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Investigation Of Nanoparticle-Based Delivery System Containing Plant Extract Of Abrus Precatorious L

Mohd Faisal[1], Dr Ruchi Tiwari[1], Dr Naveen Gupta[1], Dr. Dharmendra Singh Rajput[1]
[1]Department of Pharmacy, Madhyanchal Professional University, Bhopal, M.P.
*Corresponding Address
Mohd Faisal
Department of Pharmacy, Madhyanchal Professional University, Bhopal, M.P.
mfaisal.faisal316@gmail.com

Abstract:
Medicinal plants can be important source of previously unknown chemical substances with potent therapeutic effects. Herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs. Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic effects. The novel drug delivery systems and nanotechnology have proven to increase the chances of herbal-based therapies being implemented due to boosting the potential of medication action, promoting the sustained release of active ingredients, reducing the required dosage, and improving biological activity.
Keywords: *Nanoparticles, Solid lipid nanoparticles, Herbal formulation, Abrus precatorious, Ayurvedic formulation*

INTRODUCTION:

Herbal medicines have been used from years throughout the world; especially in India, herbal medicines are in high demand. The use of herbal medicines has increased because of their ability to treat different diseases with fewer side effects. The development of novel drug delivery system (NDDS) is of considerable importance to overcome various constraints like poor bioavailability, in vivo stability, aqueous insolubility, intestinal absorption and unspecific site of action. The integration of the Nano science as a NDDS in traditional system of medicine enriches the potential of herbal drugs for treating chronic diseases such as cancer and ravaging diseases [1]. Ayurveda is one of the ancient medical sciences practiced in India. Herbal medicines have been recognised by physicians and patients due to their potential therapeutic effect and also their fewer side effects as compared to other medicines, at the same time it also increases the bioavailability of the medicine. The modern phyto-pharmaceutical research can solve the scientific needs of herbal medicines in developing novel drug delivery systems, such as nanoparticles, micro emulsion, matrix system, solid dispersion, liposomes and solid lipid nanoparticles [2]. Nanotechnology and Nano science studies have emerged rapidly during the past years in a broad range of product domains. It provides opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits [3]. Nanoparticles currently have a highly attractive raised area or a diverse range of biological applications. Nanotechnology has been increasingly applied to enhance the effectiveness of herbal medications, addressing key challenges such as poor solubility, low bioavailability, and limited therapeutic efficacy [4]. Thus, nanoscale drug delivery systems are used through polymeric nanoparticles, solid lipid nanoparticles, and nanostructured lipid carriers, and the active ingredients are protected, stabilized, and released subsequently. These systems have demonstrated substantial potential in treating chronic diseases, enhancing the therapeutic effects of herbal remedies, and expanding their applications across healthcare and the food industry [5]. Herbal remedies were chosen as feasible drug molecule for delivery through nanocarriers as a promising delivery system; the main reasons for the popularity of herbal medicines. As per the World Health Organization (WHO), in developing countries, around 80% of the world populations at present utilize herbal medicine for primary health care. Presently, the scientific community is focused on the study associated with the bioactive compounds, its chemical composition, and pharmacological potential of a variety of plant species, to fabricate pioneering active ingredients that present moderately minor side effects than existing molecule [6]. Nano-size particle or nanoparticle is a spacious class of materials that enclose particulate substance which has not as much of 100 nm in size. It is a well-known field of research of this century and it has a wide range of revolutionary developments in the field of nanotechnology such as treatment, monitoring, diagnosis, and control of biological systems [7]. Nanoparticles or nanomaterials have gained prominent advancements in nanotechnology due to their tunable physiochemical and biological performance over

their counterparts. The major drawbacks of conventional are nonspecific, lack of solubility, and inability to enter inside the cells which offer a great opportunity for nanoparticles to play significant roles [8]. Herbal medicines have been extensively used in the region of the world since antique times. In india herbal medicines or traditional system of medicines such as Siddha and Ayurveda use herbal preparations. Nowadays, herbal drugs dwell in a leading position in the pharmaceutical industry as their effects are known and side effects are very negligible [9]. Moreover, the herbal drug has a symmetrical way of interest to fabricate nanoparticles compared to synthetic drugs. Even though the herbal drug has enormous pharmacological actions toward many diseases, it has been shown an only limited effect on the human biological system due to their less kinetic performance such as low absorption, inability to cross lipid membrane, high molecular size and weight, or poorly absorbed, resulting in a reduction of bioavailability and efficacy over the biological system. Moreover, some of the extracts are not used clinically because of the abovementioned hinders. To overcome such related issues, carriers have been used as an alternative approach to amend and improve the kinetic and dynamic parts of a drug molecule on a biological system [10-11].

