IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2018 Vol.:13, Issue:3

Development, Characterization and *In-Vitro* Evaluation of Azithromycin Niosomes

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Senthil S.P*, Parthiban S, Jacob John P, Gomathi A.R, Ganesan V.

Department of Pharmaceutics, The Erode College of Pharmacy and Research Institute, Perundurai main road, Veppampalayam, Erode-638112, Tamilnadu, India.

Submission: 21 September 2018
Accepted: 27 September 2018
Published: 30 October 2018

Keywords: Azithromycin, Span 60, Cholesterol, Dicetyl phosphate, Niosomes, Handshaking method.

ABSTRACT

Azithromycin is a semisynthetic antibiotic belonging to the macrolide subgroup of azalides used to treat STDs due to chlamydia and mycobacterium ovium complex (MAC) in patients with advanced HIV diseases. Azithromycin binds to the 50s subunit of the 70s bacterial ribosomes and therefore inhibits RNA depends on the protein synthesis in bacterial cells. The aim of this study was to assess the potential of niosomes to improve the pharmacokinetics of Azithromycin with the primary goal of enhancing in bioavailability and sustain release. Co-encapsulated azithromycin niosomes were prepared by handshaking method (lipid layer hydration). The prepared neosomes (F1-F6) were found to have encapsulation efficiency ranging between 65.2 $\pm$ 1.3 to 87.5 $\pm$ 1.8. The prepared neosomes had particle size range 646.5 $\pm$ 1.38 to 752.8 $\pm$ 1.18nm. The *in-vitro* release profile was found to be a highest of 82.164 $\pm$ 1.2 % for formulation. It is a least of 42.716 $\pm$ 1.2 % for formulation F6 after 24 hours. The formulated niosomes were found to be stable with enhancing bioavailability.

INTRODUCTION:

Niosomes are one of the best carriers. The self-assembly of non-ionic surfactants into vesicles was first reported in the 70s by researchers in the cosmetic industry. Niosomes (non-ionic surfactant vesicles) obtained on hydration are microscopic lamellar structures formed upon combining non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class with cholesterol. The non-ionic surfactants form a closed bilayer vesicle in aqueous media based on its amphiphilic nature using some energy for instance heat, physical agitation to form this structure. In the bilayer structure, hydrophobic parts are oriented away from the aqueous solvent, whereas the hydrophilic heads remain in contact with the aqueous solvent[3,4].

The general method of preparation of niosomes involves evaporation to produce a lipid film followed by hydration with the hydration medium. However, there are four methods to prepare the niosomes[5].

1. Handshaking method (Lipid film hydration)

2. Reverse phase evaporation

3. The Bubble method

4. Microfludisation.

The aim of this work is to develop and formulate Niosomes containing Azithromycin as a model drug. The main objective of the present study is to carry out formulation and evaluation of niosomes by using suitable polymers Phosphatidyl Choline, Dicetyl Phosphate, Cholesterol and nonionic surfactants like Span60 were selected to formulate the vesicles of niosomes by handshaking method is also known as lipid film hydration method[1,2].

The objectives of the present study as follows:

- Pre formulation studies for selection of suitable excipients to develop the dosage form based on physicochemical properties of drug and excipients.
- Screening of excipients for compatibility and efficacy for developing the formulation.
- Carry out Preformulation study of Azithromycin drug.
- Preparation of Niosomal drug delivery system.

- Evaluation of Niosomal drug delivery system, including *in- vitro*, Optimize the formulation using experimental design technique regarding particle size, particle size distribution, Scanning electron microscopy, stability, release profile, etc.

- Study the stability of the formulation following ICH guidelines.

MATERIALS AND METHODS:

Methods:

Preparation of standard curve:

Preparation of stock I Solution:

50 mg of Azithromycin was weighed accurately, transferred into 50 ml volumetric flask. The volume was made to 50 ml with PBS pH 7.4[6] to give 1000 mcg/ml solution.

Preparation of Stock II Solution:

0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml, 1.5ml of the stock I so was transferred to 10mlvolumetric flasks individually and made up the volume to 10 ml with PBS pH 7.4.

