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Comparative study of proniosomal drug delivery system of flurbiprofen

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ABSTRACT

In present study, an attempt has been made to evaluate a Comparative study of powder v/s gel of proniosomal drug delivery system of flurbiprofen. Flurbiprofen, are nonsteroidal anti-inflammatory drug (NSAIDs) is used for the relief of pain and inflammation associated with rheumatoid arthritis and osteoarthritis. It exhibits anti-inflammatory, analgesic and antipyretic activities. It will be also effected the transdermal system rate because of its size, nature and chemistry, these systems give better drug permeability from biological bioavailability membranes and helps in solubilization of some practically insoluble drugs and hence solve problems of many drug. In the present study we are using various surfactants like span 20, 40, 60, 80, and brij 35 and studided in various proniosomal powder and gel formulation and studied in both result in following formulation. In this result proniosomal gel of formulation brij 35 show better entrapment and highest in vitro drug release. The best result obtain in which formulation which having equimolar ratio(1:1) of brij 35 and cholesterol.The release mechanism was explained with zero order, first order, higuchi equations. Thus it can be concluded that the proniosomes gel posses higher entrapment efficiency and utilizes alcohol, which itself act as penetration enhancer. The elicited an increase of the percutaneous permeation of flurbiprofen both in-vitro and in-vivo. In addition, in vivo experiments showed that flurbiprofen proniosomes gel can ensure a sustained release of the drug and hence a prolongation of its therapeutic activity, which can be related to an accumulation of flurbiprofen in the skin.

Keywords: Proniosomes powder & gel, transdermal drug delivery, flurbiprofen in-vitro drug release, stability studies.

INTRODUCTION

Conventional chemotherapy for the treatment of intracellular infection is no more effective due to limited permeation of drug into cell. This can be overcome by the use of vesicular drug delivery system. Encapsulation of a drug in vesicular structure can be predicted to prolong the existence of the drug in the systemic circulation and thus enhance penetration into target tissue and reduce toxicity [1]. Vesicular delivery of the drug also improves the bioavailability of medications especially in the case of poorly soluble drug. They can incorporate both hydrophilic and lipophilic drug. This system also solves the problem of drug like insolubility, instability and rapid degradation. Colloidal particulate carriers such as liposome's or niosomes have been widely employed in drug delivery systems and producing them from a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used in various drug delivery Proniosomes are dry product which could be hydrated immediately before use would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (aggregation, fusion, leaking) could be minimized

[2]. These dry formulations of surfactant-coated carrier can be measured out as needed and rehydrated by brief agitation in hot water [3]. They are water-soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media. Reported methods for preparation of proniosomes were the spraying of surfactant on water-soluble carrier particles and the slurry method. This 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 systems like drug targeting, controlled release and permeation enhancement of drugs. But there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form**.** [9]

Non-ionic surfactant of wide variety of structural types has been found to be useful alternatives of phospholipids in the fabrication of vesicular systems. Non-ionic surfactants form a variety of aggregates form micelles to large vesicles, which can be used for drug delivery. Steroids are important components of cell membrane and their presence in membrane brings about discernible changes in regard to bilayer fluidity and permeability. Cholesterol can be incorporated in bilayers at significantly higher molar ratios; however by itself it does not form bilayers. Thus, it could be used to manipulate the membrane characteristics [4]. It is ampiphilic in nature thus it aligns itself in a manner that OH group faces the aqueous phase while the aliphatic chain aligns parallel to hydrocarbon chain of surfactant. Cholesterol acts as a fluidity buffer, since below the phase transition temperature it makes the membrane less ordered while above it the membrane becomes more ordered [8].

EXPERIMENTAL SECTION

MATERIALS:

Flurbiprofen gift sample obtained from F. D. C. Ltd. Mumbai. cholesterol, and dialysis tubing were purchased from Hi-Media Laboratories (Mumbai, India). Span 20, 40, 60, 80 and Brij 35 and maltodextrin were purchased from Central Drug House.