Efficacy of Abrus precatorious in various diseases including cancer has been very well established. The pharmacological safety and efficacy of Abrus precatorious makes it a potential compound for treatment and prevention o f a wide variety of human diseases. The single most important reason for this reticence has been the reduced bioavailability of orally administered Abrus precatorious. This drug candidate suffers from poor oral bioavailability, thus obstructing its use as an anticancer agent. The current work proposes the development of systems with enhanced oral bioavailability to overcome these problems.

MATERIAL AND METHODS

Procurement and Authentication of material (Abrus Precatorious L): A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. Seeds of Abrus Precatorious L was collected from fresh and fully grown plants from local area, India. It was authenticated Botanist. The seeds of Abrus Precatorious L was separated and dried under shade, powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies.

Macroscopical study: The macroscopical description of seeds of Abrus Precatorious L include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied**.**

Physicochemical Evaluation: Proximate analysis: Physicochemical analysis helps to set up certain standard for dried crude drugs in order to avoid batch-to-batch variation and also to judge their quality. Their studies also give an idea regarding the nature of phytoconstituents present. Proximate analysis of Abrus Precatorious L seeds powders were carried out using methods prescribed in the Ayurvedic pharmacopoeia of India by subjecting them to various determinations like Total Ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive value, Water soluble extractive value, Loss of moisture content and Swelling index

Preparation of calibration curve of Gallic acid: A stock solution of gallic acid was prepared by transferring 100mg of gallic acid into a 100 mL volumetric flask, dissolved in 10 mL distilled water. It was sonicated for 10 minutes and volume was made up to 100 mL with distilled water to get stock solutions containing 1mg/ml. Take aliquots of this stock solution to make 0.1ug/ml to 1 µg/ml solution. Absorbance was recorded at 260 nm. Each concentration was measured in triplicate. The corresponding absorbance was plotted against the concentration of the standard to get calibration curve of gallic acid. Calibration curve established with ten dilutions of standard, at concentrations ranging from 0.1 to 1 µg/ml. The double beam shimatzu UV spectrophotometer was used with diluted solution of extract in distilled water. Distilled water was used to calibrate the instrument at the wavelength of 400 nm. The aliquot of 5µg/mL of each extract was diluted with distilled water and was measured the absorbance. The absorbance of the extract was determined within the visible region (400– 800 nm) and the wavelength of maximum absorption (λmax) of each extract was extrapolated from the graph

Preparation of sample solution for determination of gallic acid content in extract by UV spectrophotometer: Accurately weighed 100 mg of dried extract was transferred in to a 10 mL volumetric flask seperately, dissolved in 1 mL of water, sonicated for 10 minutes, contents of the flask were filtered through Whatman No. 1 paper and the final volume was made up to 10 mL with water to get concentration 10 mg/ mL. Take 1 ml of sample in 100 ml volumetric flask and volume was made up to

100 mL with water to get concentration 10 µg/ mL**.** Absorbance was recorded at 260 nm. Calculate the amount of Gallic acid in ethnaol extract of Abrus Precatorious L seeds from calibration curve equation of gallic acid.

Preparation of Solid Lipid Nanoparticles (Abrus Precatorious L extract SLNs):

Preparation of lipid films: Solid lipids phosphatidylcholine (PC), triglycerides (TG), in different ratio were dissolved in minimum quantity of chloroform containing 3-4 drops of methanol in 100 ml round bottom flask. The lipid mixture was evaporated under reduced pressure using a rotary flash evaporator at 40°C. The evaporation process was regularly used until complete dryness to form thin lipid film on walls of the round bottom flask. The removal solvent was collected at other side of collector.

Preparation of hydration fluid Phosphate buffer saline (pH 7.4): Accurately weighed 1.38g anhydrous disodium hydrogen orthophosphate, 0.19g potassium di-hydrogen orthophosphate and 8.0 g of sodium chloride were dissolved in sufficient quantity of water and volume was made up to 1000ml.

