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Full Paper

Formulation and characterisation of valsartan proniosomes

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Abstract: Non-ionic surfactant vesicles of valsartan, an angiotensin II inhibitor, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and in vitro drug release. Stability study was carried out to investigate the leaching of drug from the proniosomal system during storage. The results showed that valsartan in all the formulations was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of valsartan from proniosomes were observed upon varying the type of surfactant and cholesterol content. The encapsulation efficiency of proniosomes prepared with Span 60 was superior to that prepared with Span 40. A preparation with 9:2:9 ratio of Span 60, cholesterol and lecithin gave maximum encapsulation efficiency (71.50%) and release results (Q_{24h}= 75%) as compared to other compositions. Proniosomal formulations showed fairly high retention of valsartan inside the vesicles at refrigerated temperature (4-8^{o}C) up to 1 month.

Keywords: valsartan, proniosomes, niosomes, encapsulation efficiency, drug delivery

INTRODUCTION

Several classes of medications collectively referred to as antihypertensive drugs are available for treating hypertension. One such class is angiotensin II antagonists. Valsartan belongs to this category and is used as a choice for patients with heart failure who are unable to tolerate angiotensin converting enzyme (ACE) inhibitors in the management of hypertension. It is currently available as tablets and hard gelatin capsules in the market. The drug is rapidly absorbed following oral administration with a bioavailability of about 23% [1]. Valsartan is poorly soluble and the aqueous solubility is reported to be less than 1 mg/mL. It has a low molecular weight (435.5 g/mol) and melting point (116-117^{o}C) with a low partition coefficient (4.5) [1-2].

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Some efforts have been made to enhance the solubility of valsartan to study its effect on the bioavailability of the drug. Valsartan/hydroxypropyl-β-cyclodextrin complex has been reported to significantly increase solubility and decrease the rate of valsartan degradation [3]. A Gelucire 50/13-based dispersed granule formulation has also been reported very recently [4]. The effect of various terpenes including a diterpene, forskolin (a putative penetration enhancer), on skin permeation of valsartan was investigated by Rizwan et al [5]. A self-microemulsifying drug delivery system (SMEDDS) has been developed by Dixit et al. [6] to enhance the diffusion rate and oral bioavailability of valsartan.

Vesicular carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms because these particles can act as drug reservoirs. In recent years, non-ionic surfactant vesicles, also referred to as niosomes, have been studied as an alternative to conventional liposomes in drug delivery [7-9]**.** Compared to liposomes (phospholipid vesicles), they offer higher chemical stability, lower cost and greater choice of surfactants. However, even though niosomes exhibit good chemical stability during storage, there may be problems of physical stability in niosomal dispersions. Aqueous suspensions of niosomes may exhibit aggregation, fusion, leaking of entrapped drug or hydrolysis of encapsulated drug, thus limiting the shelf life of the dispersion [10].

The proniosomal approach minimises the above-mentioned problems, as it involves a dry product or a liquid crystalline gel that can be hydrated immediately before use [11-12]. Ease of transfer, distribution, measuring and storage makes proniosomes a versatile delivery system. Proniosomes are water-soluble carrier particles that are coated with surfactant and can be hydrated to form a niosomal dispersion immediately before use on brief agitation in hot aqueous media [13].

Various studies have demonstrated the successful use of proniosomes for delivery of antihypertensive drugs. For instance, Blazek-Welsh and Rhodes [14] prepared proniosomes of alprenolol hydrochloride using Span 60, dicetyl phosphate and cholesterol by slurry method. Maltodextrin was used as carrier that produced proniosomes with greater drug loading. Gupta et al. [15] investigated the potential of proniosomes as a transdermal drug delivery system for captopril. Proniosomes were found promising for transdermal delivery of this drug leading to a reduction in side effects. Thakur et al. [16] reported a proniosomal transdermal therapeutic system for losartan potassium .

The aim of this study is to investigate the feasibility of formulation of proniosomes of valsartan. Vesicles prepared were characterised by optical, scanning and transmission microscopy for vesicle formation and morphology. Drug encapsulation efficiency and release studies were carried out. Finally, a stability study of proniosomal formulations was also performed to investigate the leaking of the drug during storage.

