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ORIGINAL ARTICLE

Targeted drug delivery of Rifampicin to the lungs: formulation, characterization, and stability studies of preformed aerosolized liposome and in situ formed aerosolized liposome

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Abstract

Purpose: This study aimed at the preparation and characterization of preformed and in situ formed liposomes for sustained delivery to the lungs. *Methods*: Two different liposome formulations were prepared and subjected to characterization of physical parameters and drug release profile (% cumulative drug release and % drug retained). Formulations were then subjected to accelerated stability studies as per ICH guidelines. *Results*: In situ formed liposome showed better sustained release profile than the preformed liposome as it released sufficient amount of drug while retaining considerable amount of drug. Upon subjection to accelerated conditions for 60 days, preformed liposome lost the objective of being controlled release formulation.

Key words: *Aerosol; alveoli; in situ; liposome; Rifampicin; tuberculosis*

Introduction

Tuberculosis (TB) is one of the most important infectious diseases worldwide and its incidence is increasing due to the AIDS epidemic. Its most common form is pulmonary tuberculosis in which a large number of bacteria reside in the alveolar macrophages[1]. Even if adequate blood levels of antitubercular drugs are achieved by long-term oral treatment, it is hard to kill all these bacteria, probably because the drugs are not efficiently taken up by alveolar macrophages. Also, a long-term oral therapy is known to cause hepatotoxicity[2].

Rifampicin is one of the most effective antitubercular agents and is often used to treat HIV/TB patients in combination with anti-HIV agents[3]. But it suffers from many drawbacks such as, high cost, short half-life, adverse effects, pH-dependent degradation, and bioavailability problems. The degradation of the Rifampicin in acidic pH of the stomach depends on the initial amount of Rifampicin released in the stomach. With formulations having faster release rate of Rifampicin in dissolution medium, more Rifampicin is released in short time and is exposed to the acidic environment of the stomach for longer time. However, for the formulation showing slower initial release of Rifampicin, a lesser amount of Rifampicin is available for degradation in the acidic medium and hence shows higher concentrations of Rifampicin as the time progresses. This prompts the researchers for the development of targeted sustained release formulations[4].

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Rifampicin is also a strong inducer of hepatic microsomal enzyme CYP3A4 that leads to drug interactions with protease inhibitors. Accordingly, a formulation that delivers the drug more efficiently to the target site might reduce the total dose and the frequency of administration. It might also allow HIV/TB patients to take the drug simultaneously with protease inhibitors[5].

In this aspect, improved drug delivery to its target (lungs) has become an area of increasing interest. There are extensive efforts to define the factors that influence the deposition of drug directly to the lungs and develop strategies to improve drug delivery to respiratory tract system.

For the first time, Purohit et al. determined the plasma concentration of aerosolized free drug (Rifampicin) within 2 hours of exposure[6]. In the same year, Saito and Tomioka examined intraperitoneal (I.P.) administration of liposome-entrapped Rifampicin against experimental *Mycobacterium avium* complex infection in mice. It showed a greater action of liposomal formulation over the free drug with marked reduction in bacterial growth in the lungs and spleen of infected mice. Entrapment of Rifampicin in liposome targeted the drug toward macrophages and increased the activity of the drug against *M. avium* complex phagocytosed into the macrophages[7]. This fact was further strengthened by Deol et al. They found that liposome-encapsulated drugs at and below therapeutic concentrations were more effective than free drugs against tuberculosis. Liposomal drugs also showed marginal hepatotoxicities. The elimination of mycobacterium from the liver and spleen was also higher with liposomal drugs than with free drugs[8]. Furthermore, they found that modification of surface of liposome by tagging with *O*-stearylamylopectin resulted in the increased affinity of this liposome toward lung tissues of mice rather than reticuloendothelial systems of normal and tuberculous mice. They demonstrated the slow and controlled release of encapsulated contents along with less toxicity to peritoneal macrophages[9].

Bermúdez et al. studied the effect of Rifampicin on lipid bilayers because it is an amphiphilic drug and was found to modify the surface charge of lipid vesicles. The extent of these modifications depended on the nature of both the bilayer constituents and the drug[10]. To increase the spectrum of therapeutics and safety, Labana et al. examined the administration of isoniazid and Rifampicin co-encapsulated in lung-specific stealth liposome. These liposomes exhibited a controlled release of drugs in plasma (5 days) and lungs, liver, and spleen (7 days) and reduced the mycobacterial load significantly[11]. Rodrigues et al. determined that Rifampicin is deeply buried inside the lipid bilayers. However, no significant changes in membrane fluidity were detected. In dimyristoyl phosphatidyl choline (DMPC) liposomes, ^{1}H NMR results confirmed the location of Rifampicin near the methylene group of the acyl chains of the lipid bilayers[12].