Preparation of SLNs of AP: Accurately weighed quantity of drug AP extract was dissolved in 10 mL with Phosphate buffer saline pH 7.4 in a separate beaker Dimethyl sulphoxide (DMSO) as a cosolvent (0.1 mL) was dissolve to 10mL with PBS, pH 7.4then volume. AP extract loaded SLN were prepared by hot homogenization followed by the ultrasonication method. AP extract and solid lipid were dissolved in a mixture of methanol and chloroform (1:1). Organic solvents were completely removed using a rotary flash evaporator. The embedded lipid layer was melted by heating to 5°C above the melting point of the lipid. An aqueous phase was prepared by dissolving the stabilizers (tween 80) in distilled water (sufficient amount to produce 30 ml) and heating to the same temperature of the oil phase. The hot aqueous phase was added to the oil phase and homogenization was performed (at 2500 rpm and 70°C) using a mechanical stirrer for 30 minutes. The coarse oil in water emulsion so obtained was sonicated using probe sonicator for 25 minutes. AP extract loaded SLN was finally obtained by cool to room temperature, and was stored at 4°C in the refrigerator.

Characterization of SLNs: Optimized formulation was characterized for size and size distribution, shape and surface morphology, entrapment efficiency and zeta potential.

Determination of vesicle size and size distribution: Maintaining constant size and size distribution for a prolonged period of time is an indication of stability of SLNs. Electron microscopy is widely used for the assessment of surface morphology, size and size distribution of SLNs. Besides the routine laboratory techniques such as gel chromatography etc. techniques based on light scattering and electron microscopy are need to be applied for statistically significant analysis of size and size distribution of the carriers. The average vesicle size and size distribution was determined by photon correlation spectroscopy using zeta sizer. The sample of dispersion was diluted to 1:9 with distilled deionized water.

Determination of Zeta potential: The zeta potential of particles is the overall charge that the particle acquires in a particular medium. The knowledge of the zeta potential of a preparation can help to predict the fate of the preparation in vivo and to assess the stability of colloidal systems. Zeta potential of emulsomes formulations were assessed by p-hoton correlation spectroscopy using Zetasizer Nanoseries using a flow-through cell.

Determination of shape and surface morphology: Shape and surface morphology of SLNs was determined by Transmission Electron Microscope (TEM) technique. The sample (10µl) was placed on the grids and allowed to stand at room temperature for 90 sec and excess of the fluid was removed by touching the edge of filter paper. All samples were examined under a Transmission Electron Microscope (Tecnai G2, Hillsboro Oregon, USA) at an acceleration voltage of 100 kV and photomicrographs were taken at 1400X.

RESULTS AND DISCUSSION

Procurement and Authentication of Plant material (Abrus Precatorious L): The morphology of crude drugs, which refers to their physical characteristics like shape, size, color, odor, and texture, is crucial for their identification and quality control. Understanding these features is essential for distinguishing genuine drugs from adulterants and ensuring the effectiveness and safety of crude drug. Abrus precatorius is a slender, perennial climber that twines around trees, shrubs and hedges. It has no special organs of attachment. The fruit (pod) is flat, oblong and truncate-shaped with sharp deflexed beak about 3-5cm long, 1.2 cm wide, and silky-textured. The seedpod curls back when it open to reveal the seeds. Abrus precatorius seeds are bright red with a black spot at the base (hilum). They are oval or ovoid-shaped and have a hard, shiny, and impermeable seed coat. Seeds are known for their uniform size, shape, and weight,

which in the past led to their use as weights for gold and silver. The black spot is located where the seed was attached to the pod.

Physicochemical parameters of Abrus Precatorious L: Loss on drying gives an idea of moisture present in the drug. Ash value of a drug provides an idea of the earthy matter or the inorganic components and other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs. The water-soluble extractive was high in the formulation. Swelling index gives the idea of gums present in the drug and foaming index tells about the saponins.

Extraction of the herbal drug: The coarse powder of the Abrus Precatorious L was subjected to successive solvent extraction using petroleum ether for defatting and ethanol for ethanol extract. After extraction the percentage yield of each extract was calculated with reference to the air-dried drug used for the study. The percentage yield of petroleum ether extract was 3.12 % and ethanol extract was 11.03%.

Preliminary Phytochemical Screening: The qualitative phytochemical screening of the extract for the presence of alkaloids, carbohydrate, reducing sugars, glycosides like anthraquinones, flavanoids, saponins, tannins, phenolic compounds, fixed oils, fats, proteins, amino acids and sterols in ethanolic extract of Abrus Precatorious L seeds were carried out.

Determination of gallic acid content by UV spectrophotometer: Gallic acid content was determined in all extract (Abrus Precatorious L seeds) by UV spectrophotometer. Gallic acid content in all extracts was calculated by calibration curve of gallic acid by UV spectrophotometer.

