



PREPARATION AND CHARACTERIZATION OF METFORMIN PRONIOSOMAL GEL FOR TREATMENT OF DIABETES MELLITUS

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ABSTRACT

The aim of the investigation was to design a proniosomal carrier system of metformin hydrochloride for the treatment of type - 2 diabetes mellitus that is capable of delivering entrapped drug over an extended period of time. Proniosomes of metformin hydrochloride were prepared by coacervation phase separation method. The potential of proniosomes as a transdermal drug delivery system was estimated by encapsulating the drug in various formulations of proniosomal gel composed of different ratios of Span 60/Span 40, cholesterol and lecithin. The prepared systems were characterized for encapsulation efficiency, size, zeta potential analysis, in-vitro drug release and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for one month. Proniosomes were also characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for shape and surface morphology. The results showed that the encapsulation efficiency of proniosomes prepared with span 60 was superior to that prepared with Span 40. A formulation (i.e. PNG2) with 9:2:9 ratio of span 60, cholesterol and lecithin gave maximum encapsulation efficiency (76.8 %), good zeta potential (-51.9) and lowest drug release percent after 24 hrs (75.9%). It is evident from the study that the metformin proniosomal gel is promising prolonged drug delivery system and has reasonably good stability characteristics.

Keywords: Metformin Hydrochloride, Proniosomes, Niosomes, Non-ionic surfactants, Drug permeation, Entrapment Efficiency and Stability.

INTRODUCTION

Drug Delivery systems using colloidal particulates carriers such as liposomes¹ or niosomes² have distinct advantages over conventional dosage forms. These carriers can act as drug reservoirs, and modification of their composition or surface can adjust the drug release rate and/or the affinity for the target site.

In a dispersed aqueous system, liposomes have problems associated with degradation by hydrolysis or oxidation as well as sedimentation, aggregation, or fusion of liposomes during storage included clinical applications of liposomes consisting sterilization problems and in large scale production to obtain a product with adequate physical and chemical stability. Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes³. From a technical point of view, Niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids⁴. Another advantage is simple method for the routine and large scale production of niosomes without the use of acceptable solvents.

Proniosomes are recent development in Novel drug delivery system. These are most advanced drug carrier in vesicular system which overcomes demerits of liposomes and niosomes. These, hydrated by agitation in hot water for a short period of time, offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route^{5, 6}.

Metformin Hydrochloride is selected for a current study which is hypoglycemic agents (antidiabetic drug) comes under the class of Biguanids. The drug is currently administered orally in divided multiple doses for short term management of type 2 diabetes 500-300mg, 2-3doses/daily^{7,8}. This frequent dosing, which results in unacceptable patient compliance, is required due to the short half life of the drug (4-6hrs). Therefore, an alternative non-invasive mode of delivery of the drug is needed^{9, 10}. Transdermal delivery certainly appears to be an attractive route of administration to maintain the drug blood levels of Metformin hydrochloride for an extended period of time.

The aim of this study is to investigate the feasibility of using proniosomes as proniosomal gel of Metformin Hydrochloride in the transdermal drug delivery system. Vesicles prepared were characterized by Optical, Vesicle Size Determination, Zeta Potential Analysis, Scanning and Transmission Electron Microscopy, In-Vitro drug release and stability testing under experimental conditions to investigate the leaking of the drug during storage.

MATERIALS AND METHODS

Materials

Metformin Hydrochloride was gifted from Cipla (Baddi, India). Soya lecithin, Cholesterol, Span 60 and Span 40 were purchased from Central Drug House, Delhi. n-octanol was purchased from SDFCL, Mumbai. Potassium Dihydrogen Phosphate and Disodium Hydrogen Phosphate were purchased from Nice Chemicals, Kochin. Dialysis tubing was purchased from Sigma Aldrich, Delhi.



