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Formulation, Evaluation and Optimization of Glipizide loaded Niosomes

Pankaj*, Satish Chander Arora and Rashmi Manchanda

Department of pharmaceutics, RKSD College of Pharmacy, Kaithal, Haryana, India

Abstract : In the present study, Glipizide-loaded Niosomes were formulated and evaluated for their *in vitro* characteristics 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 werenano sized. The *in vitro* release studies of drug from Niosomes exhibited a prolonged drug release as observed overa period of 24 h. The negative value of zeta potential indicated that the Glipizide Niosomes were stabilized by electrostatic repulsive forces. Results from stability study have shown that the drug leakage from the vesicleswas least at 4°C followed by room temperature. The Niosomes showing maximum entrapment and suitable release rate were selected for *in vitro*evaluation. In conclusion, the Niosomal formulation could be a promising delivery system for Glipizide with improved bioavailability and prolonged drug release profile.
Keyword : Niosome, Glipizide, Tween 80, Cholesterol.

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 dialkylpolyglycerol ether class with cholesterol [2]. 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

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the drug from biological environment and restricting effects to target cells [4]. 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 [5].

Niosomes behave *in vivo* like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability [6]. 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 [7]. 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.

Glipizide is one of the most frequently used sulfonylureas for the treatment of type 2 diabetes which requires twice daily administration. In the present study, Glipizide 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

Glipizide was supplied as a gift sample by Horizon Bioceuticals Pvt. Ltd. Cholesterol was purchased from LobaChemie Laboratories, Mumbai. And Tween 80 was purchased from Nice Laboratories Reagents, Kochi. All other chemicals used were of analytical reagent grade.

Construction of standard calibration curve of Glipizide:

Accurately weighed 10 mg of Glipizide was dissolved in 100 ml of Phosphate buffer of pH 7.4 to give an initial stock solution of concentration; 100 µg/ml. Further dilutions were made as per the scheme shown in **Table no. 1** given below to obtain the concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml. The absorbance of these solutions was measured at 223 nm. A standard curve between concentration and absorbance was plotted as shown in **Fig. no. 2**. Lambert-Beer's law was obeyed in the concentration range of 2 to 20 µg/ml.

