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FORMULATION AND EVALUATION OF NIOSOMES ENCAPSULATED METHOTREXATE

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ABSTRACT

The aim of the study was to investigate the feasibility of using niosomes as a drug delivery system for Methotrexate. By entrapment of drug in niosomes, dose also could be reduced. Niosomes were prepared by thin film hydration technique with rotary flash evaporator in the micro molar ratio of cholesterol and surfactant. Particle size, zeta potential, entrapment efficiency and *in vitro* drug release studies were performed. From the results of the present Methotrexate experimental investigation, it may be concluded that formulation MNF10 containing drug with 150:100(surfactant: cholesterol) ratio was showing small vesicles size, high percentage of entrapment with the desired sustained release of Methotrexate.

KEYWORDS

Methotrexate, Thin film hydration technique, Entrapment efficiency, *In vitro* release and Study.

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INTRODUCTON

Niosomes are self-assembled vesicles composed primarily of synthetic surfactants and cholesterol. They are analogous in structure to the more widely studied liposomes formed from biologically derived phospholipids. Niosomes represent an emerging class of novel vesicular systems. Niosomes formation requires the presence of a particular class of amphiphile and aqueous solvent. In recent years a comprehensive research carried over niosomes as a drug carrier. Various drugs are enlisted and tried in niosomes surfactant vesicles. Niosomes proved to be a promising drug carrier and has potential to reduce the side effects of drugs and increased therapeutic

effectiveness in various diseases. Niosomes are lamellar structures that are microscopic in size. They constitute of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The surfactant molecules tend to orient themselves in such a way that the hydrophilic ends of (Span 20) the non-ionic surfactant point outwards, while the hydrophobic ends face each other to form the bilayer. Controlled release drug products are often formulated to permit the establishment and maintenance of any concentration at target site for longer intervals of time. One such technique of drug targeting is niosomes. They behave *in vivo* like liposomes prolonging the circulation of entrapped drug and altering its organ distribution. Niosomal drug delivery has been studied using various methods of administration including intramuscular, intravenous, peroral and transdermal. In addition, as drug delivery vesicles, niosomes have been shown to enhance absorption of some drugs across cell membranes, to localize in targeted organs and tissues and to elude the reticuloendothelial system. Niosomes has been used to encapsulate colchicines, estradiol, tretinoin, dithranol, enoxacin and for application such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccines[7].

MATERIALS AND METHODS

Methotrexate, Cholesterol, Span 20, Span 40, Span 60 were procured from S.D. Fine Chem Ltd, Boisar, Chloroform from Qualigens Chem Ltd, Boisar, Dialysis bag (M.Wt : 12,000-14,000) from Himedia, Mumbai. HPLC water (Lichrosolv) Acetonitrile (HPLC grade), Methanol (HPLC grade) procured from Merck, India. All the chemicals used were analytical grade.

FORMULATION OF METHOTREXATE NIOSOMES

Niosomes were prepared by thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform methanol mixture ratio (2:1v/v) in a 100ml round bottom flask. The weighed quantity of drug, dicetyl phosphate was added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm[1]. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 6ml phosphate buffer saline of pH7.4 at a temperature of 60°C for a period of 2hrs until the formation of niosomes. All the batches were subjected to sonication process for 2 min using probe sonicator. The ratios of the formulations were of cholesterol: surfactant was given in Table No.1.

REMOVAL OF UNENTRAPPED DRUG FROM NIOSOMES

The unentrapped drug from niosomes was removed by dialysis method. Niosomes suspension was placed in 3cm×8cm long dialysis bag whose molecular weight cut off was 12,000. The dialysis bag was then placed in 200ml beaker containing phosphate buffer saline of pH 7.4 with constant stirring by means of a magnetic stirrer. Dialysis was carried out for 24 hour by replacing the buffer with fresh buffer for every 1hour[7].

