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Preparation and Evaluation of Ramipril Niosomes Prepared Using Sonication Method

Navin Kumar Dahiya[1], Rekha Rao[*1], Sanju Nanda[2]

[1]Department of Pharmaceutics, M.M. University, Mullana, Ambala, Haryana, India.
[2]Department of Pharmaceutical Sciences, M.D. University, Rohtak, Haryana, India.

Abstract

In present study an attempt was made to formulate ramipril niosomes using Span 40 and Span 60 in combination with cholesterol. According to optical and transmission electron microscopy results, niosomes were found spherical in shape. Particle size distribution analysis showed that ramipril loaded niosomes had a particle size distribution in nm range. *In vitro* drug release studies indicated that drug release is prolonged when Span 40 and Span 60 surfactant were used as non-ionic surfactants. It was also observed that type of surfactant affects release rate of ramipril.

Keywords: Niosomes, Span 40, Span 60.

Introduction

Niosomes carriers were first reported in 1970's. Nowadays, these have been extensively studied as drug carriers and drug delivery vesicles. Niosomes are non-ionic closed bilayer vesicular carrier systems which can entrap both hydrophilic and hydrophobic drugs. They are analogues to liposomes but provide several advantages over them like greater stability, low cost of production, biodegradable, biocompatible and non immunogenic in nature (Uchegbu and Florence 1995). They can prolong the circulation of entrapped drugs due to the presence of non-ionic surfactants. Niosomes can be prepared using different non-ionic surfactants. In niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as Span 60, Span 40 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate (Buckton 1995). They may be multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), or small unilamellar vesicles (SUVs) depending upon the method used for preparation. Several techniques for preparation of niosomes have been reported in literature. Among these, thin film hydration, sonication, reverse phase evaporation and microfludisation are most common. Thin film hydration method produces MLVs or LUVs, while sonication method produces vesicles with a smaller size.

Ramipril, 2-[N-[(S)-1-ethoxy carbonyl-3-phenyl propyl]-L-alanyl]-(1S, 3S, 5S)-2-azabicyclo [3,3,0]- octane-3-carboxylic acid [M.W. 416.51] is an angiotensin converting enzyme inhibitor (ACE). It is used in the treatment of hypertension, heart failure and myocardial infarction.

* **Corresponding author:** rekhaline@gmail.com

It is also used to reduce the risk of cardiovascular events in patients with certain risk factors. (Franz 1995, Warner and Perry 2002). But, it has several drawbacks such as low oral bioavailability and short biological half-life.

Niosomes are versatile carrier system that can be administered through various routes of drug delivery. They have been well reported to enhance penetration of the entrapped substances through skin. Transdermal delivery has been used to explain the ability of niosomes to modulate drug transfer through skin, e.g. (1) adsorption and fusion of niosomes on the surface of skin leading to high thermodynamic activity gradient of drug at the interface, which is the driving force for permeation of lipophilic drug, (2) reduction of the barrier properties of stratum corneum resulting from the property of vesicles as a penetration enhancer. Several drugs such as estradiol, tretinion, dithranol, enoxacin, acetazolamide, timolol maleate have been successfully encapsulated in niosomes for topical application. However, these systems have not been explored for systemic delivery through skin.

In order to improve the bioavailability of ramipril, the niosomes of ramipril were prepared. The work described is a part of a larger study, which is concerned with the transdermal delivery and skin penetration of ramipril. Consequently, ramipril niosomes were prepared by sonication method using span 40 and 60 as non-ionic surfactants. The vesicle suspensions were characterized by optical microscopy for vesicle formation and transmission electron microscopy (TEM) for morphology; photon correlation spectroscopy for mean size and polidispersity index. *In vitro* drug release was studied through a synthetic membrane as a prerequisite to the investigation of transdermal studies.

Material and Methods

Material

Ramipril was supplied as a gift sample by Ranbaxy, Ponta Sahib, H.P (India). Span 40 and 60 were purchased from Shah Scientific, Mumbai (India). Cholesterol was purchased from Central Drug House Pvt. Ltd, New Delhi (India). Dialysis membrane (MW cut off 8000-10,000) was purchased from Himedia Laboratories Pvt. Ltd, Mumbai. All other chemicals and solvents were of analytical grade.

Preparation of niosomes

The niosomal formulations were prepared by sonication technique in various ratios as reported in Table-1 (Cable 1989, Baillie et al. 1986). Drug, surfactant and cholesterol were weighed and dissolved in 10 ml of chloroform. An aqueous phase phosphate buffer saline (pH 7.4) was added to the above solution in a glass vial. The mixture was then sonicated using probe sonicator for 3 minutes. The resultant niosomal suspensions were characterized for further studies.

