ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Nihala Nazeer et al /J. Pharm. Sci. & Res. Vol. 11(10), 2019, 3526-3535

Development of Formulation and *In Vitro* Evaluation of Sterically Stabilized (Stealth) Liposomes Containing Selected Anti-Arthritic Drug.

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Abstract

Liposomes are a novel lipid based drug delivery systems. They are microvesicles composed of a bilayer of lipid amphipathic molecules, enclosing an aqueous compartment. Besides its numerous advantages the major limitation in the therapeutic use of conventional liposomes upon intravenous administration lies in its early recognition and fast elimination from the blood circulation by the reticuloendothelial system (RES). This led to the development of surface modified sterically stabilized liposomes or the stealth liposomes. The present study deals with the comparison of conventional and stealth liposomes prepared by thin film hydration technique. The obtained dispersions of both the conventional and stealth liposomes were evaluated and compared for particle size, surface pH, percentage yield, percentage drug content, percentage entrapment efficiency, zeta potential, *in vitro* drug release, release kinetics, *in vivo* anti-inflammatory analysis and stability study. From the results it was observed that the stealth liposomal formulation showed better results in most of the evaluations. The data obtained from *in vitro* release study were fitted to various mathematical models and it followed zero order kinetics with a better stability results. The *in vivo* analysis also showed that the decrease in edema percentage by the stealth liposomes was much more than conventional liposomes. The stability study results also indicated that stealth liposomes were more stable over three months than conventional liposomes.

Key Words: Drug delivery; Liposome; Mononuclear phagocytic system; PEG;

INTRODUCTION

Nowadays the research scholars are focusing on lipid based drug delivery systems (LBDDS), as it provides the suitable means to overcome the formulation challenges of poor water soluble drugs as well as enabling a site specific and controlled delivery of drugs. Moreover to meet the product requirement as per the disease condition, route of administration, product stability, toxicity and efficacy the lipid formulations can be modified in various ways [1]. From the past many years microparticulate and nanoparticluate lipoidal drug delivery systems are being very widely used because of their ability to be used as a carrier for the improved delivery of broad spectrum of agents including chemotherapeutic agents, imaging agents, antigens, immunomodulators, chelating compounds, haemoglobin and cofactors, lipids and even genetic material [2]. Liposomes are micro vesicular lipid based drug delivery systems composed of a bilayer of lipid amphipathic molecules, enclosing an aqueous compartment. The main constituent of liposomes is phospholipid which contains a hydrophobic tail and hydrophilic head group which enables the liposomes to organize into spherical bilayer orientation in aqueous media as well as entraps both water soluble and lipid soluble substances. The ability of these nanosized (macromolecular) formulations to alter the drug pharmacokinetics has enhanced their application as a tool for drug delivery and diagnostics. These macromolecules thus have a different biodistribution profile with a prolonged plasma half-life and reduced metabolism of the encapsulated drug. The major reason for the common use of nanomedicine is the ability of the macromolecular compounds to exploit the enhanced permeability and retention (EPR) effect to specifically target tumor and inflamed tissues. Besides its numerous advantages the major limitation in the therapeutic use of conventional liposomes upon intravenous administration lies in its fast elimination from the blood and recognition by the reticuloendothelial system (RES). The certain circulating proteins (like laminin, fibronectin, C-reactive proteins, immunoglubulins) in the blood, binds specifically to the surface of conventional liposomes as a result of innate immune response through the process of opsonization resulting into a cascade of inflammatory and complex adverse reactions (Complement Activation- Related Pseudo Allergy or CARPA). These interactions result in destabilization and rapid clearance of the conventional liposomes from the circulation. Moreover, the rapid release of the encapsulated drug into the plasma without even reaching the targeted site due to the interaction of liposomes with high and low density lipoproteins (HDL and LDL) is another limitation. Upon various studies inorder to circumvent the low-systemic circulation time of conventional liposomes, surface engineered long circulating stealth liposomes has been developed by coating the outer liposome surface with polymers like polyethylene glycol (PEG), polyvinyl pyrrolidine (PVP), polyacryl amide (PAA) etc.. These stealth liposomes resulted with increased liposome stability, increased blood circulation times and improved biodistribution after systemic administration [3].

The stealth liposomal concept is similar to the stealth bombers designed by the Germans to destroy British command centers were adopted. The British officials failed to tackle the killer stealth bombers. They were helpless and destroyed to its bits. Similarly stealth liposomes are used to fool the phagocytes and thus they fail in recognition, enhancing the blood circulation time [4]. The basic concept is that a hydrophilic polymer or a glycolipid occupies the periliposomal layer (the space

adjacent to the liposome surface) and excluding other macromolecules like for example blood opsonins protein from binding on the surface. Various methods were introduced for the stealth behavior of liposomes like the development of cholesterol-rich liposomes, incorporation of polyvinyl-pyrrolidine, polyacrylamide lipids, glucoronic acid lipids into the liposomes, also coating of liposomes with proteins, polysaccharides, glycolipids of red blood cells ganglioside G_{M1} and hydrogenated phosphatidyl inositol (HPI) [5]. After various approaches due to the unique physical properties like unlimited water solubility, large excluded volume and high degree of conformational entropy, polyethylene glycol (PEG) was used to improve the stability and biological performance of the colloidal carrier. PEG of certain molecular weight and graft density prevents the adsorption of specific proteins as well as the steric behaviour of PEG prevents the aggregation of colloidal carriers and thus enhancing their stability. The ability of PEG coated liposomes to increase the blood circulation time depends on the amount of PEG incorporated and also the length or molecular weight of the polymer. The more extended the PEG chain, the more the blood residence time [6].