Table 1: Formulation Codes of various Proniosomal Gel

Formulation code	Drug (mg)	Span 60 (mg)	Span 40 (mg)	Soya Lecithin (mg)	Cholesterol (mg)
PNG1	100	1800	-	1800	200
PNG2	100	1800	-	1800	400
PNG3	100	1800	-	900	200
PNG4	100	1800	-	900	400
PNG5	100	-	1800	1800	200
PNG6	100	-	1800	1800	400
PNG7	100	-	1800	900	200
PNG8	100	-	1800	900	400

Method of development of Proniosomal gel

Proniosomal gel was prepared by a Co-acervation Phase Separation Method. Precisely weighed amount of surfactant (Span 60 and Span 40), lecithin, cholesterol and Drug (Metformin Hydrochloride) were taken in a clean and dry wide mouthed glass vial of 5ml capacity and alcohol (2.5ml) was added to it^{11, 12}. After warming, all the ingredients were mixed well with a glass rod; open end of the glass bottle was covered with a lid to prevent the loss of solvents from it and warmed over water bath at 60°C-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the Phosphate Buffer Solution PBS (pH 7.4) was added and warmed on a water bath till clear solution was formed which was converted into proniosomal gel on cooling¹³⁻¹⁵. The gel so obtained was preserved in the same glass bottle in dark conditions. Compositions of proniosomal gel formulations are given in table 1.

Evaluation of Proniosomal gel

Determination of pH and determination of Viscosity of proniosomal gel

The pH of all proniosomal gel formulations of metformin hydrochloride was measured using pH meter before and after incorporation of the drug. Viscosity of proniosomal gel of metformin Hydrochloride was determined using Brookfield Viscometer¹¹.

Optical microscopy

Small amount of the formed niosomes hydrated from proniosomal gel were spread on a glass slide and examined for the vesicles structure and the presence of insoluble drug crystals using optical light microscope with varied magnification power (10x * 40x). The average sizes of vesicles were measured using calibrated ocular and stage micrometer in the microscope¹².

Vesicle Size Determination

The proniosomal gel (100mg) was hydrated in a small test tube 10 ml of PBS (pH 7.4) with manual shaking for 5 minutes. The dispersion was observed under optical microscope at 10x * 40x magnification. The average sizes of vesicles were measured using calibrated ocular and stage micrometer fitted in optical microscope (Olympus CKX41)^{13, 14}.

Scanning Electron Microscopy (SEM)

For Scanning Electron Microscopy, 0.2 gm of the proniosomal gel in a glass tube was diluted with 10 ml of PBS (pH 7.4). The niosomes were mounted on an aluminum stub using double sided adhesive carbon tape. Then the vesicles were sputter coated with gold palladium (Au/Pd) using a vacuum evaporator and examined using a Scanning Electron Microscopy (HITACHI 7500, JAPAN) equipped with a digital camera at 10.0kV, 10.8mm * 2.50K SE accelerating voltage^{15, 16}.

Transmission Electron Microscopy (TEM)

For Transmission Electron Microscopy, the morphology of hydrated niosomal dispersion prepared from proniosome was also determined using transmission electron microscope. A drop of niosome dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min. to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 35 sec. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried^{17, 18}. The sample was observed with a Hitachi 7500 Transmission Electron Microscopy at having different magnification of 60000x, 100000x, 120000x, and 200000x of 80kV.

Zeta potential analysis

Zeta potential analysis will be done for determining the colloidal properties of the prepared formulations. The suitably diluted proniosomes derived niosome dispersion will be determined using zeta potential analyzer based on Electrophoretic light scattering and laser Doppler Velocimetry method. The temperature will set at 25° C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements will be obtained directly from the measurement^{19, 20}.

Entrapment Efficiency (EE %)

To 100mg of proniosome formulation, weighed in test tube, was added 10ml of PBS (pH 7.4). The aqueous suspension was sonicated in a sonicated bath. The niosomal dispersion was centrifuged at 18000 rpm at 40°C for 5min to separate Metformin Hydrochloride containing niosome from the entrapped drug^{21, 22}. The precipitate consisting of the vesicular pellets was washed three times with Phosphate Buffer Solution PBS (pH 7.4).



