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FORMULATION AND EVALUATION OF MALTODEXTRIN BASED PRONIOSOMES LOADED WITH INDOMETHACIN

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ABSTRACT : This report describes the preparation of indomethacin loaded maltodextrin based proniosome by slurry method with different surfactant to cholesterol ratio. Preparation of proniosome was optimized for highest percentage drug entrapment. Microscopy confirms that all particles are uniform in size and shape. The entrapment efficiency was determined by separating the untrapped drug using dialysis. The *in vitro* release studies of drug from niosomes exhibited a prolonged release as studied over a period of 24 h. The positive values of zeta potential indicated that the indomethacin niosomes were stabilized by electrostatic repulsion forces. In the stability study it was observed that the drug leakage from the vesicles was least at 4° followed by 25° and 37°. On the basis of *in vitro* characterization, the niosome showing maximum entrapment and suitable release rate were selected for *in vivo* performance evaluation. In conclusion, the niosomal formulation could be a promising delivery system for indomethacin with improved bioavailability and prolonged drug release profile.
KEYWORDS: indomethacin, proniosome, maltodextrin, span-60

INTRODUCTION

Drug delivery system using colloidal particulate carrier such as liposomes[1] or niosomes[2] have 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 slowing drug release rate may reduce the toxicity of drug so these carriers play an increasingly important role in drug delivery. Niosome, the surfactant vesicles, are spherical lipid bilayers capable of entrapping water soluble solutes within an aqueous domain or alternatively lipid molecules within the lipid bilayers. Niosomes have been prepared from several classes of non-ionic surfactants. Niosomes may be unilamellar or multilamellar depending on the method used to prepare them. In recent year, niosome have been extensively studied for their potential to serve as carrier for delivery of drugs, antigen, hormone and other bioactive agents. The basic aim in developing delivery system is controlling the release of drugs 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.

Niosomes similar to liposomes are biodegradable, biocompatible and non immunogenic in nature and exhibit flexibility in their structural characterization[3]. In addition, handling and storage of noisome require no special conditions[4-5]. Niosomes are now widely studied as an alternative to liposome because they alleviate the disadvantages associated with liposome such as chemical instability, variable purity of phospholipids and high cost[6]. However, even though niosomes exhibit good chemical stability during storage, there may be problems of physical instability in niosome dispersions. Like liposomes, aqueous suspensions of niosomes may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion. A 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). The additional convenience of the transportation, distribution, storage, and dosing would make 'dry niosomes' a promising industrial product. In the work reported here, we describe the preparation of dry niosomes, 'proniosomes'. Proniosomes are dry product which could be hydrated immediately before use[7-8]. These dry formulations of surfactant-coated carrier can be measured out as needed and rehydrated by brief agitation in hot water. They are water-soluble carrier particles that are coated with surfactant and can be hydrated to form 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[9]. 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[10].

Indomethacin is considered to be the first-line drug in the symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The successful treatment of arthritis depends on the maintenance of effective drug concentration level in the body, for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow release rate over an extended period of time to achieve this objective. The short biological half-life (about 4 h) and dosing frequency more than once a day make indomethacin an ideal candidate for sustained release[11-12]. The objective of the present study was to prepare proniosomes of indomethacin in order to sustained release in upper GIT, which may decreases the side effect of GI disturbance by maintaining the concentration of the drug in the blood and decrease the renal excretion as well as frequency of dosing.

MATERIAL AND METHOD

Indomethacin was obtained as a gift sample from Micro Labs Limited, Hosur, 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

Proniosome were prepared by the slurry method[13]. For ease of preparation, a 250µmol stock solution of span-60 and cholesterol was prepared in chloroform:methanol (2:1) solution. The required volume of span-60, cholesterol stock solution and drug was added to a 100ml round bottom flask containing the maltodextrin carrier. Additional chloroform: methanol solution added to form slurry in the case of lower surfactant loading. The flask was attached to a rotary flash evaporator to evaporate solvent at 60 to 70 rpm, a temperature of 45 ± 2º, 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 dessicator 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.

