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IJPBS |Volume 2| Issue 2 |APRIL-JUNE |2012|198-207

Research Article
Pharmaceutical Sciences

IN-VITRO FORMULATION AND EVALUATION OF CEFIXIME LIPOSOME FORMULATION

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ABSTRACT

Cefixime is widely used as prescription and no-prescription medicine. The aim of study, is aimed at developing and optimizing liposomal formulation of cefixime in order to improve its anti-cancer activity, to prepare cefixime liposome using thin lipid film hydration using rota evaporator (Mechanical dispersion) which is now a days considered a cost effective and simple method of manufacturing. cefixime has mild to moderate anti-cancer activity as DNA binding agent eventhough it is an antibiotic drug(3rd generation cephalosporin drug), so it has to be formulate as liposome formulation for effective targeting action with minimal side effects.

Liposomes are micro particulate lipoid vesicles which are under investigation as drug carriers for improving the delivery of therapeutic agents. Due to new development in liposome technology, several liposome based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. In this research liposome formulation of cefixime was Prepared and evaluated for vesicle shape (SEM), drug entrapment efficiency, FTIR analysis. From all the characterizations the entrapment of the drug was found to be as 95.03%.

KEYWORDS

Liposomes, cefixime, percentage drug entrapped, FTIR analysis

1. INTRODUCTION

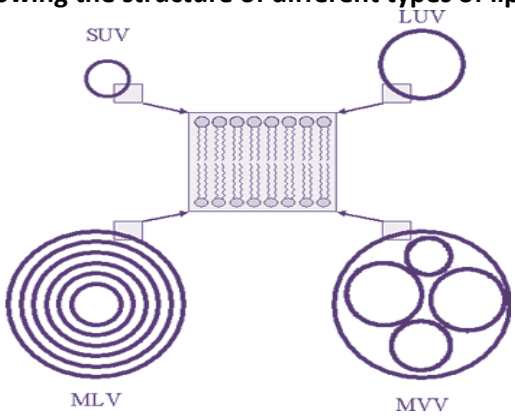
The main objective of drug delivery systems is to deliver a drug effectively, specifically to the site of action and to achieve greater efficacy and minimize the toxic effects compared to conventional drugs. Liposomal vesicles were prepared in the early years (1960's) of their history from various lipid classes identical to those present in most biological membranes. Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization[1]. Liposomes are broadly defined as lipid bilayers surrounding an aqueous space. Multilamellar vesicles (MLV) consist of several (upto 14) lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution. These vesicles are over several hundred nanometers in diameter (0.1-0.5µm). Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25-50 nm or 0.02-0.05µm (according to some authors up to 100nm) in diameter. Large unilamellar vesicles (LUV) are, in fact, a very heterogenous group of vesicles that, like the SUVs, are surrounded by a single lipid layer. The diameter of these liposomes is very broad, from 100nm up to cell size[2]. Besides the technique used for their formation the lipid composition of liposomes is also, in most cases, very important. For some bioactive compounds the presence of net charged lipids not only prevents spontaneous aggregation of liposomes but also determines the effectiveness of the entrapment of the solute in to the liposomal vesicles. Natural lipids, particularly those, with aliphatic chains attached to the backbone by means of ester or amide bonds (phospholipids, sphingolipids and glycolipids) are often subject to the action of various hydrolytic (lipolytic) enzymes when injected in to animal or human body. These

enzymes cleaves off acyl chains and resulting lysolipids have destabilizing properties for the lipid layer and cause the release of the entrapped bioactive component(s). As a result new type of vesicles, that should merely bear the name of liposomes as their components are lipids only by similarity of their properties to natural (phospho) lipids, has been elaborated. These vesicles, liposomes, are made of various amphiphile molecules (the list of components is long). The crucial feature of these molecules is that upon hydration they are able to form aggregation structures resembling an array and have properties of natural phospholipid bilayers [3].

