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# Formulation and Evaluation of Doxofylline-Loaded Polymeric Micelles for Pulmonary Administration

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Abstract

The pulmonary administration of drugs offers advantages over administration by intravenous injection. The present research work was to prepare polymeric micelles nanomicelles containing Doxofylline as dry powder for inhalation. In order to bypass the drawbacks of predictable preparations, nanotechnology-based drug delivery systems for pulmonary administration and pulmonary targeting have been oppressed. The work was aimed towards formulating the Doxofylline loaded Soluplus nanomicelles with polymer Soluplus by film hydration technique in different ratios. The nanomicelles so prepared were characterized for its particle size, ζ-potential, XRD, SEM, drug content and drug release rate. Soluplus Doxofylline Polymeric Micelles showed mean size of 70.73nm and Zeta Potential is - 8.71. The Drug Entrapment Efficiency is 87.7% and The Drug Loading Capacity is 10%. In-Vitro study reveals the drug release is 44%. Conclusion: The Doxofylline-Soluplus Polymeric Nanomicelles could have the significant value in the treatment of Asthama and COPD.

**Keywords:** Polymeric Nanomicelles, Asthama, COPD, Doxofylline, Film Hydration Technique, Lyophilisation, Dry powder for Inhalation.

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## 1. INTRODUCTION

Asthma is an obstructive pulmonary disease that affects more than 230 million public universal and is a noteworthy reason of indisposition in patients of all ages. It is an assorted illness through a complex physiopathology and phenotype. Understanding how serious the disease is important and treatment is designed to control symptoms and prevent future exacerbations. Pharmacological dealing through beta-agonists for alternating asthma and inhaled corticosteroids and a mixture of inhaled corticosteroids and long-acting beta-2 agonists for determined asthma are suggested.[1]

COPD (Chronic Obstructive Pulmonary Disease) is a rising health issues, quitting tobacco is the only effective way to prevent it. All through the extended asymptomatic stage, lung function however remains to deterioration, so numerous patients ask for health care individually once they remain in a progressive step or take knowledgeable acute aggravation.[2] Doxofylline is a Xanthine molecule that seems to be both Broncho-dilator and Anti-Inflammatory with an enhanced therapeutic window compared to conventional Xanthines such as theophylline and supporting evidence of the effects of doxofylline in the treatment of pulmonary diseases.[3]

Methylxanthines are widely used in the treatment of Asthma, as one of the few drugs that can be given orally, they are particularly useful in resource-constrained environments, It is a new derivative of methylxanthine, with similar efficacy and much lower side effects in adult animals and humans. However, there are limited studies in children with Asthma.[4]

Administering medications to the lungs takes its compensations done by another path. Inhalable powders containing of nanoparticles is ahead much attention in respirational investigate and scientific remedy. The technique of particle engineering is a significant aspect in developing breathable formulations which can effectively distribute the drug through enhanced beneficial outcome and improved targeting. Inhalable nanoparticles in solid dry powders for targeted pulmonary administration have exclusive compensations and are a new and stimulating part of investigate.[5]

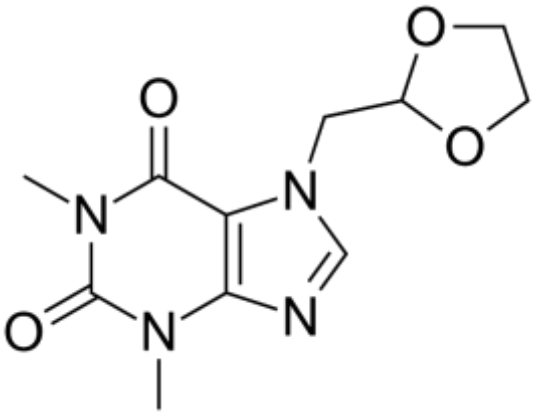

Figure 1: Doxofylline structure

The large variability of nanocarriers, Polymeric micelles characterize a striking stage for developing new drug delivery systems based on Asthma drugs. In particular, PM are nanoscale vehicles depend on amphiphilic polymers that can clump in aquatic overhead their Critical Micelle Concentration (CMC). PMs contain a hydrophobic nucleus, enclosed by a hydrophilic crown. The first is capable to encapsulate lipophilic medicines, though the last affords colloidal firmness to nano transporters.[6] The pneumonic course of association is a non-meddlesome, advantageous and effective approach to manage passing on remedial experts at the close by and primary levels. Internal breath treatment is normally used locally to treat aeronautics course diseases, similar to asthma, bronchitis, cystic fibrosis (CF) and progressing obstructive pneumonic infection (COPD).

