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Formulation and Evaluation of Maltodextrin based Proniosomal Drug Delivery System containing Anti-diabetic (Glipizide) drug

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Abstract: Glipizide loaded maltodextrin based proniosome were prepared by slurry method with different surfactant to cholesterol ratio. The proniosome formulations were evaluated for FT-IR study, angle of repose and scanning electron microscopy. The niosomal suspensions were further evaluated for entrapment efficiency, *In-vitro* release study, Kinetic data analysis, Stability study, *In-vivo* hypoglycemic Study. The result from SEM analyses has confirmed the coating of surfactant on the surface of carrier. The formulation F_4 which showed higher entrapment efficiency of 84.25 ± 1.25 and *in-vitro* release of 99.23% at the end of 24hr was found to be best among the all 7 formulation. Release was best explained by the zero order kinetics. Kinetic analysis shows that the drug release follows super case II transport diffusion. Proniosome formulation has showed appropriate stability for 90 days when compared with reconstituted niosomes by storing the formulation at refrigerator condition.
Keywords: Glipizide, Proniosome, Maltodextrin, Span-60.

INTRODUCTION

Proniosome are dry product which could be hydrated immediately before use would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (aggregation, fusion, leaking) could be minimized[1]. These dry formulations of surfactant-coated carrier can be measured out as needed and rehydrated by brief agitation in hot water[2]. They are water-soluble carrier particles that are coated with surfactant and can be hydrated to form a niosomal dispersion immediately before use on brief agitation in hot aqueous media. Reported methods for preparation of proniosomes are the spraying of surfactant on water-soluble carrier particles and the slurry method[3]. This dry, free-flowing, granular product which, upon addition of water, disperses or dissolves to form a multilamellar niosome suspension suitable for administration by oral or other routes.

The present study is aimed at overall improvement of therapeutic efficacy of antidiabetic drug through proniosomal encapsulation. Glipizide is an oral blood glucose lowering drug of the sulfonyl urea class. It is mainly used in patients with type 2 diabetes. Here we aiming to control the plasma concentrations of drug maintained throughout 24 hours dosing interval with less peak trough fluctuation than that observed with twice daily dosing of immediate release Glipizide.

MATERIAL AND METHOD

Glipizide obtained as a gift sample from Micro labs, India. Maltodextrin was procured from Himedia, Mumbai. Cholesterol and span-60 were purchased from Loba Chem Pvt. Ltd., Mumbai. All other reagents used were of analytical grade.

Preparation of Proniosome[1,4] :

The slurry method is selected for the preparation of proniosomes using maltodextrin as a carrier. The drug Glipizide dissolved in acetone solution is added to the surfactant mixture, which contains chloroform,

surfactant and cholesterol. The resultant solution is added to the 100ml round bottom flask containing maltodextrin. The flask was attached to a rotary flash evaporator to evaporate solvent at 60 to 70 rpm, at room temperature, and a reduced pressure of 600mmHg until the mass in the flask had become a dry, free flowing product. These materials were further dried overnight in a desiccator under vacuum at room temperature. This dry preparation is referred to as 'proniosomes' and was used for preparations and for further study on powder properties. These proniosome were stored in a tightly closed container at refrigerator temperature until further evaluated.
Proniosomes were prepared with different µmol ratios of cholesterol and Span 60 such as 225:25,200:50, 175:75 respectively while drug loading (100 mg) was kept constant. The prepared proniosomes were evaluated for drug entrapment.

Preparation of niosomes from proniosomes[2]:
Proniosome-derived noisome dispersions were obtained by hydrating the proniosome preparation with 80^0C distilled water and shake for a min. The niosomes were sonicated twice for 30sec using sonicator. The resulting noisome dispersion was used for the determination of the entrapment efficiency and further studies.

Measurement of Angle of repose[1]:
The angle of repose of dry proniosomes powder and maltodextrin powder was measured by a funnel method (Lieberman et al 1990). The maltodextrin powder or proniosomes powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 2.5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

Surface Morphology[1,4]:
Surface morphology was performed by scanning electron microscopy. Small amount of powder samples were placed on a stud and platinum was coated on them by Auto sputter fine coater. JFC1600, JEOL, JAPAN. Then the platinum coated samples were analysed in a cold field emission scanning electron microscope, JEOL, JSM-6701F, JAPAN and photographed.

Microscopy[4]:
The vesicle formation by the particular procedure was confirmed by optical microscopy in 400x resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera.

