World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Formulation and characterization of maltodextrin based proniosomes of cephalosporins

Preethy Cheriyan[1]*, Boby Johns George[1], Noby Thomas[1], Praveen Raj[1], Jeny Samuel[1] and Sr. Betty Carla[2]

[1]Department of Pharmaceutics, St. Joseph's College of Pharmacy, Cherthala, Kerala, India
[2]Principal, St. Joseph's College of Pharmacy, Cherthala, Kerala, India

Received: 16-12-2014 / Revised: 24-12-2014 / Accepted: 27-12-2014

ABSTRACT

Nowadays, vesicles have become the carrier of choice in drug delivery. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both hydrophilic and lipophilic drugs. In the present study efforts were taken to prepare maltodextrin based proniosomes of cefuroxime axetil. A total of seven formulations were prepared, by varying the surfactant-lipid loading in each formulation. Various evaluation techniques were employed in order to study the performance of the preparations. The micromeritics properties of each preparation were analyzed and they were also subjected to *in vitro* release study, kinetic data analysis, *ex vivo* permeation study, stability study etc. From the results of entrapment studies, it could be concluded that the formulation PN4 which is having surfactant: lipid concentration as 1:1 was the best formulation. The SEM image as well as the FT-IR spectrum of the optimized formulation was taken. The mean particle range of proniosomes was between 10.23-22.25µm.

Keywords: Cefuroxime axetil, Proniosome, Slurry method, *in vitro* drug release, *ex vivo* absorption study, SEM, stability study.

INTRODUCTION

In the past few decades, considerable attention has been focused on the development of new drug delivery systems (NDDS). Among them vesicular drug delivery systems are of much importance. Various types of vesicular drug delivery systems include, liposomes, niosomes, transferosomes, pharmacosomes, ethosomes, sphinosomes, colloidosomes, herbosomes, and cubosomes etc. The approaches like provesicular drug delivery like proniosomes, layerosomes, ufosomes etc have also been developed which have better stabilities in comparison to simple vesicular drug delivery systems. In order to counteract the stability problems associated with niosomes (degradation by hydrolysis or oxidation and sedimentation, aggregation, or fusion during storage) proniosomes were developed. Proniosome - A dry niosome which could be easily hydrated to niosome before use. Proniosomes are dry, free flowing, granular product which upon addition of water, disperses or dissolves to form a multilamellar niosome suspension suitable for administration by oral or other routes. The additional convenience of the transportation, distribution, storage and dosing would make proniosomes a promising industrial product. Among the cephalosporins, cefuroxime axetil belongs to second-generation antibiotic, having the broad spectrum of activity and is active against β-lactamase producing strains. It is an ester prodrug of cefuroxime. Its activity depends upon *in-vivo* hydrolysis and release of cefuroxime. Cefuroxime is rendered more lipophilic by esterification of the C_4 carboxyl group of the molecule by the racemic 1-acetoxyethyl bromides, thus enhancing oral absorption. Cefuroxime axetil is an orally active drug though its absorption is incomplete. Its bioavailability is only 25%. It is the axetil form of cefuroxime that is absorbed but when it is hydrolyzed to cefuroxime, its permeation is low. So encapsulation of drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation and perhaps increase the bioavailability. Also some drugs, most notably antibiotics, lose their potency in a relatively short period when prepared in a liquid dosage form. To enhance the shelf-life of these drugs, manufacturers provide products to the pharmacy in dry powder form for *constitution* (or *reconstitution*) with purified water or special diluent at the time a prescription or medication order is received. Depending on the product, the dry powder may be stable for about 24 months.

***Corresponding Author Address:** Preethy Cheriyan, Department of Pharmaceutics, St. Joseph's College of Pharmacy, Cherthala, Kerala, India; E-mail: preethycheri@gmail.com*

After constitution, the resultant solution or suspension is stable in the quantities usually dispensed, for the treatment period. In the present study an attempt is made to develop, optimize and evaluate maltodextrin based proniosomes containing cefuroxime axetil using selected surfactant and studying there *in vitro* properties.

MATERIALS AND METHODS

Materials: Cefuroxime axetil was obtained as a gift sample from Covalent Laboratories Pvt Ltd. Cholesterol obtained Qualigens Fine Chemicals Thermo Electron LLS India Pvt Ltd. Span 60 obtained from Chemdyes Corporation.Chloroform and methanol from Nice chemicals Pvt Ltd. Dialysis membrane for the study of in-vitro release obtained from Hi media Laboratories Pvt Ltd India. All other materials used and received were of analytical grade.

Method of preparation and the evaluation of proniosomes

Preparation of proniosomes: Proniosome powders were prepared by using slurry method. The composition of different proniosomal formulations is represented in Table 1. In brief, accurately weighed amounts of lipid mixture (1500 µM) comprising of span 60 and cholesterol at various molar ratios (4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4 respectively) and drug (250 mg) were dissolved in 60 mL of solvent mixture containing chloroform and methanol (2:1). The resultant solution was transferred into a 600 mL round bottomed flask and maltodextrin (1.5g) was added to form slurry. The flask was attached to a rotary flash evaporator and the organic solvent was evaporated under reduced pressure at a temperature of 45 ± 2 ^{0}C. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature so as to obtain dry, free-flowing product. The obtained proniosome powders were stored in a tightly closed container at 4^0 C for further evaluation.

EVALUATION OF PREPARED PRONIOSOME POWDERS

a) Formation of Niosomes from Proniosome Powders and Morphological Evaluation: The formation and morphology of the niosomes was evaluated by optical microscopy. The proniosome powder was placed on a cavity glass slide and few µl of water was added drop wise along the side of the cover slip. The formation of vesicles monitored through an optical microscope and photomicrographs were taken. For the morphological evaluation, proniosome powder was transformed to niosomes by hydrating with phosphate buffer (pH 7.4) at 80^{0}C using vortex mixer for 2 min. The niosome dispersion was placed over a glass slide and the vesicles formed were observed at a magnification of 450x through an optical microscope.

b)Micromeritic properties of proniosome powders: The flow properties of powder are vital in handling and processing operations. The flow properties were studied through measuring the Angle of repose, Carr's compressibility index and Hausner's ratio. The angle of repose was determined by using conventional fixed funnel method. The Carr's compressibility index and Hausner's ratio were calculated from the bulk and tapped density of the proniosome powders.

c) Number of vesicles per cubic mm: One of the important parameter to evaluate the proniosome powder is the number of vesicles formed after hydration. The proniosomes powder was subjected to hydration with phosphate buffer (pH 7.4) and the formed niosomes were counted by optical microscope using haemocytometer. The niosomes in 80 small squares were counted and calculated.

d) Vesicle Size: This is performed for characterization of vesicle's size and shape.The proniosomal powders were hydrated with phosphate buffer (pH 7.4) and subjected to bath sonication for 3 min and the resultant dispersion was used for the determination of size. Vesicle size of niosomes were determined by using optical microscopy method using calibrated optical microscope (By Ocular and Stage micrometer)

e) Entrapment Efficiency: The prepared cefuroxime axetil niosomes were separated from unentrapped drug by centrifugation. In this method, hydrated proniosomes were centrifugated at 14000 rpm for 5 minutes using the refrigerated centrifuge and the supernatant were analyzed for free drug content.

