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FORMULATION, EVALUATION AND OPTIMIZATION OF IN-SITU GEL OF AN ALPHA-GLUCOSIDASE INHIBITOR FOR MANAGEMENT OF NON-INSULIN DEPENDENT DIABETES MELLITUS

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ABSTRACT

The present study was carried out to develop an effective *in-situ* proliposomal gel containing Miglitol for treatment of non insulin dependent diabetes. The proliposomes containing Miglitol was prepared by film deposition on carrier method using vacuum rotary evaporator. Proliposomes characterized for vesicle size, surface morphology, drug content, entrapment efficiency, surface charge and yield. Proliposomes containing Miglitol were mixed into the 1%, 2% and 3% gellan gum gel to form proliposomal gels. The gels were characterized for physical appearance, pH, rhelogical properties, drug content, *in-vitro* and *ex-vivo* studies. Rheological studies of all proliposomal gels prepared with 1%, 2% and 3% w/w Gellan gum gave clear idea of concentration of Gellan gum (1%) is require for preparation of stable gel formulation. Among all Miglitol proliposomal formulations F1-F9 had maximum vesicle size and entrapment efficiency in the range 72 to 90 % and hence selected for the further study. The percentage yield of formulations was found to be increase with increase in phospholipid concentration in the range of 86.5 ± 0.265 to 95.4 ± 0.221 (mean±SD, n=3). *In-vitro* studies of proliposomal gels encapsulating drug were found to increase the skin permeation and deposition showing a sustain effect when

compared to marketed gel (conventional gel). Results showed that about 43.5% of drug from proliposomal gel and 10.8% of drug from conventional gel deposited on rat skin. This shows that proliposomes not only enhance the penetration of drug molecules but also help to localise the drug within the skin indicating sustain release of drug at site which prevents further inflammation. Hence this study indicated that proliposomal formulations containing Miglitol can be very effective in topical pharmacotherapy.

KEYWORDS: proliposome, gel, Miglitol, gellan gum, stability, penetration.

INTRODUCTION

At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery.[1] Approaches are being adapted to achieve this goal, by paying considerable attention either to control the distribution of drug by incorporating it in a carrier system, or by altering the structure of the drug at the molecular level, or to control the input of the drug into the bio environment to ensure an appropriate profile of distribution. In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering.[2-4] Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. The water soluble compounds/drugs are present in aqueous compartments while lipid soluble compounds/drugs and amphiphilic compounds or drugs insert themselves in phospholipid bilayers[5]

Proliposomes are dry, free flowing powder formulations containing water soluble carrier particles coated with phospholipids that immediately form a liposomal dispersion on contact with water in the body[6] The resulting liposomes may act as a sustained release dosage form of the loaded drugs. Proliposomes are composed of drug, phospholipid and a water soluble porous powder and can be stored sterilized in a dried state. Because of the solid properties, the stability problems of liposome can be resolved without influencing their intrinsic characteristics.[7].

Transdermal drug delivery systems (TDDS) are dosage forms involves drug transport to viable epidermal and or dermal tissues of the skin for local therapeutic effect while a very major fraction of drug is transported into the systemic circulation.[8] At present, the most

common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic metabolism and the tendency to produce rapid blood level spikes leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient.[9] To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise i.e., site specific, spatial and temporal placement within the body thereby reducing both size and number of doses.

Percutaneous absorption involves the passage of the drug molecule from the skin surface into the stratum corneum under the influence of a concentration gradient and its subsequent diffusion through the stratum corneum and underlying epidermis through the dermis and into the blood circulation. The skin behaves as a passive barrier to the penetrating molecule. The stratum corneum provides the greatest resistance to penetration and it is the rate limiting step in percutaneous absorption.[10]

Diabetes mellitus is a metabolic disorder in which carbohydrate metabolism is reduced while that of proteins and lipids is increased.[11] Enzyme α-glucosidase hydrolyzes terminal non-reducing (1→4) linked α-glucose residues to release a single α-glucose molecule. In NIDDM patients, this enzyme inhibition results in delayed glucose absorption and lowering of post-prandial hyperglycemia.[12] An α-glucosidase inhibitor, competitively and reversibly inhibits α-glucosidase in the intestines. This inhibition lowers the rate of glucose absorption through delayed carbohydrate digestion and extended digestion time. Miglitol.[13,14] is a second generation α-glycosidase inhibitor and in contrast to Acarbose (another α-glucosidase inhibitor), Miglitol is systemically absorbed. This present study was carried out to develop an effective *in-situ* Miglitol proliposomal gel formulations for treatment of non insulin dependent diabetes mellitus.

