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Research Article

DEVELOPMENT, EVALUATION, AND TARGETING OF STAVUDINE-LOADED SERUM ALBUMIN POLYMER-BASED NANOCARRIERS TO HUMAN IMMUNODEFICIENCY VIRUS RESERVOIRS

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ABSTRACT

Objective: Stavudine is an antiretroviral drug that is a part of the nucleoside reverse transcriptase inhibitor family, which is used to delay the progression of human immunodeficiency virus (HIV) infection. The present investigation involves the development and evaluates stavudine-loaded nanocarriers using natural polymer bovine serum albumin (BSA).

Methods: The desolvation technique was used to prepare nanoparticles and coated with 1% v/v polysorbate 80 to improve the targeting of drugs to the organs (HIV reservoirs). Biodistribution studies were also investigated for the best formulation to determine the targeting efficiency of nanocarriers loaded stavudine and compared with pure compound.

Results: The formulated nanocarriers have shown mean particle size below 300 nm, zeta potential in the range of −16.5 Mv, encapsulation efficiency in the range of 50.10–73.7%, and drug loading in the range of 14.73–73.84%. Cumulative percentage drug release was in the range of 24.72–71.20% and release kinetic studies showed that the mechanism of drug release was controlled simultaneously by diffusion and erosion of the matrix type formulations. The stability studies over a period of 3 months confirmed the stability of BSA nanoparticles. Biodistribution studies demonstrated that nanoparticles coated with 1% v/v polysorbate 80 were able to reach the HIV reservoirs in an amount higher than that of uncoated stavudine nanoparticles or pure drug itself.

Conclusion: The method adopted is simple and the biodistribution studies demonstrated that nanoparticles coated with 1% polysorbate 80 were able to reach the selected organs in an amount higher than that of uncoated stavudine nanoparticles or pure drug itself.

Keywords: Stavudine, Bovine Serum albumin, Albumin nanoparticles, Desolvation technique, Drug targeting.

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INTRODUCTION

Human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome pandemic is an increasing global burden with devastating health-related and socioeconomic effects and is the leading infectious disease resulting in significant morbidity and mortality. Globally, 36.9 million (31.1–43.9 million) people were living with HIV at the end of 2017. An estimated 0.8% (0.6–0.9%) of adults aged 15–49 years worldwide is living with HIV, although the burden of the epidemic continues to vary considerably between countries and regions [1]. It is characterized by immunodeficiency, opportunistic infections, and unusual malignant diseases. At present, various drugs are used as antiretroviral therapy and have contributed significantly to improve the disease management, life quality, and expectancy of infected individuals. However, limitations of currently available drug regimens and dosage forms, alongside with the extraordinary adapting capacity of the virus, have impaired further success [2].

Many antiretroviral (ARV) drugs undergo extensive first-pass metabolism and gastrointestinal degradation, leading to low and erratic bioavailability and it is found that the half-life for several ARV drugs is short, which demands the frequent administration of doses, leading to decreased patient compliance [3]. One of the major limitations is that HIV is localized in certain inaccessible compartments of the body such as the central nervous system (CNS), the lymphatic system, and within the macrophages. Majority of drugs cannot access these sites in the therapeutic concentrations required and the drug cannot maintain this concentration for the adequate duration at the site of HIV localization as a result, of which multidrug resistance against the ARV drugs is developed.

There are seven classes of the Food and Drug Administration approved ARV drugs for the treatment of retroviral infections, primarily HIV. This resulted in the development of nanocarrier drug delivery systems that can improve the efficacy of both existing and new ARV drugs.

These nanotechnology carriers are able to deliver the ARV agents in a controlled and/or targeted manner, thereby increasing the drug bioavailability and residence time at target sites with a considerable improvement in quality of HIV patients. Furthermore, by appropriate surface modification, targeting to other areas may be achieved [4-7]. In addition to targeted and controlled drug delivery, nanoparticles have also been explored for improving the efficacy of drug with physicochemical problems such as poor solubility that may lead to formulation difficulties [3]. Due to the challenges associated with its physicochemical properties and limitations in biodistribution and cellular uptake, the development of nanoparticles as a novel drug delivery system for ARV drugs has attracted significant interest. The use of nanoparticles for targeted delivery of ARVs to HIV-infected cells and to achieve sustained drug release kinetics may allow for their improved efficacy, decreased drug resistance, a reduction in dosage, a decrease in systemic toxicity and side effects, and an improvement in patient compliance.

The albumin nanoparticles are characterized by favorable pharmacokinetics, high drug delivery efficiency, and low cytotoxicity. In addition, they are biodegradable, relatively easy to prepare and non-immunogenic. Interest in the exploration of clinical applications of albumin-based drug delivery carriers, especially for those at the nanoscale, has increased in recent years [8,9]. Stavudine is an ARV drug that is a part of the nucleoside reverse transcriptase inhibitor

family [10]. It is used together with other ARVs to delay the progression of HIV infection. The drug has a short half-life, poor bioavailability, and undesirable side effects.

In this article, at formulating, albumin-based particulate systems like nanoparticles of bovine serum albumin (BSA)-loaded stavudine are formulated as drug delivery carriers, evaluated, and compared. These targeted delivery systems that can cross the physiological barriers and deliver the drug to the target sites such as the CNS, lymphatic system, and macrophages.