FORMULATION OF PRONIOSOME POWDER

Proniosomes were prepared by Slurry method [14].

Strategies for the preparation of provesicles in the preparation of proniosomes non-ionic surfactants, coating carriers and membrane stabilizers are commonly used. The non-ionic surfactants used are Span (20, 40, 60, 80, Brij 35) (Table 1). The coating carriers used is maltodextrin (Maltrin M500, M700), membrane stabilizers like cholesterol are also used. For ease of preparation a stock solution of accurately weighted quantities of surfactant, cholesterol and drug was prepared in 10 ml chloroform: methanol (2:1) solution the required volume of surfactant, cholesterol stock solution and drug was added to a 100 ml round bottom flask containing 500 mg maltodextrin carriers. Additional chloroform: methanol solution was added to form slurry in the case of lower surfactant loading. The flask was attached to a rotary evaporator to evaporate solvent at 60-70 rpm, a temperature of 45±2ºc and a reduced pressure of 600 mmhg. Until the mass in the flask had become a dry free flowing product. These materials were further dried overnight in dessicator under vacuum at room temperature. This dry preparation is referred to as Proniosomes Powder [3].

VARIABLE STUDIES

In order to obtain the best possible Proniosome formulation the various process parameter such as effect of maltodextrin, effect of cholesterol, selection of solvent and speed of rotation of flask, temperature, effect of HLB value, effect of surfactant concentration, entrapment efficiency, hydration media etc. was optimized**.**

Table 1: Composition of proniosomal powder formulation

F.code	MD (mg)	Span20 (mg)	Span40 (mg)	Span60 (mg)	Span80 (mg)	Brij35 (mg)	CHL	Drug (mg)
SKP3	500	50	-	-	-	-	50	50
SKQ3	500	-	50	-	-	-	50	50
SKR3	500	-	-	50	-	-	50	50
SKS3	500	-	-	-	50	-	50	50
SKT3	500	-	-	-	-	50	50	50

DEVELOPMENT OF PRONIOSOME GEL

Preparation of proniosome gel was adopted by the method given by Proniosome gel preparation involves mixing of surfactant, cholesterol, and the drug with a suitable alcohol. After mixing all the ingredients, it is covered with a lid

to prevent the loss of solvent and warm on a water bath at 60° - 70°C until the surfactant dissolves completely [5]. To it is added an aqueous phase, which may be purified water, dilute glycerol solution or an isotonic buffer solution like, phosphate buffer or saline solution. It is warmed again form a clear solution, which on storage for overnight under dark converts to proniosomal gel (Table 2). The ratio of surfactant, alcohol and the aqueous phase plays an important role in gel formation [15].

Table 2: Composition of proniosomal gel formulation

F.code	Drug (mg)	Span20 (mg)	Span40 (mg)	Span60 (mg)	Span80 (mg)	Brij35 (mg)	CHL	Solvent ratio(ml)
SKG3	50	50	-	-	-	-	50	0.5
SKH3	50	-	50	-	-	-	50	0.5
SKI3	50	-	-	50	-	-	50	0.5
SKJ3	50	-	-	-	50	-	50	0.5
SKK3	50	-	-	-	-	50	50	0.5

CHARACTERIZATION OF PRONIOSOMAL POWDER & GEL

Measurement of angle of repose

Angle of Repose of different formulations was measured according to fixed funnel standing method. The pure maltodextrin or proniosome powder was poured into a funnel which was fixed at 13mm outlet orifice of the funnel is 10 cm above a level black surface powder flowed down form from the funnel to from a cone on the surface, and the angle of repose was hen calculated by measuring the height of the of the cone and he diameter of its base [7].

