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DEVELOPMENT OF ANTI-DIABETIC NIOSOMES FORMULATION CONTAINING GLICLAZIDE

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Abstract:

In this study niosomal drug delivery system was developed using non-ionic surfactant incorporating Gliclazide by Thin film hydration technique. The prepared niosomal vesicles were quite stable. The formulation was subjected to Entrapment efficiency, Scanning electron microscopy, Invitro release, and Zeta potential analysis. From the results of experimental investigation, we concluded that, formulation F_{13} containing drug with300:200 µmol (surfactant:cholesterol) ratio was showing higher percentageentrapment with desired sustained release of Gliclazide. Hence formulation F_{13} wasconsidered as optimized formulation. Invitro release from optimized Gliclazide niosomal formulation (F_{13}) showedextended release for 24 hours. SEM image revealed the vesicles are exist spherical shape and uniform in size. Scanning electron micrograph shows there is no aggregation between the particles. Negative zeta potential value was observed in zeta potential analysis. This confirmed the presence of negative charge inducing agent in formulation. The formulation was checked for sterility as per I.P specification. The optimized formulation passes the sterility test. Stability study was carried out for the period of three months at various storage conditions. The results showed that the formulation remains stable at 4°c. The optimized formulation was found to follow zero order release pattern which was revealed by the linearity shown from the plot of Time Vs cumulative percentage drug release. From the drug release kinetic studies, we concluded that thedrug was released from niosome by a zero order diffusion controlled mechanism.

Key words: *Niosome, Drug Delivery System, Gliclazide, Formulation with Span60.*

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INTRODUCTION:

The concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localised on the targeted site. Hence, surrounding tissues are not affected by the drug. In addition, loss of drug does not happen due to localisation of drug, leading to get maximum efficacy of the medication. Different carriers have been used for targeting of drug, such as immunoglobulin, serum proteins, synthetic polymers, liposome, microspheres, erythrocytes and niosomes.[1]

Niosomes are one of the best among these carriers. The self-assembly of non-ionic surfactants into vesicles was first reported in the 70s by researchers in the cosmetic industry. Niosomes (non-ionic surfactant vesicles) obtained on hydration are microscopic lamellar structures formed upon combining non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class with cholesterol.[2] The non-ionic surfactants form a closed bilayer vesicle in aqueous media based on its amphiphilic nature using some energy for instance heat, physical agitation to form this structure. In the bilayer structure, hydrophobic parts are oriented away from the aqueous solvent, whereas the hydrophilic heads remain in contact with the aqueous solvent. The properties of the vesicles can be changed by varying the composition of the vesicles, size, lamellarity, tapped volume, surface charge and concentration. Various forces act inside the vesicle, eg, van der Waals forces among surfactant molecules, repulsive forces emerging from the electrostatic interactions among charged groups of surfactant molecules, entropic repulsive forces of the head groups of surfactants, short-acting repulsive forces, etc. These forces are responsible for maintaining the vesicular structure of niosomes. But, the stability of niosomes are affected by type of surfactant, nature of encapsulated drug, storage temperature, detergents, use of membrane spanning lipids, the interfacial polymerisation of surfactant monomers in situ, inclusion of charged molecule. Due to presence of hydrophilic, amphiphilic and lipophilic moieties in the structure, these can accommodate drug molecules with a wide range of solubility.[3] These may act as a depot, releasing the drug in a controlled manner. The therapeutic performance of the drug molecules can also be improved by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.[4] Noisome made of alpha , omega-hexadecyl-bis-(1-aza-18-crown-6) (Bola-surfactant)-Span 80-cholesterol (2:3:1 molar ratio) is named as Bola-Surfactant containing noisome.[5] The surfactants used in niosome preparation should be biodegradable, biocompatible and non-immunogenic. A dry product known as proniosomes may be hydrated immediately before use to yield aqueous niosome dispersions. The problems of niosomes such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing.[6]

Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability.[7] As with liposomes, the properties of niosomes depend on the composition of the bilayer as well as method of their production. It is reported that the intercalation of cholesterol in the bilayers decreases the entrapment volume during formulation, and thus entrapment efficiency.[8] However, differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol, whereas liposomes are prepared from double-chain phospholipids (neutral or charged). The concentration of cholesterol in liposomes is much more than that in niosomes. As a result, drug entrapment efficiency of liposomes becomes lesser than niosomes. Besides, liposomes are expensive, and its ingredients, such as phospholipids, are chemically unstable because of their predisposition to oxidative degradation; moreover, these require special storage and handling and purity of natural phospholipids is variable.

Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against various diseases. It can also be used as vehicle for poorly absorbable drugs to design the novel drug delivery system. It enhances the bioavailability by crossing the anatomical barrier of gastrointestinal tract via transcytosis of M cells of Peyer's patches in the intestinal lymphatic tissues.[9] The niosomal vesicles are taken up by reticulo-endothelial system. Such localised drug accumulation is used in treatment of diseases, such as leishmaniasis, in which parasites invade cells of liver and spleen.[10,11] Some non-reticulo-endothelial systems like immunoglobulins also recognise lipid surface of this delivery system.[2–8,10–12] Encapsulation of various anti-neoplastic agents in this carrier vesicle has minimised drug-induced toxic side effects while maintaining, or in some instances, increasing the anti-tumour efficacy.[13] Doxorubicin, the anthracycline antibiotic with broad-spectrum anti-tumour activity, shows a dose-dependent irreversible cardio-toxic

effect.[14,15] Niosomal delivery of this drug to mice bearing S-180 tumour increased their life span and decreased the rate of proliferation of sarcoma. Intravenous administration of methotrexate entrapped in niosomes to S-180 tumour bearing mice resulted in total regression of tumour and also higher plasma level and slower elimination. It has good control over the release rate of drug, particularly for treating brain malignant cancer.[16] Niosomes have been used for studying the nature of the immune response provoked by antigens.[17] Niosomes can be used as a carrier for haemoglobin.[18,19] Vesicles are permeable to oxygen and haemoglobin dissociation curve can be modified similarly to non-encapsulated haemoglobin. Slow penetration of drug through skin is the major drawback of transdermal route of delivery.[20] **Gliclazide** is a sulfonylurea used to treat hyperglycemia in patients with type 2 diabetes. Gliclazide binds to the β cell sulfonyl urea receptor (SUR1). This binding subsequently blocks the ATP sensitive potassium channels. The binding results in closure of the channels and leads to a resulting decrease in potassium efflux leads to depolarization of the β cells. This opens voltage-dependent calcium channels in the β cell resulting in calmodulin activation, which in turn leads to exocytosis of insulin containing secretorty granules.

MATERIALS AND METHODS:

Materials

Table:2 Materials used

S.NO	Materials	source
1.	Gliclazide	Bafna pharmaceuticals,Mumbai
2.	Potassium di hydrogen phosphate	S.D. Fine Chem Ltd, Boisar
3.	Disodium hydrogen phosphate	S.D. Fine Chem Ltd, Boisar
4.	Sodium chloride	S.D. Fine Chem Ltd, Boisar
5.	Sorbitan mono laurate (span 20)	S.D. Fine Chem Ltd, Boisar
6.	Polysorbate 20 (tween 20)	S.D. Fine Chem Ltd, Boisar

METHODS

Reagent used Preparation of release media

2.38 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8gm of sodium chloride were dissolved in sufficient amount of distilled water to produce 1000ml and pH adjusted to 7.4, if necessary.

Determination of Absorbance maximum (λ_{max})

Zidovidine was dissoled in phosphate buffer saline pH 7.4. Solution with 20 µg/ml concentration was prepared by suitable dilution.

The Gliclazide drug in solution was scanned in UV spectrophotometer from200 to 400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined as 267 nm. The drug was later quantified by measuring the absorbance at 267 nm in phosphate buffer saline pH7.4.

Standard curve for Gliclazide (by UV metohod)[73]

Preparation of primary stock solution

Gliclazide 100 mg was weighed and dissolved in phosphate buffer saline pH 7.4 in a 100 ml volumetric flask. The flask was shaken and volume was made up to the mark with phosphate buffer saline pH 7.4 to give a solution containing 1000 µg/ml.

Preparation of secondary stock solution

From the primary stock solution, pipette out 2 ml and placed into 100 mlvolumetric flask. The volume was made up to mark with phosphate buffer saline pH 7.4 to give a stock solution containing 20 µg / ml.

Preparation of sample solution

Appropriate volumes of aliquots (1 to 10 ml) from standard Gliclazide secondary stock solution were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with phosphate buffer saline pH 7.4 to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg / ml. Absorbance of each solution against phosphate buffer saline pH 7.4 as blank were measured at 267 nm and the graph of absorbance against concentration were plotted and shown in Figure.8.

Standard curve data for Gliclazide (By U.V method)Table:4

Concentrationin µg/ml	Absorbance at267 nm
2	0.124
4	0.195
6	0.290
8	0.385
10	0.483
12	0.576
14	0.671
16	0.769
18	0.864
20	0.939

RESEARCH ENVISAGED

INFRARED SPECTROSCOPIC STUDIES

IR study was carried out for identification of pure drug. IR spectroscopy (using Perkin Elmer) by KBr pellet method was carried out on drug. They are compressed under 15 tones pressure in a hydraulic press to form a transparent pellet.The pellet was scanned from 4000 to 400 cm^{-1} in a spectrophotometer and peaks obtained were identified.

