Synthesis and evaluation of solid lipid nanoparticles loaded with bovine serum albumin prepared by different methods

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Abstract: We evaluated the effect of different synthesis methods of solid lipid nanoparticles (SLNs) loaded with bovine serum albumin (BSA) on parameters including particle size, polydispersity index, loading capacity and % entrapment efficiency including release study. We investigated the binary fatty acids mixtures for test protein BSA. Different techniques were used as micro emulsion, ultrasound homogenization and double emulsification-evaporation for the BSA loading of SLNs. With the increase in BSA content from 0-10%, indicated an increase in the size and decrease in polydispersity index. The stability of SLNs loaded with BSA was examined by measuring the zeta potential and all formulations were found to be quite stable. Release study and kinetic models were applied to assess BSA release profile from different formulations of SLNs. The particle size of BSA loaded SLNs was reduced to 89.67 ± 4.88 nm when PEG 6000 and Brij were used as 0.25% and 1.5% of total formulation (F5). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier-transform infrared spectroscopy (FTIR) indicated the chemical stability of BSA which was used to load SLNs in different formulations. SLNs from the combinations of solid and liquid lipids had enhanced the physicochemical properties and permitted controlled release of BSA for up to 10 days. The study also evaluated the addition of polyethylene glycol which reduced the particles size and enhanced % entrapment efficiency. The release of BSA from SLNs was followed zero order rate kinetics and diffusion-controlled. Different mathematical models, i.e., zero order, first order, Higuchi and Korsmeyer-Peppas models were found best fit to BSA release profile of all formulations of SLNs.

Keywords: Bovine serum albumin, binary fatty acids mixture, Higuchi model; Korsmeyer-Peppas model.

INTRODUCTION

Since 1991, the long established colloidal carrier systems such as emulsions, liposomes, micelles and polymer based micro and nanoparticles have been replaced with the introduction of solid lipid nanoparticles (SLNs) (Wen *et al.,* 2018). The exceptional properties of SLNs such as small particle size, large surface area and enhanced drug loading capacity made it an attractive, efficient and non-toxic alternative lipid based colloidal drug carrier as a potential improved pharmacokinetics (Li *et al.,* 2010). The SLN offers a nanoparticulate depot system for the controlled release (Li *et al.,* 2010), gene delivery (del Pozo-Rodríguez *et al.,* 2010), topical drug delivery system (Pardeike *et al.,* 2009), versatile antimalarial drugs (Masiiwa *et al.,* 2018), in colon adenocarcinoma cell-lines (Campos *et al.,* 2018), in enhancing cytotoxicity in ovarian cancer cells (Kaur *et al.,* 2015) and improved bioavailability (Wen *et al.,* 2017) due to its biodegradable nature. The solid lipid nanoparticles (SLNs) efficiently incorporate lipophilic drugs. Secondly, a lipid matrix composed of same molecules also causes drug expulsion during storage after the polymorphic transition resulted in insufficient loading capacity. However, the lipids molecules are versatile that can fabricate structurally different solid matrices such as solid and liquid lipids mixtures resulting in a higher % entrapment efficiency and better drug release control. The SLNs fabricated from the mixtures of lipids appeared to be dependent on heterogeneity of the lipid constituents (Khalil *et al.,* 2013). In addition, it was reported by zur Mühlen *et al.,* (1998) that the fusion of a low melting point lipids in the lipid mixture increases the drug release time from SLNs. The addition of a low melting point lipid (liquid) in the formulation promotes changes in the structure of the nanoparticle which leads to a higher drug loading and better prolonged release properties. BSA is used as standard protein due to low cost, high stability and non-reactive nature in biochemical reactions. BSA is a serum albumin protein which is widely used in biochemical tests, immunohistochemistry, immunoblots and provides stability to SLNs as described earlier by Zhen *et al.,* (2010). BSA was used in the current research to determine its effect on stability of SLNs in terms of minimum size and release time of this protein from SLNs. In serum, Serum albumin act as carrier for majority of proteins and has wide integration capacity for different molecules. In

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earlier study carried out by Zhen *et al.,* (2010), SLNs prepared by only double emulsion method were investigated for BSA loading. However, in this study, the microemulsion, ultrasound homogenization, double emulsion and solvent evaporation techniques were used for the synthesis of SLNs and the bovine serum albumin (BSA) was encapsulated inside SLNs in order to check the stability of BSA loaded SLNs in terms of this protein release time and size changes. The physicochemical factors such as particle diameter, polydispersity index (PDI) values, zeta potential, percentage entrapment efficiency (%EE) and percentage loading capacity (%LC) were investigated for synthesized SLNs. Moreover, BSA release study and kinetic models were also applied to synthesized SLNs by different methods.

