Preparation of Extemporaneously Diazepam Suppositories: *In Vitro* Release and Aging Study

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บทคัดย่อ

The objective of this study was to develop a fast-release extemporaneously diazepam suppository using pulverized diazepam tablet instead of diazepam powder. Four kinds of extemporaneously diazepam suppositories were formulated: 1) a conventional suppository with Witepsol H-15 as a base, 2) a conventional suppository with mixed polyethylene glycols (PEGs) as a base, 3) a hollow-type suppository with Witepsol H-15 as a base which contained pulverized diazepam in its cavity and 4) a hollow-type suppository with mixed PEGs as a base which contained pulverized diazepam in its cavity. The results of DSC thermograms indicated that diazepam was dissolved in Witepsol base and had strong affinity to this lipophilic base while diazepam dispersed in mixed PEGs base was still in the crystalline form. From the dissolution studies, the release of diazepam from suppository formulation no. 1, 3 and 4 was very slow. Diazepam suppository from formulation no. 2 exhibited the fastest release which complete dissolved after 30 minutes and gave the similar dissolution and permeation profiles compare to diazepam suppository using diazepam powder as the active ingredient. The content of diazepam from formulation no. 2 and the dissolution profiles after storage for 2 months did not
significantly different from freshly prepared. Therefore, the extemporaneously diazepam conventional suppository which mixed PEGs as the base was found to be the most effective rapid-release formulation.

Introduction

Rectal diazepam is an effective and safe treatment for prolonged seizures outside the hospital. Rectal diazepam suppository formulations are not always available and the rectal administration of tablets, injections or retention enemas is often considered. However, rectal diazepam solutions have not been used widely because they tend to leak out of the rectum, which can lead to inaccurate dosing and treatment failure (Fitzgerald BJ. et al., 2003). Suppositories are often preferred for infants or small children, severely debilitated patients, those who cannot take medications orally and those for whom the parenteral route might be unsuitable. Since diazepam suppository is not commercially available in Thailand, it may be useful for the pharmacist in hospital to have a method of preparing this dosage form to provide for the pharmaceutical needs of certain patients. The aim of this study was to formulate the most effective rapid-release extemporaneously diazepam suppositories using diazepam tablets manufactured by Government Pharmaceutical Organization (GPO), Thailand as the active ingredient. Two types of diazepam suppositories were prepared. The diazepam conventional suppositories were prepared by incorporating the diazepam in the suppository base. Hollow-type suppositories were prepared by incorporating diazepam into the hollow cavity of suppository base. Release of diazepam from conventional and hollow-type suppositories was evaluated by in vitro dissolution and in vitro permeation tests and the data was compared with that of conventional suppository containing diazepam powder. The aging stability of extemporaneously diazepam suppositories that exhibited the fastest dissolution was also studied.

Materials and Methods

Materials

Diazepam powder was donated by Atlantic Pharmaceutical Industry (Bangkok, Thailand). Diazepam 5 mg tablets was purchased from GPO, Witepsol H-15 (Lot no. 905708) was donated by Condea Chemie GMBH (Witten, Germany). Polyethylene glycol 400 (Lot no. 99-1548) PEG 4000 (Lot no. 45-1275) were provided by BASF (Bangkok, Thailand). Dialysis membrane (Spectra/Por®, MWCO 12000-14000, 16 mm diameter) was obtained from Spectrum Laboratories (Rancho Dominguez, CA, USA). All other reagents were of analytical-reagent grade.

