Preparation and Characterization of Solid Lipid Nanoparticles for Artemisinin

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Abstract

Artemisinin is one of the effective antimalarial drugs used in the treatment of malaria. The objective of the research work was to develop solid lipid nanoparticles (SLNs) of artemisinin to enhance its bioavailability. Artemisinin SLNs formulations (ASLN1 to ASLN5) were prepared by the hot homogenization technique and evaluated for its efficacy. The results of characterization studies support the potential and futuristic applications of artemisinin SLNs in the treatment of malaria. SLN of lower particle size (225.4nm); surface charge (-18.7mV) was found to be optimum. The controlled release was achieved up to 24 hr. In conclusion, SLNs can be a promising carrier for the delivery of artemisinin at a controlled rate.

Keywords

Artemisinin, Solid Lipid Nanoparticles (SLNs), drug delivery, phospholipon 90G

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Introduction

In today's modern world, solid lipid nanoparticles (SLNs) are at the lead in phospholipid technology research with several potential applications in drug delivery (Naseri N, *et al,* 2015). Still, its application is limited in clinical and therapeutics as well as in other areas of science (Mukherjee S *et al,* 2009).

Concrete research in SLNs drug delivery may increase its applications in clinical and pharmaceutical areas. Due to its exclusive size-dependent properties, solid lipid nanoparticles propose the choice to develop a new drug delivery system in the treatment of various diseases (Montoto SS *et al,* 2020 & Xu L *et al,* 2022). The ability to incorporate pharmacologically active moieties into nanocarriers creates a new prototype in drug delivery that could be useful at different levels of drug targeting (Edis Z *et al,* 2021). Hence, the SLNs should be a great way to achieve the goals of controlled and site-specific targeted drug delivery (Satapathy MK *et al,* 2021).

SLNs may consist of drugs, genes, DNA, and proteins with solid lipids, emulsifying agents, and other required materials (Sabnis S *et al,* 2018 & Duoung VA *et al,* 2020). The formation of SLN depends on interfacial and surface tension between two liquids (Duan Y *et al,* 2020). The concept involves the formation of solid lipid nanoparticles by adhesive forces between the two phases. SLNs holds many advantages including stability, controlled and targeted release, high entrapment, biodegradability and flexibility in incorporating lipophilic and hydrophilic drugs (Khosa A *et al,* 2018). Still, the SLNs face challenges in drug loading and bioavailability.

Malaria is a dangerous disease caused by parasites. It is transmitted to people through female mosquitoes. About 320 crore people, almost half of the world's population – are at risk of malaria. It produces symptoms like cough, fatigue, headache, fever and myalgia (Bartoloni A, Zammarchi L, 2012).

Artemisinin is an antimalarial drug obtained from dried leaves or flower clusters of *Artemisia annua.* It is effective in curing malarial fever. Artemisinin reacts with hemozoin, an insoluble iron from anopheles mosquito by its peroxide group producing a radical that attacks parasite protein thereby killing the microorganism (Kannan R *et al,* 2005).

Artemisinin is effective, but the dose is high as 600 to 800mg due to its poor water solubility and bioavailability (Chen C, 2014). First-pass hepatic metabolism of artemisinin and its semisynthetic derivatives limit its oral bioavailability to 30% (Salman S *et al,* 2015). Artemisinin in general is of low toxicity, but neurotoxicity and reproductive toxicity have been reported (Medhi B *et al,* 2009). To achieve a longer half-life and to reduce toxicity, novel formulations such as solid lipid nanoparticles need to be formulated (Pires VC *et al,* 2020). The present research involves the formulation and evaluation of solid lipid nanoparticles of artemisinin.

Materials and Methods

Materials

Artemisinin, stearic acid and phospholipon 90G were obtained from Sigma Aldrich Pvt. Ltd. Tween 80 and dichloromethane were purchased from Rankem, Bangalore. The other chemicals used were of analytical grade.

