Characterization of the complexes of furosemide with 2-hydroxypropyl-β-cyclodextrin and sulfobutyl ether-7-β-cyclodextrin

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Abstract

The purpose of this study was to prepare and characterize complexes of furosemide with 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) and sulfobutyl ether-7-β-cyclodextrin (SBE-7-β-CD). Solid complexes of furosemide with 2-HP-β-CD and SBE-7-β-CD were prepared by using both a freeze–drying and kneading method. Physical mixtures were prepared for comparison. The inclusion complexes were characterized by differential scanning calorimetry, X-ray diffractometry (XRD) and 1H nuclear magnetic resonance spectroscopy (1H-NMR). 1H-NMR, and especially the use of the two-dimensional ROESY spectrum, was used to determine the position of the furosemide molecule inside the cyclodextrin cavity. 1H-NMR studies showed that furosemide fit into the cyclodextrin torus cavity with its furane ring nearest to the primary hydroxyl side.

Keywords: Furosemide; 2-Hydroxypropyl-β-cyclodextrin; Sulfobutyl ether-7-β-cyclodextrin; Complexation

1. Introduction

Furosemide is a potent loop diuretic used in the treatment of oedematous states associated with cardiac, renal and hepatic failure and the treatment of hypertension (Murray et al., 1997). Furosemide therapy, however, is frequently complicated by the apparently erratic systemic availability from the oral route (Boles Ponto and Schoenwald, 1990). The poor bioavailability of furosemide has been hypothesised to be due to the poor aqueous solubility of the compound, site-specific absorption, presystemic metabolism and other unknown mechanisms. Improvement of its dissolution properties is essential because the in vitro dissolution behavior of furosemide tablets is closely related to its bioavailability (Ammar et al., 1999; Özdemir and Ordu, 1998; McNamara et al., 1987; Kingsford et al., 1984). Furosemide, further undergoes hydrolysis and photochemical degradation (Rowbotham et al., 1976).

Cyclodextrins are cyclic oligosaccharides, which have been recognised as useful pharmaceutical excipients (Loftson and Brewster, 1996). The molecular structure of these glucose derivatives generates a hydrophilic exterior surface and a hydrophobic cavity interior. As such, cyclodextrins can interact with appropriately sized molecules to result in the formation of inclusion complexes. These complexes offer a variety of physicochemical advantages, including the possibility for increased water solubility, solution stability and bioavailability (Loftson and Brewster, 1996). Originally only the natural cyclodextrins were used, but due to problems encountered with the aqueous solubility of β-cyclodextrin, derivatives of these cyclodextrins, with increased aqueous solubility properties, have been developed (Duchêne and Wouessidjewe, 1990).

Pitha et al. (1986) and Kata and Kedvessy (1987) both showed increased solubility of furosemide with hydroxypropyl-β-cyclodextrin and β-cyclodextrin, respectively. In this study, furosemide was complexed with 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) and sulfobutyl ether-7-β-cyclodextrin (SBE-7-β-CD), a new derivative of β-cyclodextrin. These β-cyclodextrin derivatives present improved safety and solubility properties compared to the parent β-cyclodextrin. The objective of this study was to prepare furosemide (F)–cyclodextrin inclusion complexes by using a kneading and freeze–drying method. The complexes were investigated and
confirmed by differential scanning calorimetry (DSC), X-ray diffraclometry (XRD) and $^1$H nuclear magnetic resonance spectroscopy ($^1$H-NMR). The position of the furosemide molecule in the cyclodextrin cavity was determined using $^1$H-NMR.

2. Materials and methods

2.1. Materials

Furosemide was obtained from Adcock Ingram (Wadeville, South Africa). 2-Hydroxypropyl-β-cyclodextrin (Encapsin®) was purchased from Janssen Biotech (Olen, Belgium) and sulfobutyl ether-7-β-cyclodextrin was donated by CyDex L.C. (Overland Park, KS, USA). All other compounds and solvents used in this study were of analytical-reagent grade.

2.2. Preparation of the inclusion complexes

The inclusion complexes of furosemide with 2-HP-β-CD and SBE-7-β-CD were prepared by using the following two methods.