The supernatant was taken and diluted with PBS (pH 7.4). The drug concentration in the resulting solution was assayed by UV method at 230 nm. The percentage of drug encapsulation was calculated by the following equation:

$$EE (\%) = (C_t - C_f) / C_t * 100$$

Where C_t is the concentration of total drug and C_f is the concentration of free drug.

In-Vitro Release Studies

In-vitro release pattern of niosomal suspension formed by proniosomal gel was carried out by Dialysis tubing. One gram of Metformin hydrochloride proniosomes equivalent to 20 mg Metformin hydrochloride was taken from PNG1 to PNG8 in dialysis tubing (Sigma Aldrich) and was placed in a beaker containing 75 ml of PBS (pH 7.4). The beaker was placed over magnetic stirrer having speed of 100 rpm and the temperature was maintained at $37 \pm 1^\circ\text{C}$. 5 ml sample were withdrawn periodically and were replaced by fresh buffer and test was continued for 24 hrs. The sink conditions were maintained throughout the experiment²³⁻²⁵. The withdrawn samples were appropriate diluted and analyzed for drug content using U.V spectrophotometer at 230 nm keeping Phosphate Buffer Solution (pH 7.4) as blank.

Stability of Metformin Hydrochloride Proniosomal Gel

The samples were stored at $4-8^\circ\text{C}$ and 37°C for a one month. The encapsulation efficiency of all those samples was determined after one month²⁶. The encapsulation efficiency of all PNG formulations of fresh samples and samples stored at 4°C and 37°C for a one month found to be unchanged²⁶.

RESULTS AND DISCUSSION

Based on the entrapment of the drug, span 40 and span 60 were selected as non-ionic surfactants. They also give the least leaky niosomes as these span surfactants have the highest phase transition temperature. Soya lecithin was selected over egg lecithin because the former gives vesicles of larger size, possibly due to differences in the intrinsic composition of soya and egg derived lecithin. Preparations with a white semi-solid appearance were obtained with span and cholesterol. Incorporation of lecithin results in a gel like appearance. The type of alcohol affects the size of niosomal vesicles.

The Coacervation Phase Separation Method was found to be simple and suitable for laboratory scale preparation of metformin hydrochloride proniosomes. The statistical approach for optimization of formulation is a useful tool, when several variables are to be studied. The all proniosomal gel formulations of Metformin Hydrochloride were studied for physical characteristics like determination of pH as in decreasing order PNG8>PNG6>PNG5>PNG7>PNG3>PNG2>PNG1>PNG4 and determination of viscosity of proniosomal gel formulations as in decreasing order PNG7>PNG5>PNG6>PNG3>PNG2>PNG4>PNG1>PNG3 respectively. It was observed that proniosomal gel formulations showed good spreadability and viscosity¹¹.

Photomicrographs were taken for niosomes by using optical microscope (Olympus CKX41). The photomicrographs of hydrated PNG1 to PNG8 proniosomal formulations shown in figure 1 and figure 2 which is composed of span 60 and span 40 and cholesterol. The photographs reveal that the niosomes are unilamellar having spherical shape and no aggregation or agglomeration is observed¹².