OPTIMIZATION PROCESS FOR NIOSOME PREPARATION

Empty vesicles were prepared by Thin film hydration technique. A liquid phase was prepared by dissolving accurately weighed quantities of surfactant and cholesterol was dissolved in chloroform methanol mixture ratio (2:1v/v) in 100 ml round bottom flask. The solvent mixture was removed from the liquid phase using rotary evaporator at 45-60°C and the speed of rotation was varied like 75rpm, 100rpm, 125rpm and 150rpm to obtain a thin film on the wall of the flask. Simultaneously the residual solvent was completely removed by keeping the flask under the vaccum. The thin film was hydrated with phosphate buffer saline pH 7.4 by varying hydration volume 5ml, 10ml and hydration time of 30, 60 and 120 min at a temperature of 60±2°C. The vesicle size and appearance of empty vesicle were noted.

FORMULATION OF GLICLAZIDE NIOSOMES

Gliclazide loaded niosomes were prepared by Thin film hydration technique.Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform methanol mixture ratio (2:1v/v) in a 100 ml volumetric flask. Theweighed quantity of drug and dicetyl phosphate was added to the solvent mixture. The solvent mixture was removed from liquid phase using rotary evaporator at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The completeremoval of solvent can be ensured by applying vaccum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60±2°C for aperiod of 2 hour until the formation of niosomes. All the batches were subjected to sonication process for 2 min using probe sonicator. The ratios of cholesterol and surfactant used in the formulation were tabulated (table 5).

COMPOSITION OF GLICLAZIDE NIOSOMES

Table:5

Formulationcode	Gliclazide(mg)	Surfactant	Surfactant: Cholesterol (µM)
F_1	10	Span 20	100:100
F_2	10	Span 20	200:100
F_3	10	Span 20	300:100
F_4	10	Span 20	100:200
F_5	10	Span 20	200:200
F_6	10	Span 20	300:200
F_7	10	Span 20	400:200
F_8	10	Tween 20	100:100
F_9	10	Tween 20	200:100
F10	10	Tween 20	300:100
F11	10	Tween 20	100:200
F12	10	Tween 20	200:200
F13	10	Tween 20	300:200
F14	10	Tween 20	400:200

EVALUATION OF GLICLAZIDE NIOSOMES

Removal of unentrapped drug from niosomes

The unentrapped drug from niosomal formulation was separated by centrifugation method. The niosomal suspension was taken in centrifuge tube. The formulation was centrifuged at 15,000 rpm for 30 min using cooling centrifuge and temperature was maintained at 5°c. The supernatant was separated. Supernatant contained unentrapped drug and pellet contained drug encapsulated vesicles. The pellet was resuspended in phosphate buffer saline pH 7.4 to obtain a niosomal suspension free from unentrapped drug.

Encapsulation efficiency

Drug entrapped vesicles were separated from unentrapped drug by centrifugation method. 0.5 ml of Gliclazide loaded niosome preparation was added with 0.5 ml of 10% triton X 100 and mixed well then incubated for 1 hour. The tritonX 100 was added to lyse the vesicles in order to release the encapsulated Gliclazide. The solution was diluted with phosphate buffer saline pH 7.4 and filtered through whatmann filter paper. The filtrate was measured spectrophotometrically at 267 nm using phosphate buffer saline pH 7.4 and triton X 100 mixture as blank.

Percent entrapment = Amount of drug e entrapped/ Total amount of drug added× 100

In vitro release study for niosomal preparation

The niosomal formulation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 100 ml diffusion medium of phosphatebuffer saline pH 7.4. The temperature of medium was maintained at 37±0.5°C. The medium was stirred by means of magnetic stirrer at a constant speed. 1 ml of samplewas withdrawn at every 1 hour and replaced with 1 ml of fresh buffer, so that the volume of diffusion medium was maintained constant at 100 ml. The withdrawn samples were made upto 10 ml using phosphate buffer saline pH 7.4. The samples were measured spectrophotometrically at 267 nm.

Zeta potential

The zeta potential of optimized Gliclazide niosomal formulation was measured using Malvern zeta potential analyser.

Scanning electron microscopy

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM). The sample for SEM analysis was mounted in the specimen stub using an adhesive small sample was mounted directly in scotsch doubleadhesive tape. The sample was analysed in hitachi scanning electrom microscope operated at 15 kv and photograph was taken.

