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De novo synthesis of solid lipid nanoparticles (SLN) by double emulsion method and detailed biophysical characterization

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Abstract
Double emulsions are liquid dispersion systems known also as emulsions of emulsion. Attractive interest on double emulsions comes from their unique morphology enabling them to act as multifunctional carriers. Double emulsions can encapsulate both hydrophilic and lipophilic molecules in the same particle. The aim of the study was to synthesize solid lipid nanoparticles (SLNs) applicable as drug delivery systems using stearic acid as the lipid source. The study further aimed characterize the de novo synthesized SLNs by DLS, SEM, FTIR and DSC for understanding their critical biophysical characteristics. Based on these analysis, the estimated mean size, PDI and charge of NPs were computed as 404.6 nm, 0.297 and + 28.4 mV, respectively. FTIR analysis indicate no change in the chemical structure of stearic acid in SLN. DSC analysis showed a decrease in the melting point from that of pure stearic acid. The study thus established standard procedures for laboratory synthesis of solid lipid nanoparticles followed by their biophysical characterization to prove their adequacy as multifunctional carriers including drugs.

Keywords: Double emulsion method, stearic acid, solid lipid nanoparticles

Introduction
Nanoparticles are solid colloidal particles having a size of 1–1000 nm in which the drug is physically dispersed, dissolved, or chemically bounded to the polymer chains (Hillaireau and Couvreur, 2006) [7]. Nanoparticles shows high solubility, fast penetration and widely used in many dosages form due to their good solubility, less size and better penetrability (Kumari, 2018) [1]. The key advantages of nanoparticles are 1) improved bioavailability of drug 2) targeted delivery of drugs to a specific site 3) improve the uptake of poorly soluble drugs 4) easy to formulate with chemotherapeutic agents eg doxorubicin, dexamethasone and paclitaxel 5) overcome the drug resistance by inhibiting the cell's ability to pump the drug molecule out 6) may overcome the immunogenicity and other side effects of conventional methods (Singh *et al.*, 2007) [19].
Nanoparticulate systems show their promise as a potential ideal drug delivery system for poorly soluble, poorly absorbed and chemically, heat- and photo-labile substances (Florence,1998) [5]. Nanoparticles can consist of different biodegradable materials like natural or synthetic polymers, lipid, or phospholipids (Kayser *et al*., 2005) [10].
Solid lipid nanoparticles (SLN) are colloidal systems developed as an alternative to liposomes, nano and microemulsions (Araujo *et al.*, 2020). SLNs also known as solid lipid nanospheres or lipospheres are comparatively novel class of colloidal carriers. The extremely lipophilic nature of SLNs allows them to pass through the BBB with ease. These nanostructures of size between 40 and 1000 nm are constituted by a solid lipid centre dispersed in an aqueous medium and stabilized by surfactants (Souto *et al.*, 2020) [20]. Advantages of SLNs include high bioavailability, biodegradability without formation of any toxic degradation product, good tolerance, physical and biotical stability, both hydrophilic and hydrophobic drug loading capacity, easy production, no need for organic solvents like chloroform in synthesis.
Due to the lipophilic characteristic of the lipid matrix, SLN are well suitable systems for encapsulation of hydrophobic compounds. The most important methods used for the preparation of solid lipid nanoparticles are high-pressure homogenizer and ultra-sonication but they are highly sophisticated.

Double emulsion technique is widely used to reach satisfactory loading efficiency. Advantage of double emulsion method that it is a simple, inexpensive, reproducible technique, no requiring organic solvents like chloroform and limited use of surfactants. The aim of the study was to synthesize solid lipid nanoparticles (SLNs) applicable as drug delivery systems using stearic acid as the lipid source and characterize the synthesized SLNs by DLS, SEM, FTIR and DSC for understanding their critical biophysical characteristics.

