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Research Article

FORMULATION AND EVALUATION OF METHOTREXATE PRONIOSOMAL POWDER

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ABSTRACT

Methotrexate entrapped Proniosomes were prepared by Slurry method using cholesterol, the non-ionic surfactant Span 80 and the carrier maltodextrin. Preparation of proniosome was optimized for highest percentage drug entrapment. Microscopy confirms that all particles are uniform in size and shape. The *in-vitro* release studies of drug from proniosomes exhibited a prolonged release as studied over a period of 24 hrs. In the stability study it was observed that the drug leakage from the vesicles was least at 4°C followed by 37°C. Proniosomes are dry powders, which makes richer processing and packing possible. In developing proniosomes is to devise a method of producing a non-ionic surfactant-based dosage at the point of use to avoid problems of physical and chemical stability found in storage of some surfactant-based dosage forms. Thus, it can be concluded that the encapsulation of Methotrexate Proniosomes are meant for targeted drug delivery thereby reduces the toxicity associated with conventional dosage forms.

Keywords: Methodrexate, Maltodexdrin, Proniosomes, Encapsulation.

INTRODUCTION

Niosomes have gained wide attention by researchers for their use as drug targeting agents, drug carriers to have variety of merits while avoiding demerits associated with the conventional form of drugs[1, 2]. Niosomes were studied as better alternatives to liposomes for entrapping both hydrophilic and hydrophobic drugs[3, 4, 5, 5a, 5b]. The additional merits with niosomes are low toxicity due to non-ionic nature, no requirement of special precautions and conditions for formulation and preparation[6]. However, stability is a prime concern in the development of any formulation and even though, niosomes have shown advantages as drug carriers, such as being low cost and chemically stable as compared to liposomes[7]. Proniosomes are dry formulations of surfactant-coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These "proniosomes" minimize problems of niosome physical stability such as aggregation, fusion and leaking[8,9,10,11] and provide additional convenience in transportation, distribution, storage, dosing, and make proniosomes a versatile delivery system with potential for use with a wide range of active compounds. In general a limited number of studies are available which deal with the preparation and evaluation of proniosomes.[12,13,14,15,16,17] Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal suspension. In release studies, proniosomes appear to be equivalent to conventional niosomes. Size distributions of proniosome derived niosomes are somewhat better than those of conventional niosomes, so the release performance in more critical cases turns out to be superior. Proniosomes are dry powder, which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing, in which the proniosome powder is provided in capsule could be beneficial. Methotrexate is 4-amino-4-deoxy-10-methyl pteroyl-l-glutamic acid. Methotrexate has been used to treat a broad spectrum of malignant and non-malignant diseases. Today this chemotherapeutic agent is commonly used in the treatment of leukemias and lymphomas, as well as of certain solid tumours[18]. MTX, being a water soluble drug, has low dose and high toxicity. Based on this, methotrexate encapsulated proniosomes were formulated and evaluated to easy to handle transport long stability than conventional niosomes.

MATERIALS AND METHODS

Methotrexate was a gift sample from Biochem Pharmaceuticals, Mumbai, India. Cholesterol and Span 80 was procured from Loba chemicals and SD fine chemicals Mumbai, India. Maltodextrin purchased from Himedia, Mumbai, India. All other chemicals used were of analytical grade.

Preparation of proniosomes[19]

The slurry method is selected for the preparation. Maltodextrin powder as carrier is added to a 250-mL round-bottom flask and the entire volume of surfactant solution was added directly to the flask to form slurry. If the surfactant solution volume is less, then additional amount of organic solvent can be added to get slurry. The flask was attached to the rotary evaporator and vacuum was applied until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and kept under vacuum overnight. Proniosome powder was stored in sealed containers at 4°C.

Characterization of Proniosome powder

Angle of repose

The angle of repose of dry proniosome powder was measured by a funnel method [20] 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.

Surface morphology and microscopy

Surface morphology was performed by scanning electron microscopy and photography. The vesicle formation by the particular procedure was confirmed by 300x resolution. The proniosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of proniosome suspension observed for the formation of vesicles. The photomicrograph of the preparation also obtained from the microscope by using SEM.

Table 1: Composition of Proniosomes batches and Entrapment of Different Formulations

Formulation code	Drug (mg)	Span 80 (mg)	Cholesterol (mg)	Diethyl ether (ml)	Maltodextrin (mg)	% Drug entrapment
PRN 1	10	50	50	10	1000	76.1

PRN 2	10	100	50	10	1000	87.3
PRN 3	10	50	100	10	1000	85.7
CON NIO	10	50	50	10	_	76.4

Table 2: Angle of Repose

Formulation code	Angle of repose*
PRN 1	$35.2^0 \pm 1.12$
Maltodextrin	$37.4^0 \pm 0.58$

* Indicates average of three values.

Fig. 1: Proniosome powder PRN 1

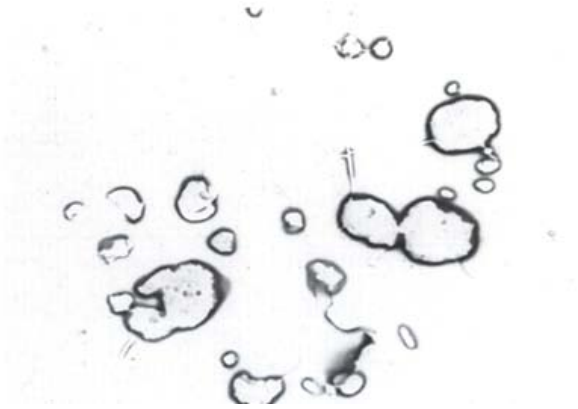

Fig. 2: SEM analysis for Proniosome powder PRN

Stability Studies

Spontaneity[12, 22]

Formulations were undergone for spontaneity study. This indicates the formation of proniosomes after a certain period of time without agitation.