Table 1. The physicochemical characterization and entrapment efficiency parameters of niosomes

Code	Surfactant	Surfactant:Cholesterol	Entrapment Efficiency	Mean Size (nm)	Polydispersity index
NF-1	SP60	1:0.5	19.79 ± 5.46	216	0.274
NF-2		1:01	62.41 ± 2.24	274.7	0.269
NF-3		1:1.5	32.27 ±4.01	348.7	0.345
NF-4	SP40	1:0.5	17.40 ± 2.19	189.7	0.581
NF-5		1:1	36.79 ± 1.38	240.6	0.278
NF-6		1:1.5	23.97 ± 1.89	294.9	0.427

Physicochemical characterization of niosomes

Optical microscopy

A drop of niosomal dispersion was spread on a glass slide and examined for the vesicle structure and the presence of insoluble drug crystals using ordinary light microscope with magnification power 40 X. Photomicrographs were taken for niosomes using digital camera (Nikon coolpix s 220) with 6 X optical / 200 m.

Particle size

Mean size and polidispersity index of niosomal formulations were measured at 25°C by photon correlation spectroscopy, using a Delsa Nano-C (Beckman Coulter Instruments). Light scattering was monitored at 25°C at a scattering angle of 90°. The polidispersity index of niosomes was performed as a measurement of the size distribution of system.

Transmission electron microscopy (TEM)

The morphology of niosomal dispersion was also determined using transmission electron microscope. A drop of niosomal dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 minute to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 35 seconds. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried. The sample was observed using transmission electron microscopy (Hitachi 7500) at 90 kV (HU and Rhodes 1999).

Determination of entrapment efficiency (%EE)

The entrapment efficiency was measured using centrifugal method. The niosomal formulations were centrifuged (18000 rpm) for 40 min. at 5^0C in order to separate unentrapped drug from ramipril niosomes. The supernatant was taken and diluted with PBS (pH 7.4). The drug concentration in the resulting solution was assayed spectrophotometrically (UV-1800 Shimadzu, Japan) at 250 nm (Nagarsenker et al. 1999). The percentage of drug encapsulation was calculated by the following equation:

$$EE\ (\%) = [(C_t - C_f)/ C_t] \times 100$$

Where C_t is the concentration of total drug and C_f is the concentration of unentrapped drug.

In vitro release study

The release of ramipril from niosomes was determined using dialysis bag (High media dialysis membrane, 8000-10,000). An accurately measured amount of ramipril niosomal formulations was pipetted out in dialysis bag, which was previously socked and washed several time with distilled water and was placed in 250 ml beaker containing 100 ml of PBS (pH 7.4) solution maintained at 37° C and stirred using a magnetic stirrer. At specified interval of time sample solution was withdrawn and the drug content was determined after necessary dilution.

Results

The prepared niosomes appeared as discrete and round in shape as seen using optical microscopy (Figure 1 and Figure 2). The TEM photographs showed the niosomes prepared using sonication method were of small size and spherical in shape with a smooth surface (Figure 3). They were observed as discrete, separate vesicles with no irregularities and aggregation. The size of the niosomes were found to be in a range of 216 - 350 nm for Span 60 and 189 - 294 nm for Span 40 (Table 1). The results showed that the size of the vesicles

increase as the amount of cholesterol used in the preparation increases. The size of Span 60 niosomes was found to be larger as compared to Span 40.

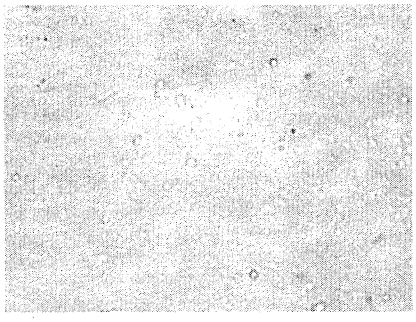

Figure 1. Optical micrographs of niosomes prepared with 1:1 molar ratio (NF-2)

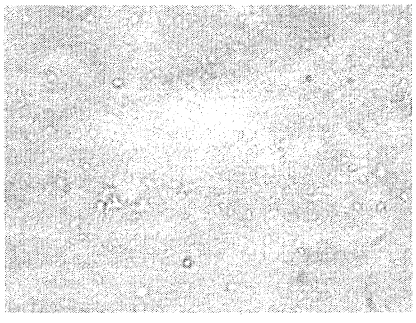

Figure 2. Optical micrographs of niosomes prepared with 1:1 molar ratio (NF-5)

