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ABSTRACT
Ondansetron HCl is a (5-HT3) serotonin receptor antagonist, used as anti-emetic drug in combination with anticancer agents. Conventional dosage forms have poor bioavailability and patient compliance. These problems can be reduced by the use of nasal niosomal thermo-reversible in situ gelling system. Niosomes were formulated using various surfactants (Span 60, Span 80, Tween 20, and Tween 80) in different ratios using the thin-film hydration technique. Niosomes were evaluated for particle size, zeta potential, transmission electron microscopy (TEM) imaging, drug entrapment efficiency, and in vitro drug release. Niosomes prepared using Span 60 and cholesterol in the ratio 1:1 (F5) showed higher entrapment efficiency (76.13 ± 1.2%) and in vitro drug release (91.76%) after 12 h was optimized. The optimized niosomes were developed into thermo-reversible in situ gel, composed of Poloxamer 407 and sodium carboxymethyl cellulose, prepared by cold method technique. Compatibility study (FTIR, DSC) was made for drugs and excipients that showed no significant interaction. The gel formulation G5 showed the most suitable gelation temperature (31°C), viscosity (1250 mpoise), bioadhesion force (5860 ± 28 dyne/cm²), and in vitro drug release (70.6%) after 12 h. Comparative in vivo pharmacokinetic study on rabbits showed a sustained release and higher relative bioavailability of the prepared nasal in situ gel compared to similar dose of oral tablets (202.4%) which make ondansetron HCl niosomal nasal thermo-sensitive in situ gel a more convenient dosage form for the administration of ondansetron HCl than oral tablets.

Introduction
The supportive care of patients receiving antineoplastic treatment has dramatically improved over the past two decades. The development of effective means to prevent nausea and vomiting arising from chemotherapy serves as one of the most important examples of this progress [1]. Patients beginning cancer treatment consistently list chemotherapy-induced nausea and vomiting as one of their greatest fears. Inadequately controlled emesis impairs functional activity and quality of life for patients increases the use of health care resources, and may occasionally compromise adherence to treatment [1]. Ondansetron HCl represents the class of (5-HT3) serotonin receptor antagonist, which is commonly employed as anti-emetic in combination with antiulcer and anticancer agents. It is indicated for the prevention of nausea and vomiting associated with cancer chemotherapy, radiotherapy or anesthesia, and surgery [2]. Intranasal administration is an attractive option for local and systemic delivery of many therapeutic agents. The nasal mucosa compared to other mucosae is easily accessible. Intranasal drug administration is noninvasive, essentially painless, and particularly suited for children. Application can be performed easily by patients or by physicians in emergency settings. Intranasal drug delivery offers a rapid onset of therapeutic effects (local or systemic) [3]. Vesicular systems are a novel means of drug delivery that can enhance the bioavailability of encapsulated drugs and provide therapeutic activity in a controlled manner for a prolonged period. Niosomes are nonionic surfactant vesicles in aqueous media resulting in closed bilayer structures that can be used as carriers of amphiphilic and lipophilic drugs [4]. To prevent rapid mucociliary clearance and improve the residence time, thermo-reversible in situ gelling system is utilized. These systems adhere to the mucous membrane and increase the residence time. This intensifies the contact between nasal membrane and the drug and facilitates the drug absorption, which results in augmented bioavailability [5]. This study aimed to prepare thermo-reversible in situ gelling system containing ondansetron HCl-loaded niosomes for nasal delivery to attain a more acceptable and convenient dosage form for patients.

Experimental section
Materials
Potassium hydrogen phosphate, disodium hydrogen phosphate, sodium chloride, potassium chloride, calcium chloride, polysorbate (Tween 80, Tween 20), and sodium carboxymethyl cellulose were purchased from El Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Sorbitan monostearate (Span 60, Span 80) and cholesterol were purchased from Loba Chemie, Mumbai, India. Poloxamer 407 was purchased from Sigma-Aldrich, KGaA, Darmstadt, Germany. Diethyl ether and 1-propane were purchased from Honeywell Riedel-de-Haën, Seelze, Germany. Poloxamer 407 purchased from Sigma-Aldrich, KGaA, Darmstadt, Germany. Acetonitrile and methanol (HPLC grade) were purchased from Fisher Chemicals, Geel, Belgium. SERVA Visking® Dialysis Tubing

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molecular weight cutoff 12,000–14,000, pore diameter 25 Å (SERVA Electrophoresis, Uetersen, Germany). Commercial product Zofran (4 mg) tablets were purchased from GSK, Egypt. Male New Zealand white rabbits were purchased from animal house (Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt).