MATERIALS AND METHODS

Materials

BSA (fraction V, with purity of 98%) was purchased from HiMedia Laboratories Ltd. (Mumbai, India). Stavudine was received as a gift sample from the Strides Arcolabs Ltd. (Bengaluru, India). Water for high-performance liquid chromatography (HPLC) and acetonitrile for HPLC were purchased from Merck Specialities Pvt., Ltd. (Mumbai, India). Mannitol, polysorbate 80, sodium hydroxide and glutaraldehyde, and all other chemicals were commercially supplied by Sigma Aldrich.

Method of preparation

Stavudine-loaded BSA nanoparticles were prepared by a desolvation method [11]. Nanocarriers were prepared using BSA containing 1:1, 1:2, 1:3, 1:4, and 1:5 of drug-to-polymer ratio and were assigned batch code as A1, A2, A3, A4, and A5, respectively. Accurately weighed quantity of polymer was added to 0.01 N NaCl solution which was made up to pH 8 with 0.1 N NaOH under constant magnetic stirring at 500 rpm. Stavudine was added to BSA solution, mixed, and incubated for 1 h at room temperature. Ethanol was added dropwise at a rate of 1 ml/min into the above solution as a desolvating agent until the solution became just turbid. Thereafter 30 min of the desolvation process, 100 ml of an 8%v/v aqueous solution of glutaraldehyde was added to induce particle cross-linking. This process was performed during stirring over a time period of 3 h at room temperature. The nanosuspensions were purified by centrifugation at 8000 rpm for 45 min and then subjected to freeze-drying after adding 2% (w/v) mannitol as a cryoprotectant for 8 h to obtain fine powder of nanoparticles. The dried nanoparticles obtained were then transferred to vials and were stored at 4°C. Coating was done for the best formulation (A1) immediately after cross-linking by adding 1% polysorbate 80 and was incubated for 30 min. Finally, the nanosuspension was centrifuged and lyophilized with 2% mannitol.

NANOPARTICLE CHARACTERIZATION

Compatibility study

Compatibility of stavudine with BSA was analyzed using Fourier-transform infrared (FT-IR) spectroscopy, Shimadzu Corporation, Japan, by the potassium bromide disc method (1:100). The differential scanning calorimetry (DSC, Mettler Toledo star 822 systems, Switzerland) thermogram of drug and lyophilized nanoparticles gives information regarding the physical properties and melting point of the drug.

Determination of percentage drug loading (DL) and drug entrapment efficiency (EE)

Freshly prepared nanosuspensions were centrifuged at 8000 rpm for 45 min and the amount of unincorporated stavudine in supernatant liquid was measured. The percentage DL and drug EE were calculated according to the following formula [12]:

$$\%DL=\left[\frac{\text{Total amount of drug added-Amoumt of unbound drug in supernatent liquid}}{\text{Total weight of nanoparticles}}\right]100$$

$$\%EE=\left[\frac{\text{Amount of drug actually present in nanopartcles}}{\text{Amount of drug actually added}}\right]100$$

Determination of particle size and zeta potential

The particle size and zeta potential of stavudine-loaded albumin nanoparticles were determined by dynamic light scattering, using a Malvern system, with vertically polarized supplied by helium/neon laser (red laser) operated at 40 mW. The samples were dispersed in distilled water and taken in clear disposable zeta cell. The experiments were performed with non-invasive backscatter technology at a temperature of 25.0±0.1°C at a detection angle of 90° to the incident beam.

Surface morphology

Scanning electron microscopy was performed to characterize the surface morphology of the prepared nanoparticles. This was done by placing freeze-dried nanoparticles on brass stub then was gold coated to render them electrically conductive and examined under the scanning electron microscope (SEM) at 20 kV (JSM 6100 JEOL, Tokyo, Japan).

In vitro drug release studies

The drug release studies were carried out by dialysis method [13,14]. A known quantity of nanoparticles equivalent to 5 mg of the drug was added to cellulose dialysis membrane (molecular weight cutoff 5 kDa, HiMedia, India) attached to one of the open ends of a diffusion tube. To this 2 ml of pH 7.4, phosphate buffer was added and it was arranged such that the surface of dialysis membrane was completely in contact with the dissolution medium in the receptor compartment, containing 100 ml of phosphate buffer (pH 7.4) on a magnetic stirrer at 37±0.5°C at 100 rpm. 2 ml quantity of sample was withdrawn at different time periods and same volume of dissolution medium was replaced in the receptor compartment to maintain sink condition for 48 h. The withdrawn samples were filtered and then the filtrate was diluted with phosphate buffer (pH 7.4). The samples were analyzed for drug release by measuring the absorbance at 266 nm using ultraviolet-visible spectrophotometer.

Release kinetics

The *in vitro* release data of the formulations A1 were fitted with various kinetic models such as zero order, first order, Higuchi model, and Korsmeyer-Peppas, to predict kinetics and mechanism of drug release [15]. The release constant was calculated from the slope of plots and regression coefficient (r^2), diffusion exponent (n) was determined.

Stability studies

A study was carried out to assess the stability of BSA nanoparticles of drug stavudine (drug-polymer ratio 1:1). The samples were stored in room temperature (15–20°C), refrigerator (3–5°C), and 37°C (Relative humidity [RH] = 75%) over a period of 3 months [16]. Samples were evaluated at 0, 1, 2, and 3 months for their drug content as well as any changes in their physical appearance. FT-IR studies were also carried out to find out any changes in the IR spectra after 3 months of storage.

