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Preparation and Evaluation of Andrographolide Containing Nanoparticle

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KEYWORDS

Solid lipid nanoparticles, Andrographolide, Nanoemulsion, High pressure homogenization, Hot homogenization

ABSTRACT:

Solid lipid nanoparticles were found to be a promising approach to improve oral bioavailability of poorly water-soluble drugs which belongs to plant derived drugs. Andrographolide was selected as a model drug for the current research as it is not well absorbed topically and oral bioavailability improvement was a major challenge. The present study has shown that the SLNs being a versatile technology have the potential to improve the biopharmaceutics properties of poorly water-soluble drug i.e. andrographolide and open up new perspectives for the formulation of drugs having low aqueous solubility and high permeability. The SLN approach was used in an attempt to increase its oral bioavailability. An optimized HA-SLN4 was prepared by hot homogenization method, using phosphatidylcholine as a lipid. This was optimized on the basis of lower particle size, minimum polydispersity index, sustained drug release from SLN, lower surfactant concentration, higher solubilization of drug in the minimum amount of lipid as well as higher bioavailability. Due to entrapment of andrographolide into SLN, it permits controlled rate of drug release and showed a sustained release effect of drug.

Introduction:

Nanotechnology has become a promising new strategy for disease diagnostics and therapeutics. A distinct advantage of nanotechnology is the ability to design and optimize the unique physiochemical properties of nanoscale materials and structures. Altering the size, shape and/ or surface chemistry of nanoparticles allows their functionalities to be tailored to meet different requirements. Nanoparticles need to reach the targeted organ or tissue in order to realize the desired function. Nanotherapeutics rely on effective cellular uptake and tumor permeability of nanoparticles, which both depend on the size of nanoparticles [1-2].

Liver disease causes about 2 million deaths annually and accounts for 4% of total deaths. Most deaths are associated with the progression of liver cirrhosis and hepatocellular carcinoma (HCC) [3]. HCC is the fourth tumor-associated death worldwide. Many factors such as the hepatitis B and C virus, alcohol abuse, and nonalcoholic fatty liver disease (NAFLD) will induce chronic cirrhosis and ultimately develop into HCC. Furthermore, with the obesity pandemic, there is increasing concern about the serious challenge of NAFLD-related HCC [4].

More than 80% of primary liver tumors are defined by HCC. As a result of the lack of effective therapeutics, the 5-year survival of HCC is only 20%. Liver transplantation will greatly prolong HCC survival, but the lack of donor organs and recrudescence after transplantation also limit this therapy. Patients who cannot tolerate surgery or chemotherapeutics generally receive systemic therapies, targeted therapy, and immune therapy [5].

The liver is the largest solid organ in the body. It removes toxins from the body's blood supply, maintains healthy blood sugar levels, regulates blood clotting, and

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performs hundreds of other vital functions. It is located beneath the rib cage in the right upper abdomen. The liver filters all of the blood in the body and breaks down poisonous substances, such as alcohol and drugs. It also produces bile, a fluid that helps digest fats and carry away waste. It consists of four lobes, which are each made up of eight sections and thousands of lobules [10]. Liver, the largest gland in the body, a spongy mass of wedge-shaped lobes that has many metabolic and secretory functions. The liver secretes bile, a digestive fluid; metabolizes proteins, carbohydrates, and fats; stores glycogen, vitamins, and other substances; synthesizes blood-clotting factors; removes wastes and toxic matter from the blood; regulates blood volume; and destroys old red blood cells [6]. Liver tissue consists of a mass of cells tunneled through with bile ducts and blood vessels. Hepatic cells make up about 60 percent of the tissue and perform more metabolic functions than any other group of cells in the body. A second group of cells, called Kupffer cells, line the smallest channels of the liver's vascular system and play a role in blood formation, antibody production, and ingestion of foreign particles and cellular debris. The liver cells synthesize a number of enzymes. As blood flows through the liver, both from the portal vein and from the hepatic artery, the cells and enzymes are filtered [7]. Blood supply and the hepatic RES promote the accumulation of nanoparticles in the liver. Systemic administration of nonmodified NPs results in preferential transport to the liver rather than to other organs. This is largely due to the anatomy and blood supply of the liver. The ideal position makes the liver a desired organ to capture pathogens through the gut and eliminate bacteria, viruses, and NPs from the blood circulation. Blood flows into the liver via the portal vein, passing through several hepatic sinusoids to the central vein. There are many resident phagocytes in the sinusoids, which screen and filter foreign materials in the blood. Thus, the liver has the capacity to eliminate oral NPs derived from the gut and systemic NPs injected into the peripheral blood circulation [8].