Methods

Preparation of ondansetron HCL-loaded niosomes

Niosomes prepared with nonionic surfactants namely Span 80, Span 60, Tween 20, and Tween 80. Drug and cholesterol in different ratios to make total lipids of 150 μmol using lipid film hydration technique (Table 1) [6,7]. Briefly, the lipid part was dissolved in 15 ml diethyl ether in 50 ml round bottom glass flask and fitted in Eyela rotary evaporator. Diethyl ether was evaporated at 25 ± 2 °C under reduced pressure overnight to ensure complete removal of residue solvent. The resulting film was hydrated by 10 ml drug aqueous solution (4 mg/ml) in phosphate buffer, at 68 °C for 60 min and 120 rpm and kept at 5 °C to mature overnight.

Determination of percentage of entrapment efficiency (E.E.%)

0.5 ml of niosomal suspension equivalent to 2 mg ondansetron HCL was centrifuged for 90 min at 21,000 g (15,000 rpm) at 4 °C. The precipitated pellets were separated and washed twice with phosphate buffer and were recentrifuged for 90 min then lysed by incubating in 10 ml of propan-1-ol for 1 h. The solution was sonicated for 15 min and then filtered through 0.22-μm syringe filter, and 1 ml of lysed solution is diluted to 10 ml of 1-propan and then measured against blank spectrophotometer at 303 nm. Entrapment efficiency was determined as follows [8,9].

\[
\text{E.E.}% = \left( \frac{\text{amount trapped}}{\text{total drug amount}} \right) \times 100
\]

Table 1. The composition, entrapment efficiency, zeta potential, and particle size of the prepared ondansetron HCL niosomal dispersion using different types and molar ratios (mol. %) of surfactants [Span 80 (S80), Span 60 (S60), cholesterol (CHO), Tween 80 (T80), and Tween 20 (T20)].

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>S80: CHO</th>
<th>S60: CHO</th>
<th>T80: CHO</th>
<th>T20: CHO</th>
<th>E.E.%</th>
<th>Charge (mV)</th>
<th>Diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>2:1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>46.33 ± 0.4</td>
<td>–47.4</td>
<td>158.1 ± 0.84</td>
<td>0.1885</td>
</tr>
<tr>
<td>F2</td>
<td>1:1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>65.10 ± 0.7</td>
<td>–51.8</td>
<td>281.7 ± 0.14</td>
<td>0.186</td>
</tr>
<tr>
<td>F3</td>
<td>1:2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>45.67 ± 0.9</td>
<td>–49.2</td>
<td>1198.5 ± 127</td>
<td>0.5865</td>
</tr>
<tr>
<td>F4</td>
<td>–</td>
<td>2:1</td>
<td>–</td>
<td>–</td>
<td>54.83 ± 0.8</td>
<td>–46.8</td>
<td>196.15 ± 1.7</td>
<td>0.164</td>
</tr>
<tr>
<td>F5</td>
<td>–</td>
<td>1:1</td>
<td>–</td>
<td>–</td>
<td>76.13 ± 1.2</td>
<td>–58.0</td>
<td>249.8 ± 55</td>
<td>0.413</td>
</tr>
<tr>
<td>F6</td>
<td>–</td>
<td>1:2</td>
<td>–</td>
<td>–</td>
<td>50.56 ± 1.2</td>
<td>–51.8</td>
<td>2486 ± 906</td>
<td>0.535</td>
</tr>
<tr>
<td>F7</td>
<td>–</td>
<td>–</td>
<td>2:1</td>
<td>–</td>
<td>13.84 ± 0.2</td>
<td>–23.9</td>
<td>303.65 ± 112</td>
<td>0.575</td>
</tr>
<tr>
<td>F8</td>
<td>–</td>
<td>–</td>
<td>1:1</td>
<td>–</td>
<td>56.22 ± 0.6</td>
<td>–14.1</td>
<td>692.7 ± 17</td>
<td>0.673</td>
</tr>
<tr>
<td>F9</td>
<td>–</td>
<td>–</td>
<td>1:2</td>
<td>–</td>
<td>44.39 ± 0.4</td>
<td>–12.9</td>
<td>660.4 ± 19</td>
<td>0.5985</td>
</tr>
<tr>
<td>F10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2:1</td>
<td>22.70 ± 1.0</td>
<td>–20.5</td>
<td>435.4 ± 20</td>
<td>0.5895</td>
</tr>
<tr>
<td>F11</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:1</td>
<td>41.26 ± 1.5</td>
<td>–20.15</td>
<td>700.6 ± 28</td>
<td>0.3005</td>
</tr>
<tr>
<td>F12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:2</td>
<td>33.24 ± 1.6</td>
<td>–26.4</td>
<td>998.2 ± 251</td>
<td>0.3595</td>
</tr>
</tbody>
</table>

1Mean ± SD (n = 3).

