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Formulation and Evaluation of Niosomes Containing an Antihypertensive Drug

Sunil Kamboj, Suman Bala, Vipin Saini

***Abstract*—**Niosomes were formulated with an aim of enhancing the oral bioavailability of losartan potassium and formulated in different molar ratios of surfactant, cholesterol and dicetyl phosphate. The formulated niosomes were found in range of 54.98 µm to 107.85 µm in size. Formulations with 1:1 ratio of surfactant and cholesterol have shown maximum entrapment efficiencies. Niosomes with sorbitan monostearate showed maximum drug release and zero order release kinetics, at the end of 24 hours. The *in vivo* study has shown the significant enhancement in oral bioavailability of losartan potassium in rats, after a dose of 10 mg/kg. The average relative bioavailability in relation with pure drug solution was found 2.56, indicates more than two fold increase in oral bioavailability. A significant increment in MRT reflects the release retarding ability of the vesicles. In conclusion, niosomes could be a promising delivery of losartan potassium with improved oral bioavailability and prolonged release profiles.

***Keywords*—**Non-ionic surfactant vesicles, losartan potassium, oral bioavailability, controlled release.

I. Introduction

DRUGS having the poor bioavailability are always become the biggest problem for the patients, physicians and researchers. All the efforts for product development and research are worthless until these make the drug bioavailable in body, in an effective concentration. Several approaches like micronization, complexation, nanosuspensions, microemulsions, solid lipid nanoparticles, liposomes, niosomes, bilosomes, etc. have been used by the researchers for improving the gastric absorption of the compounds and enhance their bioavailability [1]. Out of all the approaches, surfactant vesicles (niosomes) were found to have the distinct advantages over all other approaches, because these vesicles act as drug reservoirs and the rate of drug release can be controlled by modification of their compositions. Although liposomes have been reported as an effective vesicular drug delivery system for oral as well as transdermal delivery of drugs for improving their absorption but niosomal vesicles are more preferred over liposomes due to their higher chemical stability, economy and simple practical methods of preparation without the use of pharmaceutically unaccepted solvents etc. Niosomes are the surfactant vesicles, which are prepared by different non-ionic surfactants. These are spherical lipid bilayers capable of entrapping water-soluble molecules with in an aqueous domain or alternatively lipid molecules with in lipid bilayers. Based upon their methods of preparation, they may be unilamellar or multilamellar in nature. In the present scenario, niosomal drug delivery system is the leading topic of research for their 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. Niosomes can also alter the metabolism of the drugs, prolong the circulation, half-life and reduces the side effects of the drugs [2].

Losartan potassium is antihypertensive drug belongs to angiotensin II receptor antagonists category. It is mainly used to treat high blood pressure (hypertension). The major problem with the therapy of losartan potassium is its poor bioavailability (32%), as a reason of its limited solubility, absorption and first pass metabolism, which may be overcome by using a drug delivery system, which can make it bioavailable in the effective concentration [3].

In the present study losartan potassium, loaded niosomes were prepared and evaluated for their *in vitro* and *in vivo* characteristics in an attempt to improve its oral bioavailability and to extend its release for prolonged period.

II. Experimental

A. Materials

Losartan potassium was obtained as a gift sample from K. Pharma Chem (Ambala) Haryana, sorbitan laurate (span 20), sorbitan monopalmitate (span 40), sorbitan monostearate (span 60), sorbitan oleate (span 80), chloroform and sodium hydroxide were purchased from Qualikem specialties Pvt. Ltd., Mumbai. Dicetyl phosphate (DCP) was purchased from Sigma Aldrich, Bangalore. All other chemicals were of analytical grade and procured from the authentic sources. Dialysis membrane (42.44/25.4 mm flat width/diameter) was purchased from Himedia, Mumbai.

