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Formulation and Characterization of Drug Loaded Niosomal Gel for Anti-Inflammatory Activity

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Abstract**—**The main aim of the present research was to encapsulate mefenamic acid in niosomes andincorporate the prepared niosomes in the carbopol gel base for sustained therapeutic action. Mefenamic acid loaded niosomes were prepared by thin film hydration technique and evaluated for entrapment efficiency, vesicular size and zeta potential. The entrapment efficiency of the prepared niosomes was found to increase with decreasing the HLB values of surfactants and vesicle size was found to increase with increasing the cholesterol concentration. Niosomal vesicles with good entrapment efficiencies were incorporated in carbopol gel base to form the niosomal gel. The prepared niosomal gel was evaluated for pH, viscosity, spreadability, extrudability and skin permeation study across the rat skin. The results of permeation study revealed that the gel formulated with span 60 niosomes sustained the drug release for 12h. Further the *in vivo* study showed the good inhibition of inflammation by the gel prepared with span 60 niosomes.

Keywords**—**Mefenamic acid, niosomal gel, nonionic surfactants, sustained release.

I. Introduction

DRUG delivery systems using vesicular carriers such as liposomes, niosomes, bilosomes etc. are recently employed with the distinct advantages over conventional dosage forms because these vesicles act as drug reservoirs and the rate of drug release can be controlled by modification of their compositions [1]. Although liposomes are studied to be an effective vesicular drug delivery system for oral as well as transdermal routes but niosomes are preferred over liposomes as former exhibit higher chemical stability and economy [2]. Another advantage of niosomes is the development of simple practical method for the routine and large scale production without the use of pharmaceutically unaccepted solvents. Niosomes, or the surfactant vesicles, are spherical lipid bilayers capable of entrapping water soluble molecules with in an aqueous domain or alternatively lipid molecules with in lipid bilayers. Niosomes have been prepared from different nonionic surfactants. They may be unilamellar or multilamellar depending upon the method used for their preparation.

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In recent years, niosomes have been extensively studied for the potential to serve as carriers for delivery of drugs, antigens, hormones and other bioactive agents for an extended period of time or to a specific organ of the body [3].

The mefenamic acid is a class of NSAIDs drugs has analgesic and antipyretic properties. It is an inhibitor of cyclooxygenase. But oral administration of Mefenamic acid shows side effect such as chest pain, shortness of breath, weakness, slurring of speech and beside them a very dangerous but rare disease heart attack or stroke.

To overcome above problem associated with oral administration of mefenamic acid, topical drug delivery using surfactant vesicles such as niosomes has been used which provide distinctive advantages over conventional dosage with an increasingly important role in drug delivery, as particles can act as drug containing reservoir and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site [4].

In the present study mefenamic acid loaded niosomal gel was prepared for topical use and evaluated for its *in vitro* and *in vivo* characteristics in an attempt to improve the drug efficacy, improve the patient compliance, to minimize the side effects associated with the of the oral delivery of mefenamic acid and also to extend the drug release.

II. Materials and Methods

A. Materials

Mefenamic acid was supplied as a gift sample from Gnosis Pharmaceutical Pvt. Ltd, Kala Amb (H.P). Cholesterol (CHOL), sorbitan monopalmitate (span 40), sorbitan monostearate (span 60), sorbitan oleate (span 80), chloroform and sodium hydroxide were purchased from Qualikem specialties Pvt. Ltd., Mumbai. Dialysis membrane (35/100 mm flat width/length) was purchased from Himedia, Mumbai. All other chemicals were of analytical grade and procured from the authentic sources.

B. Experimental

1. Formulation of Drug Loaded Niosomes

Mefenamic acid loaded niosomes were formulated by using different nonionic surfactants (span 40, span 60 and span 80) grades in different drug: surfactant: CHOL ratios as 1:1:2, 1:0.75:1, 1:1:1 and 1:2:1 (Table I). Accurately weighted quantities of surfactant and CHOL were dissolved in 10 ml chloroform using a 100 ml round bottom flask. The lipid solution was evaporated by rotary flash evaporator (Perfit India) under reduced pressure at a temperature of 60±2ºC. The flask was rotated at 120 rpm until a smooth and dry lipid film

was obtained. The film was hydrated with 10ml phosphate buffer saline (PBS) of pH 7.4 containing drug for 3 hours at 60±2ºC with gentle shaking. The niosomal suspension was further stabilized by keeping at 2-8ºC for 24 hours [5].