In vitro drug release study of niosomal dispersions

Membrane diffusion method [12,13]: briefly 1 ml of niosomal suspension equivalent to 4 mg ondansetron HCL was placed in a diffusion cell (glass tube) of diameter 2.5 cm, the lower open end of the glass tube was covered with soaked cellulose membrane. SERV A dialysis membrane (molecular weight cutoff: 12,000–14,000) this cell then suspended in the beaker containing phosphate buffer pH 6.2 (60 ml). This was constantly stirred at speed 100 rpm at 37 ± 1 °C on a magnetic stirrer with a thermostat [14]. 3 ml aliquots were withdrawn at predetermined time intervals and replaced simultaneously with equal volume of fresh buffer. The ondansetron HCL concentration in the samples was analyzed spectrophotometrically. The obtained data were analyzed to determine the amount and mechanism of drug release. Free drug solution of 4 mg/ml was used as a reference.

Compatibility of ondansetron HCL with polymer and excipient using differential scanning calorimetry and Fourier transform infrared spectroscopy

Differential scanning calorimetry (DSC) of ondansetron HCL, Poloxamer 407 polymer, cholesterol, Span 60, sodium carboxymethyl cellulose, and their physical mixture was performed. Samples (3–4 mg) placed in aluminum pan and heated in the rate of 10 °C/min to a temperature of 200 °C. The instrument was calibrated with indium, and dry nitrogen used as a carrier gas with a flow rate of 25 ml/min. The drug excipient compatibility was determined by Shimadzu 8400S FTIR using KBR pellets of 0.1 mm. Samples of pure ondansetron HCL, Poloxamer 407 polymer, cholesterol, Span 60, sodium monitored at 25 °C at a scattering angle of 90° [9]. The PDI was determined for the determination of distribution of the size of niosomal vesicles. The formation of niosomal vesicles as well as their morphological aspects was evaluated by using photomicroscopy and transmission electron microscopy (TEM). Various niosomal formulations examined under optical microscope (Leica Image, Germany) and photographed at a magnification of 40×, by means of a fitted camera [10]. The shape of vesicles was examined by Hitachi H 7500 transmission electron microscope at a voltage of 25, 40, and 150 kV. The aqueous dispersion of the vesicles was drop-cast onto a carbon-coated grid and stained with 1% phosphotungstic acid as a negative stain for niosomes. The grid was dried at room temperature before loading it into the microscope [11].
carboxymethyl cellulose and physical mixtures of drug and excipients were scanned in the range of 400–4000 cm$^{-1}$.

**Preparation of thermo-reversible in situ gelling system**
Thermo-reversible in situ gels were prepared by cold method technique, the amount of niosomal suspension equivalent to 4 mg/ml ondansetron HCl was kept refrigerated at 4 °C. Then Poloxamer 407 and sodium carboxymethyl cellulose were added according to Table 2. The mixture kept refrigerated overnight until complete polymer hydration and milky white solution is formed [5,15].

**In vitro gelation temperature determination by visual inspection method**
Gelation temperature, defined as the temperature at which the liquid phase makes a transition to gel, was determined by visual inspection [16,17]. In brief, a 10 ml transparent vial containing a liquid phase makes a transition to gel, was determined by visual inspection method. The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of the measurement.

**Measuring viscosity of selected formula against temperature**
Rheological studies were performed with a thermostatically controlled Brookfield Programmable Rheometer (Brookfield LVDV III, Brookfield Engineering Laboratories, Middleborough, MA, USA) fitted with CP-52 spindle. The cone/plate geometry was used. The shear stress was controlled to maintain a shear rate of 10 s$^{-1}$. This value was chosen to allow precise determination of the gelling temperature. The temperature was increased in steps of 1 °C/min, from 5 °C to 40 °C [16].

