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DEVELOPMENT AND CHARACTERIZATION OF NANO-LIPID CARRIER FORMULATIONS FOR THE MANAGEMENT OF BACTERIAL MENINGITIS

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ABSTRACT

Solid lipid nanoparticles (SLNs) have been studied as a drug-delivery system for the controlling of drug release. Ampicillin as a β-lactam antibiotic was studied to load on SLNs for control of drug release to increase administration intervals and decrease dose of drug to increase patient compliance and decrease antibiotic resistance. The solid lipid nanoparticles of containing Ampicillin prepared by an emulsification ultrasonic-homogenization method with cinnamon oil. The in-vitro drug release profile of ampicillin from prepared SLNs was found about 12 h and releases about 98% of ampicillin during this period by a sustained behavior. No significant difference was observed and there was no burst effect from SLNs.

Keywords: antibiotics: Ampicillin, drug delivery systems, solid lipid nanoparticles

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INTRODUCTION

The discovery and therapeutic application of antibiotics have enabled the development of modern medicine. However, the abuse and misuse of antibiotics in medicine, animal health, and agriculture over the years have led to the emergence of multidrug- resistant (MDR) bacteria and the loss of efficacy of existing antibiotics. Antibiotics are broadly employed to treat infectious diseases. However, this intensive application also has developed some associated problems. According to World Health Organization (WHO), antimicrobial resistance (AMR) is one of the biggest threats to global health, and the world urgently needs to develop new tools and strategies to minimize this problem [1,2]. It has been estimated that this situation would lead to a scenario where infections caused by MDR bacteria could cause 10 million deaths each year by 2050. Moreover, this damage would also impact the economy, leading to a catastrophic situation whereby 2030 AMR could force up to 24 million people into extreme poverty [3].

Nanoscience and nanotechnology could be a realistic solution to the AMR problem, as the development and study of drug delivery systems (DDSs) have provided new possibilities to improve the effectiveness of different therapeutic drugs for other complex diseases like cancer, autoimmune diseases or pathologies in the central nervous system [4, 5]. Among all the developed DDSs, solid lipid nanoparticles (SLNs) are of particular interest not only due to their low toxicity but also because their production at a large scale is technically and economically feasible.

Nanoparticle delivery systems have been employed for the encapsulation of lipophilic, hydrophilic, and poorly water-soluble drugs [4]. The use of lipids for the formation of nanoparticles such as solid lipid nanoparticles (SLN) or nanostructured lipid carrier (NLC), offers multiple benefits compared to other materials, which is due to low cytotoxicity and controlled drug release [6]. The controlled drug release is aimed to maintain drug concentration in the blood or the target tissue at the favorable evel [7]. The controlled drug release can be applied to both hydrophilic and hydrophobic drugs.

Hydrophobic drugs in microemulsion and nanostructured lipid forms, have exhibited essential growth in the drug release compared to free dugs. To date, lipid nanoparticles have been successfully used for hydrophobic drug entrapment, though encapsulating a high content of hydrophilic drugs in these materials is challenging. The key parameters in the preparation of NLC containing hydrophilic drugs are formation method, selection of materials as solid and liquid lipids, and choice of appropriate surfactants used in organic and water phases [8].

Material and Methods

Lecithin, dichloromethane, Cholesterol, methanol, tween 80 and PVA were procured from Sigma-

Aldrich, Cinnamon oil (was purchased commercially).

Preparation of AMP loaded SLNs

The solid lipid nanoparticles of containing Ampicillin prepared by an emulsification ultrasonic-homogenization method with cinnamon oil. The oil 1ml was dissolved in 10 ml methanol, adding tween 80. The required quantity of Lecithin and Cholesterol as given in Table 1 were dissolved in 10 ml dichloromethane. Methanolic and dichloromethane solutions were mixed manually. The resultant primary organic phase was mixed with 10 ml of PVA 5% w/v solution and homogenized for 10 min at 15,000 rpm using an ultrasound probe sonicator to produce a white cloudy emulsion. The resultant o/w was subjected to a Rota evaporator at 45 °C for complete evaporation of the organic phase.

