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FORMULATION DEVELOPMENT AND EVALUATION OF REPAGLINIDE PRONIOSOMAL GEL FOR TRANSDERMAL DELIVERY

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ABSTRACT

The aim of the present study is to formulate and evaluate Proniosomal gel formulations as transdermal delivery systems of Repaglinide to improve its therapeutic effect in a controlled manner. A 2x 2factorial design have been applied for optimization, by varying surfactant and soyalecithin concentration. All the formulations were evaluated for various parameters such as vesicle size analysis, surface morphology, zeta potential, pH, stability studies. The result shows that the span40(SF_4) have better Entrapment efficiency (96.23±0.8) and also high % drug content (95.68±0.5) compare to Span60, it is evident that Span40 is better suitable surfactant to enhance the bioavailability and better therapeutic effect of Repaglinide loaded Proniosomal gel through transdermal drug delivery system.

KEYWORDS: Proniosomal gel of Repaglinide, Transdermal delivery, Antidiabetic, 2 X 2 factorial design.

INTRODUCTION

Transdermal delivery may be defined as the delivery of a drug through ‘intact’ skin so that it reaches the systemic circulation in sufficient quantity, to be beneficial after administration of a therapeutic dose. Transdermal systems are ideally suited for diseases that demand chronic treatment. Hence, anti-diabetic agents of both therapeutic and prophylactic usage have been subjected to transdermal investigation.[1]

The versatile vesicular drug delivery through transdermal route, proved to be beneficial due to the vesicles tendency to attach and adhere to the cell surface and leading to the increased permeation rate. However, the major pathways for drug permeation in the tissues is through sweat glands, stratum corneum layer and hair follicle associated with sebaceous glands.[2]

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.[3,4]

Proniosomes are vesicular systems, in which the vesicles are made up of non-ionic based surfactants, cholesterol and other additives. Semisolid liquid crystal gel (proniosomes) ready by dissolving the surfactant in a minimal quantity of an acceptable solvent, namely ethanol and then hydration with slightest amount of water to form a gel. These structures are liquid crystalline dense niosomes hybrids that can be converted into niosomes instantly upon hydration or used as such in the topical/transdermal applications. Proniosomal gels are generally present in transparent, translucent or white semisolid gel texture, which makes them physically stable throughout storage and transport.[5]

Repaglinide is a novel oral blood glucose lowering agent from the class of Meglitinide. It stimulates release of insulin from the pancreatic cell by closure of KATP channels and is rapidly absorbed and eliminated from the body. Repaglinide is developed in attempts to overcome the adverse effects associated with existing antidiabetic compounds. These include hypoglycemia, secondary failure and cardiovascular side effects.[6]

Hence, in the present investigation, an attempt is made to formulate Repaglinide proniosomal gel in order to increase bioavailability and reduce side effects by achieving transdermal drug delivery.

MATERIALS AND METHODS

Materials

Repaglinide was gifted from Biocon Ltd. Karnataka, Soya lecithin was purchased from Pharma Sonic Biochem Extractions Ltd. Indore and Span 60, Span 40 and cholesterol have been purchased from S D fine chemical, Karnataka.

Preparation of Repaglinide Proniosomal Gel

The selection of suitable surfactant have been done by applying 2x3 factorial design for the different surfactant like span 20, span 40, and span 60 by keeping – as '0'mg and + as 200mg. Based on the formation of gel and vesicle forming capacity, the suitable surfactant were selected and applying 2x2 factorial design individually for each surfcantant and changing the concentration of surfactant and soyalecithin to study the effect and interaction.

Proniosome formulation containing Repaglinide was prepared by using co-acervation phase separation method. Optimization of proniosome formulation was done by preparing varying concentration of drug, surfactants (span40/span60), lecithin and cholesterol.

Accurately weighed amount of surfactant (span40/span 60), lecithin, cholesterol and drug (Repaglinide) were taken in a clean and dry wide mouthed glass vial and alcohol (3 ml) 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 60-70 °C for about 5-10 min until the surfactant mixture was dissolved completely.

Then phosphate buffer 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.[7-9]

Table 1: Screening of surfactant by 2x3 factorial design.

Formulation Code	Drug (mg)	Cholesterol (mg)	Soya lecithin (mg)	Span20 (mg)	Span40 (mg)	Span60 (mg)	Physical appearance
1	100	50	400	-	-	-	No vesicle formation
a	100	50	400	+	-	-	Transulent gel formed
b	100	50	400	-	+	-	Glossy gel formed with vesicle
c	100	50	400	+	+	-	Transparent gel formed
ab	100	50	400	-	-	+	Transulent gel shows vesicles formation
ac	100	50	400	+	-	+	Poor vesicle with thick gel
bc	100	50	400	-	+	+	Clear translucent gel formed with vesicle
abc	100	50	400	+	+	+	Thick viscous gel with poor vesicles

+ (200mg) and - (0mg)

From the screening study we came to know that the vesicle formation and particle sizes are better with Span40 and Span60 and selected for further study. we formulated Proniosomal gel by applying 2x2 factorial design keeping Drug (100mg), cholesterol(50 mg) at constant level for all the formulation and by varying Soya lecithin(100 mg, 400mg) and Span (50mg, 200mg) for lower and upper limit.

Table 2: Proniosomal gel formulation using Span40 by applying 2 X 2 factorial designs keeping lower (-) to upper (+) limit (50, 200) respectively.

Formulation code	Soyalecithin (mg)	Span40 (mg)
SF_1	100	50
SF_2	400	50
SF_3	100	200
SF_4	400	200

Table 3: Proniosomal gel formulation using Span60 by applying 2 X 2 factorial designs keeping lower (-) to upper (+) limit (50, 200) respectively.

Formulation code	Soyalecithin (mg)	Span60 (mg)
S_1F_1	100	50
S_1F_2	400	50
S_1F_3	100	200
S_1F_4	400	200

Evaluation of Repaglinide Proniosomal Gel[10-14]

The prepared proniosomal gel were evaluated for different parameters like FTIR, Drug-Excipients compatibility, vesicle size analysis, viscosity, physical appearance, pH determination, Surface morphology, Vesicle size analysis, Zeta potential analysis, drug content, entrapment efficiency, *in-vitro* diffusion study and stability studies as per ICH guide lines.

***In-vitro* diffusion study**

In-vitro release pattern of niosomal suspension formed by proniosomal gel was carried out by using egg membrane in Dialysis tube. One gram of Repaglinide proniosomes equivalent to 20 mg Repaglinide 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°C. 5 ml sample were withdrawn periodically, the sink conditions were maintained throughout the experiment for 24 hrs.

The withdrawn samples were appropriate diluted and analyzed for drug content using UV spectrophotometer at 236 nm keeping PBS (pH 7.4) as blank. The rate and release mechanism of Repaglinide from the prepared proniosomal gel were analyzed by fitting the release data into various kinetic models.