**Determination of bioadhesive force**
The bioadhesive force of Poloxamer gel was determined by using a measuring device as shown in Figure 1. In brief [18–20], a section of nasal mucosa (1 cm$^2$) was cut from the sheep nasal mucosa and instantly secured with mucosal side out onto each glass vial (C) using rubber band. The vials with the sheep nasal mucosa were instantly secured with mucosal side out onto each glass vial (C). The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of the measurement.

Mucoadhesive strength (dyne/cm$^2$) = $M \times g/A$  \( (2) \)

where $m$ = weight (in grams) required for detachment, $g$ = acceleration due to gravity (980 cm/s$^2$), $A$ = area of mucosa exposed.

**In vitro release study of ondansetron HCl niosome gel**
Drug release from the gel was tested with nasal diffusion cell, using dialysis membrane (molecular weight cutoff 12,000–14,000) with permeation area of 0.8 cm$^2$. About 60 ml of phosphate buffer pH 6.2 was added to the acceptor chamber [21,22]. This was constantly stirred at speed 200rpm at 37 ± 1 °C on a magnetic stirrer with a thermostat. Gel containing drug equivalent to 4 mg was placed in donor compartment. At predetermined time points, a 3 ml sample was withdrawn from the acceptor compartment, replacing the sample volume with phosphate buffer pH 6.2 after each sampling for 24 h. The samples were suitably diluted and measured spectrophotometrically at 310 nm. The concentration of the drug was determined.

**In vivo pharmacokinetic study of ondansetron HCl niosome gel**
Animal handling and drug administration. Nine healthy male New Zealand white rabbits weighing between 2.5 and 3 kg were used for the study. The rabbits were randomly assigned to three groups (I, II) and control. Each contains three rabbits. All rabbits were fasted for 12h with ad libitum access to water [23]. The study conducted in accordance with animal ethical guidelines for investigations in laboratory animals and the Animal Ethics Committee of Faculty of Pharmacy, Cairo University approved the study protocol (serial no. PI 1337, valid from 29 April 2015). The rabbits were divided into three groups.

Group I received the commercial Zofran 4 mg tablets, the tablets administered at the back of the pharynx using gastric intubation tube with one tablet set on the tip of the tube and 5 ml of water administered through the tube to aid swallowing of the tablet. Group II received the developed nasal thermo-reversible gel, 200-μl nasal gel equivalent to 2 mg ondansetron HCl into each nostril (total of 400-μl equivalent to 4 mg ondansetron HCl), Control group doesn’t receive the drug, used for providing blank plasma.

Sample collection and analysis. One ml of blood was withdrawn from the marginal ear vein into commercially available plasma tubes pretreated with EDTA at time intervals of 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h. The blood was immediately centrifuged at 4000 rpm for 15 min to separate plasma and stored at −20 °C until HPLC analysis. In a 10 ml glass, tube 1 ml plasma was mixed with 50 μl of saturated sodium carbonate solution and 5 ml of

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**Table 2. Composition of the formulated niosomal thermo-reversible in situ gels using Poloxamer 407 (P407) and sodium carboxymethyl cellulose (Na CMC) in different ratios (W/V%) and corresponding gelation temperature.**

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>Niosomal suspension (W/V%)</th>
<th>P407 (W/V%)</th>
<th>Na CMC (W/V%)</th>
<th>Gelation temp. °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>10 ml</td>
<td>10</td>
<td>2</td>
<td>&gt;40</td>
</tr>
<tr>
<td>G2</td>
<td>10 ml</td>
<td>12</td>
<td>2</td>
<td>&gt;40</td>
</tr>
<tr>
<td>G3</td>
<td>10 ml</td>
<td>14</td>
<td>2</td>
<td>&gt;40</td>
</tr>
<tr>
<td>G4</td>
<td>10 ml</td>
<td>16</td>
<td>2</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>G5</td>
<td>10 ml</td>
<td>18</td>
<td>2</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>G6</td>
<td>10 ml</td>
<td>20</td>
<td>2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>G7</td>
<td>10 ml</td>
<td>22</td>
<td>2</td>
<td>21 ± 2</td>
</tr>
</tbody>
</table>

*nMean ± SD (n = 3).*

---

**Figure 1. Diagram showing device used to determine bioadhesion force.**
dichloromethane, extracted with a rotary mixer for 15 min at room temperature. The mixture was centrifuged for 5 min at 5000 g and 4.5 ml of the organic phase were transferred into a new tube and evaporated to dryness at 40 °C under a stream of nitrogen. The residue reconstituted in 100 μl of mobile phase and a volume of 50–75 μl injected into the HPLC for analysis [24].