Entrapment efficiency[5]:
Niosome entrapped Glipizide was estimated by dialysis method. The calculated amount of prepared niosomes was placed in the dialysis bag 50 (presoaked for 24 hrs). Free Glipizide was dialyzed for 30 minutes each time in 100 ml of phosphate buffer pH 7.4. The dialysis of free Glipizide always completed after 12-15 changes, when no Glipizide was detectable in the recipient solution. The dialyzed Glipizide was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 276 nm. The samples from the bulk of solution diluted appropriately before going for absorbance measurement. The free Glipizide in the bulk of solution gives us the total amount of un-entrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

$$\text{Percent Entrapment} = \frac{\text{Total drug - Diffused drug}}{\text{Total drug}} \times 100$$

***In vitro* release study[6]:**
The formulations were subjected to centrifugation prior to *in vitro* drug release study. The centrifugation was done to remove unentrapped drug in the formulation, which will affect the release study of Glipizide drug from vesicles. *In vitro* release pattern of niosomal suspension was carried out in dialysis bag method. 10 mg equivalent of Glipizide niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 75 ml of 0.1 N Hcl. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37 ± 1^0C. 5 ml samples were withdrawn periodically and were replaced by fresh buffer. After two hours, 25 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 7.4, and the test was continued for a further 22 hours. The sink condition was maintained throughout the experiment. The withdrawn samples were appropriately diluted and analyzed for drug content using U.V. spectrophotometer at 275nm keeping phosphate buffer pH 7.4 as blank. All the determinations were made in triplicate.

Drug Release Kinetic Data Analysis:
The release data obtained from various formulations were studied further for their fitness of data in different kinetic models like Zero order, Higuchi's and peppa's.

Stability Study[7]:
Physical stability study was carried out to investigate the degradation of drug from proniosome during

storage. Best three (F_3, F_4, F_5) of the optimized indomethacin proniosome formulation composed of span-60 and cholesterol sealed in glass vials and stored in refrigerated temperature (2-8^{0}C) for a period of 3 months. Samples from each batch were withdrawn after the definite time intervals and converted into noisome and the residual amount of drug in the vesicles was determined. Stability data of three formulations were further analyzed for significant difference by paired t-test.

***In-Vivo* Anti - Inflammatory Study[8]:**

It has been reported in the literatures that Glipizide have therapeutic potential as hypoglycemic agent either alone or in combination with other antidiabetic drugs. The *in vivo* efficiency tests of the pure Glipizide and optimized formulation were performed on albino wistar rats by measuring the hypoglycemic effect produced after oral administration

The study was approved by institutional animal ethical committee (proposal no. NCP / IAEC / PG / 06 / 2008-2009).

In-vivo evaluation of Glipizide niosomes (after reconstitution from proniosomes), is performed on healthy albino wistar rats weighing between 150-200g. Two groups of rats (4 in each groups) fasted for 12 hours with free access to water is used for the study. Stock suspensions of pure drug and the drug loaded niosomes containing Glipizide at a concentration of 200µg/ml, is used for oral administration. The suspensions are administered orally at a dose equivalent to 800µg/kg of Glipizide to respective groups using stomach intubation. Blood samples are withdrawn at predetermined time intervals from 0 to 24hours by tail cutting method. The blood sample withdrawn time zero is considered as control. The blood glucose level of the control and test samples is determined by using the glucose-measuring instrument.

Table I: Compositions of proniosome batches of Glipizide and entrapment of different formulation

Formulation No.	Formulation Code	Ratio (µmol) (surfactant: cholesterol)	Entrapment efficiency* %
1	F_1	225:25	57.43 ± 1.16
2	F_2	200:50	66.15 ± 0.69
3	F_3	175:75	76.90 ± 0.28
4	F_4	150:100	84.25 ± 1.25
5	F_5	125:125	65.23 ± 0.71
6	F_6	100:150	53.31 ± 0.76
7	F_7	75:175	43.73 ± 0.93

1 mg of Carrier per 1 µ mole of surfactant
Drug content used 25 mg per batch
*Average of three formulations, ± S.D.

