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Development and Characterization of Niosomal Drug Delivery of Gliclazide

Tamizharasi S, Dubey A, Rathi V[1], Rathi JC[1]

Department of Pharmaceutics, Nandha College of Pharmacy, Erode-638 052, Tamil Nadu;
[1]Department of Pharmaceutics, NRI Institute of Pharmaceutical Sciences, 3, Sajjan Singh Nagar, Opp Patel Nagar, Bhopal-462 026, Madhya Pradesh, India.

Address for correspondence: *Mr. Jagdish Chandra Rathi; E-mail: roshan_rathi2005@yahoo.co.in*

ABSTRACT

In the present study, gliclazide-loaded niosomes are formulated and evaluated for their *in vitro* as well as *in vivo* characteristic in an attempt to improve the oral bioavailability of the drug. Formulation of niosomes was optimized for highest percentage of drug entrapment. Microscopic observation confirmed that all particles were uniform in size and shape. The entrapment efficiency was determined by separating the unentrapped drug using dialysis. The in vitro release studies of drug from niosomes exhibited a prolonged drug release as observed over a period of 24 h. The positive values of zeta potential indicated that the gliclazide niosomes were stabilized by electrostatic repulsive forces. Results from stability study have shown that the drug leakage from the vesicles was least at 4°C followed by 25 and 37°C. The niosomes showing maximum entrapment and suitable release rate were selected for in vivo evaluation. In conclusion, the niosomal formulation could be a promising delivery system for gliclazide with improved bioavailability and prolonged drug release profile.

Key words: Niosomes, gliclazide, cholesterol

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INTRODUCTION

Drug delivery system using colloidal particulate carrier such as liposomes[1] or niosomes[2] has distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs. Modification of the particle composition or surface can adjust the affinity for the target site and/or the drug release rate. The slow drug release may reduce the toxicity of drug by enabling these carriers to play an important role in drug delivery. Niosomes are important from a technical viewpoint as they possess greater stability and avoid some disadvantages associated with liposomes such as variable purity of phospholipids and high cost. Another advantage of niosomes is the development of a simple practical method for the routine and large-scale production without the use of pharmaceutically unacceptable solvents. Niosomes, or the surfactant vesicles, are made up of spherical lipid bilayers capable of entrapping water soluble molecules within an aqueous domain or alternatively lipid molecules within the lipid bilayers. Niosomes have been prepared from different non-ionic surfactants. They may be unilamellar or multilamellar depending upon the method used for their preparation. In recent years, niosomes have been extensively studied for their potential to serve as carriers for delivery of drugs, antigens, hormones and other

bioactive agents. The basic aim of this study was to develop a delivery system to control the release of drug from the carrier system, in order to achieve an extended uptake in the body. Encapsulation of a drug in vesicular structure can be predicted to prolong the existence of the drug in the systemic circulation and thus enhance penetration into target tissue and reduce toxicity if selective uptake can be achieved. Niosomes alike liposomes are biodegradable, biocompatible and non immunogenic in nature and exhibit flexibility in their structural characterization.[3] In addition, handling and storage of niosomes requires no special conditions.[4-5] Niosomes are now widely studied as an alternative to liposomes because they alleviate the disadvantages associated with liposomes such as chemical instability, variable purity of phospholipids and high cost.[6]

Gliclazide is one of the most frequently used sulfonylureas for the treatment of type 2 diabetes which requires twice daily administration.[7] In the present study, gliclazide encapsulated niosomes were formulated and evaluated for their *in vitro* as well as *in vivo* characteristics and an attempt was made to improve the oral bioavailability of the drug.

MATERIALS AND METHODS

Materials

Gliclazide was supplied as a gift sample by Cadila Pharmaceutical Limited, Ahmedabad (India). Cholesterol and Span 60 were purchased from Loba Chem Pvt. Ltd., Mumbai. All other chemicals used were of analytical reagent grade.