On the other way, internal breath moreover suggestions mind boggling possible for essential association, meanwhile the lungs have a goliath superficial district obtainable for ingestion, and, bountiful vascularization.[7] Aspiratory transport of medicine has gotten an engaging goal and of monstrous sensible and biomedical interest in the clinical consideration research locale as the lung is prepared for holding drugs either for neighbourhood affirmation or for basic movement.

### 1.1 Critical micelle concentration (CMC)

This is a crucial parameter of extensively cast-off surfactants, and numerous methods have been developed to determine the CMC.[8] The nonionic surfactants form donor-acceptor complexes with iodine in aqueous medium. The spectral absorption and the move in the $\lambda_{max}$ of Iodine upon complexation have been exploited to regulate the Critical Micelle Concentration (CMC).[9] CMC is linked to the tridimensionality positioning of a surfactant or a biosurfactant in a solvent system. It is recognized that surface-active compounds obtain dissimilar structures in aqueous solution. This circumstance depends not only on their attentiveness but likewise on their composition.[10]

The CMC values found thoroughly approve by those determined by other methods, including measurements of static surface tension, differential refractive index, and iodine solubilization. The spectral characteristics of the complex salt $KI_3$ can be utilized as well to originate alike data. The CMC and the spectral move can be linked with the weight portion of the Polyoxyethylene sets and the Hydrophile-Lipophile Balance (HLB) in numerous ways, with the limitations in these relations dependent on the sequence to which the surface-active agent have its place. For both CMC and HLB be contingent on temperature, the outcomes and the relatives arise are temperature-dependent.[9]

### 1.2 Dry Powder Inhalers (DPI)

Dry Powder Inhaler (DPI) preparations suggestion a determination to possibly Ozone-depleting pMDI preparations, as they do not be sure of on propellant technology. Certainly, considerable investigate has been focused at the growth of DPI preparations completed the earlier few times. A DPI uses the air stream of the affected role own breath to produce an aerosol of a metered dose of a dry powder. DPIs can be characterized into two key classes, single- and multi-dose. [11]

## 2. MATERIAL AND METHODS

### 2.1 Materials

Doxofylline was gift sample from Ajanta Pharma, Aurangabad. Soluplus (an amphiphilic polyvinyl caprolactam polyvinyl acetate-polyethylene glycol graft copolymer) was procured from BASF India LTD india. HPLC Grade Methanol was used in the formulation and Distilled Water was collected by double Distillation.

### 2.2 Method

**2.2.1 Film hydration technique:** In RBF (Round Bottom Flask) 20 ml of HPLC grade Methanol was taken and 9.4 mg of Doxofylline and 60 ml of Soluplus is dissolved in that, Then RBF was connected to the Rota Evaporator. Process is performed at 100 rpm 37°C and 332 bar of Vaccum was applied at which evaporation of Methanol takes place. After evaporation of Methanol, Thin film was formed at bottom of RBF, Then RBF was removed and after addition of 8mL of water there was a formation of Micelles takes place. Then these micelles are Sonicated for 1 hr. Supernatant was collected after Centrifugation, after that Particle Size and Zeta potential was measured.[12]

There are 13 batches performed and batch F3 was selected as an Optimized batch.

**Table 1: Optimized Batches**

| Batch Code | Doxofylline Conc. (mg) | Soluplus Conc. (mg) | ζ Potential | Particle size (nm) | PDI |
|---|---|---|---|---|---|
| **F1** | 2.63 | 80.00 | 1.21 | 112 | 0.4 |
| **F2** | 5.00 | 100.00 | -1.24 | 103 | 0.1 |
| **F3** | 15.00 | 100.00 | -2.80 | 119 | 0.4 |
| **F4** | 10 | 51.72 | -4.41 | 100 | 0.3 |
| **F5** | 5 | 60.00 | -1.24 | 100 | 0.3 |
| **F6** | 10 | 108.28 | -1.24 | 116 | 0.5 |
| **F7** | 10 | 80.00 | -1.24 | 109 | 0.1 |
| **F8** | 10 | 80.00 | 4.72 | 109 | 0.1 |
| **F9** | 10 | 80.00 | -1.24 | 109 | 0.2 |
| **F10** | 10 | 80.00 | 9.83 | 109 | 0.3 |
| **F11** | 15 | 60.00 | 2.61 | 121 | 0.4 |
| **F12** | 17 | 80.00 | -14.2 | 128 | 0.2 |
| **F13** | 10 | 80.00 | 5.14 | 109 | 0.5 |

## 3. RESULTS AND DISCUSSION

### 3.1 Particle size analysis

Particle size of Nonomicelles is 70.78 and Polydispersity Index is 0.27. Diameter of each Micelle is 11.98.[13] Studies specify that a favorable particle size range aimed at oral absorption was beneficial to the proleptic tissue distribution and passive aiming capability of micelles in vivo. It was well recognized that nanoparticles by the sizes reaching from 10 nm to 100 nm might display an optimum cellular and nuclear acceptance in epithelial and smooth muscle cells.