f) Drug Content: Proniosomes formulation equivalent to 250mg of cefuroxime axetil was taken into a standard volumetric flask. They were lysed with 50ml propane-1-ol by shaking and 1ml of the mixture subsequently diluted with phosphate buffer (pH 7.4). The absorbance was measured spectroscopically at 281nm and drug content calculated from the calibration curve of cefuroxime axetil in phosphate buffer (pH 7.4).

g) *In vitro* release study: *In vitro* release rate of proniosomes, derived niosome dispersion was carried out and the drug in ph 7.4 phosphate buffer was used as a control. Initially the treatment of

dialysis membrane (Hi media) was done by washing in running water for 3-4 hours to remove glycerin. Then it was washed in boiling water at 60^0C for 0.5 hours. Finally the membrane was rinsed with water and stored in phosphate buffer pH 7.4. Then 2ml of niosomal dispersion was placed inside the pretreated dialysis membrane, tied at both the ends. It was then transferred to a beaker containing 100ml of phosphate buffer with 50% methanol. The assembly was stirred on a magnetic stirrer at 37^0C. 1ml samples were withdrawn at fixed intervals and replaced with equal volume of fresh media. The samples withdrawn were analyzed for drug UV spectroscopy.

h) Drug release kinetic data: In order to understand the kinetic and mechanism of drug release, the result of *in-vitro* drug release study of niosomes were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs time), Higuchi's model(cumulative % drug release vs. square root of time). r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots. The data was also subjected to Peppa's kinetic model by plotting log % cumulative drug released Vs log time plot.

i) Solid state characterization

Scanning electron microscopy (SEM): The surface characteristics of the maltodextrin and proniosome powder was investigated by scanning electron microscope (SEM) (S-4100, Hitachi, Japan). Samples were fixed on a brass tub using double sided adhesive tape and were made electrically conductive by coating with a thin layer of gold and SEM images were recorded at 15 kev accelerating voltage.

Fourier transform infrared (FT-IR) spectroscopy: Infrared spectrum of optimized proniosome powder formulation (PN4) was obtained using FT-IR spectrophotometer (shimadzu) by the conventional KBr pellet method. The scanning range was 4000–500 cm^{-1} and the resolution was 4 cm^{-1}.

j) *Ex-vivo* permeation studies: The absorption study of proniosomes formulation was done by everted sac method . The small intestine of chicken was taken; it was washed with phosphate buffer pH 7.4, 6-7 times. Then the intestine was everted using a smooth everting rod. It was then cut into 4-6cm pieces. One end of the intestine pieces was tied with thread, then 1ml of fresh buffer pH 7.4 was introduced in the intestine, and the other end was also tied. The intestine pouch was put in a beaker 50ml of niosomal dispersion (proniosome powder hydrated with phosphate buffer pH 7.4) equivalent to 2 mg of drug which is oxygenated continuously. At predetermined time intervals, an aliquot of 1 ml was collected and replenished with equal volume of medium. The samples were treated with an equal volume of methanol, centrifuged and the supernatant was quantified for cefuroxime axetil using UV spectroscopy.

k) Permeation data analysis: The cumulative amount of drug permeated (Q) was plotted against time. The steady state flux (Jss) was calculated from the slope of linear portion of the cumulative amount permeated per unit area vs. time plot.

l) Stability studies: The three formulations; PN3, PN4, and PN5 were stored in glass vials covered with aluminum foil were kept at refrigerated temperature (2-8^0c), room temperature and 40^0c (75%RH) as three different groups. Stability chamber was used for the third group. At definite time intervals (0, 30, 60 and 90 days), samples were withdrawn and hydrated with phosphate buffered saline pH (7.4) and observed for any sign of drug crystallization under optical microscope. Further the samples were also evaluated for % retention of cefuroxime axetil.

m) Test of significance: The stability data analyzed for significant difference between retention patterns of drug in three different proniosomal formulations on storage. The test value showed no significant difference ($P>0.05$) between the stability data of formulations from each other.

RESULTS AND DISCUSSIONS

Despite the advantages, niosome dispersions suffer from stability problems like aggregation, hydrolysis, drug leakage and production scale up. In this perspective, proniosome approach has resolved many stability issues pertaining to aqueous niosome dispersions. The stability of the vesicles formed after hydration with gastric fluids is also equally important for achieving maximum therapeutic benefit from the proniosomes formulations. In this regard several strategies have been employed to improve the stability of the vesicular systems. Cholesterol is the common additive used as a structural lipid to improve the stability and entrapment efficiency of vesicular formulations. The morphology and stability of the niosomes is by and large dependent on the concentration of nonionic surfactant and cholesterol and any alteration in their composition leads to disruption of vesicles which leads to leakage of free drug before drug diffusion and fusion of vesicle with gastrointestinal membrane . Keeping this in view, the effect of cholesterol was investigated by varying the composition of span 60

to cholesterol ratio keeping the total lipid constant at 3000µM. The different span types have the same polar head group with varied alkyl chain and highest entrapment could be observed with an increase in phase transition temperature of span . The phase transition temperatures for span 20, 40 and 60 are 16, 42 and 53 ^{0}C, respectively and span 80 having the lowest phase transition temperature at ˉ12 ^{0}C. Due to the high phase transition temperature, span 60 was used in our study to facilitate stable vesicle formation and to improve the oral delivery of cefuroxime axetil from proniosomes.The formation and morphology of the niosomes was evaluated by optical microscopy. The niosome dispersion was placed over a glass slide and the vesicles formed were observed at a magnification of 450x through an optical microscope and photomicrographs were taken.The formation of niosomes from proniosome powder was spontaneous as evident from figures. Initially we could notice the formation of vesicular structures on the surface of maltodextrin which is due to the swelling of nonionic surfactant bilayer and thereby upon gentle agitation they have been transformed into multilamellar vesicles acquiring spherical shape.Our results indicate small angle of repose (<30^0) assuring good flow properties for proniosome powder formulations. In addition to angle of repose, Carr's index and Hausner's ratio were also less than 21 and 1.25, respectively ensuring acceptable flow for proniosome powder formulations. The results are shown in Table 2. The maximum benefit from the proniosome formulations can be speculated when abundant numbers of vesicles are formed after hydration in the gastrointestinal tract. Among all the formulations, the proniosome formulation containing span 60 and cholesterol at a ratio of 1:1 (PN4) has exhibited good number of vesicles which is in well correlation with the size and entrapment efficiency results.Vesicle size and size distribution is an important parameter for the vesicular systems. The mean size of the vesicles was in the range of 10–23 µm. Further, the results also reveal that the entrapment efficiency is dependent on the composition of niosomes. Addition of cholesterol to the formulation seemed to increase the entrapment efficiency from PN1 to PN4. Also the rigidity thereby the stability was also increased. The increase in entrapment efficiency with addition of cholesterol could be explained by the fact that cholesterol was intercalated into the bilayers, thereby preventing the leakage of the drug through the bilayers. The permeability of the vesicles are decreased leading to the effective intercalation of hydrophobic drug within the hydrophobic core of the bilayer with an enhanced drug pay load. But a decline in the entrapment efficiency (PN5-PN7) beyond a certain cholesterol level could be attributed to the reason that when cholesterol is increased beyond the saturation limit, all the available spaces between the bilayers are filled up with the hydroxy moiety of cholesterol.

