DEVELOPMENT AND CHARACTERIZATION OF NON-IONIC SURFACTANT VESICLES (NIOSOMES) FOR ORAL DELIVERY OF NORFLOXACIN

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ABSTRACT

Controlled release drug products are often formulated to permit the establishment and maintenance of drug concentration at target site for longer interval of time. One such technique of drug targeting is 'niosome'. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Norfloxacin loaded niosomes were prepared by Lipid film hydration method with different surfactant to cholesterol ratio. The preformulations study was performed by FT-IR and UV spectroscopy to check the drug purity and stability. The niosomal suspensions were further evaluated for optical microscopy, entrapment efficiency, In vitro release study, Kinetic data analysis, Stability study. The formulation F4 which showed higher entrapment efficiency of 80.54 ±0.99. Release was best explained by the zero order kinetics. Kinetic analysis shows that the drug release follows super case II transport diffusion. Niosome formulation has showed appropriate stability for 90 days.

Keywords: Norfloxacin, Niosome, Span-60, Cholesterol, SEM, Kinetic analysis

INTRODUCTION

Norfloxacin is a synthetic chemotherapeutic antibacterial agent occasionally used to treat common as well as complicated urinary tract infections. Here we aiming to control the plasma concentrations of drug maintained throughout 24 h dosing interval with less peak trough fluctuation than that observed with twice daily dosing of immediate release norfloxacin. The short biological half-life (about 4 h) and dosing frequency more than once a day, low bioavailability (30- 40%) and low solubility of this drug make it an ideal candidate for sustained release.[1] Nowadays considerable interest has been focused on niosomes based targeted drug delivery. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. This article deals the method of preparation, characterizations, factors affecting release kinetic, advantages, and applications of niosomes.[2] 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 reticulo endothelial system [3].

The objective of the present study was to prepare norfloxacin niosomes in order to sustain the release of norfloxacin in upper GIT, which may decrease the side effect of GI disturbance by maintaining the concentration of the drug in the blood and decrease the renal excretion as well as frequency of dosing. In this work a potential drug delivery system of norfloxacin niosomes for oral delivery have been developed and characterized.

MATERIAL AND METHODS

Apparatus

Schimadzu model 800 double beam UV/Visible spectrophotometer was used to measure absorbance of all the solutions. Rotary flash evaporator (Popular India), Electronic digital balance (Schimadzu, Aux 220), Zeta Potential Probe (Zetasizer 4, France), Dialysis membrane (Hi media, India), Sonicator (PCI Analytics), Magnetic stirrer

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(REMI) and Digital pH meter (WKVI Kamal jeeth laboratory) were used in the study.

Reagents and Materials

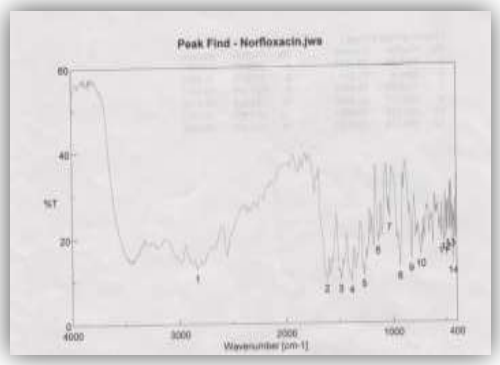
Norfloxacin obtained as a gift sample from Cipla, Goa. Span 60 were produced from National Chemicals Gujarat. Cholesterol was purchased from Merck specialties Pvt. Ltd. Mumbai. All other reagents used were of analytical grade.

Preformulation study

FT-IR Spectra and standard graph of norfloxacin shows the purity and stability of the pure drug[1]. FT-IR

Spectra of norfloxacin reveal no major interaction as it has shown in the Figure no.1.

Figure 1: FT-IR Spectra of Norfloxacin

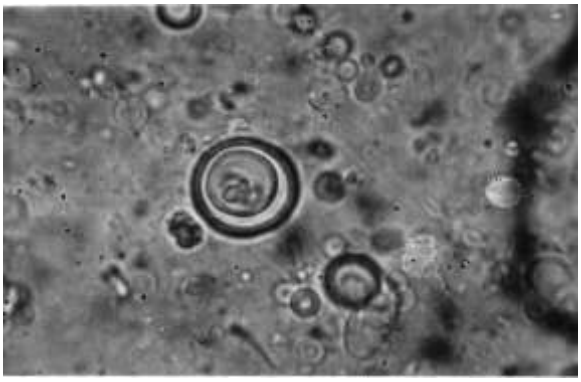


Preparation of norfloxacin noisome

The niosome formulations were prepared by lipid film hydration technique. Drug (norfloxacin) is freely soluble in 0.1N NaOH after heating. Non ionic surfactant and cholesterol were weighed (surfactant: cholesterol) in µmol) and dissolved in chloroform methanol (2:1) in a 100 ml round bottom flask. A thin lipid film was formed under reduced pressure in a rotary flash evaporator and temperature was maintained at 60^0c or above. The film was then hydrated by 10 ml of PBS* pH 7.4 at a temperature above the glass transition temperature of the surfactant with gentle shaking. The niosome suspension obtained was sonicated for 5 min which forms small sized vesicles. The stabilized MLVs were used for further studies[6].