RESULTS AND DISCUSSIONS

The λmax of the Repaglinide in Phosphate buffer pH 7.4(10 µg/ml) was found as 236 nm and the spectra was shown in Figure 1. Standards calibration curve of Repaglinide obeys the Beer's law in concentration range of 0 - 50 µg/ml in Phosphate buffer pH 7.4 with regression of coefficient (r2) of 0.999 and slope (m) of 0.017. This showed linear relationship between concentration and absorbance as shown in Figure 2. The melting point of the drug sample was found to be 128 °C by Thieles tube method and 127 °C by DSC method which complied with IP standards, thus indicating the purity of drug, is shown in the DSC Figure 3. FTIR spectra of pure Repaglinide showed sharp characteristic peaks 1087.89 cm^{-1}, 2800.73 cm^{-1}, 1774.57 cm^{-1}, 2931.90 cm^{-1} and 3309.96 cm^{-1} and the same characteristic peak were also observed in the physical mixture, confirmed no interaction between the drug and excipients. Comparative studies of FTIR graphs are shown in figure 4-6.

Based on the optimization study we came to know that the Span20 having poor characteristics to form the clear vesicles as well as the drug entrapment. The Proniosomal gel formed using Span40 and Span60 have good gelling and vesicle formation capacity hence, selected for further study.

The Repaglinide Proniosomal gels were prepared by the Co-accervation phase separation method with Soyalecithin and span as carrier. 2 X 2 Factorial design (QI Macros^{R} –DOE software) have applied individually for the different surfactant and studied the effect, main effect and interaction.

The physical appearance of prepared formulations were found to be translucent, yellowish glossy, smooth and non-greasy on application as shown in the Table 5 and 6.

The vesicle sizes observed by optical microscopy, sizes were measured for 300 particles and percentage of vesicle size distribution of different sizes were analyzed. On hydration with pH 7.4 buffer solution, the Proniosomal gel were quickly form the vesicles as shown in microphotographs from figure 7 and 8. In both soyalecithin and span containing formulation,

the maximum percentage of vesicles lies in the size range of 30-100nm and further the vesicle size were determined by Microtrac particle size analyzer for the formulation SF_4 and S_1F_3 and found that the particle size for 300 maximum number of particles were in the range of 45 - 119 nm with PDI 31.7. For SF_4 formulation in which the maximum number of particle lies in the range of 292.4 - 334.0 nm with PDI 39.16 as shown in figure 7. From these results we observed that higher the concentration of span 40 the size of particle size were slightly shifted to higher values as shown in figure 9. In case of span60 based Proniosomal gel (S_1F_3) particle size were found 51.5nm and the particles are ranges from 45-251.8nm with PDI 152 for S_1F_3 as shown in figure 10,and from result we have concluded that by lowering the concentration of Soyalecithin we can obtain the larger vesicle with increasing Surfactant concentration by applying 2 X 2 factorial design.

Viscosity measurement of all the formulations revealed optimum consistency and the results are reported in Table 5 and 6. It was found to be 11,357 cps and observed that proniosomal gel formulations showed good spreadability and viscosity.

The pH of optimised Repaglinide proniosomal gel formulation SF_4 and S_1F_3 was found to be in the acceptable limits for topical application as they were ranged from 6.4 and 6.52.

The surface morphology was studied by scanning electron microscopy (SEM). The SEM photographs of optimized Proniosomal gel formulation SF_4 and S_1F_3 as shown in figure 13 and 14. The crystalline structure of soyalecithin and span40 as well as span60 was modified and porous structure in the images confirmed the formation proniosomes that is confirmed the incorporation of surfactant and drug.

Zeta potential of optimized formulations SF_4 and S_1 F_3 of Repaglinide Proniosomal gel as was found to be -8.2 mV and 6.3mV respectively, which indicates that they are sufficient to be stable.

Uniformity in content of all Repaglinide proniosomal gel formulations was found to be 80% and it confirmed to assure uniformity in dosages. The results are reported in Table 7.

Entrapment efficiency of all formulation have been determined and the results are depicted in table 5.18, Entrapment efficiency SF_1-91.25%, SF_2-93.23%, $SF_3$90.23%, SF_4-96.23%, S_1F_1-91.54%, S_1F_2-90.56%, S_1F_3-95.32%, S_1F_4-91.56%, was observed. Highest entrapment efficiency was observed in SF_3 (- +) and S_1F_4(++).

Effect of surfactants such as span varied the % EE from low level to high level. The % EE increased in-case of Span40 from 91.45 % to 96.23 % but in Span60 the % EE have also been increased from 90.23% to 95.32%. Span40 based Proniosomal gel increases the % EE at a level of 7.47% but Span60 based Proniosomal gel showed 6.42%. On comparison the Span60 have showed better % EE efficacy.

The results indicate soyalecithin have a high drug entrapment efficiency, due to the higher fluidity of the vesicles. The results showed that increase in surfactant ratio increases the entrapment efficiency. In formulation S_1F_4 the concentration of the soyalecithin lower concentration (-) and span60 in higher concentration(+)which is having longest alkyl chain length and higher phase transition temperature so that the entrapment efficiency more dependent on surfactants compare to soyalecithin Further the effect of carrier and span-40 and span60 on % EE have been studied by 2 x 2 factorial design and observed as the increase in concentration of soyalecithin and span40 shows better effect with but in low concentration the effect in less and the increased concentration of variables will not cause the interactions as observed in full factorial design and following results as shown in the Figure 19.

The low concentration of both variables such as Span60 and Soyalecithin cause the interaction which affect the entrapment efficiency, but the increase concentration of Span60 alone does not cause the interaction with soyalecithin, and high concentration of both variables are independent and will not cause the interaction.

In-vitro release behaviour of all formulations was summarized in table 5. *In-vitro* drug release of Repaglinide Proniosomal gel in pH 7.4 was performed using dialysis tube. The release of Repaglinide from soyalecithin and span and cholesterol containing as excipients proniosome were studied and calculated using % cumulative drug release by varying the concentration of excipients. The *in-vitro* drug release study revealed that in Cholesterol and Soyalecithin based formulations cause the changes in the *in-vitro* release and in % drug release but in-case of surfactant based Proniosomal gel increased *In-vitro* release as the excipients concentration increased from lower to higher concentration and shows negative effect in absence of Surfactants.

The percent drug diffusion for SF_4 and S_1F_3, was observed at the end of 24 hrs are as follows 65.45% and 60.56% respectively. However, all the formulation releases the drug in a controlled manner for 24 hrs.