Figure I: SEM analysis for formulation F_1

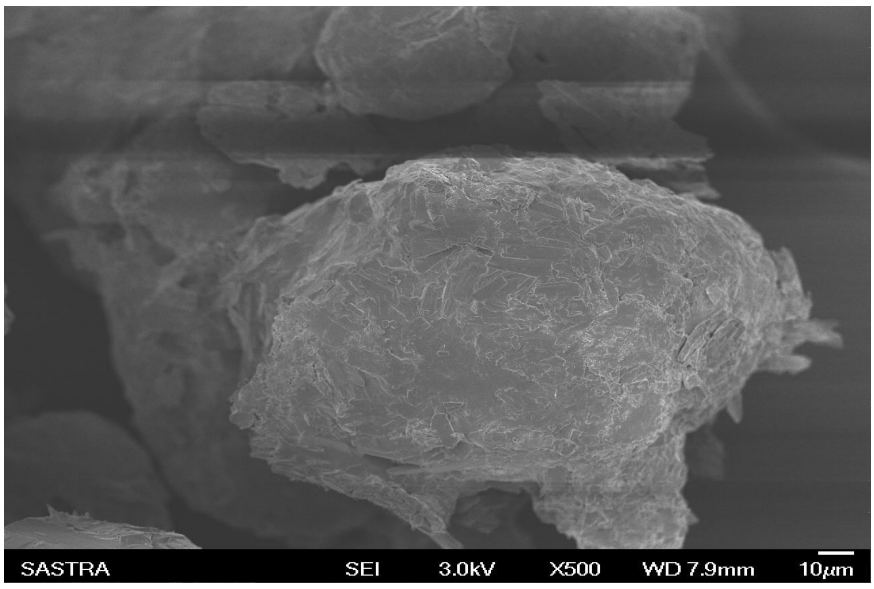


Table II: Angle of repose of uncoated maltodextrin and proniosome formulation

Preparation	Angle of Repose
Maltodextrin powder	35° 15' ± 0.42
F_1	36° 22' ± 0.19
F_4	35° 91' ± 0.15
F_7	33° 37' ± 0.25

Table III: Result of Anti hypoglycemic activity measurement

S.No	Time	% decrease std	% decrease test
1	0	0	0
2	2	49.67	17.41
3	4	37.25	24.58
4	6	28.53	42.66
5	8	10.41	40.27
6	10	4.37	35.15
7	12	4.03	31.05
8	14	4.03	25.93
9	16	3.03	21.5
10	18	2.25	15.02
11	20	2.69	8.53
12	22	2.35	5.11
13	24	2.02	2.73

