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Formulation, characterization and *in vitro* evaluation of tactically engineered proniosomes for successful oral delivery of ramipril

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ABSTRACT

The objective of our work is to formulate and characterize ramiprilbased proniosomes to improve the oral delivery of ramipril. Ramipril is used to treat hypertension and efforts are made to use provesicular approach to improve its solubilty and bioavailabilty. The drug was loaded into maltodextrin-based proniosome powders using slurry method. The powder was characterized for particle size, particle morphology and drug content. To understand the solid state properties of powder XRD was performed. The release rate of formulation was compared with marketed formulation (Ramihart5mg) using dialysis bag diffusion method. It was found that the formulation had a high encapsulation efficiencyandprovided a sustained release over 24 h.

Keywords: Niosomes; Cholesterol; ramipril.

INTRODUCTION

Ramipril is an antihypertensive drug which belongs to the class of ACE (Angiotensin Converting Enzyme) inhibitors. As the name suggests, it inhibits Angiotensin Converting Enzyme, lowering the production of Angiotensin II, causing relaxation of the arteriolar smooth muscles, resulting in decrease in the total peripheral resistance. Therefore, this drug can conveniently be used to control hypertension. Ramipril is a prodrug, which is converted to active metabolite, ramiprilat in the liver by esterase enzymes [1]. The drug has a molecular weight of 416.52 g/mol, a partition coefficient is 3.41 and is insoluble in water. It is available as tablets and capsules. Ramipril is highly lipophilic, has poor aqueous solubility and its oral bioavailability is ~ 28 %. It is rapidly distributed to all tissues. Protein binding is 73%. Side effects such as dizziness, headache, weakness, nausea and drug cough are frequently associated with the use of ramipril [2].

The aim of our study is to formulate and characterise ramipril-based proniosomes in order to enhance the bioavailability of ramipril. Provesicular approach is gaining popularity as novel drug delivery systems because of the various advantages they offer. They can be used for sustained and targeted release with a mild side-effect profile and also offers patient compliance by reduction in the dose administered. Liposomes are lipid based while niosomes are non-ionic surfactant based unilamellar or multilamellar vesicles. Niosomes are preferred over liposomes as the problems associated with liposomes such as high cost, chemical instability, variable purity of phospholipids are absent in case of niosomes and also they offer low toxicity due to their nonionic nature [3].

The aim, however, is to develop proniosomes instead of niosomes due to stability problems such as fusion, aggregation, sedimentation and leakage of drug from vesicles on storage occurring during the formulation of

niosomes. Formulation of ramipril-based proniosomes is an effective way to increase the solubility and hence the bioavailability of ramipril and thus, lowering the occurrence of side effects and increasing the efficacy of the drug.

MATERIALS AND METHODS

Material:

Ramipril was generously provided by Aarti Industries Ltd., Mumbai, India. Cholesterol, Span 60, Maltodextrin were obtained from Central Drug House (P) Ltd., India; methanol (HPLC grade), chloroform, isopropyl alcohol (IPA) were obtained from Rankem, India; sodium hydroxide, potassium di-hydrogen phosphate, sodium chloride were supplied by S.D. Fine Chemicals, India. Milli-Q water filtered through a 0.22 µm membrane filter was used for all set of experiments.

Methods:

Preparation of Proniosomes:

Proniosome powders were prepared by using slurry method [4]. Precisely, ramipril (20mg) and accurately weighed amounts of lipid mixture (Span 60:Cholesterol) in different ratios (Table 1) were dissolved in 10 mL of solvent comprising of chloroform and methanol (7:3 ratio). The resultant mixture was transferred to a 100 mL round bottom flask containing 500 mg maltodextrin powder to form slurry. The flask was attached to a rotary flash evaporator (Heidolph, Germany)and the solvent was evaporated under reduced pressure at a temperature of 45°C and60 rpm. After ensuring complete removal of the solvent, the flask was removed and kept overnight under vacuum to obtain a dry, free flowing product. The product was stored in air tight containers and further evaluated.