Methods

Preformulation Studies

Drug-Excipients Compatibility Studies

The compatibility of the drug and

excipients was determined by using DSC. DSC Spectra of pure artemisinin, excipients and a blend of artemisinin with excipients were obtained by using DSC. Samples were kept in aluminum crucible pans and scanned in the 50-300°C temperature range with a heating rate of 10ºC/min using Shimadzu DSC-60 equipment (Senthilnathan B *et al,* 2019).

Preparation of Artemisinin Solid Lipid Nanoparticles

Artemisinin SLNs were prepared by hot homogenization technique using a high-speed homogenizer (Raut ID *et al,* 2018). The artemisinin and phospholipon 90G in various concentrations (ASLN1 – ASLN5) were dissolved in dichloromethane and mixed with the melted lipid stearic acid phase. Tween 80 was used as a stabilizer. Then the organic phase was poured in 2.5% tween 80 solution. The prepared solution was placed in the water bath and stirred at 24000 rpm for 10 min. The dispersion was stirred by a magnetic stirrer until cooling for artemisinin SLNs formation. The formula used was given in table 1.

Table 1 Preparation of Artemisinin SLNs

S.No.	Ingredients	Formulations				
		ASLN1	ASLN2	ASLN3	ASLN4	ASLN5
1.	Artemisinin (mg)	100	100	100	100	100
2.	Stearic acid (mg)	300	300	300	300	300
3.	Phospholipon 90G (mg)	300	350	400	450	500
4.	Tween 80 (ml)	2.5	2.5	2.5	2.5	2.5
5.	Dichloro methane (ml)	10	10	10	10	10
6.	Purified water (ml)	100	100	100	100	100

Characterization of Artemisinin SLNs

The SLNs containing Artemisinin was characterized for various physicochemical properties.

Drug Content

The drug content in all five formulations (ASLN1 – ASLN5) was determined by using a UV spectrophotometer (Agarwal SP *et al,* 2009). 1gm of artemisinin SLNs was weighed and dissolved in 25ml of methanol. 1 ml from the above solution was taken and diluted to 25 ml with purified water. The absorbance was measured at 195nm using a UV spectrophotometer and the drug content was calculated.

Drug Entrapment Efficiency

The artemisinin in buffer solutions was exposed to 30 min centrifugation at 15000 rpm. The supernatant liquid was separated. From this solution, 1ml was diluted with buffer solution. The absorbance was measured at 195 nm by using UV Spectrophotometer.

Particle Size and Surface Charge

These two parameters play an important role in the stability of the SLNs. The particle size and zeta potential measurement were analyzed by Malvern Zeta sizer (Ferraris S *et al,* 2018). The sample was injected slowly and analyzed. Six replicates were analyzed.

Scanning Electron Microscopy (SEM)

The morphological studies of artemisinin SLNs were studied using SEM analysis (FEI-Quanto 200F) operating at 15kv (Pathak YV, Labhashetwar VD, 1993). The optimized SLNs loaded with artemisinin were mounted on a metal slab with double adhesive tape and coated with platinum under vacuum and the SEM images were observed.

***In vitro* release**

Drug release studies were performed for 24 h by Franz diffusion cell using a dialysis membrane (Salamanca CH *et al,* 2018). The formulated artemisinin SLNs preparations were placed inside the dialysis membrane and immersed in pH 6.8 phosphate buffer. The samples were withdrawn at regular intervals and analyzed by UV spectrophotometer at 195 nm. From the absorbance values, the cumulative percentage of drug release was calculated.

Results and Discussion

Drug-Excipients Compatibility Studies

Drug-Excipient compatibility studies revealed that there was no interaction between artemisinin and excipients used in the formulation. This can be observed from the DSC of artemisinin and the combination of artemisinin with excipients, which showed a sharp endothermic peak at 210.1^{0}C and 211.1^{0}C respectively. No significant changes were observed. The DSC thermograms of artemisinin and artemisinin SLNs were given in Fig 1 and 2.

