INTRODUCTION

Vesicular systems have drawn attention as promising carriers that are widely investigated for controlled delivery of pharmaceuticals to enhance their bioavailability, selectivity, and minimize toxicity. Due to their unique structure as a lamella consisting of amphiphiles, they can accommodate both the hydrophilic and hydrophobic molecules within their aqueous core and lipid bilayers, respectively. These comprise of liposomes, niosomes, ethosomes, transferosomes and bilosomes [1]. While liposomes require high formulation cost and are highly susceptible to oxidation, niosomes, on the other hand, are cheap and stable over a longer period of time in different conditions. Niosomes are made up of non-ionic surfactants and cholesterol. Surfactants commonly employed include polyoxyethylene fatty acid esters (tweens), sorbitan fatty acid esters (spans), alkyl ethers, and polyoxyethylene alkyl ethers (bijs) [2]. Niosomal formulations are able to traverse the intestinal epithelium through different routes as transcellular, paracellular transport and/or nonspecific uptake mechanisms (by M-cells and intestinal epithelial cells). Therefore, they have been extensively studied in improving oral bioavailability of poorly absorbed drugs [3].

Benazepril hydrochloride is an angiotensin-converting enzyme inhibitor. It is used in the management of hypertension and heart failure. The problem with the drug is poor oral absorption (37%) [4]. Consequently, this arises the need for developing novel vesicular carriers to improve its oral bioavailability. The aim of the present work was to formulate niosomal formulations of benazepril hydrochloride in an attempt to overcome the hurdles associated with it's poor oral absorption.

METHODS

Nine formulations were prepared with various ratios of sorbitan monostearate (span 60), sorbitan monopalmitate (span 40) and polyoxyethylene 2 stearyl ether (brij 72) as non-ionic surfactants, cholesterol as a stabilizing agent and soya lecithin as a charge imparting agent. Formulations were centrifuged at 14000 round per minute for 90 min at 4 °C in a cooling centrifuge (Hermle labortechnik, Germany) to separate the free drug from the entrapped drug. The obtained formulations were stored in 4 °C for further analysis [5].

Preparation of niosomes

Niosomal formulations were prepared using the conventional thin film hydration method. Firstly, surfactant and cholesterol in amounts given in table (1) were dissolved in 10 ml chloroform and added into a 100-ml flask of the rotary evaporator (R-210, Buchi). Then, chloroform was allowed to evaporate under reduced pressure and gentle rotation in a water bath thermostated at 60±2 °C until observing a thin lipid layer. An aqueous phase containing 10 mg benazepril HCL dissolved in 10 ml distilled water was added to the lipid phase at atmospheric pressure under gentle rotation in a water bath at 60±2 °C for 60 min, followed by sonication in a bath sonicator (SB 25-12 DTDN) for additional 60 min. Afterwards, the obtained formulations were stored in 4 °C for further analysis [5].

Characterization of benazepril hydrochloride niosomes

Vesicle size and polydispersity index (PDI)

The prepared formulations were observed under an optical microscope (Novex, Holland) to confirm vesicles formation. Microphotographs were taken by a camera attached to the microscope. Then, further analysis using image j software was undertaken to measure the mean diameter and polydispersity index (PDI) for vesicles that lie within the micro size range [6]. Measurements were carried out in triplicate for each formulation and presented as the average ±standard deviation (SD).

Entrapment efficiency (EE %)

Niosomal formulations were centrifuged at 14000 round per minute (rpm) for 90 min at 4 °C in a cooling centrifuge (Hermle labortechnik, Germany) to separate the free drug from the entrapped drug. The obtained clear supernatant fraction was then analyzed spectrophotometrically at 241 nm [7].
The percent entrapment efficiency was calculated using the below equation [8]:

\[
EE\% = \frac{\text{Total-drg-free drug}}{\text{total drug}} \times 100
\]

### Table 1: The composition of niosomal formulations

| Formulation | Benazepril HCl | Span 60 | Span 40 | Brij 72 | Cholesterol | Soya lecithin |
|-------------|----------------|--------|--------|----------|-------------|--------------|
| F1          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F2          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F3          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F4          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F5          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F6          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F7          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F8          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |
| F9          | 10 mg          | 400 mg | 400 mg | 100 mg   |             |              |

Formulations (F1-F3), (F1-F6) and (F4-F9) were elaborated to investigate the impact of surfactant type, amount of cholesterol and inclusion of lecithin on the various properties of the prepared formulations, respectively.