2.2.1. Kneading method

Solid-state furosemide complexes with 2-HP-β-CD and SBE-7-β-CD were prepared in a 1:1 molar ratio. The required amount of cyclodextrin was accurately weighed and transferred to a mortar. An ethanol–water (50:50, v/v) mixture was added to the cyclodextrin powder and the resultant slurry was kneaded for 10 min. Furosemide was added in small portions to the cyclodextrin slurry with simultaneous addition of solvent and kneaded thoroughly in a mortar and pestle for 90 min. The resultant paste was then dried in vacuo (Vismara vacuum oven, Italy) at 40±1.0 °C for 24 h.

2.2.2. Freeze–drying

Solid-state furosemide complexes with 2-HP-β-CD and SBE-7-β-CD in 1:1 molar ratios were prepared. The required amount of furosemide and cyclodextrin was accurately weighed and dispersed in distilled water and rotated in a paraffin bath at 25±1.0 °C for 24 h. The solution was then freeze–dried (Virtis Freezemobile, New York, NY, USA) for 48 h and dried in vacuo (Vismara vacuum oven) at 40±1.0 °C for 24 h.

2.2.3. Physical mixture

Physical mixtures of furosemide with 2-HP-β-CD and SBE-7-β-CD were prepared in the same stoichiometric ratio as the kneaded and freeze–dried products. The required amount of furosemide and cyclodextrin was accurately weighed, where after the components were blended in a turbula mixer (Model T 2 C, Willy A. Bachofen, Machinenfabrik, Switzerland) for 5 min.

2.3. Differential scanning calorimetry

A Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan) was used for recording DSC thermograms of the furosemide raw material, inclusion complexes prepared by the kneading and freeze–drying methods, as well as the physical mixtures. Samples (2–8 mg) were accurately weighed using a Sartorius 4503 electronic microbalance (Göttingen, Germany) and heated in closed aluminium crimp cells at a rate of 10 °C/min under nitrogen purge with a flow rate of 35 ml/min over the 30–400 °C temperature range.

2.4. X-Ray powder diffractometry

X-Ray powder diffraction patterns were obtained at room temperature using a Philips PM 9901/00 diffractometer (Philips, The Netherlands). The measurement conditions were as follows: target, Cu Kα; filter, Ni; voltage, 40 kV; current, 30 mA; slit, 0.2 nm; scanning speed, 2° per min. Approximately 200 mg samples were weighed into aluminium sample holders.

The XRD traces of furosemide raw material, inclusion complexes as well as the physical mixtures were compared with regard to peak position and relative intensity, peak shifting and the presence and or lack of peaks in certain regions of °2θ values.

2.5. $^1$H Nuclear magnetic resonance spectroscopy

The 500 MHz $^1$H-NMR spectra of furosemide as well as the inclusion complexes, prepared by the freeze–drying method, were recorded using a Bruker DRX-500 NMR spectrometer (Karlsruhe, Germany). The 500 MHz $^1$H-NMR spectrum of the inclusion complex (F/β-CD complex) of furosemide with natural β-cyclodextrin was also recorded. All the spectra were recorded in D$_2$O, except for the 500 MHz $^1$H-NMR spectra of furosemide which were recorded in DMSO-d$_6$. The two-dimensional ROESY (Rotating Frame Overhauser Effect Spectroscopy) spectrum of the F/β-CD complex in D$_2$O was recorded, using a standard pulse sequence with a mixing time of 250 ms.

3. Results and discussion

3.1. Differential scanning calorimetry

The DSC thermograms of furosemide, 2-HP-β-CD and SBE-7-β-CD, as well as their inclusion complexes (F/2-HP-β-CD complex and F/SBE-7-β-CD complex), prepared by the kneading and freeze–drying methods and their physical mixtures, are shown in Figs. 1 and 2.

Furosemide exhibits a characteristic, sharp exothermic peak at 219 °C, which is usually associated with the decomposition of the drug (Boles Ponto and Schoenwald,
1990). The degradation product of furosemide displays an endothermic peak at 268°C as is evident from Figs. 1 and 2. The disappearance or shifting of endo- or exothermic peaks of drugs is mostly an indication of the formation of inclusion complexes (Loukas et al., 1997). The absence of the characteristic peak of furosemide at 219°C (Figs. 1 and 2), is strong evidence of the inclusion of the drug into the cyclodextrin cavity. The F/2-HP-β-CD complexes show an exothermic peak on the DSC thermograms at 254°C.
and 265 °C and the F/SBE-7-β-CD complexes at 237 °C. It is clear from the DSC thermograms that a new product has formed—in this instance an inclusion complex.