PEG on surface of liposomes forms a hydrated film of water which sterically hinders opsonization of liposomes and their subsequent uptake by the mononuclear phagocyte system. Poly acrylamide (PAA), Poly (2-methyl-2oxozoline), Poly (amino) acid, Poly glycerol, Polyvinyl pyrrolidine are some other alternative polymers used for the preparation of stealth liposomes. Figure 1) shows the image of a stealth liposome

Mostly there are three ways to modify the liposome surface with polymers to enhance the blood circulation time: (1) Incorporating an Amphiphilic polymer conjugates during liposome formation (pre-insertion); (2) Inserting the polymer conjugate onto the surface of pre-formed liposomes (post-insertion); and (3) Post-modification by chemical reactions of the polymer to the liposome surface.

Arthritis is not a single disease. Inflammations in one or more joints, causing pain and stiffness are some of the symptoms of arthritis. The most common treatments for rheumatoid arthritis or arthritis include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease modifying antirheumatic drugs (DMARDs), and some biological agents. However none of the treatments available is able to achieve the ultimate goal of treatment that is drug free remission. This limitation leads to the development of new treatment strategies having an ability to deliver the drugs into the synovial cavity in proper dosage while reducing the side effects to other tissues. Amongst these the liposomes have proven to be very effective for retaining the drugs in the synovial cavity by virtue of their size and chemical composition [7]. The encapsulation of the active form of the drug into the lipid bilayer protects it against naturally occurring phenomena such as enzymatic degradation, immunologic and chemical inactivation. Therefore liposomes prevent the drug from being metabolized prior to reaching target tissues and also minimize the exposure to healthy tissues during the circulation [8]. In inflammatory conditions there is a physiological condition called the EPR (enhanced permeation and retention). This pathological condition which develops increased intraendothelial gap allowing the easy extravasation of nanoparticles into the inflamed tissue [9].

MATERIALS AND METHODS

Indomethacin, Soya lecithin and Cholesterol were obtained from Yarrow Chem Products, Mumbai. Chloroform, Methanol and PEG_{400} were obtained from Globe Scientific, Thiruvananthapuram. All other chemicals and reagents used were of analytical grades and purchased from commercial sources.

PREPARATION OF CONVENTIONAL AND STEALTH LIPOSOMES

Both the conventional stealth liposomes of indomethacin were prepared by thin film hydration technique (Table a). Accurate amount of drug and phospholipids with variable concentration of cholesterol was dissolved in the solvent system of chloroform: methanol (2:1) in a 250 ml round bottom flask connected to a rotary evaporator (Rotavapor R- 210, Buchi Switzerland). The flask was then rotated at 100 r/min in a thermostatically controlled water bath at 37 ºC under reduced pressure (200-400 mmHg) until all the organic phase evaporated and a thin lipid layer was obtained on the walls of the round bottom flask. The dried lipid film was then hydrated by adding 10 ml of phosphate buffer pH 7.4. The flask was rotated again at same speed and temperature as before, for the removal of lipid film from the walls and dispersion to from liposomes. To this suspension of conventional liposomes, 1 ml of 10 % w/v of polyethylene glycol 4000 (PEG_{4000}) was added slowly under 100 r/min rotation for the preparation of stealth liposomes. Both the suspensions were then allowed to stand for 2- 3 h for the complete swelling. Each batch was prepared similarly and was stored in the refrigerator in a suitable container [10-11].

Ingredients	Conventional Liposomes				Stealth Liposomes			
	L1	L2	L3	L4	SL1	SL2	SL3	SL4
Indomethacin (mg)	100	100	100	100	100	100	100	100
Soya lecithin (mg)	400	400	400	400	300	400	400	400
Cholesterol (mg)	50	100	150	200	50	100	150	200
Chloroform : Methanol(ml)	2:1	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Phosphate buffer (ml)	10	10	10	10	10	10	10	10
PEG_{4000} (ml)	-	-	-	-	1	1	1	1

Table a : Formulation table of conventional and stealth liposomes

CHARACTERIZATION OF CONVENTIONAL AND STEALTH LIPOSOMES

Fourier transform infrared spectroscopy

All the excipients such as soya lecithin, cholesterol, PEG_{4000}, and pure drug indomethacin, individually as well as physical mixture of drug and excipients were mixed separately with potassium bromide (KBr) in the ratio of 1 part of sample and 100 parts of KBr. Mixture was compressed to form disc using dies. This disc was placed in the sample chamber and spectrums were obtained through the software program which is then further subjected to interpretation [12, 13].

