ORIGINAL ARTICLE

Macrophage Targeting: Comparative Cytotoxicity Activity of Imipramine-loaded Polymeric Nanoparticles and Niosomes

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Abstract

Aim: The aim of the present study was to prepare niosomal and nanoparticle formulations for drug therapy of leishmaniasis. Imipramine, an antidepressant, is reported to have anti-leishmaniasis activity in the dose of 5–10 mg/kg. **Materials and Methods:** In this study, we tried an approach with imipramine and polymer chitosan to target mannose receptors on macrophages using nanoparticles as drug carrier and with span 40 as surfactant in niosomes. Formulation of niosomes encapsulated with imipramine was optimized by changing the proportions of span 40, cetrimide, and cholesterol. The imipramine encapsulated niosomal formulations based on span 40, cetrimide, and cholesterol was prepared by hand shaking method and characterized using atomic force microscopy. The nanoparticles were prepared by modified ionic gelation method, where chitosan was conjugated with mannose for facilitation of uptake by macrophages. **Results:** The Atomic Force Microscopy results showed that the particles were spherical in shape and had a size between 200 and 900 nm. The prepared nanoparticles were evaluated for particle size, encapsulation efficiency and *in vitro* drug release. The *in vitro* release of nanoparticles shows 76 ± 1% release in 6 h. **Conclusion:** Niosomes and nanoparticles cell uptake studies were evaluated using RAW 264.7 cell lines. The % viability values for niosomes and nanoparticles were 42.53% and 70.17% at the highest concentration of 100 µg/mL after the incubation period of 24 h, respectively.

Key words: Chitosan, imipramine, leishmaniasis, mannose, nanoparticle, niosome

INTRODUCTION

A protozoan parasite called Leishmania, which is spread by female sandflies, is the cause of the disease leishmaniasis. There are three primary types of leishmaniasis: Cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis (VL). The type of sickness the parasite causes relies on the infecting species and the host's immunological response. The antileishmanial medications are administered by parenteral routes because they guarantee drug delivery to all infection sites while also exposing non-target organs, which is crucial for potentially harmful medications. Studies using a range of drug delivery systems, such as liposomes, non-ionic surfactant vesicles (NIVs), nano capsule emulsions, nano discs, and nanoparticles, have widely exploited this route of administration. Since the liver and spleen, which are both abundant in macrophages, are not only the primary sites of infection but also the organs in charge of removing debris from the systemic circulation.[1]

Tricyclic antidepressant imipramine, also known as N-(c-dimethylaminopropyl)-iminodibenzyl HCl, is a member of the large class of cationic amphiphilic medicines. Based on the previous findings, imipramine was chosen as a treatment drug for experimental VL – the drug alters the proton motive force of LD's membrane, inhibits trypanothione reductase, an enzyme upregulated in SSG-resistant LD parasites, an effective immunomodulator as it induces the production of TNF-a, an important cytokine for antileishmanial defense, cationic properties favor its absorption by phagocytic cells and accumulation in phagolysosomal bodies.

These attributes of imipramine toward leishmania parasites led researchers to test its efficacy directly on LD and also in experimental infection induced by recent clinical isolates

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of SSG-S and SSG-R LD parasites with miltefosine as a reference oral drug.[2]

Liposomes were the first vesicular drug delivery technology; however, they have a number of drawbacks, including toxicity, affordability, and pH stability concerns. The drawbacks of liposomes led to a change in research focus to niosomes. Niosomes are a vesicular, new drug delivery technology that can be utilized to deliver medications in a sustained, regulated, and targeted manner.

Niosomes are named as such because they are composed of non-ionic surfactants, which also make them non-toxic. They may also include charged molecules, cholesterol, or its compounds in addition to non-ionic surfactants. The structure is made stiff by cholesterol and the charged molecule maintains the stability of the mixture. Only when surfactants and cholesterol are combined in the right ratio and the temperature is above the gel liquid transition point can these thermodynamically stable bilayered structures of niosomes develop.[3] NIVs (niosomes) can enclose a wide range of medications since they self-assemble from hydrated synthetic non-ionic surfactant monomers.[4]

Colloidal carriers known as polymeric nanoparticles have appealing physicochemical characteristics such as size, surface potential, and hydrophilic-hydrophobic balance. Chitosan is reported to be a good polymer candidate because it is biocompatible, biodegradable, and its chemical structure allows for specific modifications at the C-2 position without too much difficulty. This is in contrast to other biodegradable polymers of natural origin such as cellulose, starch, and gelatin, which are largely used as drug carriers in targeted drug delivery systems. Chitosan has also been shown to stimulate the immune system by boosting the concentration and activation of macrophages and natural killer cells, causing cytokines, and triggering cytotoxic T-cell responses.

The interaction between the mannose-containing lipopolysaccharides on the parasite cell surface and the macrophage mannose receptors determines one of the pathways for the phagocytosis of the leishmania parasite. Hence, the mannose-conjugated carriers can successfully be taken up by the cells hosting the intracellular pathogens by duplicating the same invasion process. This could boost the potential of the medicine to reach its target and to work effectively in the appropriate environment. To improve macrophage selectivity, increase cellular absorption, and to decrease toxicity, mannose-conjugated chitosan nanoparticles were investigated and formulated in the study.[5]

Reported literature information reveals that liposomal formulation results in degradation by gastric enzymes when encapsulated and given as oral formulation. As an alternative to liposomes, imipramine niosomes and polymeric nanoparticles of mannose conjugated chitosan imipramine nanoparticles were formulated and evaluated in the study.