Oral administration is the most suitable modality for patients. The gut and liver have a unique relationship in metabolic, neuroendocrine, and immune interactions, since the sinusoids of the liver and the gastrointestinal tract are connected by the portal and biliary circulation. Orally administered NPs have the tendency to be transported further into the portal vein. Nonetheless, NPs must face the challenges present in the gastrointestinal tract. Physical properties of NPs play a dominant role on their biodistributions [9]. Those properties mentioned above will be no doubts affect the fate of NPs. But, the biodegradability of NPs should also be carefully considered. The delivery goals require that NPs should be transported to the targeted tissue and targeted cells. Therefore, most NPs are stable in the blood circulation after injections. When they traveled to the diseased organs, drugs will be released to the target cells. Parts of degradation or off-self-assembly products of nanocarriers may be eliminated out of body, while the others may resident in the organs for a long time [10].

Commonly used first-line agents for HCC are Sorafenib and Lenvatinib, and their resistance limits their beneficial effects. Thus, second-line targeted agents are urgently needed for HCC treatment. Treatment with Regorafenib, Cabozantinib, and Ramucirumab as second-line agents has been evaluated in clinical trials. However, not all patients who progressed in Sorafenib are suitable for Regorafenib. Cabozantinib has the risk of high-grade adverse events. Combination with immune therapy can effectively enhance treatment, but improvements usually occur a few months after treatment. Additionally, the response rate is not optimistic for most patients [11].

The objectives of proposed work are to formulate nanoparticles to control and continue release rate of drug at the site of localization. Nanoparticles enhance drug circulation in blood, bioavailability, reduce side effects. The nanoparticle size drug delivery enhances the entire surface area of the drugs therefore allocating quicker dissolution in the blood with reduction in toxicity while maintaining therapeutic effects, thus enhanced permeation through membrane.

Material And Methods

Maximum wavelength determination: A number of phosphate buffer saline (PBS) (pH 7.4) dispersing mediums are utilized for making determination of maximum wavelength of drug andrographolide. The dilutions made according to calibration standards and their absorbance was recorded at λmax.

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Calibration curve: A number of different aqueous dispersing mediums are utilized for making calibration curves of drug andrographolide from 1µg/ mL to 10µg/ mL by adding phosphate buffer saline (PBS) (pH 7.4).

Authentication of analytical procedure: The analytical procedure employed fir the quantitative analysis of andrographolide was confirmed based on standard parameters which are follows:

Specificity: Specificity is defined as the ability to detect the analyte of interest in the presence of interfering substances.

Precision: The ICH guidelines classified precision in to two parts; repeatability and intermediate precision.

Repeatability: Standard stock solution in various dissolution media was prepared in the manner described in previous section in dissolution medium (phosphate buffer saline (PBS) (pH 7.4)) and suitably diluted with same solvents to obtain 10 µg / ml solution. The absorbance of each solution was measured separately at 236 nm in dissolution medium (phosphate buffer saline (PBS) (pH 7.4)) respectively for drug at different time intervals in replicates of ten. The percent RSD should not be more than 1 %.