### In vitro release studies

Release studies were carried out for both pure benazepril hydrochloride and the loaded niosomal formulations. Each formulation was instilled in a dialysis membrane bag with a molecular weight cut off (8000-12 000 KDa) and sealed from both sides [9]. It was then tied to the shaft of dissolution apparatus (Vanguard/USA) and immersed in 500 ml of de-ionized water at constant temperature 37±0.5 °C and rotation speed of 50 rpm. Thereafter, Samples of 5 ml were taken at predetermined intervals and replaced with fresh media so that the sink condition is maintained. Percentage drug released was assayed using a UV-Vis spectrophotometer (Shimadzu, UV-Vis 1650, Japan) at 241 nm [10].

**Results and Discussion**

#### Preparation of niosomes

Based on the collected data, It has been concluded that the type of surfactant, amount of cholesterol and inclusion of soya lecithin are influential factors that governs the vesicle size, poly-dispersion, entrapment efficiency and in vitro release profile.

#### Vesicles size and polydispersity index (PDI)

The measured vesicles diameters of all niosomal formulations are listed in the table (2). The results reveal that when other variables were fixed, the stearyl chain (C18) surfactants (span 60 and brij 72) produced significantly (p<0.05) bigger vesicles than palmityl chain (C16) surfactant (span 40). This might be explained by the effect of the alkyl chain length. Longer alkyl chains produce wider bilayers; consequently, larger vesicles are attained. Similar findings were obtained with glimepride niosomes [13].

The results also showed that increasing cholesterol content from 100 mg to 400 mg led to lowering vesicles size significantly (p<0.05). The intercalation of cholesterol within the bilayers enhances their hydrophobic nature, thereby decreasing water uptake and decreasing both the aqueous core and vesicle size [14].

On the other hand, the addition of soya lecithin produced significantly larger vesicles (p<0.05) when comparing F7, F8 and F9 with F4, F5and F6, respectively. Soya lecithin is a charge inducing agent that contains phosphatidylcholine as a major component. It imparts a negative charge on the surface of the vesicle allowing more water entrapment within the aqueous core. Therefore, larger vesicles are formed [15]. Polydispersity index (PDI) is considered a measure of the degree of the homogeneity in vesicle size distribution within the prepared formulations. PDI value below 0.3 is considered as an indication for monodisperse systems according to the literature [16]. The calculated PDI for the niosomal formulations ranged between 0.09 and 0.34.

### Table 2: Entrapment efficiency%, vesicle size, polydispersity and zeta potential of niosomal formulations

| Formulation | EE%   | Vesicle size±SD (micrometer)* | PDI     | ζ potential(mV) |
|-------------|-------|-------------------------------|---------|-----------------|
| F1          | 93.8  | 7.02±3.57                     | 0.26    | -39.14          |
| F2          | 90.4  | 4.57±2.337                    | 0.26    | -42.68          |
| F3          | 92.9  | 7.38±4.34                     | 0.34    | -12.22          |
| F4          | 86.8  | 5.01±2.1                      | 0.17    | -45.14          |
| F5          | 80.4  | 3.9±1.7                       | 0.19    | -35.03          |
| F6          | 84.7  | 5.24±1.26                     | 0.24    | -12.93          |
| F7          | 97.8  | 8.72±4.4                      | 0.25    | -32.13          |
| F8          | 94.4  | 6.21±2.5                      | 0.16    | -45.91          |
| F9          | 95.9  | 7.01±2.2                      | 0.09    | -20.12          |

*(n=3) (mean±standard deviation)
Entrapment efficiency (EE %)

The results of EE% (table 2) revealed that formulations F1 (span 60) and F3 (brij 72) gave higher (p<0.05) EE% than F2 (span 40). This may be ascribed to the entrapment of the hydrophilic drug (benazepril HCL) in the internal aqueous core of niosomes which is mainly affected by the length of the alkyl chain of the surfactant employed. These results are compatible with the vesicle diameters measurements where longer alkyl chain surfactants (span 60 and brij 72) produced bigger vesicles. As a result, provided more space for the trapped solute [17].

The increment of cholesterol content from 100 mg in (F1, F2 and F3) to 400 mg in (F4, F5 and F6) has led to significant lowering in EE% (p<0.05). This might be due to the decrease in volume diameter [18].