Shape and Surface Morphology

Scanning Electron Microscope

A drop of the sample was placed on a cover glass. Then it was mounted on a specimen stab. Dried samples were coated with platinum using a vacuum evaporator. Coated samples were then viewed and photographed in SEM (Scanning electron microscope).

pH of vesicular dispersion

The pH of the vesicular dispersion was measured using pH meter. The pH of the dispersions was determined by immersing the electrode and recording the pH.

Percentage yield

The prepared liposomes were weighed accurately. The measured weight was divided by the total amount of the drug and ingredients which were used for the preparation of liposomes. The percentage yield was calculated by the equation,

$$\text{Percentage yield} = \frac{\text{Actual weight of product}}{\text{Total drug}} \times 100$$

Percentage Drug Content

1 ml of suspension was pipetted from the dispersion and was further diluted with suitable amount of phosphate buffer pH 7.4 and the samples were analyzed spectrophotometrically at 256 nm.

Percentage Drug Entrapment Efficiency

The percentage drug entrapped was determined by centrifugation. Liposomal suspension of 10 ml was placed in the centrifugal tube and it was balanced on the other side with an equivalent weight. The centrifugation was carried out 1000 r/min for 60 min. The supernatant was removed and the concentration of the supernatant was determined spectrophotometrically at 256 nm. The percentage of drug entrapment was calculated using the equation,

$$\text{Percentage drug entrapment} = \frac{\text{Total drug} - \text{Drug in supernatent}}{\text{Total drug}} \times 100$$

Zeta Potential

Zeta potential of the liposomal formulations was determined using Zeta Sizer (Litesizer500). 1 ml of liposome suspension was diluted to 100 ml using deionized water, and the sample was placed in zeta cells and the results were recorded.

In vitro Drug Release

The *in vitro* drug release of the prepared liposomal formulations was determined using Franz diffusion cell. 50 mg equivalent indomethacin containing liposome suspension was placed on one side of the cellophane membrane in a vertical franz diffusion cell. The other side of the membrane was in contact with the dissolution medium of 22.4 ml of phosphate buffer pH 7.4. Entire dissolution assembly was placed on a magnetic stirrer at temperature of 37 ºC. Aliquots of dissolution medium were withdrawn at different time intervals from the receptor compartment, and the sample was replaced with fresh phosphate buffer saline pH 7.4 to maintain sink condition. Drug concentration in the dissolution medium was then determined by UV spectrophotometrically at 256 nm [11].

Drug Release Kinetic Study

To analyze the mechanism of the drug release kinetics of the dosage form, the data obtained were fitted to various kinetic equations of zero order, first order, higuchi model and korsemeyer-peppas model.

In vivo Anti-inflammatory analysis

Formaldehyde induced rat paw edema method was used for the *in vivo* analysis of anti-inflammatory activity. The animals were divided into 4 groups of 6 animals each. All the animals were marked on the right hind paw just behind the tibia tarsal junction to ensure constant paw volume up to the fixed mark. After the measurement of initial paw volume using vernier calipers each animals were induced with inflammation by injecting 0.1 ml of 2 % solution of formaldehyde at the paw of the rat on day 1 and day 3. The each group was then administered with the test samples until the study period of 10 days. Group I (saline treated –control group) animals were treated with 0.9 % normal saline. Group II (Standard drug) animals were administered with drug solution in the dose of 2.5 mg/kg body weight. Group III (conventional liposomes) were administered with drug loaded conventional liposomes in the dose of 2.5 mg/kg body weight. Group IV (stealth Liposomes) were administered with optimized formulation (SL2) of drug loaded stealth liposomes in the dose of 2.5 mg/kg body weight. Repeated the test and standard drug for 10 days. Measured paw volume on each day using vernier calipers. The paw volume and percentage decrease in edema were calculated using the below equation. The data are also statistically analyzed using GraphPad-InStat [14-19].

$$\text{Percentage edema decrease} = \frac{To - Tt}{To} \times 100$$

Where, T_o = Paw thickness of control and T_t = Paw thickness of test

Stability Study

The ability of liposomes to retain the drug was assessed by storing both the liposomal suspensions at different temperatures like in refrigerator temperature (4 ±1 ºC) and room temperature (25 ±2 ºC) for three months. Samples were withdrawn periodically surface pH, drug content, and *in vitro* drug release was measured.

RESULTS AND DISCUSSIONS

Fourier transform infrared spectroscopy

FTIR spectrum of indomethacin showed all the peaks corresponding to the functional groups present in the structure and there were no additional peaks. The combination spectrum of drug and excipients in figure 2) and 3) also showed no change in the peak values corresponding to functional groups of the drug when

combined with excipients, indicating that the drug is compatible with the excipients.