Chromatographic conditions

- Instrument: High=performance liquid chromatography
- Injector: Rheodyne
- Column: Phenomenex C-18 reverse phase column (250* 4.6, 5 µm)
- Mobile phase: Acetonitrile:water (9:91)
- Flow rate: 0.7 ml/min
- Injection volume: 20 µl
- Temperature: Ambient (room temperature)
- Detector: Ultraviolet detector
- Wavelength: 266 nm.

Biodistribution studies

The biodistribution studies of stavudine-loaded BSA nanoparticles were carried out on healthy adult Wistar albino rats weighing 200–250 g and after obtaining approval from the local animal ethics committee and CPCSEA (DSCP/PH.D PHARM/IAEC/49/2010–2011). All animals were provided with proper care, food, and water *ad libitum* and were maintained under well ventilated in large spacious cages throughout the

study. The rats were divided randomly into four groups of six rats each and they were fasted at least 12 h before experimentation [17]. Group 1 was injected with stavudine drug (which was dispersed in water for injection) into the tail vein of rats, Group 2 was received stavudine-loaded BSA nanoparticles (A1), and Group 3 was administered polysorbate 80 coated BSA nanoparticles (P1). Group 4 was considered as control. Rats were sacrificed after 1 h of postinjection and blood was collected by cardiac puncture in a centrifuge tube and centrifuged at 2000 rpm for 10 min. Supernatant was collected and analyzed by the HPLC assay. At the same time, selected organs (spleen, liver, kidney, lymph nodes, and brain) were excised, isolated, weighed, and will be homogenized with 2 ml of phosphate-buffered saline (PBS) (7.4) using a tissue homogenizer. To the tissue homogenate, an equal volume of 10% (V/V) trichloroacetic acid (in water) was added and vortexed for 30 s to precipitate tissue proteins. After precipitation of proteins, the drug was extracted by adding 10 ml of acetonitrile, followed by equilibration for 30 min at room temperature. The tubes were then centrifuged for 30 min at 3000 rpm. After filtration through membrane filter, the filtrate was assayed for stavudine by the HPLC assay with slight modification [18].