MATERIALS AND METHODS

Materials

Miglitol and Phosphotidyl choline was received as a gift sample from Dr.Reddy's Laboratories, Hyderabad. Drug was used without any further purification. Mannitol and Cholesterol was obtained from SD Fine Chem Pvt Ltd., Mumbai. Gellan gum was purchased from Life Expressions, Bangalore. All other chemicals and reagents used were of analytical grade. Drug permeation study was performed after obtaining the approval of the institutional

animal's ethical committee in accordance with disciplinary principles and guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Methods

Preformulation Studies

Melting point determination: Melting point of Miglitol was determined using open cup capillary method.

Preparation of calibration curve: Using absorption maxima a standard curve was prepared in the concentration range of 0.5-2.5 µg/ml with phosphate buffer pH 7.4. The absorbance of resulting solutions were measured at 270 nm and recorded. Concentration versus absorbance values was plotted.

Drug excipients interaction studies by FTIR: The compatibility between Miglitol and mannitol, phosphatidyl choline, cholesterol were detected by FTIR (Bruker α- T) spectra. The potassium bromide pellets were prepared on KBR press (Horizon WC-56). The spectra's were recorded over the wave number of 4000 to 600 cm^{-1}.

Preparation of proliposomes

The proliposomes containing Miglitol was prepared by film deposition on carrier method using vacuum rotary evaporator (Fig. 01) (Helidopath-Sonics-569-00050-00-0). There are various process variables which could affect the preparation and properties of the proliposomes. The optimization of Miglitol proliposomes was done by preparing the different formulations by varying the concentration of mannitol, phosphatidyl choline and cholesterol. Mannitol (1 g is sieved with 100 mesh) was placed in 100 ml round bottom flask which was held at 60-70 ^{0}C temperature and the flask rotated at 80-90 rpm for 30 min under vacuum. After complete drying the temperature of water bath was lowered to 20-30 ^{0}C.

Fig. 01: Rota evaporator (Helidopath-Sonics-569-00050-00-0)

Miglitol (50 mg), phosphotidyl choline and cholesterol were dissolved in mixture of organic solvents (chloroform : methanol, 6:4,v/v) and 5 ml of aliquot of organic solution was slowly introduced into the flask *via* the solvent inlet tube. After complete drying second aliquot (5 ml) was introduced. After complete dying, the vacuum was released and proliposomes were placed in a desiccator over night and then sieved with 100 mesh[15]. The collected powder was transferred into a glass bottle and stored at the freeze temperature. The various proliposomal formulations were prepared from F 1 to F18 as shown in Table No. 01 and 02.

Table No. 01: Composition of Proliposomal formulation (F1 to F9)

Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Miglitol (mg)	50	50	50	50	50	50	50	50	50
Mannitol(g)	1	1	1	1	1	1	1	1	1
Phosphatidyl choline(mg)	100	100	150	150	50	150	100	50	50
Cholesterol(mg)	150	100	50	100	100	150	50	50	150
Chloroform(ml)	6	6	6	6	6	6	6	6	6
Methanol (ml)	4	4	4	4	4	4	4	4	4

Table No. 02: Composition of Proliposomal formulation (F10 to F18)

Excipients	F10	F11	F12	F13	F14	F15	F16	F17	F18
Miglitol (mg)	50	50	50	50	50	50	50	50	50
Mannitol (g)	2	2	2	2	2	2	2	2	2
Phosphatidyl choline(mg)	150	100	150	100	50	50	100	50	150
Cholesterol(mg)	50	100	100	50	100	150	150	50	150
Chloroform (ml)	6	6	6	6	6	6	6	6	6
Methanol (ml)	4	4	4	4	4	4	4	4	4

Preparation of Gellan gum gel base

1 gm of Gellan gum was weighed and dispersed in distilled water. Then, propylene glycol was added and the mixture was neutralised by drop wise addition of 1% triethanolamine. Mixing was continued until the transparent gel was obtained and allowed to swell for 24 hours. Similarly 2% and 3% Gellan gum gels were prepared.[16]

Preparation of proliposomal gels

Proliposomes containing Miglitol (separated from the unentrapped drug) were mixed into the 1% gellan gum gel by using mortar and pestle, the concentration of proliposomes in the gel being 1%. All optimized formulations were incorporated into different gels (1%, 2% and 3%). The 1% gellan gum proliposomal gel is as shown in Fig. 02.

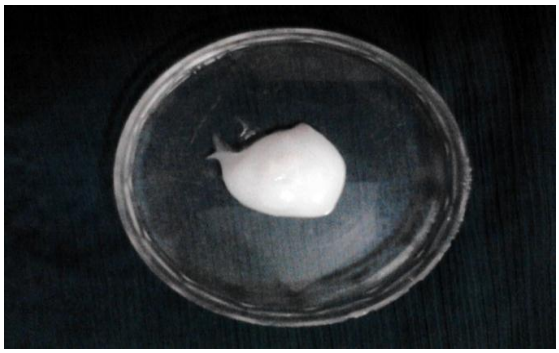

Fig. 02: 1% Gellan gum proliposomal gel

Characterization of proliposomes[17]

Vesicle size and count

Average size and size distribution proliposomes was determined using optical microscope (Metzer 5000DTM). A drop of distilled water was added to proliposome granules on a glass slide without a cover slip, and the process of liposome formulation was observed using optical microscope with 100 X magnification. Size of liposomal vesicles was measured at different locations on the slide. From the obtained results size distribution and average size of liposome vesicles was determined.

