Vaginal Delivery of Benzydamine Hydrochloride through Liposomes Dispersed in Mucoadhesive Gels

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Liposomal vaginal drug delivery systems are important strategy in the treatment of both topical and systemic diseases. The aim of this study was to develop a vaginal delivery system for benzydamine hydrochloride (BNZ) loaded liposomes dispersed into mucoadhesive gels. The delivery system was also designed for a once a day dosage and to obtain controlled release of the BNZ. For this purpose BNZ containing gel formulations using hydroxypropyl methylcellulose (HPMC) K100M and Carbopol® 974P, which are composed of polymers that show promising potential as mucoadhesive vaginal delivery systems, were developed. In addition, a BNZ containing liposome formulation was developed for vaginal administration. To improve the vaginal retention time, liposome was incorporated in HPMC K100M and Carbopol® 974P gel formulations. This system is called lipogel. The developed BNZ liposomes have a slightly negative zeta potential (−1.50±0.16 mV), a 2.25±0.009 μm particle size and a 34% entrapment efficiency. These gels and lipogels have appropriate pH, viscosity, textural properties and mucoadhesive value for vaginal administration. Lipogels were found to be the best formulations for in vitro diffusion and ex vivo mucoadhesion. The work of mucoadhesion obtained from liposomes was in the range of 0.027±0.045 and 0.030±0.017 mJ/cm², while the value obtained from lipogels was between 0.176±0.037 and 0.243±0.53 mJ/cm². N1 and N2 lipogel formulations diffused 57 and 67% of BNZ respectively at the end of 24 h. Moreover, a higher mucoadhesion, which increases drug residence time in comparison to liposomes, could improve BNZ efficacy. In conclusion, BNZ mucoadhesive vaginal lipogel formulations can be promising alternatives to traditional dosage forms for vaginal topical therapy.

Key words mucoadhesion; liposome; gel; lipogel; benzydamine hydrochloride; vaginal drug delivery

The vagina, because of its anatomical location and physiological structures, is ever more being preferred for drug delivery. Vaginal application of bioadhesive drug forms has revealed positive results in delivering drugs both locally and systemically. Vaginal drug delivery has significant advantages. These advantages can be listed as follows; wide surface area, rich vascularity, high permeability to many drugs and overcome of first-past metabolism. It should also allow self-administration, minimal interference with body functioning and daily life, and obtain high bioavailability with other medications. Among the many factors affecting the drug absorption and rate from the vaginal route are formulation factors, vaginal physiology, age of the patient and menstrual cycle. Commonly used pharmaceutical forms in the vaginal route are gels, creams, vaginal rings, tablets and suppositories. These vaginal formulations are associated with limitations such as poor retention and leakage and they cause inconvenience to users. To overcome these disadvantages, the bioadhesive polymers are used in a vaginal formulation that adheres to vaginal mucosa for longer times. Bioadhesive vaginal delivery systems have several advantages compared to conventional dosage forms: they are readily localized in the region of application, they improve the bioavailability of drugs, provide intimate contact of the formulation with the underlying absorption surface, and reduce side effects through removing the need for repeated administration of the drug. Gels are the semisolid formulations, with a degree of flexibility very similar to that of natural tissue, due to their significant water content. Natural, semisynthetic or synthetic mucoadhesive polymers can be used to form hydrogels. In some case the gels may not be able to provide enough vaginal residence time and controlled release. Therefore new vaginal drug forms with features that are more suitable for this form of drug administering needed to be developed. Various strategies have been followed to improve the delivery of drugs through the vagina, and among these is the use of nanoparticulate carriers based on lipids.