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 SLNs 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.

Determination of Entrapment Efficiency

The entrapment efficiency was determined after separation of the unentrapped drug by the use of minicolumn centrifugation method (Fry et. al. 1978; New 1990). Sephadex G-50 (1.2gm) was swelled in 20 ml of 0.9% NaCl solution for 5 hours at room temperature with occational shaking. The gel was formed and it was stored at 4ºC. To prepare the minicolumn, the hydrated gel was filled up to top in the barrel of 1mL disposable syringe, plugged with whatman filter pad. Then barrel placed in the centrifuge and centrifuged at 2000 rpm for 3 min to remove saline solution. Eluted volume was removed from the centrifuged tubes and exactly 0.2mL of emulsomal suspension (undiluted) was applied drop wise on the gel bed in the center. Columns were again centrifuged at 2000 rpm for 3 min to expel and remove void volume containing emulsomes to the centrifuge tubes. Elute was remove and 0.25 mL of saline was applied to each column and centrifuge as previously. The amount of drug entrapped in the vesicle was then determined by disrupting the vesicle using 1 ml of 0.1%v/v triton-X 100, filtering it and the drug content was determined using UV-Vis spectroscopy at 208 nm. The percentage efficiency was determined by following equation:

Percentage drug entrapment efficiency = Amount of entrapped drug / Total amount of drug 100

In Vitro Diffusion Studies

Before experiment, the cellophane membrane was washed in the running water and then soaked in distilled water for 12 h to remove glycerine present on membrane. The release of solid mass powder of SLNs was studied by dialysis method in pH 7.4 artificial skin pH. 2 ml samples were instilled in the dialysis bag which was screwed with two clamps at each end. The dialysis bag was dipped into the receptor compartment containing 35 ml of dissolution medium and stirred continuously at 100 rpm. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5°C. The receptor compartment was closed to prevent evaporation of the dissolution medium. The solution on the receptor side was stirred by externally driven teflon coated magnetic bars. At predetermined time intervals, 5 ml of solution from the receptor compartment was pipette out and immediately replaced with fresh 5 ml phosphate buffer. Samples were withdrawn at regular time intervals, and the same volume was replaced with fresh dissolution medium. The amount of drug entrapped in the vesicle was then determined by disrupting the vesicle using 1 ml of 0.1%v/v triton-X 100, filtering it and the drug content was determined using UV-Vis spectroscopy at 307 nm. Calculation of percentage drug release was done using the formula:

% drug release = (Conc. of drug (in mg) x Volume of receptor compartment) x 100 / Label claim (amount of drug in donor compartment) [7-9].

Zero order release kinetics

Zero order release kinetics refers to the process of constant drug release from a drug delivery device such as oral osmotic tablets, transdermal systems, matrix tablets with low-soluble drugs and other delivery systems. In its simplest form, zero order release can be represented as:

$$Q = Q0 + K0\ t \qquad \text{Eq. 1}$$

where Q is the amount of drug released or dissolved (assuming that release occurs rapidly after the drug dissolves), Q0 is the initial amount of drug in solution (it is usually zero), and K0 is the zero order release constant. The plot made was cumulative % drug release vs time (zero order kinetic models).

First order release kinetics

The rate laws predicted by the different mechanisms of dissolution both alone and in combination, have been discussed by Higuchi.

$$\text{Log } C = \text{Log } C0 - kt / 2.303 \qquad \text{Eq. 2}$$

where, C0 is the initial concentration of drug and K is first order constant. The equation in resemblance to the other rate law equations, predicts a first order dependence on the concentration gradient (i.e. Cs - Ct) between the static liquid layer next to the solid surface and the bulk liquid.