Table 1: types of liposomes and their sizes

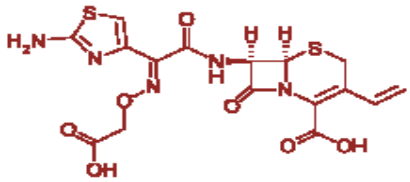
SI.NO	TYPE OF LIPOSOME	SIZE RANGE
1	Small unilamellar vesicle [SUV]	0.02-0.05 µm
2	Large unilamellar vesicle [LUV]	>0.06 µm
3	Multilamellar vesicle [MLV]	0.1-0.5 µm

Fig 1: showing the structure of different types of liposomes



Cefixime is widely used as anti-biotic (3rd generation cephalosporin anti-biotic) and is rarely used as cytotoxic drug because it inhibits the biosynthesis of nucleic acids in the tumour cells. Cefixime is mainly inhibit the protein synthesis.cefixime is a PDE4 inhibitor increasing intracellular cAMP. It also acts as inhibitor of tumour necrosis factor-alpha. But it has several drawbacks such as narrow therapeutic index, short biological half-life[4]. These factors necessitated liposomal formulation for cefixime. As this dosage form would reduce the dosing frequency hence better patient compliance.

Fig 2: chemical structure of drug cefixime



Systematic (IUPAC) name

(6R, 7R)-7- {[2-(2-amino-1, 3-thiazol-4-yl)-2(carboxymethoxyimino) acetyl] amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid.

Chemical data: Formula: $C_{16}H_{15}N_5O_7S_2$.

Phospholipids such as phosphotidylcholine (lecithin) and cholesterol were selected for the formation of liposomes into which the drug was incorporated. Cholesterol incorporated into

phospholipids membranes in very high concentration up to 1:1 or 2:1 molar ratio[5]. Cholesterol acts as a 'fluidity buffer' since below the phase transition tends to make membrane less ordered while above transition it tends to make membrane more ordered thus suppressing the tilts and shifts in membrane structures specifically at phase transition.