Vyas et al. studied the aerosolization of Rifampicin-loaded liposome for targeted delivery to alveolar macrophages after imparting negative charge (using dicetylphosphate) or by coating them with alveolar macrophage-specific ligands (maleylated-bovine serum albumin and *O*-stearylamylopectin). Percent viability of *Mycobacterium smegmatis* inside macrophages (in vitro) after administration of drug (in vivo) was in the range of 7–11% in the case of ligand-anchored liposomal aerosols, compared to 45.7% of plain drug solution[13].

Gürsoy et al. determined the effect of co-encapsulation on encapsulation percentage and extended drug release of isoniazid and Rifampicin and found a positive effect[14]. Zaru et al. prepared freeze-dried liposomes and found that DSPC/Chol (2:1) is the best composition for Rifampicin delivery in vesicular form to lungs, by nebulization[15]. Recently, Sorokoumova studied the degradation and stability of Rifampicin on storage and found that antimicrobial activity in liposomal compositions is the same as that in aqueous solutions and is well retained on storage[16]. Recently, solid-state NMR results confirmed the location of Rifampicin between the acyl chains of the phospholipids bilayer and associated with cholesterol molecules. Rifampicin in dry powder formulations was found considerably more stable compared to Rifampicin aqueous solutions and Rifampicin liposomal suspensions[17].

Currently, various aerosolized liposome formulations are available for targeted pulmonary delivery in which drug is formulated as liposome before packaging. This usually results in rupturing of vesicle structure during actuation, thereby losing ultimate motive of providing sustained release. They also have poor storage stability because of inherent instability of liposome formulations[18,19].

The focus of this article is on the development of a strategy to improve the stability of liposomal formulation of Rifampicin during aerosolization because fluctuation in drug concentration, due to rupture of liposome during aerosolization, would eventually result in resistance development. The author worked on the fact that drug–lipid mixture solubilized in chlorofluorocarbon (CFC) will form liposome upon hydration in small airways[20]. So, in this study, feasibility to exploit the anatomy and surface properties of lungs is analyzed as they have the wet surface which could provide aqueous phase for liposome formation. Two different formulations were prepared and characterized along with the determination of their stability profile. Liposomes were made and packed as aerosol and lipid mixture aerosol to form liposome in situ, thereby called as preformed and in situ formed liposomes, respectively.

However, according to Montreal protocol, the use of CFC is being phased out because of their destructive effect on ozone layer and right now is the transition period between the use of CFC and hydrofluoroalkane (HFA) propellants. CFC propellants are being used here in India as the complete phase out would be by the year 2020. FDA approved the use of HFA as a propellant in MDIs in the year 1996[21].

HFA-134a and HFA-227 have broadly similar thermodynamic properties to CFC-12, but no HFA equivalent to CFC-11 or CFC-114 is available, so excipient with lower volatility may be required to modify the vapor pressure. This issue also influences the filling methods that are feasible for HFA products and has led to the development of novel pressure filling processes[22]. Most drugs and surfactants are insoluble in fluorocarbons and HFAs because these fluorinated compounds are simultaneously hydrophobic and lipophobic. Suspensions of solid drugs in fluorocarbons lead to emission of nonhomogeneous, hence nonreproducible, amounts of drugs from the can when the valve is actuated[23]. They have high moisture ingress rate, and so stability on storage becomes important. Another concern is the cost of HFA propellants. On a different note, even HFA propellants are not entirely clean as they are grouped under greenhouse gases along with other gases by Kyoto protocol, which is, however, not ratified in the United States. For drug deposition, it was found that a substantial amount of dose (18%) is exhaled with HFA propellants whereas the exhaled amount of dose is 1% for CFC propellants[24].

Materials and methods

Rifampicin, egg phosphatidylcholine (EPC), dicetyl phosphate (DCP), cholesterol, propellants P-11 (trichloromonofluoromethane) and P-12 (dichlorodifluoromethane), methanol (95%), ethanol, chloroform, petroleum ether, acetone, potassium dihydrogen phosphate, disodium hydrogen phosphate, calcium chloride, magnesium sulfate, glucose, and sodium chloride were of pure analytical grade.

