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Research Article

FORMULATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES DRY POWDER INHALER CONTAINING TRIAMCINOLONE ACETONIDE

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ABSTRACT

Solid lipid nanoparticles (SLNs) introduced at the beginning of the 1990s represents an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro and nanoparticles. A number of route of administrations such as topical, oral, parenteral, nasal and pulmonary have been proposed for the delivery of SLNs. SLNs formulae were utilized for the release of Triamcinolone acetonide (TAA) via respiratory tract for the delivery of the drug to the lung for treatment of allergic rhinitis, an excellent alternative to their peroral application, because the dose and the incidence of local and systemic side effects can be reduced. SLNs were prepared by solvent emulsification – solvent diffusion technique using soya lecithin (Lipoid S100) as the lipid parts and poloxamer188 as surfactant. Different formulation parameters; selection of types of emulsifier, drug: phospholipid, drug: phospholipid: emulsifier, organic phase: aqueous phase ratio was studied with respect to particle size and drug entrapment efficiency. Results showed that Batch15 (B15) with composition of 1% TAA, 5% soya lecithin, which were stabilized by 1 % poloxamer188, was considered the optimum formulae as they combined small particle sizes and relatively high encapsulation efficiencies. B15 had a particle size of 339.2 ±1.859 nm with a zeta potential value of -14.7 ± 0.384 mV and an encapsulation efficiency of 58.23±1.8 %. DSC results suggested the existence of the lipids in the solid amorphous form.

Keywords: Triamcinolone acetonide, Solvent emulsification- solvent diffusion method.

INTRODUCTION

Drug delivery to the lungs using dry powder inhalers has attracted scientific and biomedical attention in recent years. [1-4] It has many advantages over other sites of administration because it has a fast onset of action, high bioavailability, avoidance of the first-pass effect, local action for pulmonary diseases, and convenience to patients when administered.[4-5]

Triamcinolone acetonide (TAA) is a glucocorticoid and is efficient in the treatment of asthma, allergic rhinitis and other inflammatory diseases. However, there is a tendency to reduce its use due to its complicated pharmacokinetic characteristics.[6] The anti-inflammatory actions of TAA are thought to involve lipocortins, phospholipase A_2 inhibitory

proteins which, through inhibition of arachidonic acid, control the biosynthesis of prostaglandins and leukotrienes. The immune system is suppressed by corticosteroids due to a decrease in the function of the lymphatic system, a reduction in immunoglobulin and complement concentrations, the precipitation of lymphocytopenia, and interference with antigen-antibody binding. TAA was used in this study as a model drug. Improvement of tissue distribution and targeting of drugs by using SLNs have been reported for some drugs including glucocorticoids.

The administration of glucocorticoids in dry powder aerosol form is one of the proposed strategies that can be adopted to reduce doses and, consequently, to reduce the side effects. [7-10] Because inhaled powders work locally in the lung as a common site of action, lower doses are needed to achieve the same therapeutic effect as the oral doses.[12-17] The aerodynamic diameter has been used for several decades to measure the intrinsic tendency of aerosol particles to deposit in the lungs, due to their shape, density, and geometric size.[18,23,24] In several studies, the most favorable particle size of aerosol particles was determined for several different drugs when given to patients. The optimal size of the particles for deposition in the lungs was found to be in the 1–5 μm range.[17,25-29] For porous particles, which

Preparation of SLN DPI of TAA [38-41]

Materials

Triamcinolone acetonide (TAA) was obtained from Zydus Cadila (Ahmedabad, India), Dynasan114 was provided by Hu¨ ls AG (Witten, Germany) and Gelusire50/13 by Gattefosse´ (Weil AR, Germany), soya lecithin was provided by Lipoid KG (Ludwigshafen, Germany), used as lipid matrix. Poloxamer188, used as emulsifier, was a gift from BASF AG (Ludwigshafen, Germany). Dichloromethan, acetone ethanol and methanol were purchased from Finar chemicals (Ahmedabad, India).