The liposome can be used as a vehicle for administration of incorporated drugs and can provide controlled release of drug. Liposomes are most often composed of phospholipids which has a potential as a vaginal delivery system. Carbopol hydrogels can act as a vehicle for the incorporation of liposomes allocated for vaginal delivery. Liposomes are classically prepared from glycerophospholipids such as phosphatidylcholine. In more general terms, they may be obtained from any amphiphilic substance that forms a lamellar phase. Liposomes are versatile systems that can be modified in size, lamellarity, surface lipid composition, volume and composition of the internal aqueous medium, in accordance with pharmacological requirements. Liposomes can be prepared by various processes including agitation, sonication, extrusion, lyophilization, freezing and defrosting, and reverse-phase evaporation, among others. Depending on the mode of preparation, many forms of lipid vesicles with diameters ranging from 400 to 3500 nm can be obtained. Liposomes have many advantages as a drug delivery system. Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, non-hemolytic and they are non-immunogenic for systemic and non-systemic administrations. Liposomes are increased efficacy of the drug, increased stability via encapsulation and they reduce the site avoidance effect. The major problem to use liposomes
applied vaginally arises from the liquid nature of the formulation. To combat this, liposomes may be incorporated into the suitable carrier systems such as methylcellulose and polyacrylic acid gels.18,19

Benzydamine hydrochloride (BNZ) is a locally-acting non-steroidal anti-inflammatory drug with local anesthetic activity. It has an alkaline pH, which means that it selectively binds to inflamed tissues and is normally free of adverse systemic effects.13 BNZ topical application can offer some advantages in vaginitis treatment in terms of a reduced administered dose, high drug concentrations placed only in the pathological site, and the reduced side effects often associated with systemic administration.16 Perioli et al.17 developed emulgel oil in water (o/w) containing the mucoadhesive polymers hydroxyethylcellulose (HEC) and sodium carboxymethylcellulose (NaCMC) for the vaginal delivery of benzydamine. Emulgel showed the best in vitro and ex vivo performances. Indeed, the benzydamine products actually available are not able to keep the drug in the vaginal area for a long period, resulting in the absorption of insufficient amounts of the drug for the treatment. To overcome these problems, it is useful to develop new formulations with increased drug retention time in the vaginal site. The use of mucoadhesive formulations represents a logical advance in this field, because they are able to prolong drug residence time. In addition the transport of BNZ loaded liposomes in such mucoadhesive gels will provide a long-term release of BNZ.

The aim of this study was to develop a vaginal delivery system for BNZ loaded liposomes dispersed into mucoadhesive gels in an attempt to improve the topical effect of the BNZ. This delivery system also designed for once-daily use of the drug and to obtain a controlled release of the BNZ. The properties of the liposomes as well the mucoadhesive gels before and after dispersion of liposomes into the mucoadhesive gels were evaluated.

**Experimental**

**Materials** l-α-Dipalmitoyl phosphatidylcholine (DPPC) and cholesterol (CH) were purchased from Sigma, U.S.A. BNZ gel formulations were prepared using different types of polymer: Hydroxypropyl methylcellulose (HPMC, Methocel K100MCR Premium, Colorcon, England) and Carbopol® 974P (Noveon, Parkoteks Chemical, Turkey). BNZ was generous gift from Abdi Ibrahim İlaç San. AŞ. Turkey.

**Preparation of Liposomes** Liposomes of BNZ were prepared according to the film formation method, using a method described previously.18,19 DPPC and cholesterol were added in 1:1 molar ratios. After being dissolved in a chloroform and methanol mixture (2:4) and the solvents were evaporated in a rotavapor. Dry film was obtained and then hydrated with phosphate buffer (pH 4.5) containing BNZ. Liposomes were formed after vortex mixing (10 min) and ultrasonication (20 min). The suspension was extruded through a polycarbonate filter (pore size, 400 nm) using a Mini Extruder (Avanti Polar Lipids). The composition of the liposomes is given in Table 1.

**Characterization of Liposomes**

Liposome Particle Size Distribution and Zeta Potential

The physical properties of the liposomes were investigated by transmission electron microscopy (TEM) (FEI Tecnai G2 Spirit Bio, U.S.A.) and appearances were framed. The particle sizes and zeta potential of the liposomes were measured by a Zetasizer Nano Series (Nono ZS, Malvern Inst., U.K.). The analysis was performed at a temperature of 25°C, using samples properly diluted with distilled water.