Table 3: Spontaneity studies

Formulation	Spontaneity*
PRN 1	$7.46 \times 10^3 \pm 0.25$
PRN 2	$8.11 \times 10^3 \pm 0.28$
PRN 3	$7.55 \times 10^3 \pm 1.14$

* Indicates average of three values

Drug Leakage studies

The formulations (PRN 1, CON NIO) were kept at 4^0C and room temperature for 30 days. The samples from each formulation were withdrawn, and the residual amount of the drug in vesicles (i.e. entrapment) was determined.

Table 3: Drug leakage studies

Formulation	Stored at	
	4^0C	37^0 C
PRN 1 (powder)	X	----
PRN1 (reconstituted)	X	----
PRN 1 (powder)	----	X
PRN1 (reconstituted)	----	X
CON NIO (1:1)	X	----
CON NIO (1:1)	----	X

Enrapment efficiency[21]

Proniosomes entrapped Methotrexate was estimated by dialysis method. The calculated amount of prepared proniosomes was placed in the dialysis bag (presoaked for 24 hr). Free Methotrexate was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 7.5. The dialysis of free methotrexate always completed after 12-15 changes, when no Methotrexate was detectable in the recipient solution. The dialyzed Methotrexate was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 304 nm. The sample from the bulk of solution diluted appropriately before going for absorbance measurement. The free Methotrexate in the bulk of the solution gives us the total amount of untrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

$$EE = \frac{\text{Amount Entrapped}}{\text{Total amount added}} \text{ X } 100$$

***Invitro* drug release[22, 23]**

In invitro studies, about 2.5ml of proniosomal preparation of Methotrexate was carried out in dialysis bag method. Dialysis bag was placed in a beaker containing 100ml of pH 7.4-phosphate buffer. Magnetic stirrer was used and the temperature was continued at37±1ºc. Samples were collected for every hour up to 24 hrs. Aliquots of 5ml samples were withdrawn; the same quantity of buffer was replaced. The collected samples were analysed at 304 nm keeping phosphate buffer 7.4 as blank.

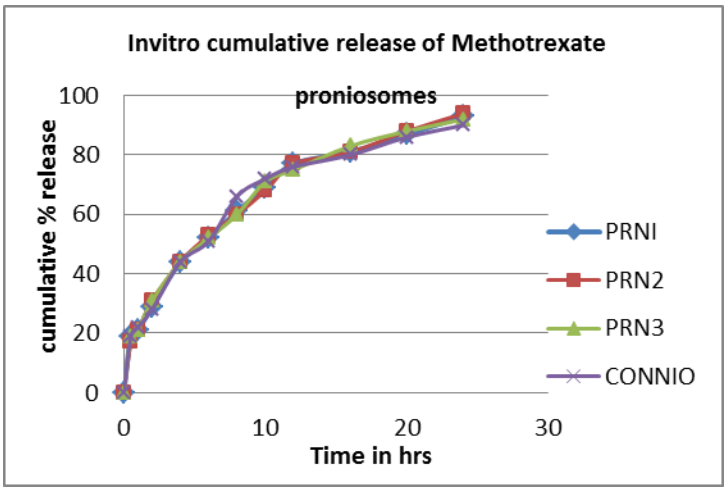

Fig. 3: Mean Dissolution profiles of Methotrexate Encapsulated Proniosomes

RESULTS & DISCUSSION

Proniosome containing Methotrexate was prepared using non-ionic surfactant span 80and cholesterol in different proportions by slurry method. Cholesterol is membrane stabilising agent in the preparation of niosomes. Increasing Span 80 concentration might increase the drug entrapment efficiency.

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.

The prepared vesicles were studied under 300x magnifications to observe the formation of vesicles. The proniosomes were observed as spherical vesicles with smooth surface. The vesicles were discrete and separate with no aggregation or agglomeration (Fig.1 &2)

In the Spontaneity study, the parameter used to compare the formations of niosomes from various prepared proniosome formulations. Its significance arises higher in the stability studies of the proniosomes.

From the leakage study, the PRN powder formulation and reconstituted dispersion, there was no change in drug entrapment when kept at 4^{0}C and 37^{0}C. There was minimal loss of drug from CON NIO stored at 4^{0}C was observed; whereas about 25% of the drug was lost from the niosomal formulations kept at room temperature. From this observation, the leakage of drug may be due to instability of bilayer vesicles at different temperature. The proniosome formulations both in powder and reconstituted form were found to be less leaky and more stable than conventional niosomes.

The entrapment efficiency was 76% for formulation PRN1whereas it was 87% and 85% formulations PRN2 and PRN3, respectively. This explains that the encapsulation efficiency was increased when Span-80 concentration was increased.

The release study was conducted for all the 3 formulations. The formulations were found to have to provide approximately 90% release within a period of 24 hours. From the graph of *in-vitro* drug release studies, Conventional niosomes in the ratio of 1:1 (Chol:Span 80) was taken as a control for comparing *Invitro* drug release with prepared Proniosomal formulations (PRN 1, PRN 2, PRN 3). The release study of proniosomes shows no much difference with conventional niosomes.

To conclude, Methotrexate was successfully encapsulated into Proniosomes by slurry method. The vesicles were quite stable and the drug release was extended up to 1 day, 24h. Proniosomes could be used as a drug carrier for Methotrexate, for producing prolonged activity.

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