The amount of drug diffused In Span40 based Proniosomal formulation (SF_1- SF_4) SF_1 was showed 67.2% SF_2 -63.56 % SF_3 -65.45% was showed 63.56% and SF_4 - 73.45%.SF_4 which was higher among the formulations. In span60 containing formulation S_1F_1 was showed 67.2% S_1F_2-63.56%, S_1F_3- 72.3% and S_1F_4-60.56%, S_1F_3 showed better drug diffusion in Span60 surfactant S_1F_1 - S_1F_4 at the end of 24 hrs.

Further the effect of carrier and Surfactants on % CDR have been studied by 2x2 factorial design for 2hrs, 6hrs and 12 as well as 24hrs time release and observed following results as shown in the figure 19 to 20.

Both the surfactants are affect the % cumulative drug release and in the same time interval the rate of drug release will be differ as shown here Span40 based Proniosomal gel SF_4 increases the % CDR at a level of %17.3 (2hrs), 32.2% (6hrs) and 43.54% (12hrs) 73.7% (24hrs) but Span60 based Proniosomal formulation showed 16.89% (2hrs), 32.55% (6hrs), 46.2% (12hrs) and 67.2% (24hrs) respectively.

The results suggested that varying the surfactant from low to high varied the % CDR from the formulation.

Effect of Span40 based formulation the increasing the level of span40 from low to high level, increasing the %CDR from 59.5% to 73.5%, Span60 based formulation the increasing the level of span60 from low to high level, increasing the %CDR from 60.56% to 67.2%.

Stability studies performed for 6 months in four different climatic zone as per ICH guidelines. The optimized formulations were observed for Physical appearance, drug content and % drug release were fixed as evaluation parameter for stability.

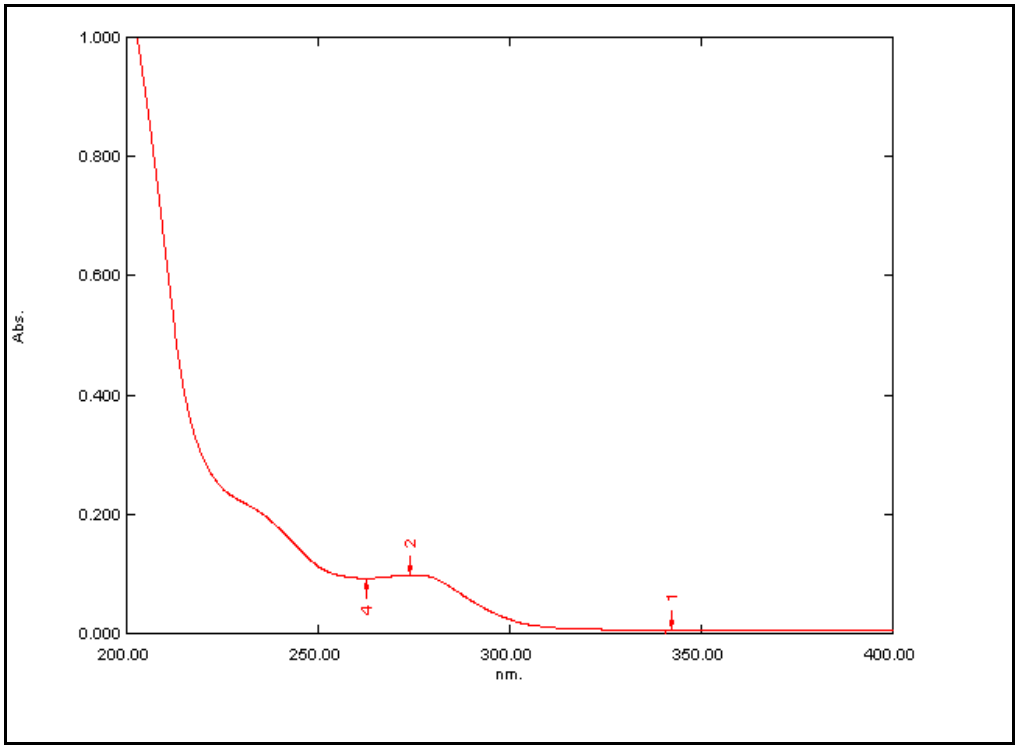

Figure 1: UV spectrum of Repaglinide at 10 µg/ml concentration.

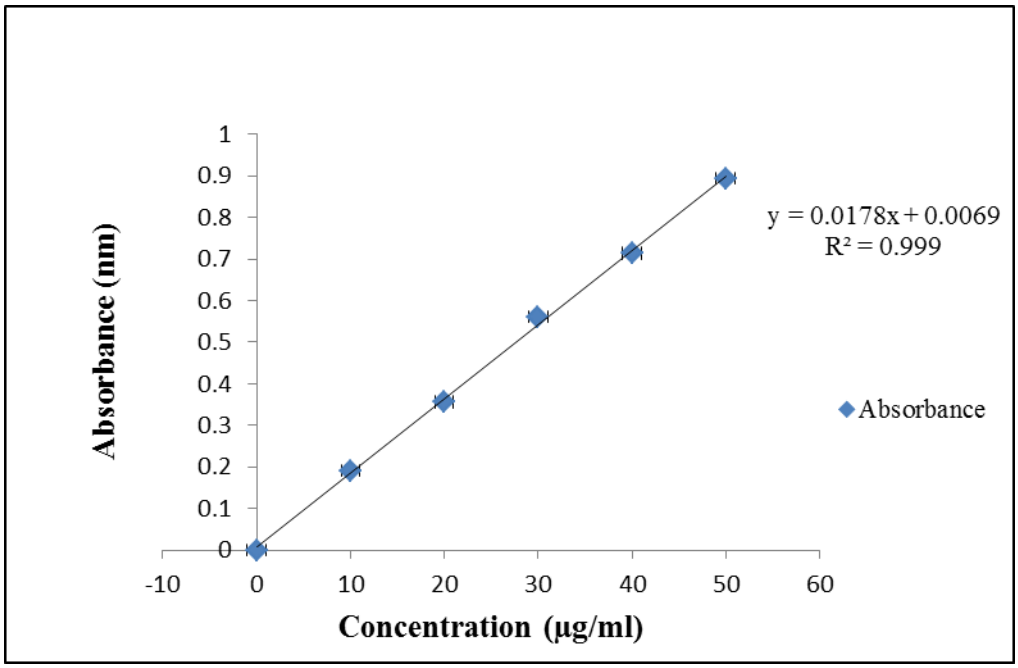

Figure 2: Standard calibration curve of Repaglinide.

Table 4: Melting point report of Repaglinide.