Sterility testing

Sterility of prepared and optimized Gliclazide niosomal formulation was evaluated by sterility test as per IP. The method selected for testing is Method I – Membrane Filtration Method.

Preparation of Soya bean Casein Digest medium (SCDM)

25 gm of dehydrated media was dissolved in 1000 ml of distilled water. The solution was boiled for 10 min. This solution was used as medium. The medium wascooled to room temperature and pH adjusted to 7.3±0.2. The medium was dispensed in suitable container and sterilized at 121°C for 15 min.

Preparation of Fluid Thioglycolate medium (FTM)

Dehydrated media 26 gm was dissolved in distilled water to get 1000 ml of themedium. It was boiled for 10 min. The medium was cooled to room temperature andpH adjusted to 7.2±0.2. The medium was sterilized at a temperature of 121°C for 15min. The sterilised media should not have more than upper one- third of the mediumin pink colour.

Preparation of Rinsing Fluid (Fluid A)

1 gm of peptic digest of animal tissue was weighed and mixed up with small amount of hot water and made upto 1000 ml. The solution was filtered and pH adjusted to 7.1±0.2. The solution was then dispensed in suitable container and autoclaved for sterilization.

Procedure

The vials containing Gliclazide niosomes were broken open under aseptic condition provided by laminar air flow work station. All precaution and preventive measures were taken to avoid contamination by the process or by the analyst. The drug solution was then passed through sterile membrane lodged on a membrane holder assembly. After passing through the solution, the membrane was rinsed threetimes with 100 ml of sterile peptone (Fluid A). The membrane was cut into two halves using sterile scissors. One half of the filter paper was introduced into the container with SCDM and the other half into the container with FTM.

SCDM containers were then incubated at 22.5±2.5°C and containers at 32.5±2.5°C. The containers were observed for turbidity or appearance of growth of microorganisms for 14 days. Positive control and negative control tests were done to validate the sterility testing procedure.

Negative control

Negative control confirms the sterility of the sterilized media. It was then inoculated and observed for 14 days. Negative control was maintained for both the media and incubated in their appropriate temperature.

Positive control

Positive control confirms the suitability of the media for the growth of microorganism. The positive control for SCDM and FTM were inoculated with Bacillus subtilis suspension and incubated at 22.5±2.5°C and 32.5±2.5°C respectively for 14 days. The growth of microorganisms witnessed by the turbidity of the medium confirms the presence of nourishments favouring the microorganisms.

Stability study of Gliclazide niosomes

The optimized Gliclazide niosome formulation was examined for stability study. The formulations were taken in a 20 ml sealed glass vial and stored in three different environments such as 4°C, room temperature and 45°c/75% RH for a periodof three months. Samples from each batch were withdrawn at the interval of one month and evaluated for entrapment efficiency and *in vitro* drug release.

RESULTS AND DISCUSSION:

Development of Gliclazide niosomes

In this study, Gliclazide loaded niosomes were prepared by Thin film hydration technique using cholesterol and non ionic surfactants such as span 20 and tween 20. Chloroform methanol mixture (2:1v/v) was used as solvent.

After evaporation of solvent from the formulation, thin film was formed. Thethin film was hydrated and removed by phosphate buffer saline pH 7.4. Size of the vesicles in formulation was reduced by sonicating the formulation in Probe sonicator.

Formulations with different ratios of surfactant and cholesterol were prepared. Several physicochemical characteristics of niosomes such as morphology, vesicle size determination, drug release profile were investigated. And stability of optimized formulation at various temperatures was evaluated.

Dicetyl phosphate (DCP) also included in the formulation as charge inducing agent. The inclusion of charge inducing agent (DCP) prevented the aggregation and fusion of vesicles. Integrity and uniformity also maintained by dicetyl phosphate.

An effective niosomal drug delivery system should posses good physical and chemical stability on storage and should incorporate high drug loading with stable encapsulation.

IR studies

Pressed Pellet Technique was used to handle the sample in FTIR spectrometer.In this technique a required amount of sample was added and mixed well with potassium bromide and the mixture was pressed with special discs under highpressure into a transparent pellet and then inserted into special holder of IR spectrometer.

The pellets were scanned from 4000 to 400 cm^{-1} in FTIR spectrophotometer and peaks obtained in both spectrums were identified. The wave number at which peaks appeared and peaks indicating functional groups are presented in table:6.

Table:6

Frequency		Group assigned
Pure drug	Physical mixture	
3402	3401	OH & NH - stretching
2852	2924	CH – stretching
1660	1654	C=O – stretching
1094	1106	CO – stretching

IR spectrums for pure drug alone and physical mixture of drug, surfactant, andcholesterol were taken. The spectrum of physical mixture was compared with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture. Hence there was no significant interaction between drug and excepients.