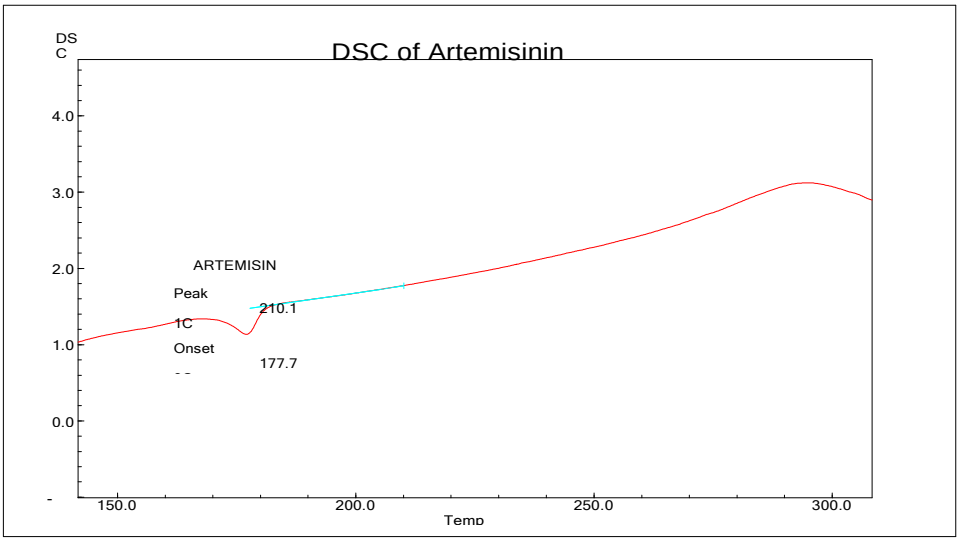

Fig. 1. DSC of Artemisinin

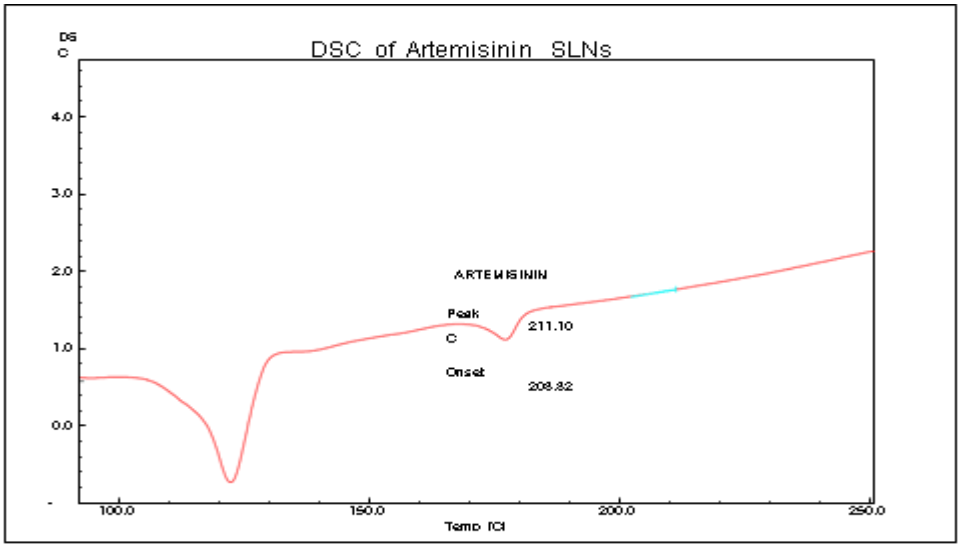

Fig. 2. DSC of Artemisinin SLNs

Drug Content

The drug content for the artemisinin SLNs was almost the same for all formulations. This result suggested that there was no drug loss during the preparation of artemisinin SLNs. The results were given in table 2.

Drug Entrapment Efficiency

The entrapment efficiency of artemisinin SLNs increased with an increase in phospholipon 90G concentration. This may be due to the availability of stearic acid to encapsulate the artemisinin, upon increasing the phospholipon 90G concentration, the number of layers of stearic acid that coat the drug decreases which increases the percentage of entrapment efficiency. The minimum and maximum percentage entrapment efficiencies for artemisinin SLNs were found to be 45.78 (ASLN1) and 89.64 (ASLN5) respectively. The results were given in table 2.