MATERIALS AND METHODS

All chemicals used in the present research work were of analytical grade and sterile. Lauric acid, oleic acid, chloroform, soy lecithin, Polyoxyethylene (23) lauryl ether (Brij35), bovine serum albumin were obtained from Sigma Aldrich and Merck-millipore (Germany). Moreover, Polyethylene glycol (PEG 6000), Polysorbate 80 (Tween 80), Polyvinyl alcohol (MW 88000-97000, 86-89% hydrolyzed) were purchased from Fisher Scientific.

Preparation of solid lipid nanoparticles (SLNs)

In current research, the solid lipid nanoparticles were fabricated from fatty acids (FA) mixtures by using three different methods. Binary mixtures of fatty acids were prepared by using natural liquid and solid fatty acids, in order to decrease the melting point of solid lipid to incorporate thermally labile drugs i.e., proteins and peptides. Lauric acid and oleic acid were used in this study. Solid FA lauric acid was heated to 55 °C above melting point (44.46 ºC) were added to liquid fatty acid oleic acid, which was maintained at the same temperature. The higher temperature was used to melt the lipids which resulted in reduced viscosity of the lipids and homogenous mixing. Their melting point was measured by using melting point apparatus (Gallenkamp apparatus). The mixtures were cooled and stored in a freezer.

Microemulsion cooling method

Binary FA mixture (100 mg) was melted to 40 ºC and added BSA solution in Phosphate-buffered saline (PBS) (2 mg/ml) in sealed glass bottle placed in a water bath. The sample was mixed for 5 minutes and solvent was evaporated under nitrogen atmosphere. The 10 ml surfactant and co-surfactant solution (1.5% tween 80 and 0.5% lecithin) was added drop-wise to melt the above mentioned mixture under magnetic agitation (1150 rpm) and continued for 15 minutes to form a melted micro emulsion. The pre-emulsion formed was ultrasonicated for 10 minutes to form a clear suspension. After sonication, the nanoemulsion formed was added to the ice cold water in 1:12 ratio under magnetic stirring. The blank SLNs were synthesized in a similar manner without the addition of BSA.

Ultrasound homogenization method

Briefly 100 mg of fatty acid mixture, BSA solution (2mg/ml prepared in sterile PBS pH 7.35) were added and was homogenized for 5min at 3000rpm and solvent was evaporated under nitrogen atmosphere. To this mixture, 10ml surfactant and co-surfactant solution (1.5 % tween 80 and 0.5% lecithin) was added drop-wise and ultra sonicated (using probe sonicator) for 30min on ice.

Double emulsion method

Briefly 100 mg BFs, 40 mg lecithin, BSA (2 mg/ml in PBS, pH 7.35) and 200 µl chloroform were mixed for 5 minutes with and without polyethylene glycol PEG (6000) 0.25%. The above O/W emulsion was added to 10 ml (1.5 %) surfactant solution and sonicated for 25 minutes on ice. The chloroform in nanoemulsion was evaporated under vacuum by a rotary evaporator at 4 ºC until the total volume remained less than 10 ml. In order to enhance the % entrapment efficiency, the bovine serum albumin was co-lyophilized with the polyethylene glycol. In brief, the bovine serum albumin and PEG 10000 solutions were prepared in a deionized sterile water separately. The BSA solution was mixed with PEG 10000 solutions in 1:5 molar ratio and freeze in -75ºC temperature. Then freeze dried at -80ºC for 24 hours. Co-lyophilisation refines the particle size of large molecular weight and enhances the % entrapment efficiency. The SLNs were prepared as mentioned above, with the addition of co-lyophilized BSA rather than BSA solution.

Percentage Entrapment efficiency (%EE) and percentage loading capacity (%LC) determination

In order to calculate percentage % entrapment efficiency (EE) and percentage loading capacity (%LC), the freshly prepared nanoparticles formulations were centrifuged at 28000 rpm using ultra centrifuge for 40 minutes to separate the nanoparticles. The supernatant collected was analysed by taking optical density (OD) at 280 nm to calculate the amount of non-entrapped protein. The % entrapment efficiency of nanoemulsion was calculated by using the following formula as described by Singh *et al*., (2016) and percentage drug loading capacity (LC) was calculated by using the formula with some modifications and was described by Dudala *et al*., (2014).

$$\%\ EE = \frac{\text{Total amount of drug per g of nanoparticles - Free drug per g of nanoparticles}}{\text{Total amount of drug per g of nanoparticles}} \times 100$$

$$\%LC = \frac{\text{Amount of protein added} - \text{Amount of free protein}}{\text{Amount of recovered SLNs}} \times 100$$

Bovine serum albumin (BSA) release and stability study (in vitro)