Bath sonicator (FS-10) and probe sonicator (FS-10) were from Frontline Electronics and Machinery Pvt. Ltd., Ahmedabad, Gujarat, India; wrist action shaker (CM101) (Remi sales and Engineering Ltd., Indore, India) and rotary vacuum evaporator (289/06) were from Buchi India Pvt Ltd., Mumbai, India; UV spectrophotometer (UV1) was from Merck Ltd., Mumbai, India; digital pH meter (DPH 115 PM) was from Kasliwal Brothers, Indore, India; and optical microscope with image analyzer (10 5810/99) was from Besto, Mumbai, India.

All glasswares used for characterization were fabricated by Rama Scientific Glassware Pvt. Ltd., New Delhi, India.

Preparation of preformed aerosolized liposome (by cast film method)

Composition of the formulations is shown in Table 1. Constitutive lipids were dissolved in minimum volume of solvent. Known amount of drug dissolved in solvent was added to the lipid solution. Solvent was slowly removed under reduced pressure, using a Buchi rotary flash evaporator. The thin lipid film formed on the inner wall of flask was dispersed in phosphate buffer saline (0.01 M, pH 7.4) by mechanical agitation using a wrist action shaker for 3 hours. The swollen lipid film was hydrated at $40 \pm 2°C$. Un-entrapped drug was removed by passing the liposome suspension through Sephadex G-50 mini column and centrifugation at $1008 \times g$ for 3 minutes[25–27].

Preformed aerosolized liposome was prepared from 10 mL of liposome formulation. Solution phase pressure packs (glass, 30 mL capacity) containing preformed liposome and CFC blend (P-11 : P-12 :: 1 : 4) were prepared using a previously reported method[28–30].

Preparation of aerosolized in situ liposome

Composition of the formulations is shown in Table 1. The solution phase pressure packs (glass, 30 mL capacity) containing phospholipids and Rifampicin in CFC blend (P-11 : P-12 :: 1 : 4) were prepared using a previously reported method[28].

The required quantities of each ingredient were accurately weighed into an aerosol container (glass,

Table 1. Composition of formulations.

		Formulation code							
S. No.	Components	L-1	L-2	L-3	L-4	IL-1	IL-2	IL-3	IL-4
1	Rifampicin (g)	1	1	1	1	1	1	1	1
2	EPC : Chol : DCP (molar ratio)	4:0:0	4:1:0.2	4:2:0.5	7:3:0.5	4:0:0	4:1:0.2	4:2:0.5	7:3:0.5
3	P-11 (g)	2	2	2	2	2	2	2	2
4	P-12 (g)	8	8	8	8	8	8	8	8

EPC, egg phosphatidylchline; Chol, cholesterol; DCP, dicetyl phosphate; P-11, trichloromonofluoromethane; P-12, dichlorodifluoromethane.

30 mL) and trichloromonofluoromethane (P-11) was added in excess. Evaporation of P-11 was permitted until the required weight was achieved. This procedure also evacuates the air foam from the container. The unit was then hermetically sealed using a mercury valve and required quantities of dichlorodifluoromethane (P-12) was added with the help of pressure burette[31–34].

All formulations were prepared by required amount of Rifampicin, phospholipids, cholesterol, and dicetylphosphate. They were mixed with small amount of solvent, and then transferred into a container containing spray valve. The containers were fitted with valve assembly and crimped to seal. Finally, the required quantities of P-12 were added with the help of pressure burette.

Characterization

Vesicle size and in situ liposome formation

Vesicle size was determined by using single-stage liquid impinger (SLI). Each aerosol unit was examined by depressing the valve to flow the cloud in SLI. Aliquots of the receptor fluid were then withdrawn with the syringe, viewed under optical microscope using an oil immersion lens (1000× magnification), and further processed with a digital image analyzer[35,36].

Appearance

Formulations were observed visually through naked eye and rated for the number of phases present as well as the homogeneity of the phases. Homogeneous systems having two phases were kept for further evaluation and rest were discarded[37].

Discharge rate

Pressure pack of known weight was allowed to discharge the contents for three actuations into a calibrated SLI [3]. Aerosol valve discharge rate was determined with the weight difference of the aerosol container before and after three actuations. Aliquots of receptor fluid from SLI were collected and the average amount of drug delivered per actuation was determined[37].