Optimization of process related variables
The prepared niosomal vesicles were influenced by some factors like speed ofrotation, hydration volume, hydration medium and vaccum. Before loading the drug, these factors should be optimized using empty vesicle.

The vaccum used for drying of thin film was 350 mmHg. Vaccum below 350 mmHg was insufficient for complete removal of solvent from the formulation and resulted in aggregation of niosomes on hydration. The vaccum above 350 mmHg resulted rapid evaporation of solvent which leads to entrapment of air bubbles on the surface of film. This caused poor entrapment of drug in niosomes and the vaccum of350 mmHg produced lipid film had appreciable drug entrapment in the niosomes. Hence 350 mmHg of vaccum was considered as optimum range.

The time of hydration of lipid film was carried from 60 -120 min. When hydration allowed to 120 min, formed niosomes were spherical in shape and existed
in desired size range. So hydration time 120
min and hydration volume 5 ml wereselected as optimum.

Thickness and uniformity of thin film was influenced by speed rotation of round bottom flask. The optimum speed was selected to 150 rpm. At this speed of rotation, thin film formed was uniform. The bath temperature of rotary evaporator was maintained at 60±2°C as optimum.

Optimization of process related variables

Table:7

Surfactant: Cholesterol	Speed of Rotation (rpm)	Hydration Time (min)	Chloroform: methanol	Hydration volume	Vesicle Size (µM)
100:100	75	30	2:1	5 ml	10.29±1.48
	100	60			9.41±1.09
	125	120			9.11±1.88
	150	120			8.69±1.88

Table:8

Volume of hydration medium(ml)	Hydration time(min)	Percentage entrapment(%)
3	60	60.74 ± 0.98
4	60	68.84 ± 0.76
4	60	73.38 ± 0.58
5	120	89.45 ± 0.88
5	180	81.45 ± 0.93

Evaluation of Gliclazide Niosomes
Removal of unentrapped drug from Niosomes
The unentrapped drug from niosomes was removed by centrifugation technique. The results are presented in following table,

Percentage drug entrapment efficiency

Table:9

Formulationcode	Surfactant: Cholesterol (µM)	Surfactantused	Percentage of free Drug (%)	Percentage entrapment Efficiency (%)
F_1	100:100	Span 20	37	63
F_2	200:100	Span 20	26	74
F_3	300:100	Span 20	32	68
F_4	100:200	Span 20	38	62
F_5	200:200	Span 20	28	72
F_6	300:200	Span 20	16	84
F_7	400:200	Span 20	29	71
F_8	100:100	Tween 20	32	68
F_9	200:100	Tween 20	19	81
F10	300:100	Tween 20	27	73
F11	100:200	Tween 20	29	71
F12	200:200	Tween 20	15	85
F13	300:200	Tween 20	8	92
F14	400:200	Tween 20	24	76

The entrapment efficiency of the niosomes is governed by the ability of formulation to retain drug molecule in aqueous core or in bilayer membrane of vesicles. After removal of unentrapped drug, the entrapment of all formulation was studied. Entrapment efficiency was varied with varying the surfactant and cholesterol ratio. Various factors like lipid concentration, drug to lipid ratio, and cholesterol content will change the entrapment efficiency.

Entrapment efficiency of formulation F_1 was found to be 63%. In formulation F_2, increasing the surfactant concentration, entrapment efficiency was increased to 74%. Further increasing the surfactant concentration in F_3, the entrapment efficiencywas decreased to 68%, due to very low concentration of cholesterol.

So, to improve the entrapment efficiency cholesterol concentration was increased to 200µM in formulations F_4, F_5, F_6, and F_7. Because, increase the amountof cholesterol will improve the entrapment efficiency. But entrapment efficiency achieved in formulations F_4, F_5, F_6, and F_7 were 62%, 72%, 84%, and 71%. The drug entrapment was not improved satisfactorily. This is due to, surfactant used in those formulations was span 20. The span 20 is more hydrophobic, hence the hydrophilic drug gets encapsulated in the aqueous core only.

The formulations were tried with tween 20 in formulations F_8, F_9, and F_{10} contained 100 µM of cholesterol and 100, 200, 300 µM of tween 20 respectively. Thereleases were accordingly, 68%, 81%, and 73%, due to low level of cholesterol concentration.

So quantity of cholesterol was increased 200 µM in formulations F_{11}, F_{12}, $F_{13}$and F_{14}, entrapment efficiency was improved to 71%, 85%, 92% and 76% respectively. The increase in the entrapment efficiency is attributed to the ability of surfactant and cholesterol to cement the leaking space in the bilayer membrane, whichin turn allows enhanced drug level in niosomes. Compared to span 20, the better entrapment efficiency was achieved in tween 20. This can be explained as the tweensare more water soluble, the hydrophilic Gliclazide drug, gets encapsulated as well aspartitions into vesicle membrane. Hence formulation F_{13} was optimized one.

