PLGA-BASED MACROPHAGE-MEDIATED DRUG TARGETING FOR THE TREATMENT OF EXPERIMENTAL VISCERAL LEISHMANIASIS

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ARTICLE INFO

ABSTRACT

The potential of PLGA-Nanoparticles as a carrier of Amphotericin B and Doxorubicin against visceral leishmaniasis was evaluated by macrophage-mediated drug targeting approach. PLGA-Nanoparticles were modified by coating them with macrophage-specific ligand- lectin. Prior to in-vitro studies, characterization studies were carried out systematically: particle size (0.160 nm), surface morphology, percent drug entrapment and percent drug release. In vitro studies were carried out in J774.1 in order to check the effective endocytotic uptake of nanoparticles by macrophages. The antileishmanial activity of PLGA-Nanoparticles and lectin-PLGA-Nanoparticles was tested in-vitro in leishmania donovani infected macrophage-amastigote system (J774A.1 cells), which showed higher efficacy of lectin grafted PLGA-Nanoparticles over plain PLGA-Nanoparticles. The proposed plain and lectin grafted PLGA-Nanoparticles based systems showed excellent potential for passive and active intramacrophage targeting, respectively and the approach could be an effective alternative to the currently available drug regimens against VL.

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is a systemic disease that is lethal when left untreated and is caused by species of the Leishmania donovani, namely L. donovani and L. infantum (Old World) and L. chagasi (New World). The causative agents could have two ways of transmission, zoonotic i.e. which means that it is transmitted from animal to vector to human or anthroponotic, which means that is transmitted from human to vector to human. The development of the disease is an importance of the dissemination of VL causing species to internal organs such as the liver, spleen and bone marrow, which results in the arrival of different symptoms including prolonged fever, anemia, loss of appetite and weight and polyclonal hypergammaglobulinaemia and, like swelling of spleen (splenomegaly), liver (hepatomegaly) and lymph nodes. However, current therapies are far away from satisfactory, especially due to the emergence of resistances, elevated toxicity and increased prevalence of human immunodeficiency virus. Thus, it is of great importance to develop alternative treatments, like nanotechnology-based drug delivery systems, which may decline toxicity and side effects of the drugs, and enhance their efficacy.[1]

Drug resistance and drug toxicity are the two major obstacles closely associated with most microbial infectious diseases. Macrophages play a key role in driving the progression of several microbial diseases affecting visceral organs such as liver and spleen. Studies on VL revealed that organs such as spleen and liver serve as safe havens for parasites residing inside the macrophages. Most of the antileishmanial drugs presently in use, fail to penetrate macrophages within which parasite creep and that derives researchers to pursue delivery systems and their engineered versions in order to be therapeutically effective.[2]

Currently surviving therapies for leishmaniasis are very toxic and also started exhibiting emergence of drug-resistant parasitic strains. For instance, pentavalent antimonials (sodium stibogluconate) and pentamidine were potent drugs used against VL;

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however, it proved to be ineffective due to the emergence of several unresponsive strains of the leishmanial parasite. 3 Miltefosine is a relatively new anti-VL drug which holds significant potential in treating VL along with other first-line therapeutics however, the clinical relevance and possible of Miltefosine are yet to be finalized. Despite all research initiatives and pre-clinical studies, active therapy for VL still remains challenge holding direct negative implications with respect to better drug targeting and overcoming drug resistance. 4 Some lipid-based amphotericin formulations are currently in clinical trials. Amphotericin B (AmB) provides substantial leishmanicidal activity as well, and its use results in fewer treatment failures and relapses. However, the important side effects, mainly nephrotoxicity, produced by this drug at therapeutic doses have often directed to its refusal as a first-choice treatment. The rational approach to the problem involves that the drug should be targeted to the macrophages in such a way that the contact of the free drug with non-target tissues could be minimized. 5 Novel drug delivery systems, such as liposomes, nanospheres, and microspheres can result in higher concentrations of AmB in the liver and spleen but lower concentrations in the kidney and lungs 1 thus declining the toxicity of AmB. Furthermore, the administration of AmB through these drug carriers can enhance the accessibility of the drug to organs and tissues otherwise inaccessible to the free drug. 6