Spray pattern

The spray pattern and area was determined on the basis of color produced over a piece of paper pretreated with methylene blue-talc mixture, kept at a distance of 15 cm[38].

Percentage of encapsulation efficiency

Percentage of drug entrapment was determined by protamine aggregation method, used for neutral and negatively charged liposome[39,40]. Liposome dispersion was precipitated with protamine solution (10 mg/mL) and centrifuged at 448 × *g*. Then supernatant and pellet (after disrupting liposome pellet with 0.6 mL of 10% triton X-100) were analyzed for drug content by using UV detection at λ_{max} 475 nm, taking 187 as the value of A (1%, 1 cm).

Deposition kinetic study

Deposition kinetics study was performed by using lung deposition of aerosolized systems model. It comprises a respirator, an artificial thorax, a perfusate reservoir, a pump to circulate perfusate from reservoir to artificial thorax and in opposite direction, and a constant temperature bath to hold artificial thorax and perfusate reservoir[41,42].

The artificial thorax was a modified 250 mL round bottom flask with four openings. The openings were made for intratracheal administration of aerosol spray, perfusate in, perfusate out, and respiratory negative tube. The openings made for aerosol administered were fitted with an artificial trachea. This was a 6 cm long glass tube with a 3 cm long brass tube.

Albino rats were anesthetized with phenobarbitone sodium (32 mg/kg, I.P.) and heparinized. An incision of 2 cm was made in the skin at the neck and trachea was exposed and cut half way through its diameter. A glass cannula was inserted and tied in place and the lungs were ventilated using a respirator. The abdomen of animal was cut horizontally to expose the vasculature. The tip of heart was cut and a glass arterial cannula was clipped into the pulmonary artery through the right ventricle. The lungs were continuously perfused with Krebs–Henseleit solution (K-H solution). When clear of blood, then lungs were trimmed free of thorax. The isolated organ was washed with K-H solution, and a rod was gently pushed through the esophagus. An artificial trachea was tied in place and the organ was suspended horizontally with the rod in artificial thorax. The respirator and perfusate pump were started[41–43].

The formulations were administered through artificial trachea. Samples were withdrawn from the perfusate reservoir according to a fixed schedule and analyzed. After each experiment, artificial trachea was washed several times with K-H solution (100 mL) to estimate the drug that did not reach the organ.

After completion of lung deposition kinetic study, artificial trachea was removed and washed with 100 mL K-H solution. Then absorbance was taken at 475 nm by using UV spectrophotometer. The amount of drug deposited into the artificial trachea was calculated from the standard curve of Rifampicin in K-H solution.

Animal studies were done in Drug Development and Industrial Pharmacy Laboratory, B.R. Nahata College of Pharmacy, Mandsaur, Madhya Pradesh, India, as per the guidelines approved by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA)/Institutional animal ethical committee (IAEC) under the registration no. 918/ac/05/CPCSEA and proposal no. 44/M.Ph/06.

Accelerated stability studies

The stability studies of formulations were carried out as per ICH guidelines. The effects of accelerated temperature and time were evaluated on the physical characteristics of the aerosolized formulation for 60 days[44–47].

Statistical analysis

The data were statistically processed to determine the level of significance. Using the two-sided *F*-ratio and *t*-test, the means of two data sets were compared. The significance was evaluated at 1% probability level ($P < 0.01$ denoting significance).

Results and discussion

Two different types of liposome formulations were successfully prepared and their physical parameters were evaluated along with their drug release profile (Table 2). The formulations were also evaluated for their stability under accelerated conditions (Table 3), as liposome formulations are known for their instability.

Vesicle size and appearance

Average vesicle size for preformed liposome and in situ formed liposome were 2.38 and 1.28 μm, respectively.

Table 2. Characterization of formulations.