Preparation of niosomes

The niosome formulations were prepared by lipid film hydration technique.[8-9] Drug, Span 60 and cholesterol were weighed (surfactant : cholesterol in micromols) and dissolved in methylene chloride (10 ml) in a 100 ml round bottom flask. A thin lipid film was formed under reduced pressure in a rotary flash evaporator. The film was then hydrated by 10 ml of PBS pH 7.4 at room temperature with gentle shaking. The niosome suspension was further hydrated up to 24 h at 2-8° C. The stabilized MLVs were used for further studies. Composition, ratio and code of the niosome formulations are given in Table 1.

Vesicle size determination

It was carried out using optical microscope (Jyoti Scientific, India) at a magnification of 400×, by means of a fitted camera (Panasonic, Japan).

Entrapment efficiency determination

The entrapment efficiency of niosomes was determined by the dialysis method.[10-11] The unentrapped drug was removed by centrifugation at 25000 rpm. Pellets were redispersed in 3 ml of phosphate buffer saline and placed in dialysis bag. This was immersed in 25 ml of phosphate buffer saline solution (pH 7.4) maintained at 37°C and stirred with a magnetic stirrer (Remi equipments Pvt ltd. Bombay). The receiver solution was completely withdrawn and replaced with fresh phosphate buffer saline (pH 7.4) at scheduled intervals of time. This was repeated till the entire drug was removed from the niosomes. The difference between the amount dialyzed and the total drug incorporated gave the entrapment efficiency, which was measured by spectrophotometeric method at 228 nm using a UV-vis spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan).

In vitro release studies

In vitro release pattern of niosomes suspension was carried out by dialysis bag method. An amount of niosomes equivalent to 100 mg pure gliclazide was weighed and filled in dialysis bag (Hi media). In the acid stage, dialysis bag was placed in a round bottomed cylindrical vessel containing 75 ml of 0.1 N HCl. The vessel was placed over magnetic stirrer (50 rpm) and the temperature was maintained at 37 $\pm$ 0.5^{0}C. Aliquots were withdrawn at predetermined time

Table 1: Compositions, entrapment efficiency and mean particle diameter of niosomal batches of gliclazide

Formulation No.	Ratio (µmol) (surfactant: cholesterol)	Span 60 (mg)	Cholesterol (mg)	Entrapment efficiency* (% w\w)	Mean particle diameter* (µm)
F 1	250:50	107.6	19.33	75.10 ± 2.31	12.34 ± 2.31
F 2	225:75	96.88	29.00	78.85 ± 3.45	13.23 ± 3.21
F 3	200:100	86.12	38.67	81.07 ± 3.74	15.34 ± 3.32
F 4	175:100	75.35	38.67	86.10 ± 5.67	15.65 ± 2.45
F 5	175:90	75.35	34.80	71.89 ± 2.65	14.32 ± 2.68
F 6	175:75	75.35	29.00	68.10 ± 1.56	13.78 ± 1.67
F 7	150:50	64.59	19.33	67.86 ± 4.32	12.73 ± 2.45

Drug content used: 100 mg per batch, *Average of three preparation ± S.D.

intervals and immediately replaced with the fresh medium equilibrated at 37°C. After 2 h, 25 ml of 0.2 tribasic sodium phosphate was added to change the pH of test medium to 7.4, and the test was continued for a further 24 h. The sink condition was maintained throughout the experiment. The withdrawn samples were diluted and analyzed for drug content using U.V. spectrophotometer at 228 nm.

Kinetic modeling

To understand the pharmacokinetics and mechanism of drug release, the result of *in-vitro* drug release study of niosomes were fitted with various p'kinetic equations like zero order[12] (cumulative % release vs time), first order[13] (log % drug remaining vs time), Higuchi's model[14] (cumulative % drug release vs. square root of time). The r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

Zeta potential

The zeta potential value of the sample was measured by a zeta potential probe model DT-300. Niosome formulation F_4 which gives best results between entrapment efficiency and drug release was studied for zeta potential determination.

Stability study

The formulated niosomes were tested for the stability. All formulations were divided into three sets and were stored at 4 ± 2°C, 25 ± 2°C/ 60 % RH ± 5 % RH and 37 ± 2°C/ 65 % RH ± 5 % RH in humidity control oven (Lab-control equipment Co. Mumbai). After 3 months, the drug content of all the formulation was determined by the method discussed previously in entrapment efficiency section.