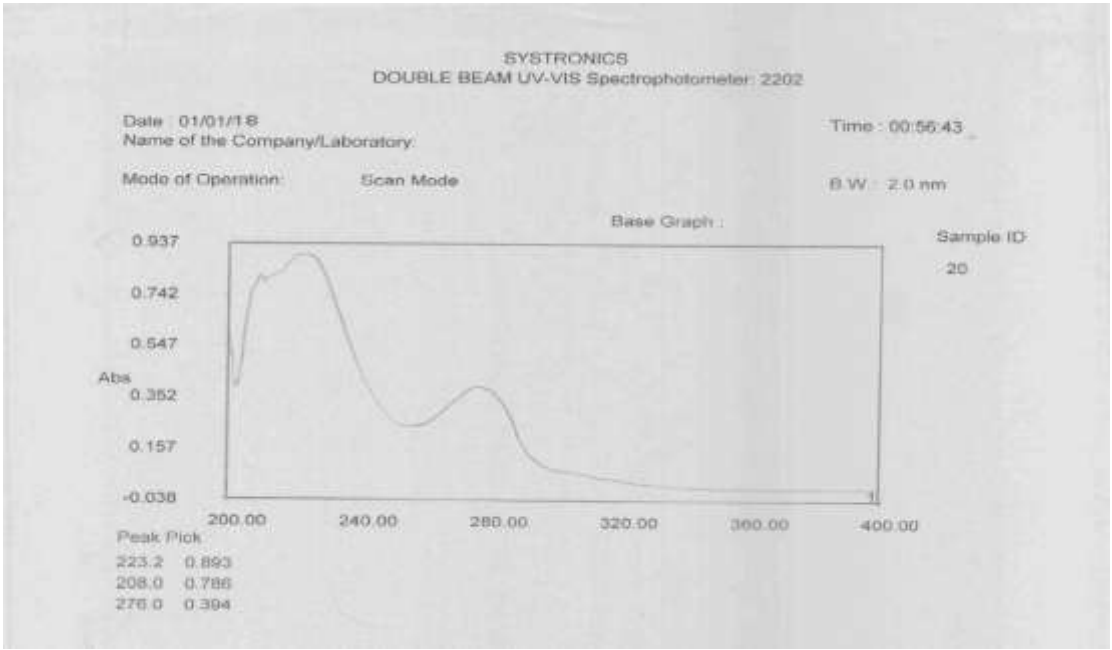

Fig. no. 1λmax of Glipizide in Phosphate buffer of pH 7.4

Table no.1 Calibration data of Glipizide at 223 nm

Sr. No.	Concentration (µg/ml)	Absorbance	Standard Parameters
1	0	0.00	
2	2	0.11 ± 0.065	
3	4	0.243 ± 0.041	
4	6	0.32 ± 0.024	
5	8	0.42 ± 0.033	
6	10	0.512 ± 0.028	Equation y = 0.045x + 0.037
7	12	0.593 ± 0.09	
8	14	0.678 ± 0.058	R^2 = 0.994
9	16	0.73 ± 0.019	Slope = 0.045
10	18	0.826 ± 0.042	Intercept = 0.037
11	20	0.946 ± 0.021	

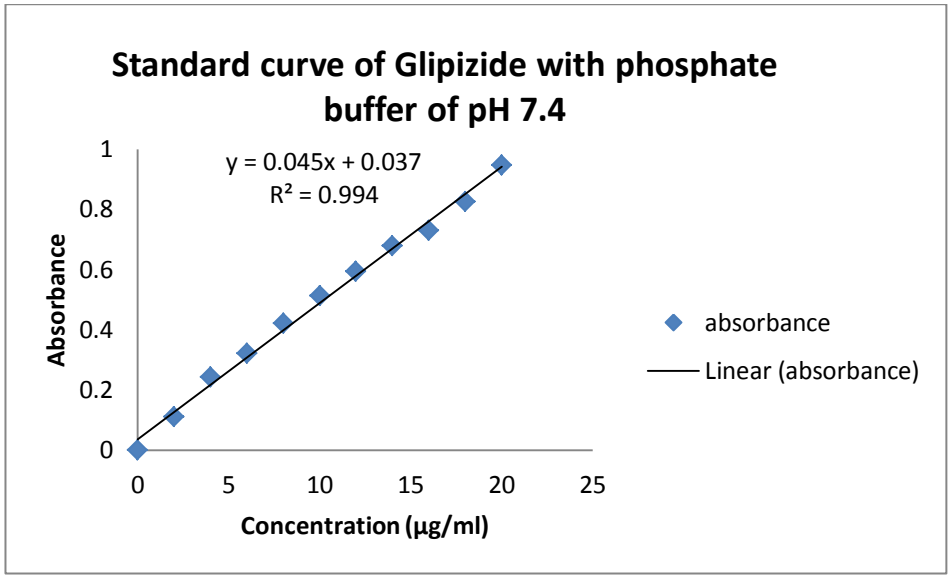

Fig. no. 2Calibration graph of Glipizide at phosphate buffer of pH 7.4 at 223 nm

Preparation of Niosomes [8]

Niosomes were prepared by the Thin film hydration method. To prepare the Glipizide, Tween 80 and Cholestrol were weighed accurately and dissolved in dichloromethane in 100 ml round bottom flask. A thin lipid film was formed under reduced pressure in a Rotary Flask Evaporator. The film was then hydrated by 6ml Phosphate buffer saline pH 7.4 at room temperature with gentle shaking. The formed Niosomes were then filtered by vacuum filtration and dried at room temperature and then stored in vacuum dessicator for further evaluation.

Entrapment efficiency

The entrapment efficiency of the Niosomes was determined spectrophotometrically. A sample of GlipizideNiosomes (10 mg) was dissolved in 10 ml of methanol and kept it for overnight. 1 ml of the supernatant was taken and diluted to 10 ml with a solution containing phosphate buffer of pH 7.4 and was analyzed at 223 nm using UV-Visible spectrophotometer. From the absorbance, the % entrapment efficiency of the Niosomes was calculated.

$$\%\, Entrapmentefficiency = \frac{observeddrugcontent}{initialdrugcontent} \times 100$$

Size Analysis by optical microscopy

A drop of Niosome suspension was placed on a glass slide and it was diluted. A cover slip was placed over the diluted Niosome suspension and evaluated the average vesicle size and shape by an ordinary optical microscope.

***In-vitro* drug release study**

In vitro release study was performed using USP Paddle method at 100 rpm and 37 ± 0.2°C in 900 ml of phosphate buffer (pH 7.4). 100 mg of the formulated Niosomes were used for the experiment. Five ml aliquots were withdrawn at 30, 60 and 120th min and the hourly intervals upto 12 hours and a last aliquot was withdrawn at 16th and 24th hour. The samples after filtration were analyzed at 223 nm. Fresh dissolution medium was replenished each time when sample was withdrawn to compensate the volume [9]. The data of *in vitro* release from various Niosomes were evaluated kinetically using various mathematical models like zero-order, first-order, Higuchi and Koresmeyer–Peppas model equations [10].

