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FORMULATION AND EVALUATION OF TRANSDERMAL PRONIOSOMAL GEL FOR GLIPIZIDE

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ABSTRACT

The aim of the present investigation was to design a proniosomal gel system of Glipizide for the treatment of type - 2 diabetes mellitus that is capable of delivering entrapped drug over an extended period of time. Skin has a very tough diffusion barrier inhibiting penetration of drug moiety which is rate limiting barrier for penetration of drugs. There are several approaches that deal with penetration enhancement across the skin. Vesicular and provesicular systems are promising amongst them. Vesicular systems including (niosomes, ethosomes, transfersomes and liposomes) are promising systems to cross this permeation barrier. But their major drawback is their unstability, which can be overcome by using provesicular approaches like proniosomes, protransfersomes and proliposomes. Provesicular approaches have been proposed to enhance the stability of vesicles. These approaches may overcome skin barrier properties and enhance percutaneous absorption. In these systems both type of drugs, hydrophilic and lipophillic drugs can be incorporated. Proniosomal gel of Glipizide was prepared by coacervation phase separation technique by varying the composition of drug, span 40, soya lecithin and cholesterol. Proniosomal gel formulations were characterized for pH, Vesicle size, Drug content, Entrapment efficiency, Surface morphology, zeta potential, *in-vitro* drug release, kinetic model, stability studies.

KEYWORDS: Glipizide, Proniosome, Entrapment Efficiency, span 40, cholesterol *etc.*

INTRODUCTION

In the past few decades, considerable attention has been focused on the development of new drug delivery system named Controlled Drug Delivery System. It has prolonged action formulations which gives continues release of their active ingredients at a predetermined rate and predetermined time. The vital objective for the development of controlled release dosage forms is to prolong the duration of action, increased safety margin of high potency drugs due to better control of plasma levels, reduces fluctuations in plasma concentration, reduces serious side effects and gives assurance for higher patient compliance.[1]

Vesicular systems like liposomes or niosomes have specific advantages while avoiding demerits associated with conventional dosage forms because these particles can act as drug reservoirs. These carriers play an increasingly important role in drug delivery because by slowing drug release rate, it is possible to reduce the toxicity of drug. Whereas provesicular system like proniosomes have advantage of minimizing the problems of physical stability related to niosomes such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing.[2]

The Proniosomes are dry formulation which is water-soluble carrier particles that are coated with surfactant and can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The resulting niosomes are very similar to conventional niosomes and more uniform in size[3]

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, on hydration for a short period of time with water, offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route.[4]

Some of the Advantages of Proniosomes include[5]

- Avoiding problem of physical stability like aggregation, fusion, leaking.
- Avoiding hydrolysis of encapsulated drugs which limiting the shelf-life of the dispersion.

- The storage makes proniosomes a versatile delivery system with potential for use with a wide range of active compounds.
- Biodegradable, biocompatible and non-immunogenic to the body.
- Shows controlled and sustained release of drugs due to depot formation.
- Furthermore, unacceptable solvents are avoided in proniosomal formulations. The systems may be directly formulated into transdermal patches and doesn't require the dispersion of vesicles into polymeric matrix.

Glipizide is a second generation, oral blood- glucose-lowering drug of the sulfonyl urea class. Glipizide has been in extensive use to treat non-insulin dependent diabetes mellitus and acts by increasing the release of endogenous insulin as well as its peripheral effectiveness. It has been associated with sever and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heart burn, anorexia and increased appetite after oral therapy, compliance problem can arise.[6]

The Glipizide is a suitable drug candidate for design into an effective transdermal system when would eliminates its first pass metabolism, ensure more uniform plasma levels, reduces side effects like gastric irritation and hence aid in patient compliance.

MATERIALS AND METHOD

Materials

Glipizide was gifted from Biocon Limited, Bangalore (India), soya lecithin was gifted from Pharma Sonic Biochem Extractions Ltd., Indore (India). Span 40, cholesterol and ethanol were purchased from SD fine chem limited, Mumbai (India). All the reagents were used without any purification. Phosphate buffer solution pH 7.4 was prepared as described in the Indian pharmacopoeia.

PREFORMULATION STUDIES

Melting point determination

Digital melting point apparatus was used for the determination of melting point of Glipizide. A few quantity of Glipizide is taken and placed in a thin walled capillary tube about 10-12 cm long and 1mm inside diameter and closed at one end, then suspended into an oil bath containing silicone oil. Apparatus was switched on and allow for heating, the temperature range over which the sample is observed to melt is taken as the melting point.