PERCENTAGE ENCAPSULATION OF DRUG

Vesicles containing Methotrexate were separated from unencapsulated drug by dialysis. Niosomal preparation of 0.5ml was taken after dialysis. To this 0.5ml of 10% triton X-100 was added and incubated for 1 hour. The triton X-100 was added to lyse the vesicles in order to release the encapsulated Methotrexate. Then it was diluted with phosphate buffer saline solution (pH7.4) and filtered through what man filter paper. The filtrate was measured spectrophotometrically at 303nm using phosphate buffer and triton X-100 mixture as blank[7].

$$\text{Percentage drug loading (PDL)} = \frac{\text{Entrapped drug (mg)}}{\text{Total Drug added (mg)}}$$

INVITRO RELEASE STUDY OF METHOTREXATE NIOSOMES BY UV METHOD

Niosomal preparation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 200 ml of diffusion medium (Phosphate buffer saline pH 7.4). The medium was maintained at a temperature of 37±0.5ºC. It was stirred by means of magnetic stirrer at a constant speed.

Sample of 1ml (diffusion medium) was withdrawn at every 1 hour for 24 hours and replaced the diffusion medium. So that the volume of diffusion medium was maintained constant at 200ml[8]. The samples were measured spectrophotometrically at 303nm.

PARTICLE SIZE AND ZETA POTENTIAL

Vesicles properties such as particle size and Zeta potential were determined by laser Diffraction Particle size analyser and Malvern Zeta size analyser[5].

RESULTS AND DISCUSSION

REMOVAL OF UNENTRAPPED DRUG FROM NIOSOMES

The percentage of drug dialyzed and Percentage entrapment efficiency can be shown in the below Table No.2.

ENTRAPMENT EFFICIENCY

After the removal of unentrapped drug by dialysis, the entrapment efficiency of all the formulations was studied. The various factors like surfactant content, cholesterol content and drug ratio will change the entrapment efficiency. The decrease in amount of cholesterol will decrease the entrapment efficiency of Methotrexate.

Effect of cholesterol content

By increasing the cholesterol ratio in the formulations MNF2, the entrapment efficiency increased compared to MNF1. From the data in the Table No.2 it is cleared that increased in cholesterol content resulted in an increase of micro viscosity of the membrane indicating more rigidity of the bilayers. Cholesterol has the ability to cement the leaking space in the bilayer membranes. Further increase of cholesterol content of 150 micromole in Formulation MNF3 reduces the entrapment efficiency. This could be due to the fact that cholesterol beyond a certain level starts disturbing the regular bilayered structure leading to loss of drug entrapment.

Effect of surfactant

The entrapment efficiencies for niosomes prepared using Span60 are superior to those prepared using Span40 and Span 20. This can be justified by many facts that Span 60 has the highest phase transition temperature and Span60 has the longer saturated alkyl chain compared to Span40 and Span20. So it produces niosomess with higher entrapment efficiency. The reason is, the longer alkyl chain influences the HLB value of the surfactant mixture which by its turn directly influences the drug entrapment efficiency. The lower the HLB of the surfactant the higher will be the drug entrapment efficiency and stability as in the case of niosomess prepared using Span 60. In MNF9 the entrapment efficiency was 88 %. But in MNF10 the entrapment efficiency is 89%. By increasing the surfactant ratio as in MNF11 the entrapment efficiency decreased to 85%. Hence the Methotrexate niosomes formulated with Span60 with ratios 100:150 micromole in formulation MNF-10 (Entrapment efficiency 89%) were found to be optimum for loading maximum amount of Methotrexate in niosomal formulations (Given in Table No.3).

***INVITRO* RELEASE STUDY OF METHOTREXATE NIOSOMES**

Results of an *in vitro* study on the release of Methotrexate niosomes prepared using Span40, Span60, Span20 and cholesterol in the micromole ratios. Methotrexate niosomal formulations were tried with different Spans (Span20, Span40 and Span60) while trying with Span20 the release of drug from the bilayer will be very fast compared to other Spans. The release of drug from the bilayer will be very fast because of less attractive force between the drug, cholesterol and Span20. For Span60 percentage amount of drug release for different ratios of (100:100µM, 100:150µM, 100:175µM, Cholesterol: Surfactant) were 88.43%, 91.95%, 90.43% in 24hrs. By increasing the surfactant content in the MNF11 formulations the release is retarded due to critical micellar concentration effect of the surfactant (Span60).

By further inspection of the data in the Table No.4 it is concluded that niosomal formulations prepared using Span60 yielded a sustain and control release of drug compared to corresponding values of Span 40. This can be explained by the fact that niosomess exhibit an alkyl chain length dependent release. The higher the chain length the release will be sustained up to 24hrs

and gives control release in formulations (MNF9, MNF10, and MNF11) (Figure No.1).

