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Research Article

DESIGN AND OPTIMIZATION OF NIOSOMES OF FAMOTIDINE

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ABSTRACT

The present study was focused on formulation and evaluation of niosomes of famotidine,with the objective to optimize the prepared niosomal formulations of famotidine on the basis of effect of type of surfactant, effect of Surfactant : Cholesterol ratio, effect of Cholesterol : di cetyl phosphate (DCP) ratio, effect of solvents and effect of Hydration media on entrapment efficiency of prepared drug loaded formulations. The effect of presence of charge inducing agent DCP on entrapment efficiency, vesicle size and size distribution studies was studied along with its effect on polydispersity index. The results indicated that the niosomes prepared with the inclusion of DCP and cholesterol showed better entrapment efficiencies as compared to niosomes that were formulated without DCP. Span 60 containing formulation NMS7 with cholesterol to surfactant ratio 1:1 formulated with DCP elicited highest entrapment efficiency with desired vesicle size range well suited for oral delivery. It was also found that the inclusion of charge inducing agent was useful in reducing the vesicle size and improving the homogeneity and stability of the niosomal formulations. Niosome formulation after proper adjustments of these formulation variables was found helpful to improve famotidine entrapment in niosomal vesicles along with controlling the vesicle size of prepared niosomes. These improvements might prove helpful in developing more effective and efficient drug delivery system.

Keywords: Niosomes, Optimization, Process Variables, Dicetyl Phosphate

1. INTRODUCTION

Nonionic surfactant vesicles also called as niosomes are formed from self-assembly of hydrated synthetic nonionic surfactant monomers which are capable of entrapping a variety of drugs [1]. Nonionic surfactants form unilamellar and multilamellar vesicles that have similar physical properties to liposomes but are relatively inexpensive drug delivery system. In niosomes, aqueous soluble drug molecules are present in the aqueous compartments between the bilayer whereas insoluble ones are entrapped within the bilayer matrix. The use of niosomes for drug delivery have a great potential to alter the biodistribution of drugs to provide a greater degree of targeting of the drug to diseased tissues, to sustained the release of encapsulated drug and to alter its pharmacokinetics [2-4]. Oral route represents the most preferable and predominant route for administration of therapeutic agents due to its easy formulation and economic administration. However, oral administration of drugs often leads to degradation due to the highly acidic gastric environment, enzymes of the mucosa or liver, before they enter the systemic circulation, some drugs may not be absorbed because of their insufficient or poor solubility [5]. Drug delivery system using novel vesicular carrier, such as liposome or niosome, has distinct advantages over microspheres, nanoparticles, and other carriers in terms of better entrapment of drugs, better target site specificity, and handling premature drug release (burst effect) [6]. Famotidine which comes under the H_2 antagonists category is prescribed in conditions like Peptic ulcer, Duodenal ulcer, gastro oesophageal reflux disease (GERD), Zollinger-Ellison Syndrome on a regular basis. Following oral administration, the absorption of famotidine is dose-dependent and incomplete. The oral bioavailability ranges from 40-50%, and the Cmax is reached in 1-4 hours post-dosing. About 25-30% of the drug is eliminated through hepatic metabolism. The elimination half-life is about 2 to 4 hours which necessitates its frequent dosing. The design of niosomal formulation for these drugs shall help to increase the absorbtion and thereby oral bioavailability of these drugs because the liquid formulations are absorbed faster as compared to solid tablet formulations which require dissolution time

prior to its absorption this in turn will also favor the absorption of drug basically from the stomach and upper part of the gastrointestinal tract which is the most suitable area for absorption of drugs like famotidine which exhibit the absorption window phenomenon, thereby increasing the fraction of the dose absorbed which in turn enhances the oral bioavailability of the drug and also to achieve sustained effect of the drug thereby reducing the frequency of dosing and thus improving patient compliance. Thus, to obtain the control release profile of famotidine, it is desirable to encapsulate the drug in the vesicular system like niosomes to prolong the existence of the drug in systemic circulation and perhaps increase its bioavailability.