Materials and Methods

Preparation of nanoparticles

The preparation of solid lipid nanoparticles was carried out by double emulsion (W/O/W) as described by Peres *et al*. (2016) [15]. The first emulsion contained stearic acid, lecithin and pluronic F-127. Briefly, 0.6 g of stearic acid was heated above its melting temperature and mixed with lecithin under magnetic stirring at 400 rpm to form the first emulsion (W1/O). 6.0 mL of warm pluronic F-127 were dissolved in the first emulsion. Then, 6.0 mL of a warm Tween 80 aqueous solution was added to the prepared first emulsion under magnetic stirring at 500 rpm for 30 min to form the second emulsion (W1/O/W2). This double emulsion was poured into 30.0 mL of cooled Milli Q water under magnetic stirring at 800 rpm for 1 hr to promote solidification of lipid nanoparticles. The obtained SLNs suspension was stored in refrigerator at 4 °C.

Biophysical characterization of nanoparticles

Measurement of particle size and zeta potential

The nanoparticles size, polydispersity and zeta potential were measured by Dynamic Light Scattering (DLS) (Malvern Zeta Sizer Nano Series) at 25 °C and at scattering angle of 90º. The sample were prepared by diluting the nanosuspension with Milli Q water and then estimating the size and zeta potential. All measurement performed in triplicates. The zeta potential was assessed by Laser Doppler Microelectrophoresis at 25 °C using disposable folded capillary cells in a Zeta sizer (Peres *et al*., 2016) [15].

Morphology

The nanoparticles were subjected to scanning electron microscopy (SEM) (SEM-JEOL 5400, Japan) to determine their shape, size and homogeneity. Lyophilized nanoparticles were mounted onto aluminium stubs using double sided adhesive tape. Samples were then made electrically conductive by coating in a vacuum with a thin layer of gold using a Polaron SC500 Gold Sputter Coater (Quotum technologies, Newhaven, 123 UK). The coated specimen was then examined under the microscope operated at an acceleration voltage of 15 kV (Yadav *et al*., 2022) [22].

Fourier Transform Infrared Spectrophotometer analysis (FTIR)

Chemical analysis of processed stearic acid particles was performed by using Nicolet Summit LITE FT-IR spectrophotometer (Thermoscientific, USA) with KBr pellet method. About 1–2 mg of sample was mixed with dry potassium bromide and the sample were examined at transmission mode over wave number range of 4,000 to 400 cm^{-1}.The spectra analysis was carried out using Origin2022, irAnalyer and eftir.com tools (Musellim *et al*., 2018) [13].

Differential Scanning Calorimetry (DSC)

Thermal behaviour of lipid matrices was assessed by DSC (TA instruments, DSC Q20). Approximately 5-10 mg of powdered stearic acid nanoparticles, sealed in a standard aluminium pan, was purged with dry nitrogen at a flow rate of 50 mL/min while the sample was heated from 50 °C to 300 °C at a rate of 10 °C/min. An empty sealed aluminium pan with pierced lid was used as a reference (Singh *et al*., 2013) [18].

Stability study

The prepared SLNs were subjected to a stability testing for six months as per International Conference on Harmonisation (ICH) Q1A guidelines (Rawal *et al*., 2017) [17]. The freshly prepared nanoparticles were transferred in amber colored glass vials, sealed with plastic tape and stored at refrigerated condition (5 °C±3 °C) and room temperature (25±2 °C/60±5% RH) up to six months. The preparations were characterized with respect to particle size and PDI after 3 and 6 months.

Results and Discussion

SLNs are prepared from lipid, emulsifier, water/solvent and different approaches exist for the production of finely dispersed lipid nanoparticle dispersions. The performance of SLNs greatly depends on the method of preparation which in turn influences the particle size, drug loading capacity, drug release, drug stability etc. Due to their unique structural, magnetic, mechanical and electrical properties, NPs are used in a wide range of applications including biosensing, drug and gene delivery, bio detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering etc.