Vesicle Size and Size Distribution Analysis

Proniosomal powder formulation was characterized for vesicle size and size distribution. The multilamellar vesicles were determined by using Zetasizer (Malvern Instruments, Malvern, UK). The formulations for vesicle size analysis using the Zetasizer were diluted with double-distilled water before analysis. The average vesicle size of samples was determined. Results of vesicle size obtain by Zetasizer [14]. The surface morphology and shape was studied by scanning electron microscopy (SEM). Particle size analysis are study by a calibrated eyepiece micrometer. (Figure 3-4)

Drug Entrapment Efficiency

Flurbiprofen entrapped within the proniosomes was estimated after removing the unentraped drug. The unentrapped drug was separated from the proniosomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi CPR-24, Mumbai, India) at 18000 rpm at a temperature of 5°C for 40 minutes, [6,8] where upon the pellets of proniosomes and the supernatant containing free drug were obtained. The proniosome pellets were washed again with distilled water to remove any un-entrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 247.0 nm using Shimadzu U-V 1700 Spectrophotometer. (Table 3-4)

Encapsulation efficiency was calculated according to the equation: [10]

Entrapment efficiency = $(D_1 - D_2) * 100/D_1$

D_1 = Amount of flurbiprofen added initially D_2 = Amount of flurbiprofen determine in the supernatant by U-V spectrophotometry. $(D_1 - D_2)$ = The amount of flurbiprofen entrapped in the formulation.

***In-Vitro* Drug Release**

The *in vitro* drug release studies were carried out by means of treated dialysis membrane. The dialysis membrane was treated before carrying out the release studies. The release rate of Flurbiprofen from proniosomal powder was carried out in using dialysis bag method. A measured amount of niosome suspension equivalent to 5.5 mg were placed dialysis bag of effective length 8 cm. Dialysis bag was placed in a beaker containing 500 ml of simulated gastric fluid. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm. The temperature of medium was maintained at 37ºC by a thermostatic control available on the magnetic stirrer. Aliquots of sample (5 ml) were withdrawn periodically and replaced with the same volume of fresh fluid, at each sampling point. The samples withdrawn were analyzed for the drug content at 247 nm spectrophotometrically. The same is also repeated

for phosphate buffer saline of pH 7.4, and volume of 500 ml. the sample withdrawn were analyzed for the drug content at 247 nm spectrophotometrically [11]. All the determination was made in three times.

In vitro release studies on proniosomal gel were performed using locally manufactured Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor compartment exposed to receptor compartment was 1.389cm^2. The dialysis cellophane membrane (MMCO14KDC) was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the membrane [12]. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1°C.

STABILITY STUDY

Stability study was carried out to investigate the degradation of drug from proniosomal gel and powder formulation during storage. The stability study of all prepared formulation were performed by storing 4ºc, 25ºc and 45ºc for a period of 45 days. Throughout the study, proniosomal formulation was stored in aluminium foil sealed glass vials [14]. The formulation was analyzed for the drug content spectrophotometrically.

RESULTS AND DISCUSSION

Proniosome containing flurbiprofen were prepared by ametho of slurry method. In this the non ionic surfactant and cholesterol are added in rotator flash evaporator with containing solvent. Maltodextrin are use as a carrier for loading of surfactant. The cholesterol plays an important role in the formation of proniosome. Formation of vesicle mainly depends on the concentration of cholesterol and surfactant ratio. Table 3 show that the entrapment efficiency of different optimized formulation. For this reason, the entrapment efficiency of flurbiprofen within the formulaion varies form as low as varies from 55.3% for span 80 vesicle (SKP3) to high as 75.42% for Brij 35 (SKT3) vesicle Table 3 show higher entrapment efficiency for Brij 35 formulation can be attributed to its length of longer side chain, and it easily diffuse into receptor membrane integrity, orientation and packaging ability.

The entrapment efficiency of proniosomal gel was found to be in the range of 40.0% -82.56 %. The entrapment efficiency of proniosomal gel was attributed due to the amphiphillic nature of the drug. The entrapment efficiency was found maximum for SKK3 formulation due to higher HLB value, of the formulation, which result in larger vesicle hence more entrapment of drug into the vesicle. Table 4 The effect of cholesterol on flurbiprofen entrapment was varied according to the nonionic surfactant used, cholesterol was found to have little effect on the flurbiprofen entrapment was obtained when 10% of cholesterol was incorporated into niosome, prepared from span 40 & span 60, followed by decrease in encapsulation efficiency of the drug upon further increase in cholesterol content.