Figure 2: Optical microscopy of formulation F_1

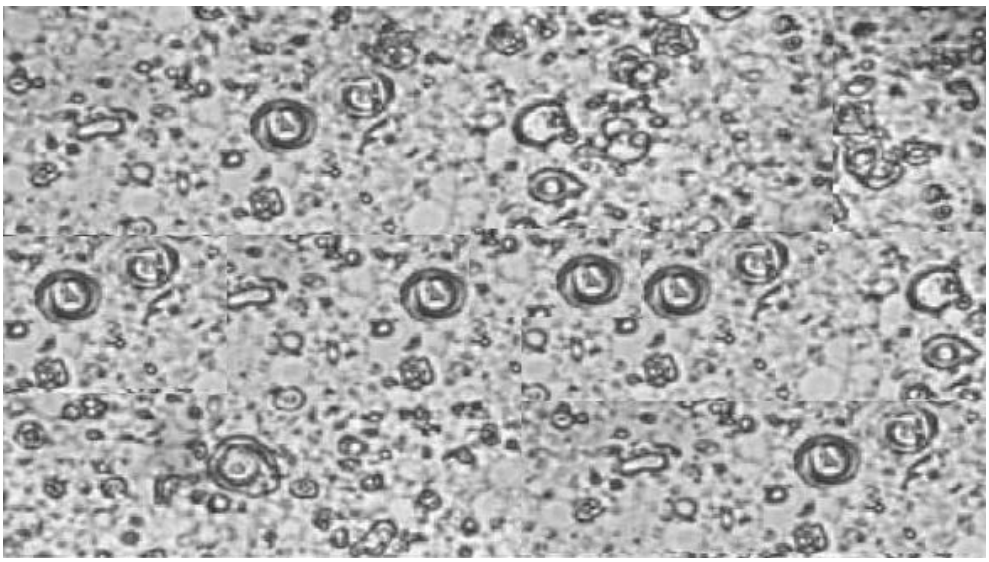


Figure 3: *In-vitro* release of all formulation

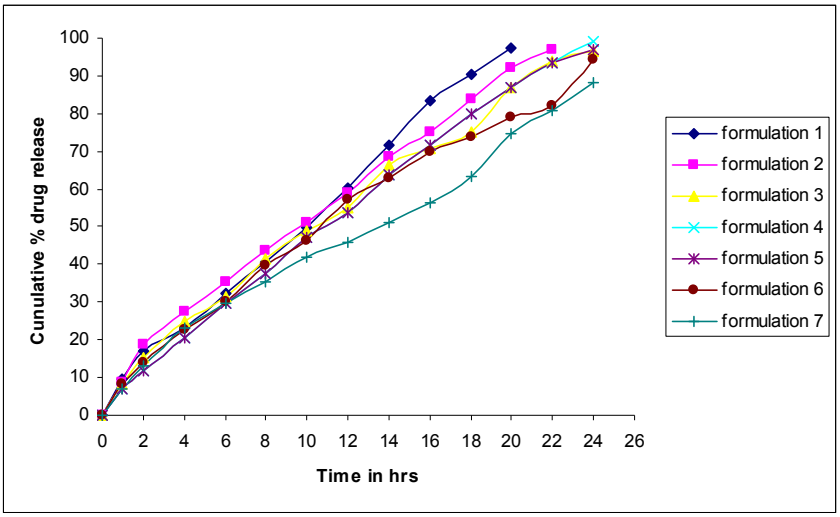
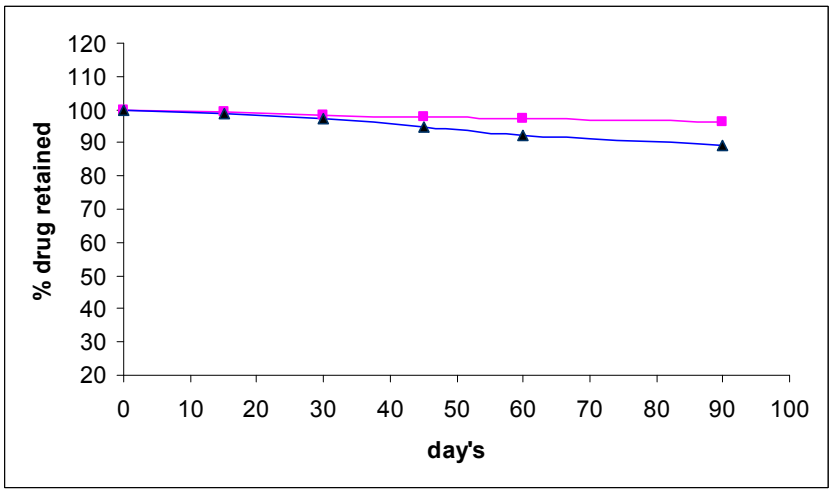


Figure 4: Percentage of Glipizide retained in the niosome formulations after storage at refrigeration temperature



RESULT AND DISCUSSION

FT-IR Spectra of Glipizide, maltodextrin, physical mixture of drug: carrier and F4 formulation were recorded. The Glipizide present in the formulation F4 was confirmed by FTIR spectra. The characteristic peaks due to pure Glipizide at 3250.16, 2943.47, 1689.70, 1651.12, 1373.36, 1159.26, 686.68 for N-H stretching, C-H Stretching, C= O Stretching, -CONH- Stretching, C-H bending, S=O Stretching, C-H bending respectively, which is shown in Table-. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between Glipizide and carrier. It also confirmed that the stability of drug during formulation.

Angle of repose of maltodextrin powder compared with proniosome formulation by fixed funnel method. Results indicate that the angle of repose of dry proniosome powder is nearly equal to that of pure maltodextrin. This shows that the prepared proniosome formulations have appreciable flow properties.

Shape and surface characteristic of proniosome examined by Scanning Electron Microscopy analysis, is shown in the Fig. Pure maltodextrin and Glipizide loaded maltodextrin Proniosome (F_4 formulation) are evaluated for surface morphology. Surface morphology confirms the coating of surfactant in carrier.

The prepared vesicles were studied under 400x magnifications to observe the formation of vesicles. Some unevenness of vesicles that observed under the study may be due to drying process under normal environment condition. The particles found to be uniform in size and shape.