S. No	Characteristics \formulation	L-1	L-2	L-3	L-4	IL-1	IL-2	IL-3	IL-4
1	Vesicle size (μm)	2.35 ± 0.23	2.36 ± 0.21	2.39 ± 0.32	2.41 ± 0.30	1.22 ± 0.22	1.27 ± 0.19	1.30 ± 0.19	1.34 ± 0.15
2	Appearance (No. of phases)	Two,Homo.	Two, Homo.	Two,Homo.	Two, Homo.	Two, Homo.	Two, Homo.	Two, Homo.	Two, Homo.
3	Discharge rate (mg/actuation)	123 ± 2.6	117 ± 3.1	120 ± 3.2	110 ± 2.4	115 ± 2.4	119 ± 2.7	127 ± 2.3	115 ± 2.2
4	Spray pattern								
	Spray area (cm^2)	12.5 ± 1.7	14.1 ± 2.2	13.2 ± 2.5	13.9 ± 1.9	14.6 ± 2.3	13.9 ± 2.1	14.7 ± 2.0	14.5 ± 1.8
	Shape of plume	Conical	Conical	Conical	Conical	Conical	Conical	Conical	Conical
5	Internal pressure (psi)	32 ± 1.5	31 ± 1.6	35 ± 1.4	34 ± 1.5	33 ± 1.6	34 ± 1.3	36 ± 1.4	38 ± 1.2
6	% Cumulative drug release	84.5 ± 2.3	80.5 ± 3.1	75.5 ± 2.1	66.5 ± 2.2	76.5 ± 2.4	73.2 ± 2.7	71.0 ± 2.1	58.5 ± 1.8
	% Drug retained	15.5 ± 2.4	19.5 ± 2.2	24.5 ± 2.0	33.5 ± 2.1	23.5 ± 1.9	26.8 ± 1.9	29.0 ± 1.5	41.5 ± 1.6
7	% Encapsulation efficiency	25 ± 2.3	32 ± 2.3	38 ± 2.8	42 ± 2.1	31 ± 2.4	34 ± 1.9	36 ± 2.0	40 ± 1.9

Values are expressed as mean ± SD ($n = 3$).

Table 3. Characterization of formulations after 60 days.

S. No.	Characteristics\ formulation	L-1	L-2	L-3	L-4	IL-1	IL-2	IL-3	IL-4
1	Vesicle size (μm)	2.72 ± 0.20	2.54 ± 0.28	2.6 ± 0.29	2.35 ± 0.22	1.20 ± 0.21	1.30 ± 0.23	1.27 ± 0.18	1.33 ± 0.18
2	Appearance (no. of phases)	Change	Change	Change	Change	Two, Homo.	Two, Homo.	Two, Homo.	Two, Homo.
3	Discharge rate (mg/actuation)	75 ± 3.1	61 ± 3.6	74 ± 2.2	72 ± 2.1	113 ± 2.2	116 ± 2.3	121 ± 2.2	111 ± 1.9
4	Spray pattern								
	Spray area (cm^2)	9.1 ± 2.9	8.5 ± 2.8	7.9 ± 3.1	8.1 ± 3.1	13.9 ± 2.1	13.6 ± 2.6	14.1 ± 2.3	14.5 ± 1.8
	Shape of plume	Irregular	Irregular	Irregular	Irregular	Conical	Conical	Conical	Conical
5	Internal pressure (psi)	31 ± 1.7	30 ± 1.9	33 ± 1.7	32 ± 1.9	32 ± 1.6	33 ± 1.3	35 ± 1.4	38 ± 1.2
6	% Cumulative drug release	93.5 ± 2.3	92.5 ± 3.1	93.0 ± 2.1	92 ± 2.4	81.5 ± 2.2	80.1 ± 2.3	79.0 ± 2.5	62 ± 2.3
	% Drug retained	6.5 ± 2.4	8.5 ± 2.2	7.0 ± 2.0	8 ± 1.6	18.5 ± 1.1	19.9 ± 1.7	21.0 ± 1.4	38 ± 2.1
7	% Encapsulation efficiency	15 ± 3.3	13 ± 2.8	16 ± 2.6	19 ± 1.7	29 ± 2.2	31 ± 1.3	33 ± 2.2	38 ± 1.9

Values are expressed as mean ± SD ($n = 3$).

All the formulations showed homogeneous biphasic system when freshly prepared. After stability studies, preformed liposome showed an increase in mean vesicle size whereas for in situ formed liposome, mean vesicle size is unaltered. After stability studies, preformed liposome showed ruptured vesicles (Figures 1–5).