Preparation of SLN DPI of TAA [38-41]

have a low density, their aerodynamic diameter can be smaller than their corresponding geometric diameter, therefore they can be used to improve drug deposition in peripheral regions.[31-34] There has been huge success in processing dry powder inhalers using a range of drug substances, including nifedipine, ciprofloxacin, tacrolimus, and budesonide.[35-37] To the best of our knowledge, this is the only study demonstrating the use of solid lipid nanoparticles of TAA as a dry powder for inhalers.

Therefore, the aim of the present investigation was to prepare and evaluate stable Triamcinolone acetonide solid lipid nanoparticle dry powder inhalation for high pulmonary deposition, thereby enhanced accumulation and absorption to a particular area like bronchi and bronchioles, thus improving therapeutic efficiency. A solvent emulsification - solvent diffusion method was chosen and was optimized to obtain SLNs with low particle size and relatively high encapsulation efficiency as well as a consistent release profile. The solid lipid nanoparticle agglomerates produced were found to have excellent inhalation properties and improved physicochemical properties compared with the unprocessed drug.

MATERIAL AND METHODS

Solid lipid nanoparticles loaded with Triamcinolone acetonide (TAA) were prepared by spontaneous emulsification–solvent diffusion (SESD) method (Fig1). To the SLN dispersion, sucrose 2% w/v was added as a cryoprotectant. The SLN dispersion was filled in 30 ml vials and sealed with muslin cloth. Lyophilization was done by manifold process. Lyophilization was then carried out for 24 hours. Firstly, prefreezing was done by freezing the mixture at −80°C and than vials was kept in adapter. The adapter was than fit into lyophilizer and dispersion was lyophilized for 24 hours in a laboratory freeze-drier VIRTIS keeping vacuum at 50 -

60mTorr and condenser temperature -60ºC for 24 h.

Preliminary Optimization

Selection of Lipid

Different types of lipids, Dynasan – 114, Gelucire – 50/13, Soya lecithin were tried:

Two types of studies were carried out for selection of lipids.

1. Solubility of drug in lipid
   For that study, 1000 mg lipid was melted and 5 mg drug was added and check the solubility of drug in lipid. Drug (TAA) was soluble in soya lecithin and Gelucire but not in Dynasan.
2. Partition-coefficient study
   1000 mg lipid and 5 mg drug was melted in glass vial.5 ml distilled water, previously heated at the same temperature was added. Mixtures were stirred for 30 minutes and cooled at room temperature. Aqueous phase was separated and centrifuged. Clear supernant was diluted with methanol and analyzed at 239nm. In the partition-coefficient study, the entire drug was partitioned in the aqueous phase in case of Gelucire. So **Soya lecithin** was selected as lipid.

Selection of Surfactant

Two different types of surfactants, Poloxamer188 and Poloxamer407 were taken at the same concentration and selected on the basis of mean particle size (MPS) and % drug entrapment (PDE).

As per shown in Table1, in case of Poloxamer188, lower MPS and higher PDE were observed as compared to Poloxamer407. Therefore **Poloxamer188** was selected as surfactant and further optimization was done using the same.

Optimization of process parameter with orthogonal experimental design

In order to study the influence of experimental parameters on the preparation of SLN is based on single factor, five factors which mainly affected the entrapment efficiency were optimized, and they are following: (1) Effect of Stirring speed; (2) Effect of cooling time; (3) Effect of concentration of Drug: Phospholipid; (4) Effect of Phospholipid: Emulsifier concentration; (5) Effect of Organic phase: Aqueous phase ratio. Individual batches were design, according to single factor variation, in order to screen optimal formulation and further formulation of prescription and artwork of preparation. Based on entrapment efficiency as an evaluation index, the factors and levels of the experimental design are mentioned in Table 2.