MATERIALS AND METHODS

Chemicals and Reagents

Sorbitan monopalmitate (Span 40) and sorbitan monostearate (Span 60) were purchased from Shah Scientific, Mumbai, India. Soya lecithin (95%) was obtained as a gift sample from Ind Swift, Parwanu, India. Cholesterol (AR grade) was purchased from Central Drug House Pvt. Ltd, New Delhi, India. Absolute ethanol was purchased from Changshu Yangyuan Chemical, China.

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Disodium hydrogen phosphate, sodium chloride and potassium dihydrogen phosphate (AR grades) were purchased from Qualigens Fine Chemicals Ltd. Mumbai, India for preparation of phosphate buffer saline (PBS, pH 7.4). Valsartan (99.8%) was obtained as a gift sample from Jubilant Organosys, Noida, India. Dialysis membrane (MW cut-off: 8000-10,000) was purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

Equipment

Valsartan was estimated in samples by a UV-spectrophotometer (UV-1800, Shimadzu, Japan). A light microscope with digital camera (Coolpix S 220, Nikon, Japan), a scanning electron microscope (JSM-6100, Jeol, Japan) and a transmission electron microscope (Hitachi 7500, Canada) were used for morphological characterisation of vesicles. The pH of PBS was set using a pH meter (Max 962-P, Max Electronics, India). A centrifuge (REMI Group, Mumbai, India) and a sonicator (Power Sonic 410, India) were used.

Preparation of Proniosomes and Niosomes

Valsartan proniosomes were prepared by coacervation phase separation method (method modified from literature by Perrett et al. [17]). The composition of different proniosomal formulations is listed in Table 1. Briefly, drug (valsartan), surfactant (Span 40 or Span 60), lecithin and cholesterol were mixed with 2.5 ml of absolute ethanol in a wide-mouth glass tube. The tube was covered with a lid and warmed for 5 min at 65±3^{o}C in a water bath. PBS (1.6 ml) was added and the mixture was further warmed for about for 2 min so that a clear mixture was obtained. It was allowed to cool at room temperature until the dispersion was converted to a proniosomal gel. The gel was transformed to niosomes by hydrating with PBS (10 ml) at 80^{o}C by gentle mixing. The niosomes were sonicated twice for 30 seconds each with a sonicator and then used for further study [14].

Table 1. Composition of proniosomal formulations prepared

Proniosomal code	Drug (mg)	Span 60 (mg)	Span 40 (mg)	Lecithin (mg)	Cholesterol (mg)
PN 1	100	1800	-	1800	200
PN 2	100	1800	-	1800	400
PN 3	100	1800	-	900	200
PN 4	100	-	1800	1800	200
PN 5	100	-	1800	1800	400
PN 6	100	-	1800	900	200

Determination of Encapsulation Efficiency

Per cent encapsulation efficiency (EE) was determined by centrifugal method [18]. The proniosomal gel was converted to a niosomal dispersion, which was centrifuged (18000 rpm) for 40 min at 5^{o}C in order to separate unentrapped drug. The supernatant was taken and diluted with PBS. The drug concentration in the resulting solution was assayed spectrophotometrically at 250 nm. The

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percentage of drug encapsulation was calculated by the following: 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.

Characterisation of Valsartan Proniosomes

Optical microscopy and vesicle size determination

A drop of niosomal dispersion prepared from proniosomes was spread on a glass slide and examined for the vesicle structure and presence of insoluble drug crystals under the light microscope with varied magnification power. Photomicrographs were taken for niosomes using a digital camera with 6X optical 200 m.

The proniosomal gel (100 mg) was hydrated with PBS (10 ml) in a small test tube by manual shaking for 5 min and the resulting niosomes were observed under optical microscope at 100 X magnification. The average size of vesicles was measured using calibrated ocular and stage micrometer in the microscope.

Scanning electron microscopy

The niosomes formed from the hydration of proniosomal gel were mounted on an aluminum stub with double-sided adhesive carbon tape. The vesicles were then sputter-coated with gold/palladium using a vacuum evaporator and examined with the scanning electron microscope equipped with a digital camera at 25kV accelerating voltage [19].

Transmission electron microscopy

The morphology of the hydrated niosomal dispersions prepared from proniosomes was also determined by transmission electron microscopy. A drop of niosomal dispersion was applied to a carbon-coated 300-mesh copper grid and left to adhere on the carbon substrate for about 1 min. The remaining dispersion was removed by a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 35 seconds and again the solution in excess was removed by the tip of filter paper. The sample was air-dried and observed under the transmission electron microscope at 90kV [13].