MATERIALS AND METHODS

Materials

Imipramine (Gift sample), Chitosan (HiMedia Laboratories Pvt. Ltd., Mumbai), Span 40 (Loba Chemie Pvt. Ltd., Mumbai), MCS (Loba Chemie Pvt. Ltd., Mumbai), Cetrimide (Yarrow- Chem Products, Mumbai), Cholesterol (Sigma Aldrich), TPP (Loba Chemie Pvt. Ltd., Mumbai), Sodium borohydride (Sisco Research Laboratories Pvt. Ltd., Maharashtra), RAW 264.7 cell lines (NCCS, Pune).

Methods

Preparation of niosomes

Accurately weighed cholesterol and non-ionic surfactant and 25 mg imipramine were dissolved in 250 mL round bottom flask with 10 mL of diethyl ether and it was hand shaken for 30 min. The film was hydrated with phosphate buffer saline pH 6.8. The formulation was evaluated for particle size, entrapment efficiency, and *in vitro* release.[6]

Design of experiment

For the purpose of statistical optimization of imipramine-loaded niosomes, a two-level, three-factor rotatable were employed using Design-Expert® software (Version 13, Stat-Ease). A total of 8 trials were conducted for the 3 factors. Based on preliminary tests and the viability of producing imipramine-loaded niosomes at these values, the amounts of cetrimide (X1), span 40 (X2), and cholesterol (X3) were chosen as independent variables in this design and varied at two levels (low and high). Particle size (Y1), entrapment efficiency (Y2), and drug release (Y3) were noted as response. The data of design of experiment niosomes are tabulated in Table 1.

Preparation of mannose-conjugated chitosan imipramine nanoparticles

Mannose-conjugation was carried out by reductive amination of chitosan with D-mannose in the presence of reductive amination agent, sodium borohydride. CS was dissolved in 1% aqueous acetic acid (pH 5.5) with continuous stirring. An aqueous solution of D-mannose and sodium borohydride was prepared and added to the viscous solution of CS while stirring slowly and left at room temperature for 48 h. The product was subjected to two cycles of overnight dialysis against distilled water to remove unreacted reagents. Mannose chitosan solution was dissolved in acetic acid solution (0.5%, v/v) and 25 mg of imipramine was dissolved in 25 mL of distilled water to obtain imipramine solution. 25 mL of imipramine solution was added dropwise into MCS solution with continuous stirring for 20 min, and then, 25 mL of TPP solution (0.2%, w/v) was dropped slowly into above mixture under stirring for another 20 min to form MCS/imipramine NPs at room temperature. All the measurement was performed

Table 1: Optimization of niosomes

Run	Formulation Code	Factor1 (Cetrimide) (mg)	Factor 2 (Span 40) (mg)	Factor 3 (Cholesterol) (mg)
1	F1	10	32	70
2	F2	0	74	110
3	F3	0	32	110
4	F4	0	32	70
5	F5	0	74	70
6	F6	10	74	110
7	F7	10	32	110
8	F8	10	74	70

on the diluted form in a low-volume disposable sizing cuvette at 25°C.[7]

Characterization

Particle size, zeta potential

The particle size of formulated nanoparticles and niosomes was determined using malvern zetasizer on the principle of dynamic light scattering and zeta potential was also measured.

Entrapment efficiency

The centrifugation method was used to assess the produced formulation entrapment effectiveness. 3mL of the imipramine-encapsulated niosomes were centrifuged for10 min at 10000 rpm.[8] 1 mL of the nanoparticle formulation was placed in an Eppendorf tube and centrifuged for 20 min at a speed of 15000 rpm. The quantity of imipramine was measured by UV-visible spectroscopy at 251 nm after the supernatant was collected.[9]

In vitro release

The *in vitro* release studies of nanoparticle and niosome formulation were carried out utilizing the dialysis sac method and pH 6.8 phosphate buffer. 1 mL of nanoparticle and niosomes formulation was placed in a dialysis bag and kept over a beaker containing 250 mL pH 6.8 phosphate buffer. At 37°C, the solution was stirred magnetically, up until 6 h, 5 mL aliquots of dialysate were taken at intervals of 30, 60, 90, 120, 180, 240, 300, and 360 min and the sink conditions were maintained by refilling the same volume of buffer. UV spectroscopy was used to examine the formulation at 251 nm.[10]

Drug release kinetics

The drug release kinetics was studied for the optimized formulation. *In vitro* drug dissolution results were plotted in various kinetics models, such as zero order, first order, Higuchi's, Hixson-Crowell, Korsmeyer-Peppas, and Weibull method to understand the kinetics and mechanism of drug release.[9]