***In vitro* release study**

The release of Gliclazide from niosomes was determined using the membrane diffusion technique. Release study was carried for 24 hours and results arenoted in following tables.

In vitro **release study of Gliclazide niosomes**

Table:24

Formulation code	Surfactant: Cholesterol (µM)	Surfactantused	Total release period (Hrs)	Cumulative percentage drug release (%)
F_1	100:100	Span 20	16	61.58
F_2	200:100	Span 20	17	72.69
F_3	300:100	Span 20	16	67.64
F_4	100:200	Span 20	24	59.57
F_5	200:200	Span 20	24	71.65
F_6	300:200	Span 20	24	81.97
F_7	400:200	Span 20	14	70.63
F_8	100:100	Tween 20	16	65.61
F_9	200:100	Tween 20	18	79.74
F_{10}	300:100	Tween 20	18	71.68
F_{11}	100:200	Tween 20	24	69.61
F_{12}	200:200	Tween 20	24	83.78
F_{13}	300:200	Tween 20	24	90.86
F_{14}	400:200	Tween 20	20	73.69

In vitro drug release was carried out for 24 hours using phosphate buffer as diffusion medium. It was found to be biphasic, and the release was controlled by lipidbilayer and dialysis membrane. Incorporation of cholesterol affected the release rate of encapsulated drug. *In vitro* drug release characteristics for formulations containing two different surfactants were compared. Gliclazide niosomes were tried with two different surfactant and cholesterol concentrations.

Drug release from formulations F_1, F_2 and F_3 were found to be 61.58%, 72.69%, and 67.64% in 16 hrs, 17 hrs and 16 hrs respectively. The release was not extended upto 24 hrs, because those formulations contained low cholesterol concentration.

Quantity of cholesterol was increased to 200µM in formulations F_4, F_5, F_6 andF_7 and release was achieved to 59.57% in 24 hrs, 71.65% in 24 hrs, 81.97% in 24 hours and 70.63% in 14 hrs. Except F_7, release from other formulation was extended to 24 hrs. This is due to higher concentration of surfactant in formulation F_7. Extendedrelease was achieved but those formulations were not satisfied with percentage drug release. Higher release was found to be 81.83%. This is due to the water insoluble nature of span 20.

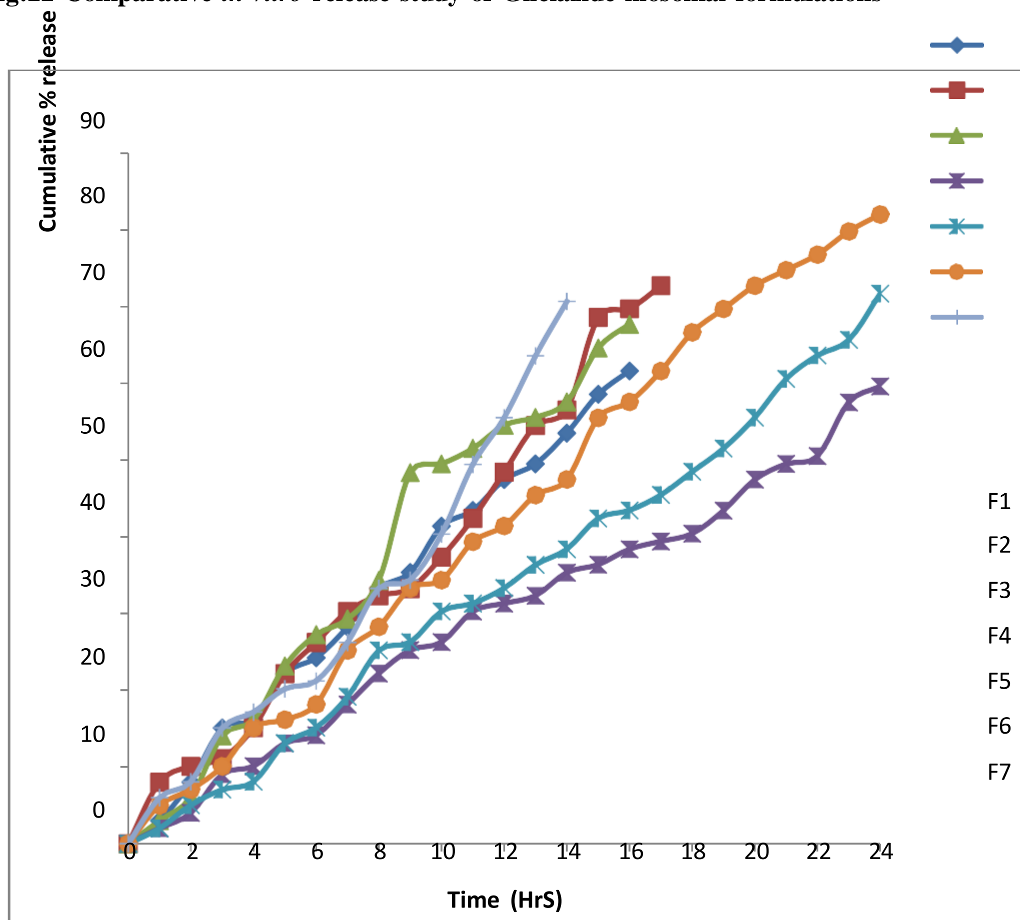
Due to lower concentration of cholesterol in formulations F_8, F_9 and F_{10} , the release were 65.61% 16 hrs, 79.48% at 18 hrs and 71.68% at 18 hrs respectively. Formulations F_{11}, F_{12}, F_{13} and F_{14} contained 200 µM of cholesterol showed 69.61% ofdrug release in 24 hrs, 83.78% of drug release in 24 hrs, 90.86% of drug release in 24hrs and 73.69% of drug release in 20 hrs. Higher release of 90.86% was found in formulation contained 300:200 µmol ratio of surfactant and cholesterol. So formulation F_{13} (300:200 µmol) was considered as optimized formulation.