Drug content: The drug content in all the seven formulations were analyzed and are included in Table 4. The results were found to be satisfactory with the optimized formulation, PN4 giving 85.3% drug content, which was the highest value of all other formulations.

***In vitro* release study:** The release study was conducted for all the seven formulations. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 80% release within a period of 24 hours. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment. The best formulation F4, was found to give a cumulative release of 88.58% over a period of 24 h.The zero order plots of formulations were found to be fairly linear as indicated by their high correlation values. Therefore, it was ascertained that the drug release from all the formulation followed either near zero or zero order kinetics. Correlation values of Higuchi's plot were in between 0.875 to 0.962 which revealed that the mechanism of drug release is diffusion. From the results we can conclude that the drug was released from niosome by a zero order diffusion controlled mechanism. The *in vitro* kinetic data subjected to log % drug remaining Vs time plot (peppa's model), all the value ranges from 0.914 to 0.952 revealed the fact that the drug release follows a diffusion mechanism.

Solid state characterization: The molecular interactions between drug and carrier were studied using Scanning electron microscopy and Fourier transform infrared spectroscopy. Maltodextrin was selected as a carrier for proniosomes powders. It is evident from the SEM images that the maltodextrin possess porous surface with high surface area which enables it to be used as an efficient carrier for the lipid loading. Further the SEM images reveal the absence of native crystalline structures of cefuroxime axetil in the proniosome powder.

FT-IR spectrum of proniosome: The FT-IR spectra of cefuroxime axetil, maltodextrin, span 60 and optimized proniosome formulation (PN4) were taken. The pure cefuroxime axetil exhibit characteristic peaks at the –NH stretching peaks

around 3400, aromatic H 2818-3000, Ketone C=O (acid) around 1734-1785, aromatic CH bending at 1580-1600, other aromatic bending were observed at 1060-700. All these peaks have appeared in proniosome formulation(PN4) at 3409.37cm-1(-NH stretching), 2919.81 cm-1, 2850.92 cm-1(aromatic H), 1738.02 cm-1 (Ketone C=O),1099.70 cm-1, 883.39 cm-1, 722.40 cm-1 (aromatic bending). This indicate no chemical interaction between cefuroxime axetil, maltodextrin, cholesterol and span 60.

***Ex-vivo* Permeation Studies:** It has been documented that in dissolved condition, the hydrolysis of the Cefuroxime axetil occurs, and it gets converted into the Cefuroxime and the absorption of the Cefuroxime as such is negligible. It is the axetil form of the Cefuroxime that is absorbed. But when the drug is entrapped in the vesicles, it can easily cross the membrane *in-vivo*, protect the drug from acid environment and additional mechanism apart from phagocytic uptake which occurs thereby enhancing the drug absorption. Hence it can be ascertained that it is the drug in vesicles that are permeated across the intestinal segment. The flux across the intestine was found to be greater for the formulation PN4 and the cumulative amount of drug permeated was 22.98% for the same. The cumulative amount permeated (CAP) across the intestine was calculated and represented in fig.9. The remarkable improvement in the permeation can be owed to the combination of several mechanisms: (i) presence of nonionic surfactant could obviate the barrier function due to the fluidization of the intercellular lipid bilayer; (ii) better membrane contact and permeation enhancement property of the non-ionic surfactants might have led to altered permeability characteristics of the membrane which otherwise result in improved partitioning of the drug into the bilayer; (iii) direct transfer of vesicles across the epithelial membrane. Thus it can be summarized that , proniosomes are the promising carriers for improved absorption of cefuroxime axetil across the biological membrane.

Stability Study: Physical stability was carried out to investigate the leaching out of Cefuroxime axetil from niosomes. Three formulations; PN3, PN4, and PN5 were kept at refrigerated temperature (2-8^{0}c), room temperature and 40^{0}c (75%RH) as three different groups. Stability chamber was used for the third group. The group which kept at refrigerated temperature showed promising results of 95% drug retained after 90 days,the group which is kept at room temperature gave 83% drug retained and the group which is kept at 40^{0}c,75% RH gave only 75% drug retained after 90 days. So the best three formulations kept at refrigerated temperature for a period of three months gave best results for storage and stability. From this it can be concluded that vesicles are stable enough to store under refrigeration temperature with least leakage.

Test of Significance: The stability data analyzed for significant difference between retention patterns of drug in three different proniosomal formulations on storage. The test value showed no significant difference (P>0.05) between the stability data of formulations from each other.

CONCLUSION

The results of all this investigation conclusively demonstrate prolongation of drug release at a constant and controlled rate, after encapsulation of cefuroxime axetil. This study suggests that niosomal formulation can provide consistent and prolonged release of cefuroxime axetil from different niosomal formulations. It will lead to sustained action of the entrapped drug that reduce the side effects associated with frequent administration of the drug and potentiate the therapeutic effects of the drug. This may also provide the high absorption inside the lumen and the same may be expected even in blood circulation. The dry granular proniosomes which has been anticipated to provide improved stability as compared to conventional vesicular delivery systems in terms of aggregation, leakage etc. For the better efficacy of the formulation refrigeration of the product is necessary. The future scopes for the work are the microbial as well as *in vivo* studies.

ACKNOWLEDGEMENTS

Thanks to the Department of pharmaceutics, St Joseph's College of Pharmacy, who provided us with valuable information during the experiment. My acknowledgements to all who helped us to carry out and finish this work

Figure 1: Prepared proniosome powder.