Preparation of standard curve for Azithromycin using PBS pH 7.4[7] :

2 mcg, 4 mcg, 6 mcg, 8 mcg, 10 mcg, 15mcg were prepared by taking 1 ml each of stock II solution and volume made up to 10 ml. The absorbances of respective solutions were determined using UV-Visible spectrophotometer at 287 nm against PBS pH 7.4 as the blank. The experiment was repeated six times in the same medium and a standard curve was determined from the mean value.

Preparation of co-encapsulated Azithromycin niosomes by handshaking method:

The niosomes were prepared using the handshaking method. 100 mg of Azithromycin, 10 mg of Dicetyl Phosphate, Span 60 and cholesterol was dissolved in chloroform in a 100 ml round bottom flask. The organic solvent was removed by rotating the flask at a temperature of 37°C under reduced pressure. The slight trace of the solvent was removed with oxygen-free nitrogen for about10 minutes.

CHARACTERIZATION OF DRUG DELIVERY SYSTEM

Determination of size distribution:

The vesicle size was determined by dynamic light scattering method (DLS) in a multimode using a computerized inspection system (Malvern Zetamaster, ZEM5002, Malvern, UK). For vesicle size measurement vesicular suspension was mixed with the appropriate medium (PBS pH 7.4) and the measurements were conducted in triplicate.

Entrapment efficiency of co-encapsulated niosomes:

One ml of prepared niosome suspension was placed in the centrifuge and centrifugation was carried out for 5min at 2000rpm. Vesicles of niosomes separate as a pellet. The vesicles that were collected were ruptured using triton-x 100 and the released drug was analyzed using UV spectrophotometer at 287 nm.

EVALUATION OF NIOSOMES:

Drug-excipient compatibility study by FTIR[8,9]

The FT-IR spectrum of Azithromycin, pure span 60, pure cholesterol, pure diacetyl phosphate physical mixture of polymers and drug were analyzed for their compatibility. By taking IR spectra we can determine the drug and polymer compatibility of various pharmaceutical preparations.

IR spectra of pure drug, excipients, all physical mixtures and all type of formulations along with drug in KBr pellets at moderate scanning speed between 4000-400 cm^{-1} was carried out using FT-IR. The peak values and the possibility of a functional group shown in spectra were compared with standard values.

In- vitro drug release studies for niosomes co-encapsulated with Azithromycin:

The Niosomal formulation filled in a dialysis tube to which a sigma dialysis sac was attached to one end. The dialysis tube was suspended in PBS pH 7.4, stirred with a magnetic stirrer and samples were withdrawn at specific time intervals and analyzed using UV spectrophotometer at 287 nm to maintain a constant volume, an amount of medium equivalent to the volume of sample is withdrawn was added immediately.

Stability studies of niosomes co-encapsulated Azithromycin niosomes[10]:

The formulation was divided into equal portions. Each portion was filled into amber colored ampoules and sealed. In case of the niosomes, the formulation and the headspace in the vials was flushed with nitrogen before sealing. The formulations were kept for stability studies at 4 ± 1º C and at 30 ± 2º C for 3 months. The samples were withdrawn at predetermined time intervals and the amount of drug still remaining intact within the vesicles was determined. At the end of the study period, the vesicles were examined for change in the size under the microscope and any changes in the same were reported.

RESULTS AND DISCUSSION:

In the present study, the causative organism is known to develop resistance if drug blood level remains below the minimum effective concentration leading to clinical failure. These drugs have various side effects like immunological disturbance, rheumatoid or lupoid syndromes, allergic rashes, eosinophilia, leukopenia, jaundice manifestations.

The present work is an attempt to provide a maximum concentration of the drug by encapsulation using different carriers to exclude undesirable side effects and minimize the risk of drug resistance.

ANALYTICAL METHODOLOGY

Preparation of standard curve of Azithromycin in PBS pH 7.4 by UV Spectrophotometer:

Table No: 1. A standard curve of Azithromycin

S.No	Concentration (µg/ml)	Absorbance at 287 nm
1.	0	0
2.	5	0.109
3.	10	0.230
4.	15	0.376
5.	20	0.446
6.	25	0.554