The BSA *in vitro* release profile from SLNs was monitored by using UV-visible spectrophotometer (model

agilent 8453). A 25 mg of prepared freeze dried SLNs were transferred to an autoclaved glass tube containing 5 ml 0.5X PBS (pH 7.35) and placed in an incubator 37 ±0.5°C at constant shaking of 150 rpm/minute. The supernatant was removed under sterile conditions after 72 hours and ultra-centrifuged at 28000 rpm for 45 minutes. The released BSA protein pellet was dissolved in 100 µl of 0.5X PBS and subjected to 12% SDS-PAGE analysis by adopting the procedure as described earlier by Ahsan *et al*., (2014). Different kinetic models were used to study drug release profile of BSA from nanoparticles. The SLNs loaded with BSA with all different formulations (F1-F5), were packed in glass vials and kept at 4ºC for 3 months. After three months these were mixed with 5 ml 0.5X PBS (pH 7.35) and supernatant was ultra-centrifuged at 28000 rpm for 45 minutes. The released BSA protein pellet was dissolved in 100 µl of 0.5X PBS and subjected to 12% SDS-PAGE analysis as described earlier. The %EE and particle size were also calculated at 0, 1, and 3 months to check the stability of BSA loaded SLNs. The BSA release profile in terms from BSA loaded SLNs prepared by double emulsion method with composition codes (F1, F2, F3, F4 and F5) in 0.5X PBS pH 7.4 at 37ºC was also determined after every 24 hours for 10 days.

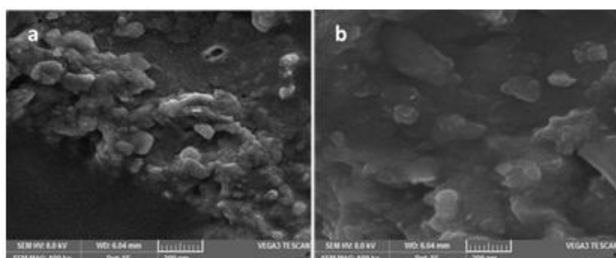

Fig. 1: SEM photographs of blank SLNs (a) and BSA-loaded SLNs with composition code F5 (b).

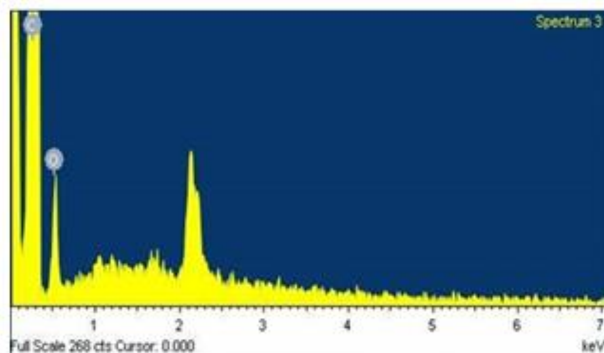

Fig. 2: EDAX spectrum of synthesized blank SLNs showing elemental composition.

Characterization of nanoparticles

Photon correlation spectroscopy was used to assess zeta potential measurement using zeta sizer nano in order to find out the size and electrophoretic mobility. A one ml of nanoemulsion was diluted to 3 ml with sterile water and was equilibrated for 10 minutes at room temperature. Particle size, polydispersity index and zeta potential measurements were performed at 25 °C. Fourier-transform infrared spectroscopy (FTIR) was used to identify different functional groups using Agilent technology model # 41630 (ATR mode). Crystalline structure of binary fatty acids, freeze dried SLNs and BSA were determined by X-ray diffraction (XRD) by PANalytical XPERT-PRO model. The instrument operated at 40 kV and 40 mA using Cu Kα radiation. The detector was scanned over a range of angle 2θ =5°-70° at a step size of 0.03. Morphology was studied by TESCAN Vega 3 LMU-Variable pressure Scanning Electron Microscope. The samples were coated with gold targets for 1.5 seconds for the analysis.

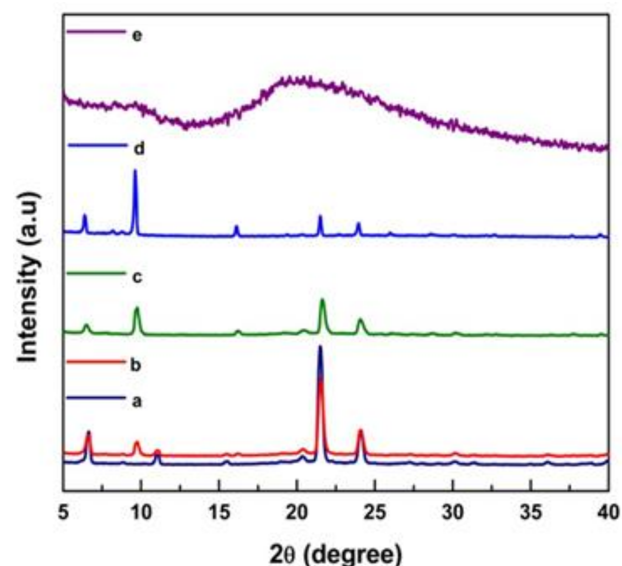

Fig. 3: XRD pattern of (a) Lauric acid, (b) fatty acid mixture of lauric and oleic acid (5:1), (c) blank SLNs, (d) BSA loaded SLNs prepared by double emulsion method with formulation code F5 (e) pure BSA.