Discharge rate and spray area

The discharge rate and spray area of both formulations were not different initially, but, after stability studies, mean discharge rate and spray area were significantly reduced in preformed liposome (Figure 6). Initially all the formulations were showing standard conical shape of spray, but after 60 days preformed liposome showed irregular spray area and reduced to mean spray area of

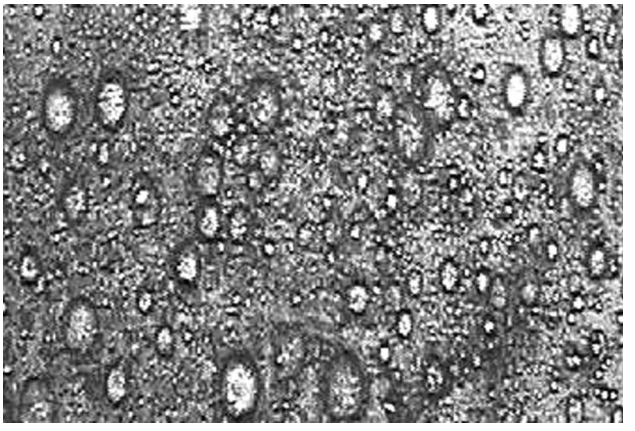

Figure 1. In situ formed liposome (IL) 4 (day 0).

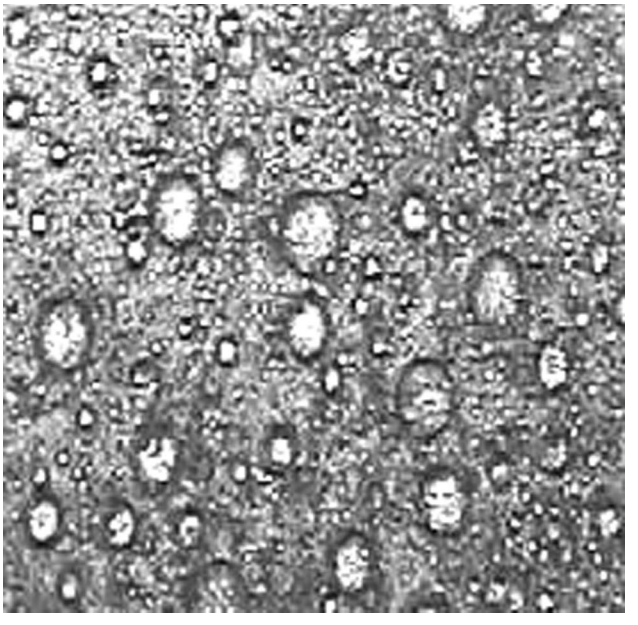

Figure 2. In situ formed liposome (IL) 4 (day 60).

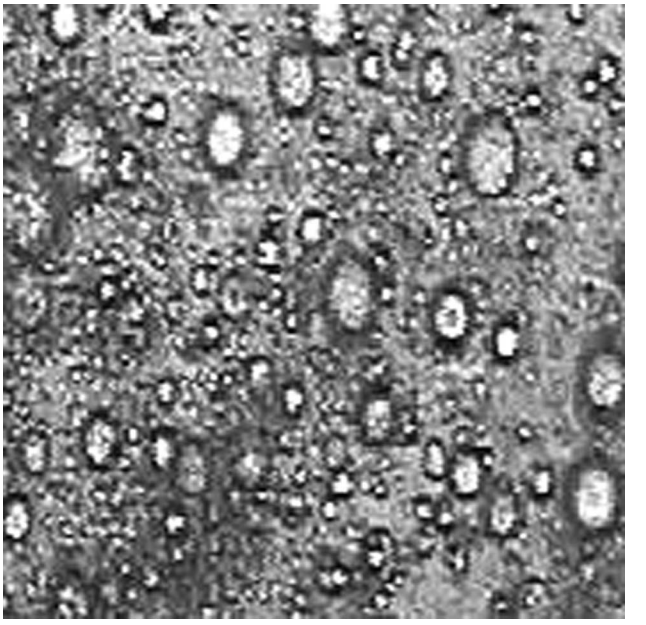

Figure 3. Preformed liposome (L) 4 (day 0).

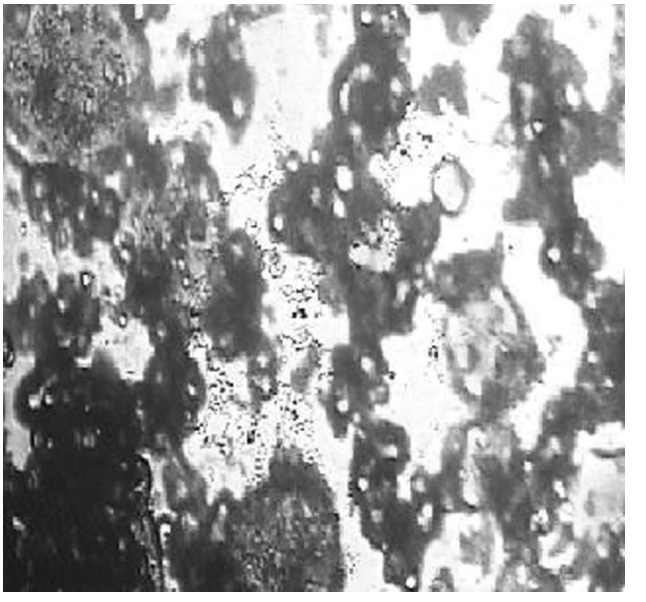

Figure 4. Preformed liposome (L) 4 (day 60).