Figure.2: Photomicrographs of cefuroxime axetil niosomes in a dry glass slide

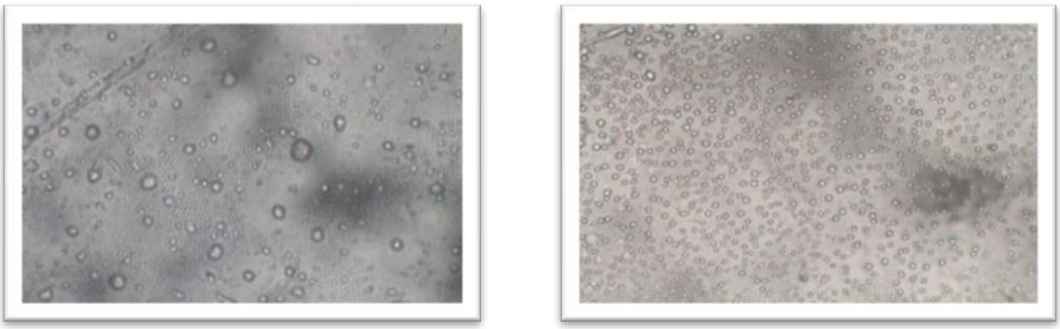
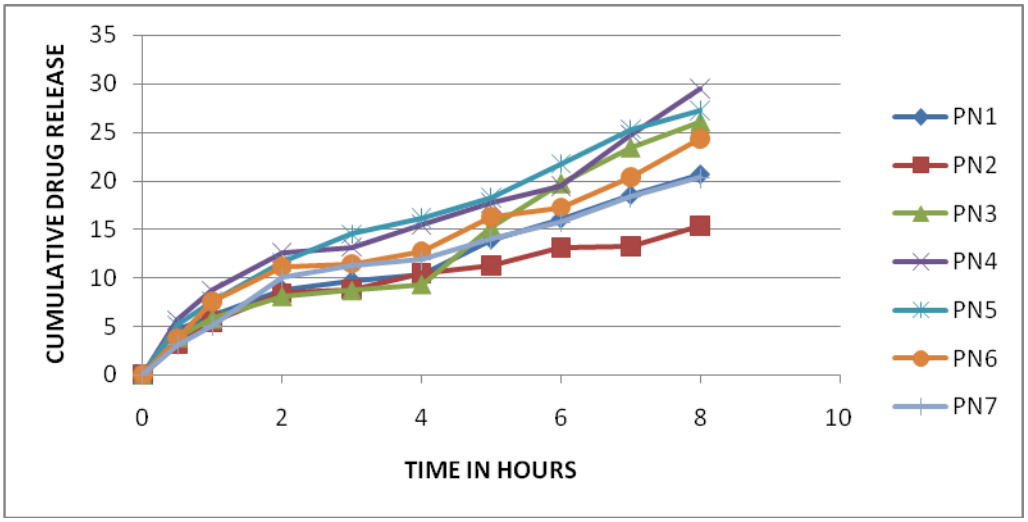


Figure.3: Percent cumulative drug release Vs time plot of in vitro drug release



DRUG RELEASE KINETIC DATA

Figure.4: Zero Order Kinetic Data Analysis Plot

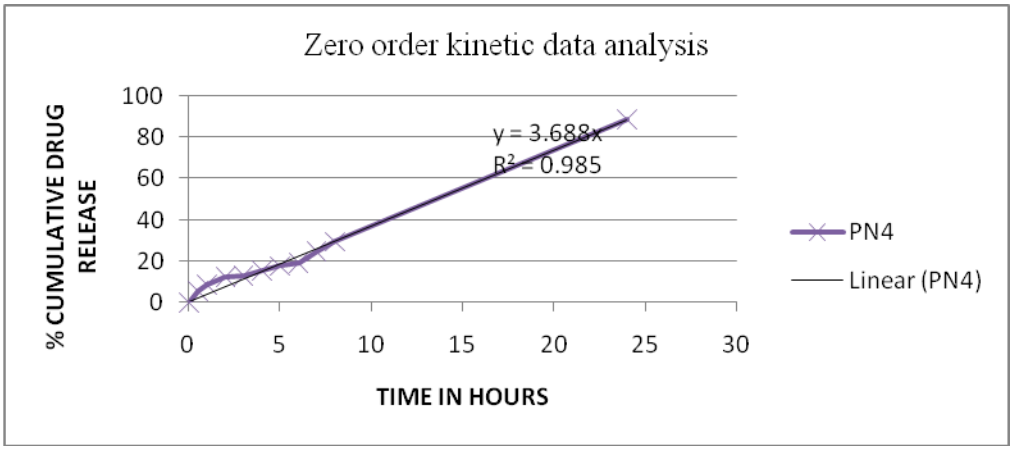


Figure.5: Higuchi model kinetic release data analysis plot

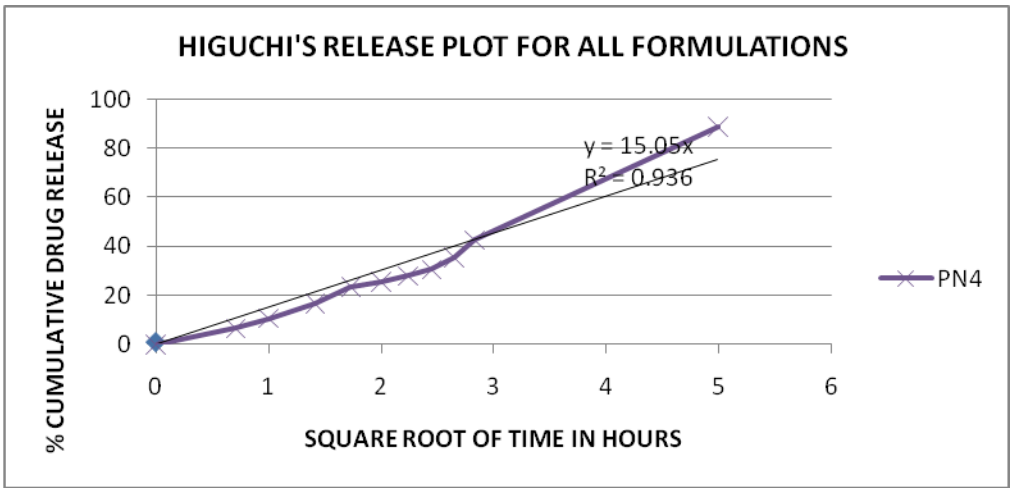


Figure.6: Peppa's model kinetic release plot

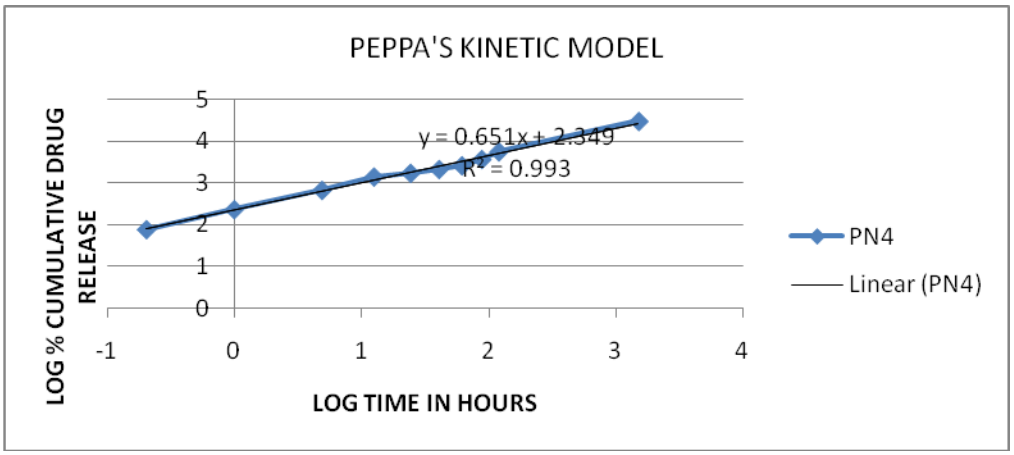


Figure.7: SEM images of pure maltodextrin powder and optimized proniosomes powder(PN4) (a),(b)- maltodextrin (c),(d) – proniosomes powder