Niosomes were prepared with different µmol ratios of Span 60 and cholesterol such as 200:200, 220:180, 240:160, 260:140, 280:120, 300:100 and 320:80 respectively while drug loading (400 mg) was kept constant as it has shown in the Table no.1.

Figure 2: Photomicrograph of norfloxacin niosome in a dry glass slide



RESULT AND DISCUSSION

Evaluation of niosomes

Microscopy

The prepared vesicles were studied under 400X magnifications to observe the formation of vesicles. Some unevenness of vesicles that observed under the study may be due to drying process under

Table 1: Compositions and entrapment efficiency of niosomal batches of norfloxacin

Formulation	Ratio(µmol) (surfactant:cholesterol)	Surfactant (mg)	Cholesterol (mg)	Entrapment efficiency %
F1	200:200	86	77.32	68.34±1.42
F2	220:160	94.74	69.6	72.88±0.44
F3	240:160	103.34	61.87	76.47±0.93
F4	260:140	111.96	54.14	80.52±0.99
F5	280:120	120.57	46.4	75.54±0.58
F6	300:100	129.18	38.67	70.65±0.88
F7	320:80	137.79	30.93	65.26±1.27

Drug content used 400 mg per batch

normal environment condition. The photomicrograph of niosomes is shown in the Figure no.2.

Entrapment efficiency of various formulations

Entrapment efficiency was studied for all the 7 formulations to find the best in terms of entrapment efficiency. Higher entrapment efficiency of the vesicles of span 60 is predictable because of its higher alkyl chain length. The entrapment efficiency was found to be higher with the F4 (80%), which may have an optimum cholesterol surfactant ratio to provide a high entrapment of norfloxacin. The niosomal formulations having high surfactant concentration (F5, F6and F7) have the higher entrapment efficiency which might be due to the high fluidity of the vesicles but it depends upon the cholesterol amount. Very low cholesterol content (F7) was also found to cause low entrapment efficiency (65%), which might be because of leakage of the vesicles. It was also observed that very high cholesterol content (F1) had a lowering effect on drug entrapment to the vesicles (68%). This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. The higher entrapment may be explained by high cholesterol content (~50% of the total lipid). There are reports that entrapment efficiency was increased, with increasing cholesterol content and by the usage of span 60 which has higher phase transition temperature. The larger vesicle size may also contribute to the higher entrapment efficiency[7,9]. Entrapment efficiency showed by various formulations is specified in Table no.1

Table 2: *In-vitro* release profile of all formulations

Formulation	Time (In hrs)	Percentage drug release
F1	24h	79.97
F2	24h	83.62
F3	24h	87.17
F4	24h	91.6
F5	24h	86.1
F6	24h	81.02
F7	24h	76.71

***In-vitro* release profile**

The release study was conducted for all the 7 formulations. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 90% release within a period of 24 hours. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment [7, 10]. The above specified three best formulations F4, F5, and F6, were found to give a cumulative release of 91.68%, 86.11% and 81.02% respectively over a period of 24 hrs, the higher release from the formulation F4 may be because of its optimum cholesterol content. Formulations F1, F2 having the highest cholesterol content showed the lowest release over 24 hours, they provide a release of 79.97%, 83.62% respectively as it shown in the Table no.2 and Figure no.4.

Drug release kinetic data

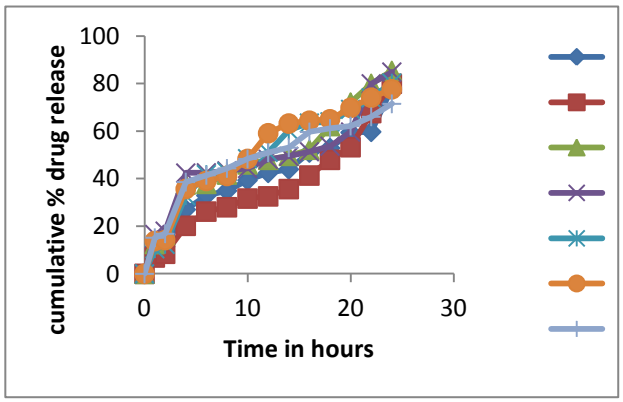
The zero order plots showed the zero order release characteristics of the formulation, which was confirmed by the correlation value. In order to find out the mechanism of drug release, the *in vitro* drug