Table 2. Drug content (%) and Entrapment Efficiency (%)

Formulations	Drug Content (%)	Entrapment Efficiency (%%)
ASLN1	98.72	45.78
ASLN2	98.71	56.89
ASLN3	98.70	71.49
ASLN4	98.72	80.71
ASLN5	98.73	89.64

Particle Size and Surface Charge

The particle size of artemisinin SLNs ranged from 225.4 to 242.8nm. Almost all the formulations possessed the optimum particle size. Still, there is a reduction in particle size

on increasing the concentration of phospholipon 90G. This may be due to the reduction of stearic acid to encapsulate the drug by the phospholipon 90G which produced less particle size. The zeta potential values of artemisinin SLNs were negative and increased from -14.7 mV to -18.7 mV. The negative value was due to phospholipon 90G. The ASLN5 formulation having -18.7 mV zeta potential was considered optimum. The results were given in Fig. 3 & 4 and Table 3.

Table 3. Particle size and Surface charge

Formulations	Particle size (nm)	Zeta potential (mV)
ASLN1	242.8	-14.7
ASLN2	235.6	-15.4
ASLN3	231.3	-16.2
ASLN4	227.8	-17.9
ASLN5	225.4	-18.7

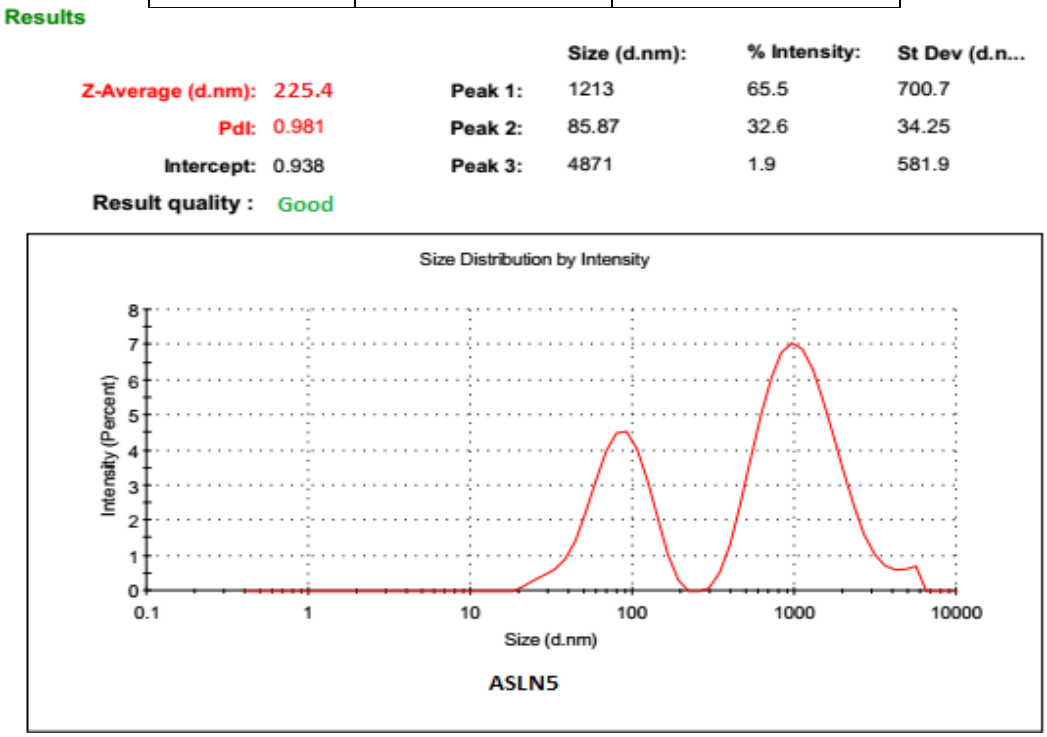

Fig. 3. Zeta size distribution of ASLN5

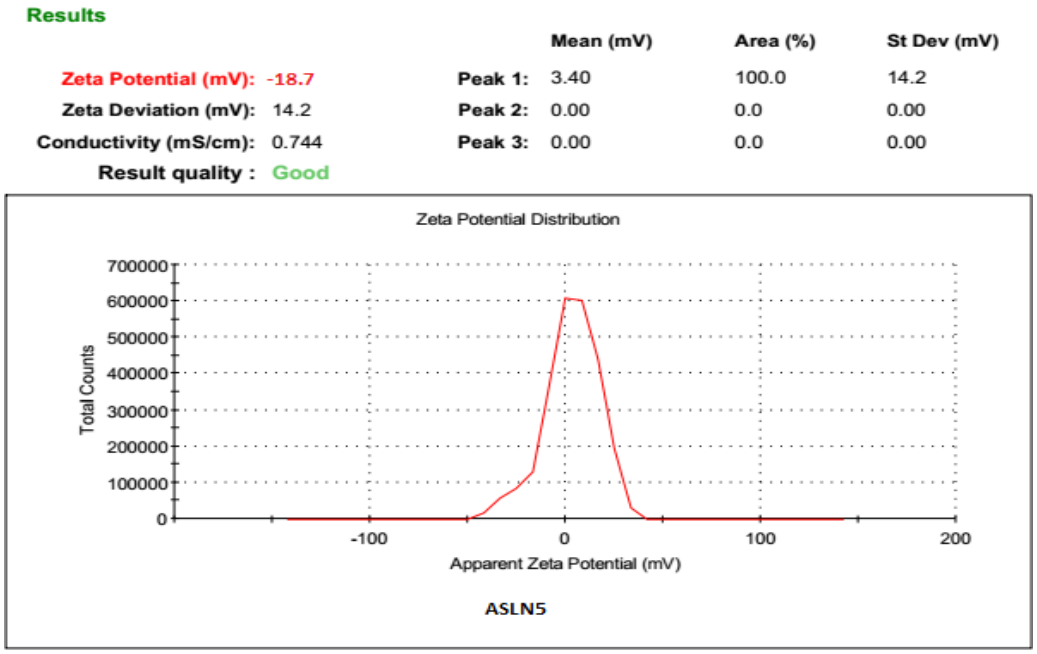

Fig. 4. Zeta Potential of ASLN5

Scanning Electron Microscopy (SEM)

The morphological properties were confirmed by scanning electron microscopy. The formulated artemisinin SLNs were spherical in shape and nano in size. No sign of drug precipitation was observed which confirmed the stability of artemisinin solid lipid nanoparticles. The scanning electron microscopy image of artemisinin SLNs was given in fig.5.

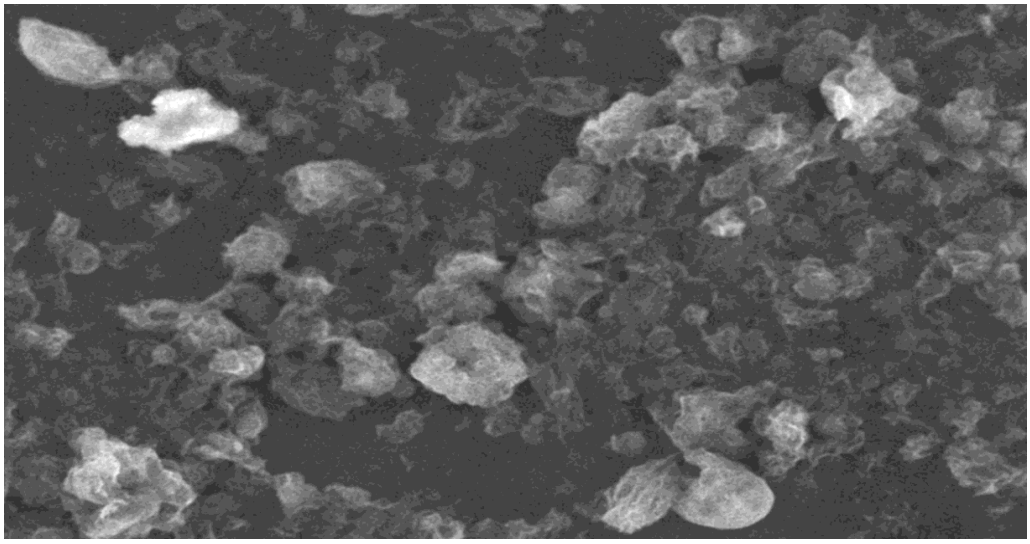

Fig. 5 SEM photo of artemisinin SLN (ASLN5)