As the Brij 35: CHL (SKT3) formulation showed the highest entrapment. It was selected for further optimization the total concentration of surfactant mixture Brij 35: Cholesterol was kept constant, the ratio of brij 35: cholesterol was varied from 10: 90 to 90: 10 to investigate the effect of this ratio on flurbiprofen. Formulation SKT3 containing 50: 50 brij 35/ CHL ratios showed the highest entrapment that is 75.4%, Table 3. The increase in entrapment efficiency with increase in cholesterol content can be explained by the fact that cholesterol intercalated into the bilayer preventing the leakage of the drug through the bilayer. This could be due to the surfactant chemical structure, all span surfactant here same head group and different alkyl chain, increasing the higher alkyl chain length is leading to higher entrapment efficiency. The entrapment efficiency order is brij 35 > span 60 > span 40 > span 20 > span80. The cholesterol content affects the membrane elasticity making the membrane rigid. A decline in the entrapment efficiency beyond a certain cholesterol level. Surfactant used to make non-ionic surfactant vesicle have low aqueous solubility, however freely soluble non-ionic surfactant such as Brij 35 can form micelles on hydration due to the presence of more polar head group in the chain, in the addition of cholesterol they abolish the more polar part present in surfactant mainly due to lipophillic in nature and help in formation of vesicle in the equimolar ratio of Brij 35 and cholesterol show better result. The proniosome formation takes place from Brij 35 with the presence of cholesterol, the length of alkyl chain show a crucial factor of permeability, Brij 35 have long lauryl(c_{12}) chain, thus the long chain influence the HLB of the surfactant and also lead to the higher drug entrapment efficiency and also show better stability of the proniosome using Brij 35. Cholesterol is one of the most important additives included in the formulation in order to prepare stable niosome. Cholesterol stabilize bilayer, prevents leakiness and retards permeation of solutes enclosed in the aqueous core of these vesicle.

Table 3: Various result of proniosomal powder formulation

S. No.	F. Code	Vesicle size (nm)	% Entrapment efficiency	% drug released
1.	SKP3	498.7	55.3±0.97	53.89±0.87
2.	SKQ3	376.4	59.3±1.63	56.23±0.87
3.	SKR3	345.3	70.1±1.51	60.43±0.62
4.	SKS3	204.3	56.0±2.02	63.32±0.43
5.	SKT3	276.3	75.4±1.51	71.23±0.89

Table 4: Various result of proniosomal gel formulation

S. No.	F. Code	Vesicle size (nm)	% Entrapment efficiency	% Amount drug released
1.	SKG3	375.2	57.3±1.97	57.78±0.45
2.	SKH3	330.6	62.6±1.65	62.75±0.87
3.	SKI3	305.6	76.0±1.98	68.12±0.86
4.	SKJ3	265.4	57.3±1.63	74.85±0.56
5.	SKK3	315.6	82.56±1.55	80.42±0.67

The release study was conducted for all the optimized formulation (formulation showing better entrapment efficiency, optimum vesicle size, Spherical surface morphology). Most of the formulation were found to have a linear release and the formulation (SKT3) was found to provide approximately 71.23% release with in a period of 24 hrs. The amount of drug release from different proniosomal powder formulation was found in order of SKT3 > SKS3 > SKR3 > SKQ3 > SKP3. In order to ascertain release kinetics, the rate constant for zero order, first order and Higuche equation kinetics were calculated for each time interval, the release constant was (Figure 2) calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. It was found that the *in-vitro* drug release of proniosome was best explained by first order kinetics for best formulation SKT3 as the plots show highest linearity. (Figure 2) The correlation coefficient (r^2) was found 0.96. It is satisfactory release kinetics of selected formulation. The best formulation SKT3 was found to give a cumulative release of 71.32% over a period of 24 hrs.