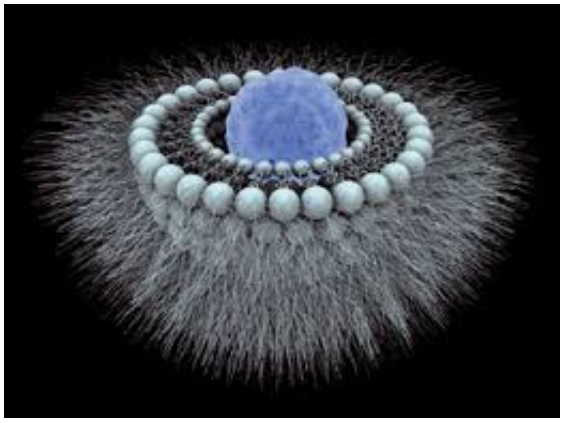


Figure 1: Stealth liposomes

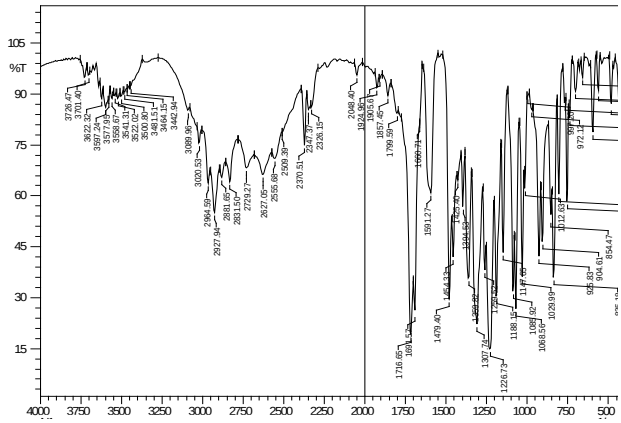


Figure 2: FTIR spectrum of pure drug indomethacin

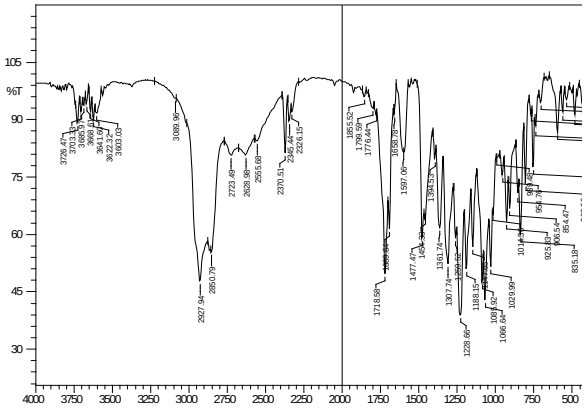
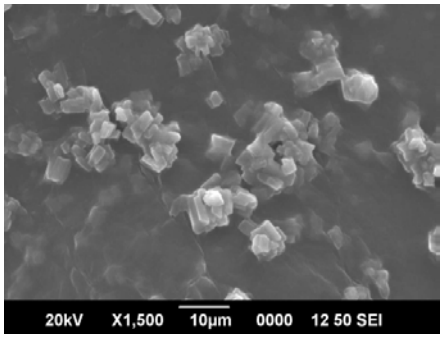


Figure 3: FTIR spectrum indomethacin + cholesterol + soya lecithin + PEG$_{4000}$

Shape and Surface Morphology

Scanning Electron Microscope

The morphological characteristics of the conventional and stealth liposomes were examined using scanning electron microscopy and the visualization of the liposomes are shown in the figure 4) and 5) respectively. From the figures it was found out that the liposomes were small in size and with a spherical shape.



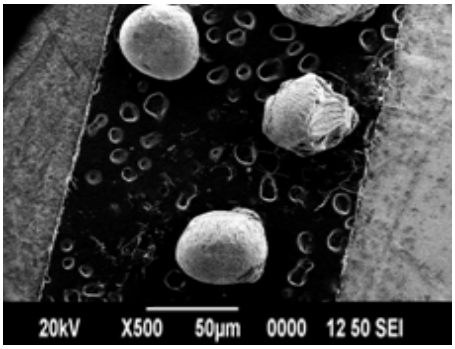


Figure 4 and 5: SEM image of conventional and stealth liposomes

pH of vesicular dispersion

The pH of all the liposomal dispersions were obtained and the values were in the range of 7.41 – 7.43 and is summarized in the table b)**.** As all the values were found to be in the range of that of blood pH, these formulations are suitable for parenteral drug delivery.

Formulation Code	pH (Mean ± SD)*
L1	7.41 ±0.005
L2	7.41 ±0.01
L3	7.42 ±0.01
L4	7.43 ±0.025
SL1	7.41 ±0.02
SL2	7.41 ±0.01
SL3	7.42 ±0.025
SL4	7.42 ±0.01

Table b: pH determination data of conventional and stealth liposomes

Percentage yield

The percentage yield of all the liposomal formulations were performed and the values obtained were in the range of 92.40% - 94.17% for conventional and 93.1% - 95.24% for stealth liposomes. The data is summarized in the table c)**.** It was observed that as the concentration of cholesterol increases the percentage yield of both conventional and stealth liposomes also increased.

Percentage Drug Content

The percentage drug content of the liposomes were performed and the values obtained were summarized in the table d)**.** The values indicated no change in the uniformity of drug distribution. Thus indomethacin was uniformly distributed in vesicular dispersions.