B. Formulation of Drug Loaded Niosomes

Drug loaded niosomal vesicles were prepared by conventional thin film hydration technique. For the preparation of niosomes, different grades of span such as sorbitan laurate, sorbitan monopalmitate, sorbitan monostearate and sorbitan oleate were used in different molar ratios of surfactant: CHOL: DCP as 2.5:1:0.1, 2:1:0.1, 1.5:1:0.1, 1:1:0.1 and 1:1.5:0.1 (total weight of lipid mixture was kept constant for all batches). Further lipid mixture: drug ratio was kept 1: 0.5 in all the batches. During the preparation, accurately weighted quantities of surfactant, CHOL and DCP

Sunil Kamboj is with M.M. College of Pharmacy, Maharishi Markandeshwar University, Mullana, Ambala, 133207, Haryana, India (corresponding author, phone: +91-8059930168; e-mail: sunilmmcp@gmail.com).

Suman Bala and Vipin Saini are with M.M. College of Pharmacy, Maharishi Markandeshwar University, Mullana, Ambala, 133207, Haryana, India.

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±2 ºC. The flask was rotated at 120 rpm until a smooth and dry lipid film was obtained. The lipid film was hydrated with 10 ml phosphate buffer saline (PBS) of pH 7.4 containing drug for 3 hours at 60±2 ºC with gentle shaking. Finally, the niosomal dispersion was stabilized by keeping at 2-8 ºC for 24 hours [4].

C. Visual Observation

All the prepared batches were visually observed for turbidity and flocculation after filling the formulations in transparent containers. Along with turbidity and flocculation, the optimized batches (with maximum entrapment efficiencies) were also analyzed for sedimentation. The studies were performed in triplicate and results were reported on the bases of bottom view of the containers having niosomal preparations (Table I).

TABLE I
BOTTOM VIEW OF CONTAINER SHOWED DIFFERENT DEGREE OF SEDIMENTATION

Bottom view of container (Differentiate from initial look)	Degree of sedimentation	Indications
No colour change	No sedimentation	-
Partially dark	Partial sedimentation (1-25%)	+
Intently dark	Near to complete sedimentation (26-75%)	++
Entirely dark	Complete sedimentation	+++

D. Vesicle Size Measurement

The average size measurement studies of the prepared niosomal vesicles were performed by optical microscope (Vaiseshika 7001-IMS). Further the vesicles size distribution studies were performed on the optimized batches (with maximum entrapment efficiency) by measuring the size of randomly selected 100 niosomes vesicles from each batch. The prepared niosomal vesicles were also analyzed by scanning electron microscopy (SEM) technique. The samples for SEM were prepared by applying the niosomal vesicles on the double-sided tape that was affixed on an aluminum stub. The aluminum stub was then placed in the vacuum chamber of scanning electron microscope (XL 30 ESEM with EDAX, Philips, Netherlands). The vesicles were observed by using gaseous secondary electron detector.

E. Zeta Potential Measurement

In vivo performance of the niosomes is mainly based on the surface charge present on the niosomal vesicles. The zeta potential of the vesicles also plays an important role in the stability of the niosomal vesicles [5]. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system. Zeta potential of suitably diluted niosomal dispersion was determined by measuring the electrophoretic mobility of the niosomal vesicles using Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK) at 25ºC.

F. Entrapment Efficiency

For performing the entrapment efficiency, the drug loaded niosomes were separated from unentrapped drug by using cooling centrifuge (Remi, C-24DL) at 12,000 rpm for 30 min. The temperature was maintained at 4ºC during the separation process. The supernatant liquid was separated and the vesicles were washed with PBS. The vesicles were suspended in 3 ml PBS and packed in a dialysis bag. The dialysis bag after tying at both the ends was immersed in 200 ml PBS, maintained at 37ºC and stirred overnight by using magnetic stirrer [6]. Drug was estimated spectrophotometrically at λ_{max} of 225 nm, against PBS as blank. The percentage of entrapped drug was calculated by applying the following equation [7]:

$$\% \text{ Entrapment} = (D_E \times 100) / (D_I) \quad (1)$$

where, D_E is the amount of entrapped drug and D_I is the initial amount of drug.