In vivo anti-hyperglycemic activity

The *in vivo* evaluation studies were conducted on the selected formulation F_4 by measuring anti-hyperglycemic activity[15-16] in normal healthy male wistar rats (weight range 250 to 300 gm each). The approval of the institutional animal ethical committee was obtained before starting the study. Two groups of wistar rats (5 in each group) fasted overnight prior to the experiments were used for the study. After collecting zero hour blood samples, one group received pure gliclazide whereas the other group received gliclazide niosomes formulation of same dose orally in a suspension from using stomach incubator. Blood sample were collected at 0, 1, 2, 4, 6, 8, 10, 12 and 24 h intervals. Blood glucose level was determined by using blood glucose measuring instrument One Touch ltra (Lifescan, Inc. Milpitas, CA 95035 U.S.A.) and percentage reduction in blood glucose level was calculated.

RESULTS AND DISCUSSION

Niosomes containing gliclazide were prepared using non-ionic surfactant Span 60 and cholesterol in different proportions by lipid film hydration technique. Preparation of niosome was optimized for highest percentage drug entrapment. The prepared vesicles were studied under 400 x magnification to observe the formation of vesicles. The niosomes were observed as spherical vesicles with smooth surface. The vesicles were discrete and separate with no aggregation or agglomeration [Figure 1]. The diameter of the formulated niosomes was found to be in the range of 12.34 ± 2.31 - 15.65 ± 2.45 μm. The entrapment efficiency was determined by separating the unentrapped drug using dialysis and it was in the range of 67.86 ± 4.32 - 86.10 ± 5.67 % w/w [Table 1]. Formulation F_4 showed highest entrapment efficiency. Very low cholesterol content was responsible for low entrapment efficiency, which might be due to leakage of the drug from the vesicles. The higher entrapment in the batch F_4 can be explained by high cholesterol content (~ 50% of the total lipid). There are reports in the literature that entrapment efficiency increases with increasing cholesterol content and by the usage of Span 60 which has higher transition temperature.

The release study was conducted for all the formulations as shown in Figure 2. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, has 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 multimellar vesicles consist of several concentric sphere of bilayer separated by aqueous

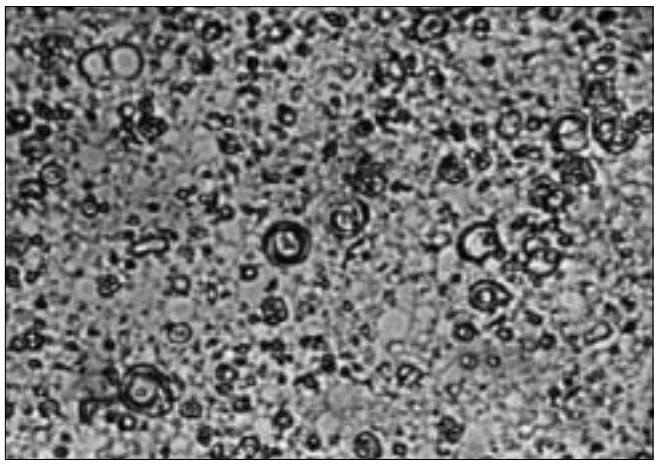

Figure 1: Photomicrograph of niosomes on a dry glass slide

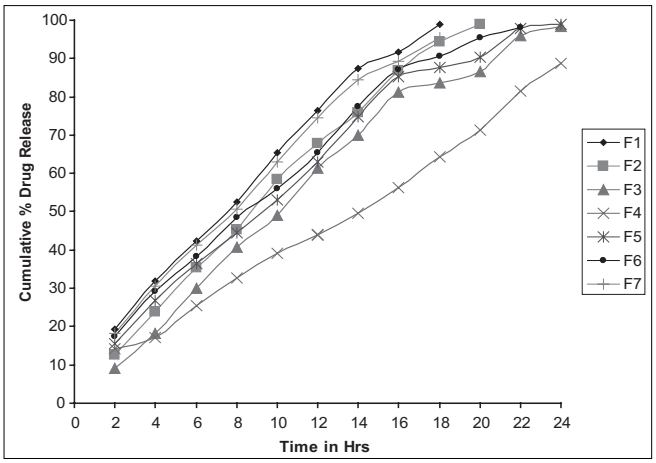

Figure 2: *In vitro* drug release profiles of gliclazide from different niosome formulations