Formulation codes of nanoparticles

Different formulation codes for nanoparticles were mentioned in the current study. The E1, E2, E3 codes represented blank SLNs produced by microemulsion technique, ultrasound homogenization and double emulsion method while E1-a, E2-a, E3-a codes represented BSA loaded SLNs produced by microemulsion technique, ultrasound homogenization, and double emulsion method under optimized condition using 2 mg/ml protein solution.

The formulation codes F1, F2 and F4 represented composition of BSA loaded SLNs involving mixture of lauric and olic acid (100 mg) and soy lecithin (0.4 mg) while for F1 surfactant used was tween 80 (1.5% of total weight of formulation) however for F2 and F4 surfactant used was brij 35 (1.5% of total weight of formulation). Formulation codes F3 and F5 represented same composition of SLNs as for F2 and F4 however only difference in these two formulations is that here 0.25 mg

PEG 6000 was also added. In all formulations (F1-F5) of SLNs, 2 mg/ml of BSA was used (table 3).

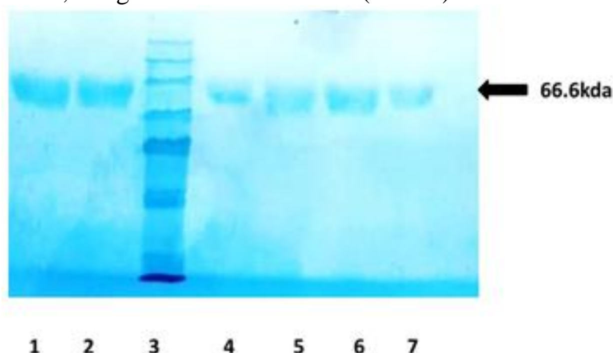

Fig. 4: SDS-PAGE analysis of BSA loaded SLNs for release studies. Lane 1: BSA standard protein (10 μg), Lane 2: BSA released after 72 hours at 37ºC in 0.5X PBS (pH 7.35) from BSA loaded SLNs prepared by double emulsion method with formulation code F5, Lane 3: Standard protein marker, Lane 4, 5, 6, 7: BSA released after 90 days from SLNs prepared by double emulsion method with formulation code F2, F3, F4 and F5 respectively.

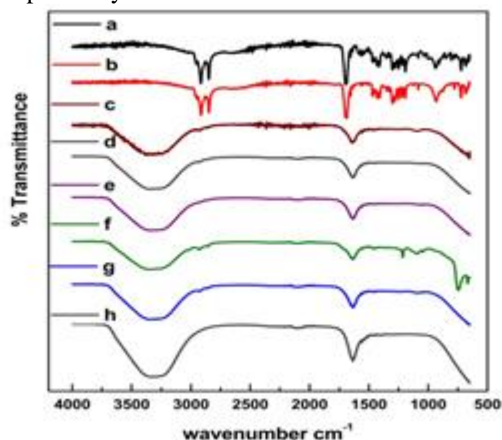

Fig. 5: FTIR spectra of (a) Lauric acid, (b) binary fatty acids, (c) SLNs (blank), (d) SLNs with composition code F2, (e) SLNs with composition code F3, (f) SLNs with composition code F4, (g) SLNs with composition code F5 and (h) Pure BSA.

RESULTS

Particle morphology and Size

Fig. 1 represents the scanning electron microscope (SEM) photographs of blank and BSA loaded SLNs produced by the double emulsion method with formulation code F5 in which least diameter of SLNs was achieved which was 89.67±4.88 nm. It can be seen from the image that particles were of semi circular morphology and monodisperse. table 1 summarizes the composition and physiochemical properties of SLNs produced through three different methods. The results indicated that ultrasound homogenization and double emulsion method produced smaller particle size however least size was achieved for BSA loaded SLNs was 91.225 nm with 0.215 PDI value. The % entrapment efficiency (%EE) of three methods for BSA loaded SLNs was 25.62%, 42.17% and 49.63% for microemulsion, ultrasound homogenization and double emulsion method respectively. On the basis of least particle size and %EE, the double emulsion-evaporation method was selected for further study. The effect of BSA concentration on particle size and zeta potential is presented in table 2. Increase in BSA amount increases the particle size, zeta potential and %EE**.** tables 3 and 4 enlist the composition of BSA loaded SLNs and their physiochemical properties. In table 3 composition of BSA loaded SLNs and non BSA loaded SLNs was described. The results indicated that addition of PEG 6000 decreases the particle size with an average diameter of 89.67 nm for BSA loaded SLNs (table 4). Influence of PEG 6000 as co-surfactant was also evaluated in this study. All formulations had PDI values lesser than 0.1, indicating that nanoemulsions were monodisperse. The zeta potential values indicated that all nanoemulsions were stable (table 4). The chemical stability of SLNs prepared by double emulsion method and loaded with BSA with formulations (F1-F5) was also assessed for three months and results were represented in table 5 which showed that the SLNs formulations were quite stable in terms of particle size and %EE. Least particle size of SLNs (89.665±5.01nm) at 0 day and after 90 days (94.4±5.49nm) was observed in case of SLNs prepared by double emulsion method and formulation code F5 (table 5).