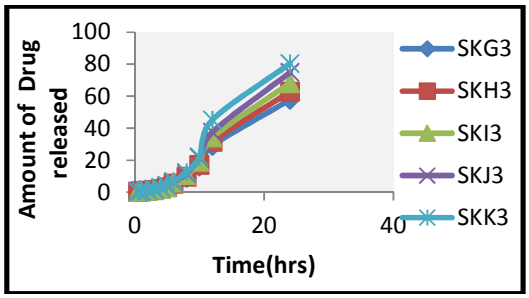

Fig. 1

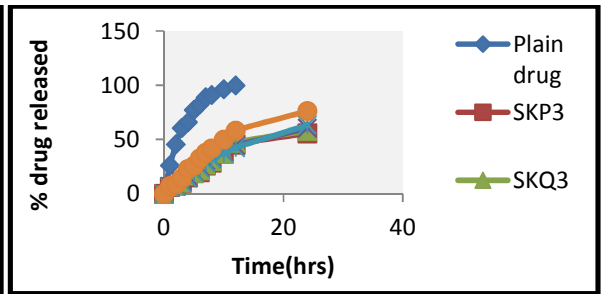

Fig. 2

Fig. 1&2: show a comparative in-vitro drug release profile of selected proniosomal gel and powder formulation