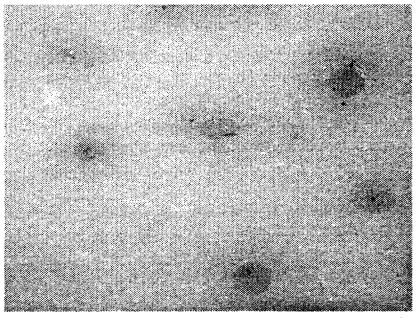

Figure 3. TEM image of NF-2

The percentage entrapment efficiency of the niosomes was reported in Table 1. The percentage entrapment efficiency increased from 19.79% (NF1) to 62.41% (NF2) for niosomes composed of Span 60 and cholesterol. Also, entrapment efficiency increased from 17.40% (NF4) to 36.79% (NF5) for niosomes composed of Span 40 and cholesterol. The entrapment efficiency for niosomes prepared using Span 60 was found superior to those prepared from Span 40. This could be due to chemical structure of non - ionic surfactants. Both Span types have same head group and different alkyl chains. It had been already

reported in literature that increase in the alkyl chain length will lead to higher entrapment efficiency (Hao et al. 2002). Span 60 has longer saturated ethyl chain (C-16) compared to Span-40 (C-14). So, it produced niosomes with higher entrapment efficiency. The larger alkyl chain influences the HLB value of non - ionic surfactant mixture. The lower the HLB value of surfactant, higher will be the drug entrapment efficiency (Naresh et al. 1994).

It is clear from our observation that formula NF2 composed of Span 60 and cholesterol (1:1 molar ratio) is most beneficial for the efficient encapsulation of ramipril as it exhibited the highest percentage entrapment efficiency (62.41%) compared to the other formulae.

In vitro release study was conducted for the formulations with the highest entrapment efficiency (NF-2 and NF-5) and results were plotted in Figure-4. Cholesterol, which has a property to abolish gel to liquid transition of niosomes, has found to prevent the leakage of drug from the niosomal formulation. The best formulations NF-2 and NF-5 were found to give a release of 81.01% and 88.09% over a period of 24 h.

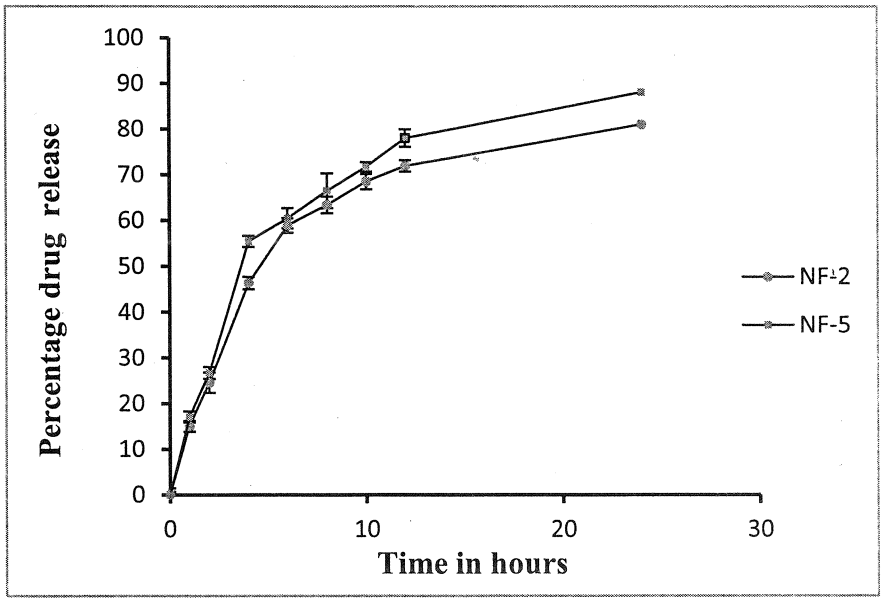

Figure 4. Release profile of ramipril niosomes

Conclusion

It is concluded that ramipril niosomes were successfully prepared using sonication method. Also, the particle size range obtained is well suited for transdermal delivery. The niosomal vesicles were spherical with good encapsulation efficiency. Moreover, niosomes prepared from Span 60 has more entrapment efficiency as compared to Span 40. Hence, ramipril niosomes can be further evaluated for transdermal delivery of drug which is in progress.

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