Surface morphology

The surface morphology of proliposomes and plain mannitol particles were examined by scanning electron microscopy (SEM).

Drug content

Miglitol content in proliposomes was assayed by an UV-visible spectrophotometer. Proliposomes (100 mg) were dissolved in 10 ml methanol by shaking the mixture for 5 min. 1 ml of the resultant solution was taken and diluted to 10 ml with methanol. Then, aliquots were withdrawn and absorbance was recorded at 270 nm using UV-visible spectrophotometer (Lab India 3200).

Entrapment efficiency

Separation of unentrapped drug from the liposomal suspension was done by centrifugation method. The entrapment efficiency of proliposomes was determined after hydration with distilled water. 10 ml of phosphate buffer (pH 7.4) was added to proliposomes granules and then subjected to sonicate for 10 mins using ultra sonicator (Citizen, India). The liposomal suspension was subjected to centrifugation on a cooling centrifuge (REMI TR-01) at 15000 rpm for 30 mins for the separation of unentrapped drug. The clear supernatant (1 ml) was taken and diluted to 10 ml with buffer and absorbance was recorded at 270 nm using UV-visible spectrophotometer (Lab India 3200).

$$\text{Entrapment efficiency (\%)} = \frac{Ct - Cf}{Ct} \times 100$$

Ct – concentration of total drug

Cf – concentratin of free drug

Surface charge

The optimized proliposomal formulation was dissolved in phosphate buffer (pH 7.4) and made the serial dilutions until the clear solution was obtained. Then the sample was analyzed for surface charge using zeta sizer (Malvern).

Yield of proliposomes

After complete drying of the proliposome powders, they were collected and weighed accurately. The yield of proliposomes was calculated using the formula

$$\text{Percentage yield} = \frac{\text{Total weight of proliposomes}}{\text{total weight of drug} + \text{weight of added materials}} \times 100$$

Characterization of gel

Physical appearance

All prepared gel formulations have been observed for their visual appearance, such as transparency, colour, texture, grittiness, greasiness, stickiness, smoothness, stiffness and

tackiness. The prepared gels were also evaluated for the presence of any particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

pH of formulation

pH measurement of the gel was measured by using a digital pH meter (Lab India SAB 5000). The observed pH values were recorded for all formulations (F1-F9) in triplicates.[18]

Rheological properties

The rheological properties of prepared gels was estimated using a Brookfield viscometer pro D II apparatus, equipped with standard spindle LV1 with 61 marking. Sample holder of the Brookfield viscometer was filled with the gel sample, and then spindle was inserted into sample holder.

All the rheological studies were carried out at room temperature (25±0.5 ^{0}C). A viscosity measurement was done in triplicate. Viscosity of 1, 2 and 3% Gellan gum gel was determined and the optimized formulation was selected.

Homogeneity

The homogeneity of Miglitol proliposomal gels were checked by visual inspection. In this regard the gels were filled into narrow transparent glass tubes and were checked in light for the presence of any particulate or lump.[19]

Drug Content

For determination of drug content, accurately weighed quantity (1 gm) of gel equivalent to 50 mg of Miglitol was dissolved in phosphate buffer (pH 7.4) and analyzed by UV-Vis Spectrophotometer (Lab India 3200) at 270 nm for drug content.

***In-vitro* studies:**

Franz diffusion cell (Fig. 03) was used in which the receptor chamber was filled with freshly prepared 30 ml 7.4 pH phosphate buffer. Proliposomal gel equivalent to 1 gm was placed on semi permeable membrane.

Fig. 03: Drug release from semi permeable membrane

The franz diffusion cell was placed over magnetic stirrer (REMI 1ML) with 500 rpm and temperature was maintained at 37±1 ^{0}C. 5 ml of samples were withdrawn periodically and replaced with fresh buffer. The withdrawn samples were periodically diluted and analysed for drug content using UV visible spectrophotometer (Lab India 3200) at 270 nm.

***Ex-vivo* studies**

Preparation of skin

Abdominal skin of male wistar rats was used in the study. Rats (250-280 g and 4-6 weeks old) were anesthetised slightly by ether and hairs removed from the abdominal skin. The rats were sacrificed and the abdominal skin of the rat was separated. The skin was stored at −20 °C until the permeation study, the skin was hydrated in normal saline at 4 °C and the adipose tissue layer of the skin was removed by rubbing with a cotton swab.