**Encapsulation Efficiency**

To calculate the encapsulation efficiency (EE) in a liposome formulation, firstly separate the free drug from encapsulated drug using centrifugation. Liposomes were ultracentrifuged at 44,803 × g for 1 h. The supernatant containing the dissolved free BNZ was used to determine the concentration by spectrophotometer at 306 nm. The EE was calculated using the following equation:19 EE = (BNZtot − BNZsup)/BNZtot × 100, where BNZtot is the total amount of BNZ and BNZsup is the amount of BNZ in the supernatant after centrifugation.

**Preparation of Gel Formulations**

Firstly Carbopol® 974P NF (2%, w/w) was dispersed in demineralised water by stirring at 800 rpm for 60 min and then 10% NaOH was added dropwise to neutralized.20 The system was mixing until a transparent gel appeared and the pH of the gel was adjusted to pH 4.5. HPMC K100M (2%, w/w) was dispersed in demineralized water by stirring at 500 rpm for 60 min.21 The gels were kept at +4°C overnight before application, in order to remove air bubbles. The reasons for the selection of Carbopol® 974P NF and HPMC K100M polymers for preparation of formulations can be explained as follows. These polymers are polymers commonly used for vaginal drug delivery systems.22 It has been found that the mechanical and mucoadhesive properties of these polymers are more suitable for vaginal drug systems than other polymers (Chitosan H, Chitosan L, Chitosan M, Poloxamer 407, HPMC K15M and Polycarbophil AA-1) in our previous work.20,21 For these reasons Carbopol® 974P NF and HPMC K100M were used in this study.

**Incorporation of Liposomes into Hydrogel (Lipogel)** Lipogels

<table>
<thead>
<tr>
<th>Code of formulation</th>
<th>Lipid composition/molar ratio</th>
<th>Carbopol® 974P NF</th>
<th>HPMC K100M</th>
<th>BNZ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 (liposome)</td>
<td>DPPC:CH/1:1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L2 (liposome)</td>
<td>DPPC:CH:BNZ/1:0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C1 (gel)</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C2 (gel)</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>0.15</td>
</tr>
<tr>
<td>H1 (gel)</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>H2 (gel)</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>0.15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipogels</th>
<th>Liposome formulation</th>
<th>Gel formulation</th>
<th>Amount of liposomes in hydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>L2</td>
<td>C1</td>
<td>50:50</td>
</tr>
<tr>
<td>N2</td>
<td>L2</td>
<td>H1</td>
<td>50:50</td>
</tr>
</tbody>
</table>
Drug Content, pH Measurements and Viscosity Studies
One milliliter of gel formulation (0.15% BNZ) was weighed and mixed with 30 mL of pH 4.5 buffered solution in a 50 mL volumetric flask. It was left in an ultrasonic bath for 15 min and after filtration of the solution the BNZ concentration was determined by spectrophotometer at 306 nm. The pH values of the formulations were measured with a pH meter (Schott CG 840, Germany) at room temperature 24 h after preparation, and discarding air bubbles. A rheometer (Anton Paar Physica MCR 301) equipped with a cone/plate accessory (spindle type CP40-2) was used to measure the viscosity of the gels and lipogels.

Drug Diffusion Studies
The diffusion of BNZ from liposomes, gels and lipogels was evaluated by placing the formulations at 37°C in the donor compartment of a Franz diffusion cell system. The diffusional sectional area was 1 cm² and receptor phase volume was 2.5 mL. A dialysis membrane (Sigma, U.S.A.) with a 12000 Da pore size was used. The receptor phase containing citrate phosphate buffer (pH 4.5) was stirred by magnetic bars at 37°C. Samples were taken periodically from the receptor phase. The receiver samples were determined spectrophotometrically at 306 nm.