**Chromatographic conditions.** Ondansetron HCL was quantified using a Shimadzu HPLC system (Duisburg, Germany) consisting of a pump (LC 10AS), an automatic sampler (SIL 10A), and a dual wavelength UV detector (SPD 10AVP). The Class LC10 software Version 1.6 Shimadzu) was used for data analysis and processing. The compounds were separated at room temperature on a Phenomenex Luna C18 column (5 μm, 250 × 4.6 mm, i.e. Phenomenex, Aschaffenburg, Germany) with a Phenomenex Luna C18 guard column (i.e. 5 μm, 4 × 4.6 mm) and quantified by UV detection at 305 nm. The mobile phase consisted of 20% of acetonitrile and 80% of 0.05 M sodium hydrogen phosphate buffer (pH 5.0) and was delivered at a flow rate of 1.5 ml/min. The analyses were quantified using peak area. Method validation was done according to FDA Bioanalytical Method Validation Guidance for Industry [25,26].

The pharmacokinetic parameters, namely maximum plasma concentration (Cmax), time to reach Cmax (Tmax), the area under the plasma concentration-time curve from 0 to the last measurable concentration (AUC0–t), area under concentration-time curve from zero to infinity (AUC0–∞), the elimination half-life (T1/2), and elimination rate constant (Ks) were conducted by PKSolver an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel directly from the data.

**Statistical analysis.** The results are reported as means ± SD [27]. The pharmacokinetic parameters, AUC0–∞, Cmax, T1/2, and Ks were analyzed statistically using independent samples t-test. The p value was calculated from the obtained F value using IBM SPSS statistics version 20 (Microsoft software). A statistically significant difference was considered when p < 0.05.

Relative bioavailability of nasal in situ gel was calculated by the following equation [28].

\[ F(%) = \frac{AUC_{0–\infty, \text{n}} \times \text{dose oral}}{AUC_{0–\infty, \text{o}} \times \text{dose nasal}} \times 100 \]  

**Results and discussion**

**Determination of percentage of entrapment efficiency (E.E.%)**

The entrapment efficiencies of all niosomal formulation are reported in Table 1. The percentage of ondansetron HCL entrapped in niosomes varied between 13.84% and 76.13%. The entrapment efficiencies varied according to the surfactant type used, Spans 60 and 80 showed higher drug entrapment than Tewees 80 and 20 with Span 60 showing the maximum entrapment efficiency. This could be due to the surfactant chemical structure, as long-chain surfactants produce high entrapment [29]. Additionally, the alkyl chain length influences the HLB value of the surfactant, which in turn directly influences the drug entrapment efficiency [10]. The lower the HLB of the surfactant the higher will be the drug entrapment efficiency [5]. Span 60 having higher TC and provides better entrapment. Span 80, Teween 20, and Teween 80 have low phase transition temperature so they form less rigid membrane that forms leaky membrane [5].

The entrapment efficiency of ondansetron HCL is directly proportional to cholesterol concentration [8]. There was an almost direct proportionality between entrapment efficiency. The 1:1 ratio [31] between the cholesterol and nonionic surfactant in the bi-layers shows an optimum ratio for the production of physically stable niosomal systems. This could be attributed to an increase in the vesicle size by cholesterol [9]. Cholesterol is known to abolish the gel to liquid phase transition of niosome systems [10], which could be able to effectively prevent leakage of drug from niosomes.

**Particle size analysis and zeta potential**

The particle sizes of different formulations presented in Table 1. Niosomes prepared from Span 80 showed the largest average vesicle size. Span 60 formed bigger vesicles than that formed from Span 80. In addition, Teween 20 formed bigger vesicles than that formed by Teween 80 as shown in Figure 2. This may suggest that when the hydrophilicity of the surfactant increases, the vesicle size increases [8] In general, the size of niosomes increases as the amount of cholesterol incorporated in the formulation increases regardless the type of surfactant used this may be due to that cholesterol is a rigid molecule with an inverted cone shape. It can be intercalated between the fluid hydrocarbon chains of the surfactant when hydrated at a temperature above the gel/liquid transition temperature so increase the size of the vesicle [32].

The zeta potential examination revealed that the surface charge of all niosomal vesicles displayed negative values (Table 1). The results revealed that niosomes prepared from Span 80, 60 gave negative zeta potential values more than (−30 mV) as shown in Figure 2 indicating stable systems [33].