Percent amount of drug release from rat skin

The amount of drug release from rat skin was determined by using franz diffusion cell. The dorsal skin of wistar rat ((250-280 g, 4-6 weeks old) was placed between donor and receptor compartments of diffusion cell with the stratum corneum side facing upwards (Fig. 04). The receptor chamber was filled with 30 ml 7.4 pH phosphate buffer. Proliposomal gel equivalent to 1 gm was applied onto the surface of skin evenly. The receptor chamber was stirred by a magnetic stirrer rotating at 500 rpm and kept at 37±1 ^{0}C. The samples (1.5 ml) were collected at suitable time interval. Samples were analyzed for Miglitol content by UV visible spectrophotometer (Lab India 3200) at 270 nm after making proper dilutions. Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of Miglitol release from proliposomal gel.

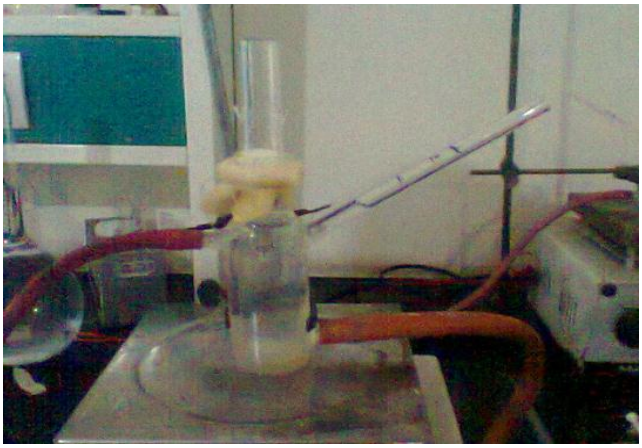

Fig. 04: Drug release from rat skin

Drug Retention Study

The skin was removed from the diffusion cells after completion of experiments. The surface of skin specimens was washed 10 times with 1 ml distilled water and dried on filter paper. The effective surface area of the skin was separated and minced with a surgical sterile scalpel then finally homogenized in a vial filled with methanol by using Homogenizer (REMI RQT-124A) at 16,000 rpm for 5 min. The tissue suspension was centrifuged for 15min at 9000 rpm using cooling centrifuge (REMI TR-01). Then filtered supernatant tissue suspension was further extracted with methanol and filtered. The filtrate was assayed for cumulative amount of drug retained on the skin by using UV-visible spectrophotometer (Lab India 3200) at 270 nm.

Stability studies

Stability studies were carried out by storing the prepared gels in tightly sealed amber colour glass bottles at various temperature conditions like refrigeration temperature (2-8 ^{0}C), room temperature (25±0.5 ^{0}C) and elevated temperature (45±0.5 ^{0}C) from a period of one month to three months. Drug content and variation in colour, morphology and consistency were periodically monitored.

***In-vitro* release kinetics**

In-vitro dissolution has been recognized as an important parameter in quality control and under certain conditions, it can be used as a surrogate for the assessment of bio-equivalence or prediction of bioequivalence.[21]

Determination of similarity factor

Moore and Flanner proposed two new indices (f1 and f2) to compare dissolution profiles of a test and a reference formulation. The concept of similarity factor (f2) has been endorsed by Food and Drug Administration (FDA); therefore, it is widely adopted in formulation and development and dossier preparation. The equation of similarity factor proposed by Moore and Flanner is represented in equation:

$f2 = 50 \times \log \{[1 / (1 + (\Sigma (Rt - Tt) 2) / N)]1/2 \times 100\}$

Where, N = Number of experimental data.

This is a widely used factor used to determine the similarity between two formulations. It is widely used when you have to check your formulation is similar to that of the marketed formulation. Values of $f2$ between 50 and 100 can be considered as superimposed dissolution profiles.

RESULTS

Preformulation studies

Melting point of Miglitol was found to be in the range of 152-155 ^{0}C. The construction of the calibration curve (Table No. 03) for Miglitol using phosphate buffer pH 7.4 is as shown in the Figure No. 04.

Table No. 03: Standard calibration curve of drug in phosphate buffer (pH 7.4)

S.No	Concentration	Absorbance
1	0.5	0.2251
2	1	0.4195
3	1.5	0.6407
4	2	0.8013
5	2.5	0.9971

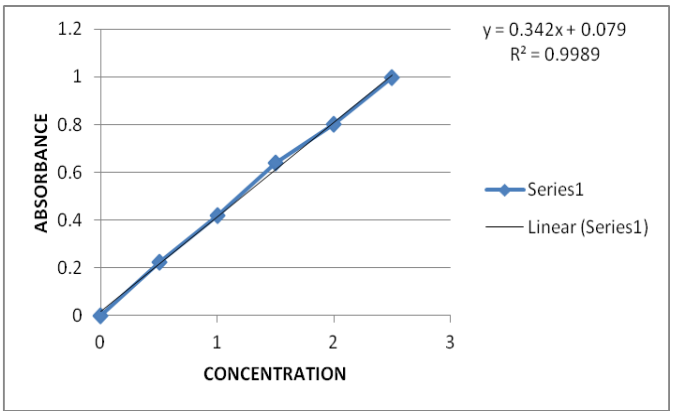

Fig. 05: Standard curve of Miglitol in phosphate buffer (pH 7.4)

IR spectra of drug, other excipients and proliposomal formulation were recorded and compared to identify possible interaction (Fig. 06 and 07).