In this study, a rational approach has been engaged wherein Amphotericin B (AmB) and Doxorubicin (DOX) encapsulated in PLGA-NPs incorporated with lectin and chosen a preferential uptake by macrophages and deliver them to the RES, which in fact, are the planned target sites where PLGA-NPs is supposed to be delivered. PLGA has been often used for drug delivery systems because of its proven safety and efficacy in sustained release. DOX is always ranked higher among other chemotherapeutics for the treatment of solid tumors'. 7 Counter intuitively, DOX was reported to be equally effective in comparison to amphotericin as an anti-VL agent based on the studies conducted by Sett et al. (1992) and other researchers. 8 Although highly potent, activity of DOX was inadequate by its fatal toxicity and cost-effectiveness. Severe toxicity concerns resulting in dermatological problems, renal failure, extravasation hazards, hyperuricemia and cardiotoxicity 7 were observed with DOX therapy. Although the potential of DOX against VL was discovered in the early 1990s, preclinical use of DOX against VL remained as an open question without any major research creativity and intensive research due to which this area remained undeveloped. Therefore, we probed into deciphering the efficacy of AmB and DOX against VL and enclosed relatively novel approaches to generate therapeutic strategies using advanced drug delivery techniques to surpass most above-mentioned ill effects of DOX by taking along with in combination with AmB against VL therapy.

In short, we developed a targeted delivery system comprising of combination of AmB and DOX PLGA-NPs with a view towards vectoring both drugs to RES via ligand mediated targeting and generated an excellent tool that can evade major issues in the treatment against VL. In the present paper, the activity of nanoparticles against L. donovani, which was tested in vitro in macrophage-amastigote system (J774A.1 cells) and in an experimental model of VL in hamsters is presented and the results of plain and ligand-appended nanoparticles entrapped AmB and DOX are compared.

MATERIALS AND METHODS
Drugs and chemicals
AmB was obtained as a gift sample from M/s Ambalal Sarabhai Enterprises, Vadodara, India. Doxorubicin Hydrochloride was received as gift sample from Sun Pharmaceuticals Industries Limited, India. PLGA 50:50 (intrinsic viscosity 0.35 dl/g) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Span 85, Tween 20, sodium chloride, potassium dihydrogen phosphate, Polyvinyl alcohol (PVA), DMSO, Isopropyl alcohol, Eagle's medium, Locke's solution and dialysis bag membrane (MWCO: 3500) were obtained from Sigma Chemicals, USA. Chloroform and all other chemicals were of pure analytical grade and used as procured.

Preparation of PLGA-NPs
Core PLGA nanoparticles were formulated according to double emulsion method with slight modification as per laboratory setup. 9 In brief, In a plastic vial Amphotericin B was dissolved in DMSO and in a separate vial doxorubicin was dissolved in water to form the aqueous phase, which was then added to a solution of 50 mg PLGA in 2 ml DMSO to give a w/o emulsion which was then sonicated and added drop wise under stirring to aqueous solution containing 0.2% PVA to form the secondary emulsion. The secondary emulsion was again sonicated to reduce the particle size and then was diluted with sufficient water to help solvent diffusion and precipitation of the polymer resulting into formation of NPs. The resulting nanoparticle suspension was used immediately for analysis or lyophilized for storage at 4 °C. 9

Optimization of PLGA-NPs
PLGA-NPs were optimized for drug content (AmB and DOX), polymer content (PLGA) sonication time. AmB and DOX to polymer content was optimized by keeping PLGA content 50 mg, and sonication time (12 min) at constant levels while AmB and DOX content was varied at different weight levels, i.e. (2, 4, 6, 8 and 10 mg) whereas for DOX (2, 5, 10, 15 and 20 mg) respectively in different formulations for determining optimum AmB and DOX content. 5, 7 Average particle size of different formulations was measured by photon correlation spectroscopy using Zetasizer Nanoseries (Nano-ZS 90, Malvern Inst. Ltd. UK) using a flow-through cell and percent drug entrapment in different formulations was also determined. 10 PLGA-NPs with optimum AmB and DOX content were optimized for optimum polymer content (PLGA) in terms of percent drug entrapment. AmB (6 mg) and DOX content (10 mg), sonication time (12 min) were kept constant while polymer (PLGA) content was varied for different formulations. PLGA-NPs with optimum AmB and DOX content and polymer content (PLGA) were optimized for optimum sonication time in terms of average particle size. PLGA content (50 mg), and both drugs (AmB-6 mg and DOX-10 mg) (optimized) were kept constant while sonication time was varied (i.e. 0, 2, 4, 6, 8, 10, 12 and 15 min) for different formulations. PLGA-NPs were evaluated for average particle size as described above. AmB and DOX content, polymer content (PLGA) and sonication time, however, were kept constant at its optimum level. PLGA-NPs were evaluated for percent drug entrapment and percent drug release as reported. 11