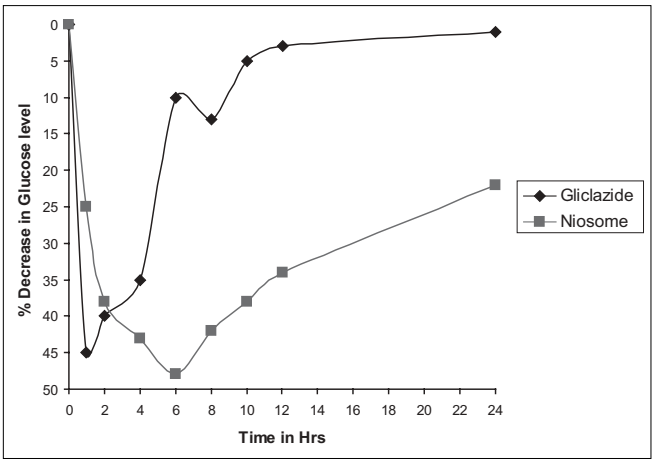

Figure 3: *In vivo* hypoglycemic activity

compartment. The best formulation F_4, was found to give a cumulative release of 88.72% over a period of 24 h. The *in-vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism. The release constant was calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. It was found that the *in-vitro* drug release of niosomes was best explained by zero order kinetics for selected formulation F_4 as the plots shows highest linearity. The correlation coefficient (r^2) was found 0.99 for F_4.

Zeta potential of gliclazide niosome (F_4) was determined and it was found to be +36 mV and positive values indicate that the gliclazide niosomes were stabilized by electrostatic repulsive forces. Stability studies of all prepared niosomes were performed by storing at 4, 25 and 37°C for a period of three months. The residual drug content was determined at the end of third month. It was observed that the drug leakage from the vesicles was least at 4°C followed by 25 and 37°C. This may be attributed to phase transition of surfactant and lipid causing vesicles leakage at higher temperature during storage. Hence, it is concluded from the obtained data that the optimum storage condition for niosomes was found to be 4°C.

In vivo evaluation of the selected formulation was carried out in healthy normal wistar rats by measuring hypoglycemic effect produced after oral administration of gliclazide niosomes in comparison to gliclazide (pure drug) at same dose. When pure gliclazide suspension was administered, a rapid reduction in blood glucose levels was observed and maximum reduction of (45%) was observed within 2 h after oral administration. Blood glucose levels recovered rapidly to the normal level within 7-8 h. In the case of gliclazide niosomes, the reduction in blood glucose levels was slow and reached maximum reduction within 4-5 h after oral administration. This reduction in blood glucose levels was sustained over longer periods of time (12 h). A 25% reduction in blood glucose levels is considered as a significant hypoglycemic effect. The hypoglycemic effect (25%) was maintained only for 2-3 h after oral administration of gliclazide, whereas in the case of gliclazide niosomes, significant hypoglycemic effect (25%) was maintained for a period of 2 to 12 h [Figure 3]. The sustained hypoglycemic effect observed over a longer period of time in the case of niosomes is due to the slow release and absorption of gliclazide over longer periods of time.

CONCLUSION

Recently, niosomes have been studied by many researchers as a choice of oral drug delivery system to provide better oral bioavailability consideration, high penetration property of the niosome encapsulated agents through biological membrane and their stability. The present formulation study on gliclazide is an attempt to prepare niosomal drug delivery system and evaluate its *in vitro and in vivo* performance. The formulations were prepared with different ratios of cholesterol and surfactant. An ideal or best formulation of niosome is said to be one that gives high entrapment efficiency. In this study, entrapment efficiency was found to be cholesterol: surfactant ratio dependent. Formulations were found to ensure a good oral bioavailability of the drug *in vivo*. On the basis of these facts, it can be concluded that niosomes may be a promising approach to improve the oral bioavailability of gliclazide.

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