8.4 cm^2 (Figure 7). The mean discharge rate of preformed liposome reduced by 40% after stability studies which could be attributed to clogging of valve assembly by liposome lipids whereas in situ liposome formulation showed almost unchanged discharge rate.

Encapsulation efficiency

There was a proportional increase in encapsulation efficiency in all formulations with increase in total

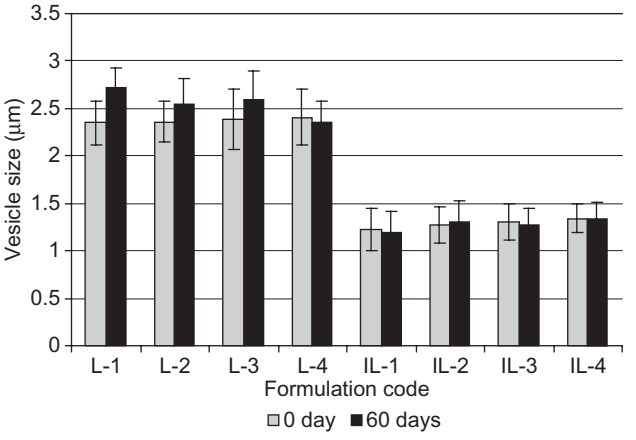

Figure 5. Comparative study of vesicle size of preformed liposome (L) and in situ formed liposome (IL) after accelerated conditions. $n = 3$, values represented as mean ± SD.

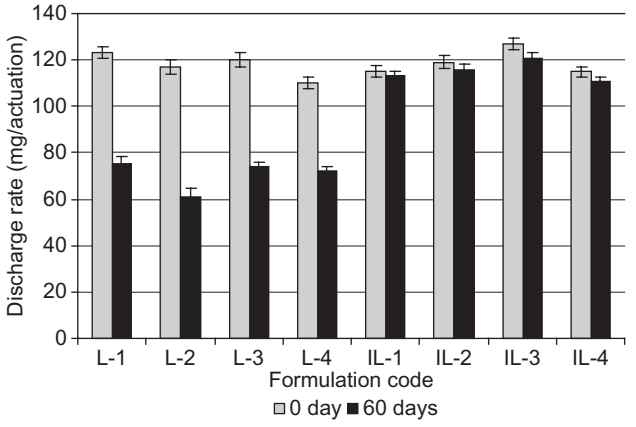

Figure 6. Comparative study of discharge rate of preformed liposome (L) and in situ formed liposome (IL) after accelerated conditions. $n = 3$; values represented as mean ± SD.

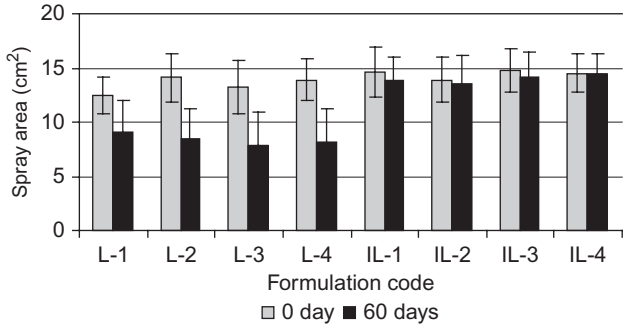

Figure 7. Comparative study of spray area of preformed liposome (L) and in situ formed liposome (IL) after accelerated conditions. $n = 3$; values represented as mean ± SD.

phospholipid concentration. This is due to the availability of more encapsulating material. After stability studies, encapsulation efficiency of preformed liposome decreased drastically to almost 44.5% (Figure 7).

Drug deposition kinetics

Amount of the drug released and retained is dependent on several factors, that is, vesicle size and integrity of vesicles. At the time of actuation of valve, preformed liposome clogged the valve and ruptured because of attrition in valve assembly. This resulted in less discharge rate and release of ruptured vesicles. Ultimately more amount of drug released in lungs, which will be immediately available for metabolism. The main aim of sustained release formulation becomes lost at this very condition (Figure 8).