In vitro release study

In vitro release pattern of niosomal suspension prepared from proniosomes (as prescribed above) was carried out using a dialysis bag (high-media dialysis membrane, 8000-10,000 MW cut-off) as a donor compartment [20]. An accurately measured amount of valsartan niosomes, equivalent to 20 mg valsartan, were taken in the dialysis membrane and placed in a beaker containing 75 ml of PBS, which acted as receptor compartment. Previously, the dialysis membrane was soaked in warm water for 10 min and both ends were sealed with closure clips after adding the niosomal preparation. The beaker was placed over a magnetic stirrer (100 rpm) and maintained at 37 ± 1^{o}C. At predetermined time intervals during 24 hr, aliquots (1ml) were withdrawn and replaced with fresh buffer. The sink condition was maintained throughout the experiment. The withdrawn samples were appropriately diluted and analysed for drug content spectrophotometrically at 250 nm using PBS as blank. The results were the mean values of three runs.

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Stability of valsartan proniosomes

A physical stability test was carried out to investigate the leaching of drug from proniosomes. The proniosomal samples were sealed in 20-ml glass vials and stored at refrigeration temperature (4-8^{o}C) and at 37^{o}C for one month. The EE of all the samples was determined in the same manner as prescribed previously after one month [21].

Statistical analysis

Statistical analyses of % EE and in vitro release of the proniosomal formulations were performed using one-way analysis of variance (1-way ANOVA) and paired t-test respectively (Graph Pad, version 3.0, San Diego, CF). The level of significance was taken at p value < 0.05.

RESULTS AND DISCUSSION

Optical Microscopy and Vesicle Size Determination

The photomicrographs of hydrated PN1 and PN2 proniosomal formulations (Table 1), composed of Span 60 and cholesterol in 9:1 and 4.5:1 ratios, are shown in Figure 2 and Figure 3 respectively. The photographs reveal that the niosomes are unilamellar vesicles having spherical shape and no aggregation or agglomeration is observed. Apparently, PN1 niosomal formulation gives vesicles of larger sizes.

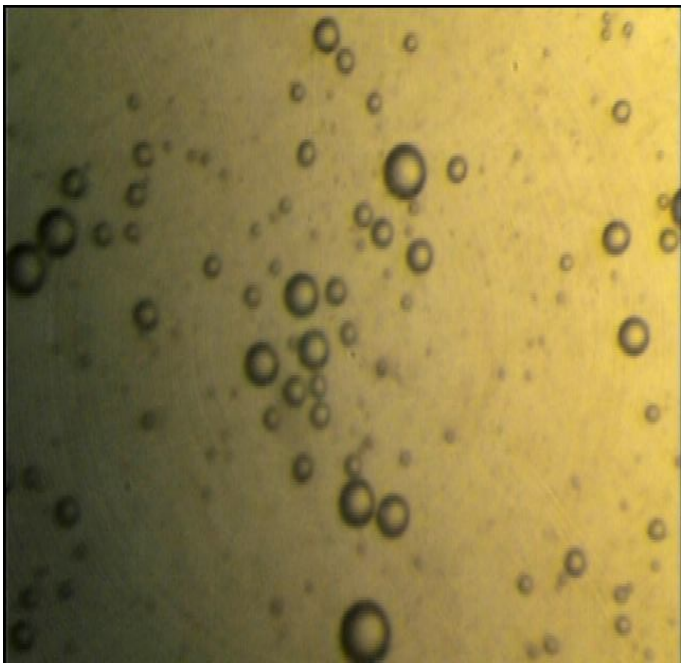

Figure 2. Photomicrograph of hydrated PN1 proniosomal formulation

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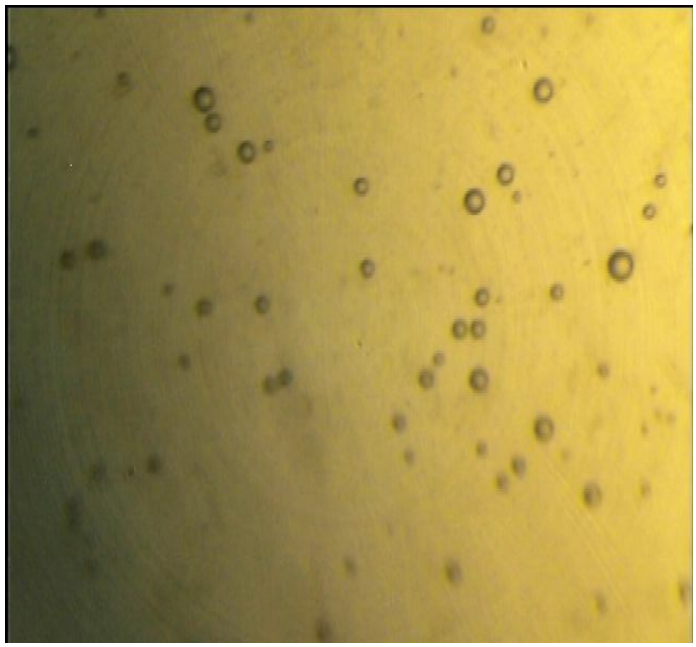