Coating with Lectin

To conjugate the surface of PLGA-NPs with lectin modified emulsion- solvent method was performed. 11 Lectin incorporated PLGA-NPs was prepared by incubating 5 ml PGNP-NPs with 1 ml lectin solution in 10 mM HEPES buffer (pH 7.4) at 37 o C for 24 hrs under sonication. The unattached lectin was removed by washing followed by centrifugation of the nanoparticle suspension; the whole process being repeated thrice to ensure complete removal. The Lectin concentration was optimized by measuring the change in zeta potential of the lectin coated PLGA-NPs dispersion in deionized water at 25 o C. 11, 12

In Vitro Characterization of PLGA-NPs

Average particle size and size distribution of PLGA-NPs and Lectin-PLGA-NPs were analyzed by photon correlation spectroscopy (PCS) at 25 o C under an angle of 90o. Zeta potential was determined using laser doppler anemometry (LDA) at 25 o C. The PCS and LDA analysis were performed using a Zetasizer NanoZS (Malvern Instrument, Worcestershire, UK), after dispersing in deionized water. 13

In order to quantify the percent drug entrapment encapsulated amount, AmB and DOX was extracted from the PLGA-NPs using dimethyl sulfoxide, diluted with methanol and analyzed using HPLC. The HPLC (LC-10ATvp, Shimadzu, Tokyo, Japan) was equipped with a Lichrosphere reverse-phase C18 column (250×4 mm, 5 µm; Merck, Darmstadt, Germany). Acetonitrile with KH_2PO_4 buffer (pH 3.5, adjusted with orthophosphoric acid), (60:40, v/v) was employed as mobile phase at 1.0 ml/min flow rate and column effluent was detected with a UV detector at 405 and 235 nm respectively. Results are expressed as AmB and DOX actual loading (drug amount encapsulated per 100 mg of PLGA-NPs) and encapsulation efficiency (EE) (ratio between drug amount entrapped in the PLGA-NPs and that added during PLGA-NPs preparation) ± SD of values collected from three different batches. 14

The in- vitro AmB and DOX release was performed using dialysis membrane diffusion technique. Briefly, AmB and DOX equivalents of total amount of drug encapsulated formulation was suspended in 1 ml of phosphate buffer solution (PBS, pH 7.4) in a dialysis bag and dialyzed against 250 ml PBS with 0.5% Tween 80 contained in dissolution apparatus (DISSO 2000, Labindia, India), thermostated at 37±1 ℃ with moderate shaking at 100 rpm. At specific time intervals, a definite volume (1 ml) of the release medium was withdrawn and replenished with fresh PBS and analyzed for AmB and DOX amount using validated HPLC method. Each measurement was performed in triplicate and reported as their average. 10

Stability study

The stability studies were performed by observing drug leaching and change in particle size following incubation of PLGA-NPs with freshly pooled rat serum at 37±10C. The drug content of the PLGA-NPs was determined by the method described previously 7 with slight modifications. PLGA-NPs formulations (1 ml) were incubated with 2 ml serum at 37±1°C for 1, 2, 4, 6 and 24 h. After specified time intervals, suspensions were centrifuged at 20,000 rpm for 15 min and supernatant was filtered through 0.22 µm membrane filter. The filtrate was analyzed for drug content by reverse phase high performance liquid chromatography (HPLC) method as described elsewhere. 6, 11 The particle size of PLGA-NPs formulations was determined after 24 h incubation of the formulations with the serum using particle size analyzer (CILAS, 1064, Marcoussis, France).

In Vitro Parasite Killing

Adherent mouse macrophage cell line J774A.1 was used for in-vitro activity against intracellular amastigotes in macrophages and maintained in Dulbecco's modified Eagle's medium (Sigma, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100U/mL penicillin and 100mg/mL streptomycin at 37 0C in 5% CO_2 in humidified atmosphere. Macrophages (1×105 cells/well) in 16-well chamber slides (Nunc, Naperville, IL, USA) were infected with promastigotes (L. donovani, Dd8) at multiplicity of infection of 10:1 (parasites: macrophage) and incubated at 37.8 0C in 5% CO_2 for 12 hrs. after which chamber slides were washed thrice with PBS (pH 7.2) to remove non-phagocytosed promastigotes and finally supplemented with complete medium. Different concentrations (0.03, 0.08, 0.13 and 0.2 mg/mL) of 100mL of free PLGA-NPs, PLGA-NPs (AmB and DOX), Lectin-PLGA-NPs in RPMI-1640 medium were added to wells in triplicate. The untreated infected macrophages were used as control. Formulations were then removed by washing after 3 and macrophages were placed in medium for an additional 20 hrs. and then examined for intracellular amastigotes under oil immersion objective of light microscope after methanol fixing and Giemsa staining of the slides. At least 100 macrophage nuclei were counted per well for calculating the percentage infected macrophages and number of amastigotes per 100 macrophages. After washing, cells were fixed in 10% formalin in PBS and observed by CLSM (Olympus IX 81, Center Valley, PA, USA) equipped with a × 60 oil objective lens. Percent parasite inhibition in treated wells was calculated using the following formula reported and published by our associated group previously. 2, 6

PI = 100-T × 100/C

Where PI is the percentage inhibition, T the number of parasites in treated samples/100 macrophage nuclei and C the number of parasites in control samples/100 macrophage nuclei.