Intermediate precision: Intra-day precision was determined by measuring the absorbance of 10 µg / ml drug solution of drug in dissolution medium (phosphate buffer saline (PBS) (pH 7.4)) at predetermined interval within a day. Inter-day precision test was determined by measuring the absorbance of 10 µg / ml drug solution in dissolution medium (phosphate buffer saline (PBS) (pH 7.4)) on three different days. The absorbance of each solution was measured separately at 314 nm in dissolution medium (phosphate buffer saline (PBS) (pH 7.4)) respectively for drug. The percent RSD of the absorbance must be less than 1 %. The result of intra-day and inter-day precision.

Accuracy: Accuracy is the difference between the measured value and the taken value. The blend of drug and excipients was prepared by mixing the active drug as drug in to the blend of excipients at different levels of the target concentration each in triplicate. The recovery sample was analysed in dissolution medium (phosphate buffer saline (PBS) (pH 7.4)) at 236 nm for drug as per the test method and the amount of drug recovered at each spike level and percent recoveries at each spike level are determined. [12]

Formulation of Andrographolide loaded solid lipid nanoparticles: High pressure homogenization or hot homogenization is a technique in which homogenization of sample takes place at a temperature higher than the melting point of lipid used. In this method first the lipid was melted at a temperature above its melting point. The drug being lipophilic in nature was dissolved in the melted lipid. Another phase containing purified water mixed with surfactant was prepared and heated to the same temperature as that of drug loaded lipid phase. The melted phase was dispersed in hot aqueous surfactant mixture drop wise and homogenised at high speed to make primary o/w type emulsion. This type of primary emulsion has coarse size of particles. This emulsion was again homogenized at high pressure above the temperature of melting point of lipid to convert the coarse emulsion of drug loaded lipid in nanoemulsion form. This hot nanoemulsion was kept a side for some time to cool at room temperature where lipid solidify at room temperature and resultant mixture was filtered through membrane filter to get solid lipid nanoparticles. High temperature during the process decreases the viscosity and produces the smaller size particles but this may also cause the degradation of heat liable drugs. Here, high pressure homogenization increases the sample temperature, approximately by 10 °C at 5 ×107Pa. In most cases, 3–5 homogenization cycles at 5 ×107 Pa to 5 ×109 Pa pressure is sufficient to have better products in hands [13].

Characterization Parameters for andrographolide SLNs

Determination of entrapment efficiency and drug loading: Entrapment efficiency is the study of drug amount that is encapsulated in the lipid matrix and quantity of drug present in supernatant layer received after the process of centrifugation at very high speed of 16000 rpm for half an hour. The entrapment efficiency is the ratio of actual amount of drug loaded and theoretical amount of drug loaded in lipid nanoparticles. The loading of drug can be measured by subtracting the free drug amount from the total quantity of drug used in the formulation. The entrapment efficiency and drug loading can be calculated using the formulas given below:

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%EE = free drug amount / total weight of drug *100

% Drug loading= drug entrapped in SLNs/ weight of vehicle *100

Determination of yield of SLNs: Yield of the formulation indicates the quantity of solidlipid nanoparticles achieved after the preparation. The yield is derived from gravimetric analysis. In this process a 10 mL suspension of drug was dried until the weight was constant to express the ratio of lipid present after drying and used initially. The yield was calculated in percentage. [14]

Particle size distribution: The particle analysis in solid lipid nanoparticles was investigated by photon correlation spectroscopy method. For this study, the dispersion of solid lipid nanoparticles was diluted with purified water in 1:2 ratios and final dispersion was filtered using membrane filter of 0.45µm. The angle for light scattering study was fixed at 90°C. The study was carried out at room temperature (25°C). Three reading were taken for calculating average mean to avoid any errors.

Zeta potential determination: The zeta potential studies for prepared formulations were carried out utilizing Zeta sizer instrument. For the preparation of sample, the drug loaded solid lipid nanoparticles were diluted with purified water in the ratio of 1:2. The sample were analysed three times and average mean was taken into consideration.

in-Vitro Dissolution: In-vitro dissolution studies were performed using membrane dialysis method. The dialysis membrane was made of cellulose. It was treated with specific treatment before performing dissolution studies. The sample was subjected to a shaker apparatus maintained at 37±1°C. The speed of strokes was fixed at 5o min-1. The samples in 2 mL quantity from the vial were taken out at time hour of 0, 0.5, 1, 2, 4, 8, 12, 16, 20 &24h. The sink conditions were maintained by replacing the amount of sample with fresh media. The samples were analyzed by UV spectroscopy method at 236 nm. The release of drug from SLNs was compared with the release of drug from pure drug suspension [15-16].