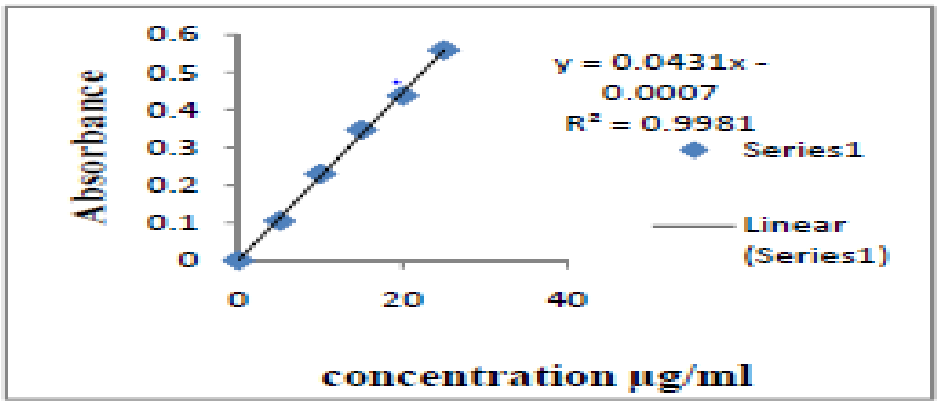

Figure No: 1. Standard graph of Azithromycin

CHARACTERIZATION OF DRUG DELIVERY SYSTEM

The formulated niosomes was characterized for size analysis, entrapment efficiency, an effect of lipid concentration of Span 60, the effect of cholesterol concentration and *in -vitro* release.

Vesicle characterization and Size distribution:

Increase in surfactant concentration leads to a decrease in vesicle size. This is observed due to the decrease in surface energy with increasing hydrophobicity. Whereas increase cholesterol content increases the vesicle size. Cholesterol increases the width of the lipid bilayer and consequently increases the vesicle size by altering the fluidity of chains in bilayers. Diacetyl Phosphate helps in disaggregation of the vesicles and thereby results in stabilizing the formulation.