A. In Vitro Drug Release Study

In vitro release studies were carried out, only on the formulations, which have shown the best entrapment efficiencies, such as LP4, LP9, LP14 and LP19. The *in vitro* release studies were performed by using the buffer solutions of different pH to simulate stomach and blood pH and to evaluate the effect of pH on drug release. Dialysis tube containing the measured amount of drug loaded niosomal dispersion (an equivalent to 10 mg of losartan potassium) was placed in magnetically stirred 200 ml of 0.1 N HCl at 37±5 ºC, for a period of 2 hours, then the test media was replaced with PBS pH 7.4. The test was continued for a total period of 24 hours. Aliquots with 5ml dialysate samples were withdrawn periodically at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours and immediately replenished with the same volume of buffer medium. The drug content was determined spectrophotometrically at 225 nm. Results are the mean of three runs.

B. Release Kinetics Modeling

For the characterization of the release kinetics studies and to determine the release mechanism of drug, the results of *in vitro* release studies were fitted with several kinetics models, such as Zero order rate equation, First order rate equation, Higuchi model and Hixson-Crowell model. The *in vitro* release data of all niosomal formulations was also fitted into Korsmeyer and Peppas equation to find out the mechanism of drug release [8].

C. In Vivo Study

Based upon the results obtained from sedimentation behavior, release kinetics modeling and stability studies (not reported), the formulation code LP14 was considered as the best formulation and further selected for *in vivo* studies in the rat model. *In vivo* study was performed according to the method reported by and in accordance with the protocol approved by the Institutional Animal Ethical Committee of M.M. College of Pharmacy, M.M. University, Mullana (protocol no. MMEC-IAEC/12/10).

Eighteen male albino rats (Sparague Dawely strain) weighing 150±5 g were selected for study. The animals were divided in to three groups, each group containing six animals. The animals were fasted overnight for 12 hours. On the study day the first group was fed with PBS of pH 7.4 orally. Second and third groups were treated with pure losartan potassium solution in PBS and losartan potassium loaded niosomal formulation (equivalent to 10 mg losartan potassium per kilogram of body weight) respectively by oral route. Blood samples were withdrawn from retro-orbital plexus of eye at 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours after dosing, in eppendorf tubes containing 1-2 drops of 10% EDTA solution. The blood samples were centrifuged by cooling centrifuge at 2000 rpm for 10 min. The temperature was maintained at 4 ºC during the centrifugation. The clear plasma samples obtained were stored at refrigeration temperature (4 ºC) until analysis. Plasma samples were prepared by taking 0.5 ml plasma into eppendorf tubes to which 0.5 ml of protein precipitating agent (perchloric acid: acetonitrile in 50% v/v each) was added and vortexed for 30 sec. The samples were then centrifuged by cooling centrifuge at 3000 rpm for 10 min. The temperature was maintained at 4 ºC during the centrifugation. The supernatants were collected and drug concentration was analyzed by double beam UV-Visible spectrophotometer at 225 nm.

III. Results and Discussion

A. Visual Observation

All the formulated batches were found turbid and whitish in colour. The niosomal formulations with higher entrapment efficiencies were additionally evaluated for sedimentation while storage at 4±2ºC for 3 months, in transparent containers. The results revealed that in all the batches, except LP14, sedimentation started after 30 days of the storage but the niosomes, formulated with sorbitan monostearate was found with good dispersible form, even after 90 days of storage, indicated the good physical stability (Table II).

TABLE II
Degree of Sedimentation of Drug Loaded Niosomes after Storage at 4 ºC for 3 Months

Formulation code	0 day	15 days	30 days	45 days	60 days	90 days
LP4	-	-	+	+	++	+++
LP9	-	-	+	+	+	++
LP14	-	-	-	-	-	-
LP19	-	-	+	+	++	++