Characterization of TAA SLNs

Determination of TAA Percentage Entrapment Efficiency

To determine drug entrapment efficiency, the freeze dried SLNs were dissolved in methanol and Phosphate buffer saline pH 7.4 (PBS) under water bath at 65ºC for 30 min and then cooled to room temperature to preferentially precipitate the lipid. Drug content in the supernatant after centrifugation (6500 rpm for 15 min) was measured at 239 nm against the blank by UV-VIS spectrophotometer (Shimadzu 1700). The drug entrapment efficiencies are calculated from following equation.

$$\%\text{Entrapment Efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug added}} * 100$$

Measurement of size and zeta potentials of SLN

The mean particle size diameter measured in solution directly after synthesis using Malvern laser light scattering Masterizer model 4700

(Malvern Instruments Ltd., UK)(Table3). TAA solid lipid nanosuspensions (2 ml) were added to the quartz cell of the photon correlation spectroscope. Measurements were taken at 90° opposite the incident light source and mean droplet size was calculated from intensity. Zeta potential values were measured by a Zetasizer 2000 (Malvern instrument Ltd., Malvern, U.K.). Samples were placed in clear disposable zeta cells and results were recorded (Table3).

Solid state characterization of TAA SLN Dry powder

Angle of repose (θ) was calculated by forming a pile that was carefully built up by allowing the powders to fall through a funnel until the tip of the funnel was 2 cm apart. The angle of repose was calculated using following equation results are shown in Table4:

$$\tan \theta = h/r$$

where, height (h) and the radius (r) of the pile formed.

Both Bulk density (BD) and tapped density (BD) was determined. A quantity of 2 gm of API powder was introduced in to 10 ml measuring cylinder. After that the initial volume was noted and the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at second intervals. Tapping was continued until no further change in volume was noted. BD and TD were calculated using the following equations results are shown in Table4.

BD = Weight of powder blend / Untapped volume of the packing

TD = Weight of powder blend / Tapped volume of the packing

The Compressibility Index of the powder blend was determined by Carr's compressibility index. The formula for Carr's Index is as below and results are shown in Table 4

Carr's Index (%) = [(TD-BD) x 100] / TD

Particle morphology

Particle morphology of the TAA SLN-DPI is performed by Scanning Electron Microscopy (SEM) for determining the surface morphology, size and shape of formulation and to observe the aggregation property of SLN with carrier particles.

DSC Studies

TAA powder, Poloxamer188, soya lecithin, TAA SLNs (4 mg) were sealed in the flat-bottomed aluminum pan of the differential scanning calorimeter (DSC TA-60WS, Shimadzu, Japan). A standard empty pan was inserted along with each pan to account for the heating of pure aluminum. The sample and the blank were continuously purged with nitrogen gas at a flow rate of 25 mL/min. Data collection was carried out at a temperature range of 20–320°C, and the heating rate was 10°C/min. The melting and transition point measurements were performed using the software provided with the device.

Aerodynamic characterization

Aerosol performance of formulations was assessed using an eight stage, nonviable Anderson Cascade Impactor with a preseparator (Graseby-Andersen, Atlanta, USA) From drug deposition data the emitted dose, fine particle dose, fine particle fraction, % emissions were calculated according to USP 27 NF 22.

In Vitro Drug Release

In vitro release was evaluated by using a dialysis bag diffusion technique under sink condition. The drug release from TAA-SLNs was performed in Phosphate buffer saline (PBS) (pH 7.4) using dialysis diffusion bag. TAA-SLNs suspension was placed in dialysis bags (MWCO 12000 Da, Sigma Aldrich) and the dialysis bags were subsequently placed in flasks containing 25 ml dissolution medium (PBS pH 7.4) and stirred at 100 rpm in a 37°C water bath. Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to the flask to maintain a constant volume. Drug concentration in the dissolution medium was determined using UV/Vis Spectrophotometry (Shimadzu 1700) at 239 nm. All experiments were carried out in triplicates. Results are expressed in table 3. The release profile of TAA from SLNs along with the mechanism of the drug release found by inserting the data of TAA-SLNs to curve fitting data of various equation like zero order, first order, Higuchi release and Korsmeyer-peppas release, Hixson Crowell release mechanisms.