Fig 3: liposome for drug delivery

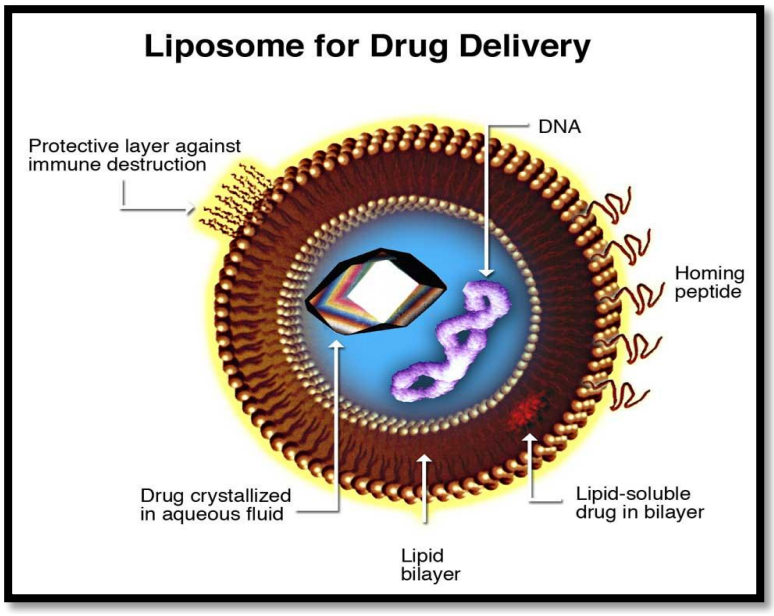


Fig 4: structure of liposome

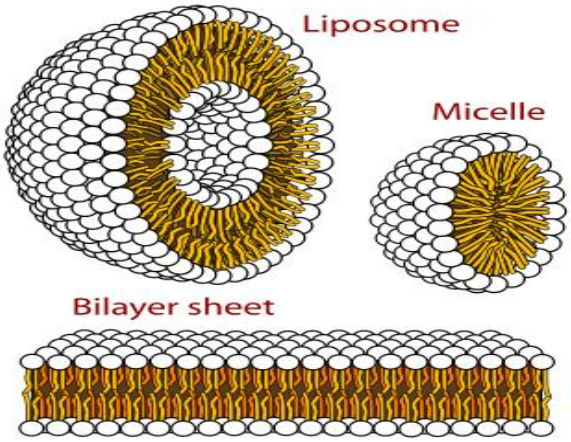
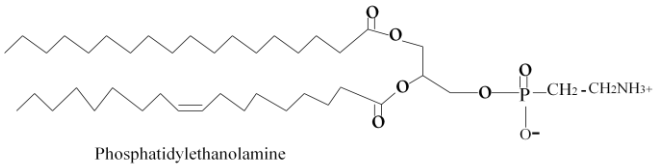
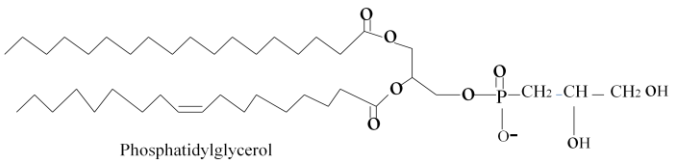


Fig 5: eg of commonly used synthetic phospholipids





The present study is aimed with the formulation of liposomes of cefixime followed by the evaluating parameters such as encapsulation efficiency, particle shape and size (SEM), FTIR analysis and *in vitro* drug release.

2. MATERIALS AND METHOD

Cefixime was obtained as a gift sample from Bakul Pharma Pvt. Ltd., Mumbai. Phosphotidylcholine was obtained as gift sample from Perfect Biotech, Nagpur. Cholesterol was purchased from Loba chemie. All other chemicals, reagents and solvents used like potassium chloride, potassium dihydrogen phosphate, acetone, chloroform, and methanol were of analytical reagent grade.

A. Evaluation of raw materials

Identification and standardization of drug and other excipients were carried out as per the official procedures mentioned in respective monographs.

B. Preparation of liposomes

Liposomes were prepared by mechanical dispersion method (Film hydration method using Rota Evaporator) using different ratios of lipids. In this method the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over round bottom conical flask of Rota Evaporator kept at, an angle of 45^0, rpm 115 for a period of 30 to 40min. The solution was then evaporated at a temperature of 40^0c. The hydration of lipid film form was carried out with aqueous medium phosphate buffer (P^H 7.4). For this inclined to one side and medium containing drug (cefixime dissolved in mixture of acetone and methanol in the ratio 2:1) to be entrapped was introduced down the side of flask and flask was rotated slowly. The fluid was allowed to run gently over lipid layer and flask was allowed to stand for 2 hr at 37^0C 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. Different batches of liposomes were prepared to select an optimum formula. All batches of liposomes were prepared as per the general method described above and composition of lipids for the preparation of liposomes given **in Table 2**.

Table 2. Composition of lipids for preparation of liposome

Sl.no	Formulation no.	Phosphotidylcholine	Cholesterol
		PARTS	
1	F1	9	1
2	F2	8	2
3	F3	7	3
4	F4	6	4
5	F5	5	5

Each formulation contain 200 mg of drug

able 3. Optimized formula for liposome preparation

Sl.no	Constituents	Quantity
1	Phosphotidylcholine	270 mg
2	Cholesterol	30 mg
3	Solvent (chloroform)	10 ml
4	Drug (Cefixime)	200mg
5	Phosphate buffer p^H 7.4	10ml

CHARECTERIZATION OF LIPOSOMES

1. Drug entrapment efficiency of liposome

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[6]. The clear supernatants were removed carefully to

separate non-entrapped cefixime and absorbance recorded at 261 nm. Amount of cefixime in supernatant and sediment gave a total amount of cefixime in 1 ml dispersion.