Solubility analysis

Solubility analysis was carried out for Glipizide samples were in various solvents. 10 mg of Glipizide was dissolved in 10 ml of different solvents *i.e.*, water, phosphate buffer pH 7.4, methanol, methylene chloride and acetone. Solubility was determined on the basis of physical appearance.

Determination of λmax

Glipizide 10 µg/ml concentration was prepared in phosphate buffer pH 7.4. The solution was scanned from 200-400 nm by UV spectrophotometer and a spectrum was observed for absorption maxima.

Standard calibration curve of Glipizide

- 100 mg of accurately weighed Glipizide was dissolved in 100 ml of methanol.
- 10 ml of the above solution was diluted to 100 ml phosphate buffer pH 7.4.
- From the above solution 5, 10, 15, 20, 25 and 30 µg/ml were prepared and analyzed by UV spectrophotometer at λmax 276 nm.

The graph of absorbance *v/s* concentration in µg/ml was plotted and r^2 value of this graph was calculated to check the linearity of the absorbance against concentration.

Drug-Excipient interactions studies by FTIR

Drug excipient compatibility studies were carried out using FT-IR. Infrared spectrum of pure drug, cholesterol, soya lecithin, span 40 and the physical mixture of drug:cholesterol:soyalecithin:span40 in 1:1:1:1 ratios were recorded in between 400 to 4000 cm^{-1} by using liquid sampling technique.

Drug excipient compatibility studies by DSC

Drug-excipients compatibility study was performed by Differential Scanning Calorimetry (DSC). Thermal characteristics of the pure drug and physical mixture of drug, span 40, cholesterol, soyalecithin (1:1:1:1) were performed by using an automatic thermal analyzer system (Mettler DSC 823, Germany). The entire samples were run at a scanning rate of 10 °C per min from 25-300 °C.

METHOD

Preparation of proniosomal gel by Coacervation phase separation

Accurately weighed amount of surfactant (Span 40), lecithin, cholesterol and drug (Glipizide) were taken in a clean and dry wide mouthed glass vial and alcohol (3ml) was added to it. 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 5-10 min until the surfactant mixture was dissolved completely. Then the Phosphate Buffer Solution (pH 7.4) was added and warmed on a water bath till clear solution was formed which was converted into proniosomal gel on cooling. The obtained gel was preserved in the same glass bottle in dark conditions Table 1.[7-8]

Evaluation of Glipizide proniosomal gel[9-10]

Physical examination

The prepared proniosomal gel formulations were examined visually for physical appearance of color,

consistency texture and greasiness. These all features were done for proniosomal gel formulation.

Determination of pH

The pH of all proniosomal gel formulations of Glipizide was measured using pH meter before and after incorporation of the drug. The glass electrode first calibrated with two standard buffers pH 4.0 and 7.0 solution then measurement of pH of each formulation was done in triplicate and average values were calculated.

Viscosity

Viscosity of proniosomal gel of Glipizide was determined using Brookfield Viscometer.

Surface morphology by scanning electron microscopy (SEM)

Surface morphology of the proniosomes was determined by using a Scanning electron microscope. Proniosomes was coated with Gold-palladium alloy of 120°A Knees on the sample sputter coating unit (Model E5 100 Polaron UK) and their surface morphological were photographed with Jeol JSM-T330A, Japan Scanning electron microscope.

Vesicle size analysis

The proniosomal gel was hydrated in a small test tube with few ml of PBS (pH 7.4) with manual shaking for 5 min. The dispersion was observed under optical microscope at 10X, 40X magnification. About 300 niosomes were measured individually, average were taken. The average sizes of vesicles were measured using calibrated ocular and stage micrometer fitted in optical microscope.

Zeta potential analysis

Zeta potential analysis was done for determining the colloidal properties of the prepared formulations. The suitably diluted proniosomes derived niosome dispersion was determined using zeta potential analyzer based on Electrophorectic light scattering and laser Doppler Velocimetery method. The temperature was set at 25 ℃. Charge on vesicles and their mean zeta potential values were measured.

Drug content

Proniosomes formulation equivalent to 10 mg of Glipizide was dissolved in 10 ml methanol. After suitable dilution with phosphate buffer solution pH 7.4, absorbance was measured by UV spectrophotometer against blank at λmax 276 nm and the drug content was calculated.