Table:25 *In vitro* drug release studies for formulations containing Span 20

Time (Hrs)	Cumulative percentage drug release (%)						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
1	3.0	8.0	3.0	2.0	2.0	5.0	6.0
2	8.03	10.08	6.03	4.02	5.02	7.05	8.06
3	15.08	11.10	14.06	9.04	7.05	10.07	15.08
4	16.15	15.11	16.14	10.09	8.07	15.10	17.15
5	22.16	22.15	23.16	13.10	13.08	16.15	20.17
6	24.22	26.22	27.23	14.13	15.13	18.16	21.20
7	28.24	30.26	29.27	18.14	19.15	25.18	26.21
8	33.28	32.30	34.29	22.18	25.19	28.25	33.26

9	35.33	33.22	48.34	25.22	26.25	33.28	34.33
10	41.35	37.33	49.48	26.25	30.26	34.33	40.34
11	43.41	42.37	51.49	30.26	31.30	39.34	49.40
12	47.43	48.42	54.51	31.30	33.31	41.39	55.49
13	49.47	54.48	55.54	32.31	36.33	45.41	63.55
14	53.48	56.51	57.55	35.32	38.36	47.45	70.63
15	58.53	68.56	64.57	36.35	42.38	55.47	-
16	61.58	69.68	67.64	38.36	43.42	57.55	-
17	-	72.69	-	39.38	45.43	61.57	-
18	-	-	-	40.39	48.45	66.61	-
19	-	-	-	43.40	51.48	69.66	-
20	-	-	-	47.43	55.51	72.69	-
21	-	-	-	49.47	60.55	74.72	-
22	-	-	-	50.49	63.60	76.74	-
23	-	-	-	57.50	65.63	79.76	-
24	-	-	-	59.57	71.65	81.97	-

Fig:22 Comparative *in vitro* release study of Gliclazide niosomal formulations



Scanning electron microscopy

The surface characteristics of Gliclazide niosomal formulation were studied by scanning electron microscopy. SEM image of prepared niosome formulation shows that the coating of surfactant cholesterol mixture on drug particles. Some particles in the images are broken, which might be due to handling and processing. Most of the vesicles are spherical and discrete sharp boundaries. The appearance of niosome vesicles in scanning electron micrograph is smooth, which indicates a thin and uniform coating over the drug. Based on the scale of micrograph, no significant change in size of particles is seen. The observation clearly shows that, there is no aggregation between the particles, due to surfactant coating.

Zeta potential

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized Gliclazide niosome formulation was measured and found to -27.3 mv. The negative zeta potential observed with niosomes reflects the presence of negatively charged DCP on the surface of vesicles. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable. The particles being suspended. The formulation was found to be very effective for parenteral administration.

Sterility test

The optimized Gliclazide niosomal formulation was subjected to sterility test. The test was carried out as per I.P specification. Both Soya bean casein digest medium (SCDM) and Fluid thioglycollate medium (FTM) were used. The method followed is Method A – Membrane filtration method. The positive control was prepared from standardised Bacillus subtilis suspension. The samples dipped in SCDM and FTM incubated for 14 days. The absence of turbidity of the test indicates the sterility of the formulation and passed the sterility test.