Statistical analysis

Results were expressed as mean ± S.D. Three sets of experiments were performed for in vitro antileishmanial activity testing. The data were statistically processed by one-way analysis of variance (GraphPad; Prism software program) followed by post-hoc Tukey's multiple comparison test to determine the level of significance. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Preparation and In-Vitro Characterization of PLGA-NPs

PLGA-NPs formulations, having combination of dual agents (AmB and DOX) were prepared by modified double emulsion method with slight modification as per our laboratory set up. 9 PLGA-NPs were optimized for various parameters. These include the drug content (AmB and DOX), polymer content (PLGA), and sonication time. At higher concentrations, (10-20 mg of AmB and 15-20 mg of DOX) particles do not formed within Nano-size range. However, as the concentration of AmB

and DOX was gradually lowered, relative numbers of particle size decreased and nanoparticles were formed (Table I (a) & b). Similarly, as the concentration of AmB and DOX was gradually lowered, particle size was decreased while percent drug entrapment was increased. When concentration of AmB and DOX was used at 6 and 10 mg respectively, PLGA-NPs formulations were free of other undesired structures, average particle size measured was 356.2 ± 0.04 nm and entrapment efficiency was recorded to be 78.4 ± 2.01 for AmB and 70.06 ± 0.14 for DOX respectively. On the basis of minimum particle size and maximum percent drug entrapment formulations PLGA-NP4 was considered to be optimum. Formulations with optimum AmB and DOX content were subjected to sonication for different time periods to optimize the sonication time. Fig. 1 shows that as the sonication time was increased from 0 to 15 min, average particle size was recorded to be decreased. From this hypothesis, optimum sonication time was recorded to be 15 min, which gave particle size of 356.2 ± 0.04 nm. On further increasing the sonication time (i.e. at 15 min) beyond the optimum limit the particle size was recorded to be 134.8 ± 0.02 nm and PLGA-NPs might have localized in hepatocytes apart of macrophages, the target site. Formulation having optimized AmB and DOX, sonication time were subjected to optimization of polymer content (PLGA). Fig. 2a & b shows that with an increase in PLGA content in PLGA-NPs, distinctive percent entrapment of AmB and DOX were recorded. As the PLGA content was increased from 50-200 mg drug entrapment was also recorded to be increased (Fig. 2a & b). However, it may be attributed to the subsequent decreased stability of the PLGA-NPs with the increase in PLGA content and initial fast release of drug from these unstable particles. 3, 11 Optimum PLGA content was found to be 50 mg which could entrap maximum amount of drug 78.4 ± 2.01 for AmB and 70.06 ± 0.14 for DOX respectively.

After optimizing the process parameters, PLGA-NPs were coated with macrophage specific ligand Lectin. Moreover, Lectin possessed a positive charge, while PLGA-NPs were negatively charged which further facilitated the adsorption process and resulted in a reduction of the zeta potential of the dispersion. This determinant variable was critically utilized in determining optimal ligand density through optimization of process variables (lectin content and incubation time). For PLGA-NPs the initial positive value of the zeta potential was decreased on addition of cationic ligand lectin and approached towards a minimum value of 8.44 mV at 03:02 lectin: polymer (w/w) ratio (Fig. 3). It was apparently related to the extent of the covering of the surface charge by the lectin. On further addition of lectin especially beyond this optimum ratio (3: 2 w/w) no significant change in the zeta potential was occurred for PLGA-NPs. Hence optimum ratio of lectin: polymer was recorded to be 3:2 (w/w) for PLGA-NPs. This indicated that at an optimal 3:2 w/w ratio of lectin: polymer, the integration of lectin was occurred at saturation level. For optimization of incubation time, the PLGA-NPs formulations using the optimum lectin: polymer ratio were incubated with lectin for different time periods (0, 1, 2, 3, 4 and 6, 7, 8 h) and the change in zeta potential was recorded (Fig.4). Zeta potential value declined steeply from its initial value of 20.36 mV for PLGA-NPs, which might have been attributed to the charge quenching of the surface associated with free lectin. With a longer incubation time (beyond 7 h) the amount of residual free lectin was decreased and the change in zeta potential was not significant. This indicated that at the end of 7 hrs. the interaction and interdigitation of added lectin could have completed. The morphology and presence of coating on the PLGA-NPs surface could be appreciated from the TEM, which indicates surface intervening and anchoring of mannose terminating ligand (data not shown). Table II shows the average particle size and percent drug entrapment of optimized formulation of PLGA-NPs and lectin-PLGA-NPs. The increase in average particle size in case of formulation lectin-PLGA-NPs as compared to formulation PLGA-NPs is an indication of coating, which can be distinguished by dark black boundary of the formulation lectin-PLGA-NPs. Percent drug entrapment of optimized PLGA-NPs and lectin-PLGA-NPs formulation was recorded to be 75.8 ± 1.21 (AmB) and 71.20 ± 0.04 (DOX) and 72.4 ± 2.01 (AmB), 68.04 ± 0.12 (DOX) respectively, revealing that lectin anchoring did not result in significant lowering of the percent drug entrapment. Preformed PLGA-NPs were used for anchoring of ligand and this may presumably be the reason for the insignificant change recorded in the percent drug entrapment value. Relatively high entrapment of AmB and DOX in the PLGA-NPs could be attributed to the lipophilic nature of the drug, since the entrapment was dependent upon lipid: aqueous phase ratio.