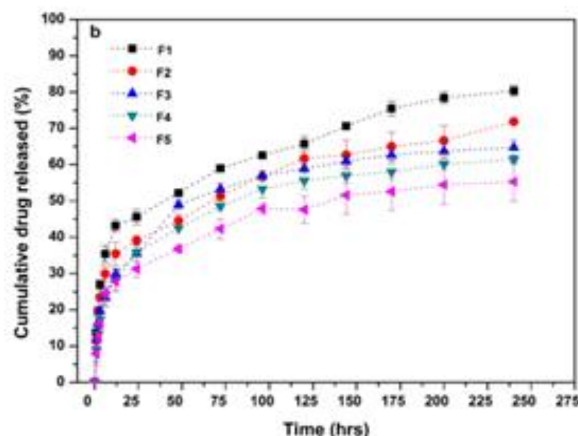

Fig. 6: BSA release profile from BSA loaded SLNs with composition codes (F1, F2, F3, F4 and F5) in 0.5X PBS pH 7.4 at 37ºC.

Energy dispersive X-rays analysis (EDAX)

Fig. 2 presents the EDAX spectrum of blank SLNs. The EDAX data of % weight of synthesized SLNs were as follows: 91.33% and O: 8.67%. The results of EDAX spectrum showed only C (carbon) and O (oxygen) elements without any impurities. Therefore, the EDAX analysis confirmed that the synthesized solid lipid nanoparticles were in perfect stoichiometry.

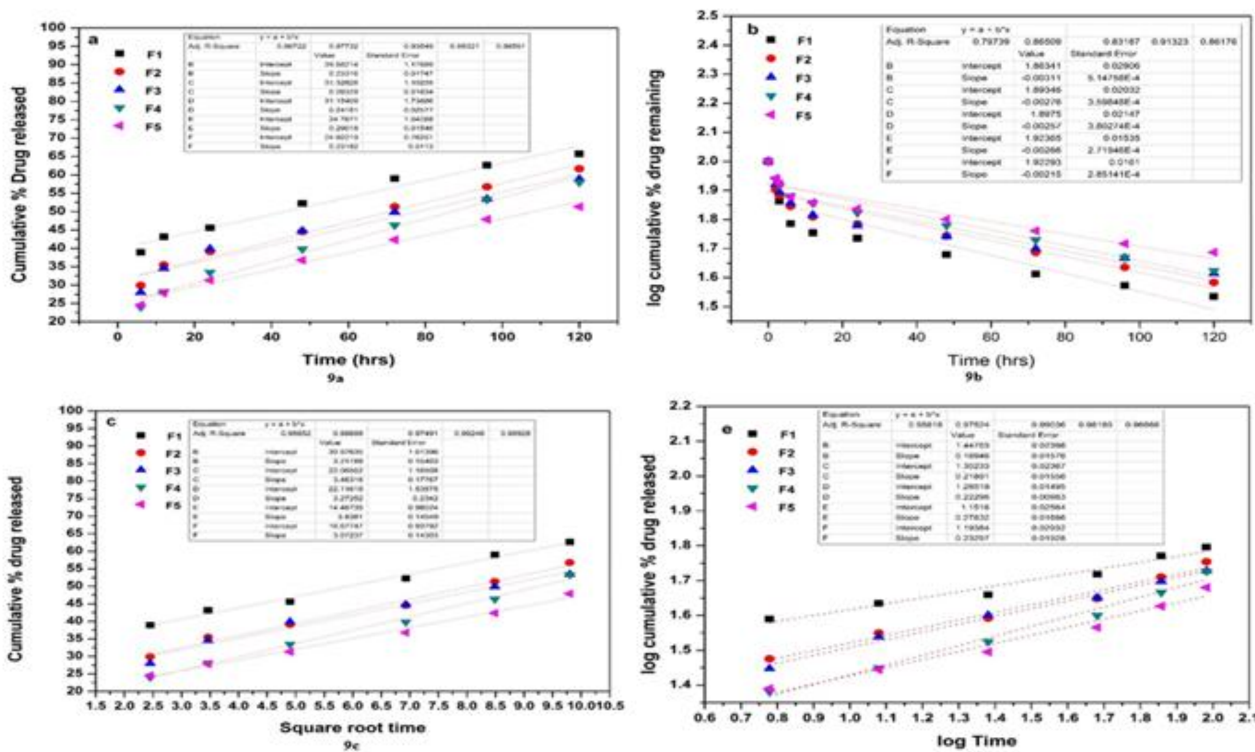

Fig. 7: Kinetic models, (a) zero order, (b) 1st order, (c) Higuchi model and (d) Korsmeyer-Peppas model.