Characterisation of Proniosomes:

Formation and morphological evaluation of vesicles:

The formation of niosomes was evaluated using digital microscope (BA 210 Motic, China). The proniosome powder was placed on a glass slide and few microlitres of water was added drop wise. A cover slip was placed and the formation of vesicles was observed using optical microscope. For morphological study the proniosome powder was hydrated with phosphate buffer (pH 7.4) using vortex mixer. The dispersion was placed as few drops on the glass slide and observed under optical microscope. Photomicrographs were taken.

Table 1: Composition of ramipril loaded maltodextrin based proniosome powders.

Name	Surfactant:Cholestrolratio	Surfactant (mg)	Cholestrol (mg)
F1	150:100	129	77.2
F2	125:125	107.6	96.4
F3	175:75	150.6	57.8
F4	75:175	64.4	135
F5	100:150	86	115.8

Maltodextrin: 500 mg
Drug: 20 mg

Scanning electron microscopy (SEM):

The surface characteristics of niosomes was found using SEM(LEO 235, Carl Zeiss, Germany) at 15 kV. The niosomal dispersion was mounted on an aluminium stub with double-sided adhesive carbon tape. The vesicles were then sputter coated with gold using a vacuum evaporator and examined with scanning electron microscope at 15 kV accelerating voltage [5].

Determination of vesicle size:

The dry, free flowing powder was hydrated with phosphate buffer and shaken for about a minute to obtain the niosomal dispersion. The niosomes were then sonicated for 60 seconds in a bath sonicator and were analyzed byphoton correlation spectroscopy (PCS)usingZetasizer Nano ZS (Malvern Instruments Ltd., USA) at 25°C at an angle of 173°. Each experiment was executed in triplicate.The PDI relates with the width of vesicle size distribution, as its small value (<0.1) point toward homogeneity, while a high value (>0.3) indicate high heterogeneity [6].

Entrapment efficiency (%):

The entrapment efficiency of proniosomes was estimated using centrifugation method (indirect method). The niosomal dispersion prepared earlier by hydration of the proniosome powder is centrifuged at 4000 rpm for 40 minutes [7]. The supernatant is taken out and diluted with phosphate buffer. The resultant solution is assayed at 207nm using UV spectrophotometer. The entrapment efficiency of the vesicles was found out by subtracting the amount of unentrapped drug from the total drug.

$$EE = \frac{\text{Total drug used} - \text{Unentrapped drug}}{\text{Total drug used}} \times 100 \quad \text{.....(1)}$$

Micromeritic properties:
The flow properties of proniosomal powder were determined by calculating the angle of repose (by funnel method)and Carr's consolidation index.

X-Ray diffractometry:
The powder XRD patterns of the pure drug, optimized formulation and maltodextrin were obtained using X-Ray diffractometer. The measuring conditions were Ni-filtered Cu-Kα radiation; 40 KV voltage: 30 mA current, scan speed 2°/min in terms of 2θ angle.

Release rate profile:
The dialysis bag diffusion method was used to study the release rate of ramipril proniosomes. The prepared proniosome powder (equivalent to 5 mg of ramipril) was placed in a capsule andenclosed within the dialysis bag with marketed formulation, separately (high-media dialysis membrane, 10,000-14,000 molecular weight cut-off). Bags were then immersed in 0.1N HCl which acts as the receptor compartment. The entire system was kept at 37° C. With continuous magnetic stirring at 100 rpm, samples were withdrawn at specific intervals and replaced continuously by fresh medium. The withdrawn samples were assayed using UV spectrophotometer at 207 nm. After 2 hours, 0.2M tribasic sodium phosphate solution was added to the receptor compartment to make an alkaline solution and sampling was continued upto 24 h [8].

RESULTS AND DISCUSSION

Proniosomes were prepared by slurry method. The composition of different proniosomal powders is depicted in Table 1. Maltodextrin was used as the coating carrier and Span 60 was the non-ionic surfactant used in the formulation. Cholesterol was added to increase the entrapment efficiency and stability of the vesicles. Solvents used were chloroform and methanol because the drug is soluble in methanol. The influence of cholesterol was studied by changing the span 60 to cholesterol ratio while keeping the total lipid constant at 250 µM. Span 60 as a result of high phase transition temperature facilitates the formation of stable vesicle and improve the oral delivery of ramipril from proniosomes [4]. Niosomes formation due to hydration of proniosome powder was spontaneous. At the start formation of vesicular structures over the surface of maltodextrin was observed, might be due to the swelling of span 60 bilayer and with the gentle agitation transformed into multilamellar vesicular structures.