The method was adopted for having advantages such as non-requirement of organic solvent, easy to adapt in laboratory and are economical (Bodmeier *et al*., 1992) [2]. On the other hand, other approaches such as high-pressure homogenization, ultrasonication, solvent emulsification evaporation methods are cumbersome and require more facilities etc (Jain, 1997) [9]. In the present study, stearic acid was used as lipid matrix for the fabrication of nano formulations. In this study we used Pluronic F68 and Tween 80 as the water surfactants and lecithin as lipid the surfactant. Earlirer studies reported a similar applicability of these surfactants and lipid matrix for obtaining stable nano formulations. Similar findings have been reported in previous study by Moze *et al*. (2023) [12].

Particle size, PDI and zeta potential

The particle size is an important parameter as it has direct effect on the particle stability (Hosseini *et al*., 2019) [8]. In present study, mean particle size, PDI and zeta potential values for SLNs were 404.6 nm, 0.297 and + 28.4 mV, respectively. Previous study reported the optimum size for SLNs were 277 – 550 nm (Peres *et al*., 2016) [15]. The polydispersity index (PDI) is degree of non-uniformity of a size distribution and also known as heterogenicity index (Nobbmann, 2014) [14]. This index is dimensionless and value of PDI ranges from 0.0 (for a perfectly uniform) to 1.0 (for a highly polydisperse). Since PDI value lower than 0.3 for lipid-based nanoparticles has been reported as ideal index (Putri *et al*., 2017) [16]. The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersions. Generally, zeta potential values range from + 30 mV to – 30 mV. As the value of zeta potential was higher

than, the stability of NPs suspensions was assured.

SEM analysis

The morphological evaluation of the particle can be visualized by transmission electron microscopy and scanning electron microscopy. In present study, SEM images shows that SLNs were in nano range size with spherical to oval shape and smooth surface (Fig. 3). The particle size of the nanoparticles was also measured, which was matched with the zetasizer data. These findings are similar to the previous study (Ghaderkhani *et al*., 2019 and Subroto *et al*., 2022) [6, 21].

FTIR analysis

FTIR is a quick and robust method to identify specific functional group of any organic compounds. There are two regions in IR spectrum group frequency region (4000 to 1300 cm^{-1}) and fingerprint region (1200 to 700 cm^{-1}). The IR spectrum of SLNs shows the peaks at 3800 to 3269 cm^{-1} due to the stretching vibration band of O–H group and asymmetrical stretching of the N–H bend. The peaks at 2849 and 2916 cm^{-1} attributed to -CH2- band asymmetric and symmetric stretching vibrations, respectively. The peak at 1636 cm^{-1} due to the presence of unsaturated (C= C) stretching vibrations. Peak in region 1507-1541 cm^{-1} due to (N-O) bend, 1386 cm^{-1} due to (C-H) bending. The absorption peaks in region 1101-1351 cm^{-1} due to carboxylic acid groups of long chain fats (Fig. 4). The chemical structure of stearic acid shows that it is composed of two important group: carboxylic head (COOH) and hydrocarbon tail. The absorption peaks at 2849 and 2916 cm^{-1} belongs to stearic acid. This indicates that there is no change in the chemical structure of stearic acid. This work supported by Zhu *et al*. (2016) [23] and Hosseini *et al*. (2019) [8].

DSC analysis

Differential Scanning Calorimetry is a suitable thermal analysis technique for determining the purity, the polymorphic forms, crystallinity and the melting point of lipids. Thermogram of SLNs shows only one pronounced melting point peak at 74.2 °C was observed. The DSC analysis of pure stearic acid showed a peak at …… which is decrease in the melting point from prepared SLNs. The lower melt point observed for processed stearic acid may be due to its lower crystallinity. Same result with our study was presented by Chokshi *et al*. (2018) [3]; and Hosseini *et al*. (2019) [8].

Stability study

In present study, the developed nanoparticles remain stable under refrigerated conditions (5±3 °C) and slight decrease in the stability was seen at room temperature (25±2 °C/60±5%RH). (Table 1).