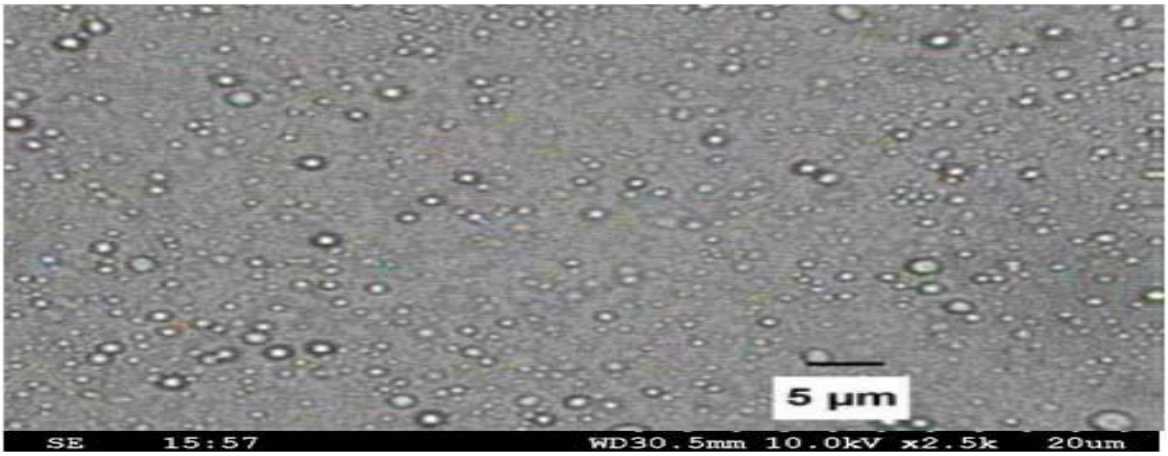

Figure No: 2. Particle shape analysis of F2

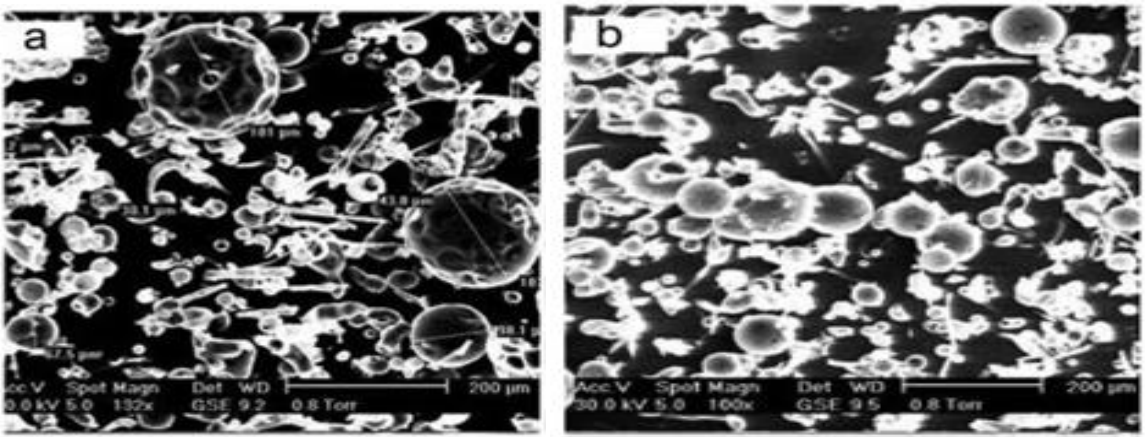

Figure No: 3. Particle shape analysis of F2

Table No: 2. Entrapment Efficiencies of Various Formulations

Formulation	Preparative method	Drug (mg)	Span 60 (mg)	Cholesterol (mg)	Dicetyl Phosphate (mg)	Entrapment efficiency %
F1	Hand Shaking Method	100 mg	50	0	10 mg	65.2±1.3
F2			50	50		84.4±1.2
F3			75	50		75.5±1.2
F4			100	50		79.5±1.7
F5			50	75		83.4±1.1
F6			50	100		87.5±1.8

EVALUATION OF NIOSOMES:

Drug-excipient compatibility study by FTIR

In the present study, the possible interaction between the drug and excipients was studied by FTIR spectroscopy. The spectra of pure compound results revealed no considerable changes in the FTIR peaks when mixed with excipient compared with pure compounds. We were observed for the presence of characteristic peaks for the respective functional group in the compound and no identical changes have been observed.