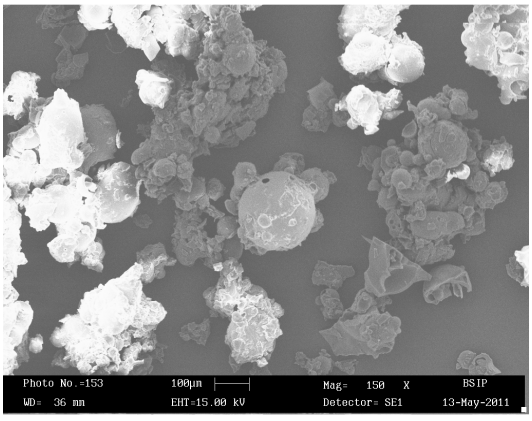

Fig. 3: Scanning micrograph of SKT3

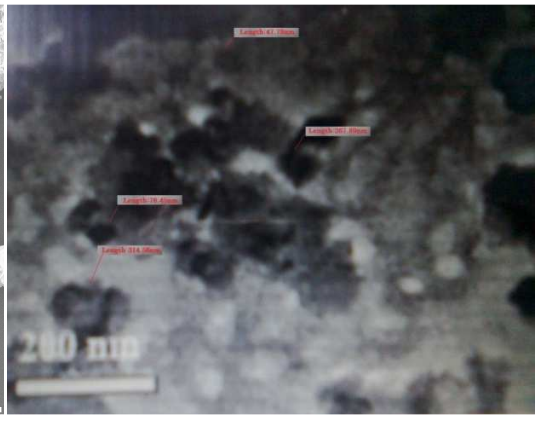

Fig. 4: Scanning micrograph of SKK3

In the case of proniosomal gel, Fig.1 the *in-vitro* drug release was done by locally fabricated Franz diffusion cell. This is used for measurements to investigate the transport enhancement potential of proniosomal gel. The ability of ethanolic lipid vesicles to deliver flurbiprofen was investigated by determining the flux of flurbiprofen. The data of percent cumulative amount of flurbiprofen permeated per unit area across dialysis membrane via various formulations was given in Table 2,4 . The amount of drug released from different proniosomal gel formulation was found in order of SKK3 > SKJ3 > SKI3 > SKH3 > SKG3. Fig. 2 show *in-vitro* release profile of flurbiprofen encapsulated proniosome gel. It was found that SKK3 showed a controlled release property from 10-24 hrs. The result of cumulative % drug release 10^{th} hour was found to be 45.12% & 80.42% at 24 hour. The release profile was found constant between 10-24 hour. So the formulation was found to exhibit a zero order controlled release profile. Other formulation SKG3, SKH3, SKI3, SKJ3, also show good controlled release property. The *in-vitro* release of flurbiprofen proniosomal gel was limited by two barriers, namely phospholipids bilayer & dialysis membrane. The data indicate that values of transdermal flux depends on surfactant cholesterol concentration, as the concentration of surfactant: cholesterol increases up to 50% results in increase in transdermal flux of flurbiprofen and further increase in cholesterol concentration significantly decreased the transdermal flux. The reason for this is the deteriorating effect of cholesterol on the lipid bilayer at higher concentration of cholesterol. The formulation Table 4, SKK3 (50% Brij 35 and 50% cholesterol) showed highest amount of % drug released (80.42%). The enhanced % drug release obtained from the proniosomal gel system could be justified on the basis of dual function performed by ethanol present in the proniosomal formulations, length of side chain, i.e. fluidizing both the vesicular lipid bilayer and greater malleability to the vesicles and enhancing permeability of the skin. Overall, the data clearly indicate that the proniosomal gel formulation SKK3 (50% w/w cholesterol and 50% w/w Brij-35) showed the highest entrapment efficiency (82.56 %), optimum size (315.6 nm), highest cumulative amount of % drug released (80.42%).

KINETIC DATA TREATMENT

To find out the kinetics and mechanism of drug released from all the formulations of flurbiprofen encapsulated proniosomes, In the case of proniosomal powder the data were treated according to zero order, first order and Higuchi's equation pattern. The correlation coefficient of the formulation (SKT3) was found 0.901 in zero order equation pattern when the data were plotted according to first order equation, the correlation coefficient was found to be 0.965, and in Higuachi equation the correlation coefficient found to be 0.938. Hence the formulation (SKT3) follows first order equation.

In the case of proniosomal gel the correlation coefficient of the formulation (SKK3) was found 0.951 in zero order equation pattern. When the data were plotted according to first order equation, the correlation coefficient was found to be 0.908. Hence the formulation (SKK3) follows zero order kinetics. These results pointed to sustained release delivery of drug. This slow release pattern of entrapped drug may indicate the high stability of the proniosomal formulation.

STABILITY STUDIES

In order to determine the percent drug remaining entrapped in vesicles and percent drug lost from Proniosome powder and gel subjecting at temperature 4±2^{o}C, 37±2^{o}C and 45±2^{o}C for 45 days, were determined drug lost at time interval of 15 days. On the basis of entrapment efficiency and controlled release property, formulation SKT3 & SKK3 were selected for the stability studies. Stability study was carried out in term of % drug release. Results showed that proniosomal gel formulation was quite stable at refrigeration and room temperature. In this condition not much leakage of drug was found at there temperature. Percent drug retained at 45ºC might have decreased due to the melting of surfactant and lipid present in the formulation to the proniosomal gel formulation can be stored at refrigeration and room temperature. Result of stability studies for proniosomal powder formulation was more promising than the proniosomal gel formulation. As at all sampling points significantly higher drug retention was observed in case of proniosomal powder.

Thus it can be concluded that the shelf life of proniosomal powder formulation is more than the proniosomal formulation. Because in dry surfactant can be avoided, by forming the suspension as needed, precipitation and aggregation can also be avoided.