Result and Discussion

Particle size

The evaluation of particles using Malvern zeta sizer (ZEN3600) showed a normal distribution of particle size, the results showed that more than 70% of particles were smaller than 126.11 nm in size. This result confirmed that lyophilization of ampicillin SLNs did not show any effect on particle size of prepared particles. Comparison of particle size of SLNs after lyophilization, shows that cinnamon oil act on affect of cryoprotectant yileds the smallest particles.

Zeta potential and PDI

The result concluded that as the concentration of solid core varies and the amount of lipid content increase the particle size increase, thus increase the PDI and zetapotential action. The variations of result of all parameters also showed the optimization of double layer. The optimization of sonication time was improving the particle size of AMP-SLN4, because of double layer of SLNs and showed smaller size

than the other formulations. Thus the result of particle size distribution, PDI and zetapotential action was because of amount of solid lipid core more amounts of LC and less amount of CH. The change of vesicles size and drug entrapment efficiency also correlated with each other, due to thickness of wall of lipid layers. The formulation AMP-SLN4 has more than 95 % drug entrapment with addition of tween 80 in 10% concentrations. All the result of dependent variables concluded that the formulation AMP-SLN4 was selected for the optimization of effect of various surfactants in different concentration to identify the penetration rate or drug entrapment efficiency inside the solid lipid core. The optimization of sonication time also evaluated for identification of effect vesicular size and shape.

SEM study

Figures 3 showed the morphologies of the SLNs formulations and concluded that no significant enlargement of particle size was happened after storage.

Drug loading efficacy

Our results showed 85.17±2.3% drug loading efficacy. The percentage of drug loading was used for calculations of drug release profile as well.

Drug release studies

The in-vitro drug release profile of ampicillin from SLNs for about 12 h and releases about 98% of ampicillin during this period by a sustained behaviour. No significant difference was observed and there was no burst effect after SLNs.

Table 1: Various composition of prepared AMP SLNs

Formulation Code	Solid Lipid content		Cinnamon Oil (ml)	Surfactant (%) (Tween 80)	Sonication time (Min.)
	Lecithin (LC) (mg)	Cholesterol (CH) (mg)			
AMP-SLN1	0	100	1	10	10
AMP-SLN2	25	75	1	10	10
AMP-SLN3	50	50	1	10	10
AMP-SLN4	75	25	1	10	10
AMP-SLN5	100	0	1	10	10
AMP-SLN6	75	25	1	10	10
AMP-SLN7	50	50	1	10	10
AMP-SLN8	25	75	1	10	10

Table 2: Characterization of various compositions of AMP SLNs

Formulation Code	Particle size (nm)	Layers	Zeta potential (mV)	PDI	Drug Entrapment (%)
AMP-SLN1	127.21±1.11	Double	-24.21±1.09	0.216±0.95	81.37±0.8
AMP-SLN2	129.01±0.91	Double	-22.91±1.03	0.219±0.05	78.13±1.1

AMP-SLN3	129.99±0.65	Double	-22.16±1.05	0.221±0.27	76.03±0.3
AMP-SLN4	126.11±1.09	Double	-24.81±1.19	0.215±0.02	85.17±0.3
AMP-SLN5	128.11±1.03	Double	-23.01±1.03	0.217±0.35	79.98±1.2
AMP-SLN6	131.78±0.88	Double	-21.91±0.95	0.229±0.07	78.11±0.9
AMP-SLN7	130.19±1.11	Double	-22.02±1.04	0.228±0.05	80.17±0.7
AMP-SLN8	128.01±0.91	Double	-23.01±0.96	0.226±0.95	82.97±0.8