The purpose of the present research was to evaluate the formulation variables that critically affect the development of niosomes with respect to entrapment efficiency, vesicle size, size distribution, vesicle charge, homogeneity and in vitro release in delivery of famotidine through niosomal carriers. There is little information in the literature on optimizing the different formulation related variables that are important in the formulation of famotidine niosomes for development of an improved drug delivery system. In this research article we attempted to optimize the prepared niosomal formulations of famotidine on basis of entrapment efficiency and vesicle size, size distribution studies. We studied the effect of type of surfactant, effect of Surfactant : Cholesterol ratio ,effect of Cholesterol : di cetyl phosphate (DCP) ratio , effect of solvents and effect of Hydration media on entrapment efficiency of prepared drug loaded niosomal formulations. The effect of presence of charge inducing agent DCP on entrapment efficiency, vesicle size and size distribution studies was studied along with its effect on polydispersity index to investigate the effectiveness and efficiency of the prepared drug delivery system.

2. MATERIAL AND METHODS

Pure drug Famotidine was gift sample from Glenmark Pharmaceutical Ltd. India, surfactants like span 20, span 40, span 60, span 80 and chloroform were obtained from Central drug house, cholesterol was procured from Loba Chemie Pvt Ltd India, di cetyl phosphate (DCP) was procured from Sigma Aldrich and dialysis membrane from Himedia (India). All materials and chemicals were of analytical grade and used as received.

2.1. Preparation of niosomes

In conventional thin film hydration technique as described by shreedevi et al 2016 [7] , weighed quantity of surfactants (table 1) and drug famotidine and cholesterol were dissolved in chloroform / methanol mixture and then transferred into a round bottomed flask. DCP (a negative charge inducer) was also added to the above mixture. The formation of thin film was produced by using rotary evaporator (EYELA, USA) with vertical double helix condenser under vacuum at temperature of 60ºC at 120 revolutions per minute (rpm) until a smooth, dry lipid film was obtained afterwards the thin film was allowed to dry completely by keeping the preparation flask in vacuum desiccator for complete removal of chloroform/ methanol mixture. Then the preparation underwent the hydration process with phosphate buffer solution (PBS) pH7.4 at room temperature for 3 hrs with continuous shaking to produce drug loaded niosomal suspension. The size reduction step was excluded to investigate the effect of method of prepration on noisome characteristics. Further these preparations were optimized on the basis of entrapment efficiency and particle size determinations [8].

Table 1: Formulation table of niosomal formulations of famotidine

Formulation code	Surfactant(mg)	Cholesterol	DCP(mg)	Chloroform/ Methanol (ml)	Drug(mg)
NML*6	50	50	--	4:1	5
NML7	47.5	47.5	5	4:1	5
NMP*6	50	50	--	4:1	5
NMP7	47.5	47.5	5	4:1	5
NMS*6	50	50	--	4:1	5
NMS7	47.5	47.5	5	4:1	5
NMO*6	50	50	--	4:1	5
NMO7	47.5	47.5	5	4:1	5

NML is Span 20, NMP* is span 40, NMS*is span 60, NMO* is span 80 containing formulations*

2.2. Morphological analysis of niosomes using optical microscope

Freshly prepared niosomal suspension of famotidine was observed under light microscope with magnification of 40x using Olympus BH-2 microscope (model BH-2 Olympus) .A small droplet of the vesicle suspension was placed on a glass microscope slide, diluted with a few drops of distilled water and covered with a glass cover slip. The samples were examined for vesicle formation, crystal formation and vesicular size [9].The formation of niosomal vesicles was confirmed by observation under this light optical microscope.

2.3. Scanning Electron Microscopy (SEM)

Surface morphology of niosomal vesicles were determined by scanning electron microscopy (SEM) [10]. Model (Carl ZEISS microscopy Ltd) at central instrument facility at IIT-BHU (Banaras Hindu University). The obtained photomicrographs of formulations prepared with DCP and without DCP are shown in fig.1.

