	5	25	75.04±1.01
T9	MS: Eudragit RS100	50	50	5	25	73.51±1.62

2.2 Preparation of dry powder for oral inhalation:

The prepared microparticles blended with inhalable grade lactose (lactohale) to enhance aerosol characters during inhalation [16-20]. This powder filled in to the size three hard empty gelatin capsules.

2.3 Fourier Transform Infrared Spectroscopy (FTIR):

Infrared spectrum produces fingerprint and characterization of the SS, AH and MS to indentify functional groups of the molecules. The band intensities are proportional to the concentration of the compounds. Infrared spectroscopy was used to determine various functional groups of the drug molecule.

2.4 Scanning Electron Microscopy (SEM):

The diameter, structural and surface morphology of prepared encapsulated particles were obtained through scanning electron microscope. The scanning electron microscopy (VEGA3) with maximum magnification 1 000 000*X and a resolution 3 nm at a voltage of 30 kV were used and investigated surface morphology and shape of the prepared microparticles. The specimens were examined with maximum vacuum and with accelerating voltage of 5-15 KV [21-24].

2.5 Entrapment Efficiency:

Drugs were extracted from the microparticles with 0.1M sodium hydroxide after dissolving it in acetonitrile. After suitable dilutions, the drug content was measured in a UV-Vis spectrophotometer (Jasco) at 278nm (SS), 257nm (AH), 351nm (MS). Entrapment efficiency was calculated using the following formula:

Entrapment efficiency was calculated using the following formula:

$$\text{Entrapment efficiency} = \frac{\text{Estimated \% drug content}}{\text{\% drug content (theoretical)}} \times 100$$

Percentage Yield:

The percentage yield was calculated by using the following formula.

$$\text{Percentage yield} = \frac{\text{Actual wt. of microspheres}}{\text{Wt. of starting materials}} \times 100$$

2.6 Particle size evaluation by laser diffraction:

The particle sizes of microparicles were estimated by laser diffraction method. The Helos particle size determination with Sympatec GMbh systems were used for estimation of particle size. The dry powder 100mg was used to achieve the required obscuration of 5%. The particle size data obtained were represented as $D_{0.5}$.

2.7 In vitro deposition studies:

The anderson cascade impactor dealings particle size in accord with pharmacopoeia specifications [25-26]. The eight stages of Andersen Cascade Impactor (ACI) used for measuring the aerodynamic particle size distribution generated by dry powder inhaler such as rotahaler. The samples were collected on the surface of the collection plates, the middle was the preseparator connect with the glass expansion chamber and the right were the sieves, which filter the particles

according to their size [27-31]. The collection plates were layered with silicone oil to prevents bouncing of the particles while performing the studies. The outlet of the ACI is connected to a critical flow controller to create flow during testing. The airflow was 60 L/min. The ACI operation conditions were specified in Table 2.

Table 2. Operating conditions and theoretical cut-off diameters of ACI

ACI Parameters	ACI Results
Flow rate (L/min)	60
Time per actuation (s)	4
Volume per actuation (L)	4
Cut off diameter (µm)	
Stage 0	5.7
Stage 1	4.5
Stage 2	3.4
Stage 3	2.3
Stage 4	1.4
Stage 5	0.8
Stage 6	0.40
Stage 7	0.12
Stage 8	Filter

2.8 In vitro drug release:

The dialysis bag dispersion technique used to calculate *in vitro* release of SS, AH and MS from the microparticles [32-36]. The diffusion medium phosphate buffer saline (PBS) pH 7.4 and a dialysis membrane of 14 kDa molecular weight (Himedia, Mumbai, India) were used. An aqueous dispersion of 10mg of drugs kept in a dialysis bag and sealed at both ends. The dialysis bag was immersed in 250 ml of diffusion medium (sodium phosphate buffer, pH 7.4 at 37^{0}C) and stirred at 100 rpm. Samples were withdrawn at predetermined time intervals, and the receptor phase was replenished with same volume of phosphate buffer saline after each sample was withdrawn. Samples were filtered through 0.46 µm filter and appropriately diluted with pH 7.4 phosphate buffer saline. Absorbance of the samples was determined by UV/Vis spectrophotometry with pH 7.4 phosphate buffer saline as blank. The cumulative percent drug at various time intervals was determined and plotted against time. All experiments were done in triplicate.

2.9 Filter paper disc diffusion method:

The disc diffusion method was used to evaluate antibacterial and antifungal activity. The test samples were prepared with the solvent dimethyl formamide. The 6 mm diameter sterile paper disc impregnated with extracted test samples of microparticles deposited on the surface of agar medium. The standard used was the ciprofloxacin (100mcg/ml) to study actimicrobial activity of microparticles prepared. The filter paper disc diffusion method was used to test by employing 24 hours cultures of the respective microorganisms. The sterile nutrient agar used as medium and test organisms were seeded by homogenously mixing 1ml of inoculums with 20 ml sterile melted nutrient agar colled at 50ºC in a sterile petridish to get solidified. All the test samples and standard were impregnated in whatman filter paper disc and kept above the medium in the petridish and petridish kept undisturbed for 2 hours at room temperature. The petridish were incubated for 24 hours at 37^{0}C and inhibition zone (mm) was measured. Antibacterial efficiency at different concentration (250 mcg/ml, 500 mcg/ml, 1 mg/ml) were determined by using the filter paper disc diffusion method. The null bacterial growth of minimum dimension of the zone around the filter paper disc was measured and calculated the zone of inhibition.

2.10 Method of MTT assay process

The alveolar epithelial cancer cell line A549 was used in the cell viability assay. The MTT reagent (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) used to know cell viability. 500-10,000 cells were plated in a 96 well plate by Leaving the 8th wells empty for controls. The seeded cells where incubated (37 °C, 5% CO_2) overnight to allow the cells to attach to the wells. 2µl (depends on the concentration) of drug of interest dissolved in DMSO to each well. Placed on a shaking table, 150 rpm for 5 min, to thoroughly mix the samples into the media. Incubated (37 °C, 5% CO_2) for 1-5 days to allow the drug/toxin to take effect. 20µl MTT(5mg/ml in DMSO) solution added to each well and placed on a shaking table, 150 rpm for 5 min, to thoroughly mix the MTT into the media. Plates are incubated (37 °C, 5% CO_2) for 1-5 h to allow the MTT to be metabolized. Dump off the media and Resuspend formazan (MTT metabolic product) in 150µl DMSO with shaking table, 150 rpm for 5 min, to thoroughly mix the formazan into the solvent. The process detail presented in Table 3. Read optical density at 560 nm and subtract background at 660 nm.

Table 3. MTT assay process

S. No	Reagents	Test Sample (T1-T9)									Blank (B)
		T1	T2	T3	T4	T5	T6	T7	T8	T9	B
1	Cell culture	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	-
	Incubate at 37° C for Over night										
2	MTT Reagent	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
3	PBS	-	-	-	-	-	-	-	-	-	100 µl
	Incubate at 37° C for 2-4 hrs										
4	DMSO	150µl	150µl	150µl	150 µl	150µl	150µl	150µl	150 µl	150µl	150µl
	Incubate at 37° C for 15mins in dark										

3. RESULT AND DISCUSSION

In the present study, microparticles were prepared by modified solvent evaporation method. The drugs were encapsuled with polymers in aqueous:organic phase. Encapsulation process involves vibracell sonication, homogenization, centrifugation and then freeze drying to yield microparticles [37-41]. Kept organic aqueous solution ratio as 1:5 (30 ml of organic:aqueous solution as 5:25ml respectively mixed with 1:1 drug polymer ratio). Presence of polymer ensures good yield of microparticles [42-43].

Particle size analysis, entrapment efficiency of microparticles, weight variation and drug content of DPI:

The particle size, and entrapment efficiency were evaluated for microparticles and demonstrated in Table 4. The size distribution of microparticles (laser diffraction) was observed for formulations T1-T9. The mean particle size of microparticles of formulation T1, was better than other formulation T2-T9. Entrapment efficiency for formulations with PLGA (50:50) were better than PLGA (75:25) and Eudragit RS100. The Table 5 described on the weight variation and drug content of capsule contains homogenous mixture of Drug:lactohale (1:19) of formulations T1-T9. The average weight of filled capsules was 20 mg containing 1 mg of drugs (SS, AH and MS). In all formulation measurement, the uniformity of weight and drug content in the capsule was achieved.