Results and discussion: The absorption maxima (λ-max) of andrographolide (10 µg / ml) in phosphate buffer saline (PBS) (pH 7.4) were found to be at 236 nm. The calibration curves show excellent linearity of data as evidenced by the values of correlation coefficients that were found to be greater than 0.99. The curves were found to be recti-linear in the concentration range 0 µg / ml to 100 µg / ml for the drug. The result of specificity showed specific in nature. The result of repeatability by Inter-day and Intra-day precision of the UV spectrophotometric methods was evaluated for drug in same concentration i.e. 10 µg / ml with variability in time period of estimation. The percent relative standard deviation was found to be less than 1 % i.e. the method was precise. The recovery of drug from phosphate buffer saline (PBS) (pH 7.4) was estimated and quantitative recoveries were recorded for the drug, ranging from 99.03 to 99.78 %. The estimation procedures for drugs were found to be sensitive, precise and reproducible.

Entrapment efficiency is the study of drug amount that is encapsulated in the lipid matrix and quantity of drug present in supernatant layer received after the process of centrifugation at very high speed of 16000 rpm for half an hour. The entrapment efficiency is the ratio of actual amount of drug loaded and theoretical amount of drug loaded in lipid nanoparticles. Drug loading end entrapment efficiency of solid lipid nanoparticles were dependent of drug lipid matrix and physico-chemical properties of drug and lipid used in the formulation. Yield of the formulation indicates the quantity of solid lipid nanoparticles achieved after the preparation. The yield is expressed as the ratio of lipid present after drying and used initially. The study of particle size and its distribution in the developed formulation is an important tool to give information of existing size range of the particles formed in the formulation. In current study, the particle analysis in solid lipid nanoparticles was investigated by photon correlation spectroscopy method. Photon correlation spectroscopy reports the data on the basis of intensity using Z average with PDI. The PDI is the representation of “broadness” of particle size distribution. The mean particle size of optimized formulation of SLNs showed particle size below 200 nm. Small particle sizes and narrow size distribution are also indicators of the effectiveness of preparation methods involving several factors. Surface topography studies by scanning electron microscopy (SEM) involves focused beam of electrons through the surface of sample. These electrons interact with the particles

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present in the sample and generate signals. The signals produced gave information regarding topography of surface.

Summary:

Chronic liver diseases represent a global health problem due to their high prevalence worldwide and the limited available curative treatment options. They can result from various causes, both infectious and noninfectious diseases. The application of nanoparticle (NP) systems has emerged as a rapidly evolving area of interest for the safe delivery of various drugs and nucleic acids for chronic liver diseases. Currently, NP therapy for liver fibrosis is updating fast, and hopefully, it can be the future remedy for liver fibrosis.

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Table 1: The different batches of androgapholide SLNs by High pressure homogenization technique

Formulation Code	Lipid content (X_1)			Amount of surfactant (X_2) (%) (Tween 20)	Addition of sonication time (X_3) (Min.)
	Lecithine (CH) (mg) X_a	Phosphatidylcholine (PC) (mg) X_b	Almond oil (AO) (mg) X_c		
HA-SLN1	150	0	150	10	10
HA-SLN2	0	150	150	10	10
HA-SLN3	150	50	100	10	10
HA-SLN4	50	150	100	10	10
HA-SLN5	150	100	50	10	10
HA-SLN6	50	100	150	10	10
HA-SLN7	100	150	50	10	10
HA-SLN8	100	50	150	10	10

Table 2: Parametrs of prepared androgapholide SLNs by high pressure homogenization