Whereas in situ liposome formulation, being devoid of any structured vesicle at the time of valve actuation, provides no hindrance in discharge that results in optimum discharge rate and standard shape of spray. After stability studies, in situ formulation does not show any adverse effect on discharge patterns. As it forms liposome in situ, there is no indication of ruptured vesicles. This ultimately showed in almost similar deposition kinetics for in situ formulation before and after stability studies (Figure 9).

As per initial characterization, in situ formed liposome appears almost similar to the preformed liposome but the difference is marked when deposition characteristics and stability parameters are taken into consideration. There is an average of 7% difference in the amount of drug released immediately after preparation (76.75% preformed liposome and 69.8% for in situ liposome) but, after subjection to accelerated condition, drug

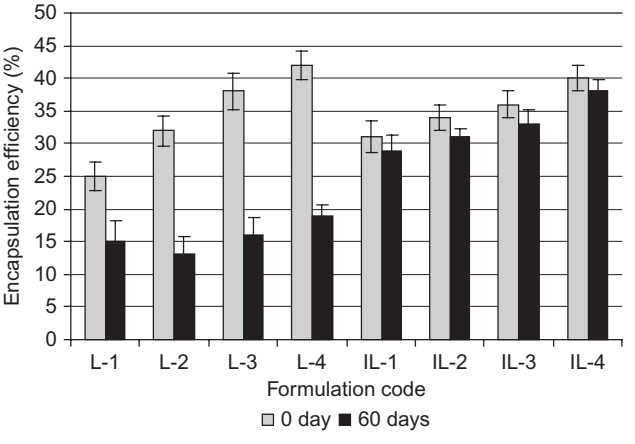

Figure 8. Comparative study of encapsulation efficiency of preformed liposome (L) and in situ formed liposome (IL) after accelerated conditions. $n = 3$; values represented as mean ± SD.

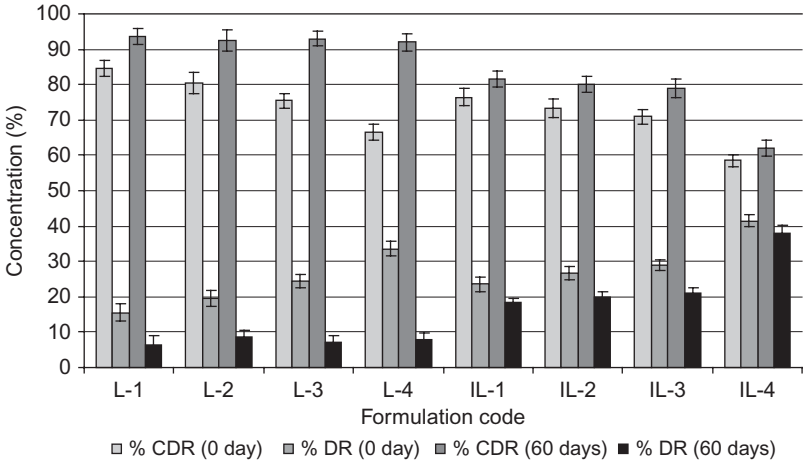

Figure 9. Comparative study of deposition kinetics of preformed liposome (L) and in situ formed liposome (IL) after accelerated conditions; (% CDR, % cumulative drug release; % DR, % drug retained). $n = 3$; values represented as mean ± SD.

release increases to 92.75% for preformed liposome whereas deposition kinetics for in situ liposome is altered very less as the drug fraction released increases up from 69.8% to 75.65% only.

Conclusion

This study clearly indicates that in situ liposome can overcome the problem of instability of liposome. In situ formed liposome has finer vesicle size (mean diameter 1.28 μm), which is important for appropriate targeting of formulation. Absence of vesicular structure also helps in smooth flow of aerosol mist and there is no vesicle rupture. Self-assembly of propellant solution of lipids in vesicle protects the drug from metabolism and also provides for sustained release. In situ formation of liposome has remarkable effect on the stability of liposome, which is evident from the results of accelerated stability studies. In situ formed liposome maintained mean vesicle size and other important formulation parameters, for example, discharge rate and encapsulation. Also, there is almost negligible effect of accelerated conditions on drug release. Statistical analysis showed the result to be significant ($P = 0.01$).

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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