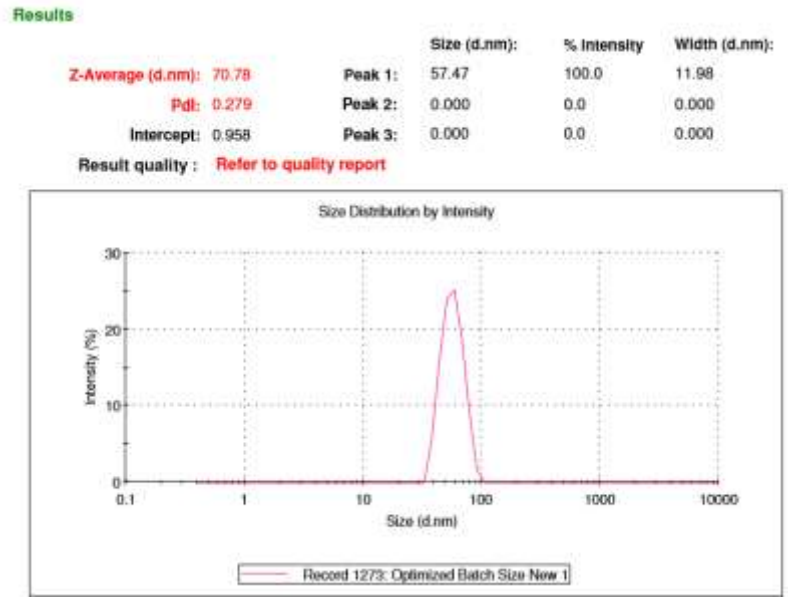


Figure 2: Particle size of optimized DOX-SOL Nanomicelles

So, DOX-SOL-PMs in small size were informal to be occupied in bowels and might avoid quick metabolism and elimination, by the constancy and extended flow period guaranteed. Also, the nanosized micelles remained described for the enhanced penetrability and retention (EPR) outcome, that is, passive aiming capability. Therefore, DOX-SOL-PMs in small size with enhanced permeability and retention were suitable for Bronchoconstriction.[14]

### 3.2 Zeta Potential Determination

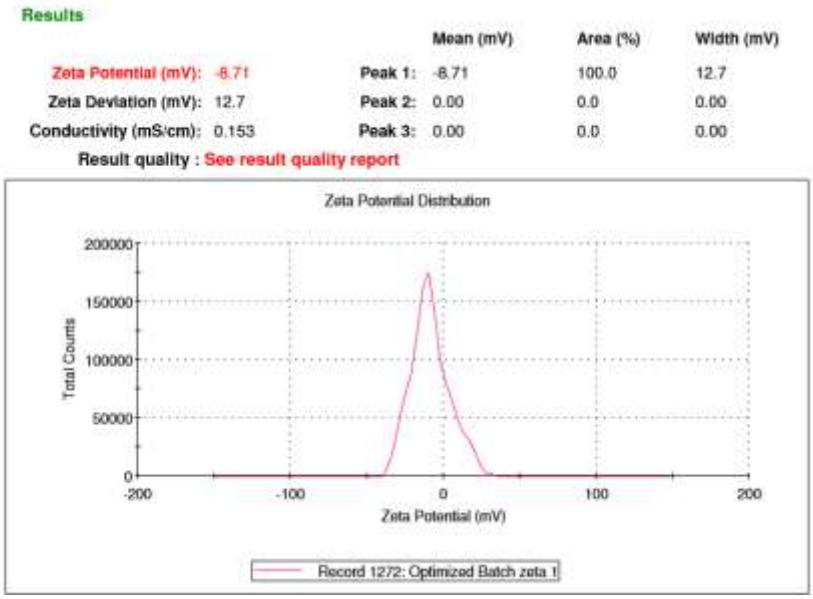


Figure 3: Zeta Potential of optimized DOX-SOL Nanomicelles

Zeta Potential of Nanomicelles is found to be -8.71. Zeta Deviation is 12.7 and Conductivity is 0.153. The average zeta potential measured was-8.71mV by negative surface charge, which positively increased the constancy of DOX-SOL-PMs in dispersal. The electrostatic repulsion and steric hindrance, as well as hydrophilic interactions between hydrophilic chains of the micelle system, might prevent the accumulation of the micelles and provide significant properties on constancy of the colloid system.