Table 1: Composition of SLNs produced by three different methods and their size, PDI (polydispersity index) and %EE (entrapment efficiency)

Formulation code	Formulation Method	BFs /surfactant	Particle Size (nm) ± SD	PDI ± SD	%EE
E1	Microemulsion	1:1.5:0.5	125.67±4.22	0.241±0.01	---
E1-a	Microemulsion	1:1.5:0.5	148.23±3.55	0.326±0.015	25.62±2.065
E2	USH	1:1.5:0.5	74.63±3.18	0.221±0.009	---
E2-a	USH	1:1.5:0.5	99.05±1.55	0.266±0.014	42.17±1.38
E3	W/O/W	1:1.5:0.4	67.83±2.055	0.204±0.007	---
E3-a	W/O/W	1:1.5:0.4	91.225±2.55	0.215±0.01	49.63±2.14

Note: E1, E2, E3 blank SLNs produced by microemulsion technique, ultrasound homogenization and double emulsion method & E1-a, E2-a, E3-a were BSA loaded SLNs produced by microemulsion technique, ultrasound homogenization, and double emulsion method under optimized condition using 2 mg/ml protein solution.

X-Ray diffraction (XRD) analysis

The diffractogram of pure BSA, lauric acid, lauric/oleic acid mixture, blank SLN, BSA loaded SLNs prepared by double emulsion method with formulation code F5 are shown in fig. 3. The two sharp peaks of lauric acid were appeared in 2ϴ range of 20 to 25º. The diffractogram fatty acid mixture of lauric and oleic acid has shown peaks in the same region with low intensity due to less crystal formation in binary fatty acid mixtures. The pure BSA peaks appeared in 2ϴ range of 9.78º and 19.90º but could not detected on the BSA-loaded SLN, demonstrating that the BSA was successfully encapsulated to an inner core of SLN.

Fourier-transform infrared spectroscopy (FTIR) and Sodium dodecyl sulphate -polyacrylamide gel electrophoresis (SDS-PAGE) analysis

To assess chemical integrity of BSA loaded SLNs, the SDS-PAGE analysis of BSA released after 72 hours at 37ºC in 0.5X PBS (pH 7.35) from SLNs prepared by double emulsion method with formulation code F5 in which least size of nanoparticles was achieved, was studied. The results indicated that integrity of BSA protein was remained unchanged after incorporation to SLNs as shown in fig. 4. There was no difference in the migration pattern of BSA, indicating that protein was maintained its integrity after its entrapment and release from SLNs.

Table 2: Effect of BSA concentration on size, PDI and zeta potential of SLNs.

BSA concentration (mg/ml)	Average diameter (nm) ±SD	PDI values ±SD	Zeta Potential (mV)±SD
0	67.83±2.055	0.225±0.015	-28.84±2.06
2	93.95±3.83	0.241±0.029	-32.28±3.39
4	96.17±3.61	0.246±0.005	-32.82±2.96
6	99.56±2.78	0.274±0.003	-33.34±3.44
8	102.34±4.44	0.206±0.006	-34.38±2.18
10	105.72±4.16	0.210±0.01	-35.12±2.67

Table 3: Composition of BSA loaded SLNs produced through double emulsion technique

Code	Amount of lipid mixture LO (mg)	Amount of surfactant (% wt)	Amount of soy lecithin (% wt)	Amount of PEG 6000 (% wt)	Amount of BSA (mg/ml)
F1	100	1.5[t]	0.4	-	2
F2	100	1.5[b]	0.4	-	2
F3	100	1.5[b]	0.4	0.25	2[c]
F4	100	1.5[b]	0.4	-	2
F5	100	1.5[b]	0.4	0.25	2[c]

Note: LO is lauric/oleic acid mixture in 5:1 ratio, t: tween 80, b: brij 35, c: BSA co-lyophilized with PEG.

Table 4: The size, PDI, zeta potential, %EE and %LC values of prepared SLNs by double emulsion method with different formulations.

Formulation code	Average diameter (nm)	PDI	Zeta potential (mV)	%EE	%LC
F1	109.56±6.11	0.225±0.012	-40.16±1.18	37.99±2.01	0.498±0.011
F2	107.12±5.45	0.281±0.01	-39.51±1.83	41.89±1.78	0.577±0.012
F3	98.01±5.55	0.254±0.013	-33.84±3.94	49.63±2.14	0.694±0.016
F4	94.65±3.24	0.186±0.005	-33.01±1.56	70.06±1.50	1.047±0.053
F5	89.67±4.88	0.176±0.007	-29.62±1.84	73.67±1.79	1.121±0.11

LC: Loading capacity.