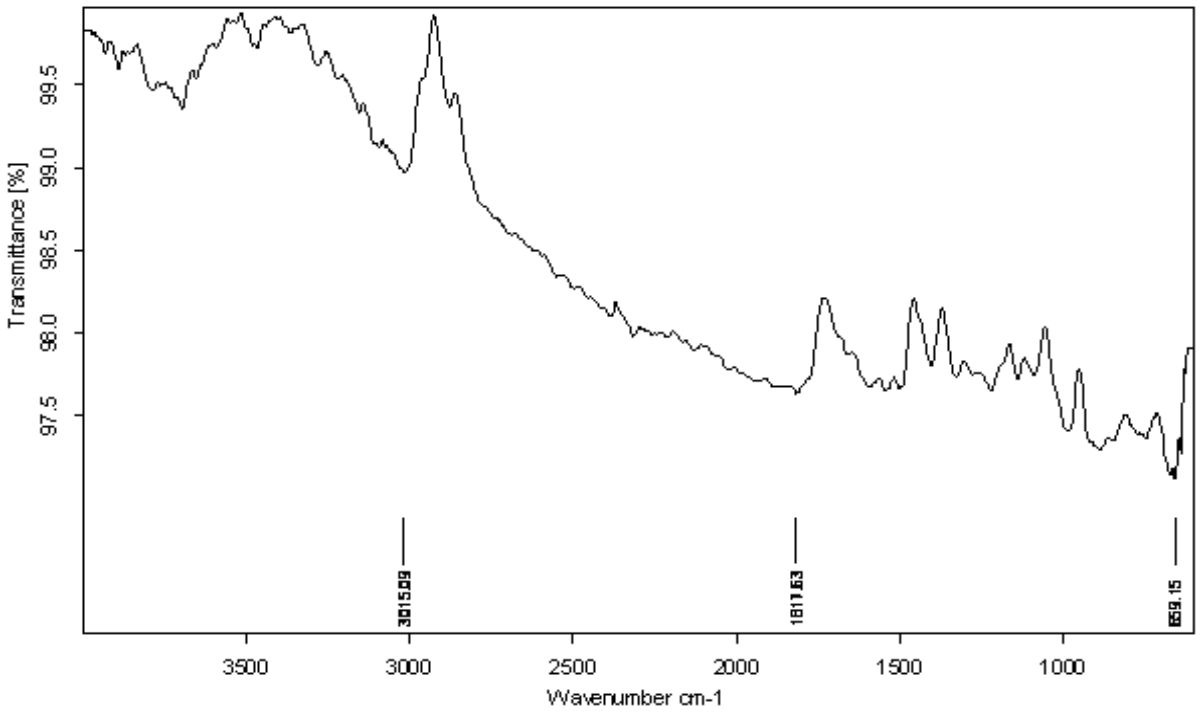

Figure No: 4. IR spectra of pure Azithromycin

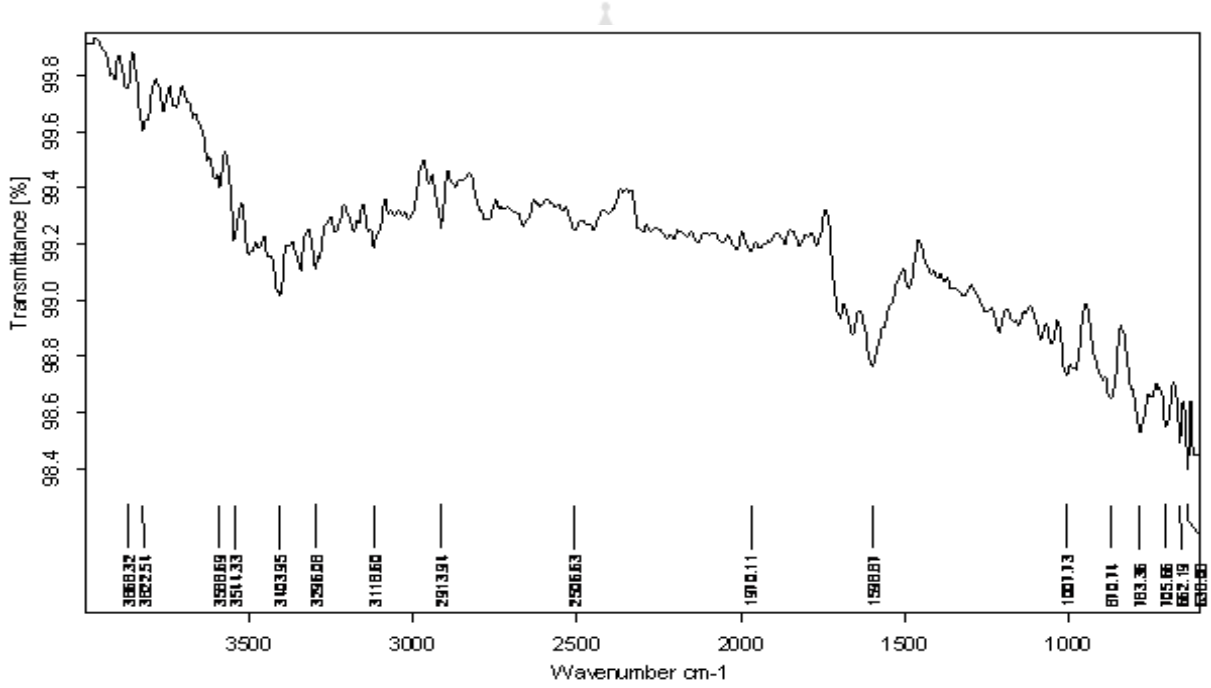

Figure No: 5. IR spectra of the physical mixture of polymers and drug.

IN -VITRO RELEASE KINETICS OF NIOSOME FORMULATION

In- vitro, drug release profile of niosome encapsulated Azithromycin in PBS pH (7.4)