The intensity of these exothermic peaks (Figs. 1 and 2) is smaller than those observed on the DSC thermograms of furosemide and cyclodextrin components before complexation. This lower intensity can possibly be attributed to the sum of the individual components and can also be indicative of the formation of an inclusion complex.

3.2. X-Ray powder diffractometry

X-Ray powder diffractograms of furosemide, 2-HP-β-CD and SBE-7-β-CD, their physical mixtures as well as their inclusion complexes as prepared by the kneading and freeze–drying method, are shown in Figs. 3 and 4.

The diffractogram pattern of an inclusion complex clearly differs from that of uncomplexed cyclodextrins (Bekers et al., 1991) when complex formation is indicated. It is necessary to compare the diffractograms of the assumed complex with that of the physical mixture, as well as that of the guest compound under investigation. The guest compound, however, must also be exposed to the same conditions as the assumed complex, because the complex preparation process, such as the freeze–drying method, may have an influence on the crystallinity of the guest compound.

The X-ray powder diffraction patterns of furosemide shown in Fig. 3 displayed crystallinity. The freeze–drying
process did not have any influence on the crystallinity of the furosemide raw material. Amorphous patterns, however, were observed for both 2-HP-β-CD and SBE-7-β-CD. The diffractograms of the physical mixtures mostly showed the amorphous cyclodextrin character, while some of the furosemide crystalline characteristics are noticeable (Figs. 3 and 4). The peak of high intensity at \(\sim 38 \, ^\circ\) appearing in some of the diffractograms in both Figs. 3 and 4 is probably due to diffraction from the planes of the aluminium sample holder. This is a common error incurred in the recording of X-ray diffraction patterns and is not an indication of potential crystalline characteristics of a compound.

Comparing the diffraction patterns of furosemide, 2-HP-β-CD and SBE-7-β-CD with the diffractograms of the inclusion complexes, a definite difference from those of the amorphous 2-HP-β-CD and SBE-7-β-CD is observed. The diffractogram of the freeze–dried 2-HP-β-CD complex (Fig. 3) shows mainly amorphous as well as some crystalline characteristics. This diffractogram differs from the diffractograms of furosemide, 2-HP-β-CD and of the physical mixture indicating complex formation. The diffractogram of the complex formed by the kneading method differs much from that of the freeze–dried complex as well as that of furosemide, 2-HP-β-CD and of the physical mixture, also indicating complex formation.

The diffractogram of the freeze–dried complex of furosemide with SBE-7-β-CD (Fig. 4) shows mostly amorphous characteristics and is very similar to that of the SBE-7-β-CD raw material, however without the crystalline characteristics of furosemide. It also differs much from the diffractogram of the physical mixture indicating complex formation. In this case the diffractogram of the kneading method shows more crystalline characteristics. According to Williams et al. (1998) lack of crystallinity is added evidence for the formation of an inclusion complex.

### 3.3. \(^1\)H Nuclear magnetic resonance spectroscopy

The structure of furosemide with the proton numbering used and a side view as well as a view from the secondary hydroxyl side of the β-cyclodextrin molecule are presented in Figs. 5 and 6, respectively. The assignments and the chemical shift values of the various protons of furosemide are given in Table 1. A two-dimensional ROESY spectrum of the freeze–dried F/β-CD complex in D\(_2\)O is presented in Fig. 7.

### Table 1

Assignments of the chemical shifts to the various protons of furosemide

<table>
<thead>
<tr>
<th>Proton</th>
<th>Chemical shifts* (ppm)</th>
<th>(\text{H-H coupling constants (Hz)})</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>7.602 d</td>
<td>1.8 (H1, H2)</td>
</tr>
<tr>
<td>2</td>
<td>6.406 dd</td>
<td>3.1 (H2, H3)</td>
</tr>
<tr>
<td>3</td>
<td>6.356 d</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.571 d</td>
<td>4.9 (H5, H6)</td>
</tr>
<tr>
<td>8</td>
<td>7.050 s</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>8.391 s</td>
<td></td>
</tr>
<tr>
<td>6 (NH)</td>
<td>8.606 s</td>
<td></td>
</tr>
<tr>
<td>NH(_2)</td>
<td>7.300 s</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Structure of furosemide with the corresponding proton numbering.