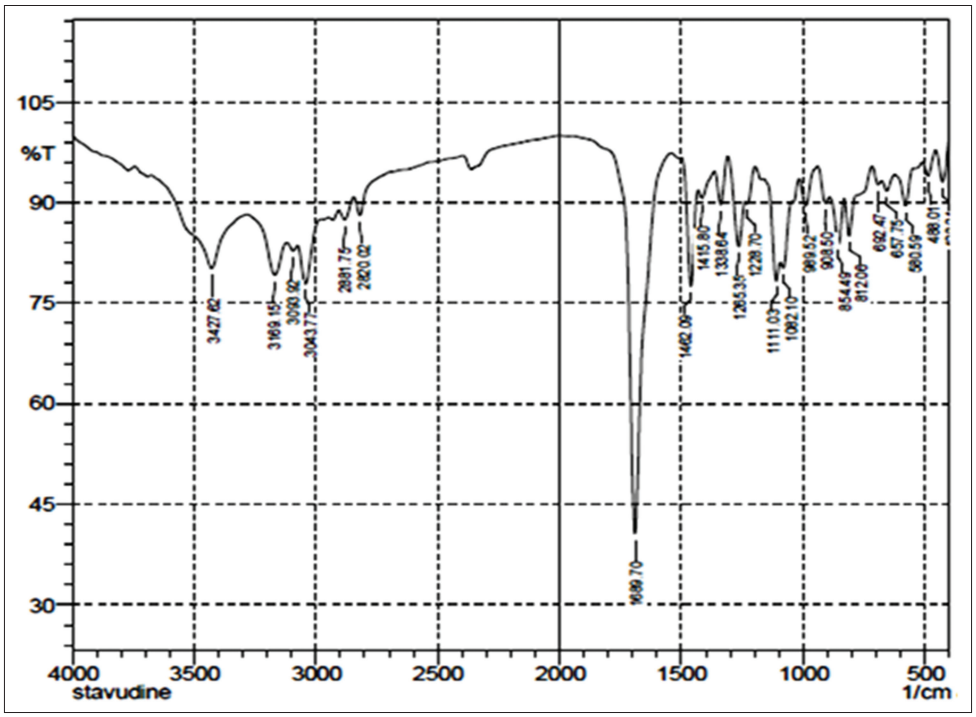

Fig. 1: Fourier transform infrared spectrum of pure drug stavudine

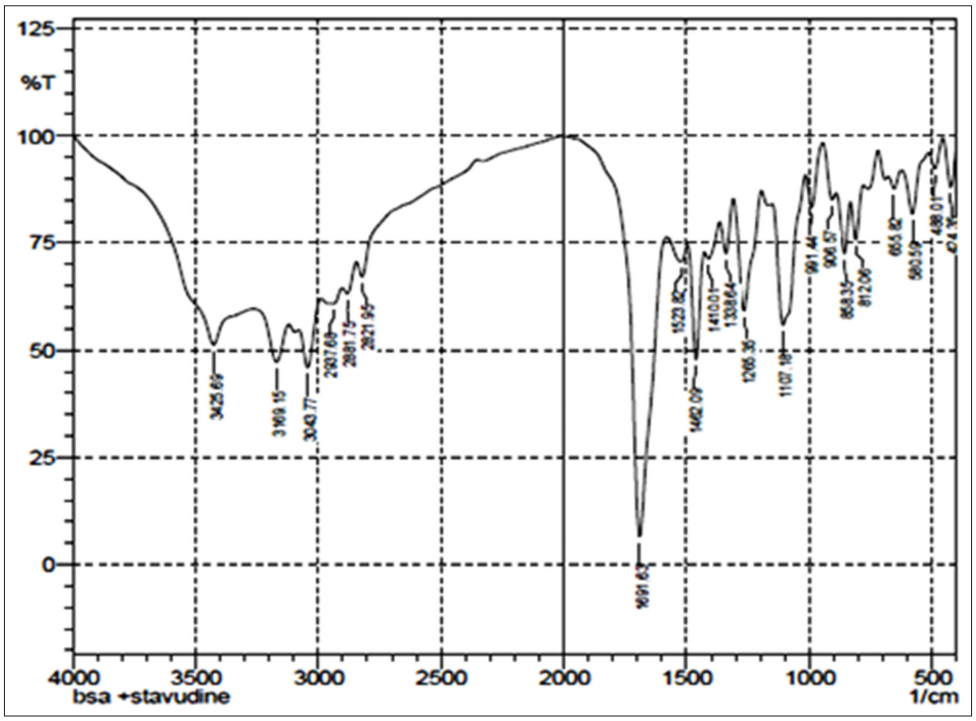

Fig. 2: Fourier transform infrared spectrum of physical mixture of bovine serum albumin and stavudine

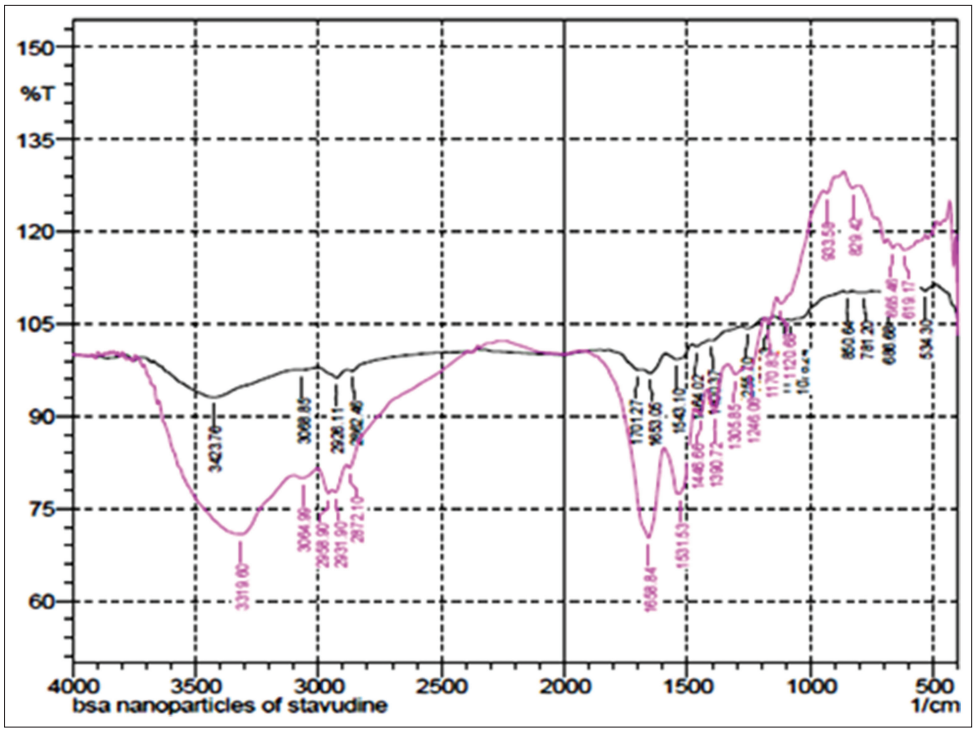

Fig. 3: Merged Fourier transform infrared spectra of bovine serum albumin (BSA) and BSA nanoparticles of stavudine

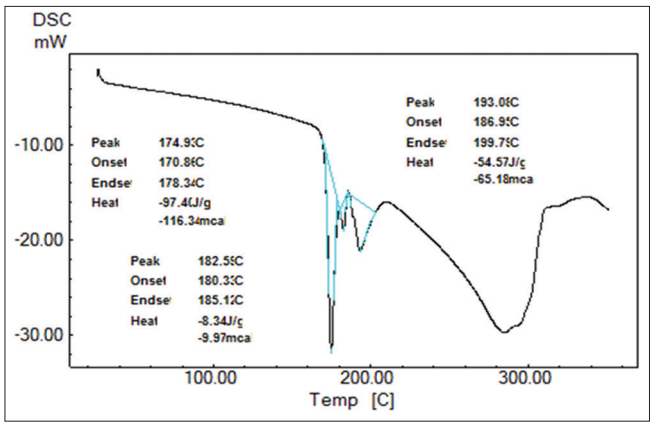

Fig. 4: Differential scanning calorimetry thermogram of stavudine-loaded albumin nanoparticles