### 3.3. Drug entrapment

$$\text{Drug entrapment} = \frac{\text{Amount of drug added - Amount of drug in supernatant}}{\text{Amount of drug added}} X\ 100$$

$$= \frac{9.4-6}{9.4} X\ 100$$

$$= \frac{3.4}{9.4} X\ 100$$

$$= 0.36\ X\ 10$$

$$= 360$$

The entrapment efficiency (EE) and release *in vitro* remain very significant physicochemical features of Dox-SOL-PMs. The Film Hydration Technique was executed for encapsulation of water unsolvable Doxofylline in Soluplus micelles. Dox-Sol-PMs were ready by dissimilar theoretical ratios of Doxofylline to Soluplus, that is, 0.5:10, 1:10, and 1.5:10, to control the optimal percentage of Doxofylline in nanocarriers. The drug loading affected significantly the particle size and encapsulation efficiency of Dox-SOL-PMs.[16]

### 3.4 Drug Loading

$$\text{Drug loading (\%)} = \frac{\text{Amount of the drug in micelle}}{\text{Amount of the drug in feed}} \times 100$$

$$= \frac{9.7}{0.9} \times 100$$

$$= 10.77 \times 100$$

$$= 10\%$$

The Film Hydration Method was applied for encapsulation of water unsolvable Doxofylline in Soluplus micelles. Dox-Sol-PMs remained ready by various theoretical ratios of Doxofylline to Soluplus, that is, 0.5:10, 1:10, and 1.5:10, to regulate the optimum percentage of Doxofylline in nanocarriers. The drug loading affected significantly the particle size and encapsulation efficiency of Dox-SOL-PMs.[16]

### 3.5 *In Vitro* Drug Release Study

**Table 2: *In Vitro* Drug Release**

| T(min) | %CDR Micelles |
|---|---|
| **0** | 0 |
| **5** | 82.42957 |
| **15** | 46.15321 |
| **30** | 15.73391 |
| **45** | 13.61174 |

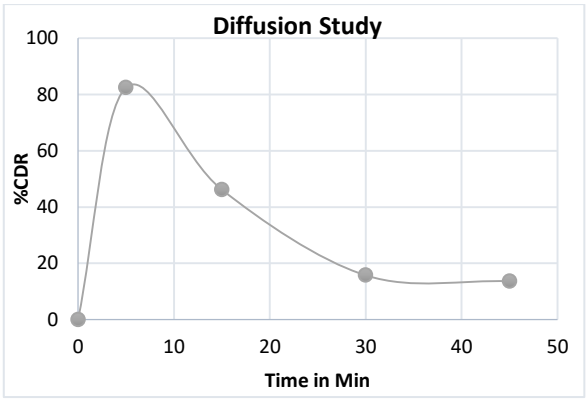


**Figure 4: Diffusion Study of optimized DOX-SOL Nanomicelles**

The prepared micelles placed in a dialysis bag to regulate *in vitro* release of micelles The carrier remained locked on both edges through pins, and immersed in a glass container comprising 100 mL of release medium (PBS 7.4) The container remained placed in a horizontal shaking incubator at 37°C and 100 rpm. About 1.0 mL of the release medium remained reserved at various time intermissions, and the quantity of sample was determined from the UV absorption at 337 nm. During the early 8 h of sampling, the reserved release medium remained substituted by fresh buffer. Later, release medium remained totally substituted each time through fresh buffer subsequently sample collection.

% Cumulative release = Amount of micelles in the medium (g)/ Amount = μ t of micelles loaded in the micelles (μg) × 100.[17]

### 3.6 CMC Study

**Table 3: CMC Study**

| Conc. | Abs. |
|---|---|
| 0.001 | 0.949 |
| 0.002 | 2.512 |
| 0.003 | 2.912 |
| 0.004 | 3.898 |
| 0.005 | 3.656 |
| 0.006 | 3.898 |
| 0.007 | 3.898 |
| 0.008 | 3.898 |
| 0.009 | 3.898 |