Table 1: In present study, the developed nanoparticles remain stable under refrigerated conditions

Stability testing conditions		Mean particle size (nm)	Poly dispersity index (PDI)
Refrigerated conditions (5±3 °C)	1 month	404.8	0.299
	3 months	407.1	0.298
	6 months	409.2	0.304
Room temperature (25±2 °C/60±5%RH)	1 month	405.8	0.301
	3 months	406.4	0.304
	6 months	409.9	0.308

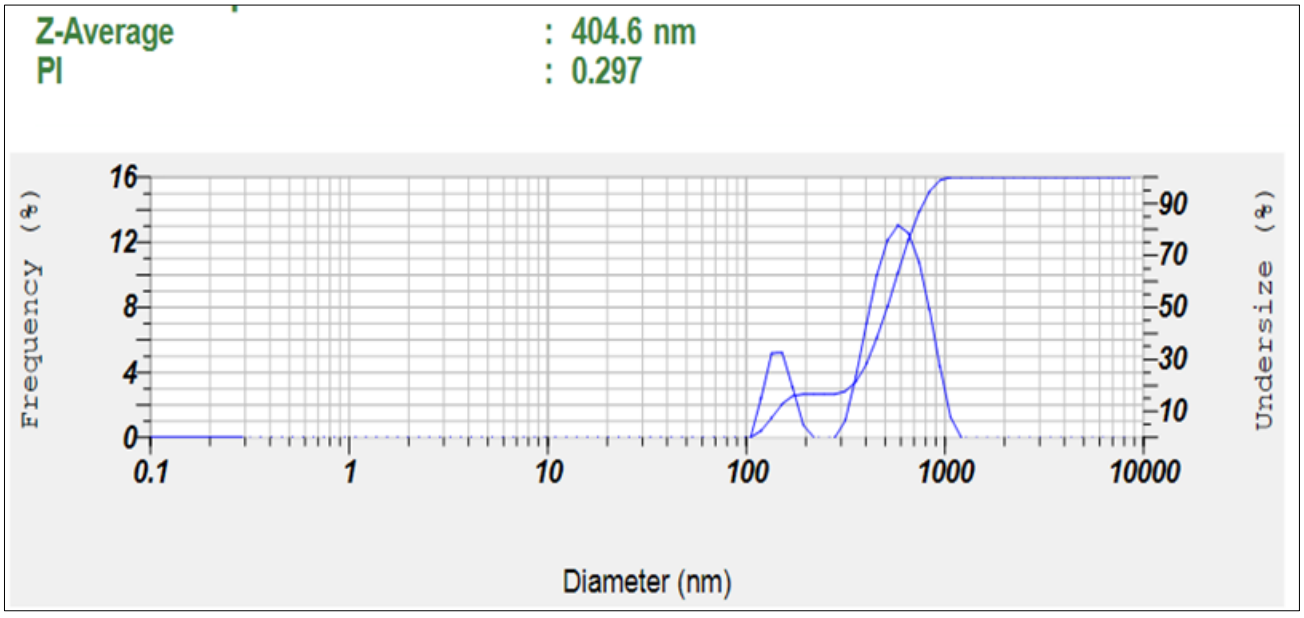

Fig 1: Analysis of particle size distribution and PDI by DLS method

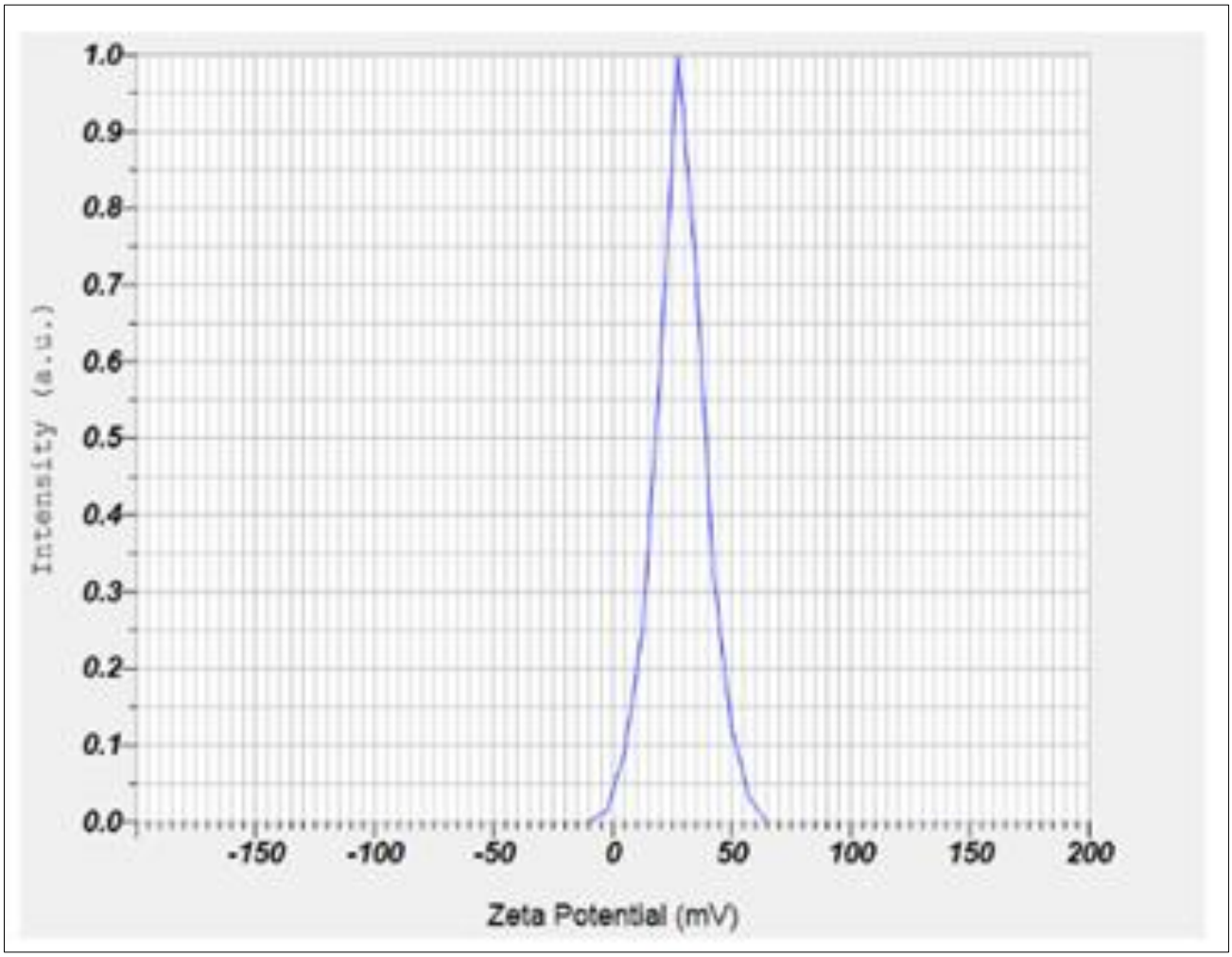

