AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and *In-Vitro* Evaluation of Salbutamol Sulphate Liposomes

***U. D Shivhare.*[1]*, *P. B Suruse.*[1] *V. S. Thombare.*[1]**

1. Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur-(MS), India.

ABSTRACT

The present investigation deals with the preparation and evaluation of sustained release system of Salbutamol sulphate for the treatment of Asthama. The liposomes of Salbutamol sulphate were prepared using physical dispersion method by using soyalecithin and cholesterol. The ratio of soyalecithin and cholesterol was found to be important factors for achieving sustained release pattern. Factors studied influenced the lag time and *in-vitro* drug release of formulations. Dissolution studies of liposomes in phosphate buffer with pH 7.4 shows the drug release in colon could be modulated by optimizing the concentration of soyalecithin and cholesterol (6:1). The results of *in-vitro* dissolution studies indicated that formulation F1 is the most successful formulation of the study and exhibited drug release 96.24% in 12 h and the total release pattern was very close to the theoretical release profile of sustained release system. The study showed that the optimised batch F1 fulfills the requirement of good liposomal formulation. Stability study of the optimized formulation indicates no significant difference in release profile after a period of two month.

Key words: Sustained release system, Soya lecithin: Cholesterol, Salbutamol sulphate, Liposomes

*Corresponding Author Email: udshivhare@gmail.com

Received 14 July 2012, Accepted 30 July 2012

Please cite this article in press as: Shivhare UD *et al.*, Formulation and *In-Vitro* Evaluation of Salbutamol Sulphate Liposomes. American Journal of PharmTech Research 2012.

INTRODUCTION

Liposomes have been used as drug carriers for several drugs to reduce toxicity or to deliver the drug at the site of infection and have now been formulated into various dosage forms. Structurally liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules that are usually phospholipids.[1]

Liposomes are formed spontaneously when the lipids are dispersed in aqueous media that gives rise to a population of vesicles ranging in size from 10's of nanometer to 10's of microns in diameter. They can be formulated to entrap materials both within their aqueous compartment as well as their lipid membrane. Lipophilic drugs show a very high degree of entrapment, which nears almost to 100%. The size and shape of the liposomes can be varied by changing the mixture of phospholipids, the degree of saturation of fatty acid side chains and conditions of formation.[2]

The amphipathic nature of phospholipids and their analogues render them the ability to form closed concentric bilayers in the presence of water. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. These hydrated lipid sheets detach during agitation and close to form large MLV, which prevent interaction of water with the hydrocarbon core of the bilayer at the edges.[3] Once these vesicles are formed, a change in the vesicle shape and morphology requires energy input in the form of sonic energy (sonication to get small unilamellar vesicles) and mechanical energy (extrusion to get large unilamellar vesicles).

Asthma is a common chronic inflammatory disease of the airways, characterized by hyper responsiveness to a variety of stimuli. Asthma affects 14 to 15 million persons in the US. An estimated 4.8 million children have asthma, which makes it the most common chronic disease of childhood.

Salbutamol is a short-acting, selective beta2-adrenergic receptor agonist used in the treatment of asthma and COPD. It is 29 times more selective for beta2 receptors than beta1 receptors giving it higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart. Salbutamol is formulated as a racemic mixture of the R- and S-isomers. The R-isomer has 150 times greater affinity for the beta2-receptor than the S-isomer and the S-isomer has been associated with toxicity. This lead to the development of Levalbuterol, the single R-isomer of Salbutamol. However, the high cost of Levalbuterol compared to Salbutamol has deterred wide-spread use of this enantiomerically pure version of the drug. Salbutamol is generally used for

acute episodes of bronchospasm caused by bronchial asthma, chronic bronchitis and other chronic bronchopulmonary disorders such as chronic obstructive pulmonary disorder (COPD). It is also used prophylactically for exercise-induced asthma.[3]

The literature survey revealed that in case of drugs having narrow absorption window in liver and stomach, it is ideal to formulate the dosage form which will keep the system above the absorption window. The ideal drug delivery system delivers drug at rate indicated by need of body over period of treatment and deliver active moiety only to site action. Liposomes have several advantages over conventional non-vesicle formulations which allow incorporation of wide variety of hydrophilic and hydrophobic drugs. Lipsomes mask the ontoward adverse effects associated with the drug and also improve the absorption of drug. These carriers are devoid of any antigenic, pyrogenic or allergic reaction and their component can be utilized as component of biological membrane.

