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Biosome forming matrices of glibenclamide: Effect of soya lecithin: Stearyl alcohol ratio on *in vitro* release

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ABSTRACT

A lipid carrier system for oral hypoglycaemic agent glibenclamide comprising of two lipid components chosen from classes of different polarity wherein one of the lipid components is amphiphatic, bilayer forming and the other is non polar. Discrete lipid particles were formed spontaneously when introduced into aqueous medium without any physical or chemical treatment or initiation. The spherical lipid bilayers thus formed in vivo are referred to as biosomes and the lipid matrix is referred to as biosome forming matrix.

Key words: Biosomes; Biosome Forming Matrix; Biosomes; Stearyl Alcohol; Soya Lecithin.

INTRODUCTION

Glibenclamide (GLIB) is an oral hypoglycemic agent of the sulfonyl urea group that is frequently prescribed for treatment of non-insulin dependent diabetes mellitus [1]. It is a low dose, poorly soluble drug with possible dissolution rate-limited bioavailability [2, 3]. Several techniques have been reported to enhance the dissolution rate of GLIB. These include comelt dispersion in polyethylene glycol (PEG) 6000 and/or 4000 [4], sorbitol and/or mannitol [5], coprecipitate formation with polyvinylpyrrolidone (PVP) or poloxamer [6] and lyophilization [7]. All these techniques have their own advantages and disadvantages.

Liposomes shall be beneficial when particularly associated with oral hypoglycaemic agents like glibenclamide as one of the components is soya lecithin which has various advantages in diabetes and its associated heart disorders like hyperlipidemia and hypercholesterolemia.

Several studies [8-10] have indicated that the administration of soybean lecithin/ phosphatidylcholine to animals with experimentally induced hypercholesterolemia decreases the level of plasma LDL-cholesterol and increases that of HDL-cholesterol when compared to a similar supply of polyunsaturated fatty acid. It has been reported that polyunsaturated soybean phospholipids induce a significant reduction in plasma cholesterol levels in hypercholesterolemic patients [11-14], but they induce no change in normolipidemic subjects [15, 16].

In another study, soya lecithin has been found to have no significant hypocholesterolemic effect on serum lipid profile in humans [17, 18].

Soya lecithin contains choline which was found to be useful in prevention and treatment of neurological complications associated with diabetes [19].

Due to the specific demand in terms of physical properties of the drug molecule in order to form stable liposome structures, only a limited number of drug candidates have been shown to be applicable in liposomes formed *In Vitro*.

Also the current well known liposome technology where the systems are prepared *In Vitro* before administration, suffers from disadvantages that the systems are quiet unstable and factors such as temperature or other constituents present in the formulation may dramatically change the nature of liposomes by irreversibly damaging the bilayers. Further, cholesterol, another component of liposomes, which is not good for health in patients associated with cardiovascular diseases, is replaced by other non polar lipids.

It is envisaged that the above mentioned needs can be met through by a biosome (Liposomes to be formed in the body) forming matrix (BFM) system [20-25]. These systems are easy to manufacture and scale-up with minimum process and formulation variables. The lipids used for the BFM consist of a mixture of lipid classes characterized by different polarities. Polar lipids such as phospholipids and non polar lipids such as mono-, di- and tri- glycerides with long chain fatty acids or long chain fatty alcohols are the main components.

In the present study, stearyl alcohol was investigated as non polar components of biosome forming matrices and the *In Vitro* dissolution rates of the prepared biosome forming matrix systems were compared with that of pure drug.

MATERIALS AND METHODS

Materials

Glibenclamide was a gift sample from Sun Pharmaceutical Industries Limited, Silvassa, India. Soya lecithin and tween 80 were procured from Himedia, Mumbai, India. Stearyl alcohol and stearic acid were from Central Drug House, New Delhi. All other chemicals used were of IP/AR grade.

Formulation composition

The soya lecithin and stearyl alcohol ratio was varied from 1:1, 1:1.5 and 1:2 fixing the amount of Tween 80 and glibenclamide at 0.5% and 1% of total formulation component. The formulation composition is shown in Table 1.