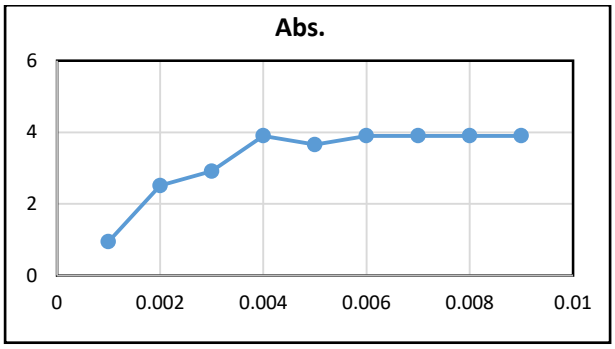


**Figure 5: CMC Study of optimized DOX-SOL Nanomicelles**

Around 0.5 g of iodine ($I_2$) and 1.0 g of potassium iodide (KI) were dissolved in 50 mL of DI water to prepare the KI/$I_2$ standard solution. Various concentrations (in the range of 0.00001%–0.1%) of the overhead combination of polymers were prepared. Twenty-five microliters of KI/$I_2$ standard solution were added to each of the polymer combinations of various concentrations. The combinations were left in the dark at room temperature for 12 h and formerly the absorbance remained measured at 366 nm. Each experiment remained repetitive three times and the average absorbance remained determined. The absorbance and the logarithm of polymer concentration were plotted. The CMC value of the polymer mixture resembles to the polymer concentration once a quick rise in the absorbance was detected.[18]

### 3.7 FTIR

The FTIR spectrum of bulk Doxofylline showed sharp characteristic peaks as presented in Figure 8.4. The observed peaks are listed in Table 1. The obtained peaks are in full support of the given chemical structure of the drug.

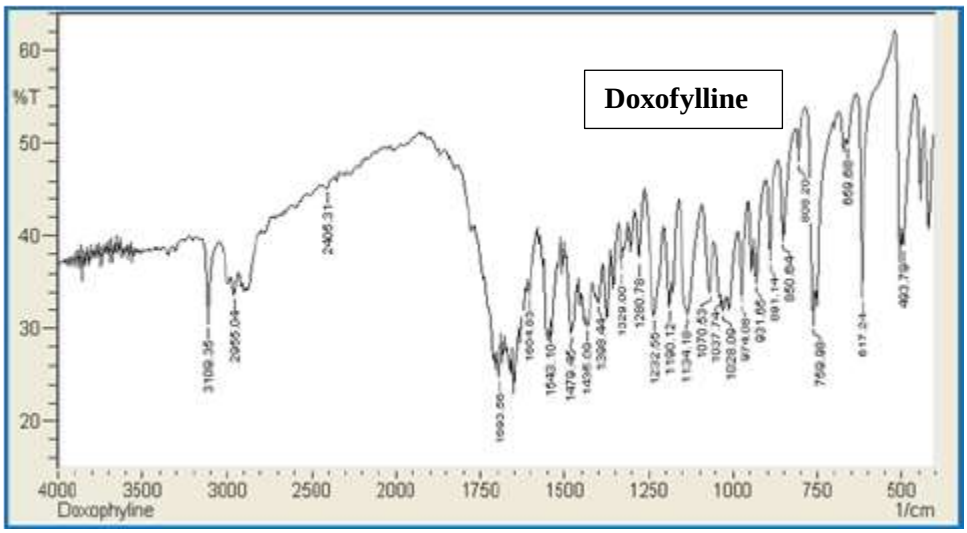


**Figure 6: FTIR of Doxofylline**

**Table 4: FTIR spectra of Doxofylline**

| Observed Peak | Reported Peak | Interpretation of Functional group |
|---|---|---|
| 3109.35 | 3000-3700 | O-H |
| 1693.50 | 1600-1700 | C-C |
| 1543.10 | 1500-1700 | N-H |
| 2955.04 | 2700-3300 | C-H |

The FTIR spectra of Doxofylline are presented in Figure 4. The characteristic peaks of the pure Doxofylline were observed in the FTIR spectra of doxofylline indicating the different functional groups of Doxofylline from 3109 to 1693.