2.4. Transmission electron microscopy.

Transmission electron microscopy (TEM) (Jeol JEM1400 Tokyo Japan) was used to determine the morphology and structure of niosomes [6]. TEM was carried out at Central Drug Research Institute (CDRI, Lucknow). Photomicrographs at suitable magnification were also obtained by negative staining technique shown in fig. 2.

2.5. Optimization on basis of entrapment efficiency

Freshly prepared famotidine loaded niosomal dispersions without DCP and with DCP were diluted with 10% triton X 100. This dissolved the niosomes and produced a clear solution, which was further centrifuged to get clear supernatant. This supernatant was diluted with PBS pH 7.4 and analyzed for drug content using UV spectrophotometer to calculate the amount of entrapped drug in the niosomal vesicles [11]. Percentage entrapment efficiency was calculated using following equation and the results are listed in table 2.

$$Entrapment\ efficiency\ \% = \frac{amount\ of\ entrapped\ drug}{Initial\ amount\ of\ drug\ added} \times 100$$

The effect of different variables (type of span, cholesterol content, inclusion of DCP solvent system used and hydration medium) upon encapsulation efficiency was also studied.

2.5.1. Effect of Type of surfactant on entrapment efficiency of formulations

As formulation number 7 of all the prepared batches showed good entrapment efficiencies these four formulations NML 7, NMP7, NMS7, NMO7 were selected to study the effect of type of surfactant used on the entrapment efficiencies. All variables like concentration of span, concentration of cholesterol, concentration of DCP and amount of drug were kept constant except the type of span used were changed like (Span 20, 40, 60, 80) to study its effect on entrapment efficiencies of prepared niosomal formulations of famotidine (table 3).

2.5.2. Effect of Span 60 : Cholesterol ratio on entrapment efficiency

As formulation containing span 60 *i.e.* NMS7 showed the best entrapment it was selected for further optimization. Here the total concentration of surfactant Mixture (span 60, cholesterol and DCP) was kept constant and the ratio of span 60 to cholesterol was changed to investigate the effect of this ratio on entrapment of Famotidine (table 4).

2.5.3. Effect of Cholesterol : DCP ratio on entrapment efficiency

The concentration of span 60 was kept constant at 47.5 and the molar ratio of cholesterol to DCP was changed over a narrow range (52.5:0, 50.5:2.5, 47.5:5, 42.5:10) as shown in table 5,the concentration of famotidine was kept constant ie 5mg this was basically done to evaluate the effect of cholesterol : DCP ratio on entrapment efficiency.

2.5.4. Effect of solvent system used

To study the effect of solvent system used to prepare the niosomal formulations various solvents were used alone as well as in combination for dissolving the surfactant, cholesterol etc for the preparation of thin film given in table 6. The solvents used were chloroform alone, di ethyl ether alone and combination of chloroform: methanol.

2.5.5. Effect of Hydration media

Only two media ie distilled water and phosphate buffer pH 7.4 were used for the hydration of thin lipid film formed on the surface of round bottomed flask to prepare niosomal suspension. Their effect on entrapment efficiency of formulation was also studied in table 7.

2.6. Optimization on basis of vesicle size and size distribution studies

Further the above prepared niosomal formulations of famotidine were optimized on the basis of vesicle size and size distribution studies that were carried out at central instrument facility at Banaras Hindu University (IIT-BHU). The surface charge of niosomes was obtained by measuring the zeta potential of niosomes [12]. The measurements were performed by using using Beckman coulter delsa^{TM} nano zeta potential and submicron particle size analyzer. Zeta potential, Size, size distribution measurements and polydispersity index measurements were obtained automatically. The vesicle size, polydispersity index and zeta potential values of famotidine loaded niosomal formulations without DCP and with DCP are reported in table 8.