Table 4. Particle size, entrapment efficiency of microparticles

Trial Code	Particle size ($D_{0.5}$ nm) Mean ±SD	Entrapment efficiency
T1	1.94±0.6	76.62±0.32
T2	1.92±0.2	73.12±0.41
T3	1.82±0.8	71.16±0.54
T4	1.93±0.5	75.14±0.21
T5	1.91±0.3	74.24±0.12
T6	1.88±0.6	72.17±0.15
T7	1.91±0.1	72.12±0.36
T8	1.90±0.5	70.06±0.47
T9	1.86±0.7	69.09±0.26

Table 5. Weight variation and drug content of dry powder inhaler

Trial Code	Weight Variation (mg ± SD)	Drug Content (%)

T1	20.06±0.05	99.2-100.5
T2	20.11±0.12	99.0-101.4
T3	19.94±0.20	98.9-102.5
T4	20.09±0.08	99.1-100.8
T5	20.21±0.22	99.0-100.2
T6	19.93±0.23	98.5-102.0
T7	20.09±0.08	98.1-100.2
T8	20.01±0.10	99.7-101.2
T9	19.93±0.20	98.4-100.9

Drugs compatibility in formulations by FTIR spectra:

The interaction of drugs in formulations T1-T9 were evaluated in Fourier transform infrared spectroscopy (FTIR-Perkin Elmer of scanning range 450-4000 cm^{-1}) was presented in Fig. 1-12. Spectral graphs were given as wave number (cm^{-1}) against Transmittance (%). SS produces sharp bands at wavelength of about 1000 cm^{-1}, as spectra of formulation also show bands at same wavelength, but somewhat less intense. AH shows peaks at 3350, 3400, 1590, 1540 and 700 cm^{-1} [44-45]. The peak of AH at 3400, 3345 and 1074 cm^{-1} becomes relatively narrow, which could be attributed to the stretching vibration of phenolic hydroxyls (OH bond), and confirms the interactions of phenolic hydroxyl groups [46]. MS exhibits many aromatic CH peaks observed from 2900cm^{-1} to 3000$cm^{-1.}$ There is no difference in the positions of absorption bands was observed in the spectra of drugs (SS, AH and MS) and formulation trials T1-T9, demonstrating no chemical interaction between drugs and polymer in solid state. From the observation drugs were found to be stable in formulation trials T1-T9.

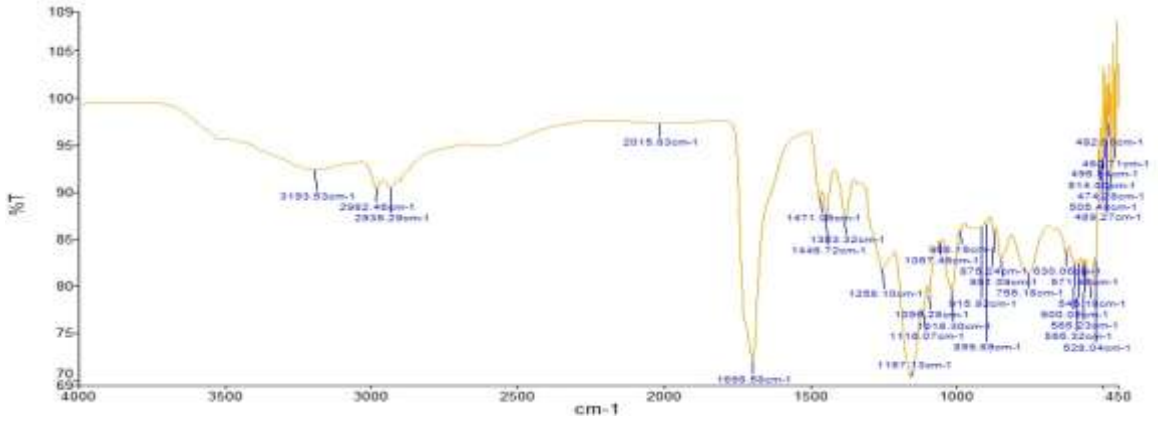

Fig. 1. FTIR graph of salbutamol sulphate

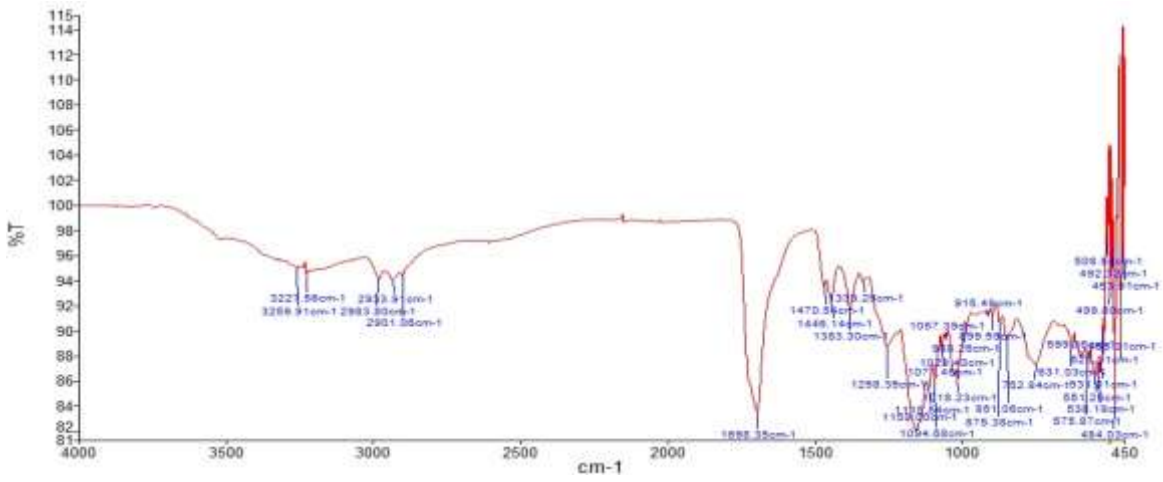

Fig. 2. FTIR graph of formulation trial T1

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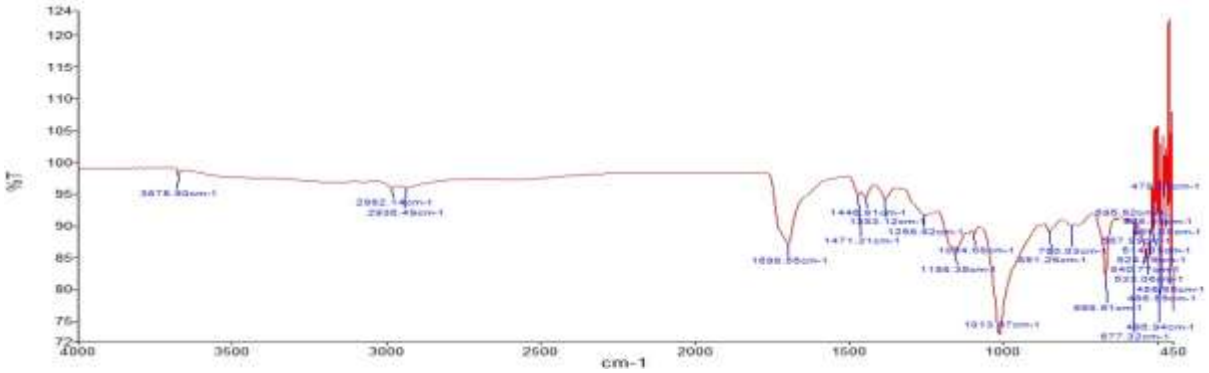