Formulation Code	Percentage Yield (%) (Mean±SD)*
L1	92.7 ±0.037
L2	93.48 ±0.020
L3	94.03 ±0.020
L4	94.47 ±0.036
SL1	93.1 ±0.269
SL2	93.87 ±0.578
SL3	94.18 ±0.390
SL4	95.24 ±0.463

Table c: Percentage yield determination data of conventional and stealth liposomes

Formulation Code	Percentage drug content (%) (Mean ±SD)*
L1	91.28 ±0.372
L2	92.89 ±0.275
L3	91.35 ±0.361
L4	91.02 ±0.358
SL1	92.05 ±0.147
SL2	93.97 ±0.138
SL3	91.58 ±0.152
SL4	91.12 ±0.184

Table d: Percentage drug content determination data of conventional and stealth liposomes

Formulation Code	Percentage drug entrapment (%) (Mean ±SD)*
L1	56.05 ±0.104
L2	77.66 ±0.508
L3	72.23 ±0.376
L4	69.18 ±0.204
SL1	64.04 .±0263
SL2	79.54 ±0.647
SL3	74.11 ±0.312
SL4	70.32 ±0.562

Table e: Percentage drug entrapment efficiency determination data of conventional and stealth liposomes

Percentage Drug Entrapment Efficiency

Percentage drug entrapment efficiency of the liposomal formulations were obtained and the drug entrapment efficiency of the formulations was in the range of 56.04% -77.88% for conventional liposomes and 64.04% -79.54% for stealth liposomes. The data obtained is summarized in the table e).

The drug entrapment depends on the concentration of cholesterol and lipid. From the table it is observed that the formulation L2 and SL2 showed more percentage drug entrapment than formulation L1 and SL1 as they have more concentration of cholesterol. When the concentration of cholesterol increases the hydrophobicity in the central region also increases favoring the inclusion of hydrophobic drugs. Whereas, after a particular point with the increase in cholesterol concentration the drug encapsulation decreases because of the limited space in the hydrophobic region (between the acyl chain of the phospholipids), is might be the reason for the decrease in drug entrapment percentage in remaining formulations.

Zeta Potential

The zeta potential of the optimized formulation L2 and SL2 was obtained. It was in the range of -22.8mV for the formulation L2 and -35.3.mV for the formulation SL2. The graphs obtained from both the formulations conventional and stealth liposomes are shown in figure 6) and 7) respectively. From the graph it was found that the vesicles had sufficient charge to inhibit aggregation.

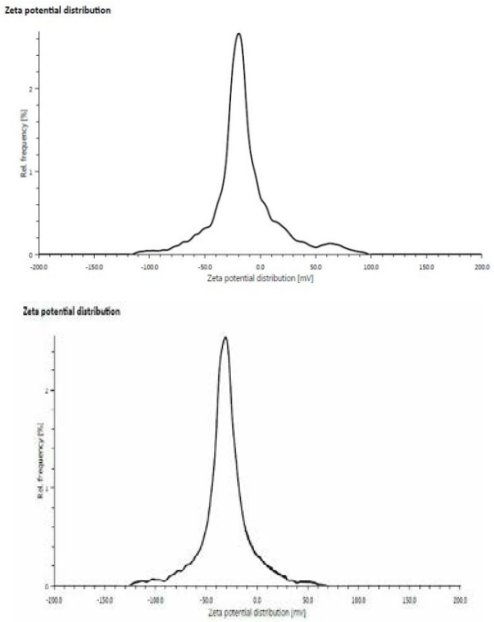


Figure 6 and 7: Zeta potential determination data of conventional and stealth liposomes

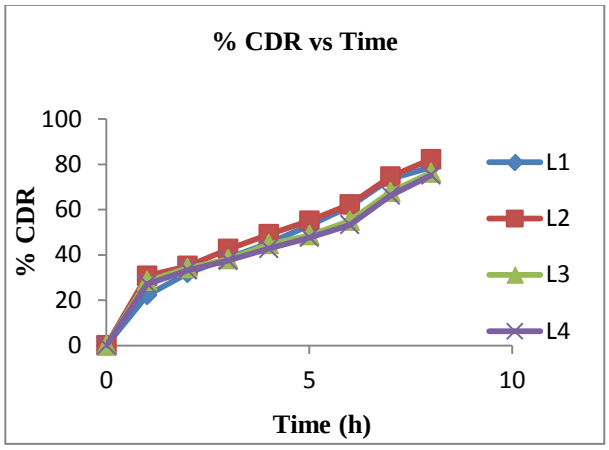
***In vitro* Drug Release**

The *in vitro* drug release profiles of all the liposomal formulations were performed. From both the results it was observed that about 30% of the drug was released at first 2 h from both the liposomal formulations due to the initial burst release. This characteristic initial burst release is common for liposomes and then after it follows a slower release rate. The initial fast release rate is commonly due to the drug detachment from liposomal surface while the later slow release results from sustained drug release from the inner lamellae. Also it is observed that as the concentration of cholesterol increases the drug release increases and then after a particular concentration of cholesterol the drug release decreases. In case of stealth liposomes the presence of a hydrophilic polymer coating over the liposomes had significantly influenced the drug release. The stealth liposomal formulation SL2 showed an increased drug release of 90.03% after 24 h compared to conventional liposomal formulation L2 with 82.17% after 8 h, indicating that the drug would be more stable in the blood circulation with a slow release rate at the site of inflammation from the stealth liposomes. The graphical representations of %CDR vs time of both conventional and stealth liposomes are shown in figure 8) and 9) respectively.