Moreover, BSA loaded SLNs prepared by double emulsion method with formulation code F1-F5 stored at 4ºC were analyzed after 3 months by dissolving them in 5 ml 0.5X PBS (pH 7.35) and supernatant was ultra-centrifuged at 28000 rpm for 45 minutes. The released BSA protein was pellet down and was dissolved in 100 µl of 0.5X PBS and subjected to 12% SDS-PAGE analysis. The results indicated that there was no change in mobility pattern of BSA protein on SDS-PAGE gel indicating that protein was maintained its integrity after its entrapment and release from BSA loaded SLNs in case of all five formulations (F1-F5) (fig. 4). FTIR analysis is a trustworthy tool to assess the chemical integrity of formulation components. FTIR spectrum of simple fatty acid, fatty acid mixtures, pure BSA, BSA loaded SLNs prepared by double emulsion method (composition codes F2, F3, F4 and F5) and blank SLNs were performed to check any chemical change after encapsulation. The BSA absorption peaks of C=N and C-O stretching at 1650 cm^{-1} and 1105 cm^{-1} was observed as presented in fig. 5. The FTIR results indicated that there was no any change in spectrum was observed after encapsulation of BSA in SLNs indicating the stability of BSA loaded SLNs with composition codes (F2, F3, F4 and F5) fig. 5.

In vitro release study of BSA loaded SLNs

This experiment was carried out to determine release of BSA from BSA loaded SLNs prepared by double emulsion method with formulation codes F1-F5. The dissolution of all nanoparticles formulation was carried at 37ºC in 0.5X PBS (pH 7.35) and a graph was plotted between cumulative % drug release vs. time (hours). The initial burst release may be due to the adsorption of BSA on surface SLNs. After burst release up to 12 hours, all formulation was maintained with a sustained release of BSA from SLNs due to the diffusion and slowed down the aqueous media penetration to the lipid nanoparticles fig. 6.The results indicated that the SLNs formulation F1, F2, F3 has up to 50% release in first 48 hours. The SLNs formulations F4 and F5 have less than 50% release up to 72 hours and further sustained release was up to 10 days fig 6.

Kinetic models for drug release

The release behaviour of BSA from BSA loaded SLNs with all five formulations (F1-F5) was estimated using different kinetic models. In this study, the rate and mechanism of BSA released from all formulations was assessed using kinetic modelling. The R^2 values (regression coefficient) of release data of all formulations

Table 5: Stability study of SLNs prepared by double emulsion method with different formulations for particle size and %EE assessment over three months.

Formulation	0 day		30 days		90 days	
	Size (nm)	%EE	Size (nm)	%EE	Size (nm)	%EE
F1	109.56±6.11	35.03±2.75	114.67±3.22	31.17±3.5	117.23±1.56	31.12±1.22
F2	107.12±5.45	37.22±1.56	11262±3.72	33.32±3.45	115.22±3.67	32.45±0.89
F3	98.01±5.55	39.14±1.76	101.78±3.89	38.06±1.28	104.34±4.55	36.67±3.11
F4	94.65±3.24	72.21±1.32	96.95±4.5	70.62±1.84	98.62±2.94	68.84±0.05
F5	89.665±5.01	73.56±2.11	93.24±4.32	72.23±3.44	94.4±5.49	71.17±2.39

Table 6: R^2 values of zero-order, 1st order, Higuchi and Korsmeyer-Peppas kinetic models for BSA loaded SLNs.

Formulation	Zero Order (R^2)	First Order (R^2)	Higuchi model (R^2)	Korsmeyer-Peppas model	
				Release exponent (n)	R^2
F1	0.967	0.797	0.989	0.169	0.958
F2	0.977	0.865	0.987	0.219	0.975
F3	0.935	0.832	0.975	0.223	0.990
F4	0.983	0.913	0.992	0.278	0.982
F5	0.989	0.862	0.989	0.233	0.987

obtained by curve fitting method for five kinetic models are reported (table 6). To eliminate initial burst release effect, kinetic modelling was conducted on drug release data after 20% of the BSA was released. The R^2 values for zero order kinetic model (0.967-0.989) were larger as compared to 1st order kinetic model (0.79-0.86) for all nanoemulsions. The R^2 values for Higuchi model were in the range of 0.98-0.99, demonstrating BSA release from nanoparticles was a diffusion controlled. The R^2 values of Korsmeyer-Peppas model were between 0.96 and 0.987. The values of the release exponent (n) were less than 0.5, which also indicated a diffusion-controlled release of BSA from SLNs. fig. 7 (a-d) show kinetic models graphs for zero order, first order, Higuchi model and Korsmeyer-Peppas model. These models, i.e., zero order, first order, Higuchi and Korsmeyer-Peppas models were found best fit to BSA controlled release profile of all formulations of SLNs.

