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INFLUENCE OF BIOENHANCERS ON THE RELEASE PATTERN OF NIOSOMES CONTAINING METHOTREXATE

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ABSTRACT

The aim of present study was to prepare sustained release formulations of niosomes of methotrexate (MTX) alone (N1 to N10) and along with bioenhancers (NB1 to NB9) by thin film hydration technique using span 60 as surfactant, cholesterol as membrane stabilizing agent, curcumin and piperine as bioenhancers and dicetyl phosphate (DCP) as charge inducing agent. All the formulations of niosomes were characterized on the basis of physical appearance and entrapment efficiency. The invitro release studies of optimized formulation of niosomes of MTX alone and along with bioenhancers were performed and compared with pure drug released. The entrapment efficiency of MTX in optimized formulation of niosomes containing MTX along with bioenhancers was found to 56.9% and entrapment efficiency of bioenhancers curcumin and piperine was found to be 40.30% and 69.1% respectively. In vitro drug release of optimized formulations of niosomes of MTX without and with bioenhancers (F3) was found to be 98.89% and 60.97% at the end of 12 h respectively. Results concluded that Niosomes of MTX containing bioenhancers followed sustain release pattern.

KEYWORDS: Methotrexate, Thin Film Hydration Technique, Entrapment efficiency, *Invitro* study.

INTRODUCTION

Cancer still remains as one of the fatal disease in spite of outstanding improvements in molecular biology, genetics, and chemotherapy. Treatment of cancer involves the use of chemotherapy, radiation therapy, and surgery.[1,2,3] As antitumor agents have high potential to induce side - effects and toxicity, localization of the drug to the tumor site would certainly

optimize the therapy. The concept of targeted drug delivery is designed for attempting to concentrate drug in the tissues of interest and thereby reducing the relative concentration of medication in the remaining tissues.[4] Certain carriers like liposomes, niosomes, microsphers, nanopaticles, cellular carriers like erythrocytes and lymphocytes may be used to ferry the drug to the required site. Ideally, such carriers should be targeted the pathological area to provide the maximum therapeutic efficacy. Niosomes have gained increasing importance as a means of targeting of drugs. Niosomes have received attention for their potential as drug delivery vehicles due to advantages like higher flexibility, better bioavailability, increased efficacy, and therapeutic index.[5,6] Bioavailability of drug encapsulated in niosome can be enhanced by encapsulating the drug along with bioenhancers in the niosomal vesicles. The coadministration of bioenhancer like piperine with MTX inhibiting the P-glycoprotein and cytochrome p-450 enzymes enhances the efficacy of drug, makes drug more effective against cancer and transporter inhibitors like curcumin increases the intracellular drug accumulation and restores the chemosensitivity.[7,8] MTX is an antimetabolite and antifolate drug. It acts by inhibiting the metabolism of folic acid. MTX is a standard chemotherapeutic agent which exhibits a dose dependent toxicity. The most common problem encountered with MTX is the development of resistance to tumors. Relatively small increase in drug resistance in cancer cells is thus sufficient to render the drug ineffective. Hence there is a need to improve its acceptability by minimizing the intensity of side effects and thus increasing the therapeutic efficacy of the drug. The aim of the present study was to utilize the principles of niosomal drug delivery systems to formulate a sustained release system for MTX alone and along with bioenhancers (a mixture of piperine and curcumin) by thin film hydration technique such that an increased entrapment with prolong the release of drug from niosomes and also provided better stability to the formulation.

MATERIALS AND METHODS

Materials: MTX was a gift sample from Khandelwal laboratories Pvt, Ltd. (Mumbai, India). Span 60(surfactant) was obtained from Lobachemie Pvt. Ltd. (Mumbai, India). Methanol, hydrochloric acid and chloroform were obtained from Merck India Ltd, (Mumbai, India). Cholesterol and potassium dihydrogen phosphate were obtained from HiMedia Laboratories Pvt, Ltd. (Mumbai, India). Curcumin extract and piperine extracts were obtained from Green Grover's Pvt Ltd. (Bangalore, India). DCP was obtained from Sigma Aldrich Chemicals, (Bangalore, India). Sodium chloride, sodium hydroxide and disodium hydrogen phosphate

were obtained from CDH Laboratory Ltd. (Delhi, India). All chemicals used were of analytical grade.