Methods

Preparation of Diazepam Suppository

Four formulations of extemporaneously diazepam suppositories and one formulation of diazepam suppository were formulated. The compositions of these formulations are presented in Table 1. Diazepam tablets were pulverized using the mortar and pestle. Conventional suppositories were prepared by fusion method. The Witepsol
H-15 or mixed PEGs (blending of PEG 400 and PEG 4000 in the ratio of 1:1) as the suppository base was melted over the water bath. Then, pulverized diazepam was added with stirring until a homogeneous mass was produced. The mixture was poured into a metal suppository mold at a temperature just above the congealing point of the suppository base and cooled. Hollow-type suppositories, shown in Figure 1, were prepared by the method reported by Watanabe et al. (Watanabe et al., 1986) In brief, Witepsol H-15 or mixed PEGs base was melted at approximately 40 °C and 65 °C, respectively, poured into the suppository mold equipped with cylindrical tube in the center of the mold and allowed to solidify for 2 hours at room temperature. After construction of a hollow cavity of the solidified base, pulverized diazepam was added to each cavity. The opening at the hind part of the suppository was sealed with the melted base. Each suppository contained 10 mg of diazepam equivalent. Diazepam conventional suppository with mixed PEGs as a base using diazepam powder, formulation no. 5, was also prepared by fusion method as described above.

<table>
<thead>
<tr>
<th>Formulation no.</th>
<th>Active ingredient</th>
<th>Suppository base</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pulverized diazepam from tablet</td>
<td>Witepsol H-15</td>
<td>Conventional suppository</td>
</tr>
<tr>
<td>2</td>
<td>Pulverized diazepam from tablet</td>
<td>Mixed PEGs</td>
<td>Conventional suppository</td>
</tr>
<tr>
<td>3</td>
<td>Pulverized diazepam from tablet</td>
<td>Witepsol H-15</td>
<td>Hollow-type suppository</td>
</tr>
<tr>
<td>4</td>
<td>Pulverized diazepam from tablet</td>
<td>Mixed PEGs</td>
<td>Hollow-type suppository</td>
</tr>
<tr>
<td>5</td>
<td>Diazepam powder</td>
<td>Mixed PEGs</td>
<td>Conventional suppository</td>
</tr>
</tbody>
</table>

**Figure 1** Compositions of diazepam suppositories
Evaluation of Physical Properties of Suppositories

The prepared suppositories were evaluated for disintegration according to method described in the British Pharmacopeia (BP). The mean values were calculated from six parallel measurements.

Differential Scanning Calorimetry

The thermal properties of pulverized diazepam tablet, Witepsol H-15, mixed PEGs and diazepam suppositories were studied on a differential scanning calorimeter (DSC) (Perkin-Elmer DSC7, Norwalk Connecticut, USA). Samples (2-8 mg) were accurately weighed and heated in closed aluminium crimp cells at the rate of 10 °C/min under nitrogen purge with a flow rate of 35 mL/min over 20 °C to 160 °C temperature range.

Dissolution Studies

The dissolution rates of extemporaneously diazepam conventional and hollow-type suppositories compared with conventional suppository with diazepam powder were determined. A rotating basket dissolution apparatus was used at 50±1 rpm at a temperature of 37±0.5 °C. Five hundred milliliters of phosphate buffer solution pH 7.4 (modeling the rectal pH) was used as the medium. At appropriate time intervals, 4 mL samples were withdrawn, filtered and the amount of diazepam was determined by ultraviolet spectrophotometry (Spectronic Genesys 5, Milton Roy, Rochester, New York, USA) at 232 nm using appropriate blank solutions. Diazepam concentration was calculated and expressed as percentage of drug released from the mean of six determinations.

In Vitro Permeation Studies

The extemporaneously diazepam suppositories and conventional suppository with diazepam powder were inserted into a semipermeable membrane tube. Both sides of the tube were tied up with a thread to prevent leakage. Then the semipermeable tube was placed in a dissolution flask containing 500 mL of phosphate buffer solution pH 7.4. Drug release test was performed at 37±0.5 °C using the paddle speed at 100 rpm. At appropriate time intervals, 4 mL of the medium was sampled and filtered. The filtrate was analyzed by ultraviolet spectrophotometry at 232 nm using appropriate blank solutions. Diazepam concentration was calculated and expressed as percentage of drug released from the mean of six determinations.