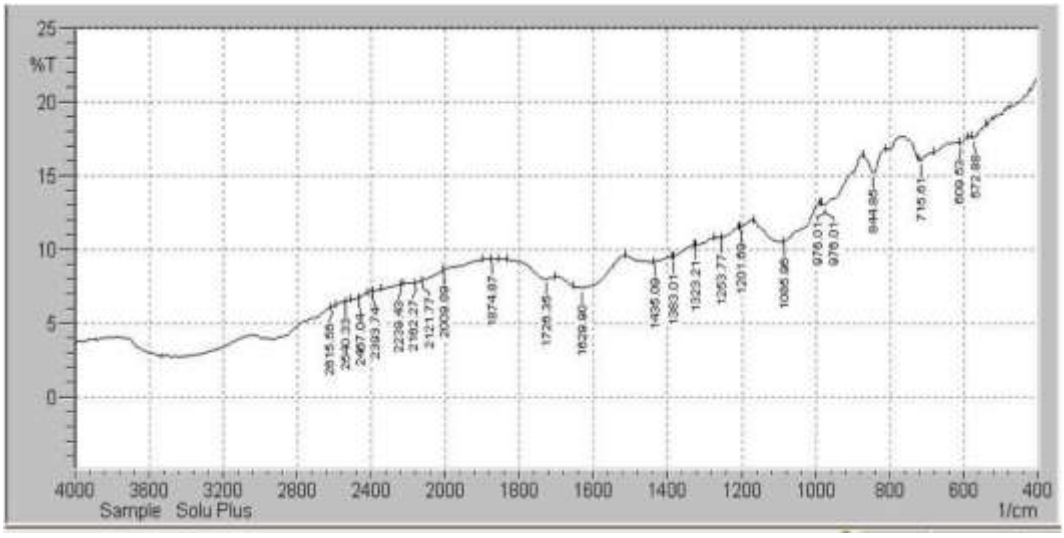


**Figure 7: FTIR of Soluplus**

**Table 5: FTIR spectra of Soluplus**

| Observed peak | Reported peak | Interpretation of functional group |
|---|---|---|
| 3109.35 | 3000-3700 | O-H |
| 1543.10 | 1500-1700 | N-H |
| 2955.04 | 2700-3300 | C-H |

The FTIR spectra of Soluplus are presented in Figure 5. The characteristic peaks of the pure Soluplus were observed in the FTIR spectra of Soluplus indicating the different functional groups of Soluplus from 3109 to 2955.

### 3.8 DSC

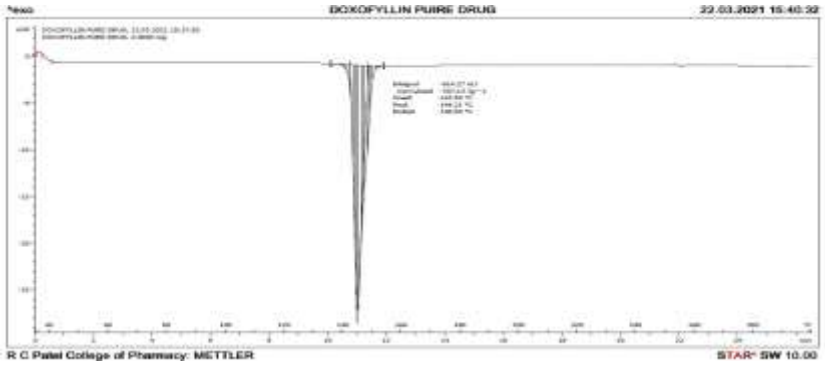


**Figure 8: DSC Graph of Soluplus**

The thermal event of Doxofylline arises in the range of 144-148 °C, which accounts for the evaporation of water and succeeding weight loss. The weight loss as a significance of evaporation of water appears to be contingent on the number of charge sites existing on the polymer chains. The complete thermal decomposition of Doxofylline, is accomplished at temperatures above 148 °C. Differential Scanning Colorimetry is performed by Star software on Differential Scanning Calorimeter (Mettler). Expriment was performed on tempreture range 200-400. Melting point was obtained on 144-148 °C.[19]