Reported	Method	Observed
126-128°C	Thiele's tube method	128 °C
	DSC	127 °C

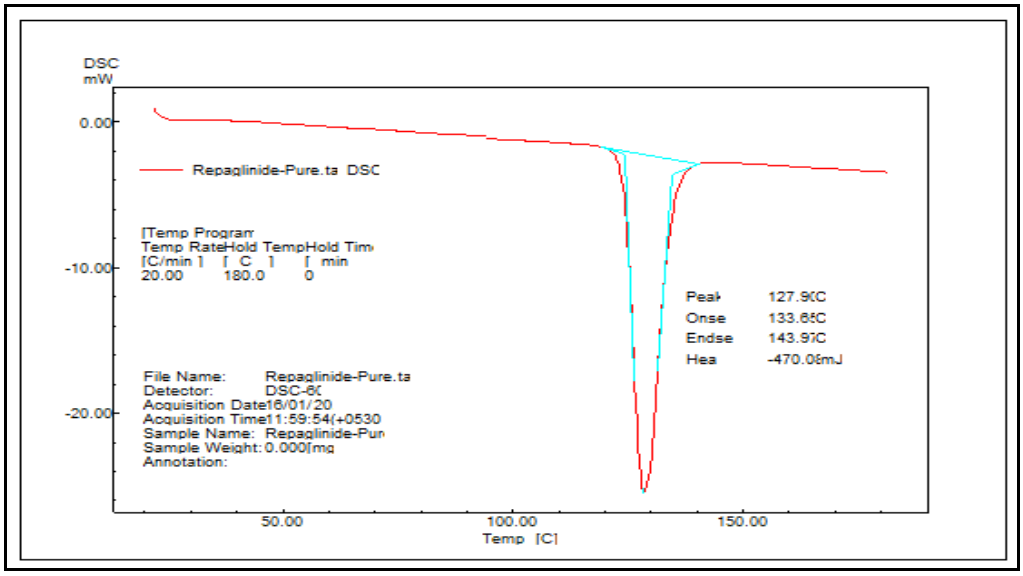

Figure 3: DSC Thermograph of Repaglinide.

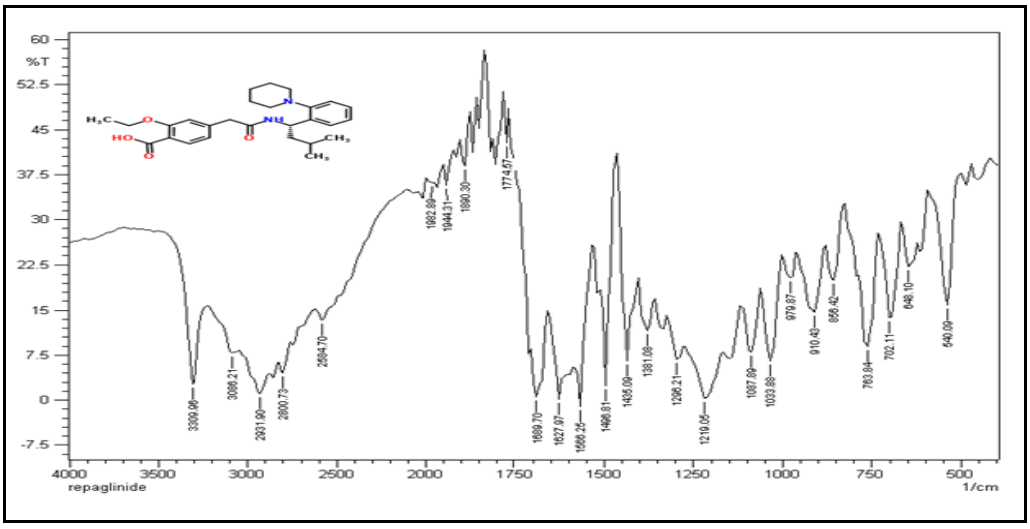

Figure 4: FTIR spectra of Repaglinide.

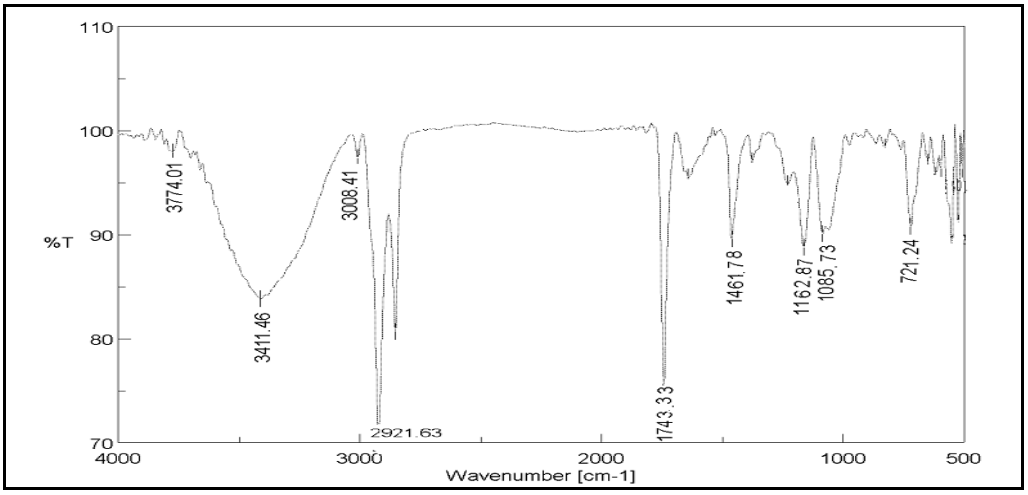

Figure 5: FTIR spectra of physical mixture SF_4.

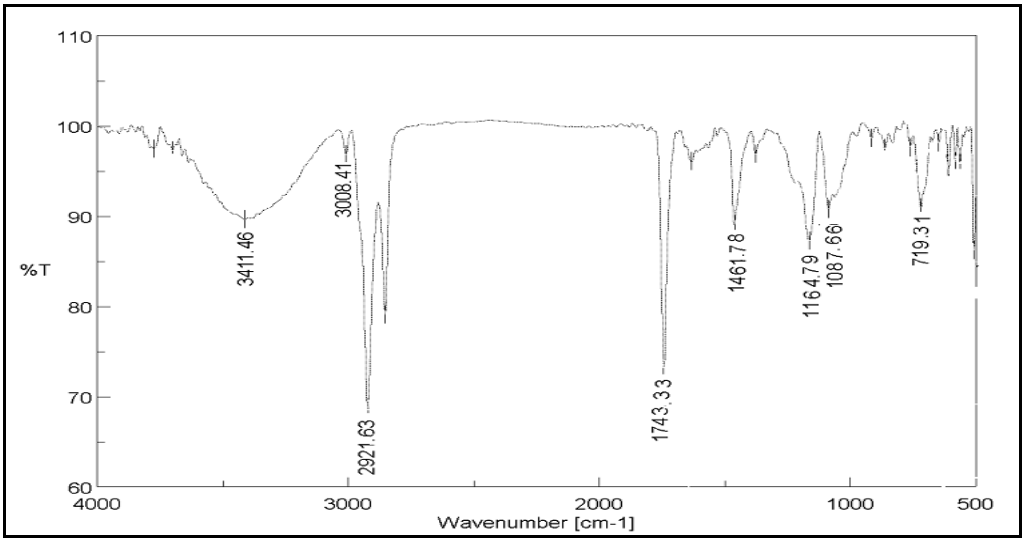

Figure 6: FTIR spectra of physical mixture S_1F_3.