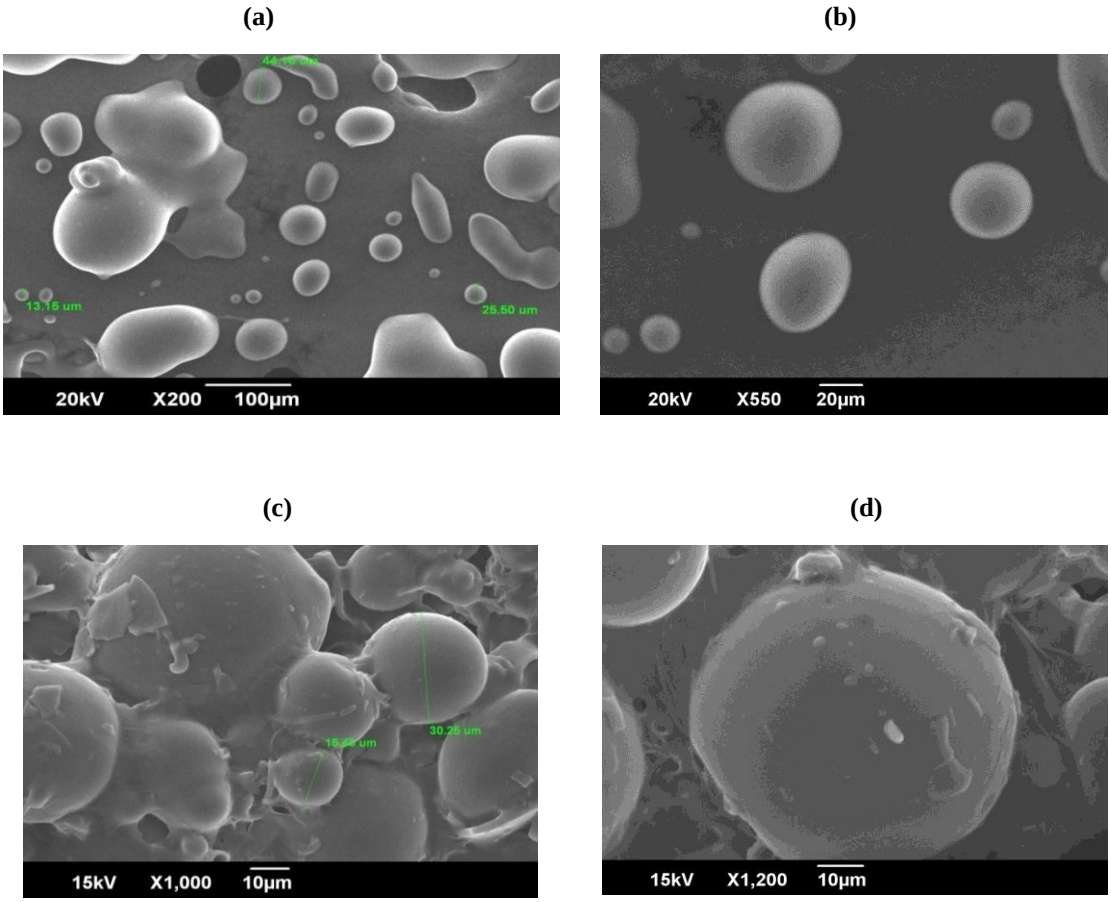


Figure.8 : IR spectrum of proniosomes powder.

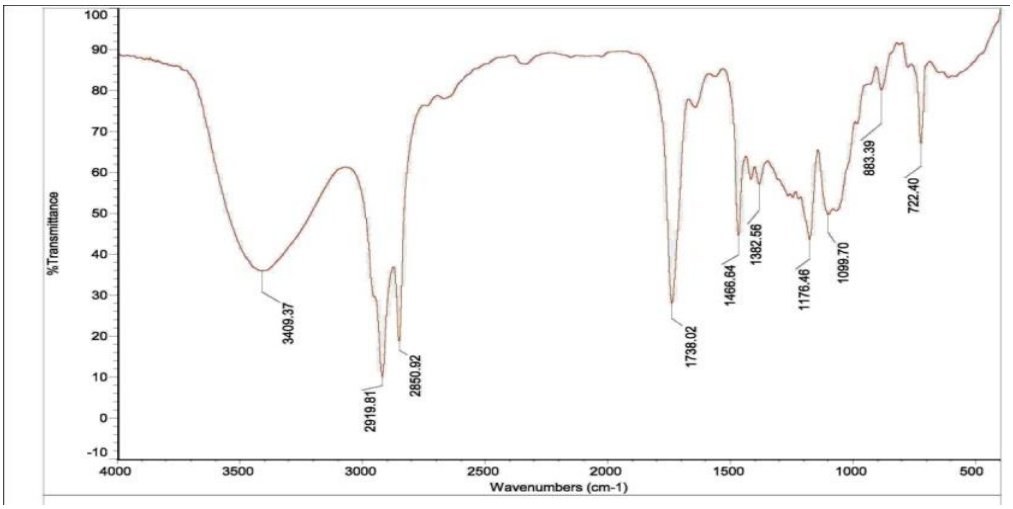


Figure 9: Cumulative amount of drug permeated Vs time plot

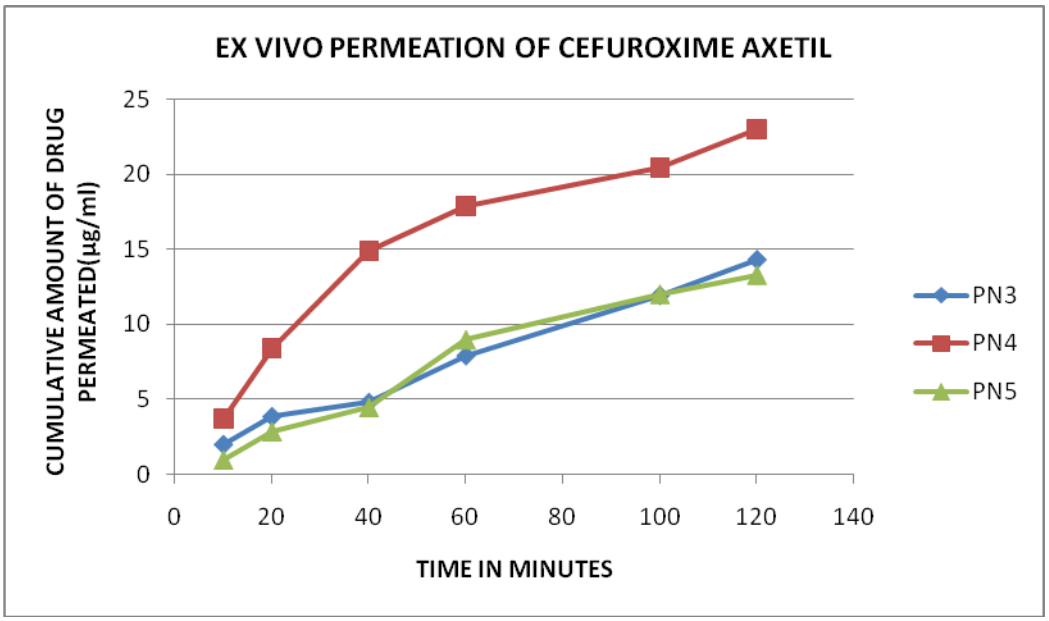


Figure .10: Graphical representation of stability of proniosomes at various temperatures