FTIR spectrum

FT-IR analyses were performed for the pure drug, drug-polymer physical mixture combinations, niosome, and nanoparticle formulations. In the presence of an IR lamp, potassium bromide was combined in a 1:1 ratio with the drug and/or polymer. Mixing can be affected by grinding in a smooth mortar. Thereafter, the mixtures were placed in the instrument's sample container, and spectra were obtained. The wave numbers for the spectra ranged from 4000 cm^{-1} to 400 cm^{-1}.[11]

Surface morphology

Atomic force microscopy was utilized to study the surface morphology of both niosomes and nanoparticles. The nanoparticle suspension was diluted 10 times in deionized water before being applied in a few drops (50 mm/mL) to a small area of a clean microscope slide. The sample was placed on the microscope scanner and allowed to air dry at room temperature to create a film (25°C).[12]

Stability studies

The particle size, PDI, and zeta potential of both the formulation were assessed during the storage of the samples at room temperature (25°C), refrigerated temperature (8°C), and stability chamber (40°C) for up to 2 months, and hence, the stability of the optimum formulation was determined.[10]

Statistical analysis

Statistical analysis was performed by student t-test using GraphPad Prism software. Level of significance defined at $p<0.05$.[13]

Differential scanning calorimetry

Thermograms were taken using a TGA/DSC1, STAR® system (Mettler Toledo, Switzerland) and an auto-cool attachment to study the thermal characteristics of mannose conjugated chitosan imipramine nanoparticles. Each sample, which weighed around 6 mg and was enclosed in an aluminum pan, was heated at a rate of 10°C/min while being continuously nitrogen-purged from 25°C to 300°C.

The reference was an empty, loosely covered metal pan. The maximum denaturation temperature was recorded.[14]

Phase contrast microscopy

With the help of phase contrast microscopy, niosome vesicles and nanoparticles were visualized. Average size vesicles were determined.[15]

In vitro cytotoxicity assay

MTT colorimetric assay was used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. Accurately 200 µL cell suspension was seeded in a 96-well plate to achieve desired cell density (20,000 cells per well), without the test agent. The cells were allowed to grow for about 24 h. The appropriate concentrations of the formulations were added and the plates were incubated for 24 h at 37°C in a 5% CO_2 atmosphere. After the incubation period, the plates were removed. To the media, MTT reagent was added and the final concentration was of 0.5 mg/mL of total volume. The plates were wrapped with aluminum foil to avoid exposure to light. Then, the plates were returned to the incubator and incubated for 3 h. The MTT reagent was removed, and then, 100 µL of solubilization solution (DMSO) was added. Then, it was stirred in gyratory shaker and the absorbance was recorded in a spectrophotometer at 570 nm wavelength.

% Cell viability is calculated using below formula:

% cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] × 100.[16]

RESULTS AND DISCUSSION

Using DoE of 2^3 factorial design, 8 niosome formulations were formulated, and the obtained particle size, entrapment efficiency, and *in vitro* drug release are tabulated in Table 2.

Formulation F4 has low concentrations of span 40, cetrimide, and cholesterol, which results in lower entrapment efficiency and *in vitro* release. In contrast, F7 has low concentrations of span 40 and high concentrations of cetrimide and cholesterol, thus it exhibits acceptable particle size.

The polynomial equation for particle size is

Y1 (Particle Size) = +648.63–221.88B

In a regression equation, a positive coefficient value (+) denotes a favorable influence (synergistic effect), whereas a negative coefficient value (–) denotes an antagonistic interaction between the factors and the output. According to statistical equation, where A is cetrimide, B is span 40, and C is cholesterol concentration. The decrease in particle size is due to the influence by span 40.

Polynomial equation for entrapment efficiency is

Y2(Entrapment Efficiency) = +66.29+0.0375B+0.6625C-0.8875BC

Span 40 has a positive impact on entrapment efficiency, but when both span 40 and cholesterol were mixed together it has a negative impact on the entrapment.

Polynomial equation for *in vitro* drug release is

Y3(*in vitro* drug release) = +21.04+0.6375B-0.5125C-1.26BC

In vitro drug release is decreased due to the presence of cholesterol, in converse to span 40 which has a positive impact on the drug release, but when mixed together, they decreased the drug release.

The formulation had a desirability value of 0.91. The desirability obtained according to design of experiment is shown in Figure 1. Based upon the desirability value, the final niosome formulation was optimized with the composition of cetrimide – 5 mg, span – 40–70.8 mg, and cholesterol – 72 mg.

The observed particle size according to DoE was 460 nm but the size obtained was 451 nm, which showed a percentage

Table 2: Particle size, entrapment efficiency, and *in vitro* release of optimized niosome formulation

Formulations	Particle size (nm)	Entrapment efficiency (%)	*In vitro* release (h)
F1	890±2	65.6±0.41	20.7±0.36
F2	292.6±2.51	66.8±0.52	21.2±0.92
F3	959.6±3.05	67.7±0.25	22.3±0.75
F4	653±2	65.4±1.44	20.2±1.55
F5	510.6±2.08	67.9±0.60	25±0.85
F6	449.3±3.51	67.1±1.02	20.5±0.95
F7	980±1	67.2±1.10	21.1±0.65
F8	457.5±1.32	66.6±0.96	22.6±0.85