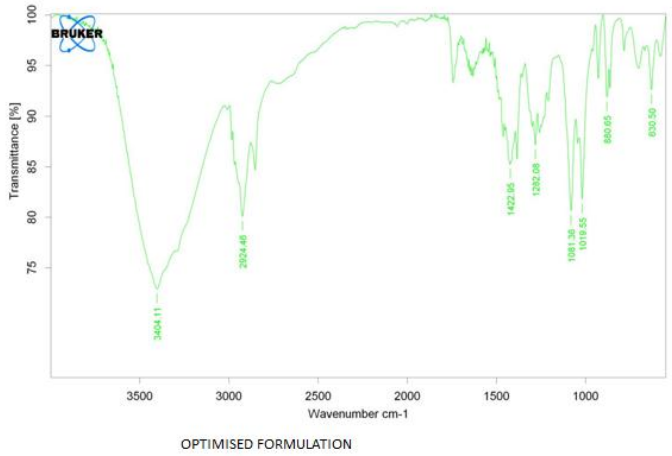

Fig. 06: FTIR spectra of optimized formulation (F4)

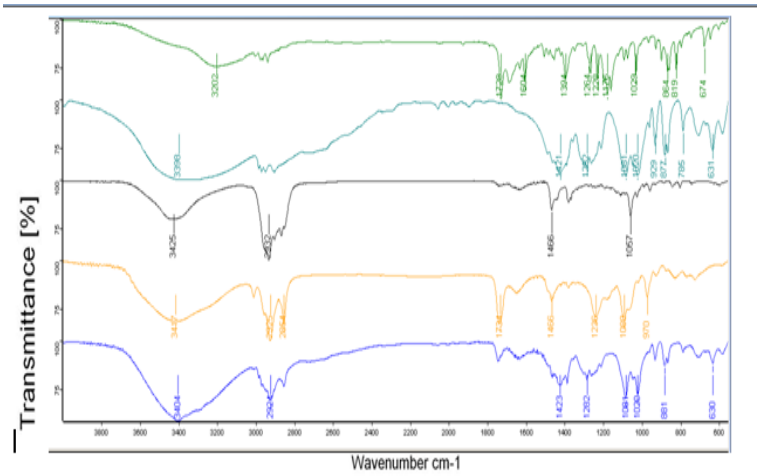

Fig. 07: FTIR spectra of drug, excipients and optimized formulation

Characerization proliposomes and proliposomal gels

Determination of vesicle size: The formation of vesicle was observed within the liposomal dispersion. The average vesicle size and distribution were calculated for count and distribution (Table No. 04).

Table No. 04: Particle size of proliposomal formulations

S.No	Formulation	Average particle size (µm) for 100 particles
1	F1	5.34±0.023
2	F2	4.43±0.123
3	F3	2.65±0.076
4	F4	6.06±0.012
5	F5	4.34±0.231
6	F6	5.12±0.167

7	F7	3.21±0.221
8	F8	2.69±0.148
9	F9	2.34±0.321
10	F10	3.45±0.321
11	F11	5.02±0.198
12	F12	4.01±0.082
13	F13	1.05±0.675
14	F14	2.67±0.321
15	F15	2.23±0.432
16	F16	1.55±0.123
17	F17	3.08±0.234
18	F18	3.09±0.134

Determination of entrapment efficiency Determination of entrapment efficiency is an important parameter in case of liposomes as it majorly effects the drug release and skin deposition. Entrapment efficiency is expressed as the fraction of drug incorporated into liposomes relative to total amount of drug used (Table No. 05).

Table No. 05 Entrapment efficiency of proliposome formulations

S.No	Formulation	Entrapment efficiency ± SD
1	F1	94.9±0.244
2	F2	85.12±1.48
3	F3	91.02±0.613
4	F4	96.5±0.205
5	F5	92.7±0.249
6	F6	94.1±0.509
7	F7	88.1±2.19
8	F8	89.2±0.817
9	F9	86.02±2.90
10	F10	78.7±0.899
11	F11	81.1±0.946
12	F12	86.6±0.821
13	F13	82.06±1.16
14	F14	77.6±2.39
15	F15	74.6±1.24
16	F16	80.6±1.40
17	F17	77.5±1.06
18	F18	72.4±0.984

Drug content estimation: The Miglitol content in the proliposomes were observed in the range of 86.4% to 96.8% at various drug to phospholipid ratios (Table No. 06).