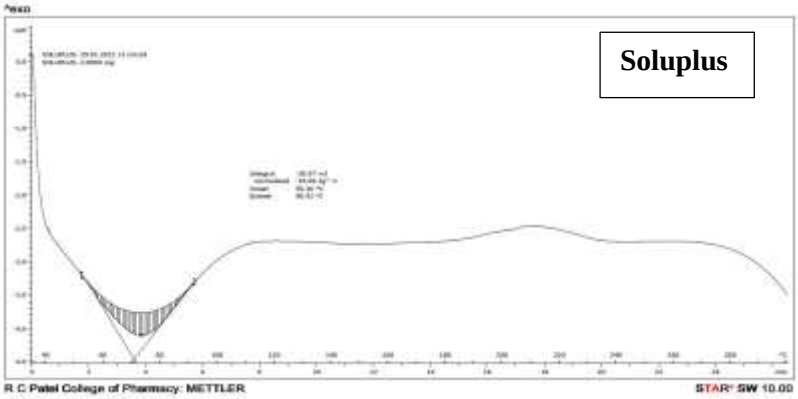


**Figure 9: DSC Graph of Soluplus**

The thermal event of Soluplus arises in the range of 55-90 °C. The thermal event of Doxofylline occurs in the range of 55-90 °C, which accounts for the evaporation of water and succeeding weight loss. The weight loss as a significance of evaporation of water appears to be contingent on the number of charge sites existing on the polymer chains. The complete thermal decomposition of Soluplus, is accomplished at temperatures above 90 °C. Differential Scanning Colorimetry is performed by Star software on Differential Scanning Calorimeter (Mettler). Expriment was performed on temperature range 200-400. Melting point was obtained on 55-90°C.[19]

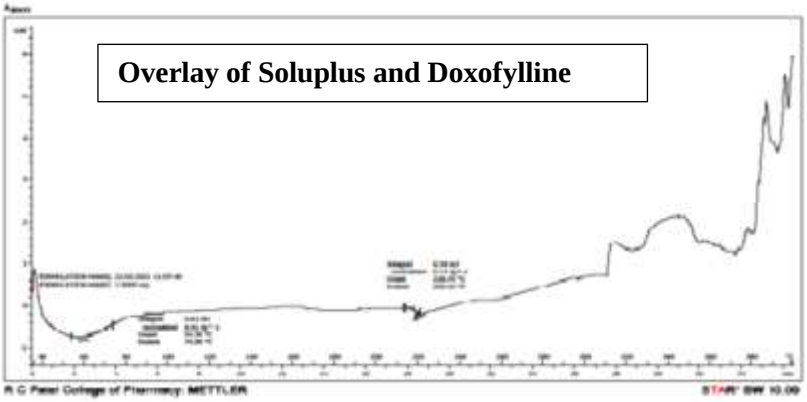


**Figure 10: DSC Graph of Drug & Polymer**

The initial thermal event arises in the range of 53-74 °C for Soluplus, which significant for the disappearance of water and succeeding weight loss. The weight loss as a significance of disappearance of water seems to be contingent on the number of charge sites existing on the polymer chains. Another thermal event arises in the range of 144-148 °C for Doxofylline.[19]

### 3.9 SEM

The scanning electron microscope (SEM) usages an attentive beam of high-energy electrons to produce a variability of indications at the surface of solid specimens. The signals that originate from electron-sample interactions disclose data about the sample with external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most

applications, information are composed over a designated area of the surface of the sample, and a 2-dimensional image is produced that shows spatial differences in these possessions. Areas ranging from around 1 cm to 5 microns in width can be imaged in a scanning mode by means of conventional SEM techniques (enlargement ranging from 20X to around 30,000X, spatial resolution of 50 to 100 nm). The SEM is also accomplished of execution investigates of designated point locations on the sample.[20] Morphological features, as demonstrated by SEM analysis, revealed the high crystallinity of Doxofylline Soluplus Nanomicelles.