Mechanical Properties
The mechanic properties of all formulations was determined in triplicate with a TA.XTplusTexture Analyzer (Stable MicroSystems, Godalming, U.K.), using the back extrusion test, performed according to the equipment instructions. Test was carried out in standard size back extrusion container (50 mm diameter), approximately 75% full, immediately after removal from storage at a 25°C. The extrusion disc (35 mm diameter) was positioned centrally over the sample container filled with samples up to a height of 40 mm. The starting distance of the disc was set at 30 mm above the top of the sample surface. The disc penetrated into the sample to a depth of 10 mm at 1 mm/s, and returned at a speed of 20 mm/s. When the probe was returning to the start position (i.e., pulling out of the sample), the container was held to prevent it from lifting. The parameters measured consisted of firmness, consistency, cohesiveness and viscosity index, obtained by using the Exponent software version 4.0.6.0 (Stable Micro Systems). The important parameters can be used to compare those of the mechanical properties of the formulations and will bring new data in to the literature.

Ex-vivo Mucoadhesion Studies
Ex-vivo mucoadhesion testing of liposomes, gels and lipogels was conducted using a TA-XT Plus Texture Analyzer with a mucoadhesive holder as previously work. According this work freshly cow vaginal mucosa was frozen at −20°C and then a 2 mm thick section was taken from the inner part of the surface of this mucosa and attached to the lower end of the probe (P 0.5 Perspex: 12.5 mm) of the instrument with cyanoacrylate glue. The mucosa was dipped into the vaginal fluid simulant and kept for 10 min until the start of the experiment. The instrumental parameters specified in a previous study21 were used to evaluate the mucoadhesive potential of the vaginal gel formulations.

The area under the curve was calculated from a force-distance plot as the work of mucoadhesion. The Eq. 1 was used to calculate the work of mucoadhesion per cm² (mJ·cm⁻²). (πr²: the area of the mucosal surface being in contact with hydrogel)
Lipogel formulations (N1, N2) have lower viscosities than gel formulations (C2, H2) because of the addition of liposomes into the gel as a suspension. The viscosity of the liposomes could not be measured because they were in suspension and their viscosity was too low. High viscosity formulations can give better results in terms of duration of vaginal retention and ease of application. In this study, all the prepared gels had pseudo-plastic flow behavior, presenting an immediate flow after stress application (Fig. 2).

**Drug Diffusion Studies**  Liposome made from DPPC and cholesterol was used for the BNZ diffusion studies. Figure 3 shows the diffusion profiles of BNZ from the liposome. All of the drug was diffused from the liposome formulation (L2) by the end of 24h. It was therefore difficult to obtain controlled diffusion properties with liposomes. Similar results are also reported in the literature. Among the most frequently used are polyacrylates, cellulose derivatives, chitosan, hyaluronic acid and its derivatives, starch, pectin, natural gums, and sodium alginate. In addition to increasing the retention time of vaginal formulations, and therefore promoting therapeutic duration and efficacy and improving comfort and patient adherence, these excipients also allow a controlled drug release, thus improving local pharmacokinetics. Figure 3 shows the diffusion profiles of BNZ from Carbopol® 974P (C2) and HPMC K100M (H2) gels. When the diffusion profiles were examined it was shown that the diffusions of BNZ were similar. However H2 and C2 formulations diffused 79 and 70% of drug at the end of 24h, respectively. Since the viscosity of the C2 formulation is higher than H2, the drug release may be less. Similar results were obtained in our previous study. The aim of this study was to use the BNZ gel formulations once a day and to obtain a controlled release of the drug. For this purpose, the BNZ liposome formulation was prepared and incorporated in the gel. It was observed that, the N1 and N2 lipogel formulations released about 57.4±6.1 and 67±4.5% of BNZ at 24h respectively. Thus, considering the release of