Table 5: Vesicle size, viscosity, physical appearance of Proniosomal gel using Span40 as surfactant.

Formulation code	Average vesicle size in nm	Viscosity (cps)	Physical appearance
SF1	82.5	6.5	Yellowish glossy, non-greasy
SF2	78	8.9	Translucent, yellowish glossy, non-greasy
SF3	90	7.8	Translucent, yellowish glossy, non-greasy
SF4	30	7.1	Translucent, yellowish glossy, non-greasy

*Each value was the average of 300 Vesicles

Table 6: Vesicle size, viscosity, physical appearance of Proniosomal gel using Span60 as surfactant.

Formulation code	Average vesicle size in nm	Viscosity (cps)	Physical appearance
S_1F_1	70	6.5	Translucent, yellowish glossy, non-greasy
S_1F_2	30	8.2	Yellowish glossy, non-greasy
S_1F_3	70	7.8	Translucent, yellowish glossy, non-greasy
S_1F_4	30	8.1	Translucent, yellowish glossy, non-greasy

*Each value was the average of 300 Vesicles

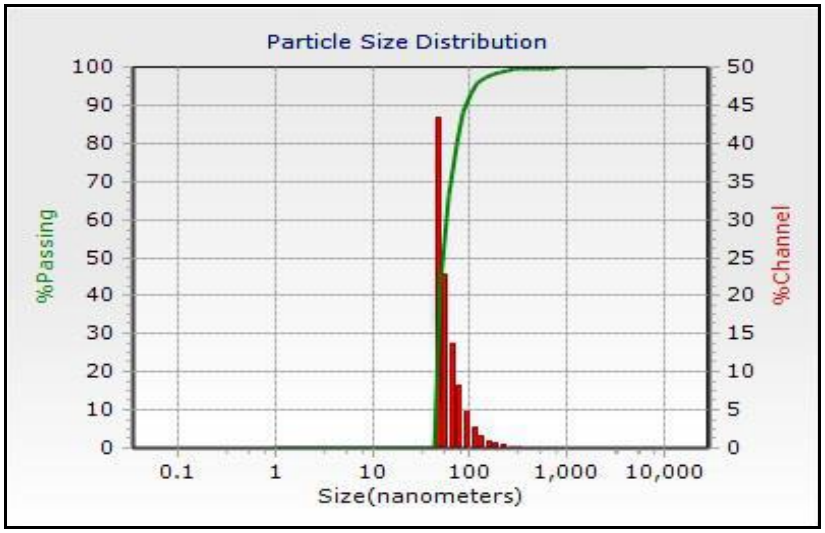

Figure 7: Particle size of Proniosomal formulation SF_4.

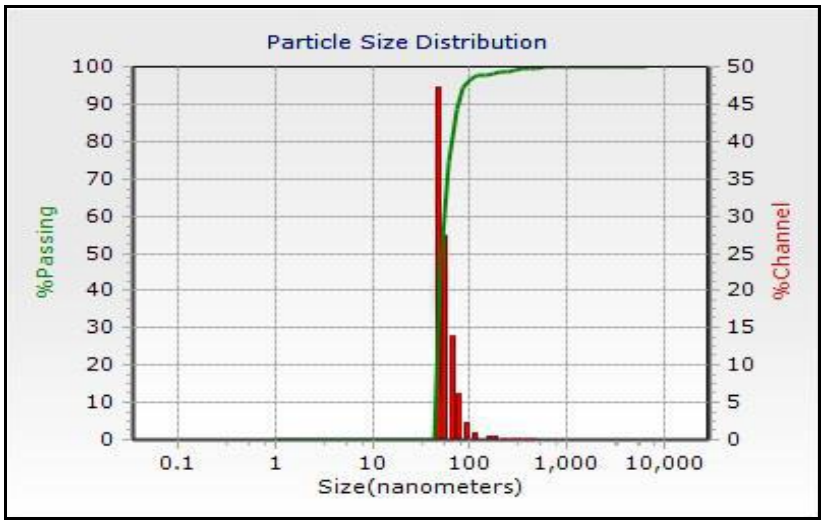

Figure 8: Particle size of Proniosomal gel formulation S_1F_3.

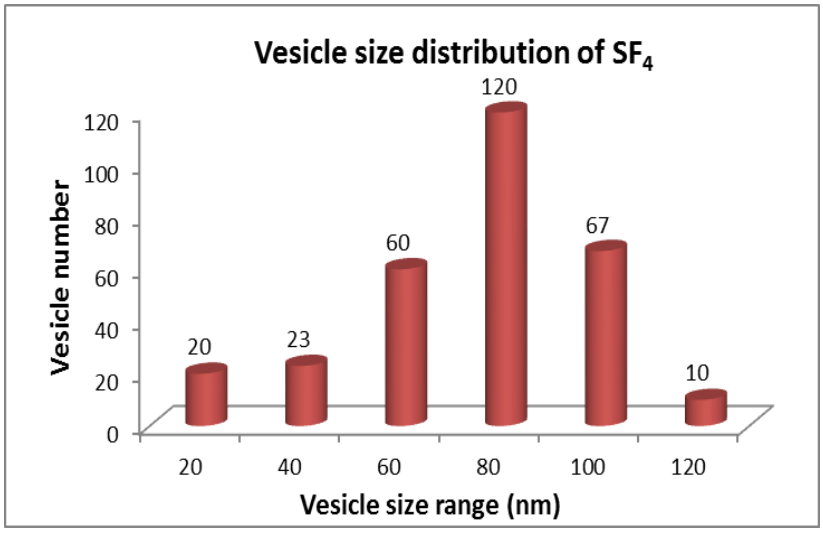

Figure 9: Vesicle size distribution of Proniosomal gel formulation SF_4.

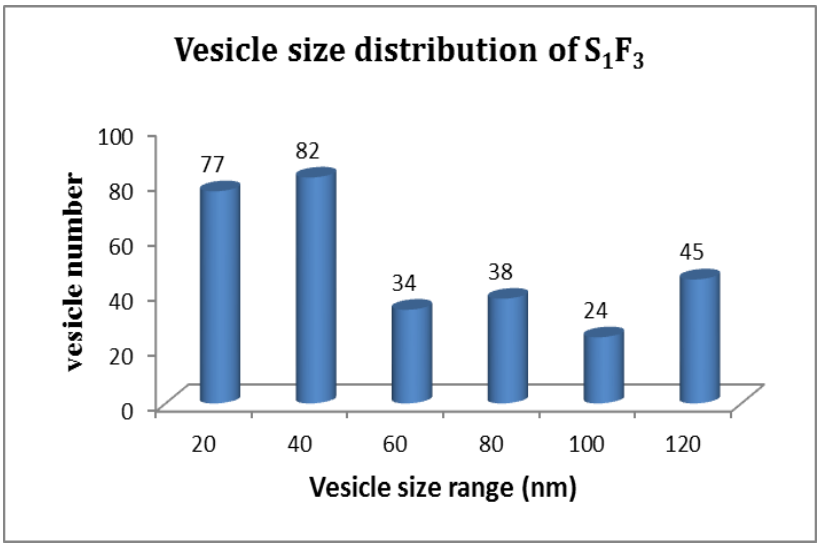

Figure 10: Vesicle size distribution of Proniosomal gel formulation S_1F_3.