Table 1. Formulation composition of BFM of glibenclamide

Formulation Code	L (mg)	S (mg)	L:S Ratio	T[#] (mg)	Glibenclamide* (mg)
B13	2462.5	2462.5	1:1	25	50
B14	1970	2955	1:1.5	25	50
B15	1641.7	3283.3	1:2	25	50

Where L = Soya lecithin and S = stearyl alcohol T = Tween 80
T and Glibenclamide were fixed at 0.5% and 1% of total formulation content (L+S+T+Glibenclamide)

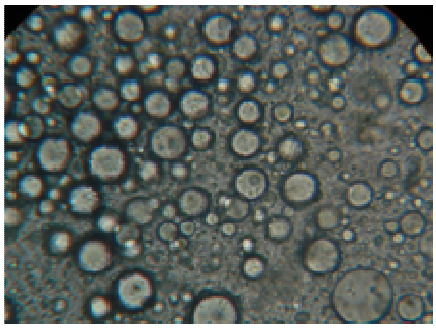
Preparation of formulations

The accurately weighed polar and non polar components i.e. soya lecithin and stearyl alcohol respectively were gently stirred at 60°C ± 10°C for about 4-7 hours (till homogenization of the two types of lipids occurs). Then accurately weighed amount of the drug was added to that and the stirring was continued for another hour at the same temperature [20-25].

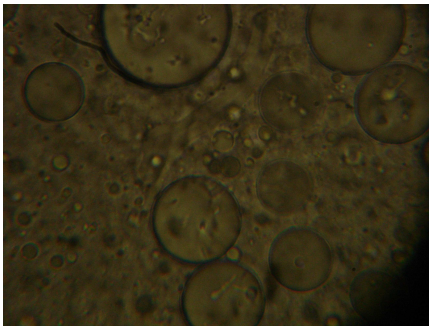
Characterization of the formulations

Appearance

The formulations equivalent to their unit dose were dispersed by gentle stirring in phosphate buffer pH 7.4 and kept aside for 5 minutes. The resulting dispersions were observed visually as well as under light microscope at 40X and 100X magnifications and images were acquired (Figure 1)).



B13 (a)



B13 (b)

Figure1: Optical micrographs of various formulations (a) 40X magnification (b) 100X magnification

Particle size distribution
Particle size distribution is an important factor for drug release in such formulations. It has been shown that the drug release is more uniform when the particle size distribution is uniform [26]. The selected formulations equivalent to their unit dose were dispersed by gentle stirring in 900 ml water and kept aside. The particle size distribution of the *In Vitro* formed biosomes was determined by zeta sizer after 5 minutes and after 120 minutes.

Estimation of drug content
For estimation of total drug content, a known accurate weight equivalent to 10 mg of the drug was transferred to a volumetric flask of 100 ml capacity. The formulation was lysed with 50 ml ethanol by sonication for 15 minutes and the volume was made up upto the mark with the same solvent. The resulting solution was suitably diluted with phosphate buffer pH 7.4 and analyzed by the appropriate method as per our previously developed method [27].

***In vitro* release studies**
The primary objective of the formulation design was to release the drug with enhanced dissolution profile. The esophageal transit time is 5-15 seconds depending on posture and swallowing difficulties. Mean gastric residence time is dependent on the size of the formulation or whether or not the formulations are taken with meal. Multiparticulate dosage forms empty more slowly. Also the gastric emptying depends on the calorific value of food and dosage form. More the calorific value, slower will be the gastric emptying [28]. Our formulation will be in the particulate form after entering into the aqueous medium. So gastric emptying will be delayed from normal emptying by about 1-1.5 hours emptying in about 3-3.5 hours. The small intestinal transit time is 3.5-4.5 hours.

The drug, glibenclamide, is reported to be absorbed from the small intestine [29]. So the release of this drug must be completed within a total of 6.5-8 hours (a total of 5-15 sesonds, 3-3.5 hours and 3.5-4.5 hours for esophageal, gastric and small intestine transit time respectively). Further, it was found that the developed formulations do not release the drugs in the gastric environment, so the release should be completed within 3.5-4.5 hours.