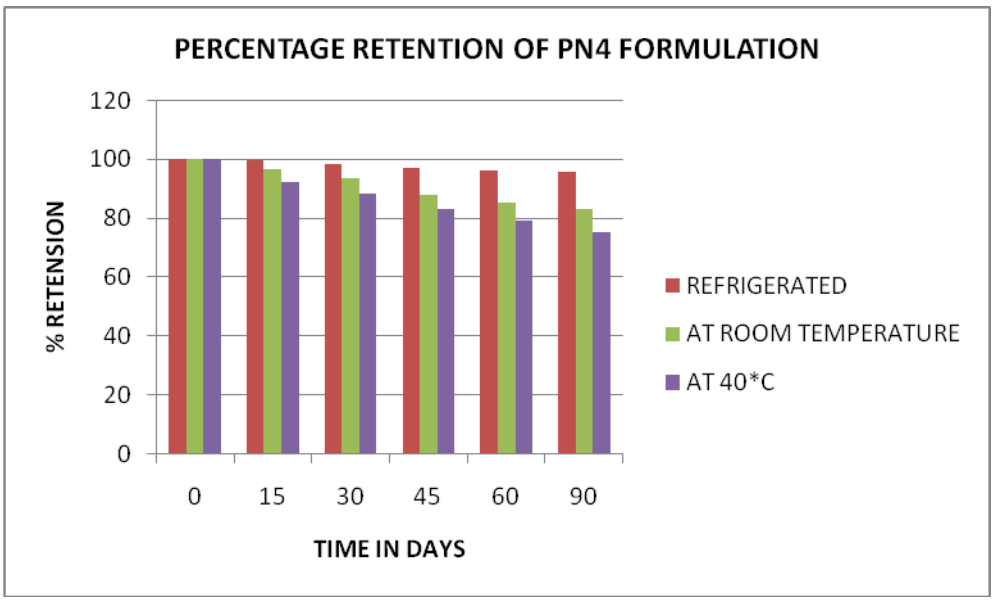


Table 1: Composition of cefuroxime axetil loaded maltodextrin based proniosome powders

Formulation	Drug (mg)	Carrier(g)	Span 60:Cholesterol (µ mol)	Span 60(mg)	Cholesterol (mg)
PN1	250	1:5	1200:300	516.74	116.00
PN2	250	1:5	1125:375	484.45	144.99
PN3	250	1:5	1000:500	430.62	193.33
PN4	250	1:5	750:750	322.97	289.99
PN5	250	1:5	500:1000	215.31	386.65
PN6	250	1:5	375:1175	161.48	434.98
PN7	250	1:5	300:1200	126.19	463.98

1 g of carrier per 1 mM of total lipid. Each formulation contains 250 mg of cefuroxime axetil

Table 2: Micromeritic properties of various proniosome powder formulations

Formulation	Angle of repose (h)	Compressibility index	Hausner's ratio
MALTODEXTRIN	36.13±0.12	19.34±0.43	1.24±0.39
PN1	31.8±0.16	12.23±0.14	1.15±0.05
PN2	30.11±0.03	12.32±0.22	1.14±0.08
PN3	29.25±0.21	10.72±0.25	1.12±0.14
PN4	27.47±0.32	9.96±0.22	1.11±0.33
PN5	26.57±0.31	10.71±0.09	1.12±0.44
PN6	26.05±0.29	11.61±0.16	1.13±0.23
PN7	28.01±0.25	8.33±0.11	1.01±0.43

*Average of three determinations ± SD.

Table 3: Physico-chemical characterization of various proniosome powder formulations

Formulation	Size(µ m)	Entrapment efficiency (%)	No. of vesicles per mm^3 $x10^3$
PN1	10.23	94.59	1
PN2	13.50	94.78	2
PN3	11.00	96.28	3
PN4	10.40	98.48	6
PN5	13.30	97.30	1
PN6	14.12	95.79	3
PN7	22.25	92.84	4

Table 4: Drug content in various formulations

Formulation	Asorbance	Concentration (µg)	Drug Content (%)
PN1	0.0192	0.3848	96.15
PN2	0.0189	0.3776	94.32
PN3	0.01908	0.3816	95.08
PN4	0.0187	0.3744	93.52
PN5	0.0184	0.368	92.01
PN6	0.0181	0.360	90.01
PN7	0.0187	0.3743	93.58

Table 5: Cumulative drug release of various formulations in *in- vitro* release study

S.No.	Time in hrs	Cumulative drug released(mg)						
		PN1	PN2	PN3	PN4	PN5	PN6	PN7
1	0.5	0.015	0.011	0.013	0.019	0.017	0.012	0.010
2	1	0.021	0.018	0.019	0.029	0.025	0.025	0.017
3	2	0.029	0.028	0.0269	0.042	0.0386	0.0369	0.0333
4	3	0.032	0.029	0.0289	0.0438	0.0484	0.0381	0.0373
5	4	0.034	0.035	0.031	0.0514	0.0536	0.0423	0.0394
6	5	0.046	0.037	0.051	0.0589	0.0609	0.0542	0.0464
7	6	0.053	0.0435	0.066	0.0647	0.0725	0.0574	0.0525
8	7	0.062	0.0439	0.078	0.0825	0.0843	0.0677	0.0609
9	8	0.069	0.051	0.087	0.0982	0.0909	0.081	0.068
10	24	0.27	0.247	0.287	0.2949	0.283	0.269	0.264

Table 6: Permeation flux of different formulations

Time(Minutes)	Permeation flux µg/ml/min^{-1}		
	PN3	PN4	PN5
10	0.196	0.372	0.099
20	0.191	0.421	0.1435
40	0.1190	0.372	0.112
60	0.1313	0.298	0.1494
100	0.119	0.204	0.120
120	0.119	0.192	0.1107