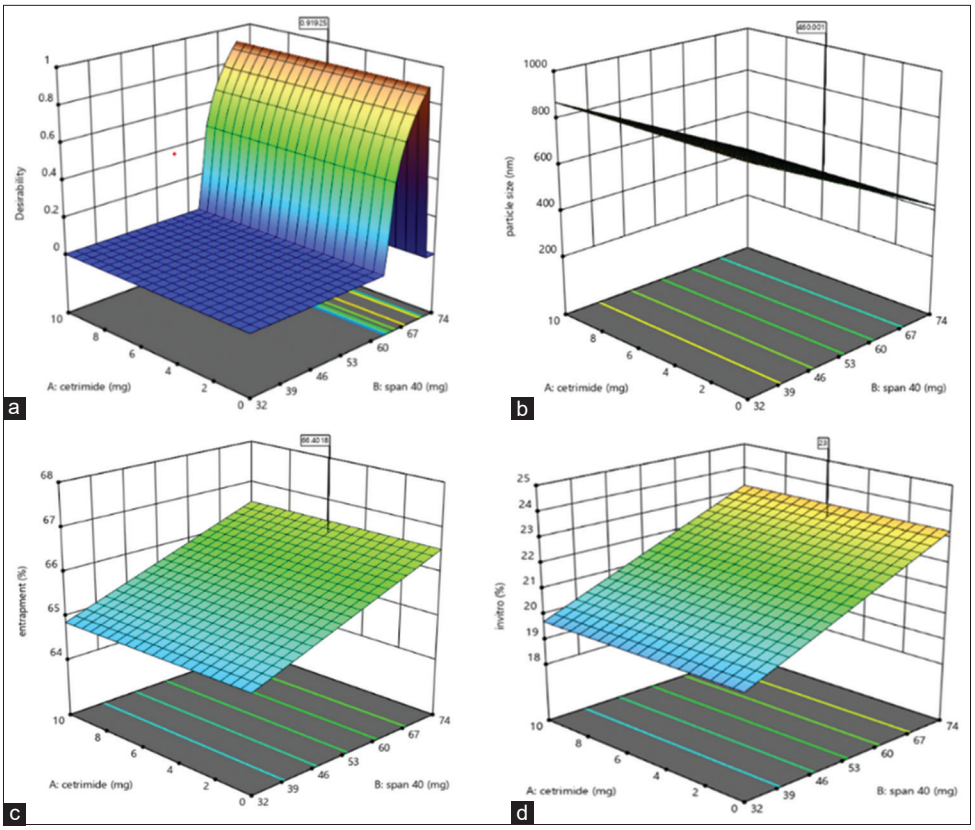

Figure 1: Three-dimensional contour plot of (a) desirability and its response surface shows effect of X1, X2, and X3 on (b) particle size Y1, (c) entrapment efficiency Y2, and (d) *in vitro* release Y3

deviation of 1.9%. For entrapment, the observed value was 66%, the obtained value was 62.4%, and the percentage deviation was calculated to be 5.4%. The percentage deviation of 2.6% was obtained for *in vitro* release, where the observed value was 23% and the obtained value was 22.4%.

The optimized niosome formulation was then prepared which had a particle size of 451±1 nm, which was greater than that reported by Mukherjee *et al*.; they reported that the liposome formulations of imipramine were heterogeneous in size and their diameter ranging from 40 to 300 nm; the difference between the 8 DoE niosomes formulation was statistically significant according to one-way ANOVA ($P < 0.0001$).

Entrapment efficiency was calculated to be 62.4 ± 0.89%, which was not in agreement with Mukherjee *et al*.; they reported a entrapment efficiency of 74 ± 4% because of the difference in the method of preparation as they employed sonication method for preparation in comparison to the method employed in this study; the entrapment was statistically significant according to "one-way ANOVA" ($P < 0.05$).

In vitro drug release was shown to be 22.8 ± 0.76% and the respective graph is shown in Figure 2. This difference was statistically significant according to "one-way ANOVA" ($P < 0.05$). The comparison graph of the 8 niosome formulations is shown in Figure 3.

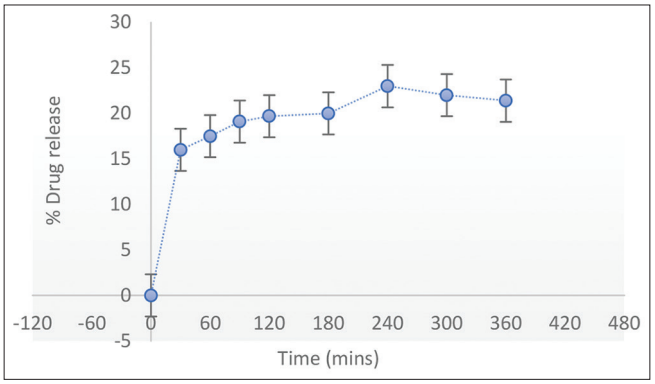

Figure 2: *In vitro* release of optimized niosomes (bars represent±standard deviation, *n*=3)