<table>
<thead>
<tr>
<th>Code</th>
<th>BNZ (%)±S.D.</th>
<th>Viscosity (Pa·s)±S.D.</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>—</td>
<td>6.73±0.19</td>
<td>4.96</td>
</tr>
<tr>
<td>C2</td>
<td>101±0</td>
<td>6.28±0.11</td>
<td>4.54</td>
</tr>
<tr>
<td>H1</td>
<td>—</td>
<td>4.71±0.13</td>
<td>4.31</td>
</tr>
<tr>
<td>H2</td>
<td>102±0</td>
<td>3.25±0.29</td>
<td>4.12</td>
</tr>
<tr>
<td>N1</td>
<td>101±0</td>
<td>4.16±0.17</td>
<td>5.51</td>
</tr>
<tr>
<td>N2</td>
<td>101±0</td>
<td>3.01±0.10</td>
<td>5.27</td>
</tr>
</tbody>
</table>

The data represent the mean±standard deviation (S.D.), n=6.

Table 2. Liposome Formulation Characteristics: Measured Particle Size, Zeta Potential, Polydispersity and at the End Encapsulation Efficiency of for BNZ Liposomes

<table>
<thead>
<tr>
<th>Code of formulation</th>
<th>Mean diameter (µm)</th>
<th>Zeta potential (mV) (after extruded)</th>
<th>Polydispersity index (after extruded)</th>
<th>Encapsulation efficiency (EE) (%) (after extruded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Before extruded: 3.08±0.035</td>
<td>−4.66±0.07</td>
<td>0.870±0.054</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>After extruded: 1.35±0.055</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L2</td>
<td>Before extruded: 5.71±0.078</td>
<td>−1.50±0.16</td>
<td>0.421±0.038</td>
<td>34.3±1.71</td>
</tr>
<tr>
<td></td>
<td>After extruded: 2.25±0.009</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

Fig. 1. TEM Image of L1 and L2 Liposomes before Extrusion and after Extrusion at 22000× Magnification
lipogels during vaginal administration, as well as the controlled release of BNZ, hydrogels made of Carbopol® 974P and HPMC K100M would be the proper choice of vehicle. Research groups working independently have already shown that the inclusion of different drug loaded liposomes in a Carbopol® 974P bioadhesive gel improved liposome stability in a medium simulating the vaginal environment while delaying the release of clotrimazole and metronidazole.30–32

Mechanical Properties

There are some difficulties in the application of vaginal dosage forms. Syringe or applicator is required for successful administration of the vaginal gel. Moreover, the rapid discharge of cervical mucus from vaginal systems is a disadvantage for pharmaceutical dosage forms.33 For this reason some textural tests are frequently used to identify formulations that may be suitable for clinical application.30 The back extrusion test has been used as an interesting technique in order to determine the mechanical properties of vaginal liposomes, gels and lipogels. This test can provide some important parameters such as the firmness, consistency, cohesiveness and index of viscosity of gels and lipogels. It can be said that this study will be original, as using the back extrusion test on pharmaceutical preparations has not been found in the literature. Data collection and calculations were performed using the Texture Exponent 4.0.6.0 software package of the instrument. Based on the resultant force-time plot, mechanical parameters, such as firmness, consistency, cohesiveness and index of viscosity of the gel and lipogel formulations were determined (Fig. 4).