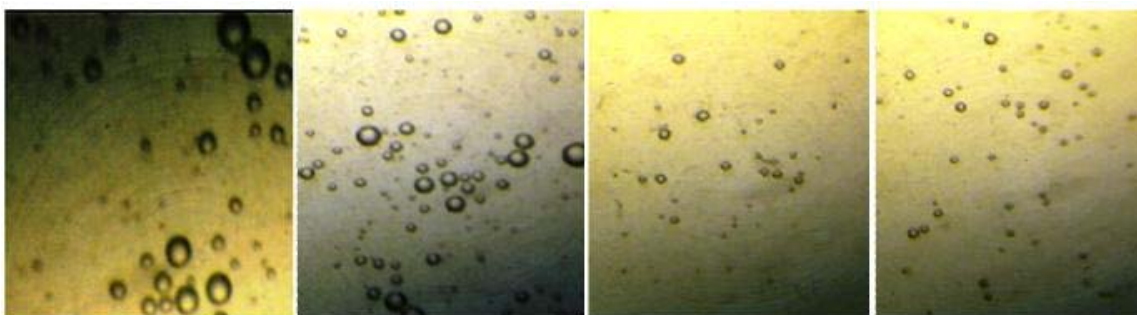


Figure 1: Photomicrographs of Formulations PNG1, PNG2, PNG3 and PNG4 Containing Span 60 as a surfactant

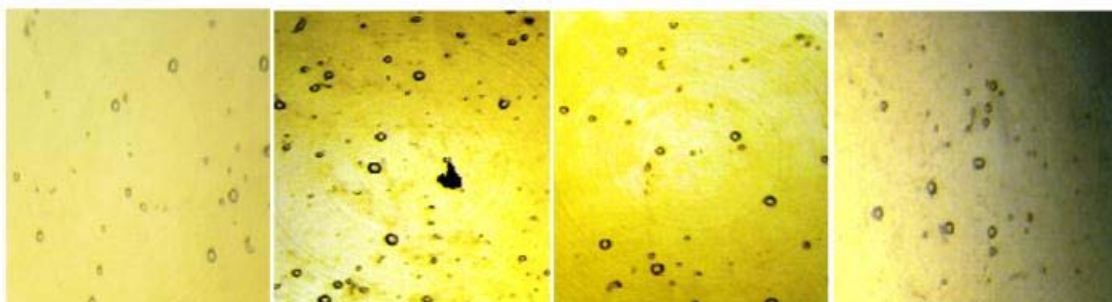


Figure 2: Photomicrographs of Formulations PNG5, PNG6, PNG7 and PNG8 containing Span 40 as a surfactant