Entrapment Efficiency (EE %)

To 100mg of proniosomal gel formulation, weighed in test tube, was added 10 ml of phosphate buffer solution (pH 7.4). The aqueous suspension was sonicated in a sonicated bath. The niosomal dispersion was centrifuged at 18000 rpm at 5-6 ℃ for 30 min to separate Glipizide containing niosome from the entrapped drug. The precipitate consisting of the vesicular pellets was washed three times with 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 276 nm. The percentage of drug encapsulation was calculated by the following equation:

EE (%) = Total drug – Diffused drug/ Total drug × 100

***In-vitro* release studies**

In-vitro release pattern of niosomal suspension formed by proniosomal gel was carried out by using egg membrane in Dialysis tube. One gram of Glipizide proniosomes equivalent to 20 mg Glipizide was taken 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±1℃. 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 experiment. The withdrawn samples were appropriate diluted and analyzed for drug content using UV spectrophotometer at 276 nm keeping PBS (pH 7.4) as blank.

Stability study

Accelerated stability testing studies was performed for 6 months. The optimized formulations were kept at 40 ± 2 ºC and 75 ± 5% RH. Physical appearance, drug content and % drug release were fixed as evaluation parameter for stability study.

RESULTS AND DISCUSSION

The melting point of the obtained drug sample was found to be 208 ℃, which complied with IP standards thus indicating the purity of drug. Glipizide was soluble in methanol and dimethyl formamide. Slightly soluble in methylene chloride and acetone. Practically insoluble in water. Springily soluble in phosphate buffer solution pH 7.4.

The λmax of the Glipizide in phosphate buffer pH 7.4 was found to be 276 nm and the curve was shown in Fig 1. Glipizide obeys the Beer's law in concentration range of 5-30 µg/ml in phosphate buffer pH 7.4 with regression of coefficient (r^2) 0.9999 and slope 0.0226. The calibration data is given in Table 2 and calibration curve was constructed in Fig 2.

FTIR spectra of pure Glipizide showed sharp characteristic peaks at 3248.23, 2939.61, 1689.70, 1442.80, 1157.33 cm^{-1}. FTIR characteristic peaks of pure drug are also observed in the spectra of physical mixture indicating no modification for interaction between the drug and excipients. This proves that there is no potential incompatibility with the drug and the excipients used in the proniosome formulation. Comparative study of FTIR graphs are showed in Fig. 3 and 4.

The thermo gram obtained was shown in (Fig. 5, 6). The endothermic peak of pure drug was retained in the physical mixture of drug and excipients and confirmed that there was no interaction between drug and excipients.

The physical appearance of prepared formulations were found to be translucent, yellowish glossy, smooth and non-greasy on application and the pH of the optimized F4formulation was found to be 6.4 . Determination of viscosity of proniosomal gel formulations as in increasing order F1>F2>F3>F4 respectively due to increasing the surfactant ratio. It was found to be 11,357 cps and observed that proniosomal gel formulations showed good spreadability and viscosity.

The photomicrographs of hydrated F1-F4 proniosomal formulations by optical microscope shown in Fig. 7 which revealed that niosomes with spherical shape were formed and no aggregation or agglomeration is observed. The surface morphology was studied by Scanning electron microscopy (SEM). The SEM photographs of optimized proniosomes formulation F4 as shown in Fig. 8. SEM photographs showed that the niosomes formed were spherical in shape.

The vesicle sizes were found in the range of 0.880 µm and 1.8 µm From F1-F4. From the results we observed increased vesicle size by increasing the concentration of surfactant (span 40). The average vesicle sizes of formulation F1-F4 were found to be 0.880 µm, 1.10 µm, 1.45 µm and 1.8 µm, respectively Table 3.

Zeta potential of optimized formulation F4 of Glipizide proniosomes as shown in Fig. 9 and it was found to be – 44.1 mV, which indicates that they are sufficient to be stable.

The percentage of drug content and entrapment efficiency of Glipizide in different gel formulations with different drug, cholesterol, soyalecithin and surfactant ratio was found to spectrophotometrically. The results are given in the following Table 3. The highest drug content was observed in F4 (92.90%). Highest entrapment efficiency was observed in F4 with 94.40%, respectively. The high drug entrapment may be observed due to the higher fluidity of the vesicles and larger vesicles size also contributes to the higher entrapment efficiency. The results showed that increase in surfactant ratio increases the entrapment efficiency.