% entrapment of drug was calculated by the following formula:

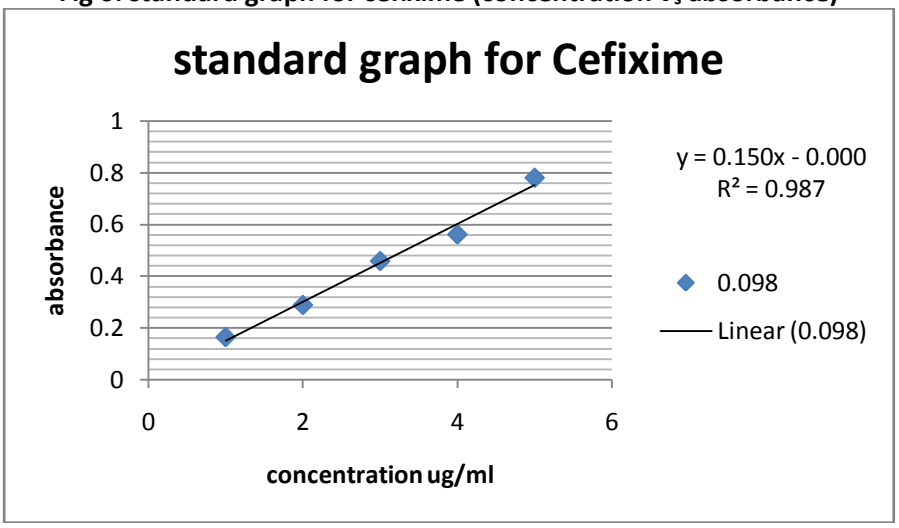
$$\% \text{ Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

∴ Entrapment efficiency (%) = ((total amount of the drug - amount of the free drug) / total drug) x100.

Table 4: Readings for formulation F1 (concentration and absorbance)

CONCENTRATION	ABSORBANCE
0.5	0.098
1	0.165
2	0.289
3	0.459
4	0.562
5	0.781

Fig 6: standard graph for cefixime (concentration v_s absorbance)



RESULTS

Cefixime λ_{max} – 261 nm

Unknown concentration of liposome after five serial dilutions – 0.926

% drug entrapment efficiency after calculating with formula was found to be as – 95.03%

2. Particle size analysis

The particle size of liposomes was determined by using motic digital microscope (model no.DMW) or by SEM. All the prepared batches of liposomes were viewed under microscope to study their size[7]. Size of liposomal vesicles from each batch was measured at location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.