PARTICLE SIZE AND ZETA POTENTIAL

Particle size results reveals that the niosomes prepared using Span60 shows size range of 310-420nm, the larger vesicle size is due to the longer alkyl chains. This accounts for higher entrapment efficiencies. The Zeta potential of the formulation showed zeta potential of 20.3mV which confirmed that the particle of the formulation remains stable in suspension. Zeta potential results shows good, the value increased due to the fact that the surface free energy of the Span surfactant increases with increased HLB value (Figure No.2).

Table No.1: The ratios of the formulations were of cholesterol: surfactant

S.No	Batch code	Surfactant used	cholesterol: Surfactant micro molar ratio	Dicetyl phosphate
1	MNF1	Span20	50:100	15mM
2	MNF2	Span20	100:100	15mM
3	MNF3	Span20	150:100	15mM
4	MNF4	Span20	100:150	15mM
5	MNF5	Span20	100:175	15mM
6	MNF6	Span40	100:100	15mM
7	MNF7	Span40	100:150	15mM
8	MNF8	Span40	100:175	15mM
9	MNF9	Span60	100:100	15mM
10	MNF10	Span60	100:150	15mM
11	MNF11	Span60	100:175	15mM

Table No.2: The percentage of drug dialyzed and Percentage entrapment efficiency

S.No	Formulation code	Percentage of drug dialysed (%)	Vesicle size in micrometer(µm)	Percentage entrapmement efficiency (%)
1	MNF1	43	12.89	57
2	MNF2	32	10.69	68
3	MNF3	37	8.96	63
4	MNF4	29	7.84	71
5	MNF5	26	13.12	74
6	MNF6	31	11.82	84
7	MNF7	18	6.14	82
8	MNF8	15	10.21	78
9	MNF9	12	11.82	88
10	**MNF10**	**11**	**5.51**	**89**
11	MNF11	15	9.94	85

Table No.3: Vesicle size and entrapment efficiency of Methotrexate niosomes

S.No	Formulation code	Cholesterol: surfactant ratio (micro molar)	Surfactant used	Vesicle size in micrometer	Total drug concentration entrapped	Percentage entrapped drug
1	MNF1	50:100	Span20	12.89	5.7	57
2	MNF2	100:100	Span20	10.69	6.8	68
3	MNF3	150:100	Span20	8.96	6.3	63
4	MNF4	100:150	Span20	7.84	7.1	71
5	MNF5	100:175	Span20	13.12	7.4	74
6	MNF6	100:100	Span40	11.82	8.4	84
7	MNF7	100:150	Span40	6.14	8.2	82
8	MNF8	100:175	Span40	10.21	7.8	78
9	MNF9	100:100	Span60	11.82	8.8	88
10	MNF10	100:150	Span60	5.51	8.9	89
11	MNF11	100:175	Span60	9.94	8.5	85

Table No.4: *In vitro* Release Study of Methotrexate Niosomes

S.No	Formulation Code	Surfactant used	Cholesterol: surfactant micro molar ratio	Cumulative % amount of drug release
1	MNF1	Span20	50-100	75.32%(8hrs)
2	MNF2	Span20	100-100	78.38%(20hrs)
3	MNF3	Span20	150-100	65.28%(24hrs)
4	MNF4	Span20	100-150	85.41%(16hrs)
5	MNF5	Span20	100-175	83.38%(15hrs)
6	MNF6	Span40	100-100	84.41%(24hrs)
7	MNF7	Span40	100-150	86.42%(19hrs)
8	MNF8	Span40	100-175	91.41%(20hrs)
9	MNF9	Span60	100-100	88.43%(24hrs)
10	MNF10	Span60	100-150	91.25%(24hrs)
11	MNF11	Span60	100-175	90.43%(24hrs)