DISCUSSION

Due to less toxicity and biocompatibility, the solid lipid nanoparticles (SLNs) have been widely used as therapeutic carriers. In this study, the mixtures of solid and liquid fatty acids were used in order to reduce crystallinity and increase in drug % entrapment efficiency (Sanna *et al.,* 2010; Rehman *et al.,* 2015; Akbari *et al.,* 2011). table PEGylated binary SLNs were produced with the mean particle size of 89.7 nm with homogeneous particle distribution. Microemulsion and high-pressure/shear homogenization (HPH) are the most commonly used methods of SLNs preparation, but the labile drugs are susceptible to either high shear stress or high temperature. Most often, the thermally unstable and hydrophilic drugs such as protein and peptides are loaded to SLNs using w/o/w double emulsion technique (Garcia-Fuentes *et al.,* 2005). Three different methods were used for bovine serum albumin (BSA) loading on SLNs in this study. The effect of BSA concentration and addition of PEG was also evaluated. Increase in average diameter of SLNs due to increase in BSA concentration indicated that BSA was incorporated to inner aqueous phase of SLNs.

The addition of polyethylene glycol stabilized the proteins at o/w interfaces which resulted in an increased encapsulation efficiency (Lam *et al.,* 2001; Péan *et al.,* 1999; Meinel *et al.,* 2001). The high encapsulation efficiency attained with colypholization which was indicative of the successful addition of PEG as amphiphilic surfactant in enhancing the bovine serum albumin dispersibility in lipophilic oil phase (Castellanos *et al.,* 2003). Furthermore, the addition of oleic acid in lipid phase resulted in a decrease in crystallinity lipid nanoparticle, hence increasing the encapsulation efficiency (Pardeike *et al.,* 2009; Sanap *et al.,* 2014). The study indicated that the amount of surfactant, addition of co-surfactant and sonication had a greater influence on SLNs formulation. The use of co-surfactant and sonication time produced monodisperse and stable dispersions. The results indicated that addition of PEG-6000 decreases the particle size. PEGylation of SLNs resulted in slight decrease in particle size was also reported in literature (Yuan *et al.,* 2013). The zeta potential values decreased with an addition of polyethylene glycol and indicated an increase in surface hydrophilicity (Yuan *et al.,* 2013; Wan *et al.,* 2008). PEGylation of SLNs slightly altered the surface properties such as smaller average particle size and less surface negative charge (Cu *et al.,* 2008), which might reduce the mucoadhesion.

The particle size of BSA loaded SLNs was 89.67 ± 4.88 nm with narrow particle size distribution. SDS-PAGE and FTIR analysis indicated the chemical stability of BSA after loading on SLNs. The chemical integrity of BSA loaded SLNs was confirmed by SDS-PAGE analysis and FTIR studies (figs. 4 and 5). The XRD spectrum of BSA-loaded SLNs could not show BSA peaks, which indicated that BSA was well solubilized within the lipid matrix of SLNs. The XRD spectra of BSA-loaded SLNs also indicated a low intensity in SLNs than lipid mixture due to the decreased crystallinity and less ordered arrangement of lipid matrix. There was not much difference in blank and BSA loaded SLNs diffraction pattern due entrapment of BSA in the lipid core of SLNs. The PEGylated binary SLNs had superior physiochemical properties, which enabled a sustained release of BSA up to 10 days. Initial burst release of BSA from the SLNs was demonstrated after one hour. The controlled slow release of Formulation 5(F5) was due to the addition polyethylene glycol, which gave stability and a sustained release (Péan *et al.,* 1999). The controlled slow release of BSA loaded SLNs formulation (F5) was due to the addition polyethylene glycol, which gave stability and sustained release (Péan *et al.,* 1999). In various cases, the initial burst release of proteins and peptides is due to its adsorption at the o/w interface and in the outer shell during fabrication (Müller *et al.,* 2000; Almeida *et al.,* 2007) which is commonly a combination-process of desorption and diffusion. Kinetic studies have demonstrated that a BSA release from SLNs tends to trail zero order kinetics, i.e., drug release is independent of the amount of BSA remaining in SLNs to be released. Therefore, it is concluded that BSA release from SLNs was diffusion controlled and followed zero order release rate. In previous study by Li *et al*., (2010b), BSA loaded SLNs which were prepared by double emulsion method only were described however in current research three different methods were used for SLNs loaded with BSA and further we evaluated them for controlled release of drug.

CONCLUSION

Solid lipid nanoparticles from the combinations of solid and liquid lipids had enhanced the physicochemical properties and permitted controlled release of BSA for up to 10 days. The study also evaluated the addition of polyethylene glycol which reduced the particles size, enhanced % entrapment efficiency and induced a long circulation time. The release of BSA from BSA loaded SLNs with formulation codes (F1-F5) followed zero order rate kinetics and diffusion-controlled. Different mathematical models, i.e., zero order, first order, Higuchi and Korsmeyer-Peppas models were found best fit to BSA controlled release profile of all formulations of SLNs.
Authors report no conflict of interest.

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