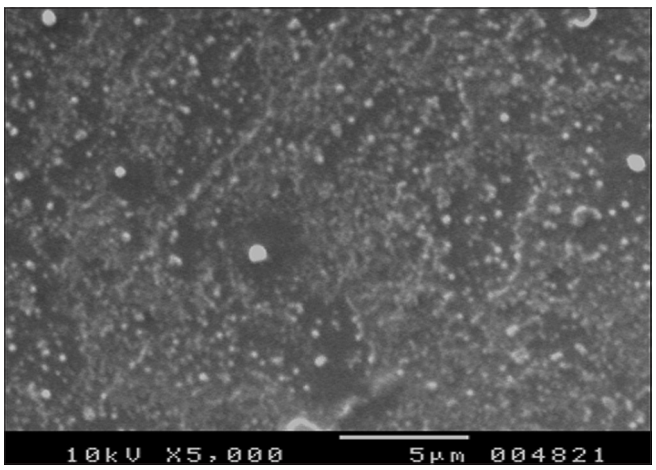

Fig. 5: Scanning electron microscope image of stavudine-loaded nanoparticles (A1)

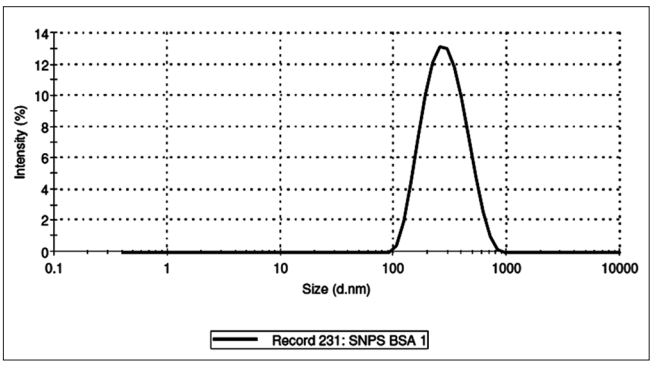

Fig. 6: Particle size distribution of stavudine-loaded bovine serum albumin nanoparticles (A1)

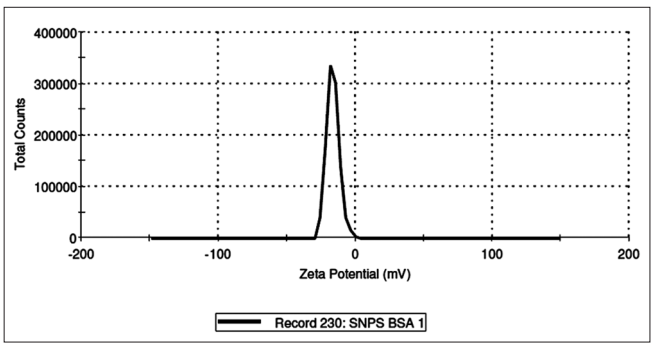

Fig. 7: Zeta potential distribution of stavudine-loaded bovine serum albumin nanoparticles (A1)

Preparation of standard stock solutions

About 10 mg of pure drug stavudine was weighed and dissolved in water for HPLC to obtain a stock solution of 1000 µg/ml. From this stock, aliquots of 10 µl, 15 µl, and 20 µl were taken in 10 ml volumetric flasks and the volume was made to 10 ml with water for HPLC to produce 1000 ng, 1500 ng, and 2000 ng solutions.

Table 1: %DL and %EE of stavudine-loaded BSA nanoparticles

Formulation parameters	%DL and %EE of stavudine-loaded BSA nanoparticles					
Formulation code	**A1**	**A2**	**A3**	**A4**	**A5**	**Polysorbate 80 coated A1 (P1)**
Drug-polymer ratio	1:1	1:2	1:3	1:4	1:5	1:1
%DL	73.84±3.2	53.44±1.7	36.70±3.6	21.95±1.9	14.73±2.6	69.80±3.7
% encapsulation efficiency	73.70±0.8	68.94±1.2	67.90±0.7	57.08±3.9	50.10±4.2	70.50±0.5

(n=6±SD). DL: Drug loading, EE: Entrapment efficiency, BSA: Bovine serum albumin, SD: Standard deviation

Table 2: Physicochemical characterization of stavudine-loaded BSA nanoparticles

Formulation parameters	Physicochemical characterization of stavudine-loaded BSA nanoparticles
Formulation code	**A1**
Mean particle size	255.9±12.59 nm
Zeta potential	−16.5±0.84 mV

(n=3±SD). BSA: Bovine serum albumin, SD: Standard deviation

Preparation of sample

About 10 µl of the filtrate (obtained by homogenization of organs, precipitation of tissue proteins, and extraction of drug) was pipetted into a 10 ml volumetric flask and the volume was made up to 10 ml with water for HPLC and 20 µl was injected onto the HPLC system.

RESULTS

Optimization of fabrication parameters

The BSA nanoparticles of stavudine were prepared by desolvation technique. The particle size and the DL and EE of nanoparticles were influenced by parameters such as stirring speed, pH, and volume of glutaraldehyde solution and so on. Furthermore, the drug-loaded nanoparticles were characterized and evaluated.

Compatibility study

The spectra obtained from FT-IR spectroscopy studies at wavelength ranging from 4000 cm^{-1} to 400 cm^{-1} are shown in Figs. 1-3. Fig. 2 illustrated that there were no changes in the IR spectra of pure drug stavudine in the presence of BSA. The characteristic peaks of BSA nanoparticles remain same with slight modifications due to other excipients present in the formulations (Fig. 3). The DSC thermogram of lyophilized nanoparticles is shown in Fig. 4. DSC curve showed the endothermic peak at 174.9°C and 182.5°C in nanoparticles represented the melting point of stavudine.