Formulation Code	Particle size (nm)	Layers	Zeta potential (mV)	PDI	Drug Entrapment (%)
HA-SLN1	122.01±1.02	Single	-20.12±1.02	0.216±0.08	81.37±0.8
HA-SLN2	123.03±1.04	Single	-20.18±1.05	0.215±0.02	78.13±1.1
HA-SLN3	129.03±0.08	Double	-22.91±1.03	0.221±0.27	76.03±0.3
HA-SLN4	127.21±1.11	Double	-24.21±1.09	0.216±0.05	85.17±0.3
HA-SLN5	130.12±1.06	Double	-22.12±1.08	0.224±0.07	79.98±1.2
HA-SLN6	128.21±1.02	Double	-23.01±1.03	0.219±0.05	78.11±0.9
HA-SLN7	131.22±1.09	Double	-22.02±1.04	0.229±0.04	80.17±0.7
HA-SLN8	130.81±1.08	Double	-22.16±1.05	0.228±0.07	82.97±0.8

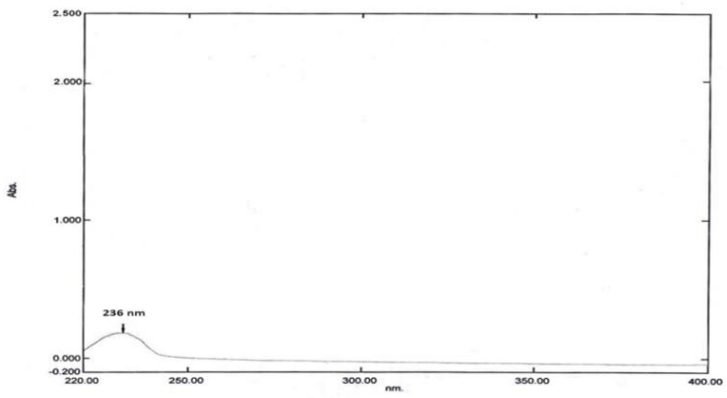

Figure 1: Absorption maxima (λ-max) of Androgapholide in phosphate buffer saline (PBS) (pH 7.4) solution (10 µg/ml)

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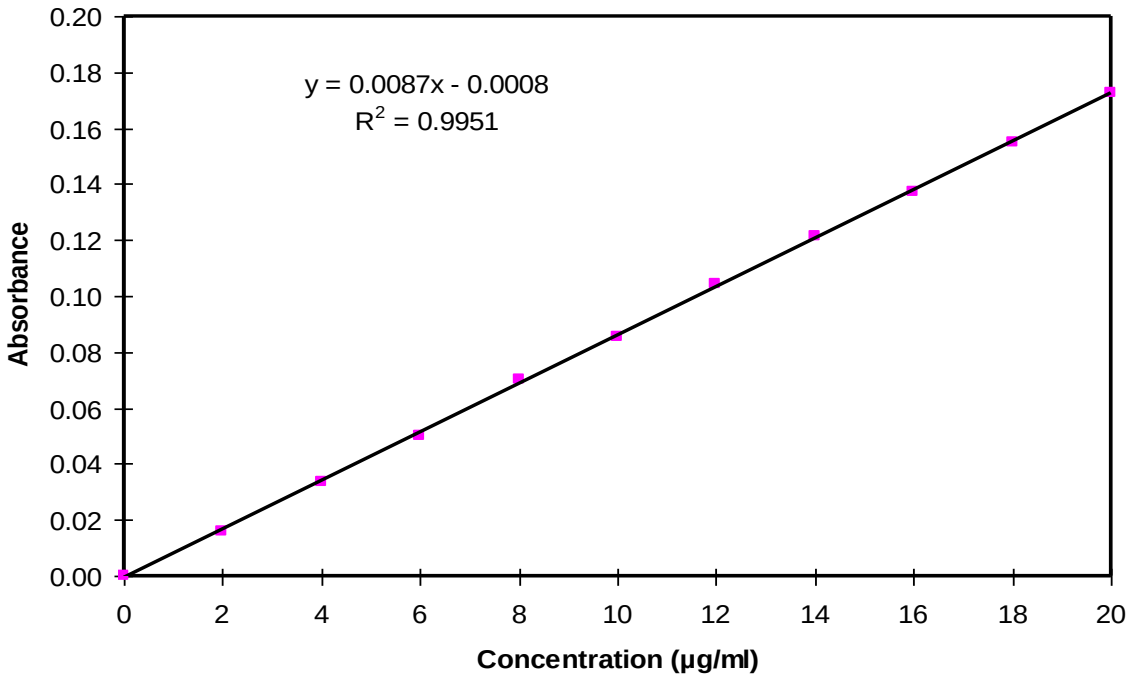