Stability studies

Stability of developed PLGA-NPs in serum was measured as percent drug leaching from PLGA-NPs and change in particle size of PLGA-NPs after incubation with serum at 4 ± 1 ºC, 28 ± 1 ºC, 37 ± 1 ˚C. The PLGA-NPs were found to be almost stable upon incubation with freshly pooled rat serum. Only 95.2 ± 1.4, 91.2 ± 2.2, 92.6 ± 2.2 and 96.1 ± 2.4, 94.8 ± 2.0, 94.0 ± 2.0 drug was leached into serum after 30 days of incubation at 4 ± 1 ºC, 28 ± 1 ºC, 37 ± 1 ˚C from formulation PLGA-NPs and lectin-PLGA-NPs respectively (Fig. 5). This may be attributed to the lipophilic nature of the prepared formulation hence prevented the drug leaching in serum. 2 Similarly, particle size analysis of PLGA-NPs did not show any significant change in the particle size of formulation PLGA-NPs and lectin-PLGA-NPs respectively after 30 days incubation with serum. There was only slight increase in the particle size of formulation PLGA-NPs and lectin-PLGA-NPs. The PLGA-NPs formulations developed for targeting to macrophages should be cleared from the circulation within a very short span of time. Therefore, this insignificant increase in particle size in vitro may not have discernible bearing on biodisposition. 12, 13

CONCLUSIONS

In conclusion, the proposed PLGA-NPs with combination of dual drugs i.e AmB and DOX systems show incredible potential for intracellular macrophage targeting. The formulations could considerably modify the pharmacokinetics of AmB and DOX, providing prolonged action at comparatively low drug doses thereby reducing the toxicity problems like nephrotoxicity, cardiac arrhythmia etc. The developed systems (plain and Lectin coated PLGA-NPs) appear promising for the treatment of VL specifically.

In summary, our findings indicated that Lectin-PLGA-NPs deliver higher amount of the drug to the desired organ sites due to being an efficient macrophage targeted drug delivery system. Targeted delivery directly reduces the drug dose, which is highly desirable for optimized therapeutic effect and diminished undesirable toxicity.

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Table I. (a) Optimization of Amphotericin B (AmB)

Formulation code	AmB content (mg)	% Entrapment efficiency of AmB	Avg. particle size (nm)
PLGA-NP1	2	65.4 ± 1.08	948.8 ± 0.06

PLGA-NP2	4	70.2 ± 1.62	781.2 ± 0.03
PLGA-NP3	6	78.4 ± 2.01	340.6 ± 0.08
PLGA-NP4	8	80.1 ± 2.04	280.7 ± 0.02
PLGA-NP5	10	81.01 ± 1.82	206.1 ± 0.08

Total polymer used-50 mg, DOX-10 mg, % PVA- 0. 2 (50 ml), sonication time-15 min; at 20% amplitude; pulse on time: 5 min, pulse off time: 5 sec for each formulation (mean ± S.D) (n=3)

Table I. (b) Optimization of Doxorubicin (DOX)

PLGA-NP5   20   78.03 ± 0.12   206.1 ± 0.08

Formulation code	DOX content (mg)	% Entrapment efficiency (DOX)	Avg. particle size (nm)
PLGA-NP1	2	50.4 ± 0.02	948.8 ± 0.06
PLGA-NP2	5	61.8 ± 0.10	781.2 ± 0.03
PLGA-NP3	10	70.06 ± 0.14	340.6 ± 0.08
PLGA-NP4	15	72.01 ± 0.10	280.7 ± 0.02
PLGA-NP5	20	78.03 ± 0.12	206.1 ± 0.08