Table No. 06: Drug content of proliposomal formulations

S.No	Formulation	%drug content ± SD
1	F1	95.03±0.543
2	F2	86.4±0.734
3	F3	93.7±0.664
4	F4	96.8±0.249
5	F5	94.7±0.984
6	F6	94.8±0.860
7	F7	92.4±1.70
8	F8	90.6±0.748
9	F9	87.5±0.953

Percentage yield of proliposomes

The results of % yield of various formulations (Table No. 07) were found to be in the range of 86.5 ± 0.265 to 95.4 ± 0.221 (mean±SD, n=3).

Table No. 07: Percentage yield of proliposomal formulations

S.No	Formulation	Percentage yield
1	F1	93.4±0.324
2	F2	90.7±0.534
3	F3	89.5±0.654
4	F4	95.4±0.123
5	F5	94.3±0.221
6	F6	94.8±0.212
7	F7	88.7±0.321
8	F8	89.3±0.187
9	F9	86.5±0.265

***In-vitro* studies**

The cumulative amount of drug release of various proliposomal formulations and conventional (Conventional) Miglitol gel after 16hrs were found to be as shown in Fig. 08 and Table No. 08.

Table No. 08: Cumulative percentage drug release of proliposomal formulations

S.No	Formulation	Cumulative % drug release
1	F1	76.01
2	F2	55.4
3	F3	55.5
4	F4	80.5
5	F5	69.8
6	F6	74.1
7	F7	60.5
8	F8	58.9
9	F9	64.4
10	Conventional gel	95.1

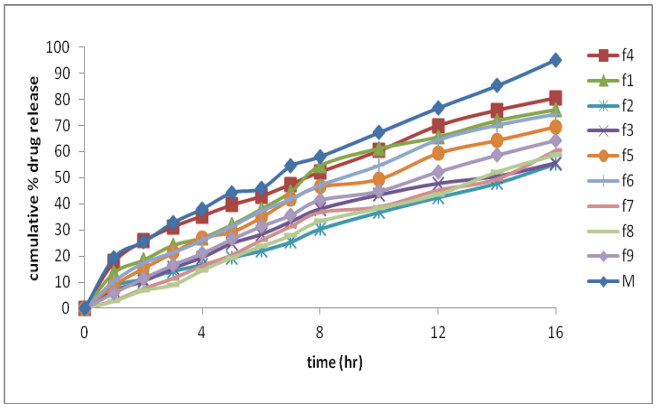

Fig. 08: Diffusion data of various proliposomal formulations and conventional gel

From the above results f4 formulation has selected as optimized one which is used for further study. The results are as shown in Fig 09 and 10, Table No. 09.

Table No. 09: Particle size distribution of optimized formulation

Range From	Range To	Count	Percentge
0	4	37	42.53
5	8	14	16.09
9	12	4	4.6
13	16	8	9.2

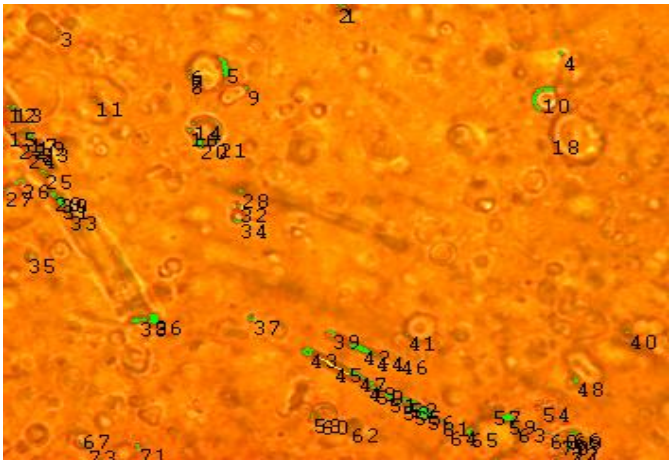

Fig. 09: Particle size distribution of optimized formulation

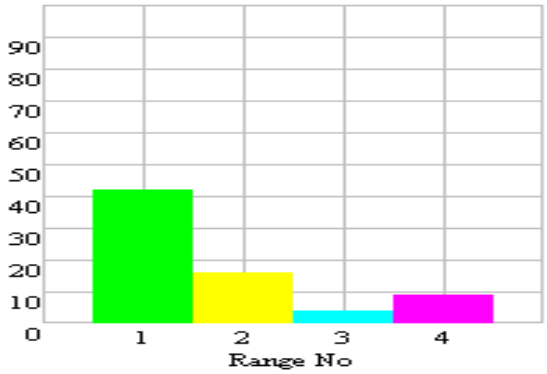

Fig. 10: Particle size range and distribution

Determination of zetapotential

Zeta potential was found to be -16mv which is sufficient to avoid aggregation of vesicles. From the reports of zeta sizer vesicle size was found to be 990 nm (Table No. 10, Fig. 11).