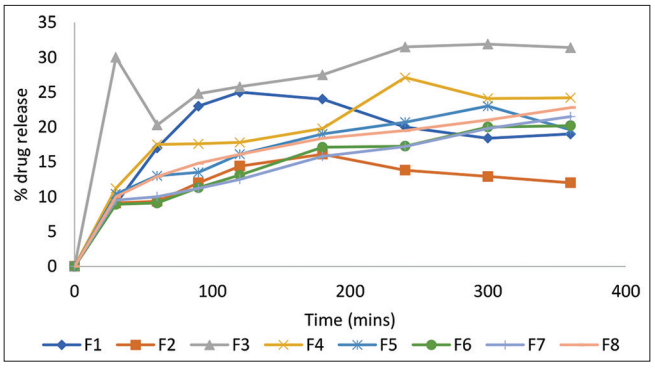

Figure 3: Comparative graph of niosome formulations

The particle size and PDI of formulated nanoparticles were determined using malvern zetasizer on the principle of dynamic light scattering (0.1–10000 nm) and the zeta potential was also measured. All the measurements were performed on the diluted form in a low-volume disposable sizing cuvette at 25℃. The particle size and zeta potential obtained are 603 ± 1.52 nm and 45.9mV, respectively, which is higher than that reported by Pramilachaubey *et al*., at 215.2 nm, the reason being the usage of rifampicin in comparison to imipramine in this study.

The entrapment efficiency of mannose conjugated chitosan imipramine nanoparticles was calculated to be 92.8 ± 0.80% which was greater than that reported by pramilachaubey *et al*., as 39.1 ± 6.3%; this may be due to the difference in the usage of drug. The *in vitro* drug release of mannose conjugated chitosan imipramine nanoparticles shows maximum drug release of 76 ± 1% in 6 h. The graph for *in vitro* is shown in Figure 4. This difference was statistically significant according to the one sample t-test ($P < 0.05$).

Drug release kinetics

Using the DD solver, the Weibull model was found to be the best suitable model for both niosomes and nanoparticle formulations. Drug release kinetics for niosomes is given in Table 3.

The regression coefficient value of 0.9074 for nanoparticles and 0.9751 for niosomes indicates that both the formulations have a super case II transport mechanism for drug release same as niosomal formulation. The table for drug release kinetics for nanoparticles is given in Table 4.

FTIR spectrum

Imipramine contains amine group, which is confirmed by the peak at 2942 cm^{-1}. C-H bending at 1466 cm^{-1} of methylene group is confirmed by the presence of cetrimide. The 1235 cm^{-1} C-O strong stretching confirms span 40.C=C bending of alkene at 970 cm^{-1} established cholesterol presence. FTIR spectrum of physical mixture of niosomes is shown in Figure 5.

The existence of NH2 and OH group stretching vibration in chitosan was shown by a band at 3283.92 cm^{-1}. Chitosan contains primary amine groups, as demonstrated by a significant N-H bending at 1595.18 cm^{-1}. Mannose's ring was opened, and then, its aldehyde group reacted with the free amino groups of chitosan to form mannose conjugation. Schiff's base (R-CH=N-R bond) is formed as a result, which was demonstrated by the presence of secondary amine N-H bending at 1558.54 cm^{-1}. The stretching at 2324 cm^{-1} showed the presence of strong isocyanate bond, and aliphatic ether strong bond was marked at 1073 cm^{-1}. The FTIR image is given in Figure 6.

Atomic force microscopy

The external morphology of imipramine niosomes and nanoparticles prepared at optimal conditions is shown in

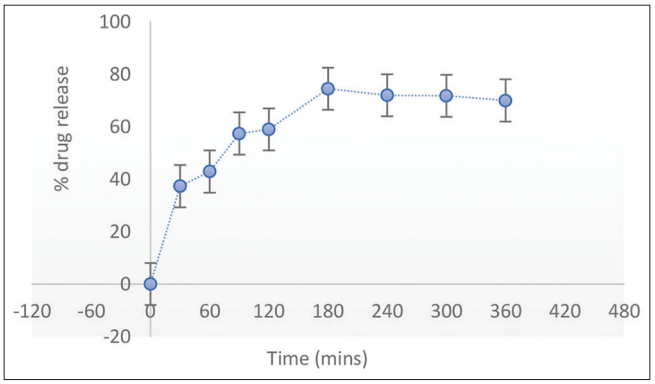

Figure 4: *In vitro* release of imipramine nanoparticles (bars represent± standard deviation, *n*=3)

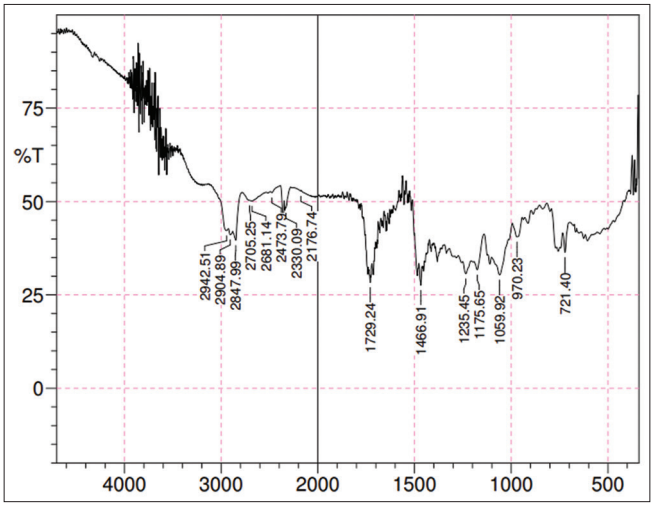

Figure 5: FTIR of imipramine niosome physical mixture

Table 3: r^2 value for the *in vitro* release fitted in kinetic models for optimized niosomes