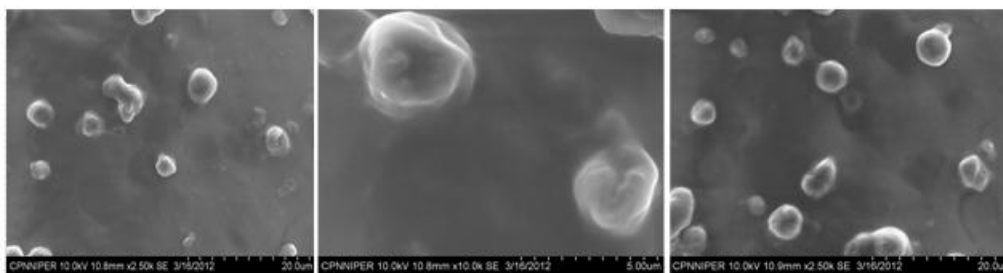


Figure 3: Scanned Electron images of Formulation PNG2

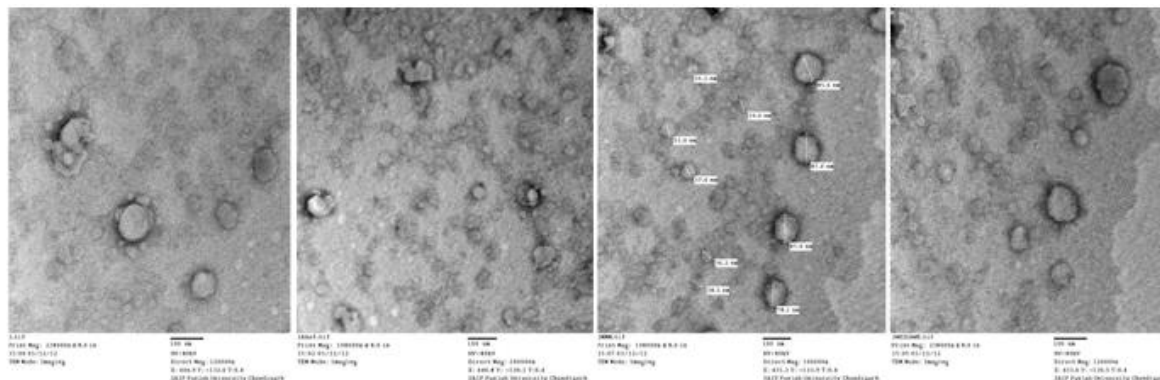


Figure 4: Transmission Electron Photomicrographs of formulation PNG2

Table 2: Zeta Potential Data for formulation PNG2

Formulation code	Zeta potential data	Remarks
PNG2	-51.9	Good

Table 3: Entrapment efficiency (%) and Vesicle Size Determination for different proniosomal gel formulation

Formulation Codes	Entrapment Efficiency (EE %)	Vesicle Size Determination (nm)
PNG1	70.2± 1.44	37.9± 1.41
PNG2	76.8± 1.36	33.7± 3.13
PNG3	66.4± 1.00	34.2± 1.04
PNG4	69.8±0.60	31.1± 1.11
PNG5	54.6±2.77	26.9± 1.81
PNG6	58.5±1.12	23.3± 2.10
PNG7	49.7±1.00	19.1± 0.75
PNG8	57.3±1.70	16.8± 0.92

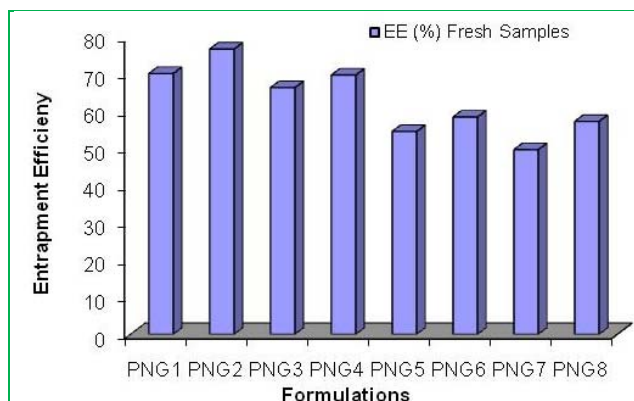


Figure 5: Comparison of Entrapment Efficiency (%) of Different formulations

The mean particle sizes of all the hydrated proniosomal formulations are shown in Table 3 which shows that the niosomes composed of span 60 are larger in sizes than those obtained using span 40. Span 60 has longer alkyl chains compared to span 40 and it was reported that

surfactants with larger alkyl chains generally give larger vesicles^{13, 14}. Increasing cholesterol content or reducing lecithin content contributed to an increase in hydrophobicity with consequent reduction in vesicle sizes. Results of Scanned and Transmission Electron Microscopic study of niosomes prepared from PNG2 proniosomal formulation is shown in figure 3 and figure 4. Most of the vesicles are well identified, spherical and discrete with sharp boundaries^{15, 16}.

Zeta potential analysis was done for determining the colloidal properties and stability of the prepared formulations. The best formulation was selected PNG2 for zeta potential analysis as they have good results in optical microscopy as well as in SEM/TEM images which is shown in table 2. The suitability diluted proniosomes derived niosome dispersion was determined using zeta potential analyzer (Malvern) based on Electrophoresis Light Scattering and Laser Droplet Velocimetry Method^{19, 20}. The temperature was set at 25°C having measurement position of 2.00 mm with cell description (Clear Disposable Zeta Cell).

For niosomes derived metformin hydrochloride proniosomes, entrapment efficiency of formulation PNG2 was found to be approximately 76.8% while the entrapment efficiency of formulation PNG7 was found to be approximately 49.7%. The entrapment efficiency of all formulations was found to be in decreasing order as PNG2>PNG1>PNG4>PNG3>PNG6>PNG8>PNG5>PNG7 shown in table 3.