Stability studies

The optimized Gliclazide niosomal formulation (F_{13}) was subjected to stability study for three months at 4^0C, room temperature and 45^0C/75% RH. At the interval of one month the niosomes evaluated for *in vitro* release and entrapment efficiency. The stability study shows that niosomal formulations are more stable at 4°C (refrigerator) when compared to room temperature and at 45^0C/75% RH

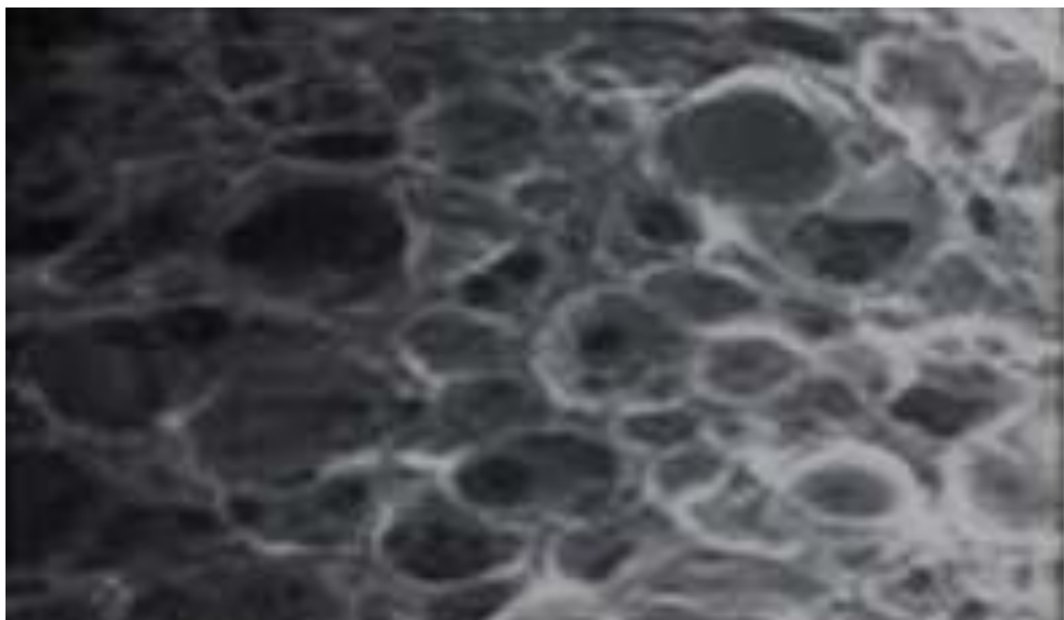
Table:27 Entrapment efficiency data

Temperature	Percentage entrapment after one month (%)	Percentage entrapment after two months (%)	Percentage entrapment after three months (%)
4°C	90	89	89
Room temperature	87	85	80
45°C/75% RH	84	81	74

Table:28 *In vitro* release data

Temperature	Cumulative % release after one month (%)	Cumulative % release after two months (%)	Cumulative % release after three months (%)
4°C	89.87	88.84	87.83
Room temperature	85.83	82.80	78.77
45°C/75% RH	81.79	75.73	70.66

Fig:24 Scanning Electron Microscopy



CONCLUSION:

In this study niosomal drug delivery system was developed using non-ionic surfactant incorporating Gliclazide by Thin film hydration technique. The preparedniosomal vesicles were quite stable.

The formulation was subjected to Entrapment efficiency, Scanning electron microscopy, Invitro release, and Zeta potential analysis. From the results of experimental investigation, we concluded that, formulation F_{13} containing drug with 300:200 µmol (surfactant:cholesterol) ratio was showing higher percentageentrapment with desired sustained release of Gliclazide. Hence formulation F_{13} was considered as optimized formulation.

Invitro release from optimized Gliclazide niosomal formulation (F_{13}) showed extended release for 24 hours.

SEM image revealed the vesicles are exist spherical shape and uniform in size.
Scanning electron micrograph shows there is no aggregation between the particles.

Negative zeta potential value was observed in zeta potential analysis. This confirmed the presence of negative charge inducing agent in formulation.

The formulation was checked for sterility as per I.P specification. The optimized formulation passes the sterility test.

Stability study was carried out for the period of three months at various storage conditions. The results showed that the formulation remains stable at 4°c.

The optimized formulation was found to follow zero order release pattern which was revealed by the linearity shown from the plot of Time Vs cumulative percentage drug release. From the drug release kinetic studies, we concluded that the drug was released from niosome by a zero order diffusion controlled mechanism.

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