Total polymer used-50 mg, AmB- 6 mg, % PVA- 0.2 (50 ml), sonication time-15 min; at 20% amplitude; pulse on time: 5 min, pulse off time: 5 sec for each formulation (mean ± S.D) (n=3)

Table II. Characterization of optimized plain and ligand anchored formulations

| S. No.	Parameters	AmB and DOX loaded PLGA-NPs	Lectin-PLGA-NPs
1	Size (nm)	340.6. ± 0.08 nm	402.2 ± 0.06
2	Polydispersity index	0.160	0.410
3	Zeta potential (mV)	-4.82	4.82 mV
4	% Drug entrapment efficiency	75.8 ± 1.21 (AmB) and 71.20 ± 0.04 (DOX)	72.4 ± 2.01 (AmB), 68.04 ± 0.12 (DOX)
5	Percent drug release	AmB release over 12 days DOX release for as long as 8 days.	AmB release over 10 days DOX release for as long as 6 days.

(mean ± SD) (n=3)
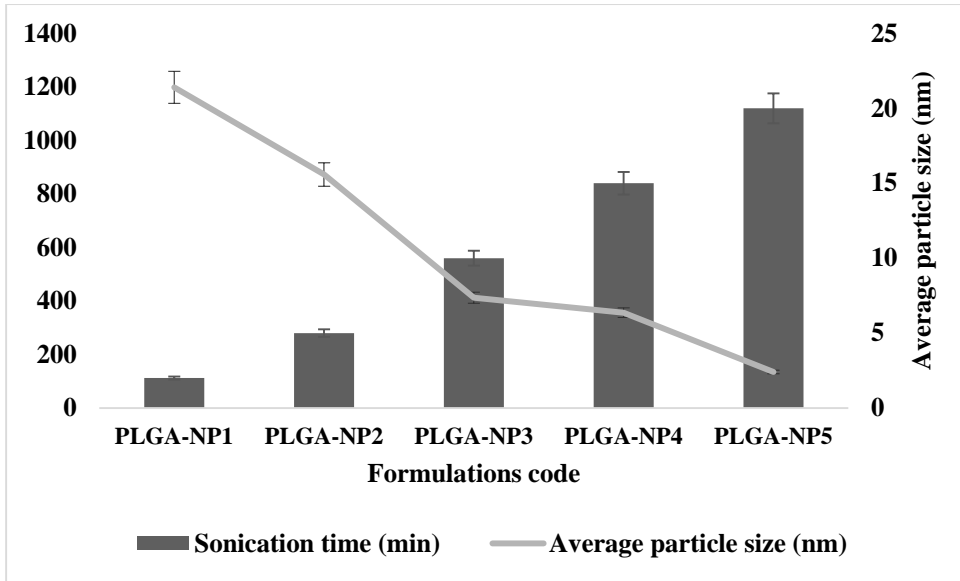
Figure 1. Optimization of sonication time in terms of average particle size.
Total polymer used - 50 mg; AmB- 6 mg, DOX-10 mg; at 40% amplitude; pulse on time: 8 sec, pulse off time: 5 sec for each formulation (mean ± S.D) (n=3)
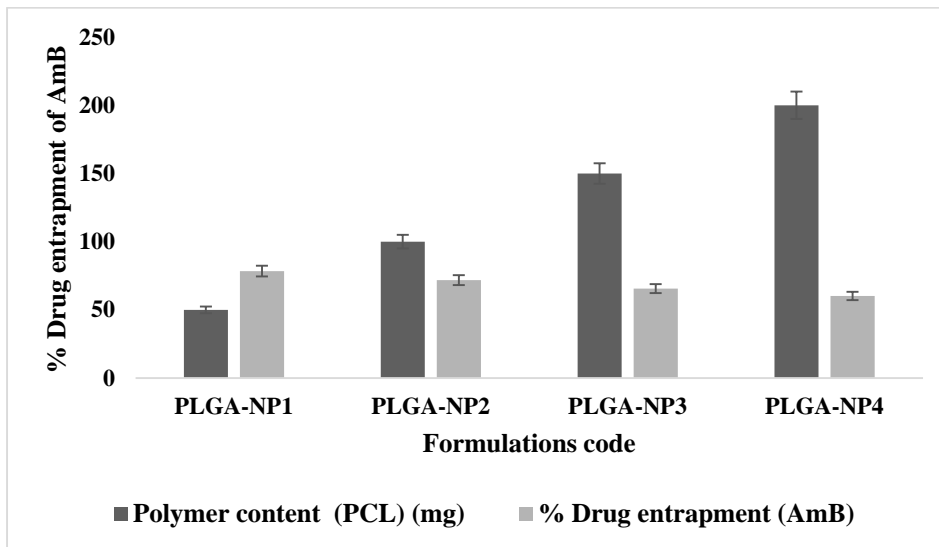