Table No: 3. *In-vitro* release of Azithromycin

Time	F(free)	F1 (50:0)	F2(50:50)	F3(75:50)	F4(100:50)	F5(50:75)	F6(50:100)
0min	0	0	0	0	0	0	0
30min	20.623±1.2	10.523±1.3	7.667±0.98	5.783±1.2	4.783±1.1	3.893±1.5	2.981±0.9
1hr	31.623±1.7	15.641±1.1	11.786±1.3	8.611±1.1	6.213±1.3	5.113±1.2	4.923±1.4
1.30hr	45.432±1.6	20.782±1.2	16.685±1.1	11.324±1.1	8.425±0.92	7.235±1.5	6.723±1.7
2hr	60.741±1.8	24.625±1.7	20.581±1.3	16.528±1.9	12.645±1.3	9.436±1.4	8.633±1.5
4hr	82.811±1.6	30.723±1.2	25.612±1.8	20.621±1.7	18.761±1.7	11.325±1.2	10.413±1.9
6hr	95.125±1.7	37.812±1.5	32.716±1.2	26.782±1.5	23.554±1.8	18.426±1.4	15.614±2.1
8hr		45.316±1.9	41.812±1.2	31.912±1.7	24.863±1.8	25.816±1.8	19.189±1.1
10hr		52.512±2.1	52.613±1.5	36.783±1.5	31.923±1.6	28.126±1.5	22.854±1.2
12hr		60.124±1.4	65.714±2.1	41.169±1.4	35.117±1.7	32.815±1.3	29.816±1.3
24hr		82.164±1.2	86.913±1.5	62.189±1.5	52.915±1.6	49.615±1.1	42.716±1.2

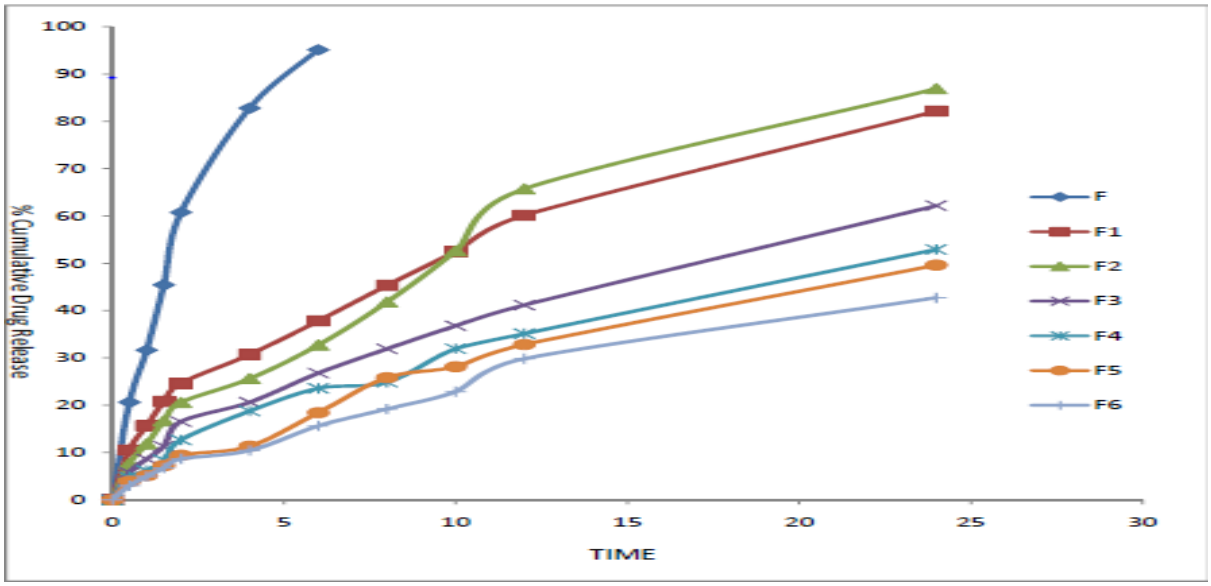

Figure No: 6. *In-vitro* release of Azithromycin

STABILITY STUDIES

Table No: 4. Stability studies of co-encapsulated Azithromycin niosomal formulation at 4 ± 1ºC and at 30 ± 2º C

Formulation code	Initial %	Percentage(%) of residual drug					
		At 4±1ºC			At 30±2ºC		
		1month	2months	3months	1month	2months	3months
F2	100	98.52 ± 2.28	95.2 ± 1.56	91.84 ± 2.82	72.5 ± 2.52	54.2 ± 2.68	39.53 ± 1.28

From the present investigation, we found that co encapsulated preparation were satisfactorily stable up to 3 months at 30 ± 2º C and 60 ± 5% RH. During storage, drug leakage and loss in a number of vesicles were observed. Lipid vesicles are self-assembles of amphiphiles into closed bilayer structures. Hydrated bilayer vesicles, however, are not considered to be thermodynamically stable and are thought to represent a metastable state in that the vesicles possess an excess of energy bilayer phospholipids, which can undergo chemical degradation such as oxidation and hydrolysis. Due to this change, vesicular systems maintained in aqueous dispersion may aggregate/fuse, and encapsulated bioactive material may tend to leak out from the bilayer structure during storage. The loss of vesicle could be attributed to the disruption/aggregation of vesicles. At 4±1ºC, a minimum loss of drug was observed, which might be attributed to the realization of the vesicles at low temperature that reduced the permeability of the drug through the membrane. Thus, from the results obtained, it can be concluded that prepared vesicular systems are more stable at 4±1ºC, as compared to storage at 30±2ºC in terms of mean vesicle size, a number of vesicles per cubic millimeter, and residual drug content.