Fig. 6. A side view of the torus of the β-cyclodextrin molecule (a) and the β-cyclodextrin molecule, with the proton 3 and proton 5 hydrogen atoms in the cavity of the torus, as seen from the secondary hydroxyl side (b).
The \( ^1 \)H chemical shifts observed for furosemide (Table 1) correspond with the proton NMR data obtained by Al-Obaid et al. (1989) on a Varian (FT 80A) NMR spectrometer. The formation of the inclusion complexes of furosemide with 2-HP-\( \beta \)-CD, SBE-7-\( \beta \)-CD and \( \beta \)-CD was indicated by upfield shifts observed for all the resonances of the ring protons of furosemide. Additionally, if the integrals of the proton resonances of the furosemide were compared to those for the cyclodextrins studied, it was evident that in solution, the cyclodextrin to furosemide ratio was at least 10 to 1 or higher, indicating fairly poor complexation formation.

Derivatisation of natural \( \beta \)-CD usually results in a variety of products, complicating the characterisation of the \( ^1 \)H-NMR spectra. As a result of this, it was impossible to assign unambiguously, the various chemical shifts to specific protons of the derivatised \( \beta \)-CD molecules. In order to overcome these complexities a complex of furosemide with \( \beta \)-CD, without any derivatisation, was prepared. All resonances of \( \beta \)-CD (Fig. 7) can easily be assigned to specific protons. It was assumed that the observed orientation of the furosemide molecule in the \( \beta \)-CD cavity was similar to the orientation of furosemide in both the 2-HP-\( \beta \)-CD and SBE-7-\( \beta \)-CD complexes, because the cavity size of \( \beta \)-CD is similar to the cavity size of 2-HP-\( \beta \)-CD and SBE-7-\( \beta \)-CD (6 Å). This assumption may be more valid for the 2-HP-\( \beta \)-CD (no charge) than for SBE-7-\( \beta \)-CD complex, because the latter is a negatively charged cyclodextrin that may alter the orientation of the furosemide molecule in the cavity.

Nuclear Overhauser Effect (NOE) measurements is a very useful tool to prove complex formation. As the magnitude of NOE values are critically dependent on the spatial distance between interacting protons, it can also provide information on the geometry of cyclodextrin complexes. Protons 3 and 5, which are in the interior of the cyclodextrin cavity, resonate at 3.904 and 3.817 ppm, respectively. Protons 2 and 4, which are on the exterior of the cyclodextrin cavity resonate at 3.595 and 3.582 ppm, respectively (Fig. 7). The NOEs observed between the furosemide and the \( \beta \)-CD protons, as detected in the two-dimensional ROESY experiment (Fig. 7), could only arise if a F/\( \beta \)-CD complex has formed. As shown in Fig. 7 NOEs are observed between protons 1, 2, 3, 8 and 11 of furosemide and protons 3 and 5 of \( \beta \)-CD. Protons 1, 2, 3, 8 and 11 of furosemide gave bigger NOE values to proton 5.

**Fig. 7.** A two-dimensional ROESY spectrum of the furosemide \( \beta \)-cyclodextrin complex in D₂O.
of β-CD compared to proton 3 of β-CD, with $3 > 2 > 8 > 1 > 11$. This data suggests that the furosemide molecule associates with its furan ring (protons 1, 2 and 3) and a part of the aromatic ring mainly to the primary face of the cyclodextrin cavity, where proton 5 (Fig. 6) is situated. The depth of the inclusion of furosemide into the β-CD cavity is indicated by the NOEs observed to the β-CD 3 proton. As is evident from Fig. 7, proton 11 of the aromatic ring of furosemide has a small NOE compared to proton 5 of β-CD, suggesting that proton 11 must be close to the primary rim of the cyclodextrin cavity.

4. Conclusion

Complexation of furosemide with 2-HP-β-CD and SBE-7-β-CD was accomplished using both the kneading and freeze–drying methods. The products found after freeze–drying showed more amorphic characteristics, causing characterization with X-ray diffractometry to be less effective. The complexation of furosemide with the β-cyclodextrin derivatives studied, was confirmed by DSC and $^1$H-NMR spectra. Using the NOEs observed in the two-dimensional ROESY experiment, it was possible to determine that the geometry of the complex was such that the furan ring of furosemide was situated deep into the torus cavity of the cyclodextrin proving complexation. Although complexation took place, the complex was not that stable if the ratio of furosemide to cyclodextrin found, is considered.

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References