Table No. 10: Zeta size distribution by intensity

Results	Peaks	Diameter(nm)	% Intensity	Width (nm)
Z-average(d. nm) -990	**Peak 1**	91.50	93.3	13.07
PDI-0.82	**Peak 2**	13.91	6.7	1.404
Intercept-0.432	**Peak 3**	0.000	0.0	0.00

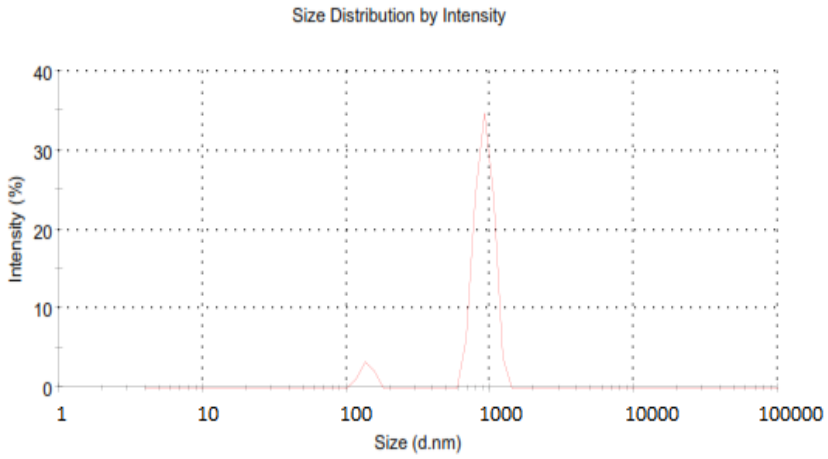

Fig. 11: Zeta size distribution by intensity

From the above results f4 formulation has selected as optimized one which is used for further study.

Surface morphology

The surface morphology of proliposome granules, pure drug and mannitol granules was examined by scanning electron microscopy (SEM) and the images were photographed at 100 resolutions (Fig. 12, 13 and 14).

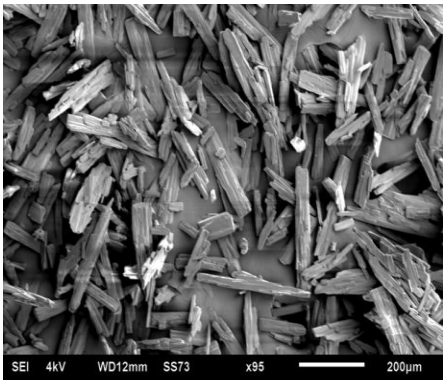

Fig. 12: SEM image of Mannitol

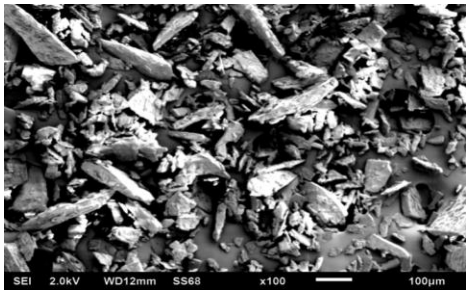

Fig. 13: SEM image of Miglitol

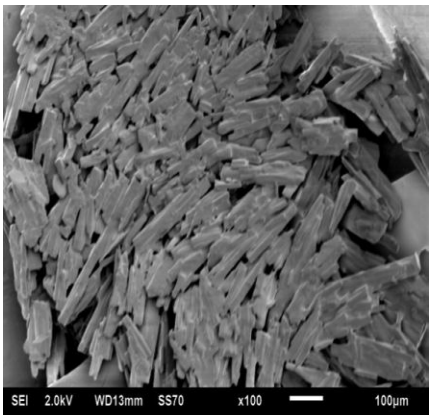

Fig. 14: SEM image of Miglitol proliposomes

The surface morphology of proliposome powder was different as compare to plain mannitol powder as shown in SEM.

Viscosity measurement

Viscosity of proliposomal gel showed 1156 cps at 100rpm.

pH measurement

The P^H values of prepared proliposomal gels were within the limits of 5.5 to 5.8.

***Ex-vivo* studies - Drug permeation studies using rat skin**

Results obtain from ex-vivo permeation studies for optimized Miglitol proliposomal gel formulation and conventional gel after 16 hrs were found to be 56.7% and 89.4% respectively (Fig. 15).