2.7. *In vitro* drug release studies of optimized niosomal formulations of famotidine

On basis of above optimization, four optimized famotidine loaded niosomal formulations namely NML7, NMP7, NMS7and NMO7 were selected for the in vitro release studies using a dialysis bag (Himedia dialysis membrane) as a 'donor compartment'. Measured amount of niosomes containing entrapped famotidine were placed in nesselers cylinder which was fitted with dialysis membrane at its lower end which served as the donor compartment. This was suspended in 500ml of phosphate buffer PBS pH 7.4 which acted as receptor compartment [13]. Samples of receptor medium were withdrawn hourly and replaced with fresh buffer and famotidine absorbance at 266 nm was measured using PBS pH 7.4 as blank. Results were the mean values of three runs given in figure 3.

3. RESULTS AND DISCUSSION

3.1. Visual observation& Optical microscopy

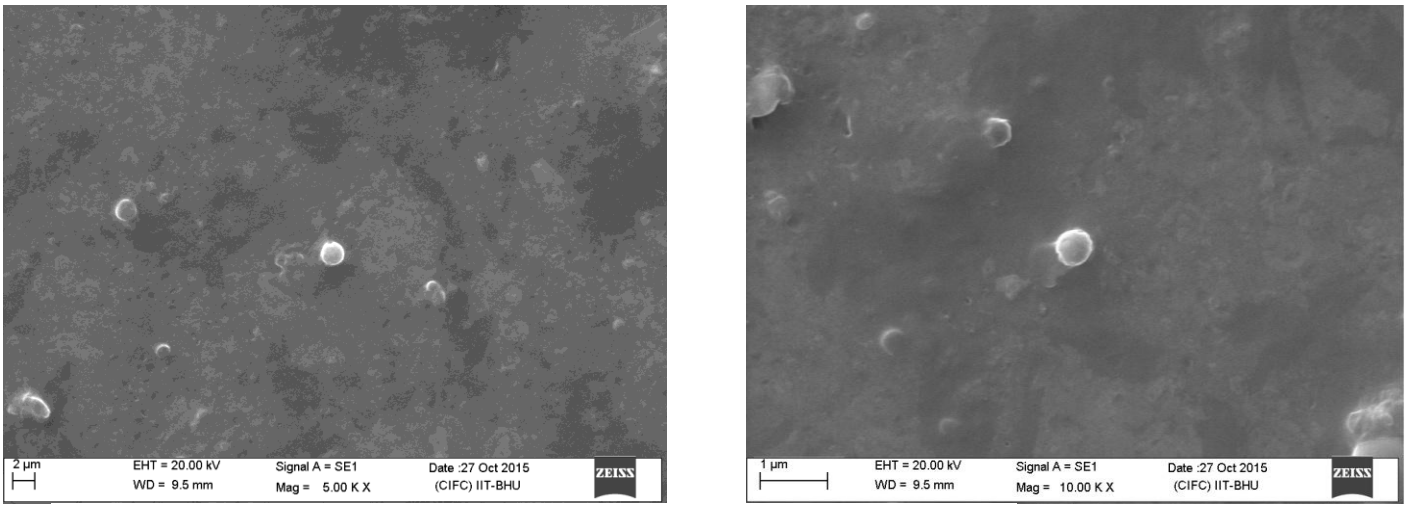
All niosomal formulations except formulations containing DCP appeared as translucent white dispersion form with no sedimentation. While the formulations containing DCP and cholesterol were more turbid and whitish. The preliminary information regarding the shape and morphology of niosomal formulation was determined by optical microscopy which revealed the spherical shape of niosomes and confirmed the formation of niosomes.

3.2. Scanning electron microscopic images

SEM Photomicrographs revealed that niosomes were spherical in shape and discrete. The SEM images of famotidine loaded niosomes prepared without inclusion of DCP(unoptimized) and with inclusion of DCP (optimized) clearly shows that the vesicle size of the formulations without DCP are larger in size range as compared to the formulations prepared with DCP which are in nanometer size range (figure 1).