Zero order	First order	Higuchi	Korsmeyer -Peppas	Hixson	Weibull
0.2403	0.5662	0.5505	0.9672	0.7192	0.9751

Table 4: r^2 value for the *in vitro* release fitted in kinetic models for nanoparticles

Zero order	First order	Higuchi	Korsmeyer -Peppas	Hixson	Weibull
0.4129	0.3375	0.8457	0.8641	0.4482	0.9074

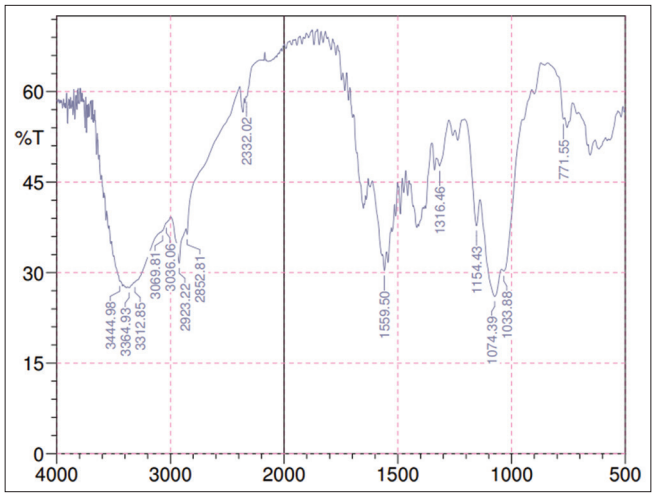

Figure 6: FTIR data of chitosan nanoparticles

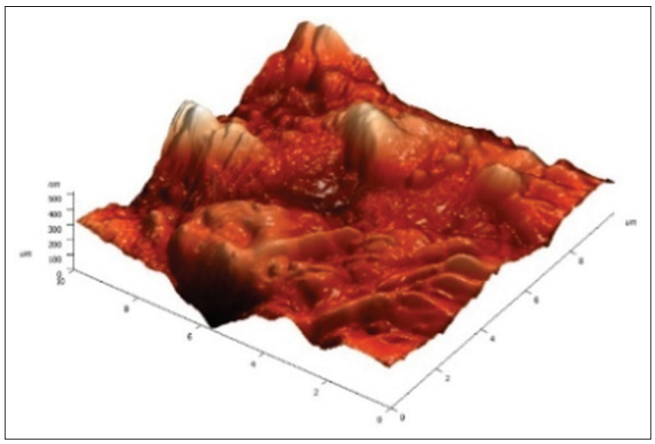

Figure 7: Morphological determination of optimized niosome formulation-3D image

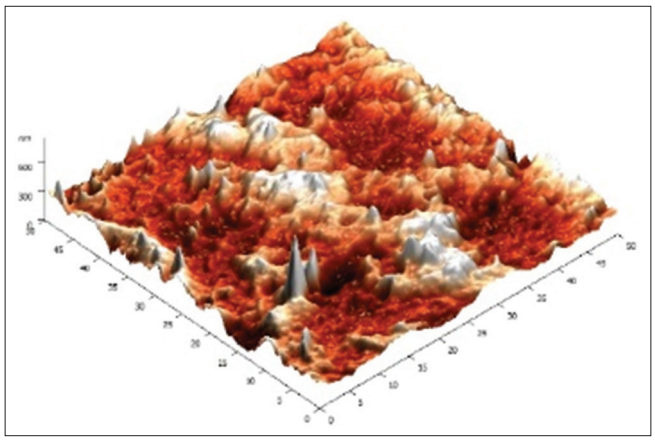

Figure 8: Morphological determination of optimized nanoparticle formulation-3D image

Figures 7 and 8, respectively. The average height of niosomes was found to be 522 nm and had an average diameter of 483 nm. The imipramine niosome average roughness and root mean square were evaluated to be 45.0158 nm and 58.9399 nm. The surface skewness and surface kurtosis were found to be 0.237654 and 1.30911, respectively.

The average height of mannose conjugated chitosan nanoparticles was found to be 395 nm and had an average diameter of 307 nm. The nanoparticles average roughness and root mean square were evaluated to be 27.7829 nm and 36.1073 nm, and the surface skewness and surface kurtosis were found to be 0.0736848 and 1.14164, respectively.

Stability studies

The vesicle size and zeta potential of the imipramine-loaded niosomes and nanoparticles were assessed during the storage of the samples at room temperature 25 ± 1°C, refrigerated temperature 4 ± 1°C, and in stability chamber 40 ± 1°C for 2 months, and hence, the stability of the optimum formulation was determined.