Determination of percentage DL and percentage EE

Percentage DL and percentage EE of nanoparticles depending on the drug-polymer ratio are shown in Table 1. The percentage DL was found to be ranging from 14.73 to 73.84%. The percentage encapsulation efficiency was found in the range of 50.10–73.7%.

Determination of surface morphology, particle size, and zeta potential

Fig. 5 depicts the SEM image of nanoparticles and Fig. 6 shows particle size distribution of stavudine-loaded BSA nanoparticles. The mean particle size of stavudine-loaded BSA nanoparticles was found to be 255.9 nm. The mean zeta potential was found to be −16.5 mV, indicating high degrees of stability due to interparticle repulsions and is shown in Fig. 7 and Table 2.

***In vitro* drug release studies**

Fig. 8 shows that the comparative graph of *in vitro* release profiles of stavudine from nanoparticles and the cumulative percentage release of stavudine from BSA nanoparticles were varied from 24.72± 71.20%.

Release kinetics

The *in vitro* release data obtained from formulation A1 were fitted to various kinetic models to reveal the drug release mechanism from nanoparticles. The n and r^2 values of different kinetic models are represented in Table 3. Diffusion-controlled drug release was observed with higher r^2 value in Higuchi model for A1 formulation. The diffusion exponent (n) value is used to characterize different release mechanism in Korsmeyer-Peppas model. The n value was found to be in the range of 0.45 and 0.89, indicating that drug release was controlled by anomalous diffusion, i.e., the mechanism of drug release is controlled simultaneously by diffusion and erosion of the matrix-type formulations.

Stability studies

The results of stability studies are shown in Table 4. There were no changes in their physical appearance. It was observed that there were no significant changes in the physical as well as chemical characteristics of the formulations. No significant changes were observed in the IR spectra of the formulations after 3 months of storage. Based on the observations, it was concluded that the developed BSA nanoparticles of drug stavudine are physically and chemically stable and retain their pharmaceutical properties at various temperature and humidity conditions over a period of 3 months.

Selection of an ideal batch

Among the different batches of nanoparticles prepared with BSA, the formulations with drug-to-polymer ratio 1:1 (A1) was found to be the best formulations after considering its DL capacity and *in vitro* drug release.

Biodistribution studies

From this study, concentration of stavudine (ng/ml) from polysorbate 80 coated, uncoated formulation was measured in various organs of Wistar albino rats and compared with free drug in PBS. The HPLC chromatograms of standard drug stavudine (Fig. 9) and P80 coated stavudine formulation (P1) in spleen and brain are shown in Figs. 10 and 11. Table 5 shows the mean concentration (ng/ml) of stavudine in various organs from polysorbate 80 coated, uncoated and free drug solutions after 1 h of intravenous (i.v) administration. It was found that higher concentration of stavudine reached in macrophage-rich organs from group which has received polysorbate 80 coated nanoparticles than Group 2 (uncoated nanoparticles) and Group 1 (the free drug solution). Furthermore, the polysorbate 80 coating significantly increased the uptake of stavudine into the brain. The concentration of stavudine in brain, liver, and spleen from polysorbate 80 coated nanoparticles was increased by 6.16-, 2.24-, and 2.67-fold in comparison with the free drug solution after 1 h of i.v injection (Fig. 12).

DISCUSSION

The drug excipient compatibility was confirmed by FT-IR and DSC thermogram; both the studies were indicating no interaction between the drug excipients and drug-polymer used in this study. Albumin nanoparticles containing stavudine were prepared by desolvation technique with different concentrations of polymer and keeping all other parameters constant. Stavudine with albumin in 1:1 ratio resulted in good nanoparticle formation. Ethanol was added to facilitate the formation of nanoparticles. Glutaraldehyde solution was added as cross-linking agent and also hardened the coacervates. The stirring time of 3 h after the addition of cross-linking agent was sufficient to cross-link the formed nanoparticles. The optimized method of desolvation technique was able to produce smooth, spherical, stable nanoparticles. From DSC profile, it was concluded that stavudine was present in the formulated nanoparticles in the amorphous state and might have dispersed uniformly in the polymer.

Table 3: Release kinetics of stavudine-loaded BSA nanoparticles

Formulation code	Drug release kinetics, correlation coefficients (r^2)			Korsmeyer-Peppas	
	Zero order	First order	Higuchi	r^2	n
A1	0.7265±0.0125	0.8454±0.0452	0.9216±0.1205	0.9127±0.1401	0.6795±0.1109
P 80 coated A1 (P1)	0.7328±0.2016	0.8282±0.2014	0.9237±0.3102	0.9270±0.127	0.6808±0.1376

(n=3±SD). BSA: Bovine serum albumin, SD: Standard deviation

Table 4: Stability studies of stavudine-loaded BSA nanoparticles (A1)

Temperature	Evaluation parameters	Observation (months)			
		0	1	2	3
15–20°C	Physical appearance	Cream-colored powder	No change	No change	No change
	% drug content	26.90±0.51	26.79±0.83	25.16±0.10	25.12±0.07
	FT-IR	Done	No significant change	No significant change	No significant change
3–5°C	Physical appearance	Cream-colored powder	No change	No change	No change
	% drug content	26.90±0.51	26.89±0.81	26.87±0.12	26.87±0.23
	FT-IR	Done	No significant change	No significant change	No significant change
37°C (RH=75%)	Physical appearance	Cream-colored powder	No change	No change	No change
	% drug content	26.90±0.51	26.87±0.01	26.85±0.32	26.84±0.41
	FT-IR	Done	No significant change	No significant change	No significant change