**Morphological examination by optical microscope and transmission electron microscope**

The niosomes were prepared with the mixture of the surfactants and cholesterol at different molar ratios. All used surfactants formed niosome suspensions in the presence of cholesterol. The results of TEM revealed that vesicles are well identified and present in a nearly perfect sphere-like shape, and have a smooth surface, a uniform size and having a large internal aqueous space and a smooth vesicle surface. It also revealed well-stained niosomal vesicles, where the outer lipophilic domain is black-stained and the inner hydrophilic domain is light-stained as shown in the micrographs of Figure 3.

**Figure 2.** Representative sketches for size and zeta potential of prepared niosome formulations.
In vitro drug release study of niosomal dispersions

In vitro drug release of niosomal dispersions shown in Figures 4 and 5 showed that Span 60, Span 80, Tween 80, and Tween 20 show reduction in in vitro drug release in 4 h compared with drug in solution (Figures 4 and 5). Span 80 showed an average reduction of 42.7%, Span 60 showed an average reduction of 24.7%, Tween 80 showed an average reduction of 42.7%, Tween 20 showed an average reduction of 64.1%.

The initial drug release in 3 h from all the formulation ratios was for Span 80 (28–35%), Span 60 (45–52%), Tween 80 (35–39%), and Tween 20 (18–26%). Fast drug release in the initial hours may be due to the release of adsorbed drug from the lipophilic region of niosomes, which will help to achieve the optimal loading dose [8].

Drug release after 12 h from Span 80, Span 60, Tween 80, and Tween 20 was found to be 81.4%, 90.7%, 89.9%, and 71.16%. The entire amount of loaded drug was not released from the niosomes. This may be due to entrapment of the drug in the lipophilic region [8].

Differences in the in vitro release profiles may be due to vesicle size, lamellarity, and membrane fluidity as a function of chain length of surfactant and cholesterol content [8].

Compatibility of ondansetron HCL with polymer and excipient using differential scanning calorimetry and Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectrum of pure drug and mixture of drug and polymers are shown in Figure 6. From the spectral study, it was observed that there was no significant change in the peaks of pure drug and drug excipients mixture. The characteristic carbonyl peak at 1624 cm⁻¹, NH stretching...
absorption 3500–3220 cm$^{-1}$, Hence, no specific interaction was observed between the drug and the excipients used in the formulations.

Thermal profile of pure product (Figure 7) exhibited a single endothermic effect corresponding to the melting of ondansetron HCl ($T_\text{m} = 185.62 \degree C$, $\Delta H = 119 J/g$). The DSC curve of physical mixture showed progressive broadening and lowering of drug melting temperature and concomitant reduction of its enthalpy.

Rheological studies

**Determination of gelation temperature of the prepared in situ gel by visual inspection method**

The results stated in Table 2. The gelation temperature decreases by increasing the concentration of Poloxamer 407 [34]. In general, the gelation temperatures have been considered suitable if they are in the range of 25–37 $\degree C$. If the gelation temperature of a thermo-reversible formulation is lower than 25 $\degree C$, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37 $\degree C$, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the administered drugs at an early stage. As the temperature of the nasal cavity is 34 $\degree C$, so G5 was chosen as a suitable candidate formulation [16].

**Measuring viscosity of selected formula against temperature**

Table 3 shows that the same concentration of Poloxamer 407 has different viscosity at different temperatures where the viscosity increased with increasing temperature, until it reaches a maximum point where the viscosity is no further increased with increasing the temperature. This may be attributed to the fact that Poloxamer, being nonionic PPO tri-block copolymers, aggregate into micelles. This micellization is due to the dehydration of polymer blocks with temperature. It has been shown that gel formation is a result of micellar enlargement and packing and that the gel is more entangled at higher Poloxamer 407 concentrations. As a result of these micelle entanglements, they cannot separate easily from each other, which accounts for the rigidity and high viscosity of gels containing high concentrations of Poloxamer [17].

**Determination of bioadhesive force**

Mucoadhesive force is an important and crucial physicochemical parameter for in situ nasal gels since it prevents the formulation from rapid drainage and hence prolongs its residence time. Results of the determination of mucoadhesive forces of all the formulations are collected in Table 4. This table shows that the prepared in situ gelling formulations with Poloxamer 407 possessed satisfactory adhesive properties. Increasing the polymer concentration significantly increased the mucoadhesive force [17].