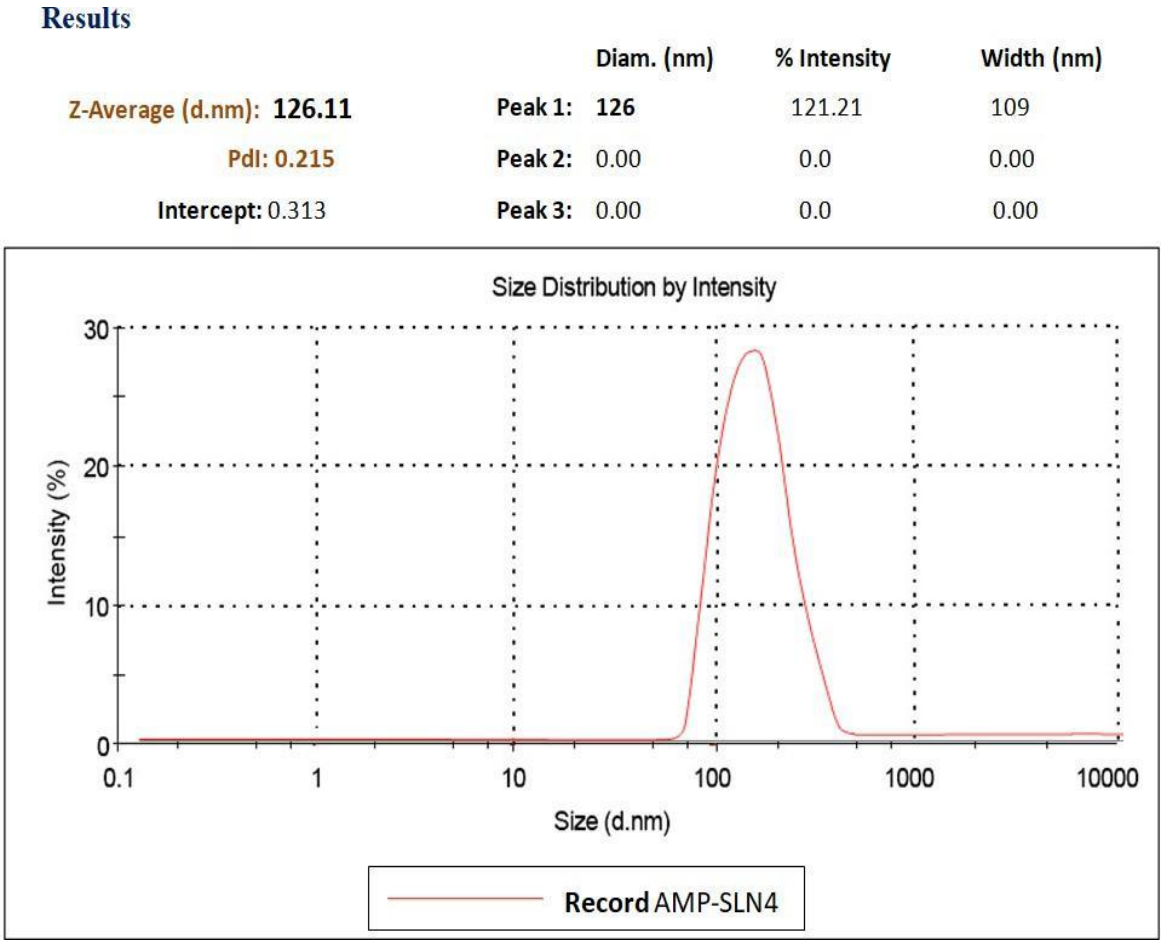

Figure 1: Particle size and PDI of prepared AMP SLNs

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -24.81	**Peak 1:** -24.81	100	5.79
Zeta Deviation (mV): 78.29	**Peak 2:** 0.00	0.0	0.00
Conductivity (mS/cm): 0.331	**Peak 3:** 0.00	0.0	0.00