The objective of the present work was to prepare Salbutamol sulphate liposome and study the *in -vitro* drug release and stability studies of prepared liposomes. [4]

MATERIAL AND METHOD

Materials

Salbutamol sulphate was received as gift sample from Zim Pharmaceuticals Pvt. Ltd., Kalmeshwar, Nagpur. All other chemicals and solvents used in this study were of Analytical grade.

Identification test of Salbutamol sulphate

The melting point of the Salbutamol sulphate was determined by capillary method and found to be 152° - 154° which was compiles with melting point reported in British Pharmacopoeia. The UV scanning of Salbutamol sulphate was performed in 7.4 pH phosphate buffer and λ_{max} was found to be 276 nm which was compiles with the λ_{max} reported in British Pharmacopoeia.

Preparation of liposomes

Liposomes were prepared by physical dispersion method using different ratio of lipids. In this method the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over flat bottom conical flask. The solution was then evaporated at room temperature without disturbing the solution. The hydration of lipid film form was carried out with aqueous medium phosphate buffer (pH 7.4). For this the flask was inclined to one side and aqueous medium containing drug to be entrapped was introduced down the side of flask and flask was slowly returned to upright orientation. The fluid was allowed to run gently over lipid layer and flask was

allowed to stand for 2 h at 37° for complete swelling. After swelling, vesicles are harvested by swirling the contents of flask to yield milky white suspension. Then formulations were subjected to centrifugation. All batches of liposomes were prepared as per the general method described above.

Evaluations of liposomes

Drug entrapment efficiency of liposomes

Entrapment efficiency of liposomes was determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3500 rpm for a period of 90 min.[5] The clear supernatants were removed carefully to separate nonentrapped Salbutamol sulphate and absorbance recorded at 276 nm. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded at 276 nm. Amount of Salbutamol sulphate in supernatant and sediment gave a total amount of Salbutamol sulphate in 1 ml dispersion. Percentage entrapment of drug was calculated by the following formula:

$$\%\,\text{Drug Entrapped(PDE)} = \frac{\text{Amount of Drug in Sediment}}{\text{Total amount of drug}} \times 100$$

Particle size analysis

The particle size of liposomes was determined by using motic digital microscope model no. (DMW 201). All the prepared batches of liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.[6]

Phase transition study

Differential scanning calorimetry (DSC) thermograms of the Salbutamol sulphate, soya lecithin and cholesterol were recorded on a differential scanning calorimeter. Thermograms of both blank and Salbutamol sulphate liposomal dispersions were recorded individually. The liposomal dispersions were weighed in an aluminium cuvette and sealed with an aluminium lid. The cuvette was placed in the DSC and heated from 20° to 200° at a heating rate of 10°/min in nitrogen atmosphere. The scan was recorded and plotted showing heat flow (w/g) on the Y-axis and temperature on the X-axis.[7]

***In -vitro* drug release study**

The release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 250 ml beaker. The beaker was assembled on a

magnetic stirrer and the medium was equilibrated at 37±5°. Dialysis membrane was taken and one end of the membrane was sealed.

After separation of non-entrapped Salbutamol sulphate liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 5ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.[8]

Drug release kinetics study

For finding out the mechanism of drug release, the dissolution data obtained from the above experiments were treated with the different release kinetic equations.