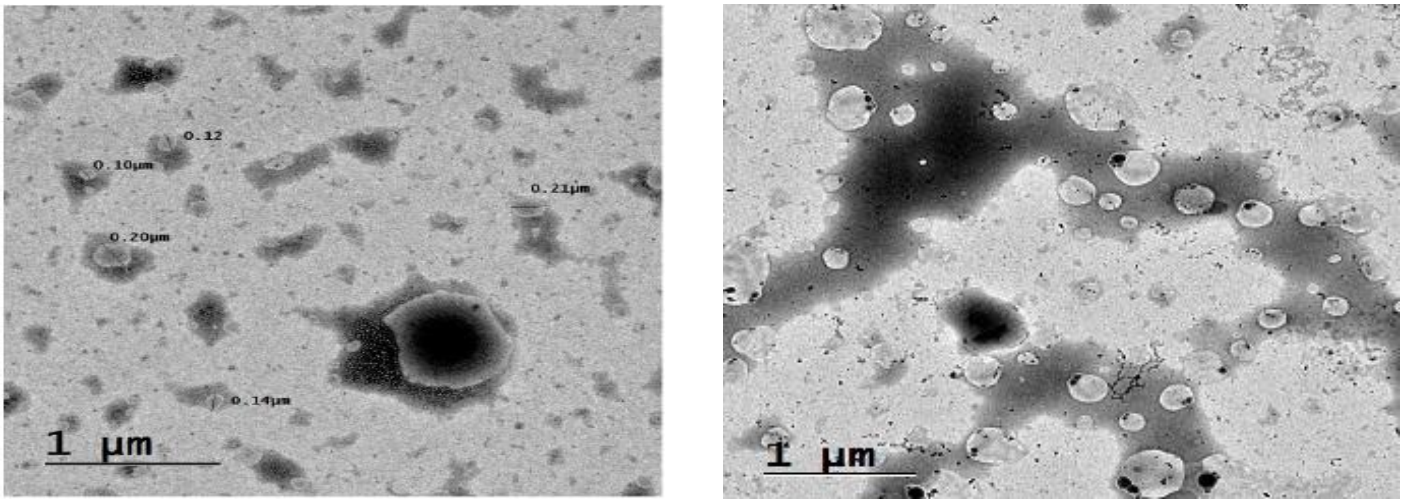
3.3. Transmission electron microscopy (TEM)

The vesicle formulations were examined by transmission electron microscopy (Jeol JEM1400 Tokyo Japan) in order to determine size, shape and lamellarity. The prepared drug loaded niosomes were spherical large unilamellar vesicles. These results were in accordance to Junyaprasert et al 2008 [9]. The TEM photomicroghaphs of formulations prepared with DCP and without DCP are shown in figure 2 which shows that the niosomes prepared without DCP were larger in size as compared to niosomes prepared with DCP.



(a) **(b)**

Fig. 1: (a) SEM images of unoptimized niosomal formulations, (b) SEM images of optimized niosomal formulations



(a) **(b)**

Fig.2: (a) TEM photomicrographs of niosomes prepared with DCP and (b) without DCP

3.4. Optimization based on entrapment efficiency

The entrapment efficiency of formulations prepared with DCP (NML7, NMP7, NMS7, NMO7)were in the range of 65.086±0.938 to 73.234±0.365 that was higher as compared to the entrapment efficiencies of formulations that were prepared without the inclusion of DCP(NML6, NMP6, NMS6, NMO6) and were in range of 51.753±0.124 to 62.444±0.606. The increase in the entrapment efficiency is attributed to the ability of cholesterol (CHO) to cement the leaking space in the bilayer membranes, which in turn allow enhanced drug level in niosomes [14]. Inclusion of cholesterol increases the viscosity of the formulation indicating more rigidity of the bilayer membrane [15]. As formulation containing span 60 NMS7 showed highest percentage entrapment (table 2) it was selected for further optimization on following basis:

Table 2: Percentage entrapment efficiency of various formulations of Famotidine prepared without DCP and with DCP

Formulation code	NML	NMP	NMS	NMO
6	56.715±0.446	60.814±0.486	62.444±0.606	51.753±0.124
7	69.333±0.925	71.3085±0.688	73.234±0.365	65.086±0.938