(n=3±SD). BSA: Bovine serum albumin, SD: Standard deviation, FT-IR: Fourier transform infrared

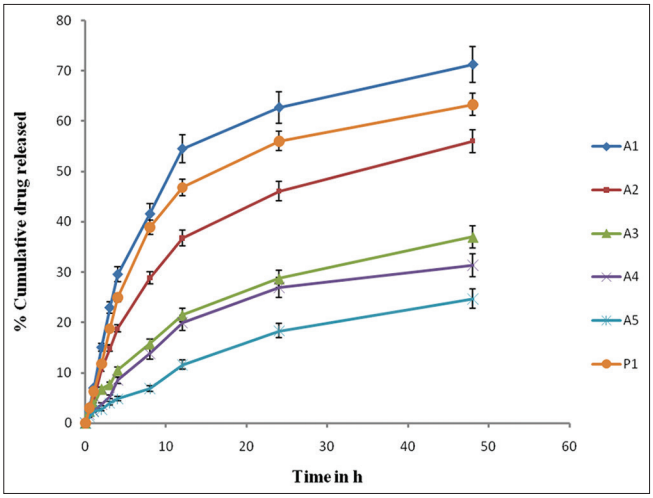

Fig. 8: *In vitro* drug release study of bovine serum albumin (BSA) nanoparticles of stavudine. Each experiment was carried out triplicate (n=3 ± standard deviation). P1 is polysorbate 80 coated BSA nanoparticles

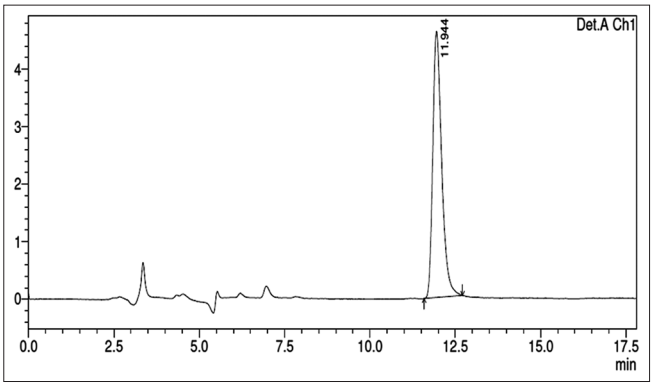

Fig. 9: High-performance liquid chromatography chromatogram of standard drug stavudine

The percentage DL was found to be decreased with increase in concentration of BSA because the concentration of stavudine was kept constant. The percentage encapsulation efficiency was decreased with respect to drug-polymer mass ratio due to limited affinity of the drug molecule to the macromolecular material. Particle size analysis showed that the formed particles were in nanosize and the mean zeta potential studies demonstrated that the nanoparticles possess a negative surface charge which indicates high degrees of stability due to interparticle repulsions. The morphological properties and surface appearance of stavudine-loaded BSA nanoparticles were spherical, smooth surface.

The drug release profile of all the nanoformulations exhibited a biphasic release pattern with an initial burst effect of stavudine within 1 h followed by a sustained release over 48 h. Burst release of stavudine from nanoparticles was due to the dissociation of entrapped drug molecules in the surface layer of the nanoparticles and subsequent solubilization in the release medium which is in contact with the nanoparticles. The diffusion exponent (n) value is used to characterize different release mechanism in Korsmeyer-Peppas model. Release kinetics showed that the stavudine release from the nanoparticles followed an anomalous diffusion, i.e., the mechanism of drug release was controlled simultaneously by diffusion and erosion of the matrix-type formulations.

No significant changes were observed in physical appearance, drug content, and in the FT-IR spectra of selected stavudine-loaded albumin nanoparticle formulations during their stability studies. Stability studies showed that the developed stavudine nanoparticles were stable and retained their pharmaceutical properties over a period of its shelf life. Based on biodistribution studies, it was clear that the concentration of stavudine was higher in tissue level in most of the organs from polysorbate 80 coated albumin nanoparticles, while the concentration measured in various organs with free drug solution. This may be due to rapid uptake of polysorbate 80 coated albumin nanoparticles by macrophage-rich organs in comparison to uncoated nanoparticles and free drug solutions. Stavudine concentration in brain in lymph nodes was 6-fold higher from polysorbate than that of free drug solution. The polysorbate 80 coated nanocarriers played a specific role to extend the half-life of therapeutically active drugs and also able to deliver higher drug levels in HIV reservoir sites which can provide better viral suppression by terminating HIV reverse transcriptase.