Table 3: Percentage of norfloxacin retained on refrigerated storage

S.No	Days Stored	F4 % Retained	F5 % Retained	F6 % Retained
1	0	100	100	100
2	15	97.44	96.42	98.92
3	30	97.44	91.30	95.77
4	45	94.71	87.58	94.39
5	60	85.84	84.19	89.65
6	90	80.13	79.55	76.55

release data was graphically treated according to Higuchi's equation and the graphical fit for the *in vitro* data was used to conclude the mechanism of the drug release involved in the delivery system. Correlation value of Higuchi's plot revealed that the mechanism of drug release is diffusion. The *in vitro* kinetic data subjected to log time log drug release transformation plot (peppa's model), all the value ranges from 1 to 1.2704 revealed the fact that the drug release follows a super case II transport

Figure 4: *In-vitro* release of all formulation



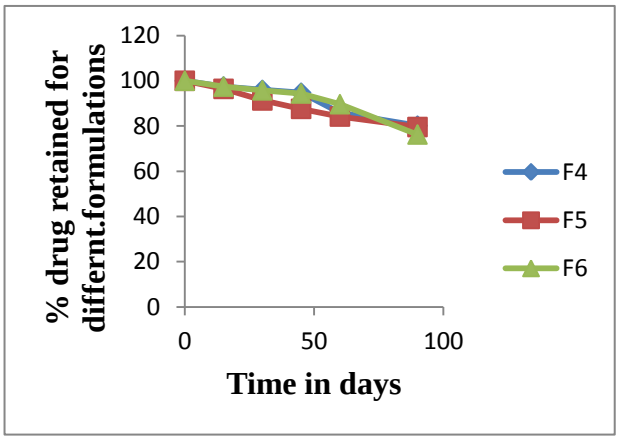
diffusion[9,10].

Stability Study

Physical stability

Physical stability was carried out to investigate the leaching out of the norfloxacin from niosomes. The best formulation F4 was kept at refrigerated temperature (2-8^{0}c), room temperature and 40^{0}c (75%RH) as three different groups. Stability chamber was used for the third group. The group which kept at refrigerated temperature showed promising results of 80% drug retained after 90 days ,the group which is kept at room temperature gave 71% drug retained and the group which is kept at 40^{0}c, 75% RH gave only 65% drug retained after 90 days. So the best three formulations kept at refrigerated temperature for a period of three months. The percentage of norfloxacin retained in the span 60 vesicle after a period of three months were 80.22% , 77.25%, 75.13% and respectively for formulations F4 (260:140), F5 (280:120) and F6 (300:100). Also the results indicate that more than 80% of norfloxacin was retained in the niosomal formulation for a period of 90 days. From this it can be concluded that vesicles are stable enough to store under refrigeration temperature with least leakage [10]. The leakage of drug from F6 may be due to its higher surfactant content and lower cholesterol which formed a leaking vesicle as it is clearly visible in the Table no.3 and Figure no.5.

Figure 5: Plot of %drug retained in different formulations v/s time in days



Test of Significance

The stability data analyzed for significant difference between retention patterns of drug in three different noisome formulations on storage [10]. The test value showed no significant difference (P>0.05) between the stability data of formulations from each other can be seen in the Table no.5.

Table 5: Test of Significance

Formulations	F4-F5	F5-F6	F4-F6
P Value	0.0743	0.9286	0.1873

Zeta potential analysis

The formulation F4 which was subjected to zeta potential analysis had a zeta value of +29mv, which is a measure of net charge of niosomes. This higher charge on the surface of vesicle produce a repulsive force between the vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension. From this it can be concluded that formulation F4 provides much stable noisome suspension [10].

CONCLUSION

This study suggests that niosomal formulation can provide consistent and prolonged release of norfloxacin from different niosomal formulations. It

will lead to sustained action of the entrapped drug that reduce the side effects associated with frequent administration of the drug and potentiate the therapeutic effects of the drug.

The concept of incorporating the drug into liposomes or niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Niosomes represent a promising drug delivery module. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multienvironmental structure. Niosomes are thoughts to be better candidate drug delivery as compared to liposomes due to various factors like cost, stability etc. Various type of drug deliveries can be possible using niosomes like targeting, oral, ophthalmic, topical, parenteral, etc. Lipid film hydration method is the best method of preparing multilamellar vesicles as it is important in the case of a hydrophobic drug. When compaired to proniosomes, niosomes occur some stability problems due to the state of the formulation but as an advantage from proniosomes, niosomes are easy to prepare and the carriers which are using for the proniosome preparation like sorbitol and maltodextrin are hygroscopic and it absorbs moisture. Selection of the carrier in the proniosomal formulation requires more attention as it affects some factors like flexibility in surfactant and other component ratio, surface area, efficient loading etc.

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