Figure 2: Standard curve of Androgapholide in phosphate buffer saline (PBS) (pH 7.4) (236 nm)

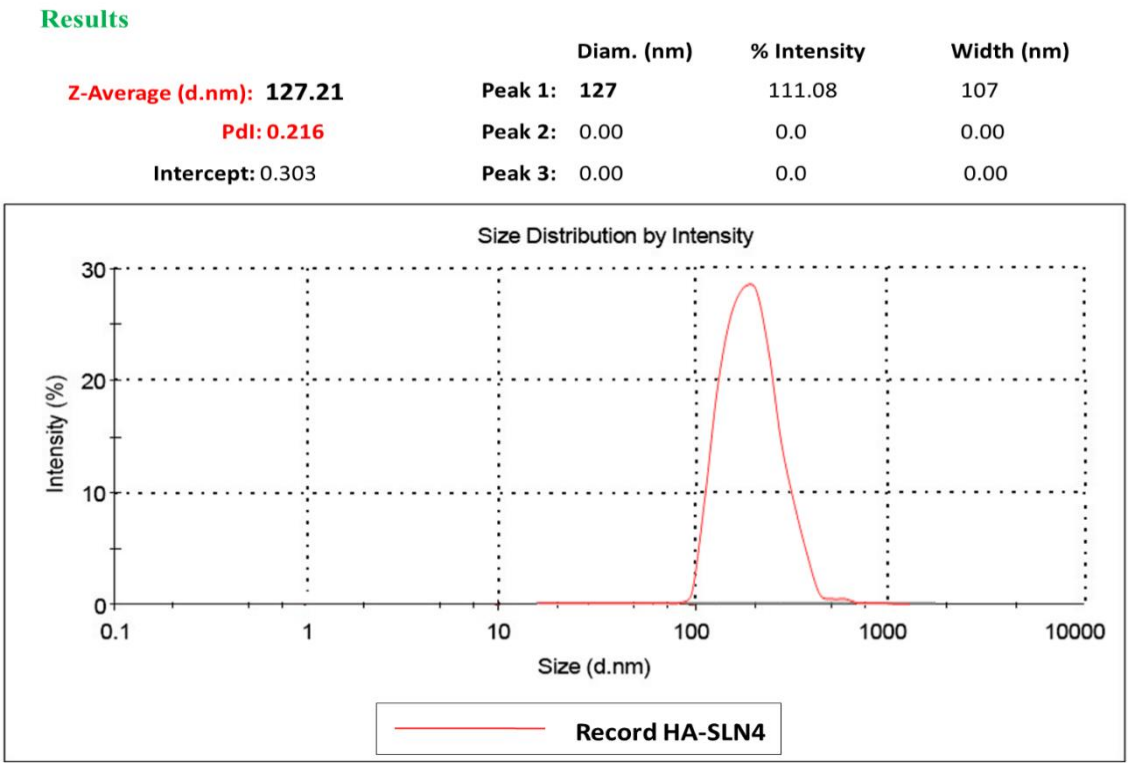

Figure 3: Particle size distribution & Polydispersity Index (PDI) of prepared androgapholide SLNs by high pressure homogenization (HA-SLN4)