Methods

1. Preparation of Niosomes of MTX alone and along with Bioenhancers

Multilamellar vesicles of MTX alone and along with bioenhancers were prepared by thin film hydration technique using rotary flash evaporator as described method of Bangham, reported by Juliano and Daoud.[9] Accurately weighed quantity of cholesterol, span 60 and DCP were dissolved in minimum quantity (about 3 ml) of a mixture of chloroform: methanol (2:1) in a 250 ml round bottom flask.[10,11] Round bottom flask was then attached to a rotary evaporator. The organic solvent mixture was evaporated in a rotary flash evaporator under a vacuum of 25 inches of Hg at 60 ± 2ºC and the flask rotated at 100 rpm until a very thin, smooth and dry film of surfactant was formed on the inner surface of the flask, The dry lipid film was slowly hydrated with 5 ml phosphate buffer saline (PBS) of pH 7.4 containing 10 mg MTX drug alone and with 10 ml PBS pH 7.4 containing 10 mg MTX drug and accurate quantity of bioenhancers at a temperature of 60 ± 2ºC for a period of 1h. It formed homogenous suspension of multilamellar vesicles (MLVs). The MLVs suspension was sonicated to form small unilamellar vesicles (SUVs) of niosomes by using probe sonicator. The final niosomal suspension was further hydrated at 4ºC for overnight to stabilize the formulation. The amount of span, cholesterol and bioenhancers to be loaded was selected on the basis of entrapment efficiency of the vesicles. The compositions of different formulation of niosomes are given in Table 1a and 1b.

2. Characterization of Niosomes of MTX alone and along with Bioenhancers

2.1. Entrapment efficiency

For determination of entrapment efficiency, unentrapped drug in the niosomal formulation was seperated using centrifugation at 20,000 rpm for 1 h at 4ºC. The supernatant contains unentrapped MTX was removed and the remaining pellet in the centrifuge tube resuspended in 0.1 N sodium hydroxide (as MTX is highly soluble in 0.1 N NaOH) and vortexed thoroughly for 3 min. After vortexing 1 ml of the suspension was taken in a micropipette and transferred to a test tube. To this added 5 ml of methanol and was further vortexed for 2 min. The absorbance of resulting solution was measured using a shimadzu UV Spectrophotometer (1700) at 292 nm after suitable 10 dilution with methanol.

2.2. *In Vitro* release study of optimized formulation

A volume of 1ml niosomal dispersion (encapsulation efficiency: 56.9%) was put in a dialysis bag (MWCO 12,000 Da, Sigma-Aldrich, USA.). The dialysis bag was suspended in 300 ml phosphate buffer pH 7.4 and maintained at 37±0.2°C. The medium was stirred continuously during the release study. At predetermined time intervals of 15 min, 30 min, 45 min, 60 min, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h,5 ml, aliquots were sampled and replaced with 5 ml fresh phosphate buffer pH 7.4. The concentration of MTX was determined by the UV spectrophotometer (Shimadzu UV 11,12 1700) at 303 nm.