Phosphate buffer pH 7.5 (900 ml), as recommended by USP dissolution media database for immediate release delivery systems of glibenclamide was used [30].

The *In Vitro* dissolution studies were carried out in paddle type USP dissolution rate test apparatus (Type II) at 50 rpm and 37±1 °C. 5 mg GLIB or an accurate weight of the formulation equivalent to 5 mg GLIB was transferred to the dissolution vessel containing 900 ml dissolution medium and the dissolution was carried out. The samples were withdrawn from the dissolution vessel at regular time intervals and analyzed as per the method described by our previously described method [27] as the effect of turbidity, present in the dissolution samples, was completely eliminated through this method and there was no need to separate the turbidity before analysis.

RESULTS AND DISCUSSION

Appearance
The optical micrographs of the biosomes formed from the formulation B13, when introduced into the aqueous medium, are shown in Figure 1.

It was observed that the biosome forming matrices formed the biosomes spontaneously when introduced into the aqueous medium. It was clear from the optical micrographs of the formulations that clear, distinct particles were formed from all the prepared batches i.e. B13, B14 and B15.

Particle size distribution
It was observed that on keeping the amount of drug and other factors constant, the particle size was increased with increase in amount of stearyl alcohol. However particle size distribution was uniform with all soya lecithin: stearyl alcohol ratios used i.e. 1:1, 1:1.5 and 1:2.

Increase in particle size was observed with time when particle size was compared at 5 minutes and 120 minutes, however, no effect of time was observed on particle size distribution. The results of particle size distribution are given in Table 2.

Drug content
The total glibenclamide content found in the various prepared formulations is given in Table 2. It was observed that the drug content in the prepared formulations was found in the range between 96.47%±0.70%-98.80±0.72% which is in the specified limits of 85%-115%.

Table 2. Drug content and particle size distribution of biosomes

Formulation code	% drug content	Particle size [Diameter in nm (% Distribution)] when in contact with aqueous medium after	
		5 Minutes	120 Minutes
B13	98.80±0.72	52.95 (100)	55.83 (89.5) and 76.86 (10.5)
B14	97.00±0.60	89.5 (79.6) and 97.64 (20.4)	99.7 (58.6) and 88.3 (41.4)
B15	96.47±0.70	123.87 (96.8) and 96.24 (3.2)	127.3 (100)

***In vitro* release studies**

The *In Vitro* release patterns of the formulations and the pure drug are compared in the Figure 2.

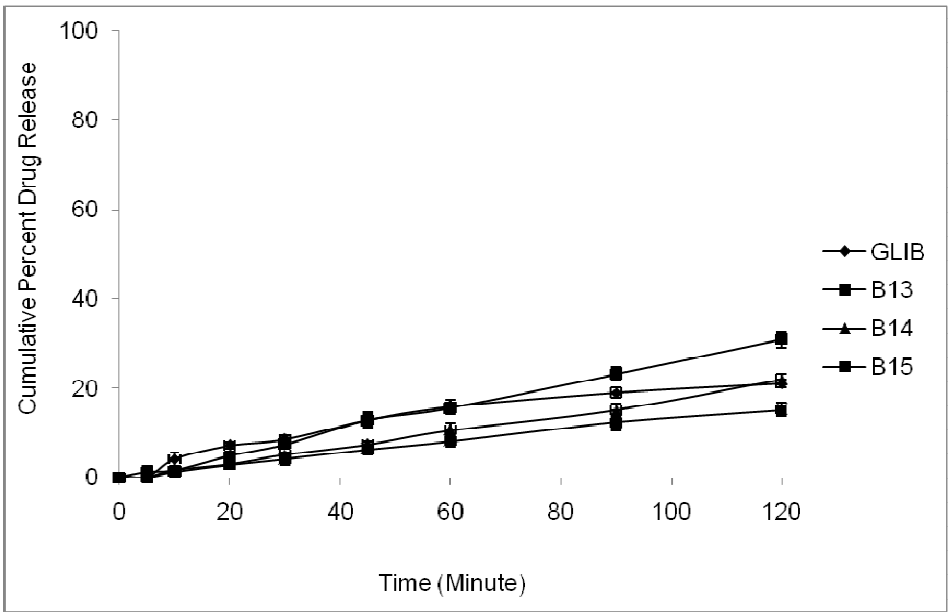


Figure 2: *In vitro* dissolution profile of plain glibenclamide and from the prepared formulations