**In vitro release study of ondansetron HCL niosome gel**

Ondansetron HCL release from the prepared in situ gel showed a reduction in drug released at 4 h. By 35.7%, compared to free drug solution and 10.4% compared to niosome formulation (F5). After 12 h, 70.65% of drug released from the niosomal in situ gel compared to 91.7% released from niosome formulation (F5), 100% released from drug solution. By a reduction, it equals 21.1%, 29.4%. The initial fast release of the drug may be due to the free drug adsorbed on the niosome surface, the following reduction in the release rate compared to free niosomes is due to the matrix effect of the Poloxamer 407 polymer forming the thermo-sensitive gel base (Figure 8).

The correlation coefficient value $R^2$ was found to be $>0.96$ (Table 5), indicating goodness-of-fit data for the Higuchi model. The model is based on the hypotheses that [35]. Initial drug concentration in the matrix is much higher than drug solubility, the drug diffusion takes place only in one dimension (edge effect must be negligible), drug particles are much smaller than system thickness, matrix swelling and dissolution are negligible, drug diffusivity is constant and perfect sink conditions are always attained in the release environment.

**In vivo pharmacokinetic study of ondansetron HCL niosome gel**

The concentration of ondansetron HCL in rabbit’s plasma was determined by a validated HPLC assay. Ondansetron HCL was well
separated under the HPLC conditions applied. Retention time was 4.6 min. No interferences were observed in blank plasma samples. Figure 9 shows the HPLC chromatograms of a blank plasma sample (I) and plasma samples after administration of ondansetron HCL (II) recorded simultaneously at 305 nm.

The linearity of the method was evaluated at six concentration levels including the LLOQ. The calibration curve was found to be linear in the range 1–800 ng/mL, with a correlation coefficient ($R^2$) of 0.999.

The inter-day and intra-day precisions were measured as relative standard deviations and expressed as percentages of the concentration of ondansetron HCL. For inter-day precision, the % CV of estimated concentrations for all four quality control samples with five replicates was within the range of 1.04–15.92%. The % mean accuracy for LLOQ, LOQ, MOQ, and HQC was within the range of 94.918–112.803%. For intra-day precision and accuracy, the % CV and accuracy results of all quality control samples were within the ranges 1.41–13.88% and 96.557–110.984%, respectively. All results are within the acceptance criteria [25].

The mean ondansetron HCl recovery % of the method was 97.41 ± 0.54 and CV% 0.55.

The bioavailability of in situ ondansetron HCl gel was determined for the optimized formulation G5 due to its suitable gelation temperature. In situ gel formula G5 was compared to commercial oral tablets having the same (ondansetron HCl) dose (4 mg) [23]. The mean plasma drug concentration-time profiles after administration of the oral as well as the in situ gel of (ondansetron HCL) are illustrated in Figure 10.

From the profile plasma, data of nasal in situ gel two peaks at 1 and 4 h are seen. The first one corresponded to direct
absorption from nasal cavity and the second to oral drug absorption that might have occurred due to portion of drug solution swallowing after conversion into gel following nasal instillation.

Table 6 shows plasma pharmacokinetic parameters for different route formulations. The $C_{\text{max}}$ values were $57.1 \pm 2.5$ ng/ml and $74.9 \pm 5.58$ ng/ml for oral tablets and nasal in situ gel respectively. It revealed that the $C_{\text{max}}$ was significantly higher in case of the nasal in situ gel ($p < 0.05$).

Concerning the rate of absorption, the results show that $T_{\text{max}}$ values were $1.96 \pm 0.06$ and $1.03 \pm 0.01$ h for oral tablets and nasal in situ gels respectively. Statistical analysis revealed that the time needed to achieve $C_{\text{max}}$ was significantly higher in case of oral formulations.

Table 3. The viscosity of the prepared thermo-sensitive in situ gel (G5) at different temperatures.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Viscosity (mPoise)</th>
<th>Torque (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>87.3</td>
<td>2.2</td>
</tr>
<tr>
<td>15</td>
<td>71.4</td>
<td>4.1</td>
</tr>
<tr>
<td>25</td>
<td>1076</td>
<td>26</td>
</tr>
<tr>
<td>30</td>
<td>1250</td>
<td>30</td>
</tr>
<tr>
<td>37.5</td>
<td>1234</td>
<td>30.8</td>
</tr>
<tr>
<td>40</td>
<td>1207</td>
<td>30.4</td>
</tr>
</tbody>
</table>

Table 4. The mean mucoadhesive force values of the prepared in situ gelling formulations.