The firmness, consistency, cohesiveness and index of viscosity were used to describe the texture characteristics of liposomes, gels and lipogels. The maximum force was taken as a measurement of firmness: the higher the value is, the firmer the sample is.34 The area under the positive part of the graph indicates sample consistency; the higher the value, the thicker
and the higher is the consistency of the sample. Cohesiveness is the maximum negative force, and the index of viscosity is the area of negative region. The results are given in Table 4 and Fig. 5. It can be seen that the texture parameters of the formulation generally increase with the increase of viscosity. The L1 formulation including 2% Carbopol® 974P showed the highest hardness value because it displayed the highest density. It can be said that the viscosity of the formulations significantly increased the value of their hardness. The formulation of L1 was observed to have a lower firmness value than that of the other formulations. The consistency value indicates the density of the product. It is evident that the higher is the value of the consistency, means the higher is the product density. The results show the same trend as in the firmness analysis of the formulations. The highest consistency value was obtained for the C1 gel formulation in parallel with is firmness values. Cohesiveness is an expression of in determining the ability of the of gel to reconstruct itself after application. A high cohesiveness value increases the performance of the product at the application site by providing full structural recovery following gel application. The maximum negative force is taken as an indication of the cohesiveness of the sample: the more negative is the value, the more cohesive is the sample. The cohesiveness results of the liposomes, gels and lipogels formulations were different, and the L1 gel formulation showed the lowest values. Depending on the formulation type, the cohesiveness of the gels was arranged in the order C1>H1>H2>C2>N1>N2>L2>L1. This data show that the cohesiveness of liposomal formulations was greatly increased by the transport within the gel, therefore, lipogel formulations (N1 and N2) were more suitable than liposome formulations (L1 and L2) in terms of application. The firmness, consistency, cohesiveness and index of viscosity of the formulation were assessed using a texture analyzer, which mainly indicates the application ability of gels and lipogels, so the formulations can be easily administered in vivo. Liposome formulations (L1 and L2) are not suitable for vaginal administration because they show very low textural values compared to other formulations. This study has shown that the vaginal administration of liposomes as lipogel formulations are possible.

**Mucoadhesive Studies** In this study mucoadhesive gel and lipogel formulations for vaginal drug delivery were developed so as to improve the topical effects of BNZ. BNZ was formulated in liposome, gel and lipogel by using the mucoadhesive polymers HPMC and Carbopol® 974P. When increase the retention time of this formulation at vaginal mucosa is increased, the effectiveness of the formulation also increases. For this purpose the mucoadhesive properties of the prepared formulations were examined using the TA·XTPlus Texture Analyzer by evaluation of the detachment force required to overcome the adhesive bond between each formulation and the vaginal mucosa. The mucoadhesion test results of the formulations prepared were given in Table 5. As seen in Table 5, gels and lipogels showed good mucoadhesive properties and their work of mucoadhesion values ranged between 0.487±0.072 and 0.176±0.037 mL/cm². In the literature, mucoadhesive formulations comprised of the anionic polymer Carbopol® and nonionic hydroxypropylmethylcellulose (HPMC) were evaluated as vaginal drug delivery systems.

This study showed that the mucoadhesion value was highest in HPMC K100M gel formulations (H1 and H2). Our results supported previous studies. However the work of mucoadhesion of the liposome formulations (L1 and L2) is lowest in all formulations: this system displayed no adhesion. So the retention time in vagina will be short. This study has developed lipogels to overcome from this problem. Lipogel formulations showed at least five times more mucoadhesive properties than liposomes, and therefore application of liposomes the vagina in the form of lipogel will prolong the retention time in vagina. The incorporation of liposomes in C1 and H1 gels further improved mucoadhesion properties and confirmed the applicability of liposomes as a vaginal delivery system for encapsulated BNZ, for the local treatment of vaginitis.
Fig. 5. Back Extrusion Test Graphs of the L1, L2, C1, C2, H1, H2, N1 and N2 Vaginal Formulations Using Texture Analyser ($n=3$)
Conclusion

The film forming method has been proven to be simple, reproducible and appropriate for the encapsulation of BNZ. Incorporation of liposomes into bioadhesive Carbopol® 974P and HPMC K100M gels (N1 and N2 formulations) further confirmed the applicability of liposomes as a novel vaginal delivery system for the controlled release of encapsulated BNZ from mucoadhesive vaginal lipogel. Lipogel formulations N1 and N2 showed the most controlled release of BNZ for the local treatment of vaginitis. It was found that these formulations confirmed the applicability of liposomes as a novel vaginal delivery system for the controlled release of encapsulated BNZ.

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Conflict of Interest

The author declares no conflict of interest.

References