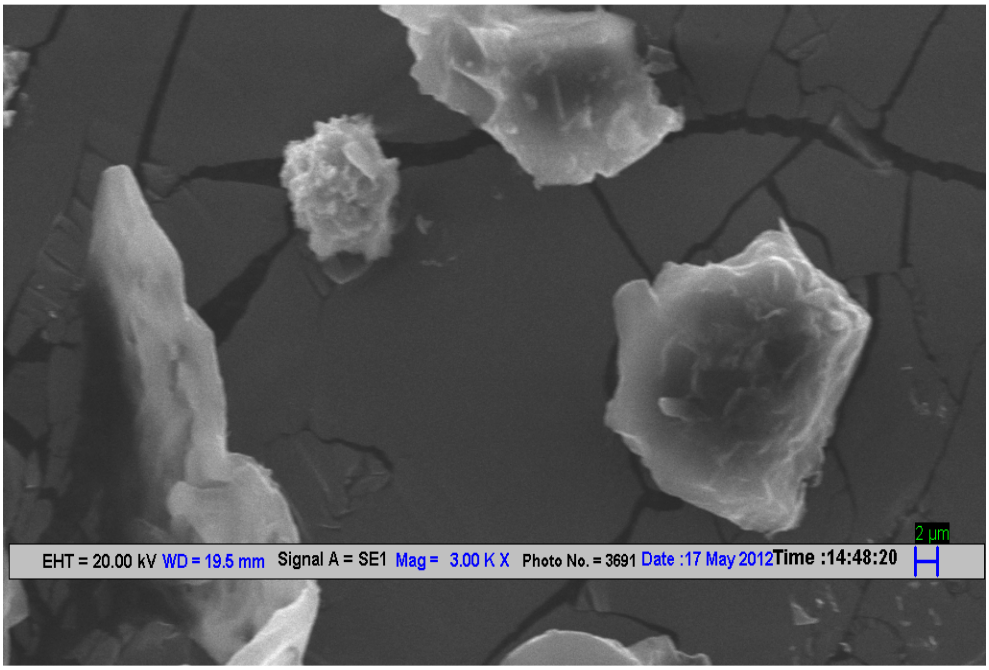

Fig. 1: Scanning electron micrographs of proniosome powder

As observed through the SEM, surface of the ramipril loaded proniosome powder (Fig. 1) was irregular due tothe coating of surfactant and cholesterol molecules over the maltodextrin surface. Micromeriticsproperties of the

proniosomal formulation is important parameter to be considered as it will affect the dose uniformity and ease of filling into capsules. The proniosomal formulation flow properties were assessed with the help of angle of reposeand Carr's index (Table 2). Results obtained pointed out that the formulation has a small angle of repose (<30θ), promising a good flow properties. Also the angle of reposeand Carr's index were in agreement to the acceptable limits. The size of the optimized formulation was 458± 20.34nm with anEE of the 83.11 ± 19.9 % (Table 3). Small value of polydispersity index (PI) (<0.1) specifies a homogenous population, while a PI (>0.3) specifies a higher heterogeneity.

Table 2: Micromeriticand carrier related properties of ramipril loaded proniosome

Name	EE (%)	Vesicle Size (nm)	Angle of repose	Compressibility index
F1	23.88 ± 8.1	845 ± 25.18	12.4 ± 2.8	2.4 ± 0.8
F2	55.64 ± 14.8	993 ± 38.83	14.6 ± 4.9	5.7 ± 1.2
F3	83.11 ± 19.9	458 ± 20.34	17.8 ± 1.6	9.1 ± 1.8
F4	63.88 ± 28.1	756 ± 64.02	22.5 ± 4.3	11.6 ± 2.2
F5	43.88 ± 18.1	818 ± 58.98	28.4 ± 2.4	14.8 ± 2.9

Value represented as Mean ±SD

Ramipril showed several characteristic high intensity diffraction peaks signifying its crystalline nature (Fig. 2). However, diffused peaks in the proniosomal formulation point towards its amorphization. EE% of ramipril depends on the composition of proniosomes [9].