The release of Glipizide from span 40 was varied according to their concentration. The progressive decrease in the amount of drug diffused through a dialysis membrane from formulations F1 to F4 attributed to gradual increase in span 40 content. It has been concluded that, if we increase the concentration of surfactant the diffusion of drug also decreases. The amount of drug diffused from formulation F4 was showed 49.5% which was lower among the formulations F1-F3 which were showing 58.73%, 55.65%, 53.6% Fig. 10. However, the results clearly showed that the gels have ability to retain the drug for prolonged period of time. All the formulations follows Higuchi model and the release mechanism was diffusion controlled. The 'n' values of all formulations were found to be more than 0.5. This indicates that the release approximates Non-Fickian diffusion mechanism. However, the results clearly showed that the gels have ability to retain the drug for prolonged period of time.

Accelerated stability studies were carried out at 40 ± 2 ºC and 75 ± 5 % RH for the optimized formulation F4 monitored for drug content, pH and dissolution profile study. The results are shown in Table 4 which indicated that all the proniosomal gels were stable during storage period.

Table 1: Formulation design for the preparation of proniosomal gel formulation

Formulation code	Drug (mg)	Span(40) (mg)	Soya lecithin (mg)	Cholesterol (mg)
F1	100	100	100	100
F2	100	200	100	100
F3	100	400	100	100
F4	100	600	100	100

Table 2: Data for Standard calibration curve of Glipizide

SL. NO.	Concentration in µg/ml	Absorbance at 276 nm
1	5	0.114
2	10	0.231
3	15	0.340
4	20	0.450
5	25	0.563
6	30	0.675

Table 3: Vesicle size, % Drug content and % Entrapment efficiency of proniosomes formulation F1-F4

Formulation code	Average vesicle size in µm	% Drug content	% Entrapment efficiency
F1	0.880	81.20	89.37
F2	1.10	85.31	90.02
F3	1.45	89.70	92.73
F4	1.8	92.90	94.40

*Each value was the average of 300 Vesicles

Table 4: Accelerated stability studies for optimized formulation of proniosomal gel F4 at 40 ± 2ºC and 75 ± 5%

Parameters	Duration in months			
	0	1	3	6
%Drug content	92.90	92.6	92.2	91.92
pH	6.4	6.45	6.5	6.54
% CDR	49.5	48.8	48.2	47.3