Stability Studies

For both SLNs dispersion and lyophilized SLNs, were stores at refrigerated condition (2-8ºC) and at room temperature, 25°C ±

2°C (ambient) for 1 month and assay were evaluated immediately after production of the SLNs and during one month (after 7, 15 and 30 days) of storage at different temperature conditions. The final formulations were also examined visually for the evidence of caking and discoloration.

RESULTS AND DICUSSION

Optimization of process parameter with orthogonal experimental design

The lowest Mean Particle Size (MPS) and highest %EE (PDE) were found at 1800 RPM stirring speed and 15 minutes cooling time. So 1800 RPM as stirring speed and 15 minutes as cooling time were optimized (Table2). That formulation were considered to explore further for investigation to see the effect of concentration of lecithin (1% to7 % w/v), drug concentration was keeping constant as 10 mg (1% w/v) and concentration of poloxamer188 (1%, 2% and 3% w/v) in SLNs of TAA shown in Table2. Further all the formulation were named with code of B11 to B23, again based on entrapment efficiency of individual formulation. Most efficient formulations were found to be B15 with concentration of soya lecithin (5%), with drug concentration (10%), B18 with concentration of poloxamer188 (1%), B22 with organic to aqueous phase ratio (1:2). All the formulation (B15, B18 and B22) with B3 considered for formulation of SLNs. In all the formulation, the entrapment efficiency was found within a range of 50-60%. Accordingly we choose the optimized formulation to prepare the TAA loaded Solid lipid Nanoparticles. The experimental design indicates that concentration of Soya lecithin and poloxamer188 are two important factors other than drug concentration affecting entrapment efficiency. An average entrapment efficiency of was achieved in TAA loaded SLNs with optimized formulation

Particle Morphology

SEM image of SLNs B15 were presented in Fig2 (A, B, C). It was clear from image A that TAA loaded SLNs were spherical in shape with the presence of some particle aggregates. The presence of aggregates might be attributed to a short redispersion time after centrifugation and drying at room temperature. The sizes observed from SEM micrographs were slightly higher than those obtained from particle size analyzer. Micrographs B & C showed irregular surface of single articles under high magnification.

DSC Study

DSC experiments are useful to understand solid dispersions like solid solutions, simple eutectic mixtures, or, as in this case, drug and lipid interactions. It is a tool that gives an insight into the melting and recrystallization behavior of crystalline materials like SLNs.26,42-44 The DSC thermograms of pure TAA, Poloxamer188, soya lecithin as well as optimized SLN formulation were shown in Fig.3. Thermogram of Triamcinolone acetonide showed exothermic peak of melting a 284.75°C and endothermic peak of melting at 290.25°C. The thermal curves of Poloxamer188 and Soya lecithin showed endothermic peaks at 53.72°C and 125.75°C, respectively.

The melting endothermic peaks of these emulsifier and lipid in the prepared SLNs were slightly shifted to a lower temperature (50.40°C and 120.50°C). The decrease in melting temperature of glyceride lipid nanoparticles compared with bulk lipids has been attributed to their small size (nanometer range), the dispersed state of the lipid, and the presence of surfactants [45,50,51]. Furthermore, the lower melting enthalpy values should suggest less ordered lattice arrangement of the lipid within nanoparticles compared to the bulk materials.[47,49] DSC thermograms of TAA - loaded SLNs also show depressed peaks of TAA compared to the pure drug, which suggests that TAA could be present in an amorphous form in the SLNs; this behavior is expected to improve the solubility of the drug in water resulting in a better bioavailability.[52,53,54]

Aerodynamic characterization

% FPF is the major criterion, which defines the efficacy of the DPI in lungs. Higher the

% FPF, higher will be the lung deposition of the DPI.[26,30,31] The % FPF values of the SLN DPI formulations prepared using sucrose as cryoprotectant were found to be highest than prepare using lactose and mannose (Table5). The % FPF values of batch TS is 36.41 ±2.330.