2. Formulation of Niosomal Gel

Based upon the results of formulated drug loaded niosomes, the batches with good entrapment efficiency were selected for further formulation of niosomal gel. The gel containing pure mefenamic was also formulated for comparison of evaluated parameters.

For the formulation of niosomal gel, the gel base was prepared by dispersing 1% w/w carbopol 940 in a mixture of water and glycerol (7:3), the dispersion is then neutralized and made viscous by addition of sufficient amount of triethanolamine [5]-[7].

The measured amount of selected niosomal formulations (MAN03, MAN07 and MAN11) were centrifuged by using cooling centrifuge apparatus for 10min at 3ºC and 8000 rpm. The semisolid mass of niosomes was separated from the supernatant and mixed in the 1% carbopol gel base by using electric homogenizer. The gel containing pure mefenamic acid (MA gel) was also formulated for making the comparison of parameters.

3. Characterization of Drug Loaded Niosomes and Niosomal Gel

i. Evaluation of Drug Loaded Niosomes

The formulated niosomes were evaluated for various parameters.

Entrapment Efficiencies Determination

Entrapment efficiencies of niosomal formulations were carried out in triplicate by centrifugation method. The niosomal suspension was centrifuged at 8000rpm for 10min at 3ºC. Then the solid mass was separated from the supernatant and the suitable dilutions were prepared with PBS (pH 7.4). The drug concentration was assayed by double beam U.V/ visible spectrophotometer method at 285 nm. The percentage of drug entrapment was calculated by the following equation [8].

$$\textbf{Entrapment efficiency (\%)} = [(C_t - C_f)/ C_t] \times 100 \quad (1)$$

where, C_t is the concentration of total drug and C_f is the concentration of unentrapped drug.

Determination of Mean Particle Size and Zeta Potential

Mean particle size and zeta potential of the selected batches of niosomal formulations were measured at 25ºC by photon correlation spectroscopy, using a Malvern particle size analyzer. Light scattering was monitored at 25ºC at a scattering angle of 90º [5].

ii. Evaluation of Topical Gel

The formulated niosomes gels and pure mefenamic acid gel were evaluated and compared for various parameters such as pH measurements, viscosity measurements, spreadability, extrudability, rat skin permeation and *in vivo* studies [3].

pH Measurements

The pH measurements were performed in triplicate by using digital pH meter (Max 962-p). Before measurements pH meter was calibrated and readings were taken by dipping the glass electrode into the gel formulations.

Viscosity Measurements

The viscosity of the gel formulations were measured in triplicate by brookfield viscometer (DV 1 prime). For this 25g of the gel was taken into a beaker and the spindle was dipped into the gel formulation, viscosity of the gel formulation was measured by rotating the spindle at 50rpm.

Spreadability and Extrudability Measurements

The spreadability of niosomal gel formulations was calculated in triplicate by using spreadability apparatus which consisted of two glass slide, the lower slide held the gel sample and upper slide exerted force to the sample on lower slide. 1g of the sample was placed on the lower slide and upper slide was placed on the top of the sample. Force was generated by adding known weight (80g) on the upper plate. The spreadability was calculated by the following equation.

$$\textbf{S} = \textbf{m} \times \textbf{L}/\textbf{t} \quad (2)$$

where S: spreadability, m: mass of the gel formulation, L: length travel by upper slide and t: time.

Extrudability is an empirical test for the measurement of force required to extrude the material from tube containing gel. The method adopted for evaluating gel formulations for extrudability was based upon the total quantity of gel present in aluminum collapsible tube and quantity of gel extruded out after application of weight in g required to extrude at least 0.5cm ribbon of the gel in 10s. The extrudability was calculated by using the following formula [5].

$$\textbf{Extrudability} = \textbf{Applied weight (g)}/ \textbf{Area (cm}^2\textbf{)} \quad (3)$$

Rat Skin Permeation Studies

Skin from male albino rat weighing 180-200g was used in the studies. The experiments were carried out under the approval of the institutional animal ethics committee, Animal ethical protocol no. MMCP/ICE/11/17. The rats were anesthetized, sacrificed and hairs were removed with the help of seizer. Then the skin was separated from the connective tissues with help of blades and was kept frozen at -200ºC [9].