It had been found that where the pure glibenclamide was released up to 21±0.97% in 120 minutes, its prepared formulations with polar:non polar lipid ratio 1:1, 1:1.5 and 1:2 released only up to 30.87±1.76%, 21.93±1.20% and 15.16±1.45% respectively in two hours (Figure 3).

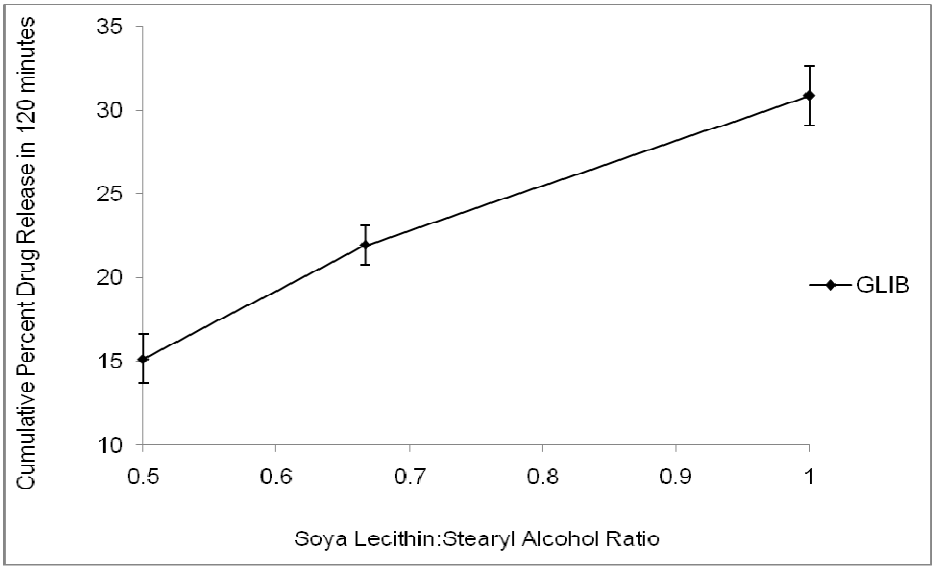


Figure 3: Effect of soya lecithin:stearyl alcohol ratio on *In Vitro* release of glibenclamide

Where 21±0.97% glibenclamide was released from the plain drug in 120 minutes, 30.87±1.76% glibenclamide release during the same period from B13 indicated that the dissolution of the drug was improved with biosome forming matrices of soya lecithin and stearyl alcohol in 1:1 ratio. A further increase in the stearyl alcohol content by

1.5 times of soya lecithin (Formulation B14) leads to decrease in the release i.e. up to 21.93±1.20% in 120 minutes. The release in 120 minutes was even lower than that from plain drug (21±0.97%) when stearyl alcohol content was further increased by 2 times that of soya lecithin in B15 (15.16±1.45%). This is probably due to too increase of non polar content in the formulation to dissolve in the aqueous medium.

CONCLUSION

The biosome forming matrices were successfully prepared from soya lecitin and stearyl alcohol as polar and non polar lipid component respectively in the ratio 1:1, 1:1.5 and 1:2. The biosomes of uniform size, irrespective of the soya lecithin:stearyl alcohol ratio used, were formed spontaneously from the formulations when they were introduced to aqueous medium. The *In Vitro* release was found to be dependent on soya lecithin:stearyl alcohol ratio. On increasing the stearyl alcohol i.e. non polar content in the formulation, the release was reduced and even lesser than that from plain drug on further increasing the stearyl alcohol content.

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