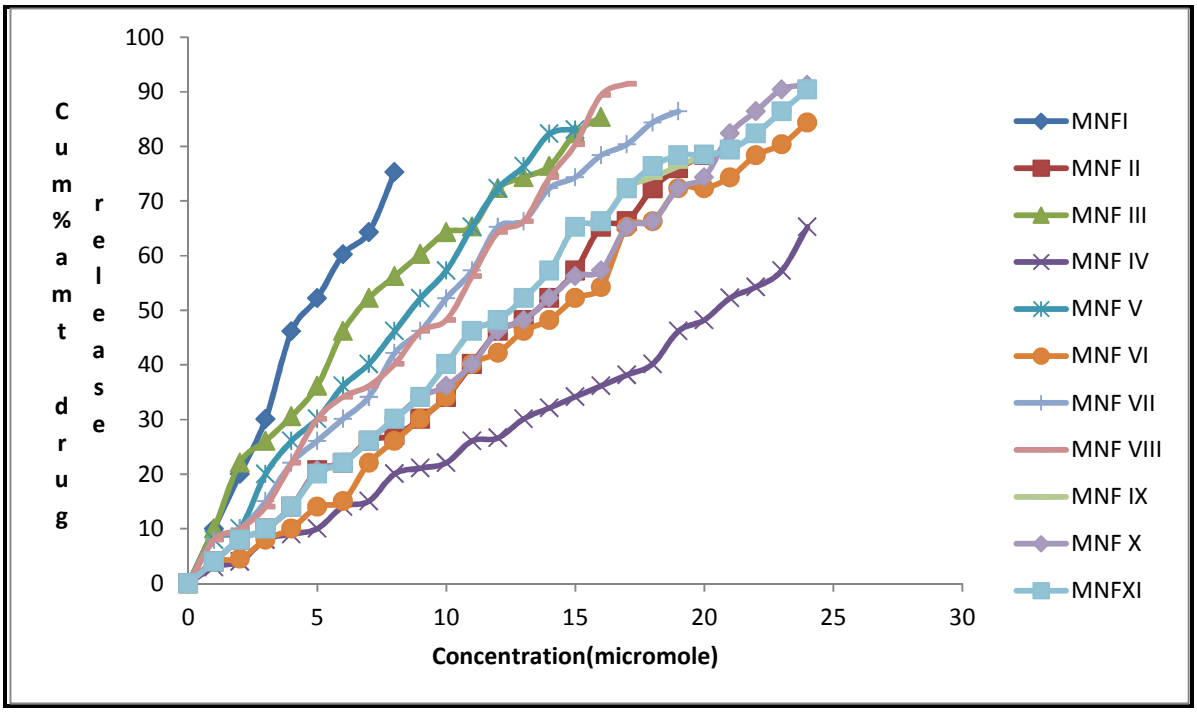

Figure No.1: Comparative *In vitro* Release Study of Methotrexate Niosomal Formulations