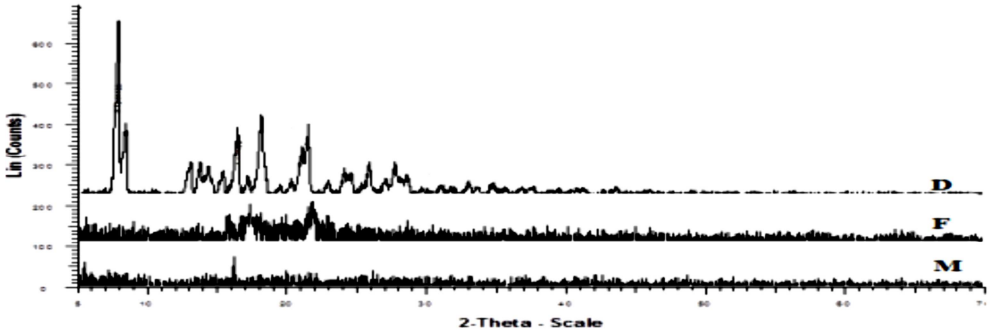

Fig. 2: The X-ray diffraction image of pure Ramipril (D), proniosome formulation (F) and maltodextrine (M)

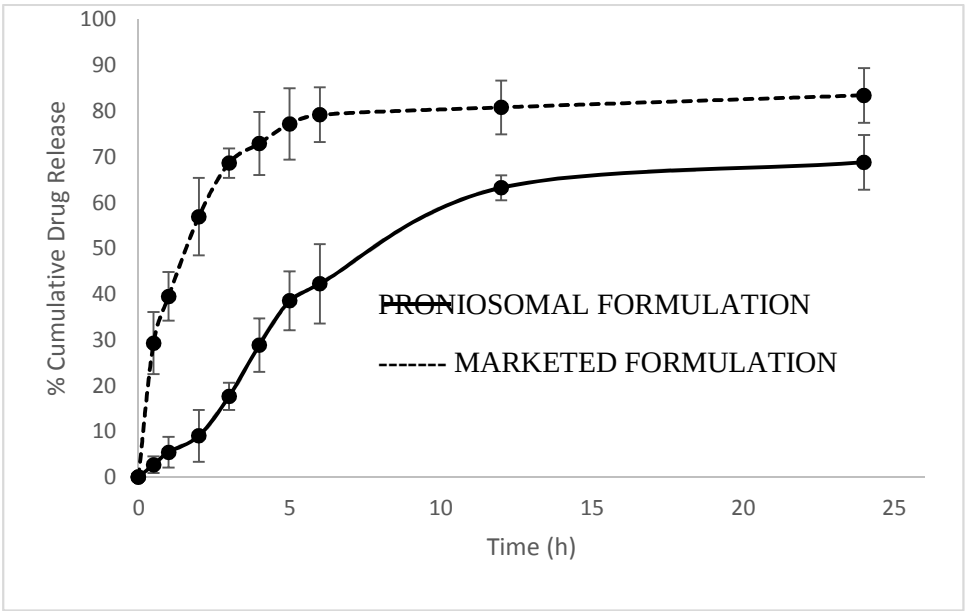

Fig 3: Dissolution profile of ramipril loaded proniosomal formulation and marketed formulation (Ramihart 5 mg)

With increase in the concentration of cholesterol EE% has been increased upto a point however further increase replaces the drug from the bilayer and this results in decrease in the EE%. The *in vitro* dissolution study proved that the optimized proniosomal formulation sustained the release of the ramipril over the 24 h in comparison to the

marketed capsule. The % cumulative drug release obtained with the proniosomal formulation was 68.76 ± 5.98 % after 24 h(Fig. 3).

Superior dissolution profile obtained with the proniosomal formulations might be a consequence of the altered physical state of the ramipril entrapped within the bilayer of niosome and enhanced effective surface area available for the dissolution medium [10]. Further these results arein agreement with XRD studies.

CONCLUSION

Ramipril-based proniosomes were successfully formulated and were found to have high encapsulation efficiency. This approach can be used to improve the efficacy of the drug as well as to alleviate the adverse effects of the drug. The sustained release of the drug further offers several advantages such as patient compliance, lower toxicity and reduction in dose. We can conclude that the treatment of hypertension using ACE inhibitors such as ramipril can greatly be advanced and improved using the provesicular approach.

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