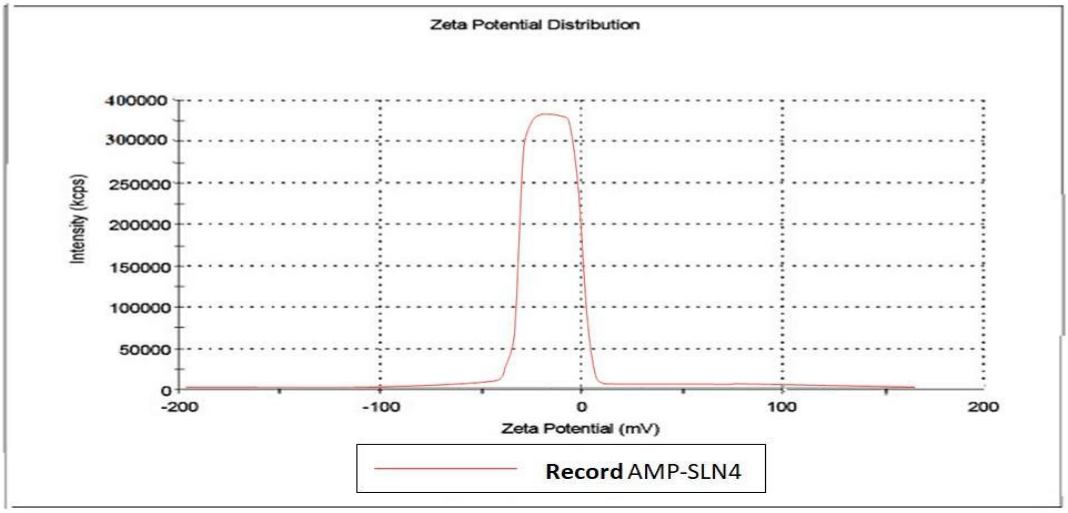

Figure 2: Zeta potential of prepared AMP SLNs

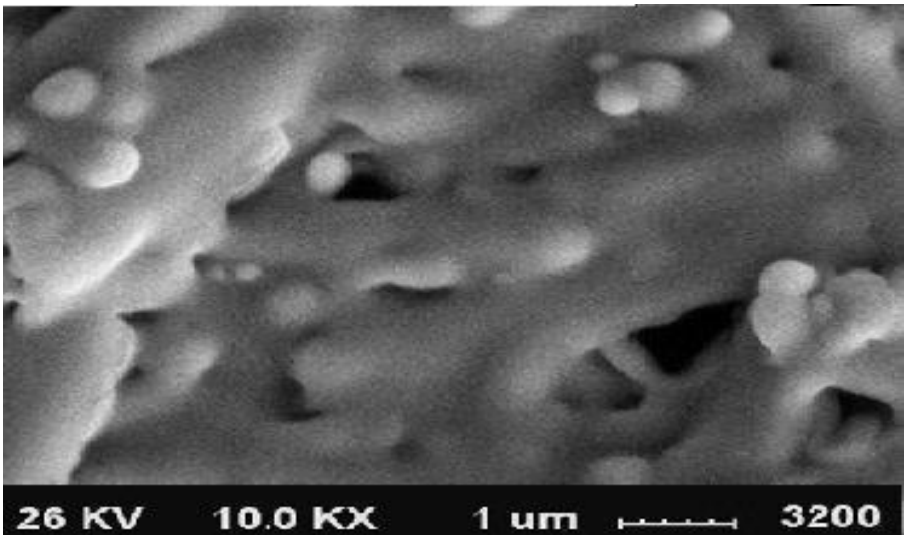

Figure 3: Scanning electron microscopy pictures of prepared AMP SLNs

Conclusions

Antimicrobial resistance is currently one of the biggest threats to global health, and we urgently need new strategies to tackle it. Considering the results of new developed formulations of antibacterial drugs represents, the improvement of the efficiency of well-known drugs by nanocarriers seems the most promising approach. In this context, solid lipid nanoparticles offer highly interesting opportunities to increase antibiotic efficiency and reverse or reduce antibiotic resistance. SLNs can improve drug stability, solubility and permeability, enhancing its bioavailability and, most importantly, drug concentration in the target site. Moreover, they have demonstrated their ability to overcome some of the basic resistance mechanisms developed by resistant bacterial strains, such as reduced drug permeation, intracellular infections or biofilm formation. The in-vitro drug release profile of ampicillin from SLNs for about 12 h and releases about 98% of ampicillin during this period by a sustained behaviour. No significant difference was observed and there was no burst effect after SLNs.

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