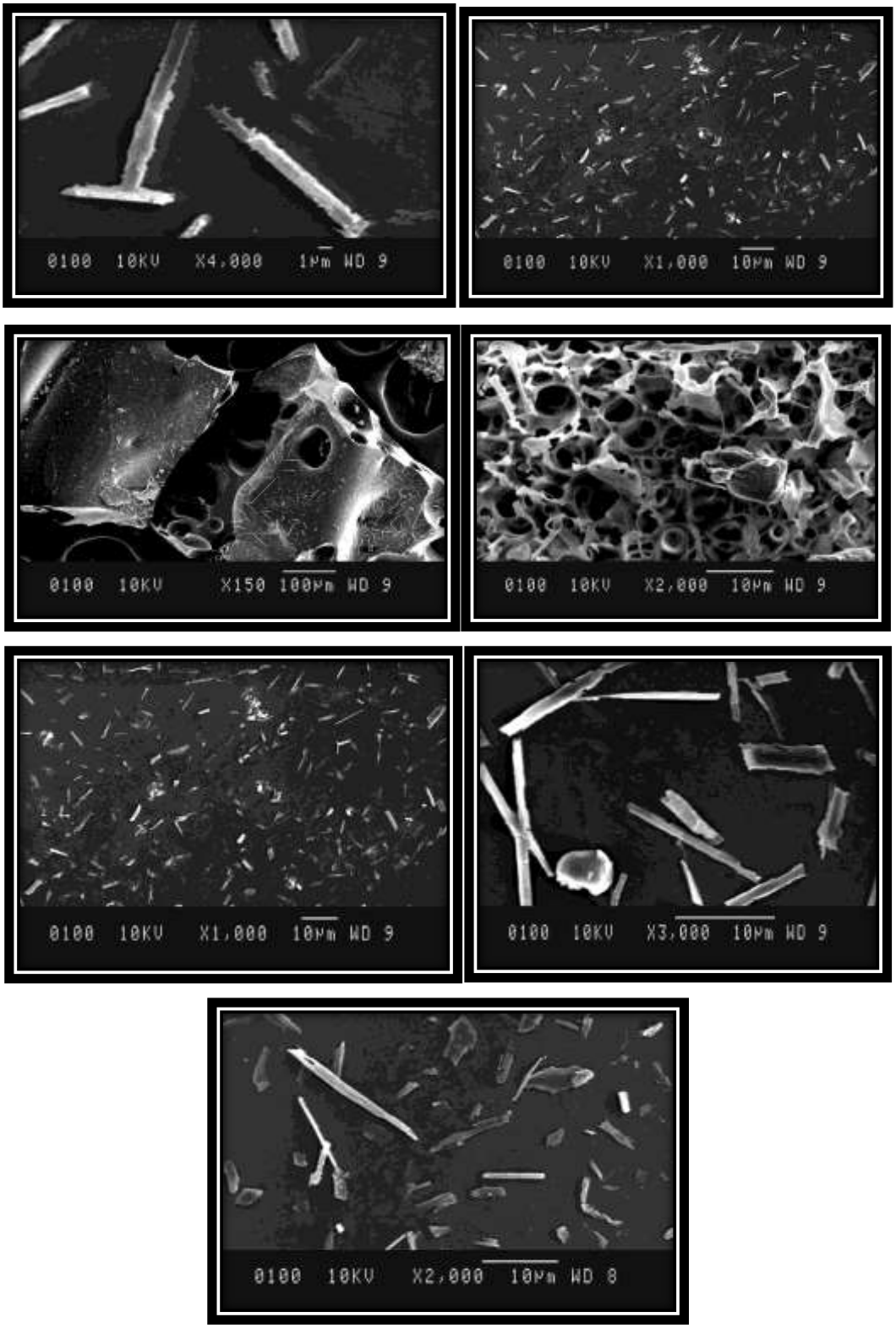


**Figure 11: SEM Images of Optimized Micelles**

### 3.10 Andersen cascade impaction Studies

*In vitro* drug release study of Doxofylline micelles was carried out with the help of Cascade Impactor, where it shows controlled drug release and Drug specificity in targeted organ.

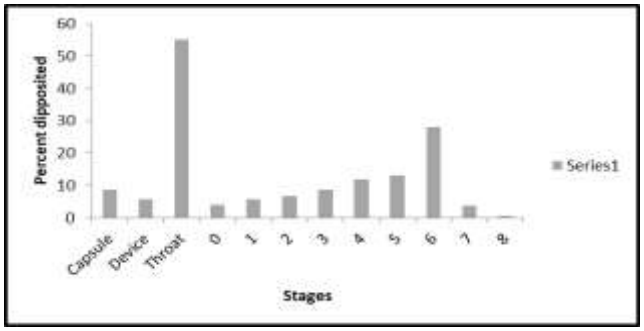


**Figure 12: Anderson Cascade Impactor**

Aerosol particle size is a key parameter for forecasting aerosol statement in the airlines of the lungs. Numerous methods of particle sizing can be used, cascade impactors and lasers existence the most common. Cascade impactors offer the major benefit of measurement the aerodynamic diameter of the drug studied.[21]

## 4. CONCLUSION

The pneumonic course of organization is a non-intrusive, fast and successful way to deal with the conveyance of therapeutants at the nearby and foundational levels. Breathed in drug treatment is for the most part utilized locally to treat aviation route infections, like asthma and bronchitis and constant obstructive pneumonic illness (COPD). Then again, inward breath additionally offers an incredible potential for foundational conveyance on the grounds that the lungs have a gigantic surface region accessible for assimilation, and, bountiful vasculature. In this context, herein doxofylline-loaded soluplus micelles formulation intended for pulmonary administration of doxofylline, as anti-asthmatic agent. The developed micelles, are found to be robust, stable on long-term storage, and safe for pulmonary administration. The experimental findings presented herein fulfill all the objectives set before the study. The developed micelles formulation could reach the shelf of commercial market, provided it should further be investigated extensively for pre-clinical lung distribution studies, lung deposition and permeability studies, and cellular uptake analysis.

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**Conflicts of Interest:** The authors declare that there is no conflict of interest.

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