The particle size and zeta potential of the formulations were measured at 1 and 2 months and compared between room, refrigerated temperatures, and stability chamber. The variation in particle size and zeta potential in room and refrigerated formulation is very less when compared to the formulation stored in stability chamber. The formulation stored at room and refrigerated temperature has a better stability than the formulation stored in stability chamber for both niosomes and nanoparticles. In niosomes, the percentage deviation of 2.81% was observed for room temperature, 1.94% for refrigerated temperature, and 2.60% for stability chamber. For nanoparticles, 2.57% for room temperature, 2.88% for refrigerated temperature, and 2.95% was calculated for stability chamber. The stability data are given in Table 5 and 6, respectively, for both niosomes and nanoparticles.

Differential scanning calorimetry

On predicting the thermogram found using differential scanning calorimetry, the endothermic peak begins at 36.08°C and ends at 49.16°C with the peak attaining at 36.19°C which confirms the presence of chitosan, another peak beginning at 107.36°C with the peak height at 131.08°C and ends at 147.90°C, confirms the melting point of mannose. The onset of melting point of imipramine is at 167.94°C and ends at 182.46°C with the peak at 171.73°C. The DSC image is given in Figure 9.

Phase contrast microscopy

According to the PCM, the niosome vesicles and nanoparticles obtained were spherical in shape and shown in Figures 10 and 11, respectively.

In vitro cytotoxicity assay

The MTT assay results suggest us that the given formulations, F1 (niosomes) and F2 (nanoparticles), were moderately toxic in nature on murine macrophages with % viability values of 42.53% and 70.17% at the highest concentration of 100 µg/mL after the incubation period of 24 h, respectively. Niosomes and nanoparticles were non-toxic till the highest concentration of 25 µg/mL and 50 µg/mL, respectively. LPS with 1µg/ml was used as a standard control for the study. For niosomes, significant viability was observed between the control and concentration according to the "one-way ANOVA" ($P < 0.0001$).

Table 5: Stability studies of niosomes

Month	Temperature conditions	Particle size (nm)	Zeta potential (mV)
0 month	Room temperature	461±3.60	−24.2±0.64
	Refrigerated temperature	462±2.51	−24.6±0.52
	Stability chamber	461±1	−24.9±0.36
1 month	Room temperature	464.6±2.51	−27.1±0.36
	Refrigerated temperature	463.6±1.52	−27.2±0.58
	Stability chamber	468±2.64	−27.2±0.52
2 months	Room temperature	474±1	−28.5±0.61
	Refrigerated temperature	471.3±1.52	−27.6±0.96
	Stability chamber	473±2	−28.5±0.4

All data are shown as mean±SD; *n*=3, nm: nanometer, mV: millivolt

Table 6: Stability studies of nanoparticles

Months	Temperature conditions	Particle size (nm)	Zeta potential (mV)
0 month	Room temperature	602.5±0.5	45.8±0.70
	Refrigerated temperature	602.6±0.76	45.9±0.9
	Stability chamber	603.1±1.04	45.9±1
1 month	Room temperature	609.5±0.5	46.6±0.45
	Refrigerated temperature	611±1	47.1±0.82
	Stability chamber	613.3±1.52	47.5±0.55
2 months	Room temperature	617.6±0.52	47.2±0.40
	Refrigerated temperature	620.2±0.68	47.9±0.36
	Stability chamber	620.9±1.1	47.6±0.55