RESULT AND DISCUSSION

Niosomes of MTX without and with bioenhancers such as curcumin and piperine were prepared by thin film hydration method by using span 60 and cholesterolas asurfactant. They are optimized on the basis of observation and max imum percentage drug entrapment (PDE). Entrapment efficiency The optimization of niosomes of MTX without bioenhancers depends on the basis of entrapment efficiency. The entrapment efficiency was varied as concentration of surfactant varied. The amount of span 60 was increased by keeping drug and cholesterol concentration constant. As the amount of span 60 increased, the PDE of drug was also increased upto the formulation N6 (1:15 ratio of drug and span60) and further increasing the amount of span 60 did not change encapsulation efficiency. The higher entrapment of span 60 may be due to their high phase transition temperature and hydrophobic in nature. The amount of cholesterol was increased by keeping drug and surfactant ratio constant. The ratio1:15:2 gave highest encapsulation efficiency due to stabilizing effect of cholesterol. The cholesterol in niosomes greatly affects the membrane properties of the bilayers by reducing the rotational freedom of hydrocarbon chains. Cholesterol also eliminates the gel to liquid phase transition of the vesicle bilayers and induces permanent transition of the gel-state bilayer to an ordered liquid crystalline state. Both these mechanisms make the bilayers more stable leading to increase in entrapment efficiency. Further increase in cholesterol concentration, decreased the fluidity of the bilayers by filling empty spaces among the surfactant molecules and results of the membrane become more rigid and ultimately decreased the encapsulation efficiency (Table 2a). Optimization of niosomes of MTX with bioenhancers was done on the basis of entrapment efficiency. The formulation in which bioenhancers were added (40mg of curcumin and 10mg of piperine) produced a uniform dispersion with lower drug entrapment. Further increase in curcumin concentration (50mg) by keeping piperine concentration constant (10mg) produced a uniform suspension with an acceptable PDE. Further increase in

concentration of piperine (>10mg)reduce the entrapment efficiency. So the formulation NB6 containing curcumin (50mg) and piperine (10 mg) as a bioenhancers was found to be an optimized formulation which gave highest drug entrapment (55.1±0.49%) (Table 2b). In Vitro Release Study: The invitro release study revealed that the release of the drug was sustained on encapsulation in niosomes. The free drug released approximately 98.77% of the drug within 60 min whereas the same percentage of drug release from niosomes of MTX was occurring at the end of 11 h. Release of MTX from niosomes was biphasic with an initial faster release for 3 h followed by a period of slow release. Thus, the study revealed that initially there was a high rate of drug release, which may be due to the release of the adsorbed drug from the lipophilic region of niosomes, which help to achieve the optimum loading dose. The drug diffuses slowly after 3 h due to the presence of cholesterol in the formulation which affects the fluidityby making it more rigid. As the amount cholesterol increased, they filled the pores of vesicular bilayers and abolished the gel-liquid phase transition of the niosomal systems. This confirms that addition of cholesterol acted as a membrane stabilizing agent that decreased the permeability and helped to sustain the release. The maximum release of drug from niosomes containing MTX along with bioenhancers was 60.9% at the end of 12 h. The reason for slower release of the drug from niosomes encapsulated complex may be the interaction of complex with the lipid/surfactant bilayers and bioenhancers. These results indicate that the release of MTX followed a sustain release pattern (Figure: 1).

Table 1a: Composition of different formulation of niosomes of MTX without bioenhancers.

Batch Name	MTX (mg)	Span 60 (mg)	Cholesterol (mg)	DCP (µmol)	Organic Solvent (ml)	Hydration volume (ml)
N1	10	50	10	7	3	5
N2	10	75	10	7	3	5
N3	10	100	10	7	3	5
N4	10	125	10	7	3	5
N5	10	150	10	7	3	5
N6	10	175	10	7	3	5
N7	10	150	20	7	3	5
N8	10	150	30	7	3	5
N9	10	150	40	7	3	5
N10	10	150	50	7	3	5

Table 1b: Composition of different formulation of niosomes of MTX along with bioenhancers.