Characterization of AP extract SLNs (ASLN1 – ASLN8)

Entrapment efficiency: Entrapment efficiency is an important parameter for characterizing solid lipid nanoparticles. In order to attain optimal encapsulation efficiency, several factors were varied, including the type and concentration of the lipid and surfactant material used. The entrapment efficiency of the SLN dispersions was found to be in the range of 72.50 to 86.40%.

Effect of surfactants on entrapment efficiency: All SLN formulations prepared with a surfactant showed higher entrapment efficiency and showed that the increase in the concentration of the surfactant increased the entrapment efficiency. This may be due to the increase in the solubility of the drug in the lipid on increasing the concentration of the surfactant. This result was in agreement with the results. Formulations containing tween 80 as surfactants showed lower entrapment efficiency compared to the other surfactants; this could be due to the lower HLB value of tween 80. Hence the entrapment efficiency of various SLNs stabilized with different nonionic surfactants, and decreased in the order of tween 80.

Influence of surfactants on in vitro drug release: Formulations prepared by using SL:CH as a lipid matrix, with Tween 80 10.0%, as stabilizers showed a higher drug release from AP extract-loaded SLN showed that the increase in the concentration of the surfactant there was an increase in the drug release from the SLN. However, this difference could not be attributed to the formulations. These formulations showed a decrease in the drug release on the increase of the concentration of surfactant, which could be due to the lower melting point.

Influence of lipids on in vitro drug release: Formulations containing soyalecithine:cholesterol as a lipid matrix exhibited higher drug release than the formulations containing soyalecithine:cholesterol as the lipid matrix, which showed a more sustained release. Soyalecithine produced less ordered crystals than cholesterol, leading to lower drug expulsion from the imperfect lattice, contributing to the prolonged release of the lipophilic drug. Moreover, SL was a lipid with a lower melting point when compared to CH and has a lower melting point could produce a controlled release from SLN. The order of percentage of drug release was SL> CH on the basis of the lipid matrix. Thus, it could be well concluded that the amount of drug released was much slower and controlled from the SLN dispersions prepared by using SL as the lipid matrix, with Tween 80 as the surfactant, than that from the AP extract -pure drug solution.

Particle size analysis: The effect of the lipid and surfactant concentration on the particle size distribution of AP extract loaded SLN prepared by using SL and CH with tween 80 %. The particle size ranged from 130.81 nm – 149.03 nm. There was a significant difference in the size of the particles, with change in the lipids.

Effect of lipids on the particle size of AP extract SLN: AP extract loaded solid lipid nanoparticles, prepared by using SL as the lipid matrix, resulted in larger particle size compared to SLN prepared by using SL:CH as the lipid matrix, with all type of surfactants studied. This phenomenon could be attributed to the melting point of the lipid, SL has a higher melting point than TS, which results in a slower lipid crystallization from the hot homogenized condition resulting in an increase in the particle size. The particle size of various SLNs prepared with different lipids was in the order of SL > CH.

Effect of surfactants on the particle size of AP extract SLN: SLN dispersion prepared using Tween 80 10% as stabilizer [ASLN4] showed stabilized particle size due to the higher molecular weight of tween 80 and higher HLB value.

Summary and Conclusion

In order to properly identify and regulate the quality of crude pharmaceuticals. Having a thorough understanding of these characteristics is necessary in order to differentiate authentic medications from adulterants, as well as to guarantee the efficacy and safety of crude pharmaceuticals. In conclusion, the present study indicates nano formulation of Abrus precatorius plant extract (AP) showed valuable effect active nature than other valuable formulations.

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Table 1: Composition of AP extract loaded solid lipid nanoparticles containing different lipids and surfactants

Formulation Code	Solid Lipid content		Amount of stabilizer (X2) (%) (Tween 80)	Addition of sonication time (Min.)
	Soy Lecithin (SL) (mg)	Cholesterol (CL) (mg)		
ASLN1	0	100	10	10
ASLN2	25	75	10	10
ASLN3	50	50	10	10
ASLN4	75	25	10	10
ASLN5	100	0	10	10
ASLN6	75	25	10	10
ASLN7	50	50	10	10
ASLN8	25	75	10	10

Table 2: Physicochemical parameters of Abrus Precatorious L seeds

S. No.	Physicochemical parameter	Value (% w/w)
1	Total ash	4.98
2	Water soluble ash	3.18
3	Acid insoluble ash	1.31
4	Foreign organic matter determination	1.02
5	Ethanol soluble extractive	11.42
6	Water-soluble extractive	13.56
7	Loss on drying (%)	3.21