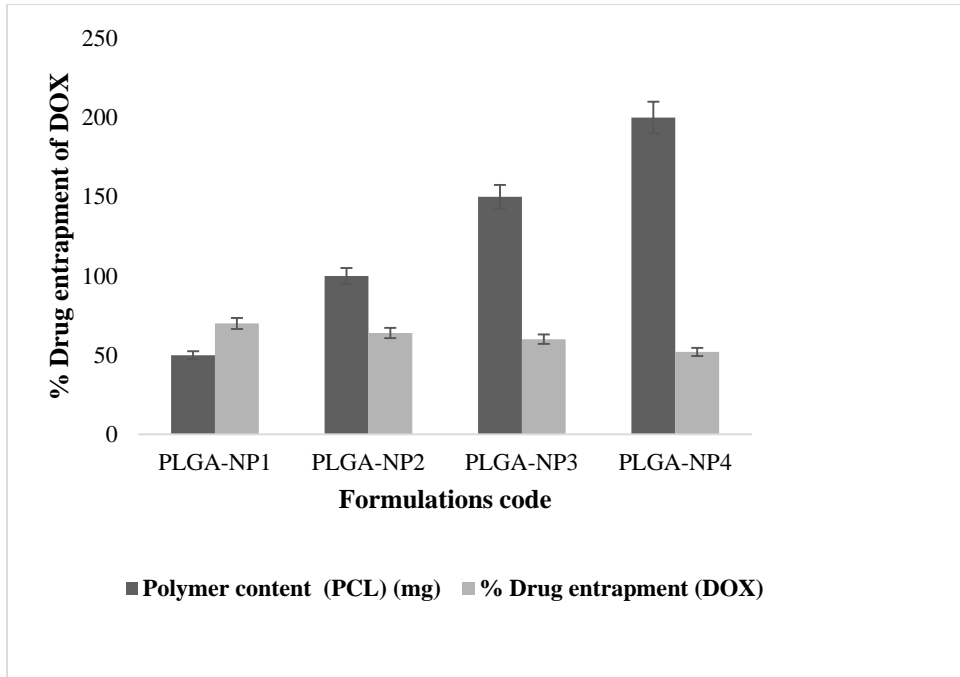Figure 2 (a). Optimization of polymer content in terms of % entrapment efficiency. Total AmB used - 6 mg, Dox - 10 mg; % PVA= 0.2%; sonication time-15 min at 40% amplitude; pulse on time: 8 sec, pulse off time: 5 sec for each formulation, as optimized at the optimum level;
(mean ± S.D) (n=3)

Figure 2 (b). Total AmB used - 6 mg, Dox-10 mg; % PVA- 0.2%; sonication time-15 min at 40% amplitude; pulse on time: 8 sec, pulse off time: 5 sec for each formulation, as optimized at the optimum level; (mean ± S.D) (n=3)
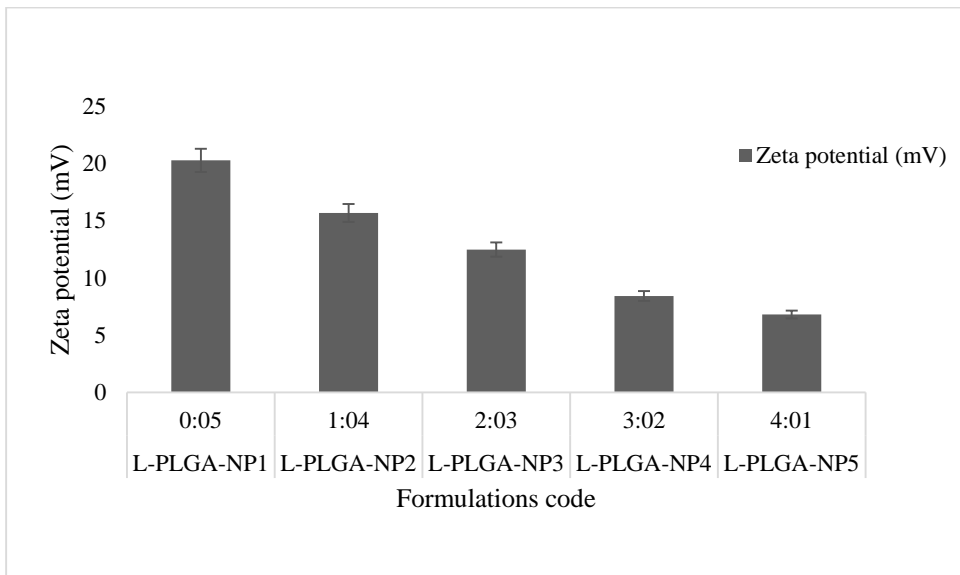