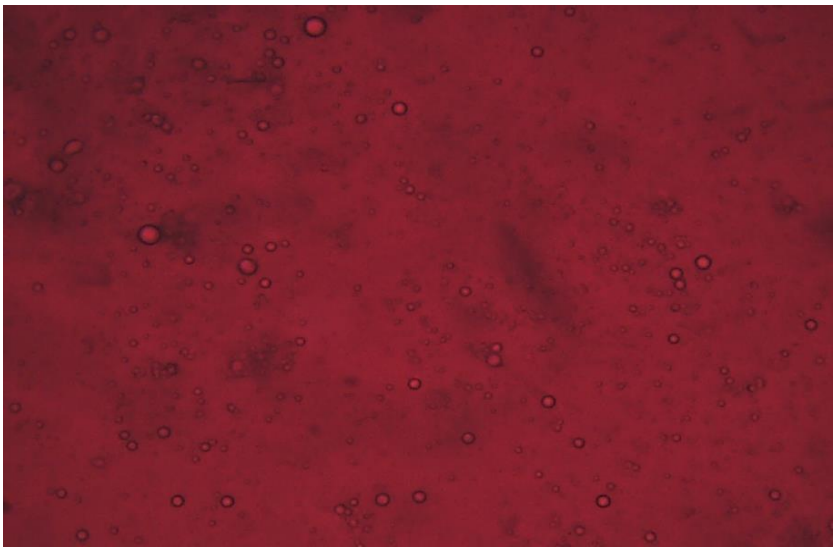

Figure 11: Microphotographs of Proniosomal gel formulation SF_4.

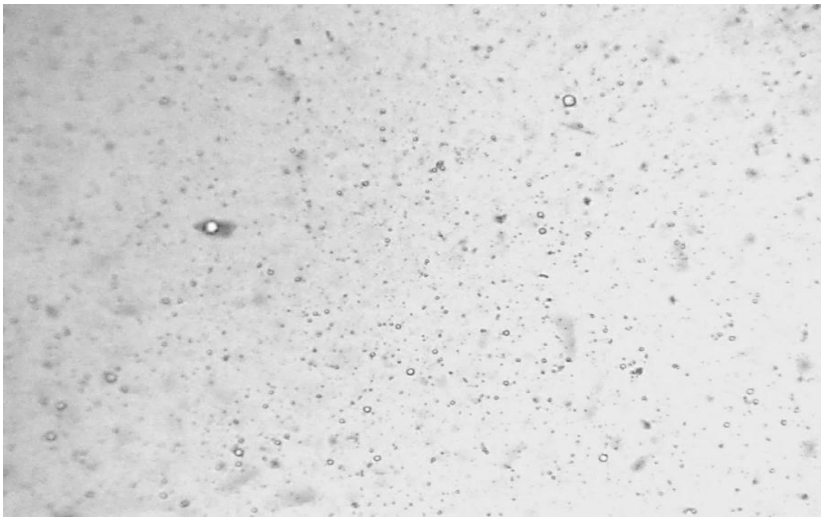

Figure 12: Microphotographs of Proniosomal gel formulation S_1F_3.

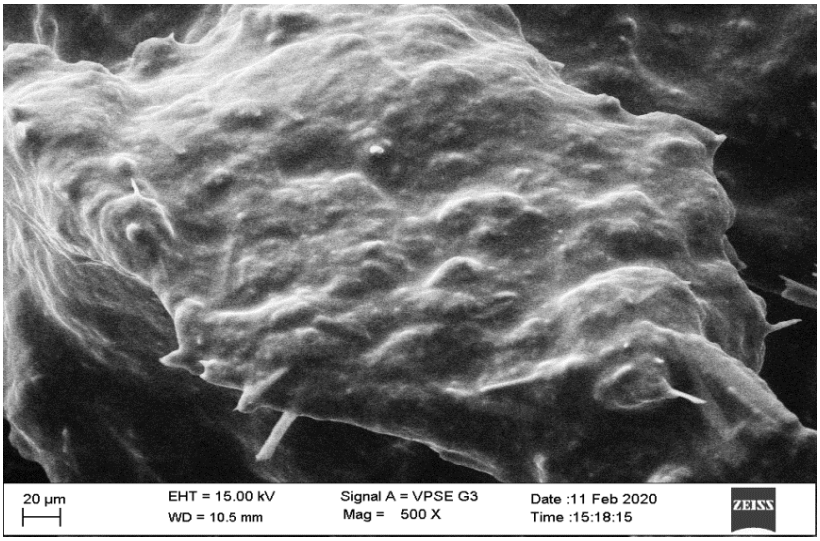

Figure 13: Scanning electron micrograph of Proniosomal gel formulation SF_4.

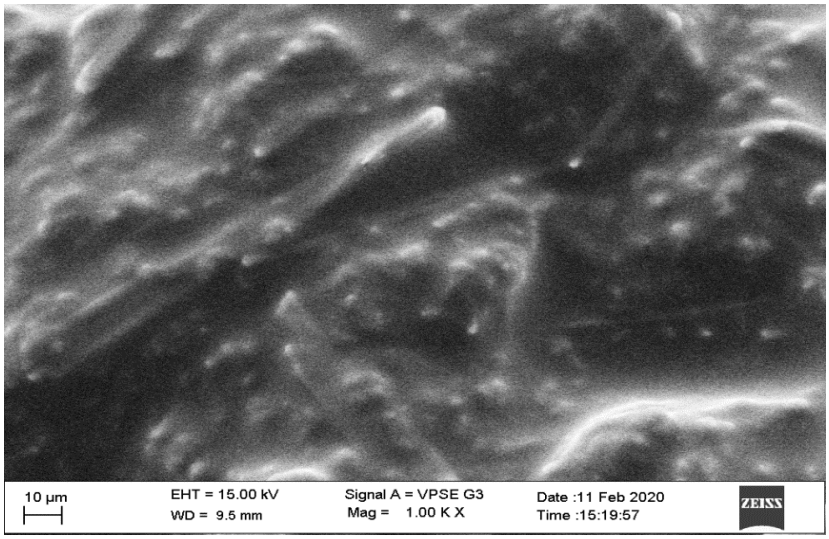

Figure 14: Scanning electron micrograph of Proniosomal gel formulation S_1F_3.