Figure 3. Photomicrograph of hydrated PN2 proniosomal formulation

The mean particle sizes of all the hydrated proniosomal formulations are shown in Table 2, which shows that the niosomes composed of Span 60 are larger in sizes than those obtained using Span 40. Span 60 has longer saturated alkyl chains compared to Span 40 [22] and it was reported that surfactants with larger alkyl chains generally give larger vesicles [23]. This seems to account for the high EE obtained with Span 60 proniosomes. Moreover, increasing cholesterol content or reducing lecithin content contributed to an increase in hydrophobicity with consequent reduction in vesicle sizes as listed in Table 2.

Scanning and Transmission Electron Microscopy

Results of scanning and transmission electron microscopic study of niosomes prepared from PN1 and PN2 proniosomal formulations are shown in Figures 4-7. Most of the vesicles are well identified, spherical and discreet with sharp boundaries having large internal aqueous space.

Encapsulation Efficiency

The EE of all proniosomal formulations are reported in Table 2. From the table, increase in cholesterol, one of the common additives for preparing stable proniosomes, is seen to increase EE of valsartan. An increase in cholesterol content has also been found to result in increase in microviscosity of the membrane leading to more rigidity of the bilayers [23]. Cholesterol seems to have an ability to cement the leaking space in a bilayer membrane [24].

All Span-type surfactants have the same head group with different alkyl chains. Increase in the alkyl chain length has been found to lead to a higher EE [25]. Span 60 has a longer saturated alkyl chain (C_{16}) compared to that of Span 40 (C_{14}). A larger alkyl chain lowers the HLB value of a surfactant and this tends to increase EE of the drug [22]. It is clear that the PN2 formula, which was

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composed of Span 60, cholesterol and lecithin in a 9:2:9 ratio, seems to be the most suitable for an efficient encapsulation of valsartan as it exhibits the highest EE (71.5 %).

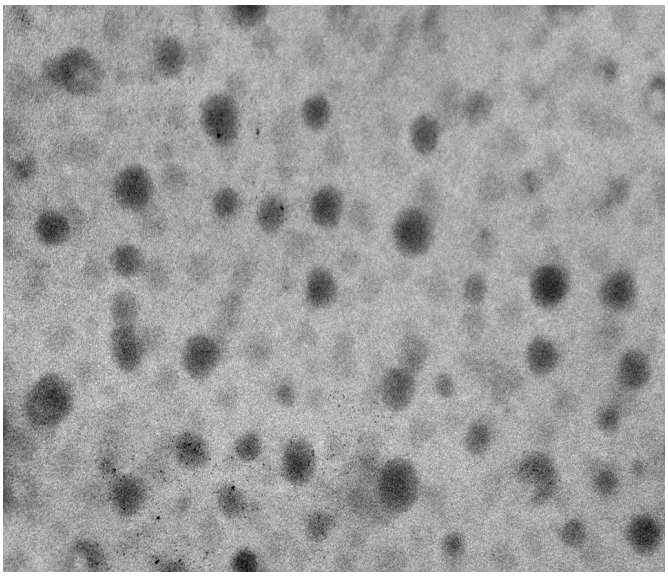

Figure 4. Transmission electron micrograph of hydrated PN1 proniosomal formulation (at 90 kV with magnification 100,000 X)