Fig. 3. FTIR graph of formulation trial T2

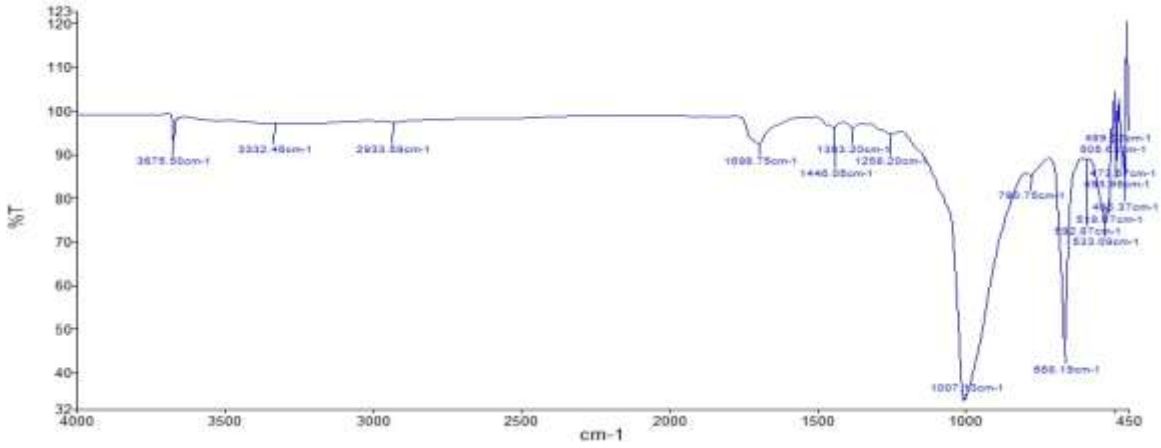

Fig. 4. FTIR graph of formulation trial T3

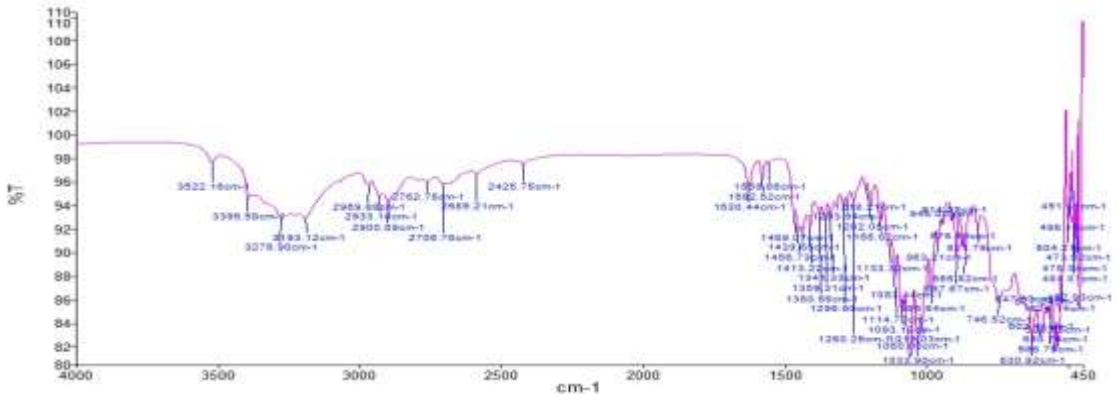

Fig. 5. FTIR graph of ambroxol hydrochloride

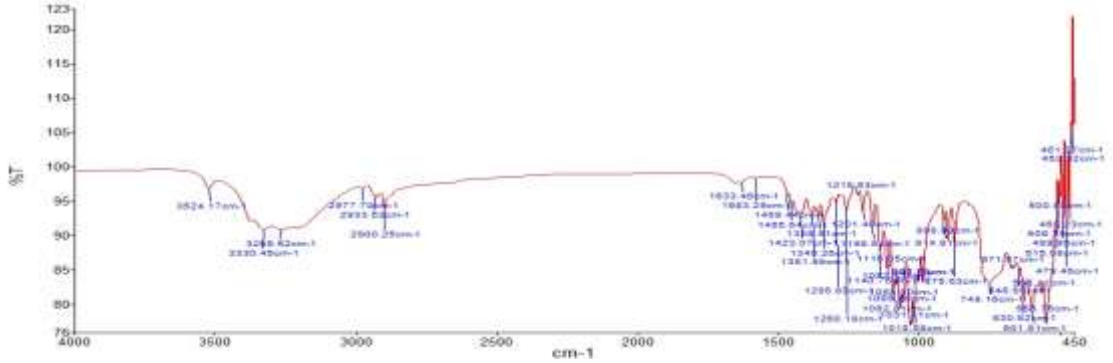

Fig. 6. FTIR graph of formulation trial T4

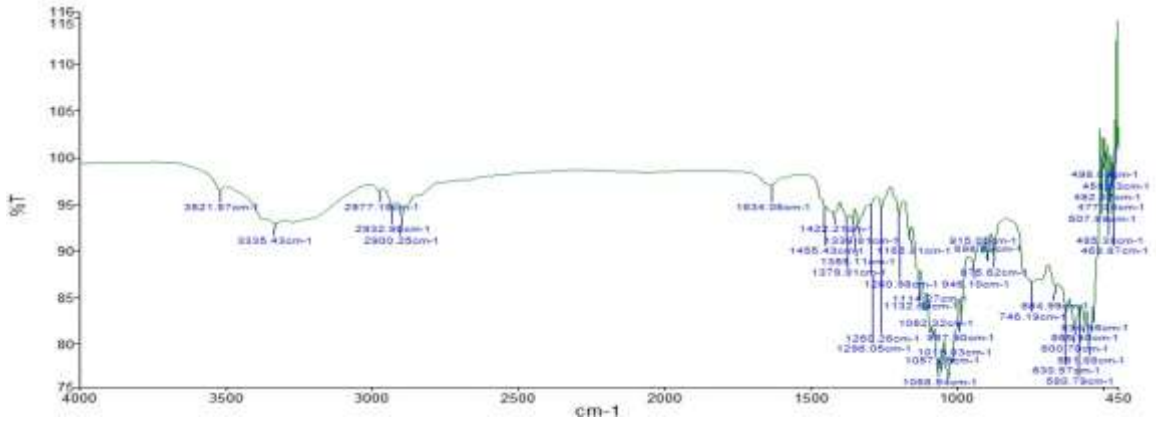

Fig. 7. FTIR graph of formulation trial T5

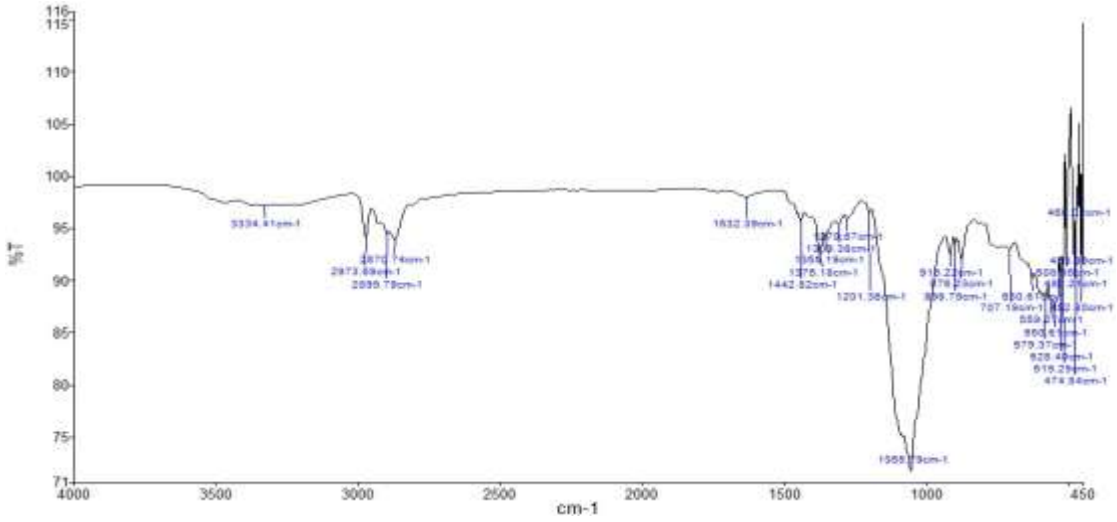

Fig. 8. FTIR graph of formulation trial T6

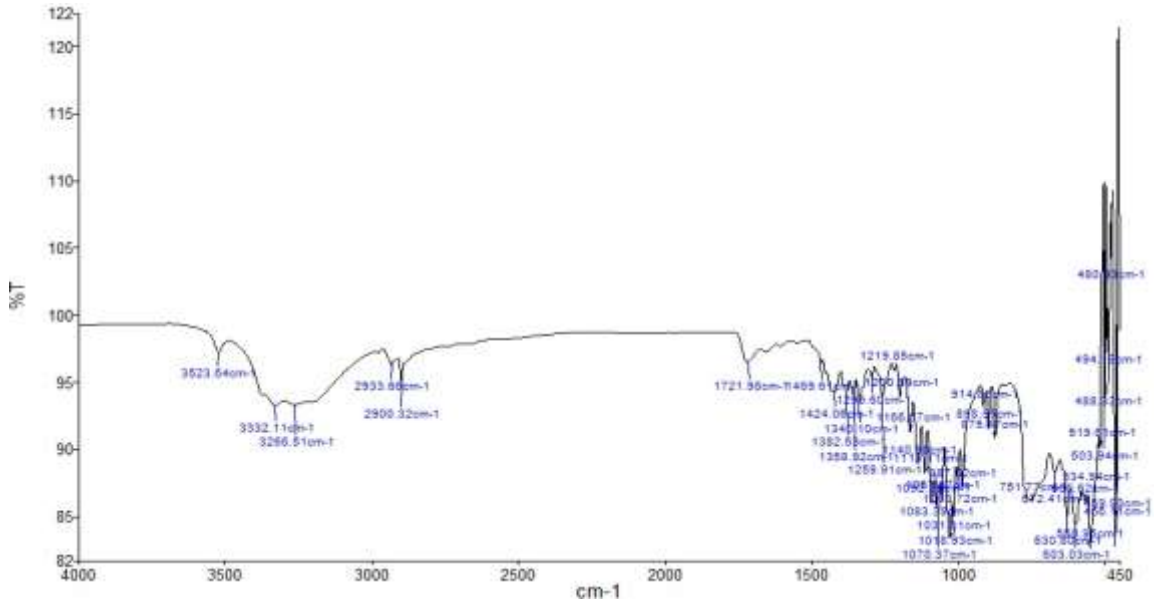

Fig. 9. FTIR graph of montelukast sodium

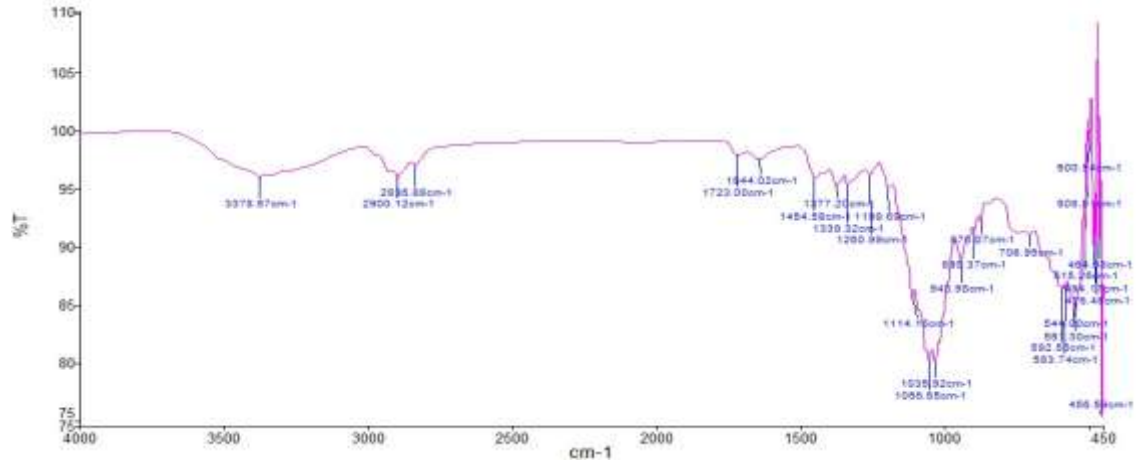

Fig. 10. FTIR graph of formulation trial T7

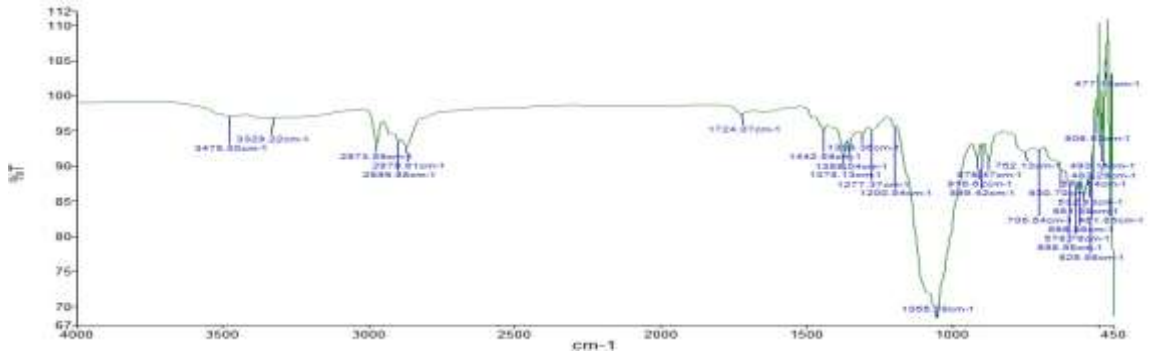

Fig. 11. FTIR graph of formulation trial T8

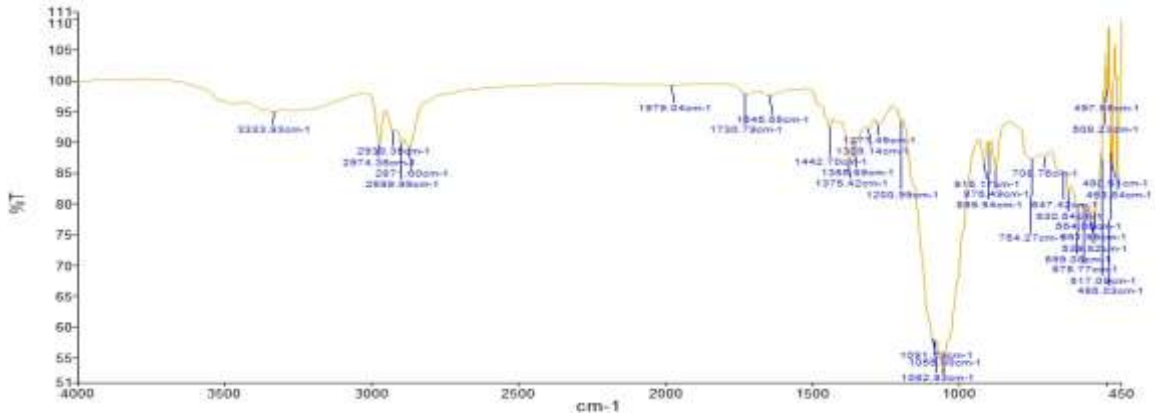

Fig. 12. FTIR graph of formulation trial T9

Scanning electron microscopy:

Scanning electron microscope (SEM-VEGA3) analysis showed the surface morphology of microparticles Fig. 13-21. The scanning range was 400-4000 cm^{-1} and the resolution was 2/cm. The SEM image showed microparticles are nearly spherical in nature.