Figure 3. Optimization of lectin to polymer ratio for complete lectin ratio for complete lectin coating over PLGA-NPs. Incubation time of 8 hrs. was kept constant.
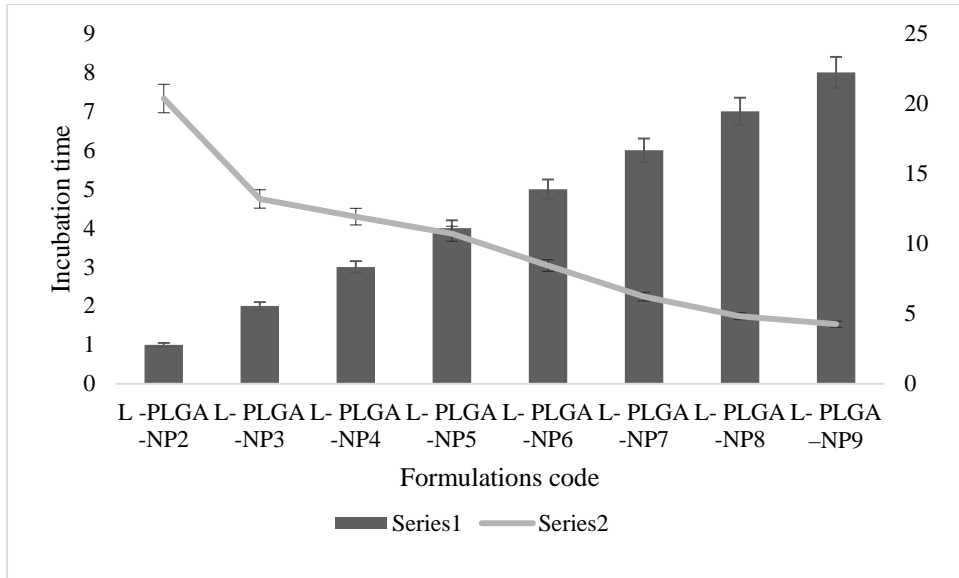
Figure 4. Optimization of incubation time for complete lectin Coating. Lectin to polymer ratio 3:2 w/w was kept constant (mean ± SD) (n=3)
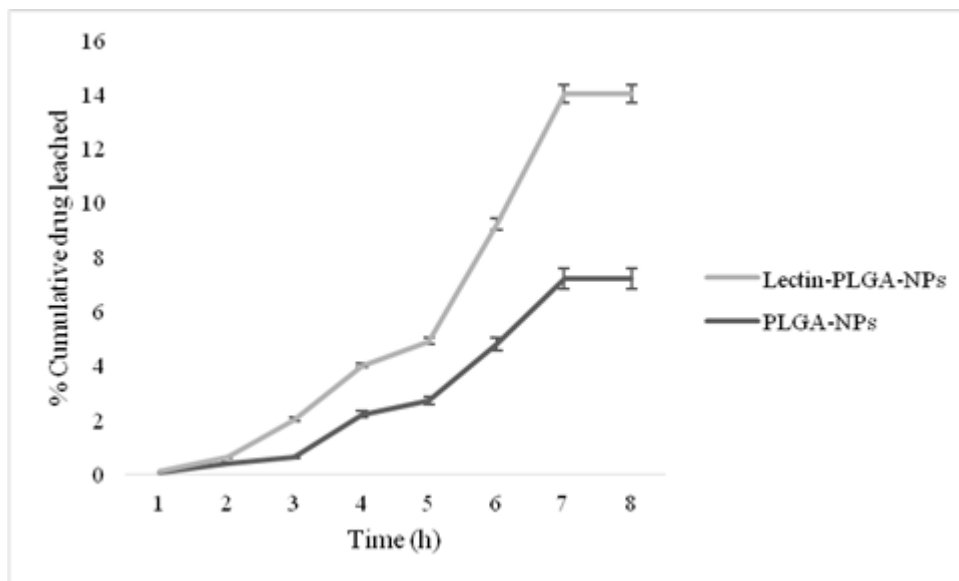

Figure 5. In vitro drug leaching in serum from plain PLGA-NPs and coated Lectin-PLGA-NPs). PLGA-NPs were incubated with serum at 37 ± 10C for different time intervals and drug concentrations in the serum were recorded (mean ± SD) (n=3)