Zero order release equation

$Q = K_0 t$ (1)

First order equation

$In\ Q = K_f\ t$ (2)

Higuchi's square root of time equation

$Q = K_H\ t\ ½$ (3)

Korsmeyer's and Peppas equation

$F = (M_t / M) = K_m\ t^n$ (4)

Where,

Q = amount of drug release at time t

M_t = drug release at time t

M = total amount of drug in dosage form

F = fraction of drug release at time t

K_0= zero order release rate constant

K_f = first order release rate constant

K_H= Higuchi square root of time release rate constant

K_m= constant depend on geometry of dosage form

N = diffusion exponent indicating the mechanism of drug release

Scanning Electron Microscopy (SEM):

Surface morphology of microspheres was investigated by Scanning Electron Microscopy (SEM) using JSM 6380A (JOEL, Japan). The liposomes, coated with Platinum by ion sputtering using Auto fine coater JFC-1600 (JOEL, Japan), for 20 S at 1.1V under argon atmosphere were

mounted onto metal stubs using double-sided carbon adhesive tape and the scanning electron micrographs were taken.[9]

Zeta potential

The Zeta potential of the various batches of liposomes was measured with the help of Electrophorometer (Electrophorosis).[10]

Stability studies

The formulations were subjected to stability studies by storing at 4°, 25° and 37° for 60 days and were analyzed for its % drug entrapped at an interval of 15 days. Stability studies were carried out for a period of two month at 4±2°, 25±2° and 37±2°. The entrapment efficiency was estimated at an interval of 15 days.

RESULTS AND DISCUSSION

The procured sample of Salbutamol sulphate was tested for its identification. The Salbutamol sulphate sample showed compliance with the data given in BP which reflects its quality and purity. The lipids such as soyalecithin and cholesterol and all other excipients provided by the suppliers confirmed by their identification test official in USNF, IP and BP. The saponification value of was found to be 195 which complies with Merck index. The Infrared spectrum of phosphatidylcholine was taken for its structural confirmation. Identification test for cholesterol was carried out along with Infrared spectrum. All the excipients showed results in compliance with standard specifications.

Physical dispersion method was widely used to prepare liposomes. The method yielded the liposomes with a heterogeneous size distribution. Also the liposomes that are formed were of multilamellar in nature. Soyalecithin and cholesterol were used to prepare liposomes in different ratio. The drug entrapment into the liposomes was dependent mainly on soyalecithin and cholesterol concentration. Different batches of liposomes were prepared in order to select an optimum formula. The compositions of different batches of liposomes were shown in Table 1.

Table 1. Composition for liposome formulations

Sr. No.	Formulation code	Constituents				
		Soyalecithin (mg)	Cholesterol (mg)	Chloroform (ml)	Salbutamol sulphate (mg)	Phosphate buffer pH 7.4 (ml)
1	F1	450	75	25	5	20
2	F2	375	150	25	5	20
3	F3	300	225	25	5	20
4	F4	225	300	25	5	20
5	F5	150	375	25	5	20
6	F6	75	450	25	5	20

Encapsulation efficiency of liposomes is influenced by many factors such as vesicle type, pH, method of preparation, liposomes composition and ratio of lipid to drug and the physicochemical properties of liposomes. The balanced concentration of cholesterol is essential to form stable liposomes. Percentage drug entrapments of liposomes made by using different ratio of lipids were shown in Table 2.

Table 2. PDE of different batches of liposome made by using different ratio of lipids

Sr. No	Formulation No.	PDE
1	F1	48.92 ± 0.81
2	F2	47.23 ± 0.92
3	F3	44.71 ± 0.53
4	F4	40.51 ± 1.02
5	F5	35.28 ± 1.07
6	F6	29.64 ± 0.63

(Mean ± S.D., n=3)

The encapsulation efficiency of liposomes was governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Soyalecithin was used to entrap drug to a greater extent due to formation of thin film. Cholesterol improved the fluidity of the bilayer membrane and improved the stability of bilayer membrane in the presence of biological fluids such as blood/plasma.