Modified Franz Diffusion (FD) cell was used to study the percutaneous absorption of mefenamic acid. The skin was mounted on the modified FD cell with dermis facing the receptor chamber. Donor side was filled with 1g of the gel and the receptor chamber was filled with 5ml of PBS of pH 7.4. Temperature of cell was maintained 37±0.5ºC. Three mlsamples were withdrawn at the time interval of 1h, 2h, 3h, 4h, 6h, 8h, 10h, and 12h from receiver chamber and replaced with a same amount of PBS of pH 7.4 to maintain a constant

volume. The samples were analyzed by double beam U.V/visible spectrophotometer at 285nm.

In Vivo Studies

In vivo anti-inflammatory activity was evaluated on the basis of inhibition of the volume of the hind paw edema induced by phlogistic agent. For the present study 0.1ml of 1% w/v carrageenan solution was used as phlogistic agent [10].

Selection and Preparation of Animal Groups

White male albino rats of weighing between 180-200g were selected for study. The animals were divided in to three groups, each consisting of six rats. Each group was treated as follows: Group I was treated with plan mefenamic acid gel (gel containing pure drug), Group II, Group III and group IV were treated with formulations MAN03, MAN07 and MAN11 gels. Study was conducted by complete cross over design.

Carrageenan Induced Paw Edema Method

The animals were fasted overnight and all groups were treated by applying 1g of respective gel formulation on the left paw of each rat. The area of application was occluded with bandages and it was left in place for next 45min. The dressing was then removed and the gel remained on the surface was wiped off with cotton. The animals were then injected with 0.1 ml of 1% w/v of carrageenan solution in plantar region of left hind paw and the paw volume was measured after 1hr, 2hr, 4hr, 5hr respectively using water displacement technique. The percent edema produced with test samples were subtracted from percent edema produced in control group to obtain percent edema inhibition by respective groups. Percent inhibition of edema is directly proportional to the anti-inflammatory activity.

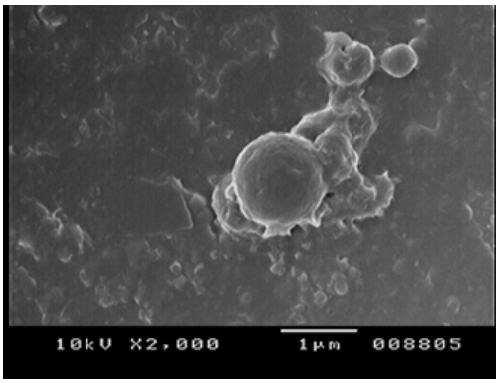

Fig. 1 Scanning electron microscopic (SEM) image of mefenamic acid loaded niosomes

III. Results and Discussion

A. Evaluation of Niosomal Gel

1. Entrapment Efficiency Determination

Entrapment efficiency of mefenamic acid loaded niosomes was determined in triplicate by centrifugation method. Entrapment efficiency of all the batches formulated with different drug: surfactant: CHOL is tabulated in Table I. Vesicle entrapment efficiency mainly dependent on the type of surfactants, amount of surfactant forming the bilayers and intrinsic properties of surfactants like HLB value, chemical structure, lipophilicity, phase transition temperature and alkyl chain length. It was found that surfactants which having low HLB value, higher lipophilicity, higher phase transition temperature and longer alkyl chain length shows higher entrapment. Thus depending upon these properties niosomes prepared with span 40 and span 60 showed higher entrapment efficiency.

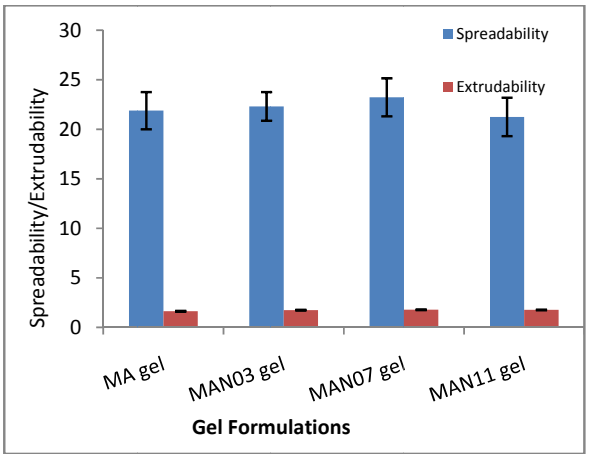

Fig. 2 Spreadability and extrudability plot of gel formulations

The entrapment efficiency of span 80 formulations was less than those of span 60, may be due to reason that spans 60 and 80 have the same head group, but span 80 has an unsaturated alkyl chain. Introduction of a double bond into the paraffin chain causes a marked enhancement in the permeability in liposomes. Values for entrapment efficiency were ranging from 85.06% to 90.74% for all the formulations.