Stability study

A well-designed stability-testing plan is essential and pertinent part of the quality assurance program. Ability of a formulation to retain properties in specified limits throughout its shelf life is referred as stability. Stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container, to retain its physical, chemical, microbiological, therapeutic and toxicological specifications [11].

The stability of finished pharmaceutical products depends on several factors. On the one hand, it depends on environmental factors such as ambient temperature, humidity and light. On the other hand, it depends on product-related factors such as chemical and physical properties of active substance and pharmaceutical excipients, the dosage form and its composition, the manufacturing process, the nature of container closure system and properties of packaging materials.

Prepared formulation was stored in amber coloured screw capped small glass bottles at 4 ± 1°C, room temperature and 40 ± 1°C and 75 ± 5% RH. Samples were analyzed for residual drug content after a period of 15, 30, 45, 60 and 90 days. Initial drug content was taken as 100 % for each formulation. The log percent residual drug content vs. time graph was plotted for the optimized formulation in order to evaluate k (specific rate constant or degradation rate constant), $t_{1/2}$ and $t_{10\%}$ of the formulation.

Results and Discussion

Size Analysis

Size analyzed performed by optical microscopy. Niosomes have spherical in nature as shown in **Fig.no.3**. Particle size analysis is performed by Malvern Metasizer and average size of particles is reported as 451.6nm

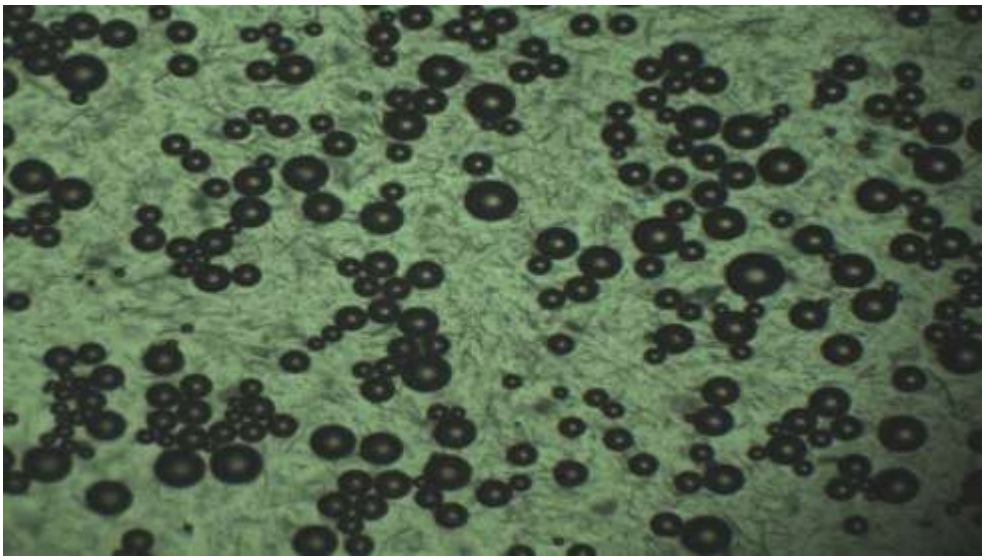

Fig. no. 3 Optical microscopic image of Glipizideloaded Niosomesformulations N6

Entrapment efficiency:

Entrapment Efficiency of nine batches (N1-N9) is summarized in **Table no. 2**.

Table no. 2: Entrapment Efficiency of Niosomes

Batch Code	Entrapment Efficiency (%)
N1	69.3 ± 0.69
N2	68.7 ± 0.45
N3	73.5 ± 1.12
N4	69.3 ± 1.45
N5	78.9 ± 1.76
N6	**82.3 ± 0.82**
N7	67.2 ± 1.14
N8	75.9 ± 0.78
N9	79.62 ± 0.81

Dynamic light scattering: Vesicle size and Zeta potential determination

Zeta potential is a measure of surface charge of dispersed particles in relation to dispersion medium. The charge of the Niosomes was determined by Malvern Metasizer. The experiment was performed using clear disposable zeta cell, water as dispersant which has refractive index (RI) -1.330 and viscosity (cPs) -0.88 and the temperature was kept constant at 25°C. The sample was analyzed for three times to minimize the error. The average zeta potential was reported as -16.6 (mV).

***In vitro* drug release and kinetic study:**

The percent cumulative amounts of Glipizide released as a function of time from Niosomes formulated was illustrated in **Table no. 3** and in **Fig. no. 4**.