Batch Name	MTX (mg)	Span 60 (mg)	Cholesterol (mg)	Curcumin (mg)	Piperine (mg)	DCP (µmol)	Organic Solvent (ml)	Hydration volume (ml)
Nb1	10	150	20	5	10	7	3	10
Nb2	10	150	20	10	10	7	3	10
Nb3	10	150	20	20	10	7	3	10
Nb4	10	150	20	30	10	7	3	10
Nb5	10	150	20	40	10	7	3	10
Nb6	10	150	20	50	10	7	3	10
Nb7	10	150	20	50	20	7	3	10
Nb8	10	150	20	50	30	7	3	10
Nb9	10	150	20	50	40	7	3	10

Table 2a: Optimization of niosomes of MTX without bioenhancers.

Batch name	Observation	%Drug entrapped
N1	Flaking	22.17±1.667
N2	Flaking	29.89±1.025
N3	Flaking	38.49±1.351
N4	Flaking	43.35±0.920
N5	Uniform dispersion	50.73±0.714
N6	Non-uniform dispersion	21.35±0.840
N7	Uniform dispersion, without flaking	56.9±1.33
N8	Uniform dispersion, lower PDE	53.85±0.818
N9	Uniform dispersion, lower PDE	51.03±0.512
N10	Uniform dispersion, lower PDE	49.48±0.918

*Data are expressed as Mean±SD. SD = Standard Deviation.

Table 2b: Optimization of niosomes of MTX with bioenhancers.

Batch Name	Observation	%Drug entrapped	%Curcumin entrapped	% Piperine entrapped
NB1	Flaking	18.90±0.21	27.66±0.66	10.05±0.36
NB2	Flaking	23.46±0.39	31.66±0.67	23.58±0.21
NB3	Flaking	25.89±0.44	32.78±0.12	24.30±0.64
NB4	Uniform dispersion	27.67±0.62	35.94±0.51	32.10±0.26
NB5	Uniform dispersion	32.24±0.51	39.01±0.21	39.00±0.82
NB6	Uniform dispersion	**55.1±0.49**	**40.30±0.67**	**64.31±0.96**
NB7	Non-uniform Dispersion	49.89±0.53	33.87±0.54	29.00±0.38
NB8	Non-uniform Dispersion	47.77±0.55	31.33±0.86	27.13±0.27
NB9	Non-uniform Dispersion	46.98±0.34	28.00±0.96	26.18±0.91

Table 2c: *In vitro* release of pure drug methotrexate.

Time(min)	Free methotrexate (percent Drug release)*
10	22.21±0.6
20	39.08±1.9
30	50.39±0.3
40	78.19±1.6
50	98.77±0.9

*Data are expressed as Mean ±SD. SD = Standard Deviation (n=3).

Table 2d: *In vitro* release profile of niosomal formulation of MTX alone and along with Bioenhancers.

Time(min)	Noisome containing MTX*	Noisome containing MTX along with bioenhancers*
0	0	0
1	28.71±1.19	15.49±1.00
2	33.79±1.27	20.57±0.15
3	54.56±0.07	29.46±0.11
4	55.65±0.24	32.63±0.59
5	68.01±0.77	44.14±0.72
6	69.92±0.06	48.62±0.32
7	72.68±0.57	50.67±0.21
8	77.81±1.67	54.68±0.01
9	86.4±0.40	54.71±0.23
10	90.57±0.16	59.7±0.12
11	98.55±0.22	60.37±0.49
12	98.89±0.10	60.97±0.66

*Data are expressed as Mean ±SD. SD = Standard Deviation (n=3).