% Emission is the criterion, which shows the amount of DPI emitted from the inhalation devices. It can also be used for the determination of efficiency of the inhalation device. More will be the % Emission better will be the DPI deposition and less will be the loss in device.[34-37] Similar results were also found in this case, % Emission values of batch TS is 73.12 ±2.034 (Table5).

In Vitro Drug Release

In vitro dissolution characteristics of Triamcinolone acetonide (TAA) of other reported article showed higher release. In this work at the 2nd hour less than 20% and at 24thhour less than 60% TAA release from the SLNs. The drug release from TAA-SLNs was found in controlled manner, these data were shown the lipophilicity of TAA and effectiveness of SLNs, and all the data were summarized in Table6**.** The results obtained until 2h for the in vitro drug release study were not considered because the of burst effect that do not correspond to the real mechanism of the drug release from SLNs.[39-41] Although the significance of burst release in controlled delivery systems has not been entirely considered, no successful theories have described the phenomenon yet. Therefore a thorough understanding of the burst effect in controlled release systems is undoubtedly necessary. From the comparative *in-vitro* study of TAA Suspension and TAA SLNs, conclude that Triamcinolone acetonide suspension followed the Peppas-Korsemeyer model because R^2 value (0.9909) was nearer to 1, indicating that there were linear correlation exits between Log % drug release and Log time. This type of drug release was controlled by combination of polymer swelling, erosion and diffusion through the hydrated matrix (Diffusion and chain relaxation).[48-52] The Hixson- Crowell equation is semi empirical equation to describe drug release from polymeric system. The regression coefficient of the plot of %drug release versus cube root of time was found to be 0.9776 for TAA SLNs. It indicates that the release of Triamcinolone acetonide from SLNs followed drug release mechanism that drug get released by constantly changing surface area.[37,39,40] In both the formulations, 'n' value was found to be between $0.5 < n < 1$ indicating non-fickian release mechanism.

Stability of SLN

From the Table7, it was found that the particle size of SLNs dispersion increased in both the cases; at refrigerated condition and also at room temperature. However increase in particle size of lyophilized SLNs was found to be relatively less than that of SLNs dispersion. In case of % Assay, the results showed that at room temperature % Assay was reduced faster in comparison to refrigerated condition. So it was found that lyophilized SLNs were more stable when stored at refrigerated conditions. Thus to avoid increase in PS and reduction in % Assay, SLNs dispersion should be lyophilized and lyophilized SLNs should be stored at refrigerated condition.

CONCLUSIONS

In the present investigation, an attempt was made to enhance the bioavailability of TAA in the treatment of Allergic rhinitis by preparing SLNs. The SLNs were prepared by spontaneous emulsification- solvent diffusion technique using Soya lecithin as lipid matrix and Poloxamer188 as surfactant. The in vitro release tests confirmed that in case of TAA SLNs the drug was released in a sustained manner. These types of release profiles of TAA SLNs resemble the drug enriched core model. In such a model, the drug enriched core is surrounded by a practically drug-free lipid shell. Due to the increased diffusional distance and hindering effects by the surrounding solid lipid shell, the drug has a sustained release profile. In case of stability study, increase in particle size and reduction in % Assay was faster at

room temperature in comparison to refrigerated condition. Thus to avoid this problems, SLNs dispersion should be lyophilized and lyophilized SLNs should be stored at refrigerated condition. Finally based on above results, solid lipid nanoparticles are suitable carriers for incorporating Triamcinolone acetonide. However, the findings of this investigation can only be settled after animal models experimentation followed by an extensive clinical evaluation.