***In vitro* release**

Drug release studies were executed for all the preparations (ASLN1 to ASLN5). The maximum percentage of drug release was observed in ASLN5(98.72%) at 24 h. The maximum percentage of drug release at 24 h for ASLN1 to ASLN4 was 75.82%, 79.85%, 81.54% and 88.47% respectively. The results indicated that an increase in the concentration of phospholipon 90G causes an increase in the percentage of drug release. The percentage of drug release for all the formulations (ASLN1 to ASLN5) was given in table 4 and fig. 6.

Table 4. % Cumulative drug release of artemisinin SLNs (ASLN1 to ASLN5)

Time (hrs)	% CUMULATIVE DRUG RELEASE				
	ASLN1	ASLN2	ASLN3	ASLN4	ASLN5
0	0	0	0	0	0
0.5	15.25	18.78	20.56	25.57	30.26
1	25.76	28.77	30.72	33.81	35.94
6	36.14	40.38	43.84	47.92	52.67
12	47.36	50.46	52.75	55.79	60.72
16	61.26	65.81	68.82	74.81	79.65
20	69.31	74.66	76.80	82.75	87.92
24	75.82	79.85	81.54	88.47	98.72

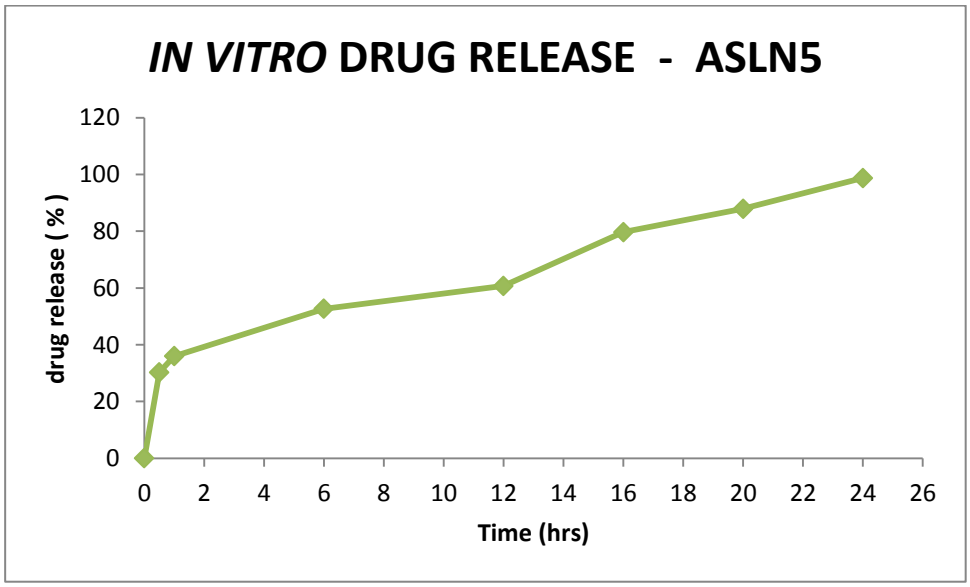

Fig. 6. *In vitro* Drug Release for artemisinin SLNs (ASLN5)

From all the formulations (ASLN1 to ASLN5), ASLN5 was selected as the optimized formulation due to its ideal particle size (225.4nm), zeta potential (-

18.7mV), high entrapment efficiency (89.64%) and *in vitro* drug release (98.72%) at 24 h.

Conclusion

The solid lipid nanoparticles containing artemisinin (ASLN5) exhibited most of the preferred characteristics required for novel drug delivery systems. The artemisinin solid lipid nanoparticles of lower particle size (225.4nm) with negatively charged surface charge (-18.7mv) have been achieved. Controlled drug release was observed for up to 24 hr. Hence it can be concluded that the newly established artemisinin solid lipid nanoparticles are ideal and effective in delivering the drug at a controlled rate.

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