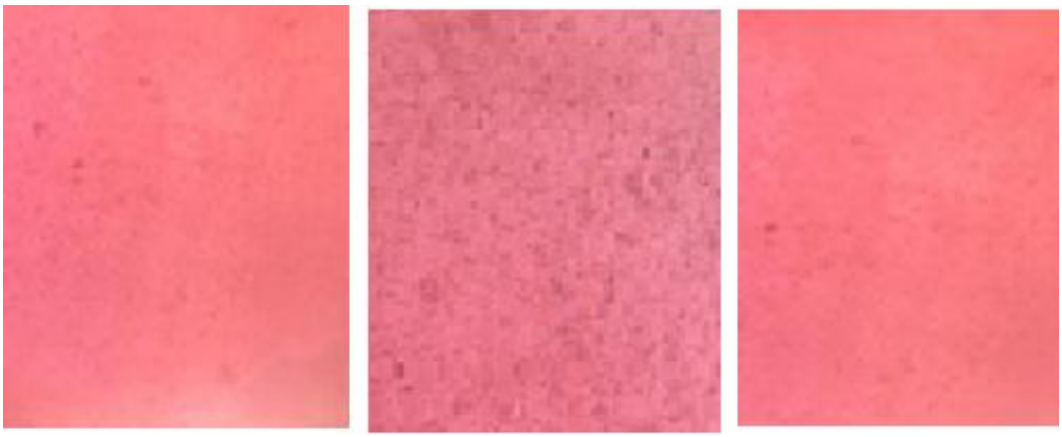

Figure 15: Microscopic observation of Proniosomal gel formulation using Span20 as surfactant.

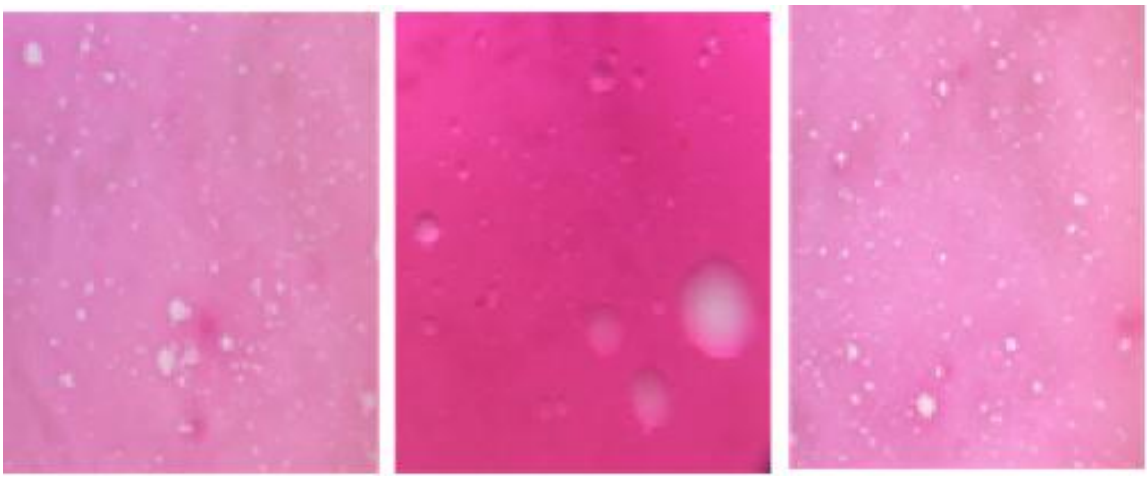

Figure 16: Microscopic observation of Proniosomal gel formulation using Span40 as surfactant.

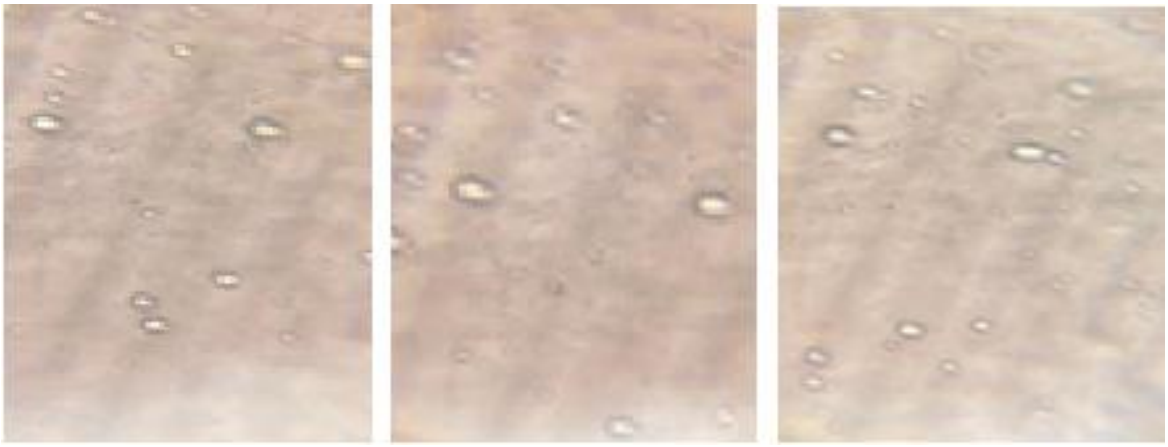

Figure 17: Microscopic observation of Proniosomal gel formulation using Span40 as surfactant.

Table 7: % Drug content and % Entrapment efficiency of proniosomes formulations.

Formulation code	% Drug content	% Entrapment efficiency
SF_1	89.5	91.45
SF_2	83.6	93.25
SF_3	86.5	94.23
SF_4	95.68	96.23
S_1F_1	80.56	90.23
S_1F_2	86.5	91.54
S_1F_3	87.32	95.32
S_1F_4	93.85	90.56

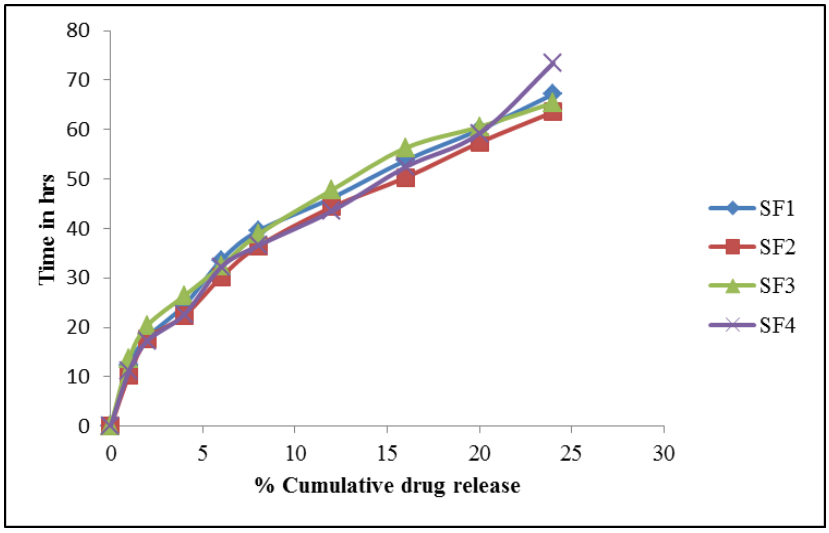

Figure 18: *In vitro* diffusion profile of SF_1 to SF_4.