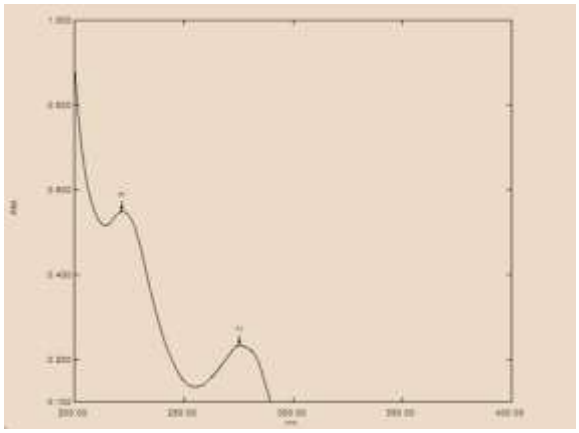

Fig.1: λmax of the Glipizide

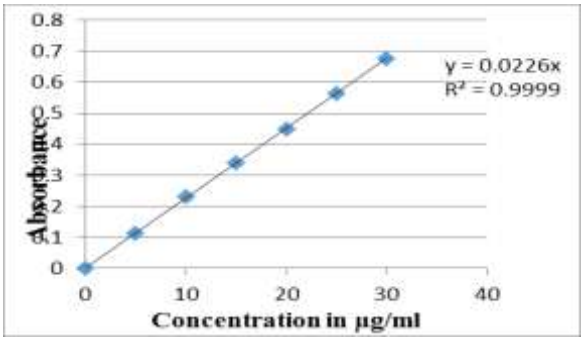

Fig. 2: Standard calibration curve of Glipizide

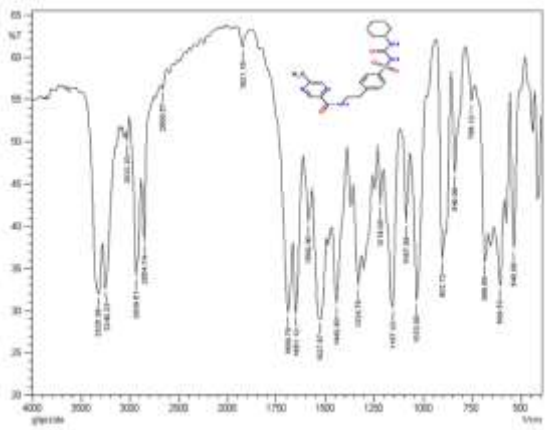

Fig. 3: FT-IR Spectroscopy of pure Glipizide

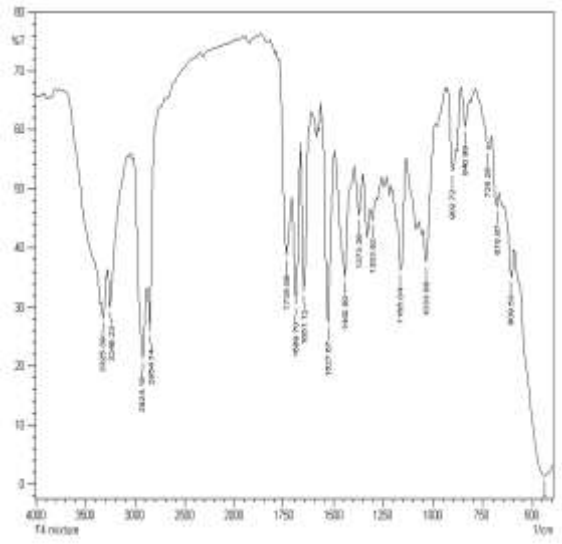

Fig. 4: FT-IR Spectroscopy of Glipizide+Span40+Soyalecithin+Cholesterol

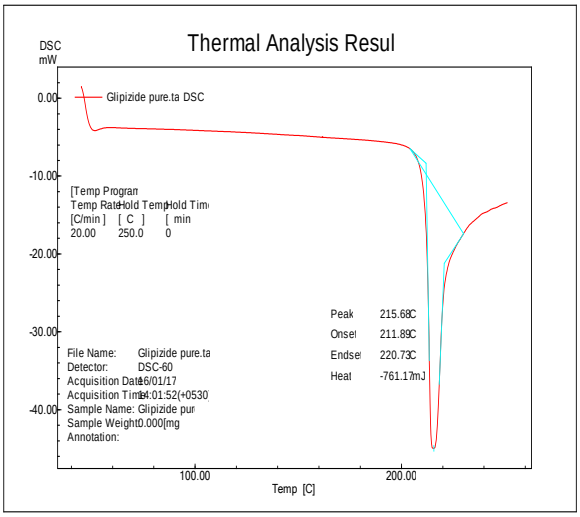

Fig. 5: DSC thermo graph of pure drug Glipizide

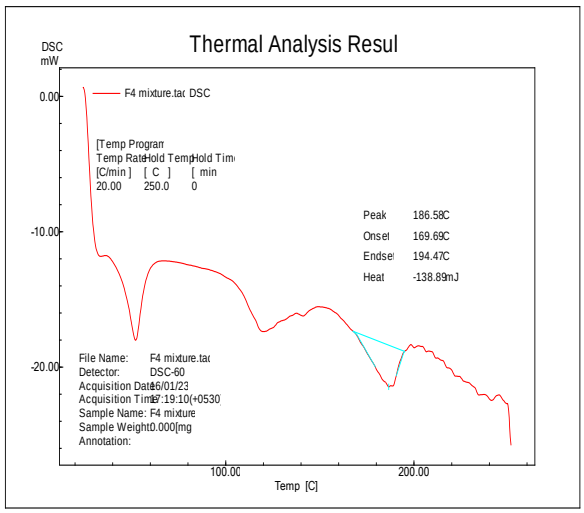

Fig. 6: DSC thermo graph of Glipizide+Span40+Soyalecithin+Cholesterol

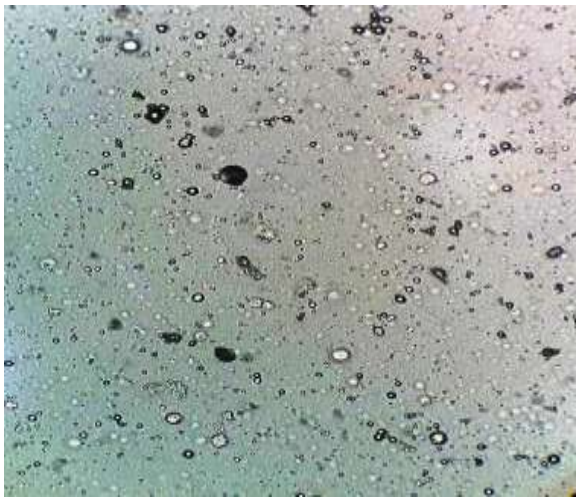

Fig. 7: Microphotographs of optimized proniosomal gel formulation F4

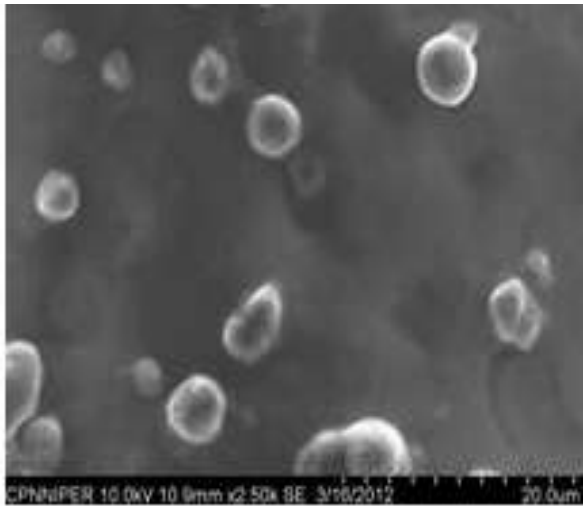

Fig. 8: Scanning electron micrograph of optimized proniosome formulation F4

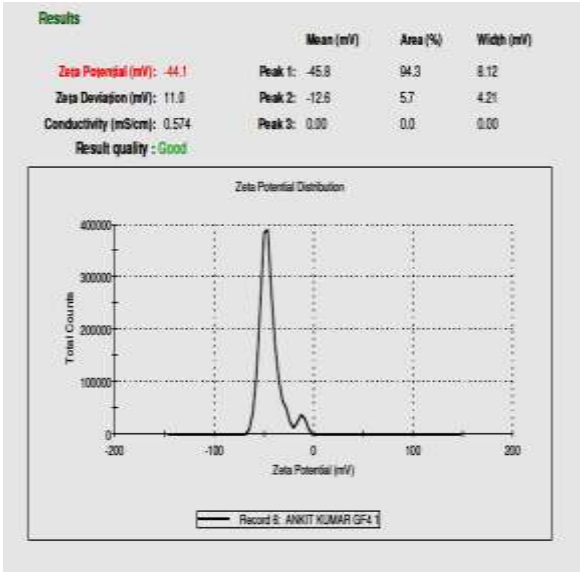

Fig. 9: Zeta potential of optimized proniosomal gel formulation F4

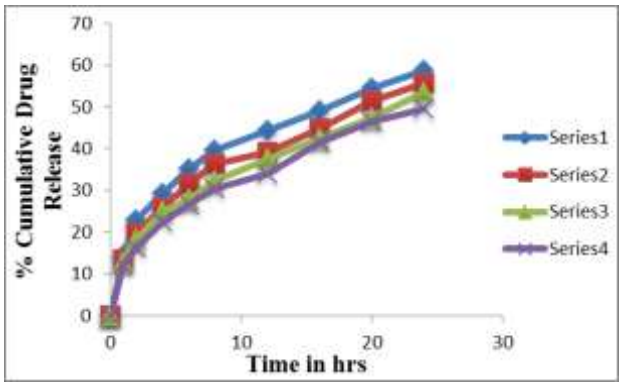

Fig. 10: % CDR of proniosomal gel formulation from F1-F4

CONCLUSION

Glipizide was successfully entrapped within the non-ionic surfactant vesicles by using Coacervation phase separation method. The above mentioned results suggest that span 40 can be successfully used for entrapping the drug. The formulation F4 showing better controlled release due to the more surfactant concentration. The inclusion of an optimal ratio of surfactant in the vesicles contributes to the enhancement of drug permeation from proniosomal gel formation. The *in vitro* permeation studies suggest that proniosomal gel formulations enhance the rate of permeation of the drug across skin. This penetration enhancement effect may be attributed to both the presence of non-ionic surfactants and cholesterol. Thus proniosomes may be a promising carrier for Glipizide due to their simple and cost effective production.

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