CONCLUSION

In this study, an attempt was made to develop nanoparticulate delivery system of an ARV drug stavudine using BSA and can be concluded that it is possible to prepare by desolvation technique. Among the batch of nanoparticles prepared, A1 was selected as the ideal formulation after considering its DL capacity and *in vitro* drug release properties. Release

Table 5: Stavudine concentrations (ng/ml) in different organs after intravenous injection of stavudine formulations

S. No.	Organ	Formulation		
		Stavudine in PBS (ng)	Uncoated BSA nanoparticles (ng) (A1)	Coated BSA nanoparticles of stavudine (ng) (PI)
1	Liver	87.79±12.56	123.07±14.23	196.80±11.56
2	Kidney	45.10±7.21	87.56±10.28	150.33±9.14
3	Spleen	32.77±4.35	65.44±8.59	87.7±9.25
4	Lungs	14.23±3.73	37.89±5.78	46.54±5.46
5	Heart	12.47±4.12	32.56±4.56	81.66±9.71
6	Brain	11.73±5.84	20.65±4.01	72.35±9.12

(n=6±SD). BSA: Bovine serum albumin, SD: Standard deviation

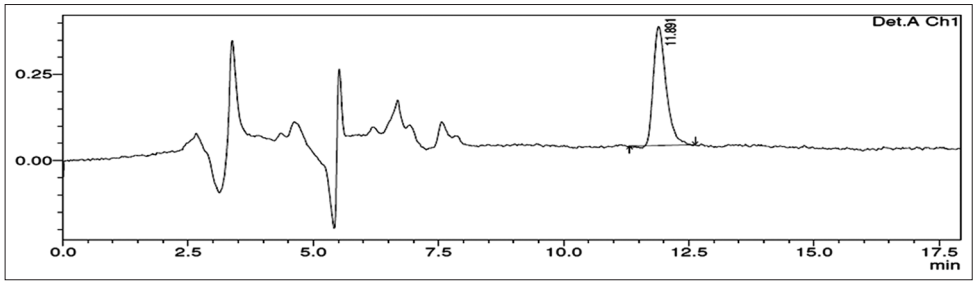

Fig. 10: High-performance liquid chromatography chromatograms of P80 coated stavudine formulation (P1) in brain of Wistar albino rats

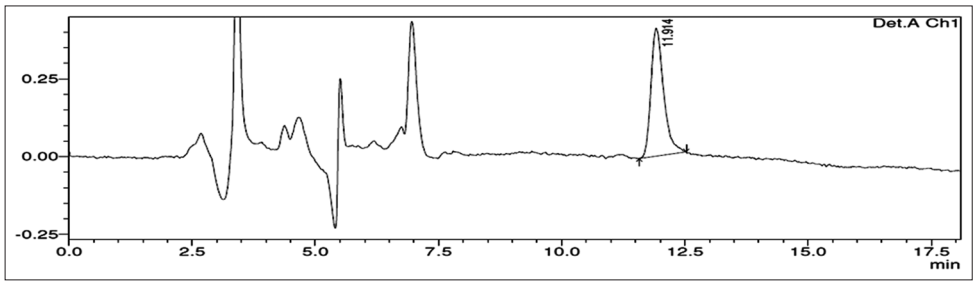

Fig. 11: High-performance liquid chromatography chromatograms of P80 coated stavudine formulation (P1) in spleen of Wistar albino rats

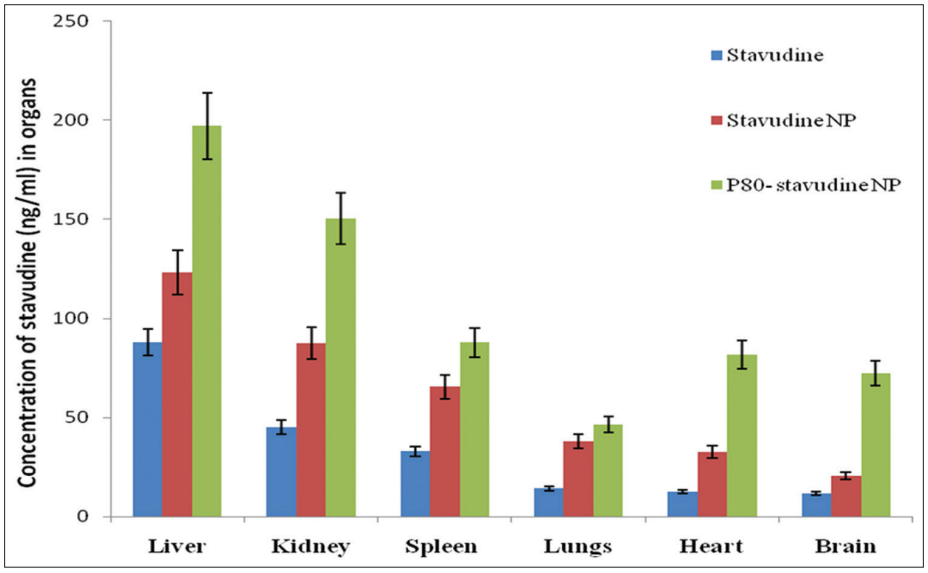

Fig. 12: Graphical representation of biodistribution studies in rat organs after intravenous injection of stavudine-loaded bovine serum albumin nanoparticles (mean ± standard deviation, n=6)

kinetics showed that the stavudine release from the nanoparticle followed an anomalous diffusion, i.e., the mechanism of drug release was controlled simultaneously by diffusion and erosion of the matrix-type formulations. Based on biodistribution studies, polysorbate 80 coated nanocarriers play a specific role to extend the half-life of therapeutically active drugs with reduced dose-related adverse effects and also able to deliver higher drug levels in HIV reservoir sites which can provide better viral suppression by terminating HIV reverse transcriptase. Results obtained by biodistribution study suggested that the developed albumin nanoparticles containing stavudine can be a promising formulation for targeting viral reservoirs to suppress and delay the progression of virus.

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AUTHORS' CONTRIBUTIONS

Josephine Leno Jenita developed and designed the study, Suparna developed analytical methods and technical work toward the research work. Shanaz Banu verified the analytical data and Manjula helped for drafting manuscript.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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