Fig 7: Size of liposomes (MLV) observed under electron microscope

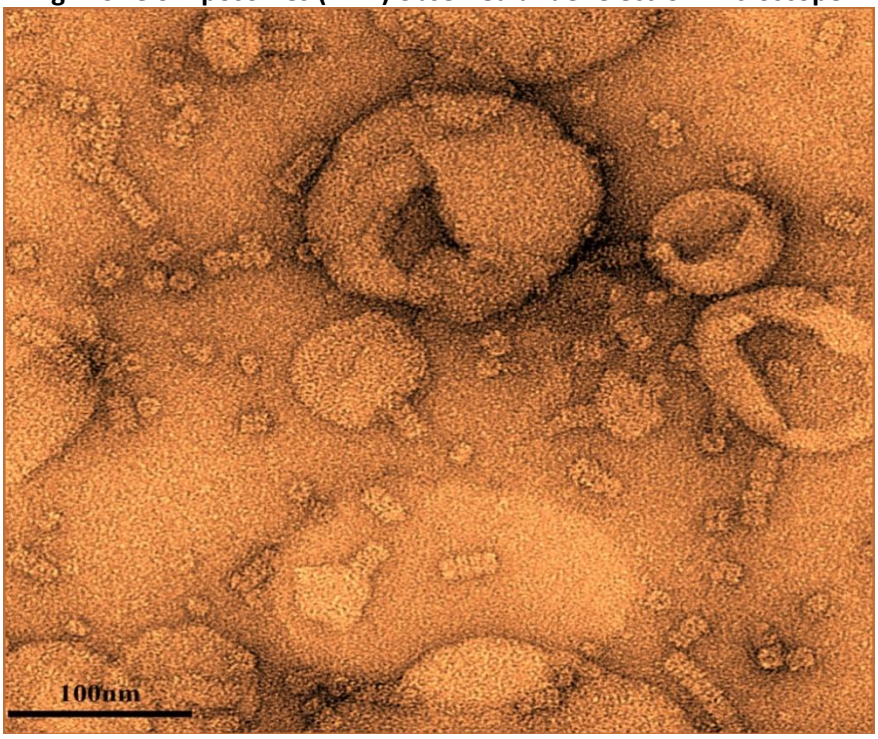


Table 5: evaluation parameters of liposome

Sl.no	Formulation no	% drug entrapped	Mean particle size µm ± SD
1	F1	48.92 ± 0.81	6.24 ± 0.09
2	F2	47.23 ± 0.92	7.14 ± 0.098
3	F3	44.71 ± 0.53	10.74 ± 0.064
4	F4	40.51 ± 1.02	12.27 ± 0.082
5	F5	35.28 ± 1.07	15.07 ± 0.105

3. FTIR analysis

FTIR (Fourier Transform Infrared) spectroscopy is a failure analysis technique that provides information about the chemical bonding or molecular structure of materials, whether organic or inorganic. It is used in failure analysis to identify unknown materials present in a specimen, and is usually conducted to complement EDX analysis. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristic to the molecule. During FTIR analysis, a spot on the specimen is subjected to a modulated IR beam. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated in to an IR absorption plot consisting of reverse peaks. The resulting FTIR spectral pattern is then analyzed and matched with known signatures identified materials in the FTIR analysis.