Preparation of niosomes from proniosomes

Proniosomes were transformed to noisome by hydrating with phosphate buffer saline pH 6.8 at 80° and by gentle mixing. The niosomes were sonicated twice for 30 sec using sonicator and than evaluated for further studies.

Measurement of angle of repose

The angle of repose of dry proniosome powder was measured by a funnel method[14]. Briefly, the pure maltodextrin or proniosome powder was poured into a funnel which was fixed at a position so that the 13 mm outlet orifice of the funnel is 10 cm above a level black surface. The powder flowed 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.

Morphology and vesicle size determination

The surface morphology and shape was studied by scanning electron microscopy (SEM). Particle size determination was carried out using optical microscopy with a calibrated eyepiece micrometer. The prepared vesicles were studied under 400 x magnifications to observe the formation of vesicles.

Entrapment efficiency

Niosomes entrapped indomethacin was estimated by dialysis method. The calculated amount of prepared niosomes were placed in the dialysis bag (presoaked for 24 h). Free indomethacin was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 6.8. The dialysis of free indomethacin always completed after 12-15 changes, when no indomethacin was detectable in the recipient solution. The dialyzed indomethacin was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 318 nm. The sample from the bulk of solution diluted appropriately before going for absorbance measurement. The free indomethacin in the bulk of the solution gives us the total amount of untrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

Percent Entrapment = (Total drug-Diffused drug / Total drug) × 100

***In vitro* release study**

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. Indomethacin niosomal suspension equivalent to 10 mg 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°. 5 ml sample were withdrawn periodically and were replaced by fresh buffer. After 2 h, 25 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 6.8, and the test was continued for a further 22 h. The withdrawn samples were appropriately diluted and analyzed for drug content using UV spectrophotometer at 318 nm keeping phosphate buffer pH 6.8 as blank. All the determination were made in triplicate.

Drug release kinetic data analysis

In order to understand the kinetic and mechanism of drug release, the result of *in-vitro* drug release study of niosomes were fitted with various kinetic equation like zero order[15] (cumulative % release vs. time), first order[16] (log % drug remaining vs time), Higuchi's model[17] (cumulative % drug release vs. square root of time). r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

Stability

The formulated proniosome was tested for its stability. All formulation divided in to three sets and were stored at 4° ± 2°, 25° ± 2°/ 60 % RH ± 5 % RH and 37° ± 2°/ 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.

Zeta potential analysis:

Zeta potential was analyzed to measure the stability of niosome by studying its colloidal property. The zeta potential value of the sample was measured by a zeta potential probe model DT-300. A niosomes which gives best results F_4 between entrapment efficiency and drug release was determined for zeta potential.

***In vivo* anti inflammatory study**

The *in vivo* release behaviour of the best formulation F_4 was studied by measuring anti–inflammatory activity in adult male wistar rats using cotton pellet granuloma method[18]. The study was approved by institutional animal ethical committee (NCP/IAEC/PG/11/2008-2009). The male wistar rats were divided in to three groups, each group consisting of 6 animals. One group served as control, second group served as standard, received 10 mg/kg of indomethacin as solution in water in two divided dose orally. While third group received niosome containing indomethacin equivalent to same dose once daily during the experiment. The rats with an average weight of 250 g were anaesthetized with ether. The back skin was shaved and disinfected with 70% ethanol. The cotton pellets each weighing 20±1 mg were prepared and sterilized in hot air oven at 120° for 3 h. Then 4 sterilized cotton pellets one each in to both the axillae and groin region were subcutaneously implanted under aseptic conditions. The animals were treated for 7 days. All animals had free access to drinking water and food *ad libitum*. The cotton pellets were removed after the period of treatment, under ether anesthesia and the wound was closed by suturing. These pellets were dried in hot air oven over night at 70° and dry weight was determined. The weight of granuloma was determined by calculating the difference. Data was analysed by unpaired student's t-test.