All data are shown as mean±SD; *n*=3, nm: nanometer, mV: millivolt

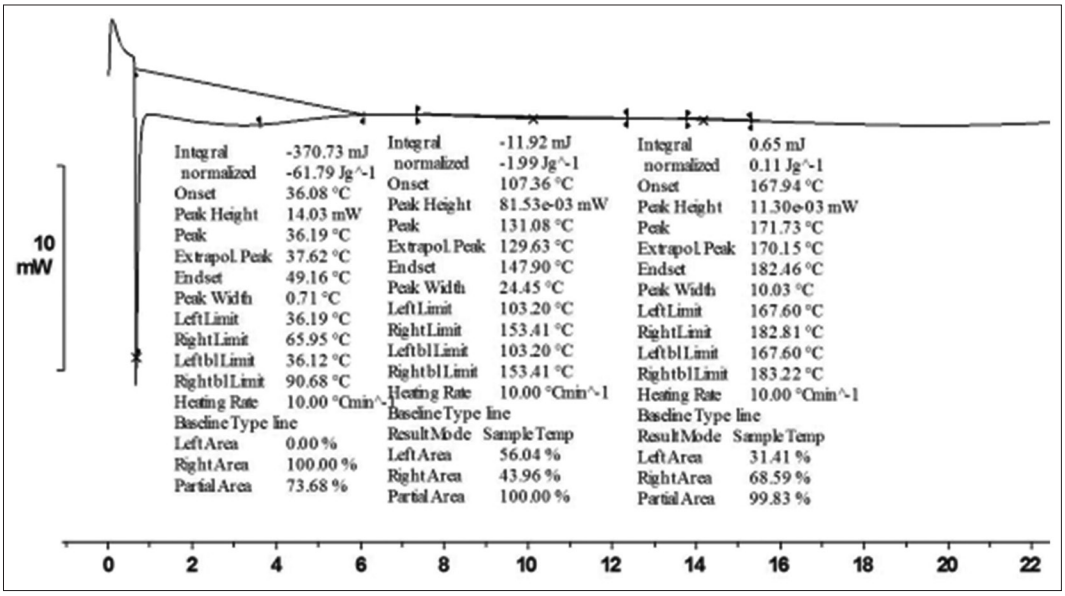

Figure 9: DSC of imipramine nanoparticles

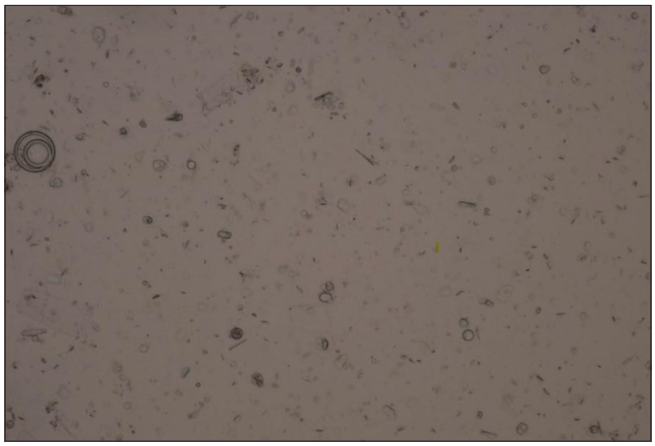

Figure 10: Phase contrast microscopy of niosomes

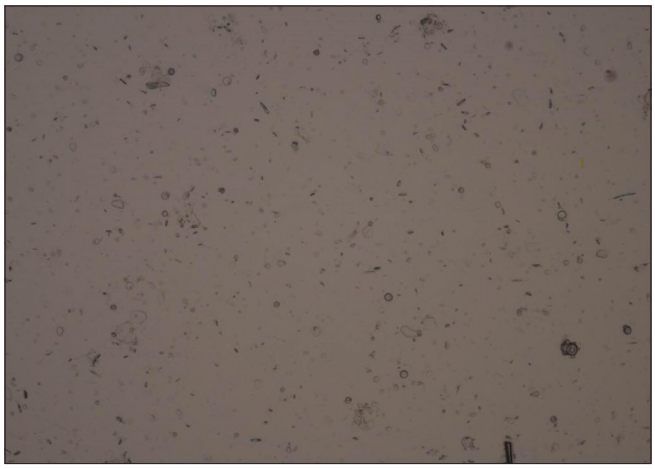

Figure 11: Phase contrast microscopy of nanoparticles

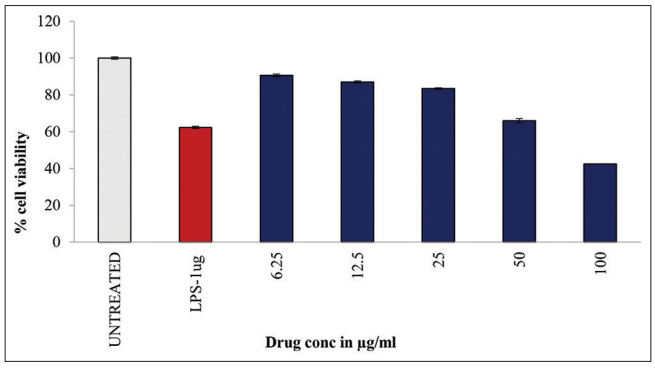

Figure 12: % cell viability values of Raw 264.7 cells treated with F1 after the incubation period of 24 h

The % cell viability of niosomes (F1) and nanoparticles (F2) is given in Tables 8 and 9, respectively. The various concentrations of niosomes and nanoparticles versus cell viability are given in Figures 12 and 13. For nanoparticles, significant viability was observed between the control and concentration according to the "one-way ANOVA" ($P < 0.0001$).

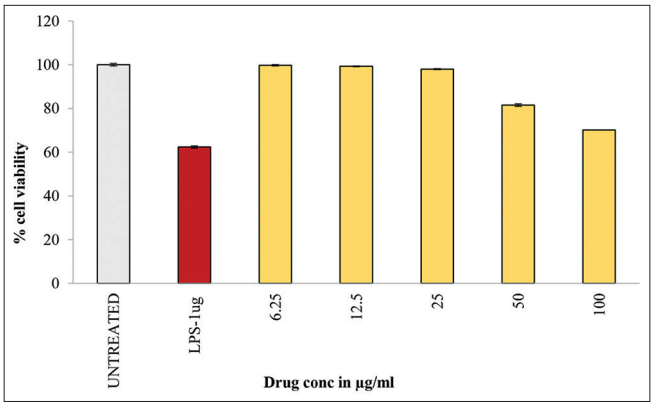

Figure 13: % cell viability values of Raw 264.7 cells treated with F2 after the incubation period of 24 h

CONCLUSION

In this study, imipramine-loaded nanoparticles and niosomes were successfully prepared. The size distribution in the formulations was >200 nm which confirms the suitability for macrophage engulfment. According to the *in vitro* cytotoxicity results, niosomes showed less viability at 42%, than nanoparticles at 70%. The selective uptake is due to the presence of chitosan and mannose, which binds to mannose receptor on the macrophages of cell line. Other reason is due to higher entrapment efficiency and *in vitro* release of drug from nanoparticles. Further studies are needed to evaluate the *in vivo*, safety of the developed niosomes and nanoparticles for leishmanicidal activity.

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