Drug Release Kinetic Study

The dissolution profile of optimized formulation SL2 and L2 was fitted to various kinetic models like Zero order,

First order, Higuchi model, and Korsmeyer Peppas model. The release kinetics data given in the table f) indicated that drug release from both the systems best fits to the zero order release kinetics and the R^2 values of the zero order kinetic equations were found to be more close to unity indicating that the release from the dispersion system is not dependent on the comcentration of drug present in the formulation. The data were also fitted to the higuchi equation which gave almost a linear plot indicating that the mechanism of drug release was diffussion. The 'n' value determined lies between 0.5 and 1.0 indicating that it follows non-fickian diffusion. These observation thus shows that all the formulations were non-fickian diffusion following Higuchi model of drug release. The SL2 formulation showed better result when compared to other formulations. The zero order kinetic release of both conventional (L2) and stealth liposomes (SL2) are shown in figure 10) and 11) respectively.



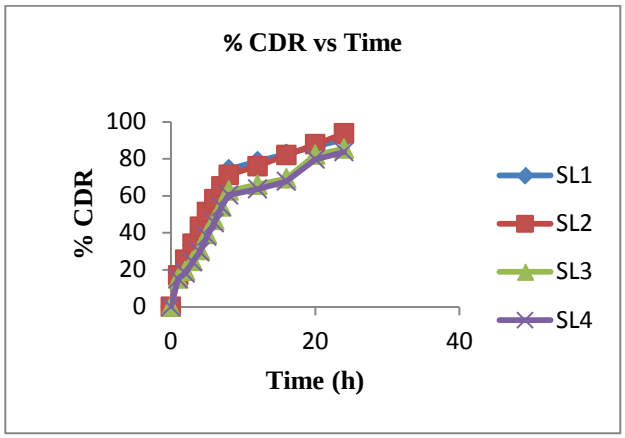
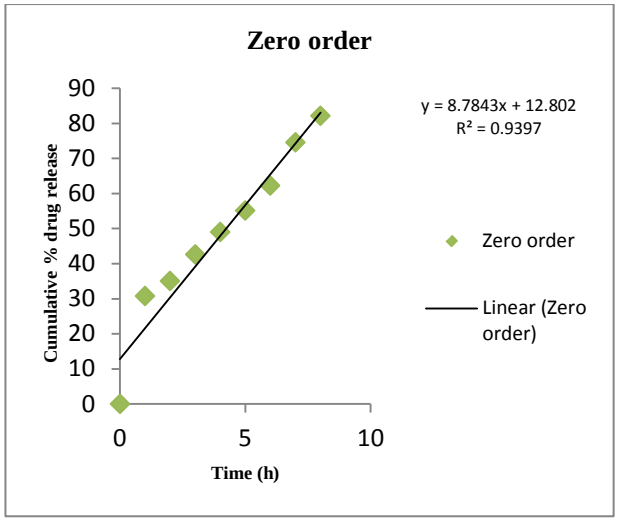


Figure 8 and 9: % CDR profile of indomethacin loaded conventional and stealth liposomes

Formulation Code	Kinetic Model				
	Zero order Model	First order Model	Higuchi Model	Korse-Peppas	
	R^2	R^2	R^2	n	R^2
L2	0.939	0.921	0.911	0.878	0.539
SL2	0.981	0.977	0.971	0.906	0.639