COMPARISION OF PRONIOSOMAL GEL AND POWDER FORMULATION

On the basis of results summarized in Table 4, Proniosomal gel formulation are found to be more promising drug carries than proniosomal powder formulation, vesicle size of proniosomal gel derived niosome were larger than the niosome derived from the proniosomal powder. Cumulative release of drug from proniosomal gel SKK3 was found

to be 3025.5µg/cm^2 at 24th hour. The release profile was constant and controlled between 10-24 hour. Thus the proniosomal gel was found to exhibit good controlled release profile. While the proniosomal powder showed controlled release from 11 to 24 hour. And the value of controlled release was 71.23% at 24 hour. Thus it was concluded that the release profile of proniosomal gel was better than proniosomal powder. Although in the stability data, proniosomal gel show good stability at room temperature and refrigeration temperature but on comparison with proniosomal powder, the stability of proniosomal powder found to be better at all define temperature than gel. It is clear from the results, obtained that the proniosomes gel have shown the minimum drug lost at refrigerated condition, and fairly high retention of drug inside the vesicles was observed. The higher amount of drug leakage at elevated temperature may be related to the degradation of lipid bilayer resulting in defects in membrane packing and loss of overall rigidity that makes them leaky. With the increase in temperature, there is also increase in the fluidity of bilayer, due to phase transition phenomenon. So it can be inferred from the above discussion that the proniosomal gel formulation should be stored at either refrigeration or room temperature to minimize the drug loss. It may be due to the presence of ethanol in proniosomal gel system which gives more stability to the vesicles than other vesicular system.

CONCLUSION

Thus it can be concluded that the proniosomes gel posses higher entrapment efficiency and utilizes alcohol, which itself act as penetration enhancer. The elicited an increase of the percutaneous permeation of flurbiprofen both *in-vitro* and *in-vivo*. In addition, in vivo experiments showed that flurbiprofen proniosomes gel can ensure a sustained release of the drug and hence a prolongation of its therapeutic activity, which can be related to an accumulation of flurbiprofen in the skin. [13]These findings are very encouraging and confirm that proniosomes are a very promising carrier for the topical administration due to the enhanced delivery of drugs through the skin thus prompting various opportunities for the development of suitable therapeutic strategies through the topical route. The formulation is easy to scale up as the procedure is simple and do not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives. It offers direct fabrication of transdermal patch and do not require dispersion of vehicle into polymer matrix. Proniosomal powder provide an effective means of delivering the drug through oral route and it can be further processed to make beads, tablets, and capsules, which increase the patient compliance.

On the basis of stability studies, it was concluded that proniosomal powder posses more shelf life as compare to the proniosomal gel.

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REFERENCES

[1] Yoshioka T, Sternberg B, Florence A. T. *Int. J. Pharm*. **1994**, 105, 1-6
[2] Lusia D. Marianecci C. *colloids and surfaces B: Biointerfaces* **2011**,82, 18-24
[3] Tangri P, Khurana S. *International journal of biopharmaceutics* **2011**, 2(1), 47-53.
[4] Abdelbary .G, El-gendy N. *AAPS PharmSciTech*, **2008**, 105 8-9105.
[5] Pardakhty A, Varshosaz J. *International Journal of Pharmaceutics* **2007**, 318, 130–141.
[6] Shahiwala A, Misra A. *J Pharm Pharm Sci* **2002**; 5(3): 220-225.
[7] Khandare JN, Madhavi G, Tamhankar BM. *East Pharm* **1994**; 37:61-64.
[8] Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. *Int J Pharm* **2001**; 215: 91-99.
[9] Hu C, Rhodes DG. *Int J Pharm* **1999**; 185(1): 23-35.
[10] Wu PC, Huang YB, Chang JJF, Chang JS, Tsai YH. *Int J Pharm* **2000**; 209: 87-94.
[11] Abubkar O Nur, Zhang JS. *Int J Pharm* **2000**; 194: 139-146.
[12] Zhou XH, Li Wan PA. *Biochem Pharmacol* **1994**; 47: 1121-1126.
[13] Tripathi KD. Essentials of Medical Pharmacology. New Delhi, India, Jaypee Brothers, **2003** pp 449-454.
[14] Jain NK, Vora B, Khopade AJ. *J Control Rel* **1998**; 54: 149-165.
[15] Nagarsenker MS, Londhe VY, Nadkarni GD. *Int J Pharm* **1999**; 190: 63-71.