The impact of charge inducing agent on EE% was investigated by the inclusion of 50 mg soya lecithin into formulations (F7, F8 and F9). As expected, EE% was enhanced significantly (p<0.05) in these formulation as a result of their relatively large vesicles which allows for more solute entrapment [19]. Besides, it might be due to the electrostatic attractions between the cationic drug and the negatively charged vesicles. Similar observations were obtained in tamoxifen citrate loaded span-based nanovesicles [20].

In vitro release studies

All formulations showed sustained release when compared to a pure drug which exhibited percent release of 99.9% within 15 min (fig. 1). This might be due to the inherent nature of niosomes as a depot that encapsulate the drug and releases it in a controlled manner. Formulations F1, F2 and F3 were elaborated using span 60, span 40 and brij 72, along with cholesterol in ratio of (4:1) to investigate the impact of surfactant structure on the release profile from niosomal formulations. The release profile followed the trend span 60<span 40<brij 72 where span 60 formulation exhibited the slowest release rate (p<0.05) among the other formulations. It has been previously reported that more tight and compact niosomes results from more hydrophobic span surfactants when hydrated in presence of cholesterol [21]. These findings agree with the previously reported results of paclitaxel niosomes [3].

Span 40 formulation exhibited a faster release rate (p<0.05) compared to span 60. This can be explained by the fact that span 40 possess a shorter alkyl chain length and smaller vesicle diameter. Thus, a shorter diffusion path for benazepril HCL [22].

Even though brij 72 share the same alkyl group (stearyl) chain with span 60, F3 showed significantly faster (p<0.05) release rate than F1. This may be attributed to the long hydrophilic polyoxyethylene head group in brij 72 which might build looser bilayered vesicles facilitating the drug escape [23].

Increasing the amount of cholesterol from 100 mg in F1, F2 and F3 to 400 mg in F4, F5 and F6 while fixing the quantities of surfactants led to a profound retardation effect (p<0.05) on the drug release rate as shown in (fig. 2). Cholesterol was employed in this study as a stabilizing agent that strengthens the cohesion and membrane rigidity. Therefore, it decreased drug permeability across the bilayered vesicles [24].

The inclusion of soya lecithin in F7, F8 and F9 also decreased the release rate significantly (p<0.05), as seen in (fig. 3) in terms of increasing membrane stability and reduced leakage [25].
Fig. 3: Benazepril HCl release from formulations (F4–F9) (n=3) (mean±standard deviation)

Formulations F7, F8 and F9 exhibited the desired sustained release profile as they released only 38.4%, 41.4% and 50.8% respectively at the end of 4 h. The least the drug released from the carrier vesicles, the higher encapsulated within to be absorbed at the absorption site in the gastrointestinal tract.

**Zeta potential (ζ potential)**

Zeta potential analyses provide a measure to the stability of colloidal dispersions. It governs the degree of repulsion of similarly charged particles, thereby preventing their flocculation. It is reported that zeta potential value of -30 to -60 mV is favourable for the niosomal stability, but if the potential is reduced below -30 mV, the particles may attract each other and aggregate eventually [26].

Zeta potential measurements shown in the table (2) clearly suggest that formulations prepared using span 60 and span 40 possess adequate stability with potential range between -32.13 to -45.91 mV, whereas all formulations prepared using brij 72 had inadequate zeta potential range between -12.22 and -20.12 mV.

Zeta potential measurements are directly related to the surface charge. Formulations (F1–F6) were prepared using non-ionic surfactants, yet they carry a negative charge due to adsorption of hydroxyl ions from aqueous medium [27].

Soya lecithin containing phosphatidylcholine as a major component was included in this study as a charge imparting agent to boost the niosomal stability. Obtained results revealed that addition of soya lecithin while fixing the quantities of both surfactant and cholesterol had increased the zeta potential from -35.03 in F5 to -45.91 mV in F8 and from -12.93 in F6 to -20.12 mV in F9. These findings are in agreement with clomipramine niosomes [28]. Fig. 4 depicts zeta potential analyses for F7 (A) and F8 (B) and F9 (C).

**Transmission electron microscopy (TEM)**

The obtained TEM images clearly elucidate the formation of spherically shaped vesicles with distinct boundaries, as observed in (fig. 5).

**CONCLUSION**

It can be concluded that benazepril HCL was successfully formulated in niosomal formulations. Among all formulations, F7 and F8 elaborated employing span 60 and span 40, respectively exhibit the desired properties with respect to size, PDI, EE%, ζ potential and in vitro release profile. Thus, they might be employed as potential carriers of benazepril HCL.
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AUTHOR CONTRIBUTION

All the work have been carried out by me

CONFLICT OF INTERESTS

Declared none

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