Table 1: Selection of Surfactant

Types of surfactant	Conc. of surfactant	Drug: lipid	Particle size (nm)*	PDE
Poloxamer 407	1%	1:5	435.7±1.290	41.68±0.521
Poloxamer 188	1%	1:5	362.4±1.041	52.53±0.792

Table 2: Optimization of Stirring speed, Cooling time, Drug: Lipid, Phospholipid:Emulsifier, Organic phase: Aqueous phase

Batch	Parameter	PDE (nm)*
Effect of Drug: Lipid ratio		
B11	1:1	23.62±1.8
B12	1:2	37.90±1.2
B13	1:3	43.12±2.9
B14	1:4	52.55±2.8
B15	**1:5**	**58.23±1.8**
B16	1:6	58.57±2.1
B17	1:7	58.89±2.6
Effect of Phospholipid: Emulsifier ratio		
B18	**1:5:1**	**58.63±1.4**
B19	1:5:2	52.21±1.7
B20	1:5:3	47.39±1.9
Effect of Organic phase: Aqueous phase ratio		
B21	1:1	47.28±2.3
B22	**1:2**	**56.34±2.7**
B23	1:3	51.68±2.8

Stirring speed (RPM)	Batch No.	Cooling time (min.)	Average size* (nm)	PDE*
1800	B1	5	585±1.519	53.23±0.534
	B2	10	478.4±2.902	54.94±0.818
	B3	**15**	**410.7±0.867**	**58.83±0.840**
1500	B4	5	579.9±0.674	48.37±0.383
	B5	10	495.1±1.553	47.58±1.172
	B6	15	434.6±1.311	46.34±0.612
1200	B7	5	648±1.126	38.82±0.932
	B8	10	526.5±1.721	40.29±0.610
	B9	15	470.9±0.721	44.04±0.853

Table 3: Particle Size and Zeta Potential of Optimized SLN Batch

	TAA Suspension	TAA lyophilized Powder
Particle size(nm)	339.2 ±1.859	425.4 ± 2.021
Zeta potential (mV)	-14.7 ± 0.384	-15.3 ± 0.427

Table 4: Solid State properties of DPI

Sample	Angle of Repose (°)	Bulk Density (g/ml)	Tapped Density (g/ml)	Compressibility Index (%)
DPI	26.23	0.213	0.286	25.52

Table 5: *In Vitro* Characterization of Aerosol Performance

Batch	Fine Particle Fraction (%FPF)	Fine Particle dose (µg)	% Emission
TS	**36.41± 2.330**	**66.55± 2.403**	**73.12± 2.034**
TL	30.64± 1.551	54.04± 3.356	70.55± 1.514
TM	35.82± 3.857	65.94± 2.924	73.64± 2.44

Table 6: *In Vitro* Drug Release of Plain TAA suspension and TAA SLN

Time (hr)	Cumulative% Drug Release*	
	Plain drug suspension	Formulation
1	14.53±0.578	6.13±0.324
2	30.53±0.202	8.90±0.249
3	46.07±0.388	11.78±0.448
4	69.4±0.551	15.71±0.240
5	72.92±0.594	21.32±0.500
6	85.31±0.394	23.11±0.301
7	93.8±0.472	27.16±0.434
9	-	33.60±0.369
11	-	38.02±0.245
23	-	48.24±0.074
24	-	50.49±0.588

	Linear Correlation Coefficient (R^2)	
	TAA suspension	TAA SLNs
Zero order	0.9761	0.8936
First order	0.9427	0.9412
Higuchi model	0.9862	0.9764
Peppas-Korsemeyer model	**0.9909**	0.9726
Hixson Crowell model	0.9763	**0.9776**

Table 7: Stability Data of TAA SLN Dispersion & TAA Lyophilized SLN

	SLN Dispersion				Lyophilized SLNs			
	Particle size (nm)		% Assay		Particle size (nm)		% Assay	
	At Refr. Temp	At Room Temp.	At Refr. Temp	At Room Temp.	At Refr. Temp	At Room Temp.	At Refr. Temp	At Room Temp.
Initial	339.09 ± 1.859		100%		425.82 ± 1.72nm		100%	
After 30 Days	435.69 ± 2.99	487.65 ± 1.56	94.72 ± 0.80	92.6 ± 0.227	465.22 ± 4.22	485.38 ± 3.25	97.57 ± 0.021	95.87 ± 0.082