2. Determination of Mean Particle Size and Zeta Potential

Mean particle size and zeta potential of selected batches of mefenamic acid loaded niosomal formulation were measured by photon correlation spectroscopy using Malvern particle size analyzer. The results shown that the niosomes formulated with span 40 have the least particle size, ranging 346.23-365.38 nm. The niosomes formulated with span 60 have the largest particles, ranging 928.83-942.73nm and the niosomes formulated with span 80 were found to have particle ranging 756.93- 772.32nm in size. The particle size of niosomes formulated with span 60 was found largest compared with the niosomes formulated with span 40 and span 80 may be due to the longest saturated alkyl chain of span 60 (Fig. 1).

The zeta potential of all the niosomal formulations were found to be in acceptable range (-13.4 mV to -23.2 mV).

B. Evaluation of Niosomal Gel

All the gel formulations were first examined physically for appearance, homogeneity and texture. The formulations were found to be white in color, smooth and homogeneous. There was no sign of grittiness.

1. pH and Viscosity Measurements

pH of all the gel formulations was measured by digital pH meter and found to be in the range of 6.4 to 6.7, which lies in the normal pH range of the skin. No formulation showed variation more than ± 0.12. The viscosity of all the gel formulations was found to be in desirable range.

2. Spreadability and Extrudability Studies of Gel Formulations

The formulation MAN07 gel has shown the better spreadability and extrudability as compared to the other formulations. The result of the spreadability and extrudability studies of all the gel formulations are given in descending order as MAN07 gel > MAN03 gel > MA gel > MAN11 gel (Fig. 2).

TABLE I
COMPOSITION OF NIOSOMAL FORMULATIONS ALONG WITH THE ENTRAPMENT EFFICIENCY, ZETA POTENTIAL AND PARTICLE SIZE DATA OF NIOSOMAL FORMULATIONS

Sr. no	Formulation code	Surfactant grade	Drug:surfactant: CHOL ratio	Entrapment efficiencies* (%)	Zeta potential* (mV)	Mean particle size* (nm)
1	MAN01	Span40	1:1:2	88.03±2.53	-13.9±0.09	365.38±4.92
2	MAN02	Span40	1:0.75:1	88.35±3.17	-14.5 ±0.13	358.94±3.38
3	MAN03	Span40	1:1:1	89.11±1.78	-14.4 ±0.21	351.64±3.94
4	MAN04	Span40	1:2:1	77.81±2.17	-15.4 ±0.92	346.23±3.98
5	MAN05	Span60	1:1:2	89.95±2.87	-13.2 ±0.46	942.73±4.01
6	MAN06	Span60	1:0.75:1	90.14±3.01	-13.5 ±0.52	932.21±4.11
7	MAN07	Span60	1:1:1	90.74±1.04	-13.7 ±0.72	931.44±3.19
8	MAN08	Span60	1:2:1	86.27±1.92	-13.4 ±0.68	928.83±5.04
9	MAN09	Span80	1:1:2	88.14±2.71	-19.5 ±0.59	772.32±2.89
10	MAN10	Span80	1:0.75:1	88.53±2.81	-21.2 ±0.25	768.04±3.82
11	MAN11	Span80	1:1:1	88.19±2.02	-23.2 ±0.29	763.30±3.39
12	MAN12	Span80	1:2:1	85.06±2.21	-23.1±0.71	756.93±3.87

*The data were reported as an average of 3 measurements (mean ± S.D.)

3. Skin Permeation Studies

The results revealed that MAN 07, gel formulation (prepared with span 60 niosomes) permeated 99.90% drug, followed by MAN 11 gel (prepared with span 80 niosomes) with 94.92% and MAN 03 gel (prepared with span 40 niosomes) with 94.13% for a period of 12 h. MA gel (prepared with pure mefenamic acid) showed 99.10 % drug permeation within a period of only 8h. It is clear from the skin permeation study that the gel containing the niosomal preparations, sustained the drug release for 12h. Further the gel containing span 60 niosomes has shown the maximum drug permeation. Gel prepared with pure mefenamic acid (MA gel) sustained for only 8h (Fig. 3).