B. Vesicle Size Measurement

The formulated niosomal vesicles were found spherical in shape (Fig. 1 (a)), ranging 54.98 µm to 107.85 µm in size. The details of vesicles size range and their distribution plots are given in Table III and Fig. 1 (b) respectively. The results have shown the effect of HLB values on vesicular size, as the HLB value of the surfactants moves towards the hydrophilicity, vesicle size was found to increase. Niosomal vesicles formulated with sorbitan laurate (HLB 8.6) showed higher vesicular size and vesicles formulated with sorbitan oleate (HLB 4.3), showed least vesicular size. Further, the vesicles formulated with sorbitan monostearate (HLB 4.7) were found to have almost similar size as with sorbitan oleate, which may be due to the nearby HLB range of these surfactants. Further, the effect of surfactants HLB values on the vesicles size could be explained in term of surface energy, which increases with increasing the hydrophilicity, also the water uptake capacity of the surfactants increases when the HLB values moves towards hydrophilic region and both reasons result in larger of vesicles [9]. Further, the effect of CHOL concentration on the vesicles size has also come forward, vesicle size increases with increasing the CHOL concentration. A key additive of the formulation, CHOL affects the physical stability of the vesicles. It provides the rigidity to the bilayer membrane. It strengthens the bilayer and diminishes the bilayer fluidity by eliminating the phase transition temperature peak of the vesicles [10].

TABLE III
Composition and Characterization of Niosomal Formulations

Formulation Code	Mean vesicle diameter (µm)*	Zeta potential (mV)*	Entrapment efficiency (%)*	% Cumulative drug released (at the end of 24 hours)*
LP1	78.12±0.75	-61.3±0.22	38.8±1.82	-
LP2	90.84±0.61	-47.4±0.18	42.3±1.02	-
LP3	96.65±1.24	-36.2±0.12	51.7±1.30	-
LP4	101.33±0.76	-29.6±0.09	76.1±2.01	84.24±3.72
LP5	107.85±0.86	-22.1±0.11	73.5±1.73	-
LP6	75.71±0.75	-52.5±0.19	51.3±1.02	-
LP7	81.01±0.96	-42.2±0.18	59.1±2.38	-
LP8	88.73±0.81	-32.7±0.21	68.5±2.86	-
LP9	98.09±1.30	-22.7±0.11	83.7±2.09	92.98±4.82
LP10	105.19±0.78	-21.9±0.14	78.4±1.99	-
LP11	56.95±0.34	-46.2±0.24	55.6±1.01	-
LP12	58.17±0.67	-39.1±0.22	67.7±1.92	-
LP13	72.24±1.16	-26.6±0.19	72.4±1.20	-
LP14	81.82±0.76	-19.3±0.16	95.2±2.89	99.19±4.78
LP15	89.72±0.86	-17.2±0.21	81.5±2.84	-
LP16	54.98±0.87	-45.9±0.18	59.1±1.89	-
LP17	57.31±0.86	-37.3±0.17	68.4±1.09	-
LP18	70.93±0.56	-25.3±0.14	75.5±3.01	-
LP19	79.31±0.75	-20.1±0.14	88.4±2.37	88.42±3.99
LP20	89.55±0.65	-17.2±0.16	79.8±2.11	-

* Data are means ± SD

C. Zeta Potential Measurement

The values of zeta potential for all the batches are presented in Table III, which were found in range of -17.2 to -61.3. The results revealed that the zeta potential of the niosomes increases on the way to negative with increasing the HLB values of the surfactants. The niosomes formulated with sorbitan oleate, found to have the least zeta potential, ranging -17.2 to -45.9 and the vesicles formulated with sorbitan laurate, found to have the higher zeta values, ranging -22.1 to -61.3. The influence of HLB values of surfactants on zeta potential can also be explained in terms of surface energy, which increases with increasing the HLB values of surfactants towards the hydrophilicity. Increase in surface energy of niosomes, also results in increasing the zeta potential of

vesicles towards negative. Further, it was also found that the zeta potential decreased with decreasing the surfactant concentration from 2.5:1 to 1:1.5 with respect to the CHOL concentration in each surfactant type. This may be supposed that the decreased surfactant concentrations and increased CHOL concentrations lead to increase the vesicle size of the niosomes and increase in vesicle size further leads to decrease the surface area and surface charge of the vesicles; finally decrease in zeta potential values [11].