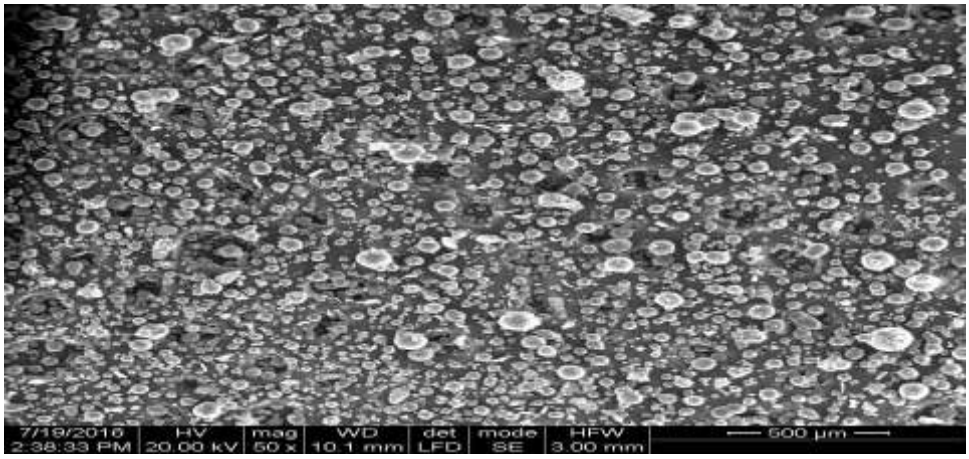

Fig. 13. SEM photograph of formulation T1

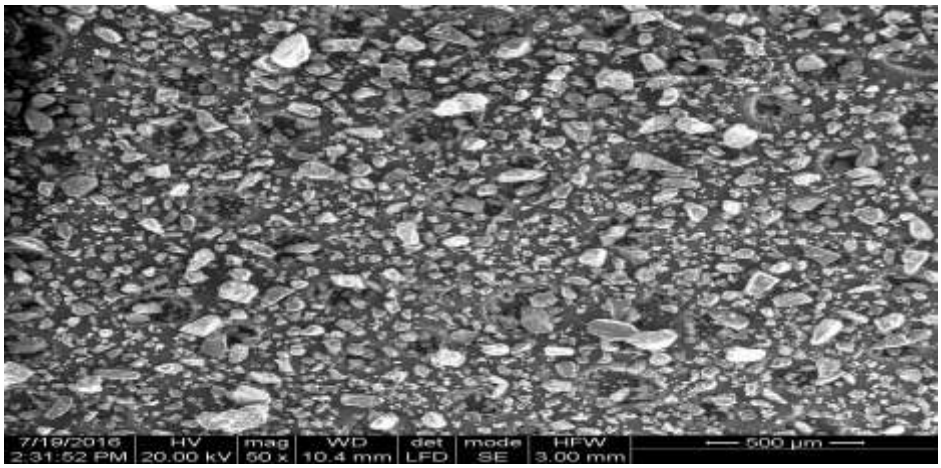

Fig. 14. SEM photograph of formulation T2

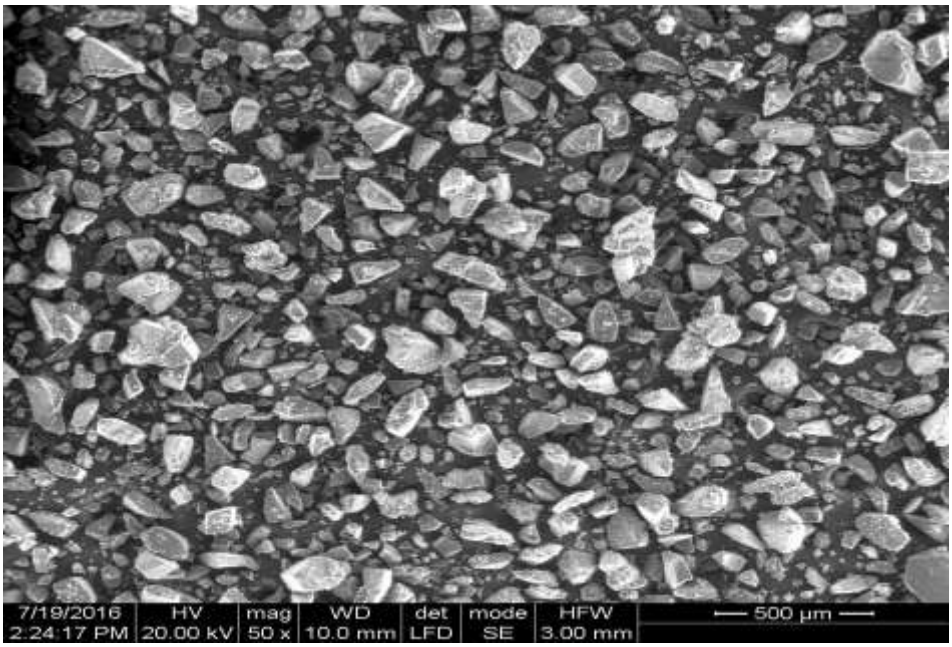

Fig. 15. SEM photograph of formulation T3

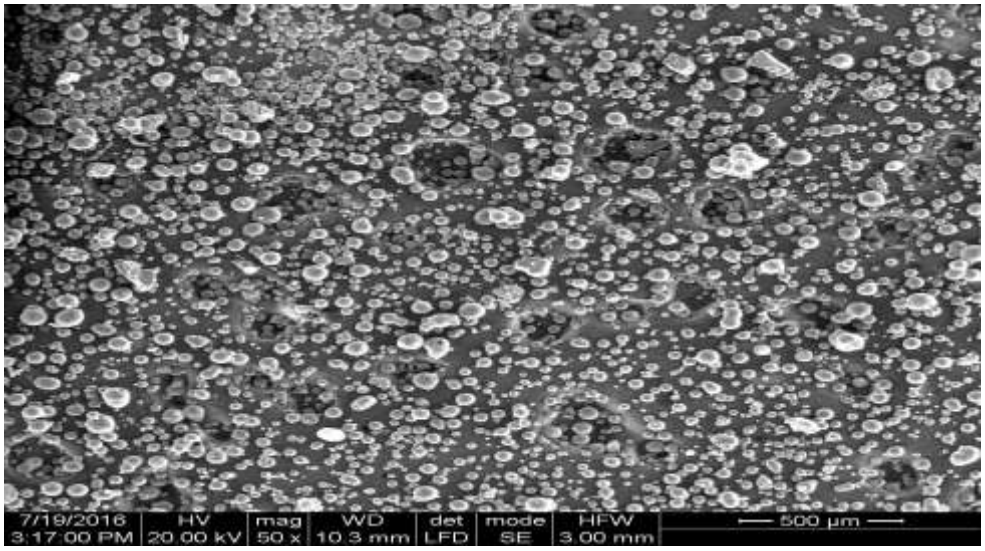

Fig. 16. SEM photograph of formulation T4

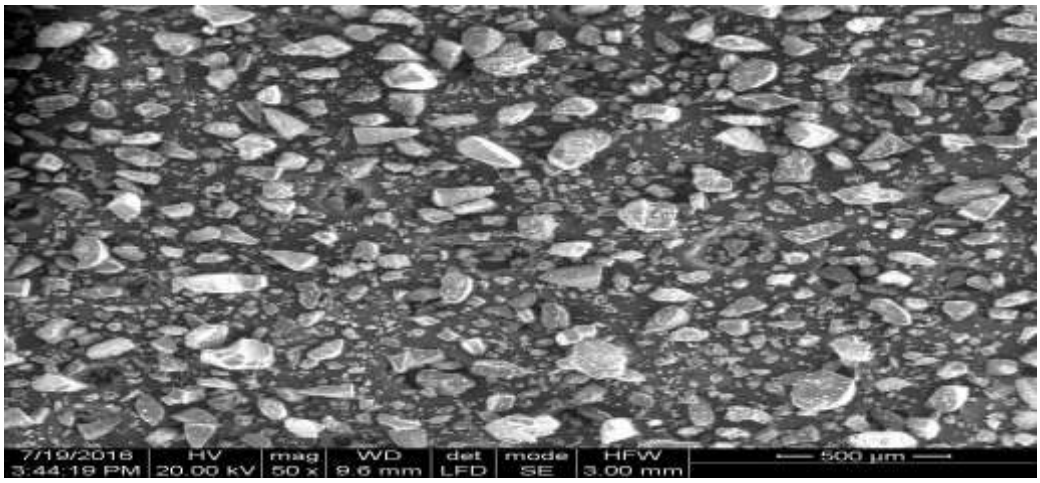