Fig 8: FTIR data for cefixime pure solid

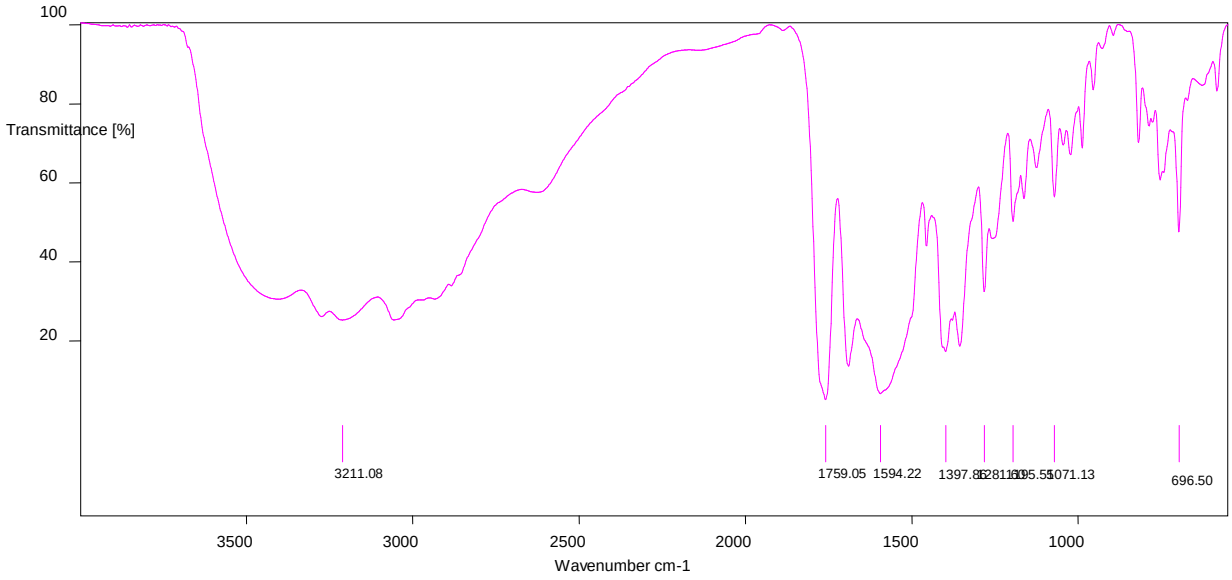
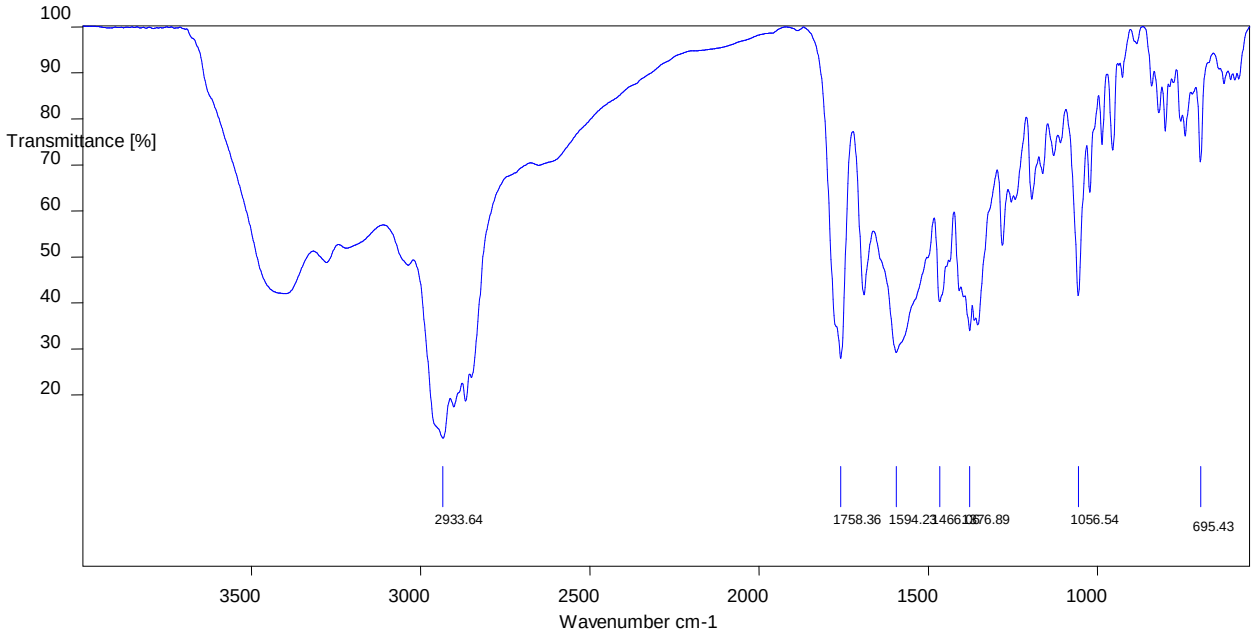
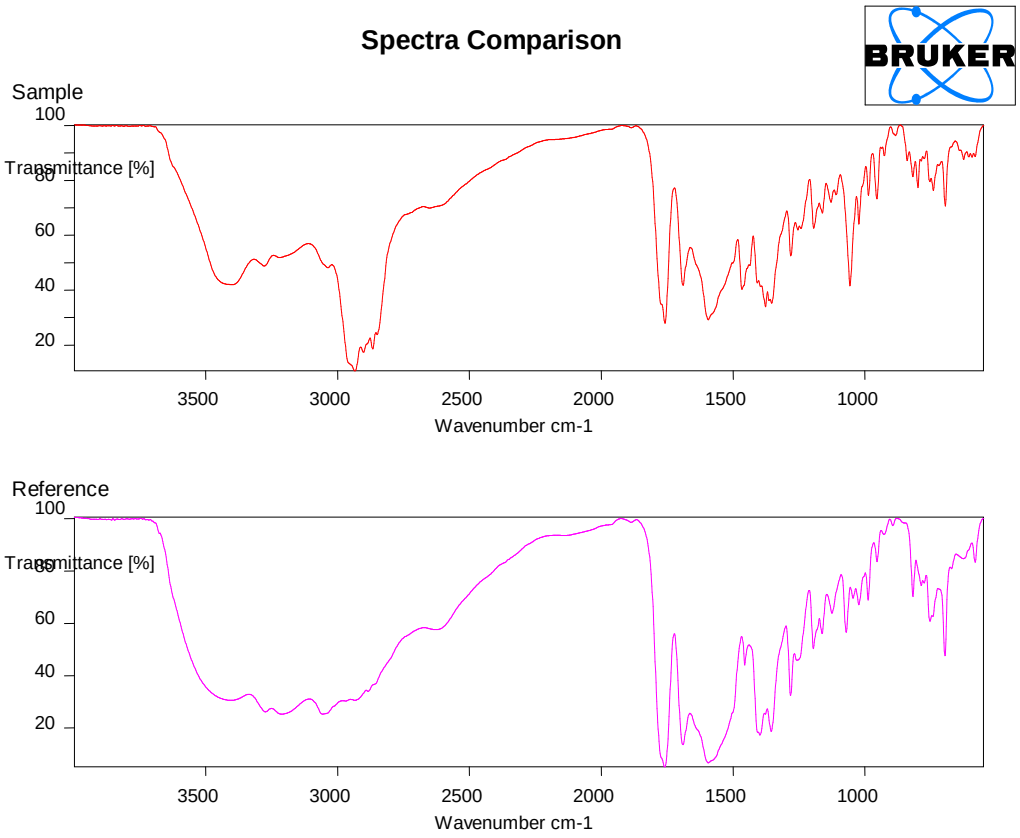