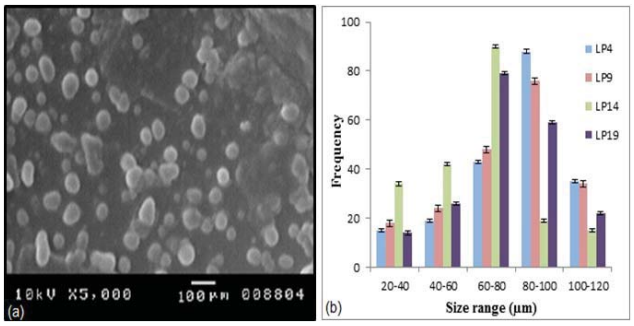

Fig. 1 (a) SEM image of drug-loaded niosomes, (b) Size distribution plots of all the optimized batches

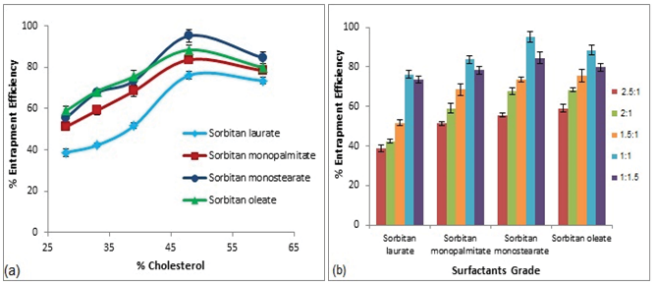

Fig. 2 (a) % Entrapment efficiency vs. % CHOL indicating the effect of CHOL concentration on % entrapment efficiency, (b) % Entrapment efficiency vs. surfactant grades with several surfactant: CHOL ratios

D. Entrapment Efficiency

The entrapment efficiency of the prepared niosomes was found in range of 38.8% to 95.2% and given in Table III. The results have shown the effects of lipid concentration and surfactant types on the entrapment efficiency of niosomal vesicles. It was found that the entrapment efficiency increased with increasing the CHOL concentration at a ratio up to 1:1 with respect to the surfactant, after which it became constant or decreased (Fig. 2). The viscosity of the prepared niosomes was found to increase with increasing the concentration of CHOL, indicates more membrane stringency and good physical stability [12]. The entrapment efficiency was found good with surfactant: CHOL ratio 1:1, with all grades of surfactants but niosomes formulated with sorbitan monostearate have shown the highest drug entrapment compared with other grades of surfactants (Fig. 2).

E. In vitro Drug Release Study

In vitro release studies of the selected batches (LP4, LP9, LP14 and LP19) were performed by using dialysis method. The results revealed that the release of losartan potassium was retarded for 24 hours. he percentage drug released at the end of 24 hours is given in Table III. As per the results, the maximum percentage of losartan potassium (99.19 %) was released from LP14 (with sorbitan monostearate), followed by LP9 i.e., 92.98% (with sorbitan monopalmitate), LP19 i.e., 88.42% (with sorbitan oleate) and LP4 i.e., 84.24% (with sorbitan laurate) at the end of 24 hours.

F. Release Kinetics Modeling

The *in vitro* drug release data was fitted to several release kinetics models to envisage the release mechanism of drug from the prepared niosomes. The results revealed that all the formulations were best explicated by zero order release kinetics (plots show highest linearity) followed by Higuchi release kinetics indicates the drug release from the vesicles was independent to the drug concentration. The formulation LP14 has shown the highest linearity among all the formulations indicates the best fit to zero order release kinetics. However, the drug release from all the formulations was also found adjacent to Higuchi kinetics, referred to the square root kinetics (Table IV), according to which drug diffuses at slower rate with increasing the distance for diffusion. Further, all the niosomal formulations have also shown good linearity for Korsmeyer-Peppas model and the values of release exponent (*n*) were found in the range of 0.45 to 0.89, which indicates the combination of diffusion and erosion mechanisms, so called anomalous release mechanism. This specifies that the drug release may be controlled by more than one mechanism [13].