Mean particle sizes (mps) of different formulation of liposomes were shown in Table 3.

Table 3. Mean particle size (mps) of different formulation of liposomes

Sr. No.	Formulation No	MPS µm± SD
1	F1	6.24 ± 0.09
2	F2	7.14 ± 0.098
3	F3	10.74 ± 0.064
4	F4	12.27 ± 0.082
5	F5	15.07 ± 0.105
6	F6	14.03 ± 0.051

(Mean±S.D., n=3)

It showed that as the concentration of cholesterol increased there was increase in the particle size due to formation of rigid bilayer structure but upto a specific concentration as there was also decrease in particle size observed in formulation F6. It was reported that small unilamellar vesicles were significantly lysed on incubation with phospholipase whereas, lower leakage rates were observed for multilamellar vesicles. Elimination half-life and volume of distribution of

drug in multilamellar vesicles were greater than drug encapsulated in unilamellar vesicles this was due to increased uptake of larger liposomes by liver. The interaction of the encapsulated drug with the lipid components of liposomes may alter the physicochemical properties of liposomes which in turn would influence the transfer of active ingredients from the liposomes.

Phase Transition Study (Figure1) showed that DSC thermograms of blank unloaded liposome dispersion had shift in the melting endotherm of cholesterol from 150.0° to 125.17° and for soyalecithin from 42.64° to 72.2° which signify that all the lipid components interact with each other to a great extent while forming the lipid bilayer. In the DSC thermogram of Salbutamol sulphate liposome dispersion, absence of the melting endotherm of Salbutamol sulphate suggested significant interaction of Salbutamol sulphate with the bilayer structure. The phase transition temperature gave good clues good liposomal stability, permeability and whether a drug is entrapped in the bilayer or in the aqueous compartments.

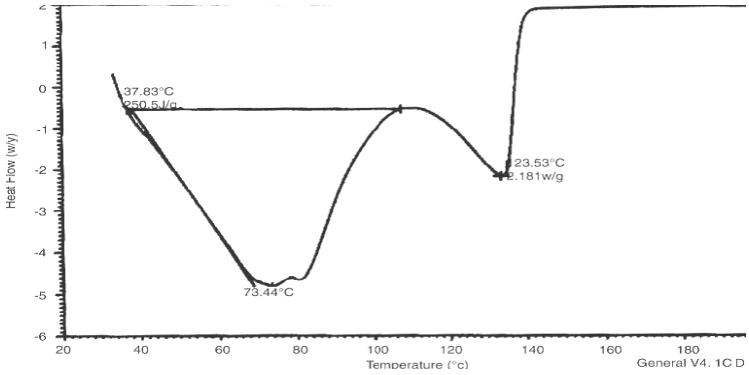

Figure 1. DSC Thermogram of formulation F1 batch

Table 4. Cumulative percentage drug release from various formulations of liposomes

Time in h	Cumulative % drug release					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0	0.00	0.00	0.00	0.00	0.00	0.00
1	14.48±1.08	19.31±0.23	12.83±0.65	18.68±1.10	15.21±0.24	13.06±0.54
2	28.79±1.27	36.25±0.65	25.55±0.56	31.75±1.23	28.13±0.37	25.93±0.34
3	43.26±0.76	47.41±0.75	38.41±0.45	43.14±0.54	39.06±0.34	36.82±0.35
4	53.85±0.83	57.49±0.10	48.27±0.23	52.80±0.43	47.98±0.16	45.71±0.12
5	63.53±1.20	66.48±0.54	55.08±0.91	60.73±0.24	54.88±0.35	52.57±1.21
6	71.30±1.10	71.98±0.78	60.40±0.45	65.06±0.78	61.85±0.78	59.50±0.77
7	76.11±0.78	75.14±0.57	65.76±0.34	69.44±0.56	66.76±0.67	63.32±0.56
8	80.97±0.87	77.73±0.45	69.61±0.24	73.85±0.76	70.66±0.34	67.18±0.46
9	84.86±0.76	80.34±1.23	77.41±0.65	79.11±0.34	77.50±0.56	70.01±0.76
10	89.11±0.98	84.82±0.96	81.36±0.28	81.77±0.56	80.43±0.24	75.74±0.35
11	92.64±1.9	88.68±0.78	86.92±0.65	84.85±0.78	82.97±0.67	78.64±0.78
12	96.24±0.37	93.21±0.56	90.48±0.24	88.71±0.23	84.24±0.56	81.78±85