KINETIC MODELING

Table No: 5. Kinetic modeling for Azithromycin:

Formulation	Zero-order R^2	First order R^2	Higuchi's model R^2	Peppa's model (n)
F1	0.915	0.449	0.995	0.577
F2	0.938	0.530	0.972	0.673
F3	0.958	0.543	0.991	0.704
F4	0.942	0.579	0.987	0.756
F5	0.959	0.647	0.970	0.808
F6	0.964	0.658	0.966	0.826

SUMMARY:

In the last few years, we have witnessed an explosion in research aimed at creating new drug delivery systems. Varieties of novel carriers differing in the degree of sophistication are available to control, sustain and target the drugs. Although delivery systems that could target drugs to specific body sites or precisely control drug release rates for prolonged times have long been dreamed of, only in recent years, the development of such systems has become practical. Yet, in the short time, new drug delivery systems have had an impact on nearly every branch of medicine including cardiology, ophthalmology, endocrinology, pulmonology, immunology, pain management, and oncology.

The design and development of formulations and methods of delivery for antibiotic agents are dependent on several variables. The relationship between the formulations, route of delivery, pharmacokinetics, toxicity and clinical indication must be carefully balanced to successfully develop a suitable drug delivery system.

The *in-vitro* results showed slow and prolonged release characteristics. The degradation pathway and their stability should be systematically analyzed and competing for degradation rates must be balanced to arrive at the most stable formulation possible. The formulation characteristics may have a dramatic impact on the *in-vivo* stability of the drugs.

The drug disposition by niosomal drug delivery proved that the drug accumulated in the visceral organs (lung, kidney, liver) was lower than free drug. This proved that the niosomal drug delivery system has lesser toxicity than free drug.

CONCLUSION:

To conclude the niosomal drug delivery of Azithromycin would act as a suitable technique to enhance the bioavailability of Azithromycin than the conventional dosage form. It is also concluded that niosomes will control the drug release, from the executed experimental results, it could be concluded that the surfactants and excipients, were suitable carriers for the preparation of Azithromycin niosomes.

REFERENCES:

1. Allen TM. Liposomal drug formulations: Rationale for development and what we can expect for the future. Drugs. 1998;56:747–56.
2. Malhotra M, Jain NK. Niosomes as drug carriers. Indian Drugs. 1994;31:81–6.
3. Udupa N. Niosomes as drug carriers. In: Jain NK, editor. Controlled and novel drug delivery. 1st edition. New Delhi: CBS Publishers and Distributors; 2002.
4. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The Preparation and properties of Niosomes-Non ionic surfactant vesicles. J Pharm Pharmacol. 1985;37:863–8.
5. Jadon PS, Gajbhiye V, Jadon RS, Gajbhiye KR, Ganesh N. Enhanced oral bioavailability of griseofulvin via niosomes. AAPS PharmSciTech. 2009;10:1186–92.
6. Indian pharmacopeia 2010 ; Vol – I ; Page no : 563.
7. Indian pharmacopeia 2010 ; Vol – II ; Page no : 857.
8. Robert M. Silverstein, Francis X. Webster. Infrared Spectrometry. In: Robert M. Silverstein, Editors. Spectrometric Identification of Organic Compounds. 6th Ed, John Wiley, and Sons. Inc. New York, Pg.No: 71–143.
9. Y. R. Sharma. Elementary Organic Spectroscopy, Principles, and chemical applications, Pg.No: 69-339.
10. Kulkarni G T, Gowthamarajan K, Suresh B, "Stability Testing of Pharmaceutical Products: An overview" Indian Journal of Pharmacy Education, 2004, Vol-38, Issue-4, Pg.No: 194-202.