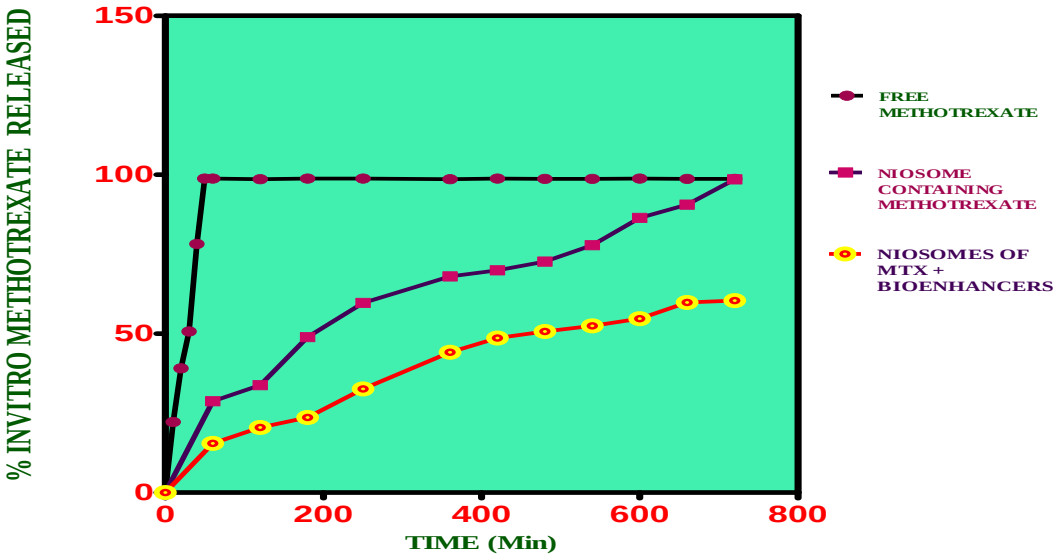

Figure 1: *Invitro* release profile.

CONCLUSION

The use of various pharmaceutical nanocarriers has become one of the most important areas of nanomedicine. Niosomes of methotrexate alone and along with bioenhancers such as curcumin and piperine were prepared by thin film hydration method by using surfactant span 60 and cholesterol that were optimized on the basis of entrapment efficiency. The in vitrostudy revealed that the release pattern of the drug was sustained in niosomes and it was further significantly sustained by addition of bioenhancers. Further *in vivo* and stability studies can be performed to see the pharmacological activity as well as the stability of the formulation because the stability of niosomes is a great issue and a major challenge in commercializing the formulations.

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REFERENCE

1. Aggarwal B B, Kumar A, Bharti A C. Anticancer potential of curcumin: Preclinical and clinical studies. Anticancer Res., 2003; 23(1A): 363 – 98. 2. #
2. Harsh Mohan. “Neoplasia” in textbook of pathology, 2005; P: 142, Jaypee Brothers, New Delhi.
3. Harvey Ladies et al. “Cancer” Molecular cell biology, 2005; P: 1267, Scientific American book, London.
4. M. Reza Mozafari. Nanocarrier Technologies: Frontiers of Nanotherapy, 2006; P: 1-16, Springer, Netherlands.
5. Torchillin VP. Multifunctional nanocarriers. Adv drug del rev., 2006; 58(14): 1533 – 9.
6. Torchillin V P. Targeted pharmaceutical nanocarriers for cancer therapy and imaging. The AAPS Journal., 2007; 9(2): E128 –47.
7. Gottesman M.M. Mechanisms of cancer drug resistance. Annual rev. med., 2002; (53): 615-27.
8. Zhang S, X, Morris M E. Flavanoids are inhibitors of breast cancer References: resistance protein (ABCG2)- Mediated transport. Mole pharmaco, 2004; 65(5): 1208-16.
9. Joshi M, Misra A. Dry powder inhalation of liposomal ketotifen fumarate: Formulation and characterization. Int. J. Pharm., 2001; 223(1-2): 15–27.

10. Singh C H, Jain C P, Kumar B N. Formulation, characterization, stability and in vitro evaluation of nimesulide niosomes. Pharmacophore, 2011; 2(3): 168-85.
11. Karki R, Mamatha G C, Subramanya G, Udupa N. Preparation, characterization and tissue disposition of niosomes containing isoniazid. Rasayan J. Chem., 2008; 1(2): 224-7.
12. Yang Z J, Huang W H, Wong Y.F, Zhao Z Z, Liu L. Development of liposomal salbutamol sulphate dry powder inhaler formulation. Biol. Pharm. Bull., 2010; 33(3): 512-7.