(Mean ± S.D., n=5)

The cumulative percent drug release curve of the drug loaded liposomes showed the drug release from the liposomes decreased as the concentration of cholesterol increased, suggesting that drug release could be controlled by varying soya lecithin: cholesterol concentration. It was attributed that increased concentration of the soya lecithin: cholesterol resulted in smaller liposomes and this in turn increased the absorption, since the drug molecules have to traverse during absorption. The highest drug release about 96.24% was found to be in formulation F1 and hence was designated as optimized batch (Table 4).

The release of drug from multilamellar vesicles was slower because it consists of several concentric spheres of lipid bilayers separated by aqueous compartments. They were found to be spherical, rounder, free flowing and of the monolithic matrix type.

The drug release kinetics for formulations F1-F6 (Table 5), showed that majority of the batches governed by peppas model. All the batches showed release up to 12 h and above 81%. Formulation F1 (96.24%) showed maximum release while other formulation showed less amount of drug release in 12 h. Formulation F1 had highest correlation coefficient (R^2=0.985) value and follows drug release by peppas model. The drug release from liposomes depends on many factors including the composition of liposomes, the type of drug encapsulated and nature of the cell. Once released, drug that normally crosses the membrane of a cell wall enter the cell. The drug is released from liposomes by one of the possible mechanism i.e., endocytosis, fusion and adsorption. Orally administered liposomes release the drug by endocytosis as gut epithelial cells take up intact liposomes by absorptive endocytosis**.**

Table 5. Drug release kinetics for the various formulations of liposomes

Formulation Code	R^2				n
	Zero order equation	First order equation	Higuchi's equation	Korsmeyer Peppas	
F1	0.975	0.978	0.937	0.985	0.724
F2	0.964	0.973	0.895	0.978	0.765
F3	0.956	0.960	0.870	0.964	0.789
F4	0.940	0.945	0.865	0.961	0.793
F5	0.927	0.897	0.857	0.954	0.819
F6	0.912	0.885	0.843	0.943	0.836

The SEM photomicrographs showed that the lipid liposomes of batch have a spherical morphology. In SEM photomicrographs, the lipid liposomes were observed at different magnification value i.e. 500X, 1500X and 3000X which showed the surface texture of liposomes. Surface texture of the liposomes was rough because of presence of crystals of drug on the surface of lipid liposomes.The liposomes were found to be spherical, rounder, free flowing and of the monolithic matrix type (Figure 2).