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Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -24.21	**Peak 1:** -24.21	104	3.91
Zeta Deviation (mV): 92.11	**Peak 2:** 0.00	0.0	0.00
Conductivity (mS/cm): 0.3090	**Peak 3:** 0.00	0.0	0.00

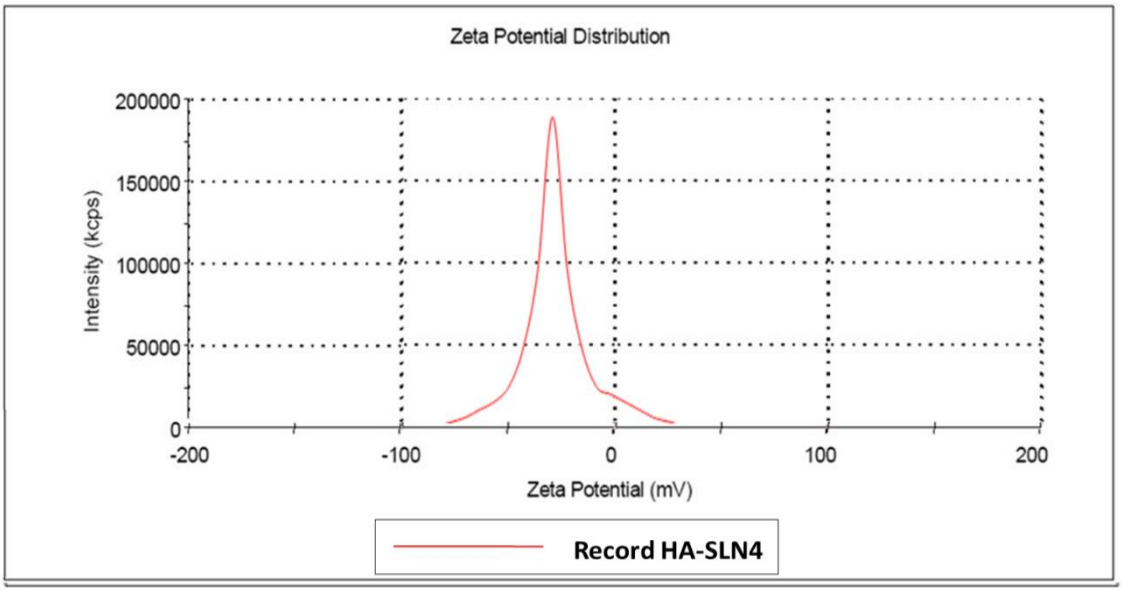

Figure 4: Zeta potential (mV) of prepared andrographolide SLNs by high pressure homogenization (HA-SLN4)