<table>
<thead>
<tr>
<th>Gel formulation</th>
<th>Bioadhesion force (dyne/cm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>4610 ± 21</td>
</tr>
<tr>
<td>G2</td>
<td>4908 ± 19</td>
</tr>
<tr>
<td>G3</td>
<td>5194 ± 23</td>
</tr>
<tr>
<td>G4</td>
<td>5672 ± 28</td>
</tr>
<tr>
<td>G5</td>
<td>5860 ± 23</td>
</tr>
<tr>
<td>G6</td>
<td>5931 ± 27</td>
</tr>
<tr>
<td>G7</td>
<td>6830 ± 32</td>
</tr>
</tbody>
</table>

*Data represents Mean ± SD ($n = 3$).

Figure 7. Thermogram of pure ondansetron HCL (A), thermogram of drug excipients mixture (B).

Figure 8. Comparative in vitro drug release between prepared in situ niosomal gel, free niosome formulation (F5), and free ondansetron HCL solution.

Table 5. Release kinetics of the prepared niosomal gel (G5).

<table>
<thead>
<tr>
<th>Release kinetic model</th>
<th>$R^2$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>0.8274</td>
</tr>
<tr>
<td>First order</td>
<td>0.8269</td>
</tr>
<tr>
<td>Korsmeyer-Peppas</td>
<td>0.5518</td>
</tr>
<tr>
<td>Higuchi</td>
<td>0.9642</td>
</tr>
<tr>
<td>Hixson-Crowell</td>
<td>0.9527</td>
</tr>
</tbody>
</table>
tablet compared to nasal in situ gel ($p < 0.05$). The high values of plasma mean residence time (MRT) of ondansetron HCL obtained from nasal in situ gels ($7.44 \pm 0.41$ h) than in case of oral tablet ($3.94 \pm 0.12$ h) indicates a prolonged drug release.

Significant difference was found between plasma (AUC$_{0-24}$) of nasal in situ gel ($618.9 \pm 78.2$ ng h/ml) and oral tablets ($305.73 \pm 11$ ng h/ml) ($p < 0.05$) indicating the nasal route achieves excellent relative bioavailability of (202.4%) for nasal in situ gel [23].

Table 6. Pharmacokinetic parameters of the drug released from the prepared nasal thermo-sensitive in situ gel (G5) and marketed oral tablet in rabbit plasma.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Oral tablet</th>
<th>Nasal gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>mg</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$K_e$</td>
<td>/h</td>
<td>0.48 ± 0.008</td>
<td>0.14 ± 0.008</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>h</td>
<td>1.96 ± 0.06</td>
<td>1.03 ± 0.01</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>ng/ml</td>
<td>57.1 ± 2.5</td>
<td>74.9 ± 5.58</td>
</tr>
<tr>
<td>$AUC_{0-24}$</td>
<td>ng h/ml</td>
<td>300.76 ± 10.81</td>
<td>596.21 ± 71</td>
</tr>
<tr>
<td>$AUC_{0-c}$</td>
<td>ng h/ml</td>
<td>305.73 ± 11</td>
<td>618.9 ± 78.2</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>3.94 ± 0.12</td>
<td>7.44 ± 0.41</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>h</td>
<td>1.42 ± 0.02</td>
<td>4.93 ± 0.2</td>
</tr>
</tbody>
</table>

*Data represents Mean ± SD (n = 3).
Conclusion
The present study represents the formulation and optimization of niosomes entrapping ondansetron HCl, using different surfactants and cholesterol in different ratios. Niosome formulation (F5) was chosen as a suitable formulation of the niosomal nasal thermo-sensitive in situ gel, for its high drug entrapment efficiency (76.13 ± 1.2%), stability (−58.05 mV), suitable and uniform size distribution (1067.55 ± 175 nm, PDI = 0.315) (G5) in situ gel formulation was found to be the most suitable formulation for its desirable gelation temperature (31°C), mucoadhesive (5860 ± 23 dynes/cm²).

In vitro and in vivo release studies showed a sustained release and higher relative bioavailability of the prepared nasal in situ gel compared to similar dose of oral tablets (202.4%) which make niosomal nasal thermo-sensitive in situ gel better and more convenient dosage form for the administration of anti-emetic drugs than oral tablets.

Disclosure statement
The authors declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

References