Aging Studies

The extemporaneously diazepam suppository was kept at ambient temperature and 2-8 °C for 2 months. At appropriated time intervals, 0, 7, 14 days, 1 and 2 months, the content of diazepam was determined according to the method described by BP for diazepam tablets (British Pharmacopoeia, 2001). In brief, diazepam suppositories were dissolved in 80 mL of 0.5%-w/ v of sulfuric acid in ethanol. The obtained solution was sonicated for 15 minutes and adjusted the volume to 100 mL in volumetric flask and then centrifuged at 4,000 rpm for 15 minutes. The supernatant was appropriate diluted and measured the drug content spectrophotometrically at 284
nm. Blank suppositories without the drug were prepared and subjected to the same analytical procedure to serve as blank for spectrophotometric determination. The dissolution of aged diazepam suppository after 2 months of storage was also investigated.

Results and Discussion
Evaluation of Physical Properties of Suppositories
The disintegration time for the suppository formulation no. 1, 2, 3 and 4 was $10 \pm 0.7$, $29 \pm 1.8$, $9 \pm 0.7$ and $27 \pm 2.1$ minutes respectively. According to the BP requirement, disintegration occurs in not more than 30 minutes for lipophilic-based suppositories and in not more than 60 minutes for water-soluble suppositories. So, all suppositories were found to satisfy the BP requirement for disintegration.

Differential Scanning Calorimetry
Figure 2 shows the DSC thermograms of diazepam powder, pulverized diazepam, Witepsol H-15, diazepam conventional suppository with Witepsol H-15 as the base (formulation no. 1), mixed PEGs and diazepam conventional suppository with mixed PEGs as the base (formulation no. 2). The thermogram of diazepam powder showed a single sharp endothermic peak at 139°C indicating the melting point of diazepam. Witepsol H-15 showed an endothermic peak at 36°C, and the mixed PEGs showed endothermic peaks at 44°C and 51°C, corresponding to the melting points of the base. The thermogram of pulverized diazepam showed two endothermic peaks at 133°C and 151°C indicating the melting point of diazepam, shift to lower temperature, and other tablet excipient respectively. The thermogram of the diazepam suppository in Witepsol H-15 showed two endothermic peaks at 36°C, 152°C indicating the melting point of Witepsol H-15 and the excipient respectively while the endothermic peak of diazepam, at 139°C, absent revealing that diazepam is completely soluble in the Witepsol base (Mura P. et al., 1996). The thermogram of the diazepam suppository in mixed PEGs showed the endothermic peaks of this base at 44°C and 51°C and the endothermic peaks of diazepam and excipient were still presented, indicating that the diazepam dispersed in this base is still in the crystalline form (Wang X. et al., 2004).