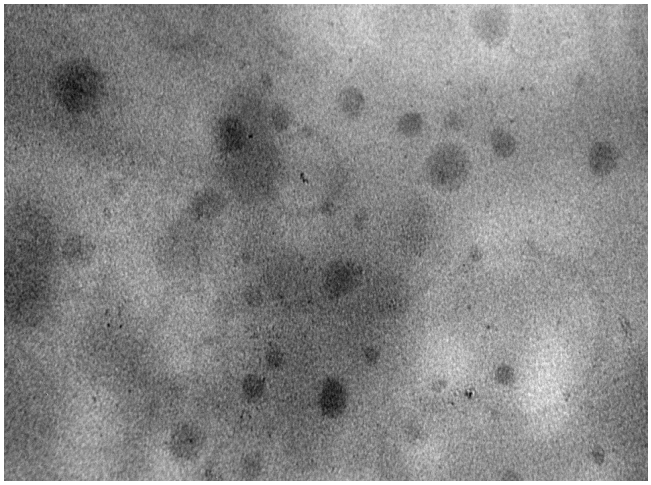

Figure 5. Transmission electron micrograph of hydrated PN2 proniosomal formulation (at 90 kV with magnification 100,000 X)

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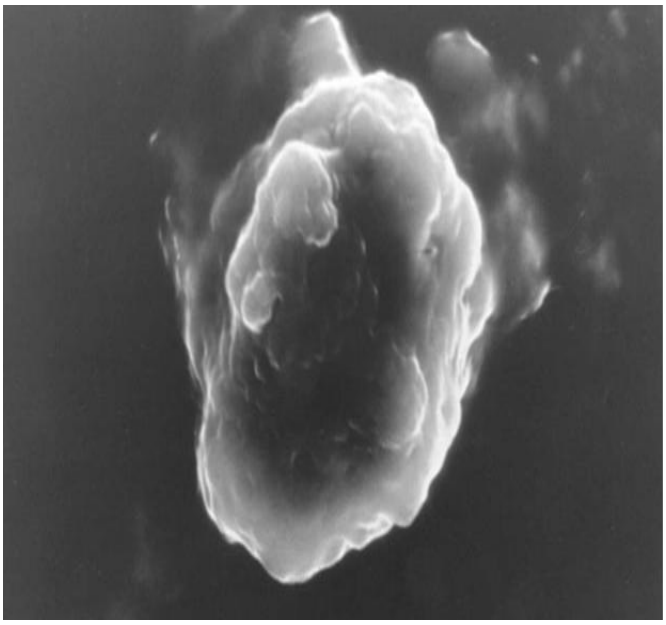

Figure 6. Scanning electron image of hydrated PN1 proniosomal formulation (at 25 kV with magnification 2,000 X)

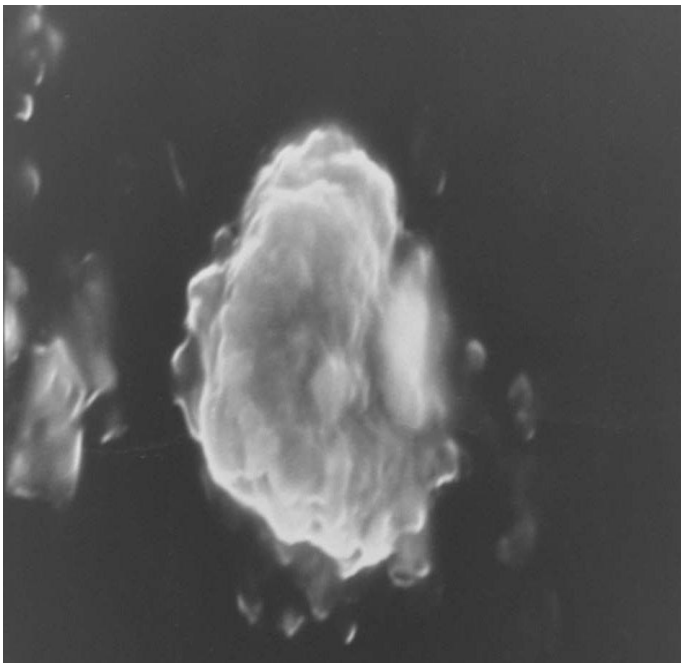

Figure 7. Scanning electron image of hydrated PN2 proniosomal formulation (at 25 kV with magnification 2,000 X)

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Table 2. EE and vesicle size of hydrated proniosomes

Formulation	Surfactant	Lecithin (mg)	Cholesterol (mg)	EE (%) ± S.D.	Vesicle size (μm) ± S.D.
PN 1	Span 60	1800	200	66.97 ± 2.35	5.26 ± 0.46
PN 2	Span 60	1800	400	71.47 ± 1.61	4.19 ± 0.082
PN 3	Span 60	900	200	69.47 ± 1.16	3.61 ± 0.105
PN 4	Span 40	1800	200	49.43 ± 2.11	3.36 ± 0.198
PN 5	Span 40	1800	400	51.83 ± 1.59	3.03 ± 0.066
PN 6	Span 40	900	200	43.34 ± 4.13	2.80 ± 0.025