Table no. 3 Diffusion characteristics of FormulationsN1-N9

Batch Code	Zero Order Kinetics	Higuchi Kinetics	Korsmeyer-Peppas Kinetics	N	First Order Kinetics
N1	0.898	0.994	0.993	0.569	0.704
N2	0.900	0.995	0.994	0.578	0.705
N3	0.909	0.994	0.983	0.572	0.702
N4	0.897	0.996	0.991	0.596	0.684
N5	0.859	0.982	0.977	0.643	0.635
N6	**0.926**	**0.990**	**0.983**	**0.589**	**0.716**
N7	0.883	0.992	0.994	0.582	0.693
N8	0.914	0.995	0.992	0.568	0.718
N9	0.901	0.997	0.987	0.596	0.677

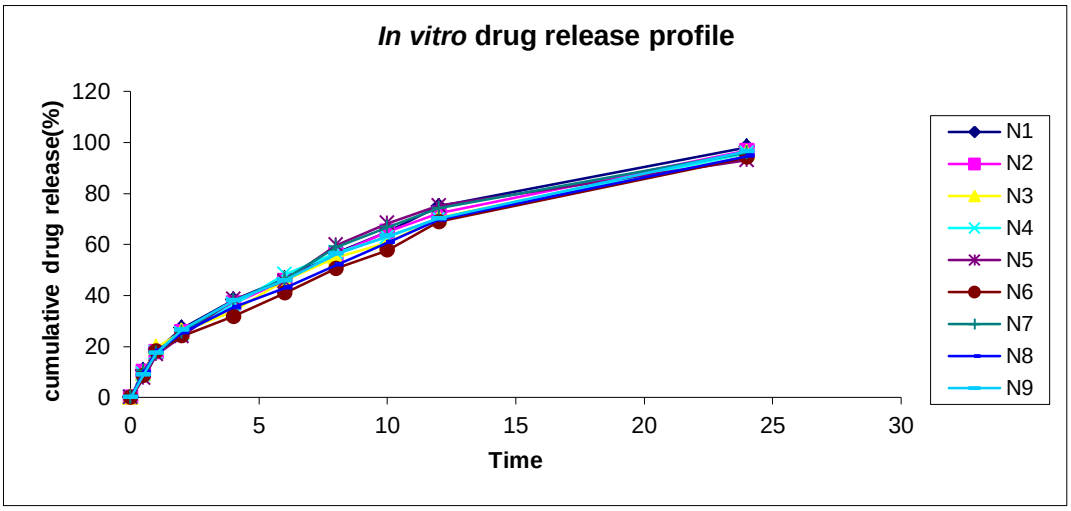

Fig. no. 4 *In-vitro* drug release graph N1 – N9

Stability Studies

Physical stability study of the prepared Niosomes was carried out to determine the comparative leakage of the drug from Niosomes stored at different conditions compared to each other. After washing and removal of the free drug, each Niosomal formulation was stored either at 4°C or at room temperature. At predetermined time intervals of 15, 30, 60 and 90 days. The percent residual drug content of optimized batch was determined as shown in **Table no. 5 and Table no. 6**.

Table no. 5 Effect of aging on residual content at 4 ± 1°C

Sr. No.	Days	Physical Change	Mean Percent Residual Drug Content (at 4 ± 1°C)
1	0	No Change	100
2	15	No Change	99.48 ± 0.06
3	30	No Change	99.12 ± 0.09
4	45	No Change	98.75 ± 0.16
5	60	No Change	98.36 ± 0.09
6	90	No Change	97.96 ± 0.12

Table no. 6 Effect of aging on residual drug content at room temperature

Sr. No.	Days	Physical Change	Mean Percent Residual Drug Content (at room temp.)
1	0	No Change	100
2	15	No Change	99.15 ± 0.10
3	30	No Change	98.46 ± 0.08
4	45	No Change	97.65 ± 0.12
5	60	No Change	96.94 ± 0.15
6	90	No Change	96.28 ± 0.14

Conclusion

Morphological investigations showed that all Niosomes were spherical in shape, having mean diameter of 451.6 nm. Selection of the appropriate experimental conditions resulted in the production of Glipizide loaded Niosomes and **N6** batch was found to be the optimized formulation having high entrapment efficiency of 82.3 ± 0.82% (w/w) and high percent cumulative drug release of 94.13 ± 0.56% (w/w) at 24thhr which showed that GlipizideNiosomes have the potential for prolonged drug release. Over three months of investigation on stability at 4 ± 1°C and room temperature, formulation shows faster degradation at higher temperature. The results indicate that the ideal storage temperature for the Niosomes is a cold place. Hence, it can be concluded that it is possible to design Glipizide loaded Niosomes for the treatment of type II diabetes where efficacy and patient compliance are of prime importance.

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