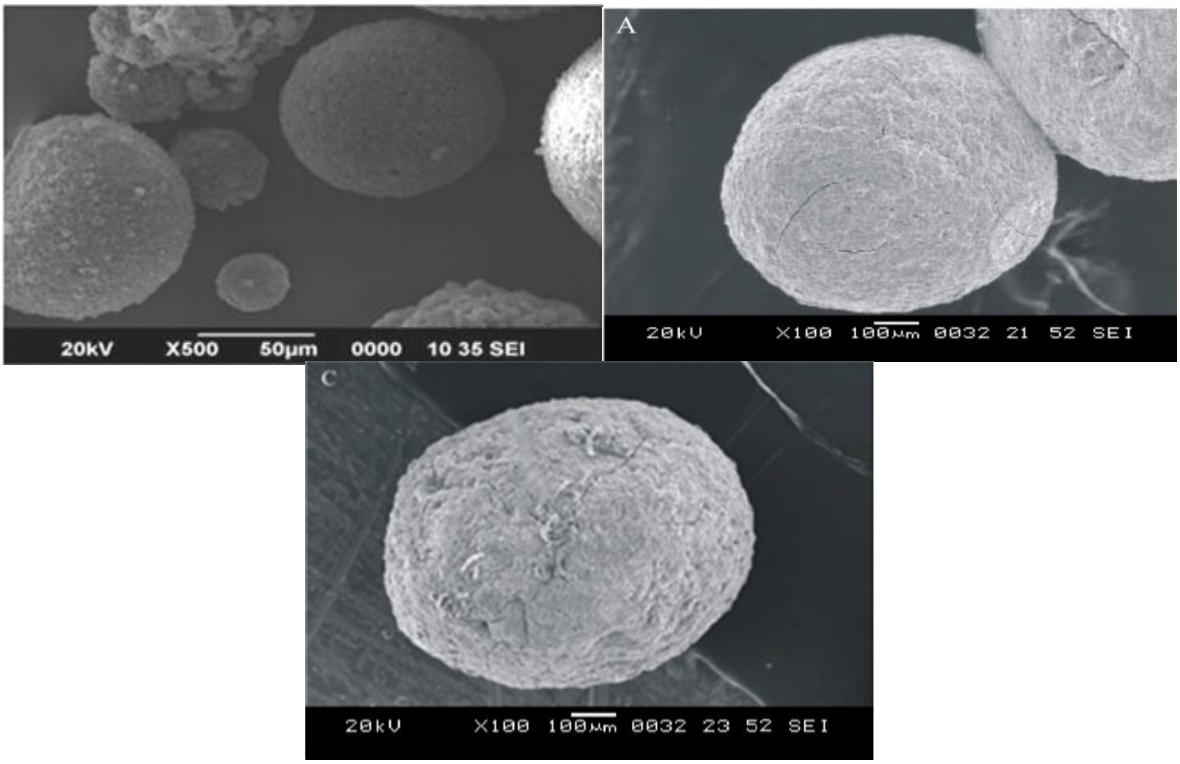

Figure.2. SEM images of drug loaded liposomes

The SEM photomicrographs showed that the lipid liposomes of batch F1 after dissolution at different magnification value i.e. 500X, 1500X and 3000X. SEM photographs showed presence of pores on the surface of liposomes and roughened surface. Surface of the liposomes was changed due to the 12 h exposure to the dissolution medium. The reason for this might be dissolution and diffusion of the entrapped drug crystals in the dissolution medium.

The measurement of zeta potential has been inextricably connected with the study and characterization of the colloidal dispersions, as this parameter was highly useful for the physical stability of the colloidal dispersion. The zeta potential indicates the degree of repulsion between the adjacent, similarly charged particles in the dispersion. Zeta potential is related to the change on the surface of the lipid vesicles which influence vesicular properties such as physical stability. The moderate physical stability is achieved when the zeta potential is in between ±30 and ±60 mV and are good to excellent ±60 and ±100mV. The zeta potential of the optimized formulation was shown in Table 6.

The results of stability studies of formulation batch F1 is shown in Table 7. Stability studies of all batches were carried out at different temperatures and these batches were evaluated for their drug encapsulation efficiency. The formulation kept at 4±2° (48.44%) showed a minimum decrease in drug encapsulation efficiency of drug as that of at formulation at 25±2° (44.45%) and 45±2° (42.14%) when compared with initial drug encapsulation efficiency of formulation. The

liposomes showed a fairly high retention of drug inside the vesicles at a refrigerated temperature while storage at high temperature leads to substantial loss of encapsulated drug from liposomes due to degradation of lipids in the bilayers resulting in defects in the membrane packing making them leaky. The liposomal dispersions stored at 25±2° showed sedimentation after fifteen days of storage while dispersions stored at 45±2° showed sedimentation within fifteen days and hard cake was formed. The liposomal dispersion stored at 4±2° showed sedimentation after thirty days which on slight shaking redispersed easily. The reduction in the percent encapsulation is occurred due to leakage and aggregation of vesicles. The changes in the particle size were also observed at different temperature.