Table 7: Test of Significance

Formulations	PN3-PN4	PN4 -PN5	PN5-PN3
P-VALUE	0.13	0.135	0.43

REFERENCES

1. Ajay BS, Jolly RP, Rajesh HP. Formulation and Optimization of Piroxicam Proniosomes by 3-Factor, 3-Level Box-Behnken Design. AAPS Pharm Sci Tech. 2007;8(4):1-7.
2. Ajay S, Jolly P, Rajesh P. Preparation, Characterization, Optimization, and Stability Studies of Aceclofenac Proniosomes. Iranian J of Pharm Res. 2008;7(4):237-246.
3. Akhilesh D, Prabhakara P. Development and Evaluation of Lornoxicam Loaded Maltodextrin Based Proniosomes. Int J Pharma Tech Res 2013; 5 (3): 865-872.
4. Alekhya Gurrapu , Raju Jukanti , Sharan Reddy Bobbala , Swetha Kanuganti , Jyothi B. Jeevana. Improved oral delivery of valsartan from maltodextrin based proniosome powders. Advanced Powder Technology 23 (2012) p 583–590
5. Almira I. Blazek-Welsh, David G. Rhodes . Maltdextrin based proniosomes. The AAPS Journal; Vol. 3 Number 1
6. Arora Sonia, Prashar Bharat, Dhamija Hitesh, Chandel Abhishek, Thakur Varun. Niosomes: the unique vesicular drug carriers. Journal of Drug Delivery & Therapeutics; 2012,p- 2(1)
7. Chandra A. and Sharma P. K. Proniosome based drug delivery system of piroxicam. African J Pharm and Ph.cology 2008; 2(9)p :184-190
8. Chawda Himmat Singh, Jain C P, Bairwa Narendra Kumar. Formulation, characterization, stability and invitro evaluation of nimesulide niosomes. Pharmacophore 2011, Vol. (3) p 168-185
9. Chintankumar JT, Borkhataria CH, Baria AH, Patel RP, Tamizharasi S, Dipen KS, Sandip DP, Ghanshyam RP. Formulation and evaluation of aceclofenac loaded maltodextrin based proniosome. Int J Chem Tech Res 2009; 1(30)p :567-573.
10. Chirag madhu, Kamal Saroha, Sachin kamboj, Meenakshi Goel. Proniosomes - drug carrier for transdemal drug delivery system. The pharma Research, Volume 8, Issue1. Page 187-195
11. D. Akhilesh, G. Faishal and JV. Kamath. Comparative study of carriers used in maltodextrin based proniosomes International research journal of pharmacy *2012, 3 (6)* P: 176-179

12. D. Akhilesh, V. N. Anoop, Dr. B.P. Rao. Formulation and Evaluation of Gliclazide Loaded Maltodextrin Based Proniosomes. International Journal of Research in Pharmaceutical and Biomedical Sciences Vol. 2(4) Oct - Dec 2011
13. Dalia S Shaker, Mohamed Nasr, Mirhan Mostafa. Bioavailability and hypocholesterolemic effect of proniosomal simvastatin for transdermal delivery. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 5, Issue 4, 2013 p: 344-351
14. Francesca Sansone, Teresa Mencherini, Patrizia Picerno, Matteo d'Amore, Rita Patrizia Aquino ,Maria Rosaria Lauro. Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. Journal of Food Engineering 105 (2011)p 468–476
15. G.V.Radha, T.Sudha Rani, B Sarvani. A review on proniosomal drug delivery system for targeted drug action. Journal of basic and clinical pharmacy 03/2013; 4(2):42-48.
16. Gannu P. Kumar, Pogaku Rajeshwarrao. Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta Pharmaceutica Sinica B, Volume 1, Issue 4, December 2011, Pages 208–219
17. Gomes Hazel, Dubey Akhilesh, Prabhu Prabhakara, Kamath Jagadish V. Development and evaluation of norfloxacin loaded International journal of life science and pharma research. Vol 2/Issue 1/Jan-Mar 2012p L 82-96
18. Hanu Priya, Harmanpreet Singh. Formulation and evaluation of niosomes containing punicalagin from peels of punica granatum. Journal of Drug Delivery & Therapeutics; 2012, 2(6), p: 56-67
19. Harini Chowdary Vadlamudi, M. Sevukarajan**.** Niosomal drug delivery system-a review. Indo American Journal of Pharmaceutical Research. 2012:2(9)
20. Jain Pritam, Patel Manish, Surana Sanjay. Development and validation of UV-Spectrophotometric method for determination of Cefuroxime Axetil in bulk and in Formulation. International Journal of Drug Development & Research | October-December 2011 | Vol. 3 | Issue 4, p: 318-322
21. Jigar Vyas, Puja Vyas, Dhaval Raval, Paresh Paghdar. Development of Topical Niosomal Gel of Benzoyl Peroxide. ISRN Nanotechnology
    Volume 2011 (2011), Article ID 503158, 6 pages
22. Karim Masud Kazi, Asim Sattwa Mandal, Nikhil Biswas, Arijit Guha, Sugata Chatterjee, Mamata Behera. Niosome: A future of targeted drug delivery systems. J Adv Pharm Technol Res. 2010 Oct-Dec; 1(4): 374–380
23. Kshitiji B. Makeshwar, Suraj R.Wasankar. Niosome: a Novel Drug Delivery System. Asian Journal of Pharmaceutical Research. Vol 3, No 1(2013), p: 16-20
24. Kumar, Kapil; Rai, A. K. Development and Evaluation of Proniosome-Encapsulated Curcumin for Transdermal Administration. Tropical Journal of Pharmaceutical Research;Dec2011, Vol. 10 Issue 6, p697
25. Lohumi Ashutosh, Rawat Suman, Sarkar Sidhyartha, Sipai Altaf bhai., Yadav M. Vandana. A novel drug delivery system: niosomes review. Journal of Drug Delivery & Therapeutics; 2012, 2(5), 129-135
26. M. Abraham Lingan, A. Abdul Hasan Sathali, M. R. Vijaya Kumar , A. Gokila. Formulation and evaluation of topical drug delivery system containing clobetasol propionate niosomes. Scientific Reviews and Chemical Communications. 1(1), 2011, p 7-17
27. M. Madhavi, C. P. Meher, B. Pochaiah, A. M. Rao. Formulation and Evaluation ofMetforminBasedNiosomes.International Journal of Pharma Research & Review, Jan 2013; 2(1): p 1-7
28. M.D.Game, D.M. Sakarkar, K.B.Gabhane and K.K. Tapar. Validated spectrophotometric methods for the determination of cefuroxime axetil in bulk drug and tablets. International Journal of ChemTech Research. Vol.2, No.2, pp 1259-1262
29. Madhav NVS, Saini A. Niosomes: a novel drug delivery system. International journal of research in pharmacy and chemistry. 2011, 1(3), P 498-511
30. Manivannan Rangasamy, Balasubramaniam Ayyasamy, Senthilkumar Raju,Sandeep Gummadevelly ,Sanaullah Shaik. Formulation and in vitro evaluation of niosome encapsulated acyclovir. Journal of Pharmacy Research Vol.1.Issue 2. Oct-December 2008, P 163-168.
31. Nazia Khanam, Md. Irshad Alam, Anupam K Sachan, Sudhir S Gangwar, Ranjana Sharma. Recent trends in drug delivery by niosomes: A review. Asian Journal of Pharmaceutical Research and Development Vol.1 (3) May– June 2013:p 115-122
32. Nirav N. Patel, Vikran, Komal Roopchandani, Arvind Gupta, Amit Gsupta. Proniosomes for improved transdermal drug delivery – A review. A Journal of Pharmac y Research, p- 62-82
33. P.U.Mohamed Firthouse, S.Mohamed Halith, S.U.Wahab, M.Sirajudeen, S.Kadher Mohideen. Formulation and Evaluation of Miconazole Niosomes. International Journal of PharmTech Research. Vol. 3, No.2, p1019-1022
34. Prakash SG, Vijay GJ. Development and targeting efficiency of irinotecan engineered proniosomes. Trop J Pharma Res 2012; 11 (1): 1-8.
35. Punitha Sundaresan, Ch.Sravanthi, Tananki Gowtham. Evaluation of aceclofenac niosomes prepared by various techniques. International Journal of Pharmaceutical Sciences Review and Research. es., 16(1), 2012;vol 13, 75-78
36. Ranjana Sharma, Ritu Mahatma, Meenakshi Bharkatiya and Anju Goyal. Niosome as a as potential drug delivery system. International Journal of Drug Research and Technology. 2012, Vol. 2 (6),p 422-429
37. Rekha Kumari, Kuldeep Varma, Aatish Verma, Girish Kumar Yadav, Sheo Datta Maurya. Proniosomes: A key to improved drug delivery. Journal of Drug Delivery and Therapeutics. p 56-65
38. Rishikesh Gupta, Santosh Kumar, Nikhil Gupta , Virendra Kumar, Dr. S K Prajapati, The proniosomes development and optimization as a surrogated drug carrier for oral delivery of gliclazide. an-overview International journal of pharmaceutical and chemical sciences. Vol. 1 (1) Jan – Mar 2012 p: 164-173
39. Rishu Kakkar, Rao Rekha , Dahiya Navin Kumar Nanda Sanju. Formulation and characterisation of valsartan proniosomes. Maejo International Journal of Science and Technology. 2011, 5(01),p 146-158
40. Ruiz-Balaquer N, Nacher A, Casabo VG, Merino M. Nonlinear intestinal absorption kinetics of cefuroxime axetil in rats. Antimicrobial Agents Chemotherapy. Feb 1997; 41(2): 445–448.
41. S. Pankaj, T. Rini, P.M. Dandagi. Formulation and Evaluation of Proniosome Based Drug Delivery System of the Antifungal Drug Clotrimazole. International Journal of Pharmaceutical Sciences and Nanotechnology. Volume 6 • Issue 1 •2013;6(1):1945-51
42. S. Srinivas, Y. Anand Kumar, A.Hemanth, M.Anitha. Preparation and evaluation of niosomes containing aceclofenac. Digest Journal of Nanomaterials and Biostructures Vol. 5, No 1, March 2010, p. 249 – 254
43. S.N.V. Sivaprasad, P. Lakshman Kumar, M. Srinivas, B. Brahmaiah, Sreekanth Nama. Proniosome: a novel approach to vesicular drug delivery system. Innovative Drug Discovery. Vol 3 , Issue 2, 2013 p 85-90
44. Satyanand Tyagi, Patel Chirag J, Tarun Parashar, Soniya. Novel Drug Delivery System (NDDS): Niosomes. Journal of Biomedical and Pharmaceutical Research 1 (3) 2012, 14-21
45. Saurabh Bansal, Chandan Prasad Kashyap, Geeta Aggarwal and SL Harikumar. A comparative review on vesicular drug delivery system and stability issues. International journal of research in pharmacy and chemistry, 2012, 2(3), p 704- 713