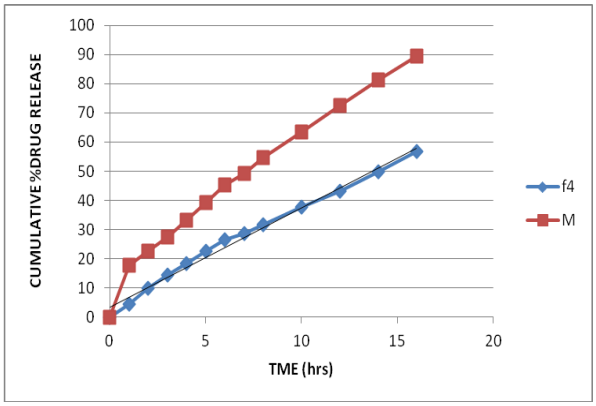

Fig. 15: *Ex-vivo* drug release profile of optimized and conventional gels

Drug deposition studies

Results showed that about 43.5% of drug from proliposomal gel and 10.8% of drug from conventional gel deposited on rat skin (Fig. 16).

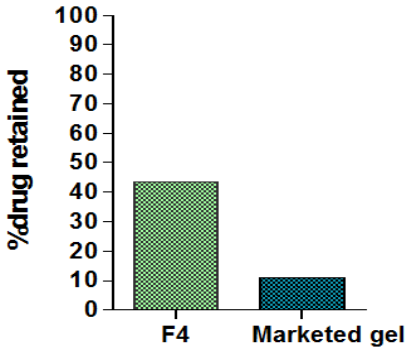

Fig. 16: Percentage drug retention of proliposomal and conventional gels

Stability studies

Stability of proliposomal formulation as well as gel formulation was carried out for 60 days at 8 ^{0}C, room temperature and 40 ^{0}C and analyzed for following parameters: visual appearance, drug content, entrapment efficiency of reconstituted liposomes (Table No. 11).

Table No. 11: Stability study of the F4 proliposomal formulation

Time (days)	Temperature(^{0}C)	Drug content	Entrapment efficiency
15	RT	95.4	94.0
15	8	95.2	93.5
15	40	94.5	92.6
30	RT	94.1	93.2
30	8	93.7	92.1
30	40	93.6	91.4
60	RT	93.5	92.5
60	8	92.3	91.5
60	40	91.2	90.7

After 2 months of storage period the Miglitol proliposomes still appeared free flow and immediately form a liposomal dispersion on contact with water.

DISCUSSION

Melting point of Miglitol was found to be in the range of 152-155 ^{0}C which compiles the standards thus indicating that purity of the drug sample. On comparison of IR spectra of proliposomes, pure Miglitol drug, mannitol, cholesterol and phospholipid it was clear that there was no significant interaction of encapsulated drug with the phospholipid and water soluble solid support (mannitol) with formulations. A positive correlation was observed for both variables phospholipid and cholesterol in case of liposome vesicle size. Thus, with increase in the concentration of phospholipid and cholesterol vesicle size was found to be increased. A positive correlation was observed for both variables phospholipid and cholesterol. Results show that with increase in the concentration of phospholipid and cholesterol entrapment efficiency found to be increased. Among all Miglitol proliposomal formulations F1-F9 had maximum vesicle size and entrapment efficiency which were selected for the further study. F4, F1, F5 and F6 formulations showed maximum drug content when compare to other formulations. The % yield of formulations was found to be increase with increase in phospholipid concentration.

The surface morphology of proliposome powder was different as compare to plain mannitol powder as shown in SEM. From SEM photographs it is clear that, the surface of mannitol

crystals becomes illegible due to deposition of phospholipid on mannitol surface. The pH of the developed formulation was in accordance with human skin pH rendering them more acceptable. Therefore formulated proliposomal gel was suitable for topical application.

The amount of drug retained in skin the skin was considerably higher in case of proliposomal gels when compared to conventional gel formulation. This shows that liposomes not only enhance the penetration of drug molecules but also help to localise the drug within the skin indicating sustain release of drug at site which prevents further inflammation. The results indicated that at elevated and freezing temperature there was slightly but insignificant decrease in drug content and entrapment efficiency for proliposomes. So the proliposomal products should be stored in refrigeration conditions, to minimize the drug leakage from the proliposomal systems compared to conventional gel formulation. The drug leakage of <1% of the initial load at refrigeration conditions is well within the limits and good for topical application. Hence the proliposomal formulations containing anti-diabetic drugs would be very effective in topical pharmacotherapy.

CONCLUSION

A sustained delivery of Miglitol can be achieved by proliposomal drug delivery system. Phospholipids, being the major component of liposomal system, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug permeation. Fusion of lipid vesicles with skin contributed to the permeation enhancement effect. The phospholipid was found to have a significant influence on the lipid matrix of the stratum corneum, suggesting a disruption of the intercellular lipid lamellar structure and act as penetration enhancer. Hence as the phospholipid concentration was increased, it would increase the permeation of drug following application on the skin. The free flowing properties of the proliposomes granules will be beneficial in formulating the proliposomes as a solid dosage form.

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