Table 3: Phytochemical analysis of Abrus Precatorious L seeds extracts

Tests of Phytoconstituents	Abrus Precatorious L
1. Alkaloids	
Mayer's reagent	+
Dragendorff's reagent	+
2. Flavonoids	
Shinoda test	+
3. Saponins	
Froth test	+
4. Carbohydrate	
Molisch's test	+
Test for gums	+
Test for mucilage	+
5. Phytosterols	
Libermann-Burchard test	+
Salkowski reaction:	+
6. Tannins and Phenolic	
With Lead acetate	+
7. Cardiac glycoside	
Borntrager's test	+
Legal's test	+
8. Coumarins	
With ammonia	-
Hydroxylamine HCl	-
9. **Proteins**	
Biuret test	+
10. Triterpens	
Vanillin sulphuric acid	-

Table 4: SLNs layers of AP extract SLNs (ASLN1 – ASLN8)

S. No.	Formulation Code	Layers	Particle size (nm)	PDI	Zeta potential (mV)	Drug Entrapment (%)
1	ASLN1	Single	142.01±1.02	0.216±0.18	-25.12±1.02	86.53±1.3
2	ASLN2	Single	143.03±1.04	0.225±0.12	-24.98±1.05	89.78±1.1
3	ASLN3	Double	149.03±0.08	0.244±0.12	-24.11±1.03	81.86±1.1
4	ASLN4	Double	147.21±1.11	0.226±0.15	-23.21±1.01	91.37±0.8
5	ASLN5	Double	140.12±1.06	0.234±0.17	-23.98±1.02	88.32±1.2
6	ASLN6	Single	148.21±1.02	0.221±0.15	-24.11±1.01	86.53±0.9
7	ASLN7	Single	141.22±1.09	0.239±0.14	-25.12±1.02	83.05±0.8
8	ASLN8	Double	130.81±1.08	0.238±0.17	-25.99±1.02	85.12±1.2

Figure 1: Abrus Precatorious L seeds

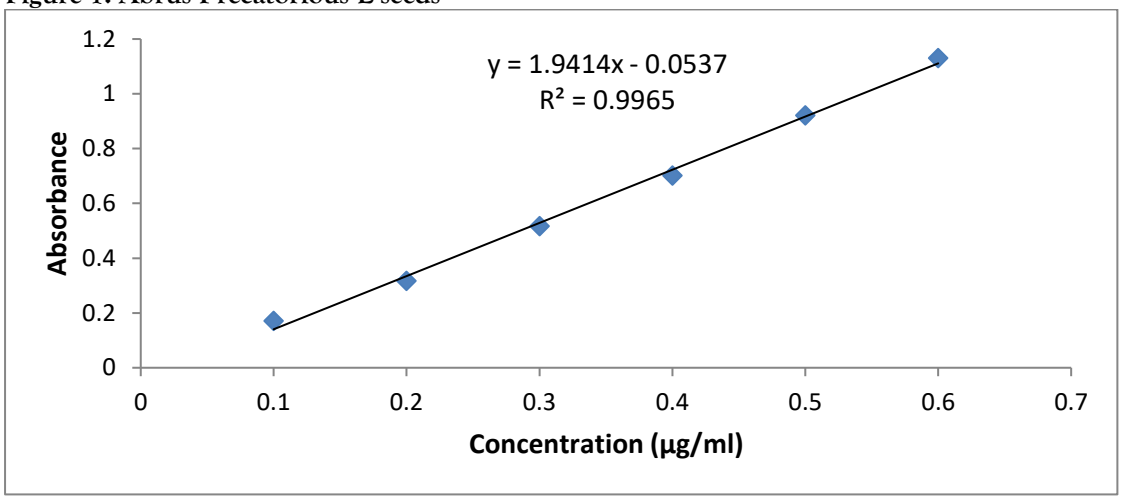

Figure 2: Standard curve of gallic acid by UV spectroscopy (260 nm)

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 147.21	**Peak 1:**	**147**	101.08	98.9
PdI: 0.226	**Peak 2:**	0.00	0.0	0.00
Intercept: 0.313	**Peak 3:**	0.00	0.0	0.00