Result and discussion

Proniosome containing indomethacin was prepared using non-ionic surfactant span 60 and cholesterol in different proportions slurry method. Preparation of proniosome was optimized for highest percentage drug entrapment. The angle of repose of dry proniosome powder is smaller than that of pure maltodextrin. If the proportion of maltodextrin to surfactants in the formulation is increased, the angle of repose of dry proniosome powder increases slightly, more closely approaching the angle measured for pure maltodextrin. In FT-IR study, the characteristic peaks due to pure indomethacin have appeared in proniosome formulation, without any markable change in their position after successful encapsulation, indicating no chemical interaction between drug and carrier. It also confirmed the stability of drug during formulation (fig.1).

The prepared vesicles were studied under 400x 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 (fig.2). The diameter of the formulation niosome was found to be in the range of 10-15 μm.

The entrapment efficiency was determined by separating the untrapped drug using dialysis Formulation F_4 showed highest entrapment efficiency of 81.28±4.38% w/w. The niosomal formulation having low cholesterol content was found to cause low entrapment efficiency, 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 increase with increasing

cholesterol content and by the usage of span 60 which has higher transition temperature. It was also observed that very high cholesterol content had a lowering effect on drug entrapment to the vesicles. 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 release study was conducted for all the formulations as shown in the fig. 3. 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 h. 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 multimellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment. The best formulation F_4, was found to give a cumulative release of 98.51% 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 best formulation F_4 as the plots shows highest linearity. The correlation coefficient (r^2) was found 0.99 for F_4.

The formulation F_4 was subjected to zeta potential analysis had a zeta value of +32mv. It is a measure of net charge of niosomes. This higher charge on the surface of vesicle produce a repulsive force between the vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension. From this it can be concluded that formulation F_4 would provide much stable niosomal suspension.

Stability studies of all prepared niosomes were performed by storing 4°, 25° and 37° for a period of 3 month. 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° followed by 25° and 37°. 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°

The release behaviour of the best formulation F_4 *in vivo* was studied by measuring anti-inflammatory activity in adult male wistar rats using cotton pellet granuloma method, in which inflammation and granuloma developed during a period of 7 days. Formulation F_4 showed 41.8% decrease in the weight of granuloma, whereas, the standard showed a decrease of 28.86% compare to control. This indicates that the prepared formulation exhibited a better efficacy than the standard preparation. It can be considered as a proof of constant release of the drug from formulation in good correlation with the *in vitro* release pattern. When data was analyzed by unpaired student's t-test, the statistical analysis showed significant difference ($P<0.001$) compare to control (Table2).

TABLE 1: Formulation and physicochemical characterization of proniosome batches of indomethacin

Formulation Code	Ratio(µmol) (surfactant: cholesterol)	Surfactant (mgs)	Cholesterol (mgs)	Carrier (mgs)	Entrapment efficiency* %(w/w)	Mean particle diameter*
F_1	210:40	90.43	15.46	210	56.19± 1.46	12.34 ± 2.31
F_2	190:60	81.81	23.19	190	64.59± 3.81	13.23 ± 3.21
F_3	170:80	73.20	30.93	170	77.33± 2.31	15.34 ± 3.32
F_4	150:100	64.59	38.66	150	81.28± 4.38	15.65 ± 2.45
F_5	130:120	55.98	46.39	130	75.13± 2.78	14.32 ± 2.68
F_6	110:140	47.36	54.13	110	66.95± 1.87	13.78 ± 1.67
F_7	90:160	38.75	61.86	90	53.94± 4.89	12.73 ± 2.45