Fig 2: Analysis of particle surface charge with zeta potential

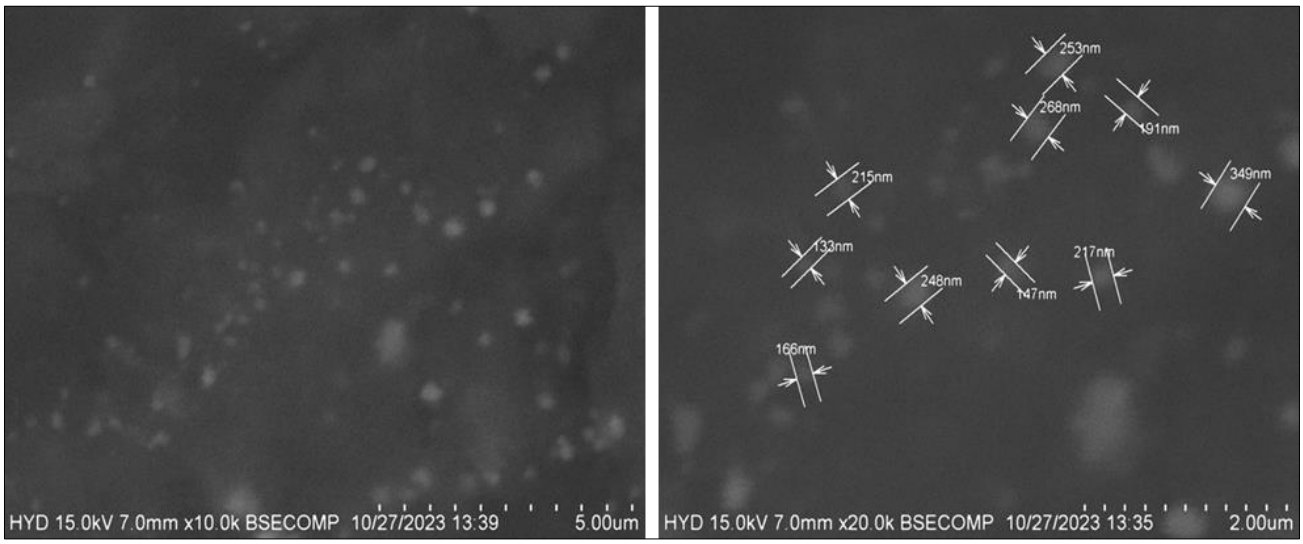

Fig 3: SEM imaging of SLNs

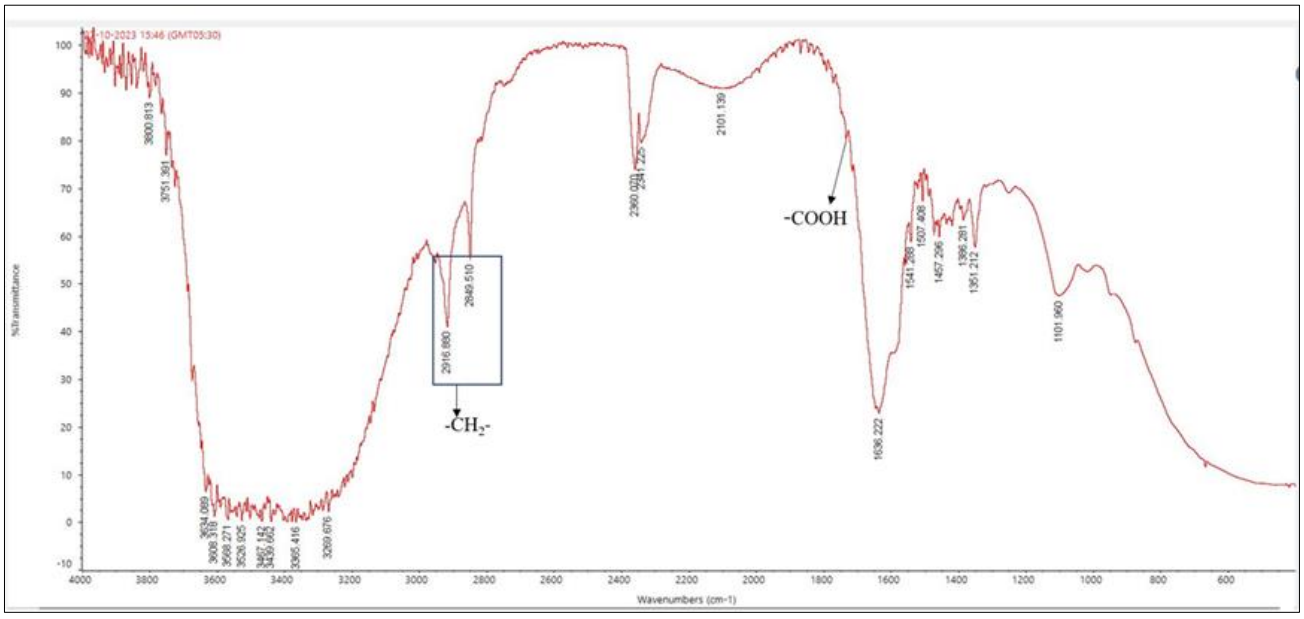

Fig 4: FTIR analysis of SLNs

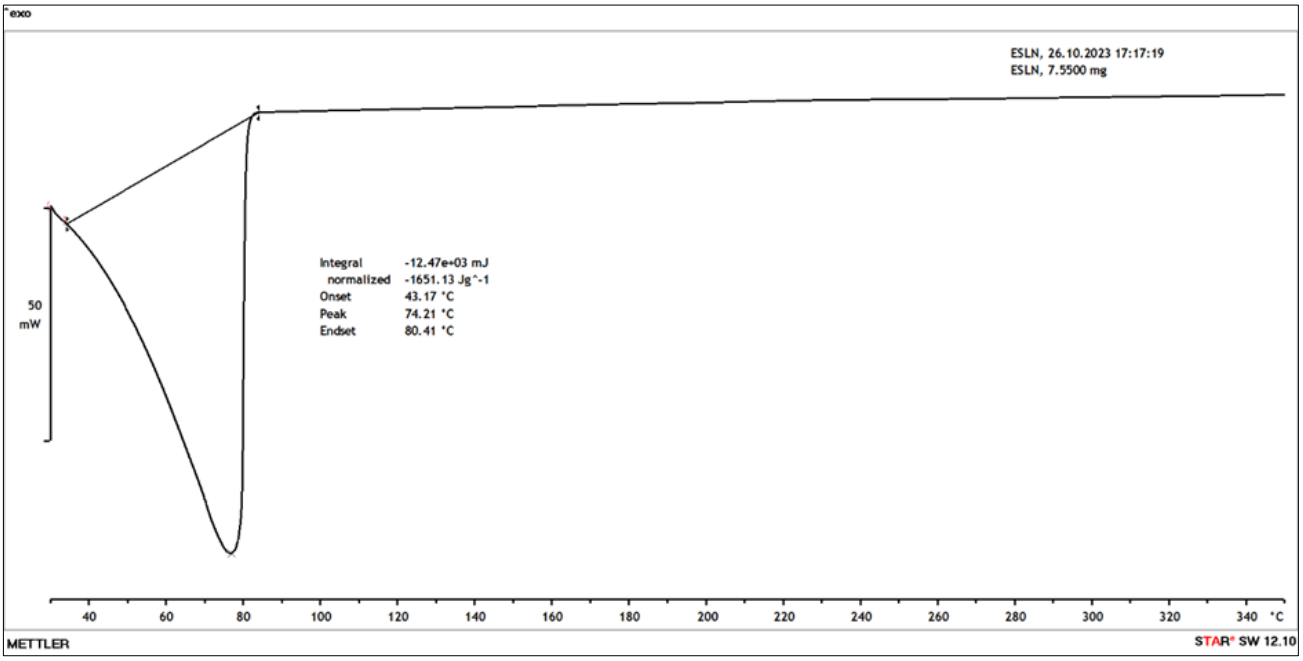

Fig 5: DSC analysis of SLNs

Conclusion

Synthesis of solid lipid nanoparticles using the double emulsion method resulted in optimal size and optimal stability. Based on FTIR and DSC analysis, no significant change in chemical composition of the stearic acid particles was detected.

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