Fig. 17. SEM photograph of formulation T5

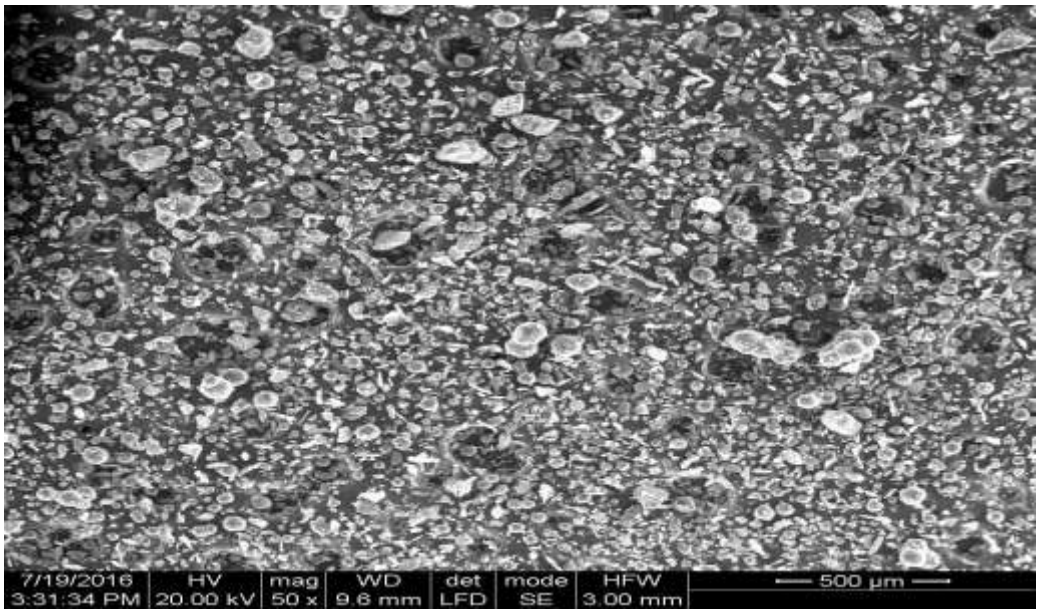

Fig. 18. SEM photograph of formulation T6

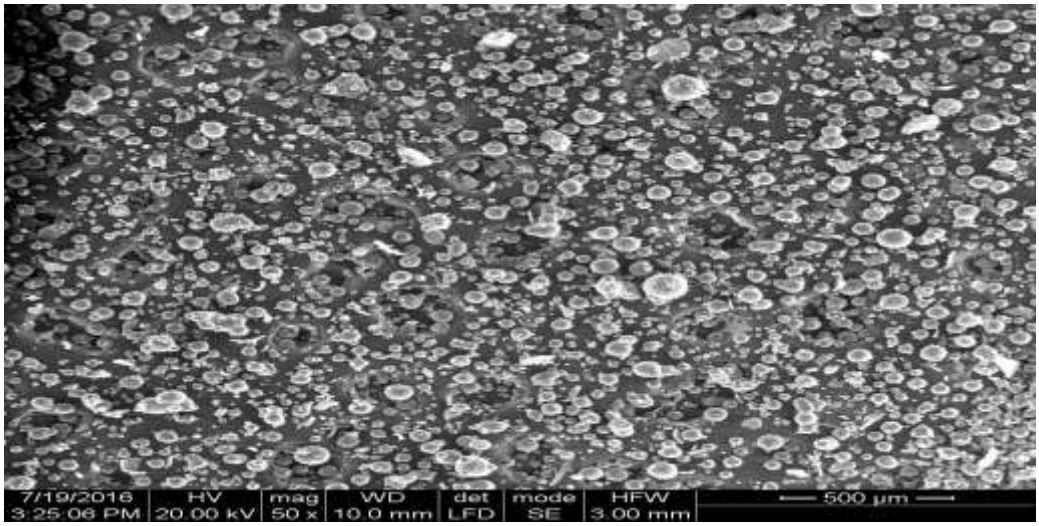

Fig. 19. SEM photograph of formulation T7

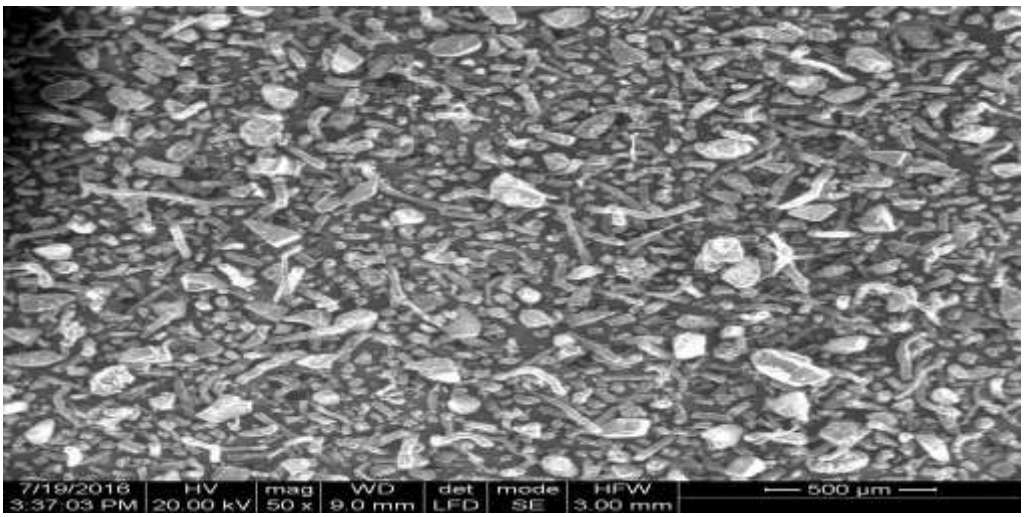

Fig. 20. SEM photograph of formulation T8

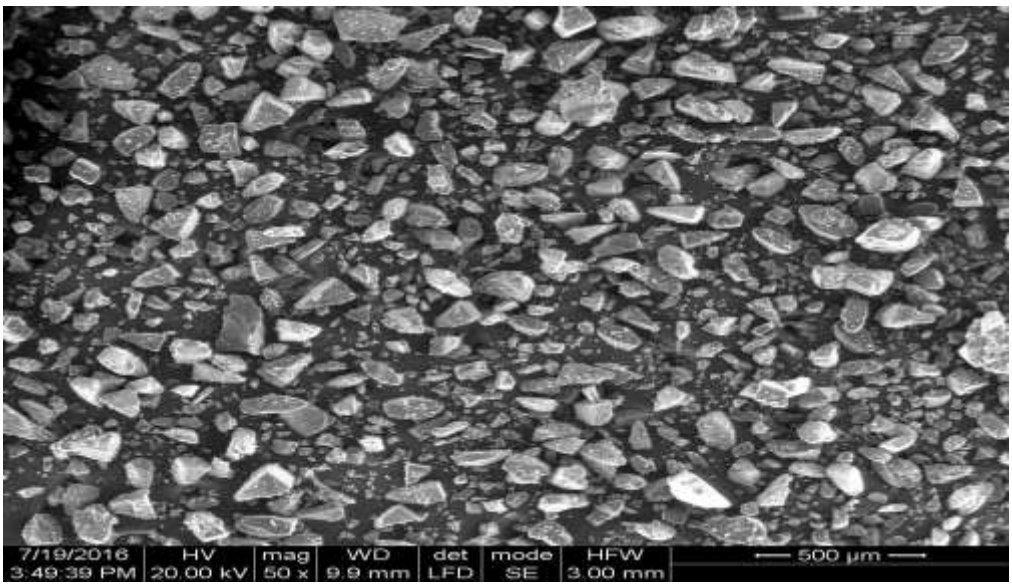

Fig. 21. SEM photograph of formulation T9

Anderson cascade impaction studies:

The In vitro depositions of powder particles in the lung were evaluated by powder particle distribution in the stages of Anderson cascade impactor (ACI, Copley Scientific)[47]. The distribution of powder particles were graphically demonstrated in Fig. 22-30, showing mean (SD) percent emitted dose deposited on each stage of the ACI using Rotahaler at 60 L min^{-1} (n = 6). The respiratory fraction[48-49] (particle deposition in stage 2-8) and mass median aerodynamic diameter of formulation T1-T3 were given in Table 6, T4-T6 were given in Table 7 and T7-T9 were given in Table 8.

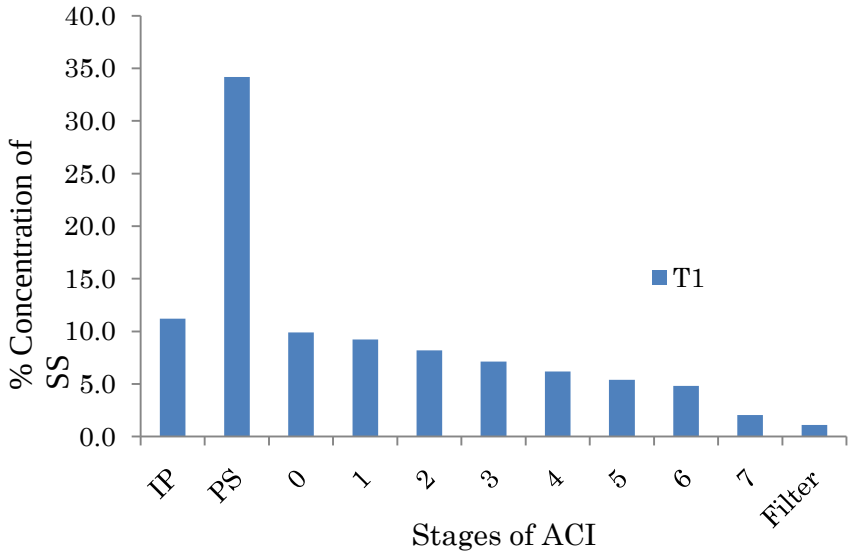

Fig. 22. ACI for % deposition profiles in each stage for formulation T1

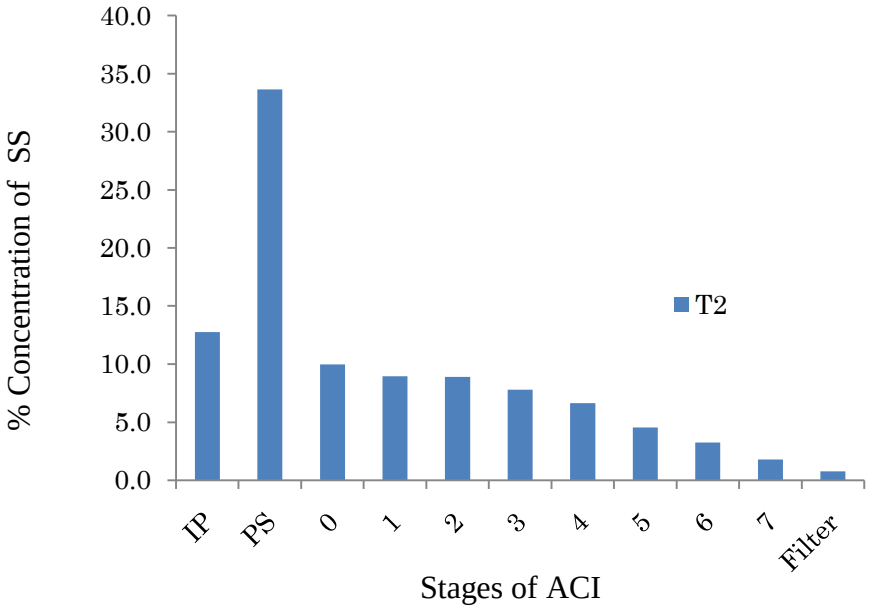

Fig. 23. ACI for % deposition profiles in each stage for formulation T2

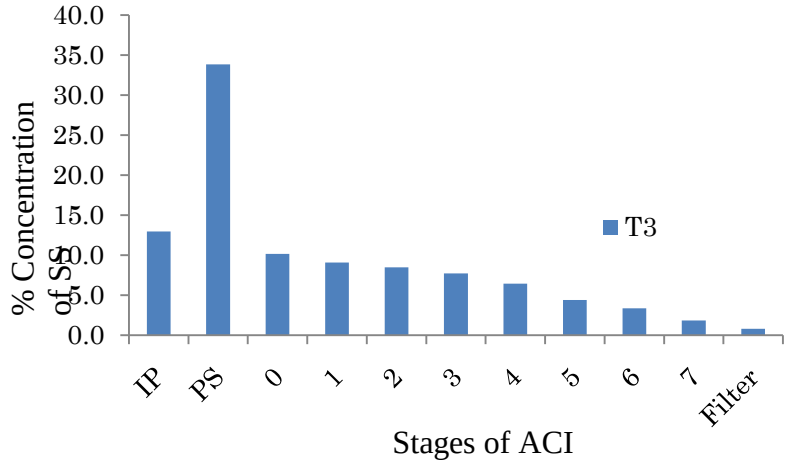

Fig. 24. ACI for % deposition profiles in each stage for formulation T3

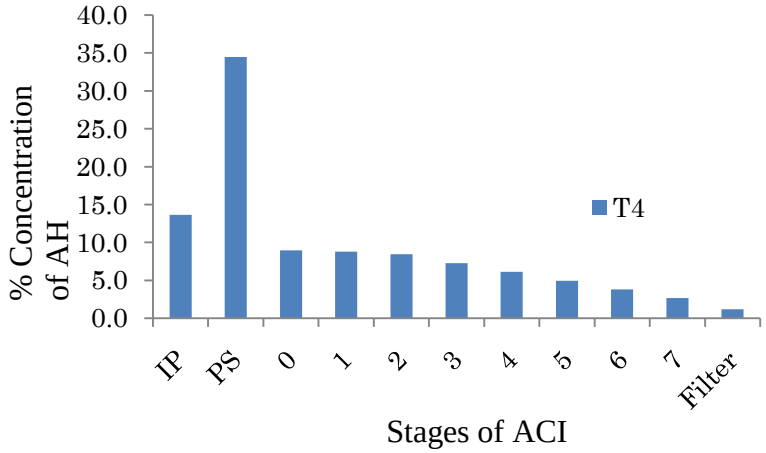

Fig. 25. ACI for % deposition profiles in each stage for formulation T4

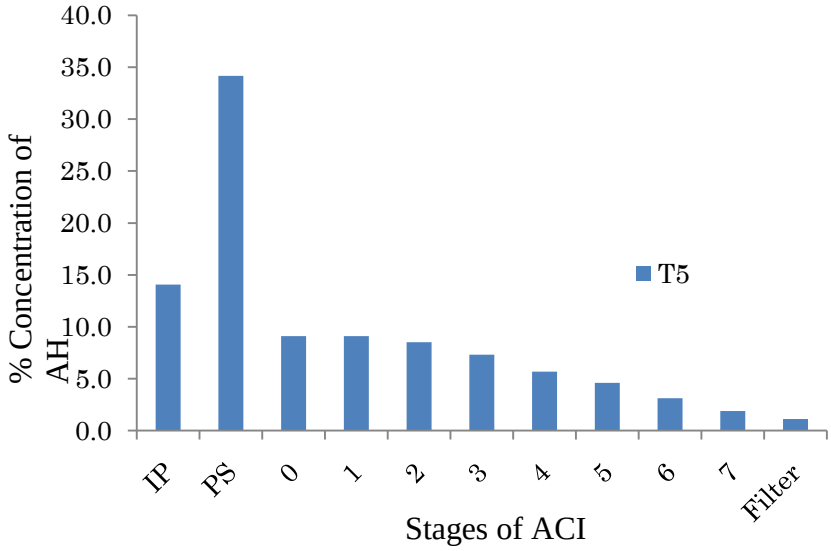

Fig. 26. ACI for % deposition profiles in each stage for formulation T5

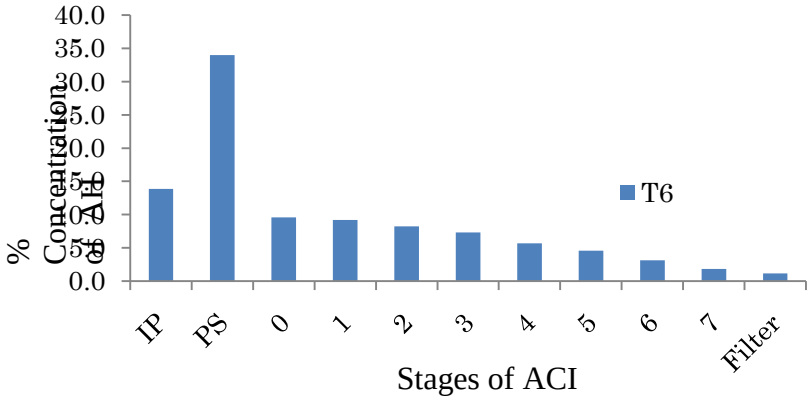

Fig. 27. ACI for % deposition profiles in each stage for formulation T6

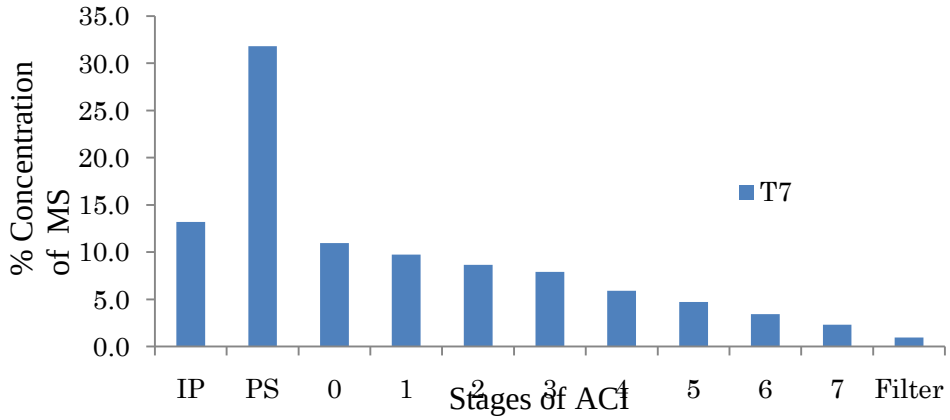

Fig. 28. ACI for % deposition profiles in each stage for formulation T7

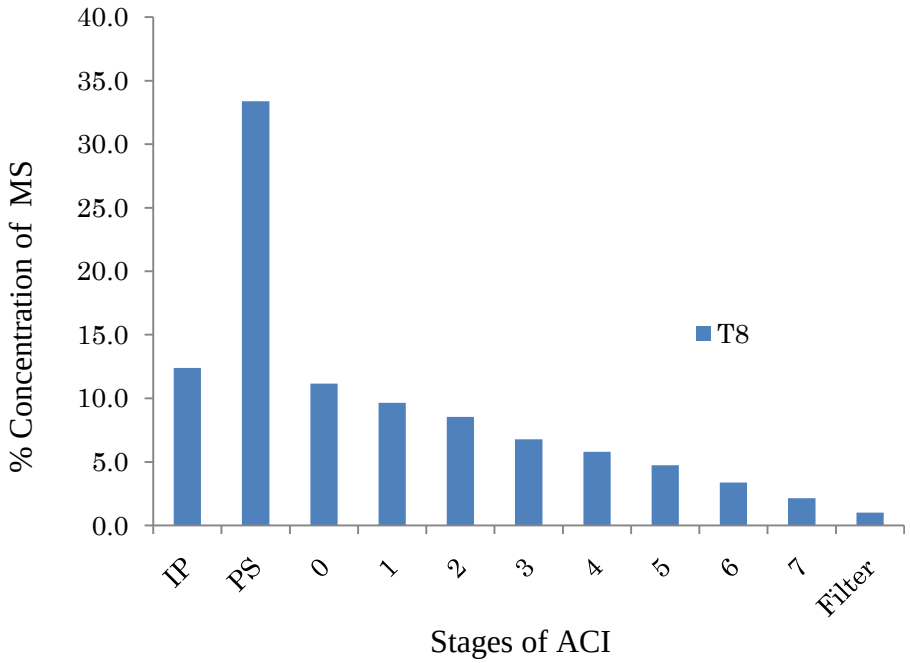

Fig. 29. ACI for % deposition profiles in each stage for formulation T8

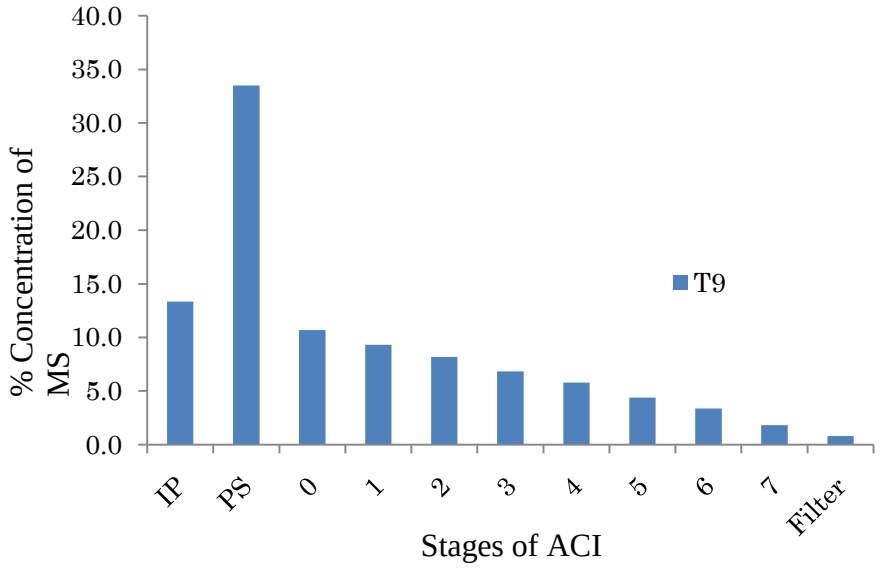

Fig. 30. ACI for % deposition profiles in each stage for formulation T9

Table 6. ACI results for the formulations T1, T2 and T3 measured using an air-flow rate of 60 L min^{-1} (n = 6).

Particle size Attributes	ACI (inhalation indices) Mean ± SD		