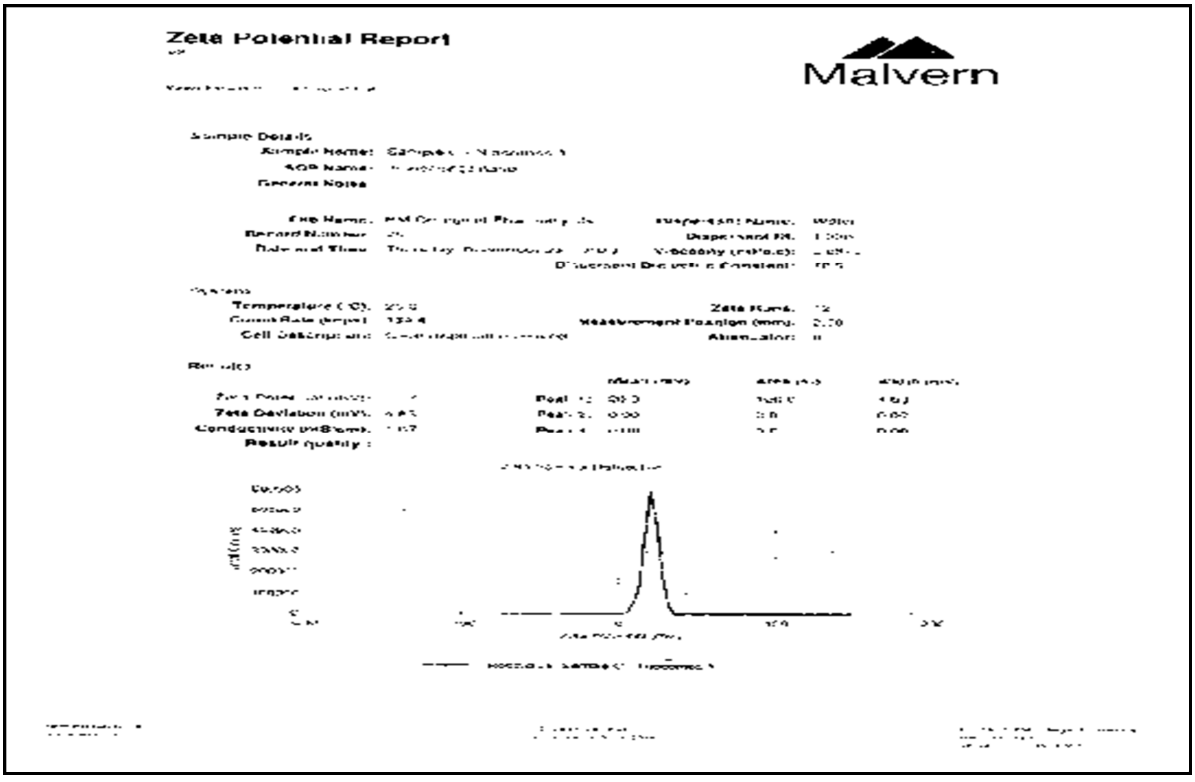

Figure No.2: Zeta Potential Report

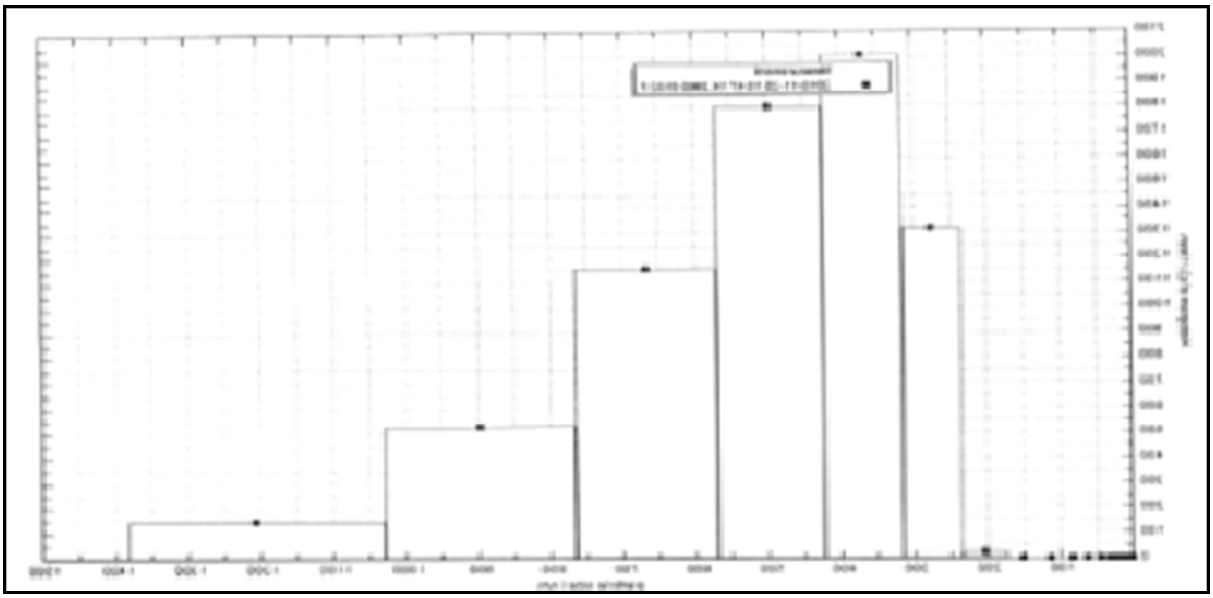

Figure No.3: Zeta potential results

CONCLUSION

In this study we have taken effort to prepare niosomal formulations of Methotrexate, the vesicles formed quite stable. The optimized ratio of 150:100 micromole, (surfactant: cholesterol) with process optimized parameters like speed of rotation 150rpm and 2hours hydration time. From the results of the present experimental investigation, it may be concluded that formulation MNF10 containing drug with 150:100 (surfactant: cholesterol) ratio was showing small vesicles size, high percentage of entrapment with the desired sustained release of Methotrexate. Hence MNF10 formulations were the optimized formulation. *In vitro* release from niosomal formulations showed extended release of drug for 24hours. So, we can conclude that niosomess could be used as drug carriers for Methotrexate, to reduce its renal, hepatic, gastrointestinal toxicity and to sustain the effect of drug release.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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