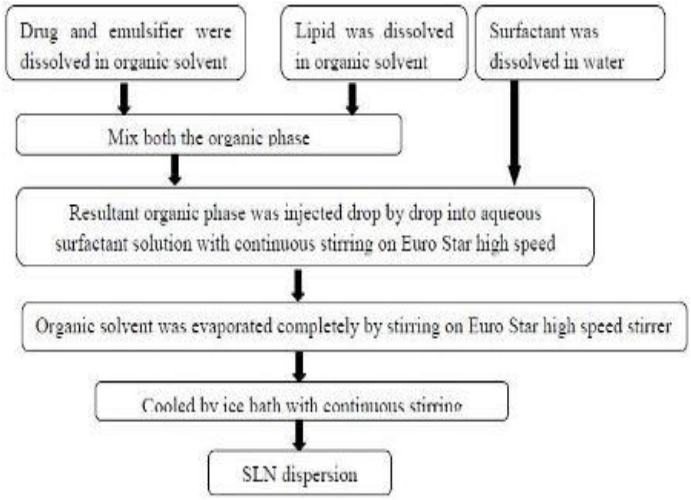

Fig. 1: Schematic Procedure of SESD Method for SLNs Production

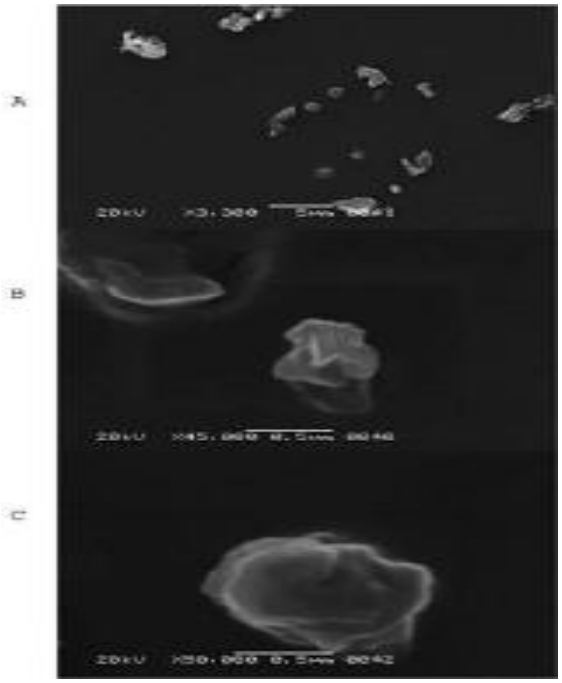

Fig. 2: Scanning electron microscopy photomicrographs for B15 SLNs: A, a field containing different particle sizes using 3,300 X magnification power, B, a field showing two single particles using 45,000 X magnification power, and C, a field containing single particle using 50,000 X magnification power.

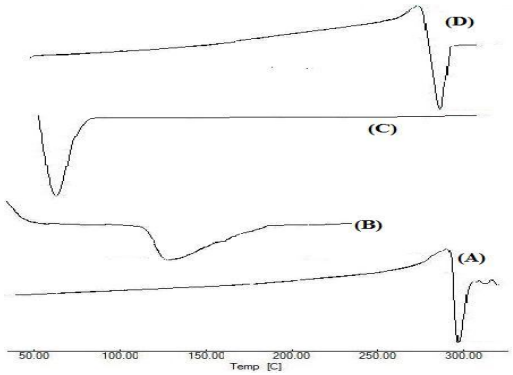

Fig. 3: Differential Scanning Calorimetry Thermograms of (A) TAA, (B) Soya lecithin, (C) Poloxamer188, (D) TAA SLN.

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