	T1	T2	T3
Emitted dose (%)	98.8 ± 2.57	98.2 ± 2.97	98.2 ± 2.96
Respiratory fraction (%)	34.9 ± 2.59	33.7± 3.08	33.1 ± 2.93
Total recovery	99.8 ± 3.02	98.9 ± 3.37	98.9 ± 2.96
Mean median aerodynamic diameter (µm)	1.75 ± 0.11	1.94 ± 0.12	2.14 ± 0.21

Table 7. ACI results for the formulations T4, T5 and T6 measured using an air-flow rate of 60 L min^{-1} (n = 6).

Particle size Attributes	ACI (inhalation indices) Mean ± SD		
	T4	T5	T6
Emitted dose (%)	98.5± 2.39	96.8 ± 2.8	96.9 ± 2.87
Respiratory fraction (%)	34.5 ± 2.17	32.2± 2.8	31.9 ± 2.70
Total recovery	99.5 ± 2.82	97.8 ± 3.1	97.9 ± 3.16
Mean median aerodynamic diameter (µm)	1.72 ± 0.3	1.90 ± 0.20	2.24 ± 0.01

Table 8. ACI results for the formulations T7, T8 and T9 measured using an air-flow rate of 60 L min^{-1} (n = 6).

Particle size Attributes	ACI (inhalation indices) Mean ± SD		
	T7	T8	T9
Emitted dose (%)	97.6± 3.09	97.9 ± 3.1	97.2 ± 3.04
Respiratory fraction (%)	33.8 ± 2.84	32.4± 2.65	31.2 ± 2.67
Total recovery	98.6 ± 3.47	98.9 ± 3.45	98.2 ± 3.38
Mean median aerodynamic diameter (µm)	1.71 ± 0.10	1.89 ± 0.3	2.14 ± 0.11

The in vitro drug dissolution:

The dissolution was carried out in PBS at pH 7.4 at 37°±0.5°C. The drug released at 12 h of formulation trials were T1, 91.23 %; T2, 86.92%; T3, 85.05 %; T4, 90.67; T5, 85.27%; T6, 84.05; T7,90.56; T8,86.91 and T9,83.67. The drug releases were found to be not less than 80% at 12 h in all trials. The Fig. 31-33 represents cumulative percent drug release

versus time in hours for formulations T1-T9. It was understood that the drug release versus time proved the sustained release of drugs in all formulations T1-T9. SS:PLGA(50:50) Formulation T1 exhibited best dissolution result, when compared to other formulations T2-T9 [50].