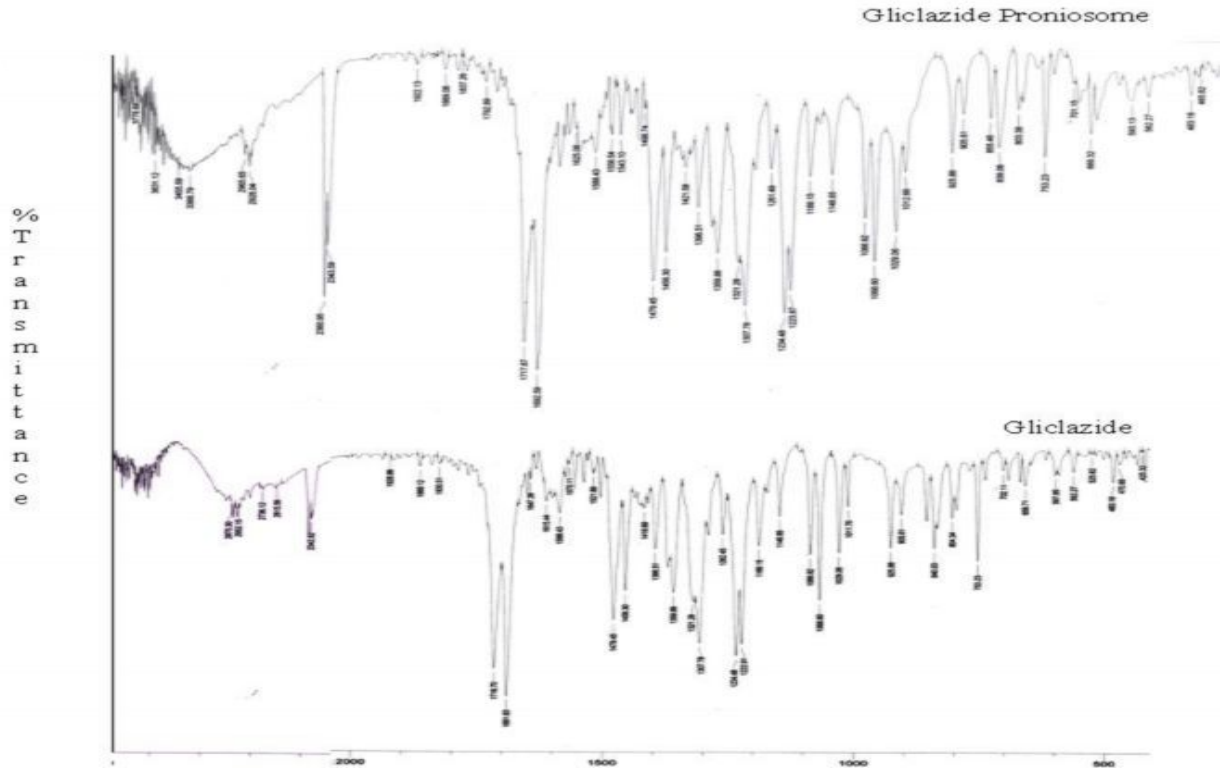
1 g of Carrier per 1 m mole of surfactant
Drug content used 25 mg per batch
* Average of three preparation ± S.D.

TABLE 2: Result of anti-inflammatory activity measurement

Treatment	Dose mg/kg	Weight of Cotton Pellet (mg)		Weight of granuloma (mg)	Percentage decrease in granuloma (%)
		Before	After		
Control	-	20.4±0.0235	63.5±0.734	43.3 ± 0.264	-
Standard	10	20.2±0.232	51.32±0.348	30.8 ±0.754*	28.86
Formulation F_4	Equivalent to 10 mg/kg	20.1±0.021	45.32±0.116	25.2 ±0.075*	41.8

values are mean ± standard error of mean . Number of data points are 24 (6 animals)
*P<0.001 when compared to control.

Fig.1 : FT-IR spectra of pure gliclazide and gliclazide loaded proniosome formulation (F_4)