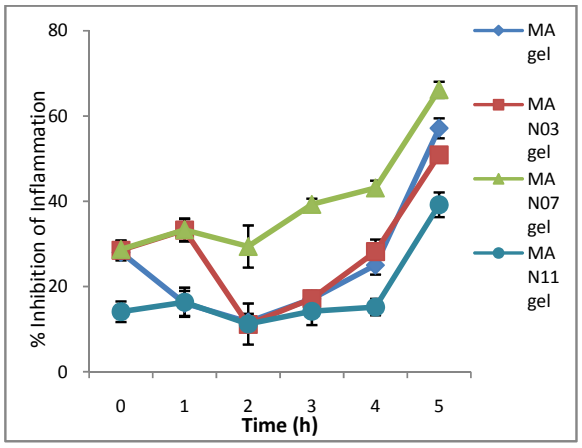

Fig. 3 Rat skin permeation profile of gel formulations

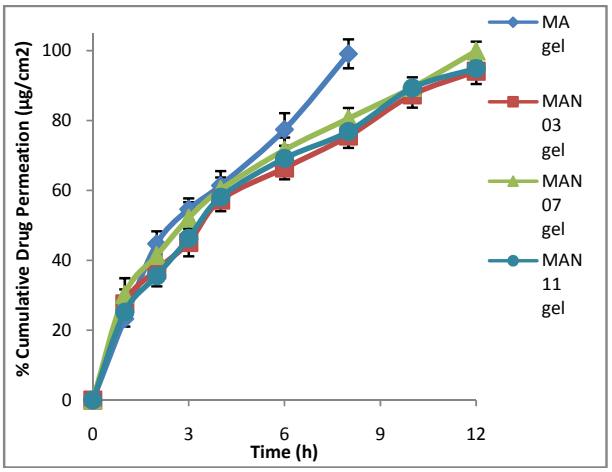

Fig. 4 Comparative values of % inhibition of gel formulations

4. *In vivo* Anti-Inflammatory Studies

The results of *in vivo* studies revealed that gel formulation (MAN07) prepared with span 60 niosomes has shown the maximum inhibition of inflammation followed by MA gel (prepared with pure mefenamic acid), MAN03 (prepared with span 40 niosomes) and MAN11 (prepared with span 80 niosomes) in 5h (Table II). The results of the percent inhibition produced by the gel formulations are given in descending order as MAN07 gel> MA gel> MAN03 gel> MAN11 gel (Fig. 4).

TABLE II
IN VIVO PERCENT INHIBITION OF INFLAMMATION BY GEL FORMULATIONS

Formulation code	Percent inhibition of inflammation*					
	0 h	1 h	2 h	3 h	4 h	5 h
MA gel	28.01±1.21	16.08±2.93	11.71±1.94	17.11±1.89	25.02±2.19	57.13±2.34
MAN03 gel	28.51±2.34	33.25±2.61	11.12±1.45	17.18±1.04	28.20±2.86	50.90±1.79
MAN07 gel	28.71±2.01	33.32±2.67	29.41±4.95	39.28±1.34	43.13±1.72	66.16±1.90
MAN11 gel	14.12±2.42	16.35±3.42	11.23±4.82	14.21±3.21	15.21±1.92	39.20±2.89

*The data were reported as an average of 3 measurements (mean ± S.D.)

IV. CONCLUSION

The mefenamic acid loaded niosomes were successfully prepared by the conventional thin film hydration technique using CHOL and different grades of spans as nonionic surfactants. The presence of CHOL and nonionic surfactants made the niosomes more stable. Niosomes formulated with span 60 have shown the best entrapment efficiency compared with the niosomes prepared with other grades like span 40 and span 80. This may be due to low HLB value, higher lipophilicity, higher phase transition temperature and longer alkyl chain length of span 60. Further the gel formulation prepared with the span 60 niosomes found to have the best skin permeation studies when studied on the albino rat skin. The results of *in vivo* anti-inflammatory study also revealed that the gel formulation having span 60 niosomes showed the best inhibition of inflammation and sustained the drug release for a period of 12 h.

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