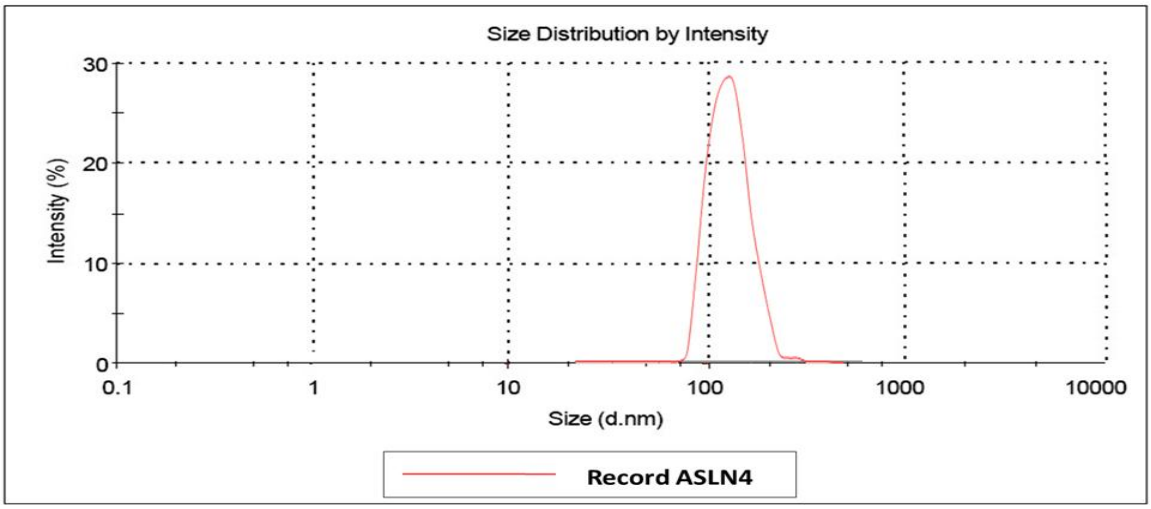

Figure 3: Particle size distribution & Polydispersity Index (PDI) of AP extract SLNs (ASLN4)

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -23.21	**Peak 1:** -23.21	111	2.91
Zeta Deviation (mV): 89.11	**Peak 2:** 0.00	0.0	0.00
Conductivity (mS/cm): 0.289	**Peak 3:** 0.00	0.0	0.00

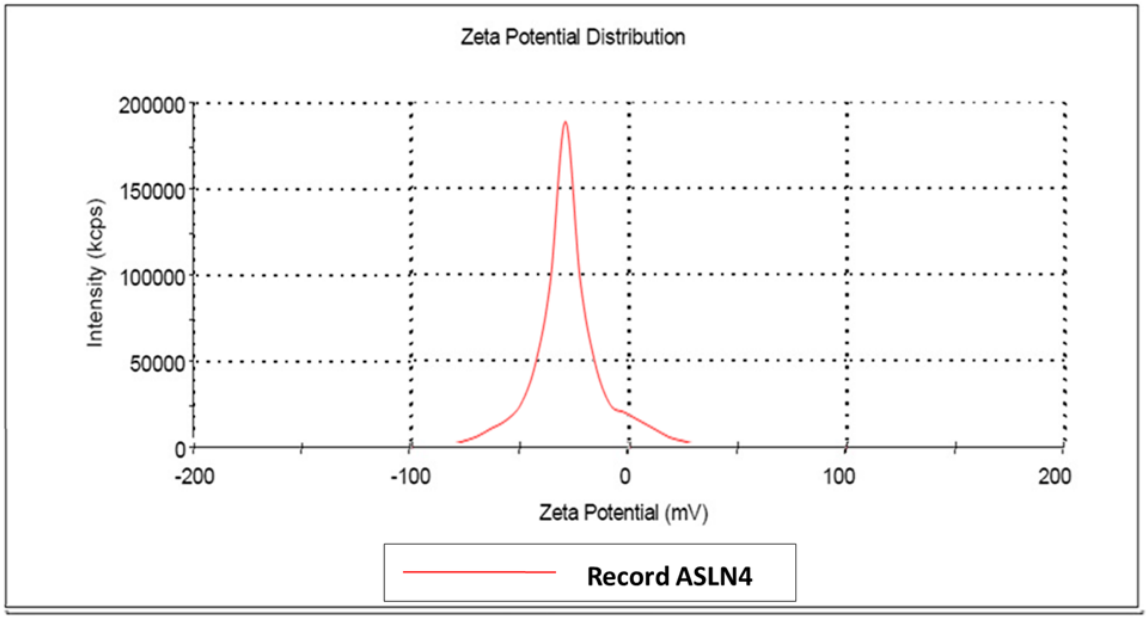

Figure 4: Zeta potential (mV) of AP extract SLNs (ASLN4)