Proniosomal formulations containing span 60 has more EE (%) than formulations containing span 40 because span 60 has the longest saturated alkyl chain (16-carbon) as compared to span 40 (14-carbon). It had been already

reported that the length of alkyl chain is a crucial factor of permeability and long chain results in highest entrapment of drug²¹. As per literature the cholesterol content of formulations decreased and entrapment efficiency of drug is also decreased. PNG1 and PNG2 has same lecithin content but PNG2 has more cholesterol content as compared to PNG1, so EE (%) of PNG2 (76.8%) is greater as compared to EE (%) of PNG1 (70.2%). PNG3 and PNG4 has same lecithin content but PNG4 has more cholesterol content as compared to PNG3, so EE (%) of PNG4 (69.8%) is greater as compared to EE (%) of PNG3 (66.4%). PNG5 and PNG6 has same lecithin content but PNG6 has more cholesterol content as compared to PNG5, so EE (%) of PNG6 (58.5%) is greater as compared to EE (%) of PNG5 (54.62%). Similarly, PNG7 and PNG8 has same lecithin content but PNG8 has more cholesterol content as compared to PNG7, so EE (%) of PNG8 (57.3%) is greater as compared to EE (%) of PNG7 (49.7%). These results can be explained by the fact that an increase in cholesterol content resulted in an increase of microviscosity of the membrane indicating more rigidity of the bilayers. Cholesterol has the ability to cement the leaking space in the bilayer membranes^{22, 23}.

Vesicle size of different proniosomal gel formulations were determined using optical microscopy. It was observed that vesicle size of PNG1 formulation (37.9nm) was largest and vesicle size of PNG8 was smallest. Decreasing order of vesicle size of different proniosomal gel formulation found to be PNG1>PNG2>PNG3>PNG4>PNG5>PNG6>PNG7>PNG8. The formulation PNG1, PNG2, PNG3, PNG4 contain span 60 as surfactant and PNG5, PNG6, PNG7, PNG8 contain span 40 as surfactant¹³. The vesicle size of proniosomal gel formulation containing span 60 was greater as compared to span 40. The vesicle size of PNG1 was as compared to PNG2 because of lesser cholesterol content in PNG1 as compared to PNG2. The vesicle size of PNG3 and PNG4 is lesser as compared to PNG1 and PNG2 because of decreased soya lecithin content of PNG3 and PNG4 as compared to PNG1 and PNG2. The vesicle size of PNG3 is greater than as compared to PNG4 because of decreased cholesterol content in PNG3 as compared to PNG4. The decreased vesicle size of PNG5, PNG6, PNG7, PNG8 were same as explained above for PNG1, PNG2, PNG3, PNG4 because PNG1, PNG2, PNG3, PNG4 formulations contain same lecithin and cholesterol as present in PNG5, PNG6, PNG7, PNG8.

Release rate was 78.6% for PNG1, whereas 75.1% for PNG2 after 24hr increasing the cholesterol content from 200mg in PNG2 to 400mg resulted in more intact lipid bilayer as a barrier for the drug release and decrease its leakage by improving the fluidity of the bilayer membrane and reducing its permeability which led to lower drug elution from the vesicles.

In PNG3 and PNG7 proniosomal formulations, the drug release was found 78.6% and 92.5% respectively after 24 h. In PNG3 proniosomal formulations, amount of

cholesterol was 200 mg and lecithin was 900 mg less than from other proniosomal formulations. Similarly, PNG7 proniosomal formulation, amount of cholesterol was 200 mg and lecithin was 900 mg.

When release rate was compared for PNG1 and PNG3 proniosomal formulations using span 60, the release rate was better in PNG3 formulations. Similarly, in PNG5 and PNG7 proniosomal formulations using span 40 release rates was more in PNG7 formulations²³. Amount of lecithin was reduced in PNG3 and PNG7 formulations. So, it is concluded that reducing the lecithin results in faster drug release. It may be due to disruption of structure of vesicles having reduced amount of lecithin.