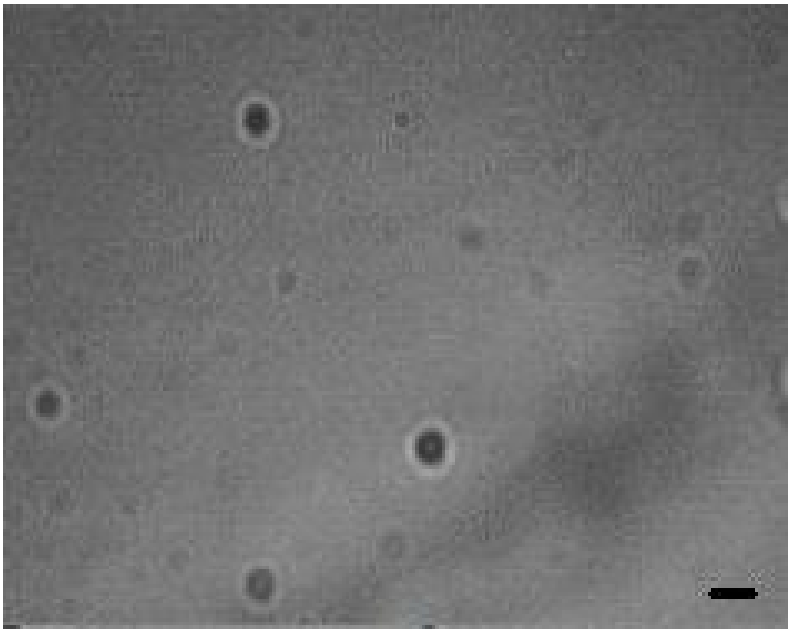


Fig.2 : Scanning electron microphotograph of gliclazide loaded proniosom formulation (F_4). Scale bar indicates 10 µm.

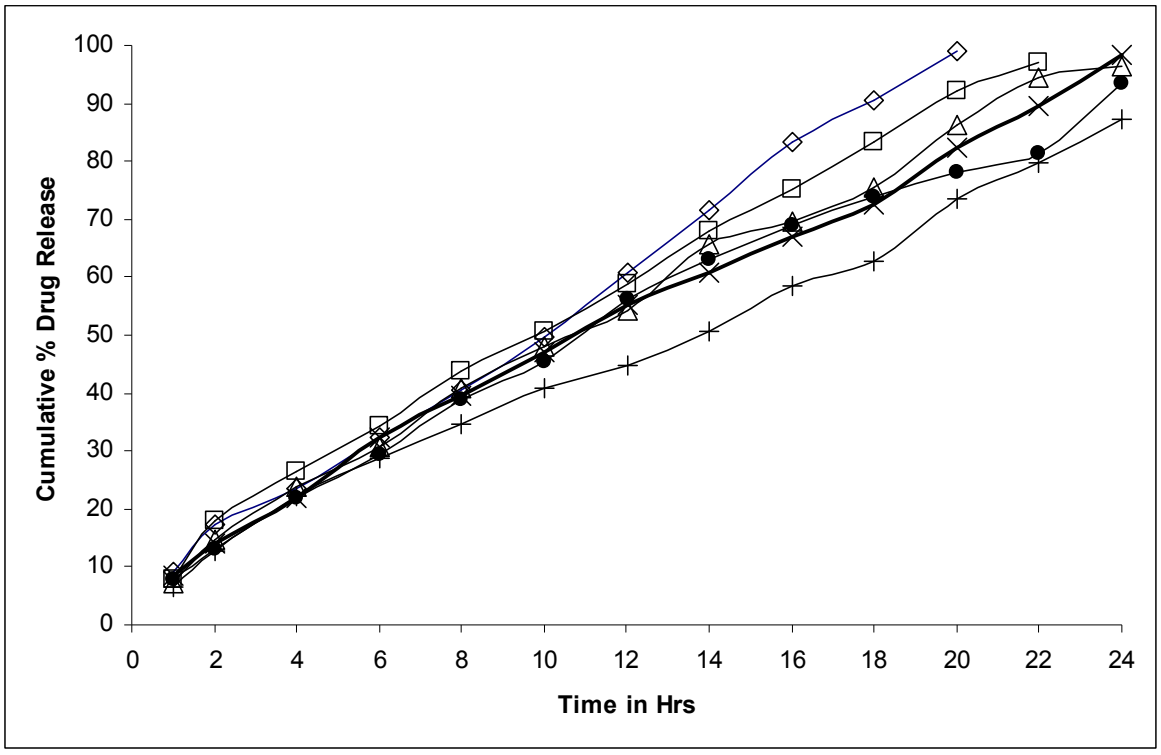


Fig.3 : *In vitro* drug release of indomethacin from niosome formulations F_1 (-◊-), F_2 (-□-), F_3 (-Δ-), F_4 (-×-), F_5 (-×-), F_6 (-✱-), F_7 (- + -)

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