Fig 9: FTIR data for (cefixime liposome) liposome solid



D:\FTIR DATA\CEFIXIME LIPOSOME.0	LIPOSOME	SOLID	26/03/2011

Fig 10: spectra comparision



Result: OK

Correlation: 95.03%
Threshold: 95.00 %

Sample: LIPOSOME.0
Compared with Reference: CEFIXIME PURE.0

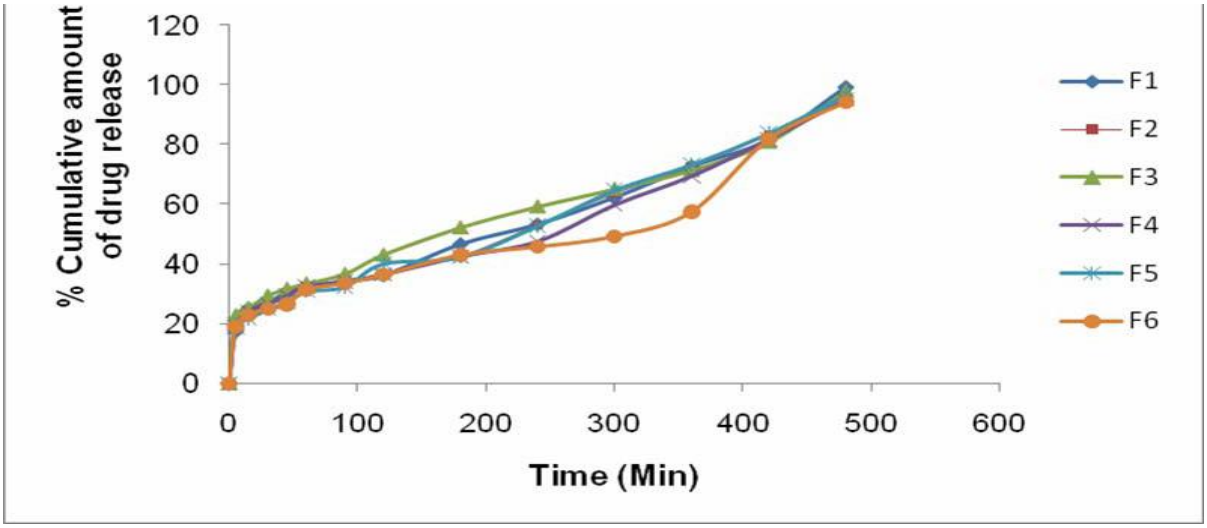
Method file: CEFIXIME.qcm (2011/03/26 11:57:40 (GMT+5))
Operator: Default
Date and time (measurement): 26/03/2011 17:26:09.450 (GMT+5)
Comment:

4. In vitro drug release study

The release studies were carried out in 250 ml beaker containing 100 ml of phosphate buffer p^H 7.4. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped cefixime, liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were

withdrawn (5 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Fig 11: in vitro drug release for various formulations



RESULTS AND DISCUSSION

Among the various methods mechanical dispersion method is widely used to prepare liposomes. This method yields the liposomes with a heterogeneous size distribution. Also liposomes that are formed are 'multilamellar' in nature.

The result of drug entrapment efficiency of liposomes (**Table 3**) indicates that as the concentration of phosphotidylcholine decreases, drug entrapment efficiency of liposomes decreases which was due to the saturation of lipid bilayer with reference to the drug where low phosphotidylcholine content provides limited entapment capacity. The encapsulation efficiency of liposomes is go by the ability of formulation to retain drug molecules in the acqueous core or in the bi layer membrane of the vesicles. Cholesterol improves the fluidity of the bilayer membrane and improves it's stability in the presence of biological fluids such as blood/plasma. From results of % drug entrapped it was observed that as the percentage of cholesterol increased there was subsequent increase in the stability and rigidity of liposomes but at the same time %drug entrapment reduced due to reduction in phosphotidylcholine. From the values of formulation F1 the % drug entrapment was calculated as 95.03%.

Results of particle size analysis showed that, as the concentration of cholesterol increases particle size increases which was may be due to formation of rigid bilaer structure.

The study of drug release kinetics showed that majority of the formulations governed by peppas model. The curve was obtained after plotting the cumilative amount of drug released from each formulation vs time. All the formulations showed release up to 8 hr and above 90% drug released with each formulation. Formulation F1 (99.23%) showed maximum release while other formulations showed less amount of drug release in 8 hr. formulation F1 has highest correlation coefficient (r=0.9552) value and follows drug release by peppas model.

CONCLUSION

The present study demonstrated the sucessful preperation of cefixime liposomes and its evaluation. Formulation F1 showed high encapsulation efficiency with minimum particle size and drug release over an 8 hr, hence

suppose to give greater bioavailability and considered as good liposomal formulation.

REFERENCES

1. D. D. Lasic, Liposomes: from Physics to Applications. Elsevier, Amsterdam, London, New York. (1993).
2. M. C. Woodle, D. Papahadjopoulos, Methods Enzymol. 171, 193 (1989).
3. New, R.R.C., Introduction and preparation of liposomes. In: New, R.R.C.(Ed.), Liposomes: A Practical Approach. Oxford University Press, Oxford,1–104 (1990).
4. K. D. Tripathi, Essentials of medical pharmacology, 6th Edn. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 536, 568 (2004).
5. G. Gregoriadis, Liposome technology: Liposome preparation and related techniques, 3rd Edn Vol. I, Informa Health care, New York, 21 (2007).
6. S. S. Bhalerao, A. R. Harshal, Drug Dev. Ind. Pharm., 29 451 (2003).
7. M. V. Ramana, A. D. Chaudhari, Indian J Pharm Sci. 69, 390 (2007).
8. Allison, A.C., Gregoriadis, G, Liposomes as immunological adjuvants. *Nature* 1974; P:252.
9. Alving, C.R., Liposomes as carriers of antigens and adjuvants. *J. Immunol. Methods* 1991;P:140, 1-13
10. Allen TM. 1994. Long-circulating (sterically stabilized) liposomes fortargeted drug delivery. *Trends Pharmacol Sci*, 15:215–20.
11. Allen TM, Brandeis E, Hansen CB, et al. 1995. A new strategy for attachment of antibodies to sterically stabilized liposomes resulting in effi cient.

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