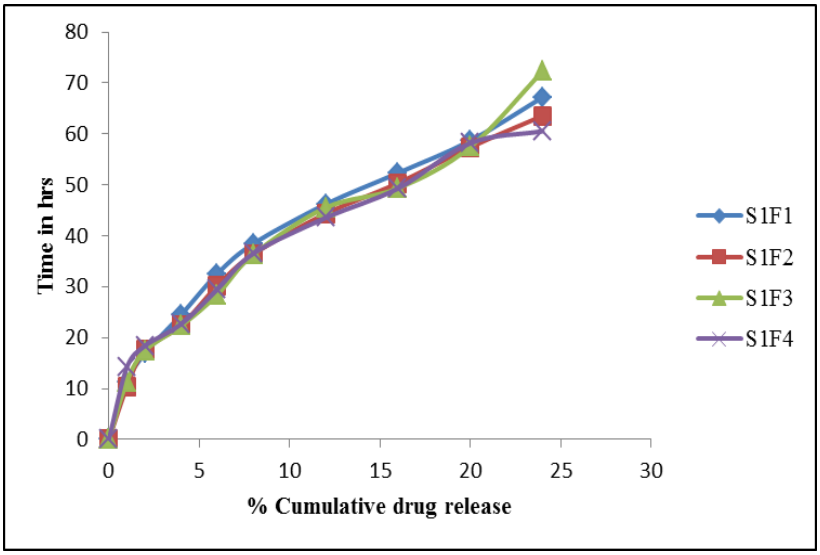

Figure 19: *In-vitro* diffusion profile of S_1F_1 to S_1F_4.

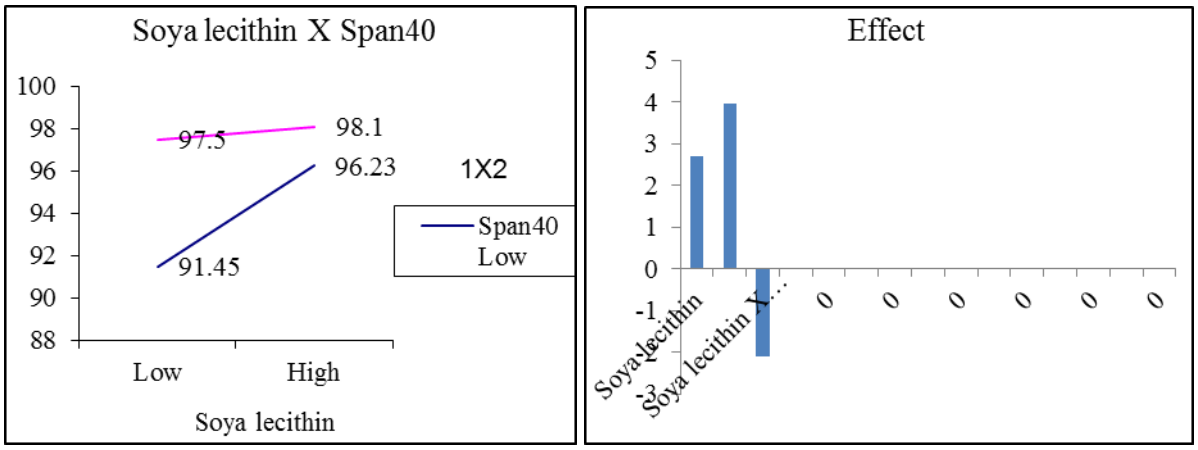

Figure 20: Effect of Soyalecithin and Span40 in Lower and Higher concentration.

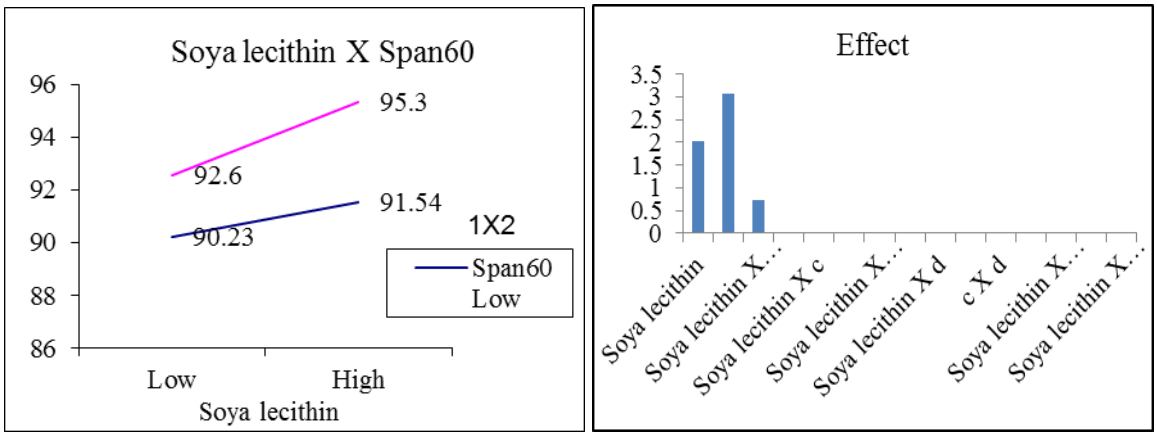

Figure 21: Effect of Soyalecithin and Span60 in Lower and Higher concentration.

Table 8: Stability studies for optimized formulations as per ICH guidelines.

Formulation	Parameter	Duration in month		
		0	3	6
SF_4	%Drug content	86.2	85.1	84.8
	pH	6.4	6.50	6.54
	%CDR	65.45	64.82	64.24
S_1F_3	%Drug content	87.32	86.62	86.22
	pH	6.52	6.64	6.67
	%CDR	60.56	59.65	59.32

CONCLUSION

A successful attempt was made to develop proniosomal gel for transdermal delivery of Repaglinide by utilizing 2 X 2 factorial design by non-ionic surfactant (Span 40 and span 60): cholesterol, soya-lecithin using co-acervation phase separation method. Repaglinide was successfully entrapped within the non-ionic surfactant vesicles. 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. Proniosomal gel system prepared with Span 40 and Span 60 exhibited optimum entrapment efficiency and has shown potential for delivery Repaglinide as antidiabetic drug.

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