PNG1, PNG2, PNG3 and PNG4 proniosomal formulations were prepared using span 60 whereas PNG5, PNG6, PNG7 and PNG8 formulations were prepared using span 40. So, when release rate was compared for all these formulations, the proniosomal formulations using span 40 showed more release than span 60 keeping cholesterol and lecithin same in both cases shown in figure 6. This can be explained by the fact that proniosomes exhibit an alkyl chain length- dependent release. Moreover, due to larger vesicle size of span 60 proniosomal formulations (PNG1, PNG2, PNG3 and PNG4) compared with span 40 proniosomal formulations (PNG5, PNG6, PNG7 and PNG8) which acted as barrier in the drug release and the release rate was less in span 60 formulations. Also, low release rate for PNG1, PNG2, PNG3 and PNG4 (using span 60) was due to high phase transition of span 60 compared with span 40.

It is to be noted that the in-vitro release results are consistent with those of entrapment efficiency (%), PNG2 proniosomes composed of span 60 with highest entrapment efficiency (%) 76.8 % showed lowest drug release percent after 24 h (75.9 %).

Results of stability studies showed insignificant difference in EE (%) when compared to freshly prepared proniosomal gel at both conditions after one month storage²⁶. However the drug leakage from vesicles was least at 4-8°C followed by 37°C. Hence, it is concluded from the obtained data that the optimum storage conditions for Metformin hydrochloride proniosomal gel was found to be 4-8°C shown in figure 7.

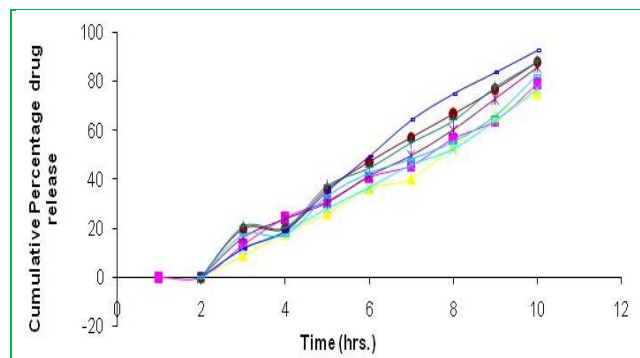


Figure 6: Comparison of *in-vitro* release of all proniosomal formulation

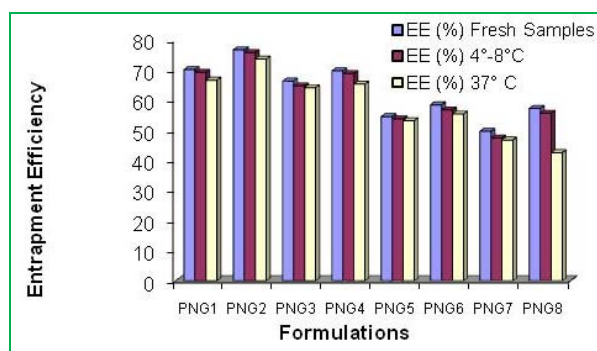


Figure 7: Comparison of EE (%) of all Proniosomal formulations of fresh samples and samples stored at 4°C and 37°C

CONCLUSION

In the present work, metformin hydrochloride has been successfully incorporated by coacervation phase separation method in proniosomal formulations which can be potentially useful for prolonged drug delivery. Results obtained during the work have shown that surfactant type and content of cholesterol and lecithin affect the encapsulation efficiency and drug release rate from proniosomes. Surfactant used in the proniosomal gel also promotes penetration enhancement. Proniosomal gel possesses high entrapment efficiency and also impart controlled systemic transdermal delivery of metformin hydrochloride for the treatment of type 2 diabetes mellitus. Proniosomes may be recognized as suitable carrier for metformin hydrochloride or other drugs due to their ease of production and the fact that they do not require the use of pharmaceutically unacceptable additives. Metformin hydrochloride can be formulated into low dose proniosomal gel for transdermal delivery that can save the recipient from harm of large doses with improved bioavailability and can be recommended for further pharmacokinetics and pharmacodynamics studies in suitable animal models.

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