TABLE IV
RELEASE KINETICS OF NIOSOMAL FORMULATIONS

F. code	Zero order	First order	Higuchi	HC Model	Korsmeyer-Peppas model	
	r^2	r^2	r^2	r^2	r^2	n
LP4	0.988	0.893	0.914	0.857	0.963	0.50
LP9	0.985	0.928	0.953	0.909	0.968	0.55
LP14	0.994	0.876	0.906	0.813	0.967	0.63
LP19	0.926	0.801	0.922	0.883	0.961	0.60

HC: Hixson-Crowell

G.In vivo Study

The average plasma drug concentration time profile in rats after a single oral dose of losartan potassium (10 mg/kg) as pure drug solution and niosomal dispersion (containing both entrapped and unentrapped drug) are shown in Fig. 3. Various pharmacokinetics parameters of losartan potassium were calculated from individual profiles, the mean values of which are given in Table IV. The niosomal formulation showed significantly ($P < 0.05$) higher values for $AUC_{0\rightarrow\infty}$, C_{max}, T_{max}, $T_{1/2}$ and MRT; and significantly ($P < 0.05$) lower values for absorption (K_a) and elimination (K) rate constants as compared with pure losartan potassium solution. The increase in $AUC_{0\rightarrow\infty}$ and MRT values and decrease in K_a value reflect the release retarding effect of niosomal formulation, which was also investigated by *in vitro* release studies in terms of controlled release [14].

The higher values of $AUC_{0\rightarrow\infty}$ and C_{max} for niosomal formulation may be due to the enhanced absorption of drug-

loaded niosomes through gastrointestinal track after oral administration and T_{max}, $T_{1/2}$ and MRT may be due to the release retarding effect of niosomal vesicles. Further the $AUC_{0\rightarrow\infty}$ value for niosomal formulation was compared with that of pure drug solution to determine the relative bioavailability and the mean ratio was found to be 2.56 (± 0.74), showed more than two fold increase in the oral bioavailability of losartan potassium by niosomal formulation [15].

TABLE V
PHARMACOKINETIC PARAMETERS OF LOSARTAN POTASSIUM IN RATS AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 10 MG/KG AS PLANE DRUG SOLUTION AND NIOSOMAL FORMULATION*

Parameters	Plane drug solution	Niosomal formulation
$AUC_{0\rightarrow\infty}$ (µg.h.ml^{-1})	7854.01 ± 564	20107.51 ± 845
C_{max} (µg.ml^{-1})	1141.21 ± 92	1482.61 ± 125
T_{max} (h)	1.22 ± 0.15	1.73 ± 0.12
$T_{1/2}$ (h)	4.75 ± 1.12	9.29 ± 2.87
MRT (h)	6.85 ± 3.55	17.03 ± 5.35
K_a (h^{-1})	1.24 ± 0.36	0.83 ± 0.09
K (h^{-1})	1.15 ± 0.07	0.08 ± 0.04

* Data are means ± SD

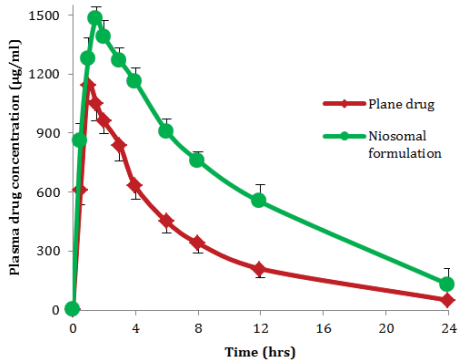

Fig. 3 Mean plasma losartan potassium concentration time profiles (± SD) in rats after oral administration of plane drug solution (●) and niosomal formulation (♦) as a single dose of 10 mg/kg (n=6)

IV. CONCLUSION

All the prepared niosomal vesicles were found, spherical in shape ranging 54.98 µm to 107.85 µm in size and have zeta values within the acceptable range. The percentage of drug entrapment was found higher, with surfactant and CHOL ratio 1:1, with all grades of surfactants but sorbitan monostearate has shown the highest drug entrapment compared with the other grades. *In vitro* study revealed that the formulation LP14 (prepared with sorbitan monostearate) has shown the maximum drug release (99.19 %) at the end of 24 hours. Further *in vitro* release profile was also fitted to various release kinetics models to predict the release mechanism of drug from the prepared niosomes and the results revealed that all the formulations were best explained by zero order release kinetics. The results of *in vivo* study have shown more than two fold increase in oral bioavailability of losartan potassium by niosomal vesicles compared with the pure losartan potassium solution, in same dose. So it is concluded that the niosomal vesicles could be the promising drug delivery system for the bioavailability enhancement of losartan potassium and also to release it in the controlled manner.

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