Table 3: Effect of type of surfactant on % Entrapment efficiency of various formulations of Famotidine

Formulation Code	Composition of surfactant Mixture (47.5 : 47.5 : 5)	% Entrapment efficiency
NML 7	Span 20 : CH : DCP	69.333±0.925
NMP 7	Span 40 : CH: DCP	71.3085±0.688
NMS 7	Span 60 : CH : DCP	73.234±0.365
NMO 7	Span 80 : CH : DCP	65.086±0.938

Values represented as mean ± SD (n = 3) ; CH = Cholesterol , DCP= Di cetyl phosphate

3.4.1. Effect of Type of surfactant on entrapment efficiency of various formulations of famotidine

The type of surfactant used showed a marked effect on the entrapment efficiencies of the formulations (table 3), Span 20, 40 and 60 have the same head group but different alkyl chain but span 80 has an unsaturated alkyl chain. The introduction of double bonds made the chain bend. This means that the adjacent molecules cannot be tight when they form membrane of niosomes. This caused the membrane to be more permeable which possibly explains the low entrapment efficiency behavior. While span 60 has the longest saturated alkyl chain due which it showed highest entrapment efficiency. The increase in the alkyl chain length of different grades of span led to increase in the encapsulation efficiency (%EE) these results were in accordance to the study done by Azeem et al and Hao & Li [16, 17].

3.4.2. Effect of Span 60 /Cholesterol ratio on entrapment efficiency

With 5 % DCP, ratio of span 60 : cholesterol was varied from 95:0 to 40:55 (table 4.) and its effect was studied. The formulation NA6 containing equal amount of span 60 and cholesterol *i.e.* 47.5: 47.5 showed the best entrapment efficiency that is 73.234±0.365. it was found that the increase in cholesterol content showed a marked increase in entrapment efficiency of the formulations but upto certain limit beyond which further increase in cholesterol content led to decrease in entrapment efficiency [6] which could be due to increase in cholesterol content which led to increase in hydrophobicity and stability of the bilayers which in turn decreases the permeability which may have caused efficient trapping of hydrophobic drug into bilayers during vesicle formation. But higher amounts of cholesterol may compete with the drug for packing space within the bilayers hence excluding the drug as the amphiphiles assembled into drugs [18]. The increase in entrapment efficiency with increase in cholesterol content can also be explained by the fact that cholesterol intercalated into bilayers preventing leakage of the drug through the bilayers. Many non-ionic surfactant forms vesicles when cholesterol is included in the bilayers to the level of 30 to 50% [19]. Incorporation of cholesterol into niosomes increased the encapsulation efficiency as cholesterol increases the viscosity of the formulation indicating more rigidity of the bilayer membranes. Moreover drug partitioning will occur more easily in highly ordered system of surfactant and cholesterol [14].

Table 4: Effect of span 60 /cholesterol ratio on % entrapment efficiency of various formulations of Famotidine

Formulation code	Span 60/CHL ratio/DCP	% Entrapment efficiency
NA1	95:0:5	39.827±0.594
NA2	85:10:5	42.938±1.305
NA3	75:20:5	49.577±1.038
NA4	65:30:5	55.975±0.752
NA5	55:40:5	64.444±0.780
NA6	47.5:47.5:5	73.234±0.365
NA7	40:55:5	62.66±1.000

Data obtained with 5% DCP, values represented as mean ±SD (n=3)

Table 5: Effect of Cholesterol : DCP ratio on % entrapment efficiency of various formulations of Famotidine

Formulation code	CHL : DCP ratio	% entrapment efficiency
NB1	52.5 : 0	61.753±0.858
NB2	50.0 : 2.5	68.148±0.707
NB3	47.5 : 5	73.234±0.365
NB4	42.5 :10	59.333±0.633

Data obtained with 47.5% span 60. Values represented as mean ± SD (n=3)

3.4.3. Effect of Cholesterol : DCP ratio on entrapment efficiency.

Only a very small concentration range of DCP was tested because a high concentration of DCP can inhibit niosomes formulation [14]. The highest entrapment efficiency was observed in formulation (NB3) containing 47.5 :5 ratio of cholesterol to DCP so it was selected for further study. This might be due to the fact that the interlamellar distance between the successive bilayers in multilamellar vesicles tends to increase due to presence of charge and leads to greater overall entrapped volume [20]. The optimal concentration of DCP in niosomes was identified based on entrapment efficiency given in table 5.