Table f: R^2 values of various kinetics models of formulations L2 and SL2



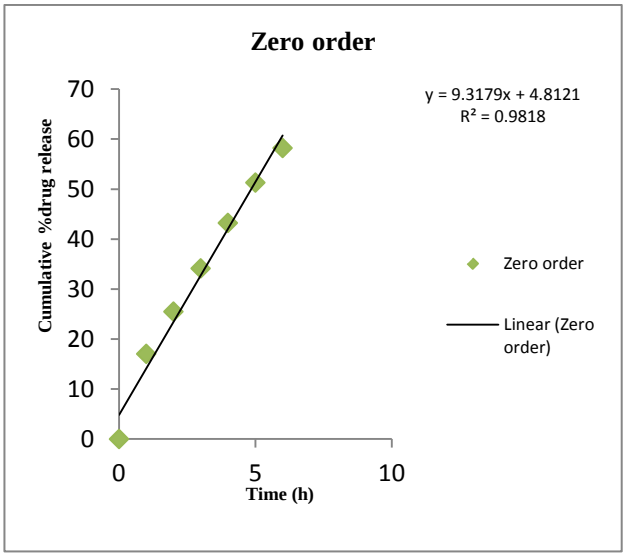
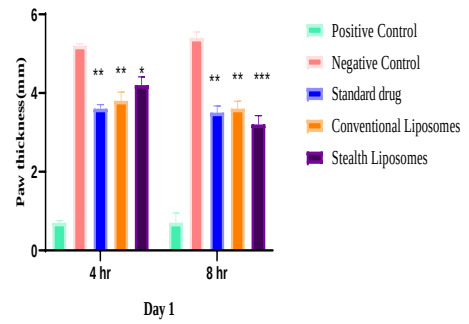
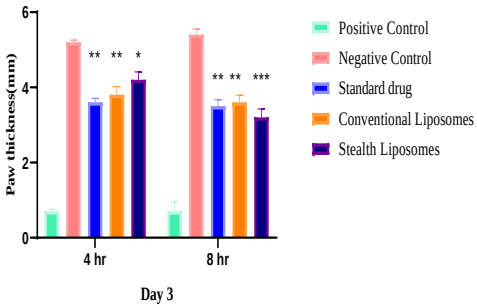


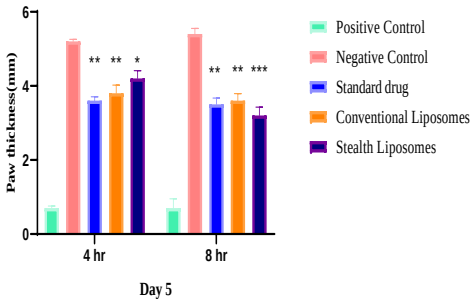
Figure 10 and 11: Zero order plot of formulation L2 ans SL2

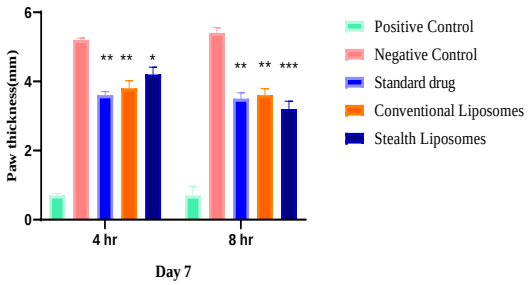
***In vivo* Anti-inflammatory analysis**

The *in vivo* analysis of the optimized formulation L2 and SL2 in comparison with the free indomethacin standard drug solution and 0.9% saline were performed in formaldehyde induced rat paw edema model. The group positive control showed a paw thickness of 0.7 mm. The P-values < 0.05 was considered significant when compared with the control group. The figures show the paw thickness of the rat paw on day 10. From results, it was observed that the percentage edema decrease shown by the stealth liposomes were more even after 8h prior administration. This indicates that the stealth liposomes are not easily identified and degraded by the mononuclear phagocytic system whereas both conventional liposomes and the standard drug solution showed a very less percentage edema decrease. The statistical representation of the data in each day is shown in figures 12), 13), 14), 15), 16). The photographs of edema decrease in paw of animals of each group on day 10 in shown in the following figures 17), 18), 19), 20), 21)