46. SC Arora, PK Sharma, R Irchhaiya, A Khatkar, N Singh, J Gagoria. Development, characterization and solubility study of solid dispersions of Cefuroxime Axetil by the solvent evaporation method. International Journal of ChemTech Research 2 (2), p 1156-116
47. Sharda Sambhakar, Bishambar Singh, Sarvesh Paliwal, Prabhat Ranjan Mishra. Sorbitol based proniosomes to improve the permeability and stability of an oral cephalosporin. International Journal of Drug Delivery 4 (2012)p 236-245
48. Shirsand, S. B.; Para, M. S.; Nagendrakumar, D.; Kanani, K. M.; Keerthy, D. Formulation and evaluation of Ketoconazole niosomal gel drug delivery system. International Journal of Pharmaceutical Investigation;Oct-Dec2012, Vol. 2 Issue 4, p201
49. Singh Pankaj Kumar, Niranjan Sunil Kumar, Irchhaiya Raghuveer. Proniosome as a sustained drug delivery system: A review. International research journal of pharmacy, 2012, 3 (10) p 27-32
50. Sudhamani.T, Priyadarisini.N, Radhakrishnan.M. Proniosomes – A Promising Drug Carriers, International Journal of PharmTech Research, Vol.2, No.2, p 1446-1454
51. Sunil Kamboj, Vipin Saini, Nancy Maggon, Suman Bala, Vikas Jhawat. Vesicular drug delivery systems: a novel approach for drug targeting. International journal of drug delivery. vol 5, No 2(2013)
52. T. Sudhamani, V. Ganesan, N. Priyadarsini, M. Radhakrishnan. Formulation and evaluation of ibuprofen loaded maltodextrin based proniosome. Sudhamani T. et al. / International Journal of Biopharmaceutics. 2010; 1(2): p 75-81
53. Tamizharasi Sengodon, Biradar Sunil, Rathi Vaishali, Rathi Jagdish Chandra; Formulation and Evaluation of indomethacin loaded maltodextrin based proniosomes; Int. journal of PharmTech Research, vol 1, No. 3, July-Sept 2009, pp 517-523
54. Tavano, Lorena · Muzzalupo, Rita · Mauro, Loredana · Pellegrino, Michele · Andò, Sebastiano · Picci, Nevio. Transferrin-conjugated pluronic niosomes as a new drug delivery system for anticancer therapy. The ACS journal of surfaces and colloids. 2013, Vol. 29, pp. 12638-12646.
55. Trupti Anil Udasi , Vikrant P. Wankhade , Latika M. Ingle, Sandeep Atram, Kiran K. Tapar. Proniosome: A novel approach to vesicular drug delivery system. International Journal of Pharmacy and Pharmaceutical Science Research, 2013; 3(1):p 1-6
56. V. C. Okore, A. A. Attama, K C Ofokansi, C O Esimone, E. B. Onuigbo. Formulation and Evaluation of Niosomes. Indian Journal of Pharmaceutical Sciences 05/2011; 73(3):323-8.
57. V.Sathyavathi1,A.Abdulhasansathali,R.Ilavarasan,T.Sangeetha.Formulationand evaluation of niosomal in situ gel ocular delivery system of brimonidine tartrate.
58. Vijay D Wagh, and Onkar J Deshmukh, Itraconazole Niosomes Drug Delivery System and Its Antimycotic Activity against Candida albicans. ISRN pharmaceutics, vol. 2012, pp. 653465, 2012.
59. Viviane F. Naggar, Safaa S. El gamal, Ahmed N. Allam. Proniosomes as a Stable Carrier for Oral Acyclovir: Formulation and Physicochemical Characterization. Journal of American Science 2012;8(9) p 417- 428
60. Vyas Jigar, Gajjar Vishal, Gediya Tejas, Christian Vishal, Upadhyay Umesh.Formulation and characterization of topical gel of erythromycin entrapped into niosomes. International Journal of PharmTech Research, , July-Sept 2011 Vol.3, No.3, pp 1714-1718
61. Vesicular & Particulate Drug Delivery Systems- R.S.R.Murthy
62. Handbook of Pharmaceutical Excipients-Raymond C Rowe