3.4.4. Effect of solvents on Entrapment efficiency

The maximum entrapment was seen in formulation NC3 *i.e.* 73.234±0.365 %. This might be attributed to the fact that the addition of chloroform to methanol led to delay in rate of evaporation of chloroform containing surfactant mixture. This delayed rate of evaporation gave more time and spacing to the alkyl chains of span to spread

more easily and evenly on the surface of round bottomed flask to produce a very thin film. This film on hydration led to the swelling of exposed layers of surfactant film that were later sheared off on agitation to form vesicles. In contrast to this formulation the other formulations (NC1 and NC2) that were prepared with chloroform or ether alone as solvent system resulted in formation of thick film with patches of surfactant mixture deposited on the surface of round bottomed flask which further on hydration led to minimum recovery of surfactant mixture and hence led to low entrapment efficiencies [21] 67.676±0.699 and 52.568±1.112 respectively (table 6).

Table 6: Effect of solvents on% entrapment efficiency of various formulations of Famotidine

Formulation code	Solvent	% Entrapment efficiency
NC1	Chloroform	67.676±0.699
NC2	Di ethyl ether	52.568±1.112
NC3	Chloroform : Methanol	73.234±0.365

Values represented as mean ± SD (n=3)

Table 7: Effect of hydration media on % entrapment efficiency of various formulations of Famotidine

Hydration media	% Entrapment efficiency
PBS pH 7.4	73.234±0.365
Water	62.195±1.042

Values represented as mean ± SD (n=3)

3.4.5. Effect of Hydration media on entrapment efficiency

The effect of hydration media *i.e.* phosphate buffer (7.4 pH) and distilled water on niosomal formulations was also studied as shown in table 7. The formulations prepared with distilled water as hydration media resulted in a low entrapment *i.e.* 62.195±1.042% as compared to formulation prepared with PBS pH 7.4 as hydration media which showed a better entrapment *i.e.* 73.234±0.365%.

3.5. Optimization on basis of vesicle size and size distribution studies

It was clearly observed from the results that the mean vesicle size of niosomes containing DCP were smaller as compared to niosomes prepared without the inclusion of DCP. The vesicle size of niosomes decreased consistently from span 20 to span 80 and are found in the following order- Span 20 > Span 40 > span60 > span 80. Results are listed in table 8. This was in accordance to Namdeo and Jain 1999 [22] when their studies revealed that the inclusion of the charged molecules tends to reduce the size of vesicles of niosomes [23, 24]. The zeta potential values (table 8) indicate that the formulations NML 7, NMP7, NMS7, NMO7have good stability. The niosomal formulations with DCP showed polydispersity index in the range of 0.114 to 0.514 (table 8) indicates the homogeneity of the formulations, whereas the formulations prepared without DCP showed greater degree of non uniformity of size distribution of vesicles with PDI values ranging between 0.378 to 0.757. This can be attributed to the fact that the Niosomes which were formulated with the charge-inducing agent; DCP, the inclusion of a charge-inducing agent in the lipid layer prevents the aggregation and fusion of vesicles, and maintains their integrity and uniformity [25].