Figure 2 DSC thermograms of diazepam powder, pulverized diazepam tablet, Witepsol H-15, extemporaneously diazepam conventional suppository with Witepsol H-15 as a base (formulation no. 1), mixed PEGs, extemporaneously conventional suppository with mixed PEGs as a base (formulation no. 2).
The dissolution profiles of extemporaneously diazepam suppositories are shown in Figures 3 and 4. The dissolution rate of diazepam from conventional suppository with Witepsol H-15 as the lipophilic base, formulation no. 1, was very slow and lower than from hollow-type suppository with Witepsol H-15 as the base, formulation no. 3. The dissolution of diazepam from the conventional suppository with mixed PEGs as the hydrophilic base, formulation no. 2, was about 45% after 5 minutes and exhibited complete dissolved after 30 minutes. The dissolution of diazepam from conventional suppository with Witepsol H-15 as the base was only 3% after 5 minutes and 10% after 120 minutes. The faster dissolution rate of diazepam from the mixed PEGs base may be due to both the low affinity of the drug to the base and the water solubility of the base, which allows the drug to be released by both diffusion and erosion mechanisms (Hosny EA. Et al., 1996). The slower dissolution rate observed in the conventional suppository using Witepsol H-15 as the base is most probably due to the strong affinity of the drug to the lipophilic base, which results in hindered migration of diazepam molecules in the dissolution medium. (Tanaka M. et al., 1998). Therefore, Witepsol H-15 is not suitable for diazepam rapid-release conventional suppository. The dissolution rate of diazepam from the hollow-type suppository with Witepsol H-15, formulation no. 3, was very slow. This may be because the drug was dissolved in the melted base. The dissolution rate of diazepam from the hollow-type suppository with mixed PEGs, formulation no. 4, was very slow because in the first five minutes only the mixed PEGs dissolved. Then outer surface of pulverized diazepam which contacted the dissolution medium formed the film that prevented the medium to penetrate into the inner portion of the pulverized drug. This may be due to the binder or other excipients of diazepam tablet interact with dissolution medium. The dissolution rate of diazepam from the conventional suppository with mixed PEGs as the base was significantly faster than that of Witepsol H-15 as the base may be due to the rapid soluble of the base, which allowed the rapid release of the diazepam. Figure 5 shows the dissolution profiles of extemporaneously diazepam suppository with mixed PEGs as the base (formulation no. 2) and diazepam suppository using diazepam powder with mixed PEGs as the base (formulation no. 5). The dissolution profiles of two formulations were quite similar and the percentage diazepam dissolved after 30 minutes and 2 hours did not significantly different (p>0.05)

![Dissolution profile graph](image)

**Figure 3** Dissolution profiles of extemporaneously diazepam conventional suppositories with Witepsol H-15 (formulation no. 1) and mixed PEGs (formulation no. 2) as a base.
that the permeation profiles of formulation no. 2 and formulation no. 5 gave faster release than the formulation no. 1, 3 and 4. This may be due to the faster dissolution rate of the formulation no. 2 and 5. The extemporaneously diazepam suppository which exhibited the fastest dissolution and permeation, formulation no. 2, was selected for aging studies.

Figure 6 The in vitro permeation profiles of diazepam suppositories (formulation no. 1-5).

Aging Studies

The content of diazepam in extemporaneously suppository, formulation no. 2, stored at ambient temperature and at 2-8 °C retained more than 90% of the initial concentration throughout the 2-month study period (Table 2). The dissolution profiles of freshly prepared extemporaneously diazepam suppository and 2 month aged sample, Figure 7, did not significantly different (p>0.05). Therefore, this suppository was stable under the storage conditions.
Table 2 Aging studies of extemporaneously diazepam conventional suppository (formulation no. 2).

<table>
<thead>
<tr>
<th>Storage time</th>
<th>% Initial concentration remaining(\text{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient temperature</td>
</tr>
<tr>
<td>1 day</td>
<td>100.4±3.1</td>
</tr>
<tr>
<td>7 days</td>
<td>99.8±2.8</td>
</tr>
<tr>
<td>14 days</td>
<td>98.7±3.5</td>
</tr>
<tr>
<td>1 month</td>
<td>96.9±2.2</td>
</tr>
<tr>
<td>2 months</td>
<td>96.6±2.8</td>
</tr>
</tbody>
</table>

\(\text{a}\)Mean+S.D., n=3

Figure 7 Dissolution profiles of extemporaneously diazepam conventional suppositories with mixed PEGs as a base (formulation no. 2); freshly prepared and after storage and after storage at ambient temperature and 2-8 °C for 2 months.

Conclusion

In this study, the extemporaneously diazepam conventional suppository with mixed PEGs as the base, formulation no. 2, give the fastest dissolution and exhibit the similar dissolution and release profiles compare to diazepam conventional suppository using the same base and diazepam powder as the active ingredient. The diazepam still stable in this suppository base within the 2 months of storage. Therefore, this extemporaneously diazepam conventional suppository may be useful as a practical rectal dosage form. However further studies are required to demonstrate the rectal absorption of the drug in humans.

References