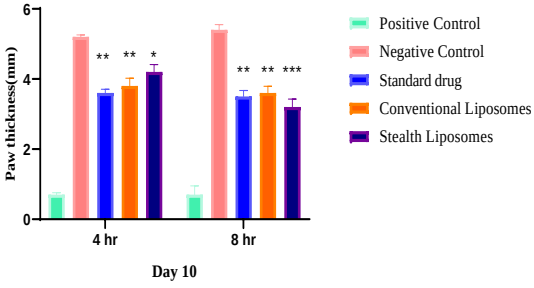


Figure 12, 13, 14, 15, and 16: Percentage edema decrease in Day 1, 3 5, 7 and 10

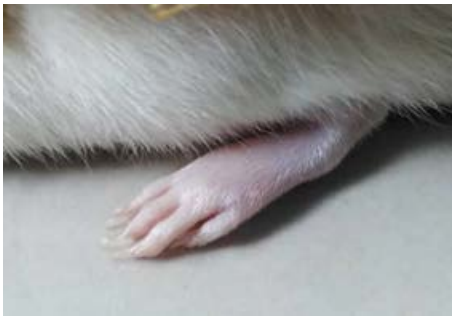


Figure 17: Group I on Day 10

Figure 18: Group II on Day 10

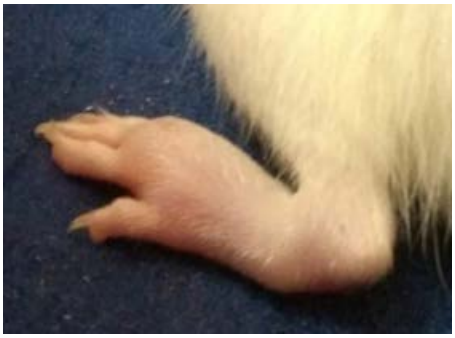


Figure 19: Group III on Day 10

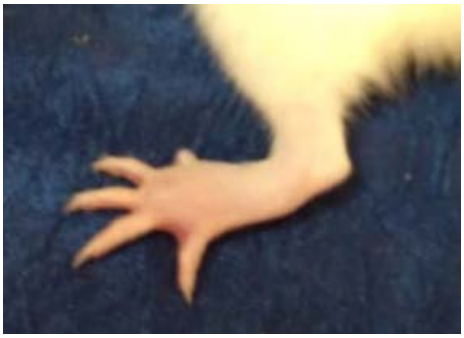


Figure 20: Group IV on Day 10

Figure 21: Group V on Day 10

Stability Study

The stability studies of both the liposomal formulations were performed. The stability study determination data of both conventional liposomes and stealth liposomes after 3 months of study period are summarized in tables g, h, i, j.. According to the data, formulations stored at refrigeration temperature were more stable than the formulations stored at room temperature. Also, there were no significant changes in surface pH, % entrapment efficiency and *invitro* drug release data of stealth liposomes when compared with conventional liposomes indicating that the stealth liposomes remained more stable than the conventional liposomes after a period of 3 months.

Parameters	At temperature 4±1ºC		
	After 1 month	After 2 months	After 3 months
Surface pH	7.41	7.41	7.41
% Entrapment efficiency	77.12%	75.79%	74.39%
Drug release	81.68%	80.26%	79.85%

Table g: Stability study determination data of liposomes at 4 ±1 ºC

Parameters	At temperature 25±2ºC		
	After 1 month	After 2months	After 3 months
Surface pH	7.41	7.40	7.40
% Entrapment efficiency	76.74%	74.73%	72.59%
Drug release	80.26%	78.60%	76.87%

Table h: Stability study determination data of liposomes at 25 ±2 ºC

Parameters	At temperature 4±1ºC		
	After 1 month	After 2 months	After 3 months
Surface pH	7.41	7.41	7.40
% Entrapment efficiency	79.11 %	78.15%	77.27%
Drug release	74.01%	73.39%	72.77%

Table i: Stability study determination data of stealth liposomes at 4 ±1 ºC

Parameters	At temperature 25 ±2ºC		
	After 1 month	After 2months	After 3 months
Surface pH	7.41	7.41	7.41
% Entrapment efficiency	78.28%	76.94%	75.53%
Drug release	73.20%	71.98%	70.74%

Table j: Stability study determination data of stealth liposomes at 25 ±2 ºC

CONCLUSION

Liposomes are one of the most studied and a novel form of drug delivery. In spite of its higher efficacy in drug delivery they have certain limitations like easy recognition and degradation by the mononuclear phagocytic system, stability problems etc.. These confinements can be overcome by the surface modification of liposomes which ultimately leads to the development of stealth liposomes. In this study both conventional and stealth liposomes were formulated and compared. Using varying concentrations of cholesterol both the liposomes were prepared by thin film hydration technique and is then evaluated for particle size, drug content, surface pH, drug entrapment efficiency, zeta potential etc… The results of these evaluations were within the prescribed limits of pharmacopoeial specifications. FTIR study results showed that there was no marked incompatibility between the drug and the excipients. *In vitro* drug release from the stealth liposomes were more sustained than the conventional liposomes over a period of 24 h. After fitting onto various mathematical models, based on the R^2 values the optimized formulation SL2 followed zero order kinetics indicating that rate of drug release is independent of concentration and followed a mechanism of non-fickian diffusion. Also, *in vivo* anti-inflammatory analysis showed a better circulation time and an enhanced mean percentage edema decrease for stealth liposomes in comparison with the conventional liposomes and standard drug, indicating that the stealth liposomes are not identified by macrophages for early degradation. Moreover, from the stability study data the stealth liposomal formulation was found to be more stable than conventional liposomes for over a period of three months. Accordingly stealth liposomes loaded with indomethacin was found to be a better drug delivery system than conventional liposomes. The results also shows that they serve as suitable parenteral dosage form and can replace the oral therapy, which has poor bioavailability, short half-life and requires frequent dosing. Also, this mode of targeting indomethacin at the sites of inflammation may also reduce the associated side effects.

ETHICAL APPROVAL

This study is not against the public interest and has been performed in accordance with the Institutional Animal Ethics Committee (IAEC – Reg.No: 974/PO/RE/5/06/CPCSEA for Research and Education Purpose on Small Animal), Ezhuthachan College of Pharmaceutical Sciences. After scrutinizing the proposal has granted permission to carry out animal study for the project titled Development of formulation and in vitro evaluation of sterically stabilized (stealth) liposomes containing selected anti-arthritic drug.

Acknowledgement

The authors wish to acknowledge Prof. (Dr). Shaiju S Dharan (Principal, Ezhuthachan College of Pharmaceutical Sciences), Dr. Mathan S (Professor and Head, Department of Pharmaceutics) Dr. Merlin (Professor and Head, Department of Pharmacology) for their logical support and guidance. The authors wish to acknowledge Ezhuthachan College of Pharmaceutical Sciences, KUHS, Thiruvananthapuram, India, for necessary motivation and support.

Conflict of Interest

The authors declare no conflict of interest.

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