In Vitro Release Study

The dialysis method was used to investigate the in vitro valsartan release from niosomes. Results are shown in Figure 8. The percentage of the drug released after 24 hr from the niosomal vesicles are shown in Table 3. Formulations which have higher cholesterol content (PN2 and PN5) are seen to have less drug release over a period of 24 hr. Hence, increase in cholesterol ratio seems to result in a more intact bilayer and consequent reduction in permeability.

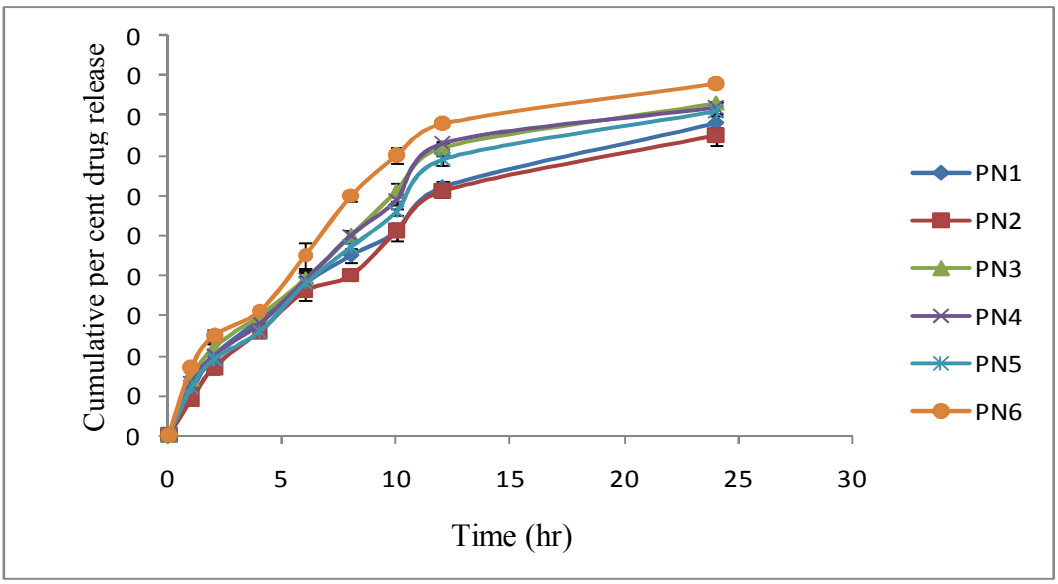

Figure 8. In vitro drug release of niosomes prepared from various proniosomal formulations

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Table 3. In vitro release of valsartan from niosomes prepared from various proniosomal formulations after 24 hr

Formulation	Per cent release ± S.D.
PN 1	78.05 ± 1.87
PN 2	74.96 ± 2.23
PN 3	83.02 ± 0.62
PN 4	82.08 ± 1.37
PN 5	81.01 ± 2.31
PN 6	88.09 ± 0.34

By inspection of the data, it is also evident that proniosomal formulation with less amount of lecithin gives a faster rate of drug release, probably owing to disruption of structure of vesicles having a reduced amount of lecithin. However, this increase in release rate was found to be insignificant ($p>0.05$) in both cases. Among all formulations, those with Span 40 showed statistically significant ($p<0.05$) increase in release cf. Span 60, keeping all other additives the same. The large vesicle size of Span 60 formulations also tends to act as barrier to the drug release thereby reducing it. It is to be noted that the in vitro release results are consistent with those of EE; PN2 proniosomes with highest EE (71.47%) show lowest drug release (74.96%) after 24 hr. Similar results were obtained by Guinedi et al. [22].

Mathematical models are commonly used to predict the release mechanism and compare release profile. For all the formulations (PN1 to PN6), the cumulative per cent drug release vs time (zero order), the cumulative per cent drug release vs square root of time (Higuchi plot), and log cumulative per cent drug remaining vs time (first order) were plotted separately (not shown here). In each case, r^2 value was calculated from the graph and reported in Table 4. Considering the determination coefficients, Higuchi model was found to fit the release data best. This demonstrates that valsartan molecules were dispersed in the proniosomes matrix and there was no interaction between the drug and proniosomes material. The first order release model fitting of the release data shows that the release rate was concentration- dependent. It is therefore concluded that the drug was released from proniosomes by a diffusion-controlled mechanism. The results are in good consistency with the experimental results observed by Guinedi et al. [22].