Entrapment efficiency was studied for all the 7 formulations to find the best, in terms of entrapment efficiency. Higher entrapment efficiency of the vesicles of span 60 is predictable because of its higher

alkyl chain length. The entrapment efficiency was found to be higher with the formulation no. F_4 (84.25%), which may have an optimum surfactant cholesterol ratio to provide a high entrapment of Glipizide. The niosomal formulations having high surfactant concentration (F_2, F_3 and F_4) have the higher entrapment efficiency which might be due to the high fluidity of the vesicles. Very low cholesterol content (F_1) was also found to cause low entrapment efficiency (57.43%), which might be because of leakage of the vesicles. The higher entrapment may be explained by high cholesterol content (~50% of the total lipid). There are reports that entrapment efficiency was increased, with increasing cholesterol content and by the usage of span-60 which has higher phase transition temperature. It was also observed that very high cholesterol content (F_6, F_7) had a lowering effect on drug entrapment to the vesicles (43.73%). This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. The larger vesicle size may also contribute to the higher entrapment efficiency.

The release study was conducted for all the 7 formulations. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 90% release within a period of 24 hours. The formulations which have high cholesterol ratio (F_6, F_7) were found to sustain the drug release. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment. The three best formulations F_3, F_4, and F_5 were found to give a cumulative release of 96.68 %, 99.23 % and 92.93 % respectively over a period of 24 hrs, the higher release from the formulation F_3 may be because of its low cholesterol content. Formulations F_6 and F_7 having the highest cholesterol content showed the slow release over 24 hours, they provide a release of 94.13 % and 88.01 % respectively.

The zero order plots showed the zero order release characteristics of the formulation, which was confirmed by the correlation value which found to be nearer to one. Correlation value of Higuchi's plot revealed that the mechanism of drug release is diffusion. The *in vitro* kinetic data subjected to log time log drug release transformation plot (peppa's model), all the value ranges from 1.009 to 1.092revealed the fact that the drug release follows a super case II transport diffusion.

Physical stability was carried out to investigate degradation effect of the Glipizide from proniosomes at refrigerated temperature. The percentage of Glipizide retained in the reconstituted vesicles after a period of three months were 90.33%, 89.43% and 85.85% respectively for formulations F3, F4 and F5. The percentages of Glipizide retained in the proniosome formulation were 95.80%, 96.50%, 96.75% respectively. Also the results indicate that more than 95% of Glipizide was retained in the proniosomal formulation for a period of 90 days. From this it can be concluded that proniosomes are stable to store under refrigeration temperature with least leakage. The leakage of drug from F6 may be due to its lower surfactant content and higher cholesterol which formed a leaking vesicle.

In vivo efficiency tests of the pure Glipizide and optimized proniosome formulation (F4) were performed on albino Wistar rats by measuring the hypoglycemic effect produced after oral administration. Pure Glipizide and the optimized proniosome formulation were administered at a dose equivalent to 800 mg kg–1. With pure Glipizide, a rapid reduction in blood glucose levels was observed with maximum reduction of 49.67±1.16% within 2 hours after oral administration. Following the drug administration, the blood glucose levels recovered to normal within 8 hours in case of the pure drug (graph 10.25). The reduction in blood glucose levels was gradual and reached maximum reduction (42.58± 3.98%) at 6 hours after the administration of optimized Glipizide proniosomes. This reduction in blood glucose levels was sustained for longer periods of time (14 hours). A 25% reduction in blood glucose levels is considered a significant hypoglycemic effect. Significant hypoglycemic effect was observed between 1 and 6 hours after oral administration of Glipizide, whereas with formulationF4, a significant hypoglycemic effect was maintained for 3 to 16 hours after oral administration.

Proniosomes are prepared with non-ionic surfactant (span 60) and cholesterol by coating maltodextrin powder as carrier, which upon reconstitution gives niosomes. Glipizide, an anti-diabetic drug is encapsulated in these formulations for the sustained action of drug. On using different ratios, 150:100 µmol ratios of the surfactants to cholesterol preparation shows the highest entrapment efficiency and good release characteristics. In this study it is found that entrapment efficiency of drug is depend upon the cholesterol: surfactant ratio. The *in vivo* studies of best formulation shows the hypoglycemic effect of Glipizide up to 14 hours.

Maltodextrin-based proniosomes are a potentially scalable method for producing niosomes for delivery of hydrophobic or amphiphilic drugs. The method of preparation is very easy when compared to conventional niosomes. Proniosomes minimize problems of noisome physical stability, such as aggregation, fusion, and leaking and provide additional convenience in transportation, distribution, storage and dosing. The optimized formulation developed using the desirability approach produced high drug encapsulation efficiency and sustained anti-diabetic activity in rats following oral administration.

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