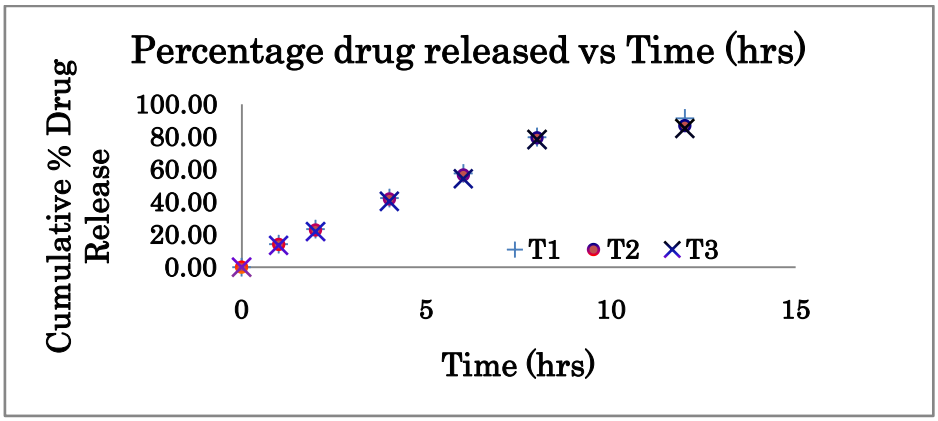

Fig. 31. Cumulative Percent drug release versus time (h) for formulations T1-T3 (n=3).

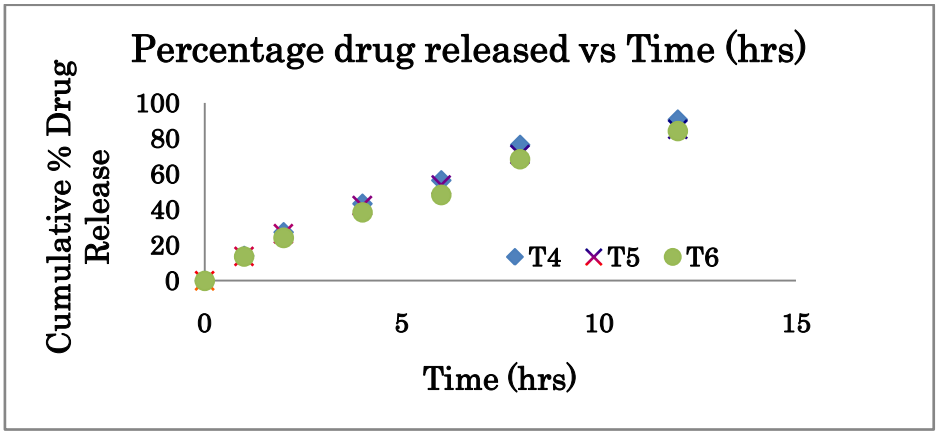

Fig. 32. Cumulative Percent drug release versus time (h) for formulations T4-T6 (n=3).

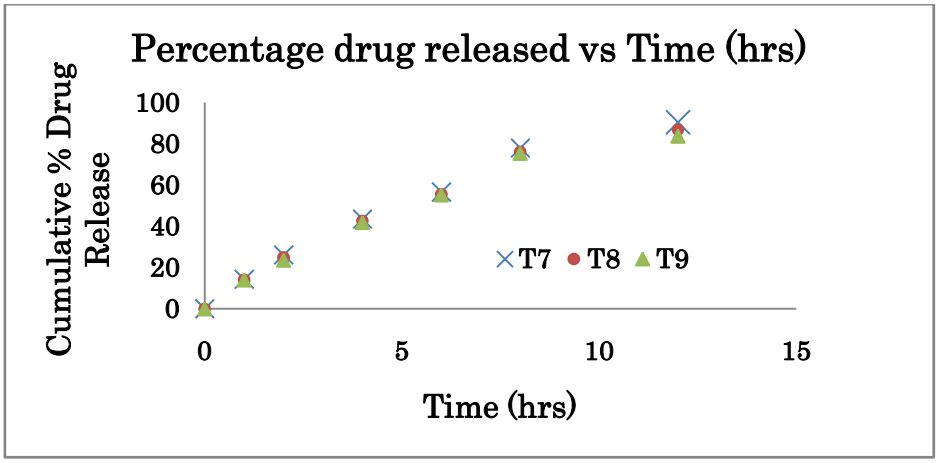

Fig. 33. Cumulative Percent drug release versus time (h) for formulation trials T7-T9 (n=3).

Antibacterial activity:

The antibacterial activity of the dry powder inhaler formulation trials T1-T9 were studied against gram positive, gram negative bacteria and fungi shown in Table 9. The three respiratory drugs were found demonstrated antibacterial activity. The minimum inhibitory concentrations were determined. Significant antimicrobial activity was found with the dry powder inhaler formulation. The microparticle possess a notable antibacterial action against all the microorganisms were tested.

Table 9. Minimum inhibitory (mm) concentration of the microorganism for Trial T1 – T9

Test Micro organisms	Zone of inhibition (mm)				
	Sample	250 µg/ml	500 µg/ml	1mg/ml	Standard*
Staphylococcus aureus (Gram positive)	T1	5	7	10	19
	T2	6	6	10	18
	T3	6	6	9	17
	T4	6	8	10	20
	T5	5	6	10	19
	T6	6	7	8	20
	T7	7	7	9	21
	T8	6	8	8	20
	T9	7	7	8	20
Micrococcus luteus (Gram positive)	T1	7	8	11	20
	T2	6	7	10	20
	T3	6	6	9	19
	T4	5	9	10	21
	T5	5	8	11	19
	T6	5	7	10	20
	T7	6	7	10	19
	T8	6	6	9	18
	T9	6	6	10	17
Escherichia coli (Gram negative)	T1	6	9	10	20
	T2	6	7	10	20
	T3	5	8	9	19
	T4	7	8	9	21
	T5	6	7	8	20
	T6	6	7	9	19
	T7	5	7	11	20
	T8	4	8	10	19
	T9	5	7	10	20
Pseudomonas aeruginosa (Gram negative)	T1	6	9	9	21
	T2	5	7	9	20
	T3	5	7	8	20
	T4	6	8	10	20
	T5	5	8	9	19
	T6	6	7	10	19
	T7	7	8	11	19
	T8	6	6	10	18
	T9	7	7	10	18

Aspergillus niger (Fungi)	T1	7	7	10	20
	T2	7	7	9	20
	T3	6	6	10	19
	T4	5	8	9	19
	T5	5	7	9	18
	T6	4	7	9	18
	T7	6	7	11	20
	T8	5	6	10	19
	T9	4	6	11	19

*Standard - ciprofloxacin

MTT assay:

The sustained release microparticles tested for by cell line studies. The Invitro cell viability assayed (MTT assay) carried out by alveolar epithelial cancer cell line A549 for formulations T1-T9. MTT assay is a colorimetric method for measuring the activity of enzymes in living cells that reduce MTT to formazan dyes, giving a purple color. The process of MTT assay given in table 8. It is commonly used to determine cytotoxicity of potential medicinal agents and toxic materials, since these types of materials are expected to stimulate or inhibit cell viability and growth. At 500 μg concentration, formulations T1-T9 showed more than 80% cell viability. The results indicated that the formulations are safe proved with alveolar epithelial cancer cell line A549 by invitro cell viability assay. Percentage of cell viability graph of T1-T9 was presented in Fig. 34.

Calculated the cell density and percentage cell viability using the following formula

Calculated the average cell density of triplicates:

$$\text{Percentage of cell viability (\%)} = \frac{\text{Optical density of sample}}{\text{Optical density of Control}} \times 100$$

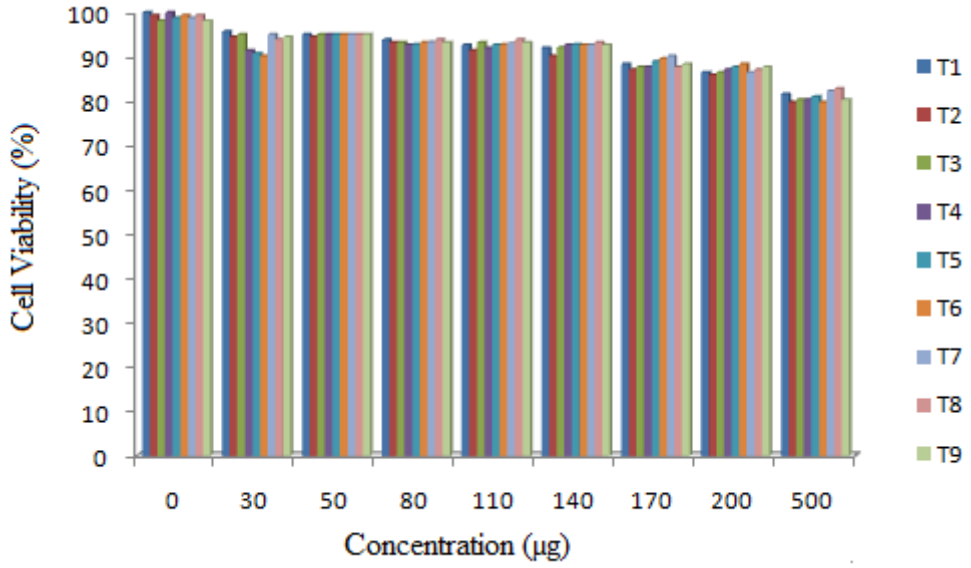

Fig. 34. Percentage cell viability against alveolar epithelial cancer cell line A549 of formulated DPI; Data are presented as mean ± SD, n = 3.

CONCLUSION

The orally inhalable dry powder formulation was prepared using drug and polymers for encapsulation. The prepared

microparticles committed for sustained drug delivery in airways through inhalation. All the formulations had extended in vitro drug release profile upto 12 hours. Formulation T1 compared with all the nine formulations based on laboratory analysis was best. In vitro drug deposition studies exhibit optimum characteristics of respirable particles. Further invivo studies will be carried out to analyse clinical characteristics of formulations.

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