Stability Studies

Physical stability of proniosomal formulations were studied for a period of one month. The EE were determined for all proniosomal formulations stored at 4-8^{o}C and 37^{o}C as shown in Figure 9, which indicates insignificant decrease in EE of proniosomes stored at 4-8^{o}C: approximately 90% of valsartan was retained in all proniosomal formulations after the one-month period. Thus, both Span 40 and Span 60 proniosomes of valsartan seemed to exhibit good stability at low temperature.

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Table 4. Kinetic analysis release data of valsartan proniosomal formulations

Formulation	Zero order (r^2)	Higuchi model (r^2)	First order (r^2)
PN 1	0.886	0.986	0.982
PN 2	0.885	0.974	0.969
PN 3	0.852	0.965	0.958
PN 4	0.849	0.958	0.944
PN 5	0.871	0.966	0.962
PN 6	0.813	0.953	0.951

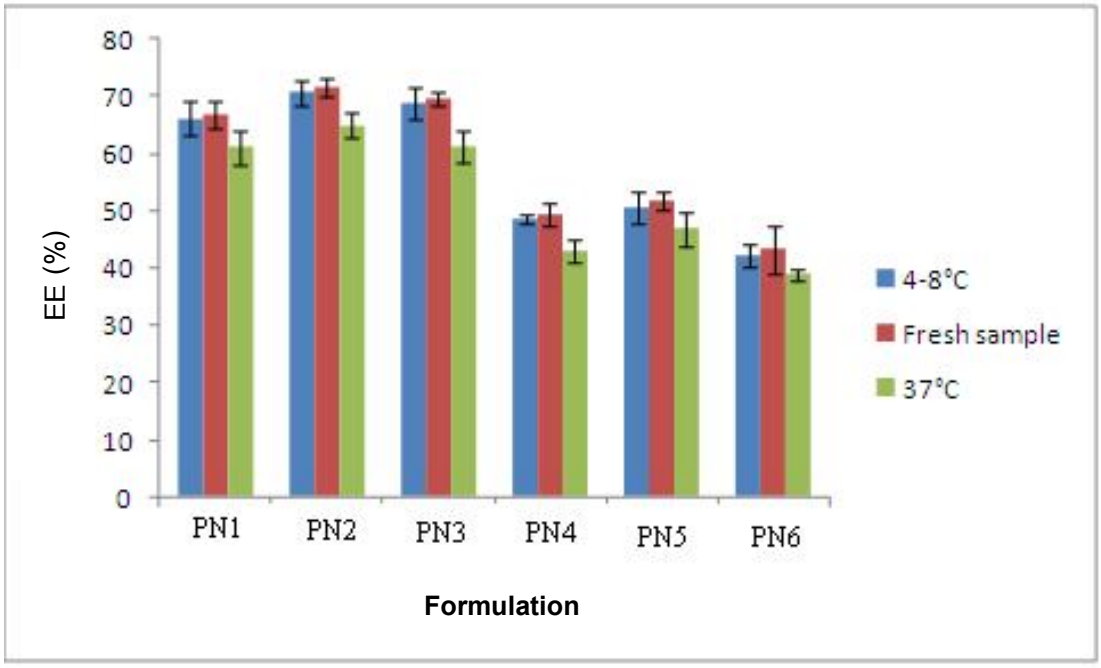

Figure 9. Comparison of EE of valsartan proniosomal formulations after one month

CONCLUSIONS

Using coacervation phase separation method, valsartan has been successfully incorporated in proniosomal formulations which can be potentially useful for delivery of this drug. Results of the present work have shown that surfactant type and content of cholesterol and lecithin affect the encapsulation efficiency and drug release rate from proniosomes. A maximum encapsulation efficiency of 71% and drug release of 88% after 24 hr have been attained. Encapsulation efficiency of proniosomes formed by Span 60 was observed to be higher compared to that obtained with Span 40. Valsartan proniosomes were also found to be quite stable at 4-8^{o}C over a one-month period. This work has established the foundation for future study on the potential of valsartan-loaded niosomes for a transdermal delivery system.

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