Table 6. Zeta potential of the optimized formulations

Sr. No.	Formulation	Zeta potential(mV)
1	F1	-9.033
2	F2	-8.393
3	F3	-6.056
4	F4	1.500
5	F5	1.765
6	F6	2.391

Table 7. Stability studies for the formulation F1

Sampling Intervals(Days)	% drug entrapped at		
	4 ± 2^0	25 ± 2^0	37 ± 2^0
0	48.92	48.92	48.92
15	48.73	47.56	46.39
30	48.44	44.45	42.14
45	48.07	41.63	38.96
60	47.82	39.13	34.12

CONCLUSION

Oral drug delivery system is one of the promising alternatives to other dosage forms especially for drugs that are subjected to the first-pass elimination such as Salbutamol sulphate. To optimize the release of drug from the ODDS as close to a desired profile as possible for the long-time period of operation, much attention has recently been focused on the development of a liposome preparation composed of lipid concentration.

The physical parameters of drug as well as excipients concluded that these were considerably good to formulate liposome using physical dispersion method. The liposomes were spherical in shape and the drug remained dispersed in the matrix at amorphous state. Scanning electron microscopy, Differential scanning calorimetry and *in vitro* drug release studies were performed to characterize the liposomes.

From the performed work it was concluded that Salbutamol sulphate possesses all requisite qualities required for liposomal drug delivery. Among the various formulation, the combination F1 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F1 comprising soyalecithin, cholesterol 6:1 ratio, fulfilled the requirement of good liposomal formulation. Formulation F1 showed 96.24% *in-vitro* drug release upto 12 h and followed peppas model. It showed encapsulation efficiency of 48.92% and particle size of 6.24 µm.

ACKNOWLEDGEMENT

The authors are thankful to Zim Pharmaceuticals Pvt. Ltd., Kalmeshwar, Nagpur for providing gift sample of Salbutamol sulphate and Principal, Sharad Pawar College of Pharmacy for providing necessary facilities to carry out the research work.

REFERENCES

1. Benita S. Microencapsulation Method and Industrial Application, Drug and the Pharmaceutical Sciences 2002; 73:213.
2. Fuji GV. Realization of the Drug Delivery Concept. New York; 1996: 491-495.
3. Gregoriadis G. Liposome Technology, Liposome preparation related techniques, 3rd ed., 2007: 21.
4. Vyas SP, Khar RK. Targeted and Controlled Drug Delivery, Novel Carrier System, CBS Publishers and Distributors; 2002: 249.
5. Bangham AD, Standish MM. Diffusion of univalent ions across the lamella of swollen Phospholipids. J Mol Biol 1965; 13: 238-252.
6. Lachman L, Lieberman HA, Kanig JL. In, The Theory and Practice of Industrial Pharmacy. Varghese Publishing House. Bombay; 1987: 417-424.
7. Vyas SP, Khar RK. Targeted and controlled Drug Delivery, Novel Carrier system, CBS Publishers and Distributors; 2002: 263-265.
8. Datta S, Karki R. Liposomes encapsulated Rifampicin and Isoniazid. Indian J Pharm Sci 2000; 62(5): 384.
9. Ramana MV, Chaudhari AD. An approach to minimize pseudo membranous colitis caused by Clindamycin through liposomal formulation. Indian J Pharm Sci 2007; 69(3): 390.
10. Qui L, Jing N, Jin Y. Preparation and *In-vitro* evaluation of liposomal chloroquine diphosphate loaded by a transmembrane pH-gradient method. Int J Pharma. 2008; 56-63.