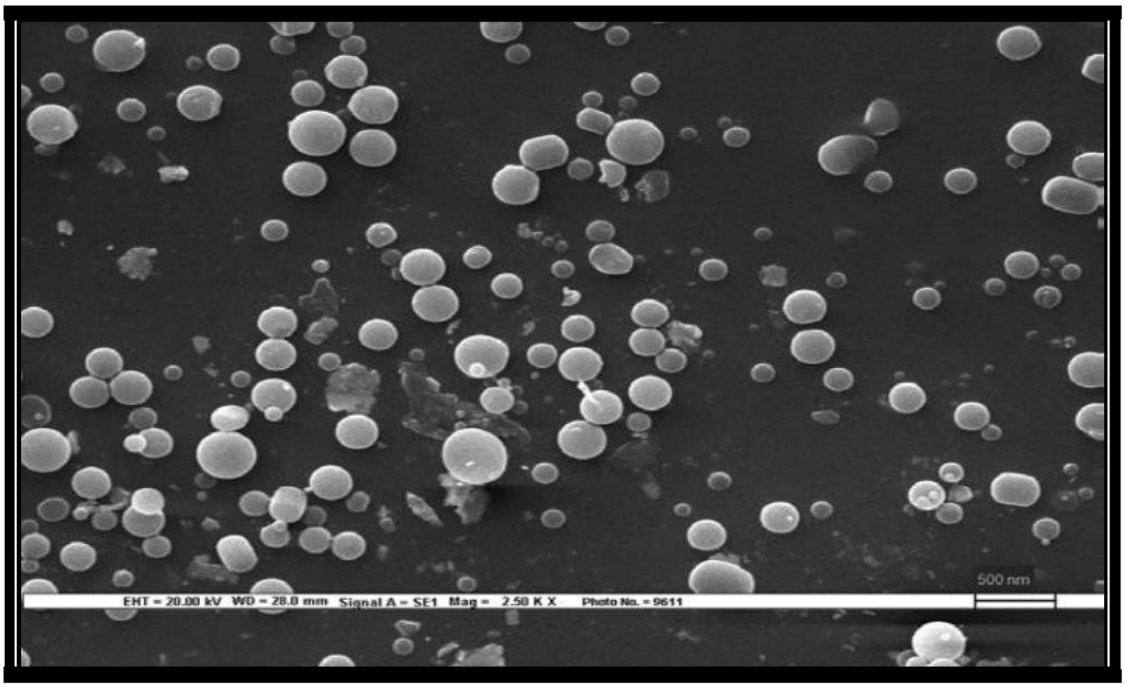

Figure 5: Scanning Electron Microscopy (SEM) image of HA-SLN4

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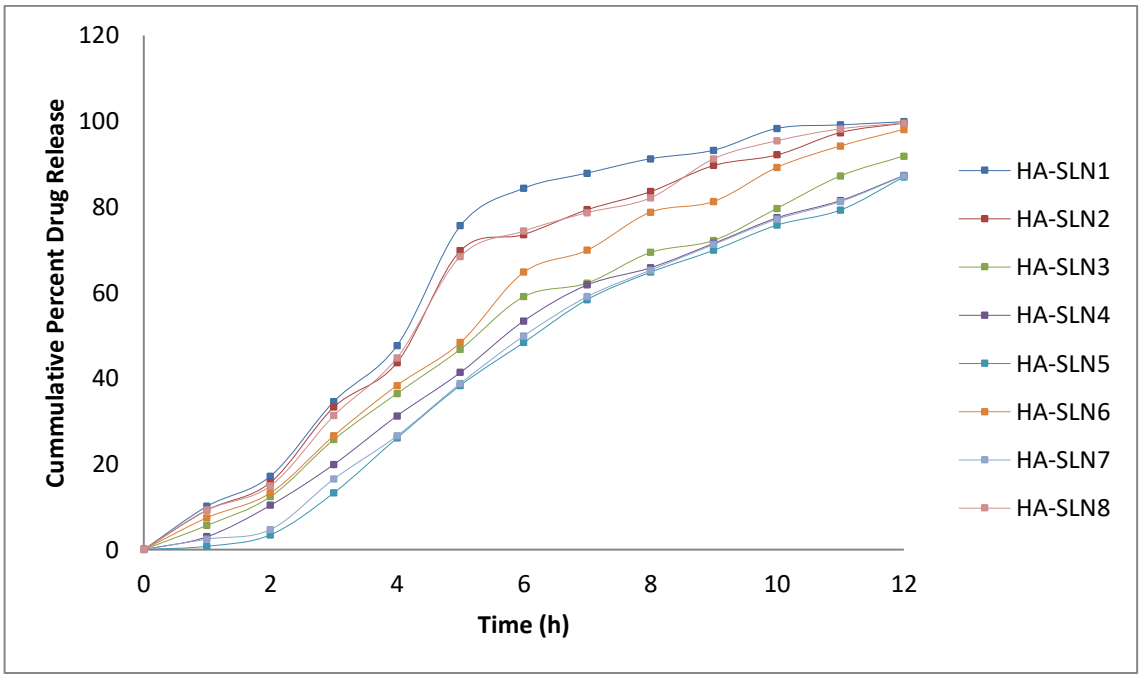

Figure 6: Zero-order plot in-vitro drug diffusion analysis of of prepared andrographolide SLNs by high pressure homogenization (HA-SLN1 to HA-SLN8)