Table 8: Vesicle size, size distribution, polydispersity index and zeta potential of niosomal formulations prepared without DCP and with DCP

Formulation code	*Vesicle size (nm)	Polydispersity index	Zeta potential
NML6	2088.4 ± 2119.2	0.757	ND*
NML7	718.7 ± 749.9	0.514	-57.45
NMP6	1523.8 ± 1534.8	0.457	ND
NMP7	487.6 ± 52.1	0.114	-49.02
NMS6	1213.8 ± 780.3	0.400	ND
NMS7	236.1 ± 65.3	0.274	-44.99
NMO6	587.6 ± 110.1	0.378	ND
NMO7	160.1 ± 69.7	0.295	-41.04

**values are represented as mean n=3, ND not determined*

3.6. *In vitro* drug release study

On the basis of results of Optimization based on entrapment efficiency and optimization based on vesicle size and size distribution studies the formulations containing DCP (NML7, NMP7, NMS7, NMO7 were selected as optimized formulations as they showed better entrapment efficiencies , good vesicle size well suited for oral delivery and polydispersity index values indicated homogeneity in formulations. These optimized famotidine loaded niosomal formulations were subjected to in vitro drug release studies in phosphate buffer pH 7.4, where there results (figure 3) indicated that the drug loaded niosomes showed constant drug release with no burst effect predicting that the drug was homogeneously dispersed. The free drug solution began to plateau only after 4 hrs but the optimized niosomal formulations continued to show drug release for more than 24 hrs without reaching plateau. So the study suggests that these famotidine loaded niosomal formulations have a potential to provide prolonged delivery of famotidine.

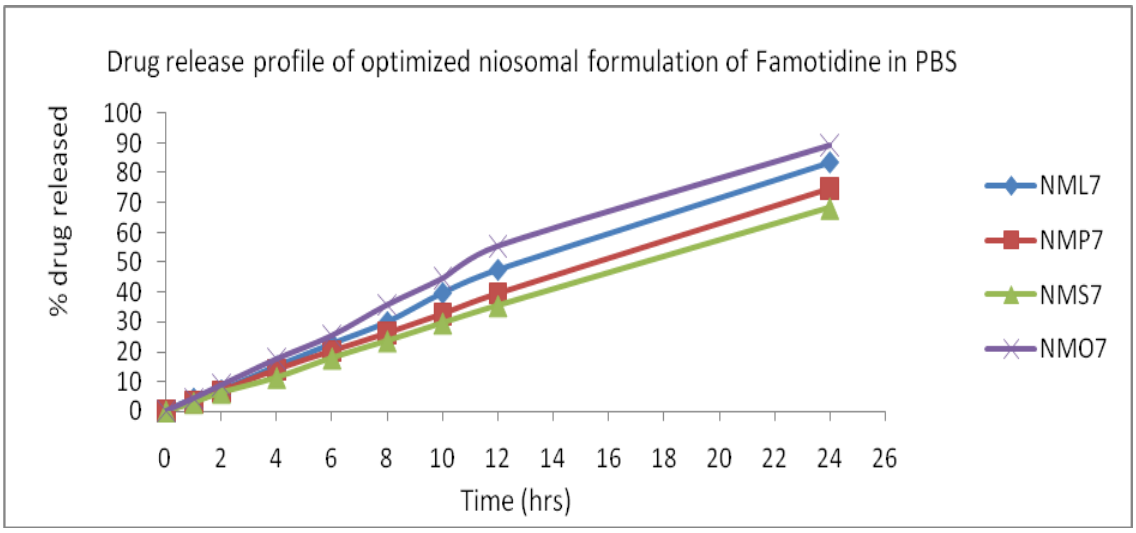


Fig.3: Drug release profile of optimized niosomal formulations of Famotidine in PBS pH 7.4

4. CONCLUSION

It can be concluded that the addition of the charged inducing agent greatly affected the particle size, zeta potential, and percentage entrapment efficiency of niosomal formulations of famotidine. With respect to stability, the addition of the DCP and cholesterol increased physical stability and entrapment efficiency of the niosomes due to the rigid bilayer membrane composed of Span 60 and cholesterol. In the present research, the findings revealed that the process variables critically affect the formulation of niosomes and need to be carefully controlled. In conclusion, our study suggests that the prepared niosomal formulations of famotidine provide prolonged delivery of drug with enhanced bioavailability.

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