

# Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray

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Received 9 May 2000; received in revised form 15 September 2000; accepted 19 September 2000

## Abstract

The cyclodextrin solubilization of three benzodiazepines, i.e. alprazolam, midazolam and triazolam, was investigated. The cyclodextrin solubilization was enhanced through ring-opening of the benzodiazepine rings and ionization of the ring-open forms. Additional enhancement was obtained through interaction of a water-soluble polymer with the cyclodextrin complexes. The ring-opening was pH-dependent and completely reversible, the ring-open forms dominating at low pH but the ring-closed forms at physiologic pH. The ring-closed forms were rapidly regenerated upon elevation of pH. In freshly collected human serum in vitro at 37°C, the half-life for the first-order rate constant for the ring-closing reaction was estimated to be less than 2 min for both alprazolam and midazolam. Midazolam (17 mg/ml) was solubilized in aqueous pH 4.3 nasal formulation containing 14% (w/v) sulfobutylether  $\beta$ -cyclodextrin, 0.1% (w/v) hydroxypropyl methylcellulose, preservatives and buffer salts. Six healthy volunteers received 0.06 mg/kg midazolam intranasally and 2 mg intravenously, and blood samples were collected up to 360 min after the administration. Midazolam was absorbed rapidly reaching maximum serum concentrations of  $54.3 \pm 5.0$  ng/ml at  $15 \pm 2$  min. The elimination half-life of midazolam was  $2.2 \pm 0.3$  h and the absolute availability was  $73 \pm 7\%$ . All mean values  $\pm$  SEM. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Solubility; Benzodiazepines; Cyclodextrin; Complexation; Ionization

## 1. Introduction

Most benzodiazepine drugs are derivatives of 2,3-dihydro-1*H*-1,4-benzodiazepine with sedative, antianxiety, anticonvulsant and muscle relaxant

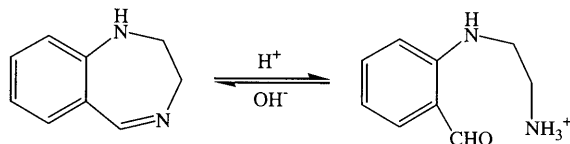
properties. In pharmaceutical formulations benzodiazepines are mainly used as the solid base, and as such they are readily dissolved in lipophilic solvents or in polar organic solvents such as ethanol. Formulation of the benzodiazepine bases in aqueous drug formulation has been hampered by their low aqueous solubility and frequently the only practical means of obtaining pharmaceutically acceptable benzodiazepine solutions is

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through the use of combinations of cosolvents (Bechgaard et al., 1997; Alvarez Núñez and Yalkowsky, 1998). Unfortunately, administration of non-aqueous drug formulations may result in pain, irritation and drug precipitation upon administration (Yalkowsky and Rubino, 1985; Way and Brazeau, 1999). Replacing the cosolvent formulations with aqueous cyclodextrin containing drug formulations may circumvent these side effects (Brewster et al., 1989; Brewster, 1991; Brewster and Loftsson, 1999). Previously, benzodiazepines have been solubilized through cyclodextrin complexation (Kraus et al., 1991; Loftsson et al., 1994). However, the complexation efficacy is frequently low and, thus, relatively large amounts of cyclodextrin are needed to solubilize small amounts of a given benzodiazepine drug. Increased complexation efficacy can be obtained by increasing either the intrinsic solubility of the drug ( $S_0$ ) or the apparent stability constant ( $K_c$ ) of the drug/cyclodextrin complex, or by increasing both simultaneously (Loftsson, 1998; Loftsson et al., 1999). In aqueous solutions, some drugs can exist in more than one structural form, e.g. equilibrium isomers or ionization stages. Although the individual forms are in equilibrium with each other, and thus not totally independent of each other, the overall aqueous solubility (or apparent  $S_0$ ) of a drug can be enhanced through formation of such multiple structural forms.

Cyclic imines, such as 2,3-dihydro-1*H*-1,4-benzodiazepine, are known to undergo reversible and pH-dependent ring-opening through formation of aldehyde or ketone and a primary amine:



Under certain conditions open and closed forms are both present in aqueous solutions. Coexistence of such forms increases the apparent solubility of the benzodiazepine. Often, the ring-open form is an intermediate which is formed during benzodiazepine degradation in aqueous solutions but in some cases, e.g. in the case of alprazolam, midazolam and triazolam, this form

is chemically stable and can contribute to the overall aqueous solubility of the drug (Cho et al., 1983; Kanto, 1985; Kurono et al., 1985; Kuwayama et al., 1986; Sbarbati Nudelman and de Waisbaum, 1995). For example, in the commercial aqueous intravenous (i.v.) solution of midazolam (Dormicum<sup>®</sup>, Hoffmann–La Roche, Switzerland) the drug is 15–20% in the ring-open form and the pH is approximately 3.3 (Gerecke, 1983). In addition, both forms, i.e. the ring-open and the ring-closed midazolam, can exist in several different ionization forms. In the aqueous i.v. solution, the ring-open form of midazolam can be characterized as a midazolam prodrug since the ring is completely closed when the pH is elevated to 7.4. Previously we have shown that low complexation efficiency can hamper the usage of cyclodextrins in certain pharmaceutical formulations and that both drug ionization and water-soluble polymers can enhance the complexation efficiency (Loftsson, 1998; Loftsson et al., 1999). Ionization of a drug molecule increases the apparent  $S_0$  and addition of a water-soluble polymer to the complexation media increases  $K_c$ .

Several investigators have attempted to use the commercially available aqueous i.v. solution for intranasal (i.n.) administration of midazolam (Björkman et al., 1997; Burstein et al., 1997). The midazolam concentration in this solution is only 5 mg/ml. Thus, relatively large amounts of the acidic i.v. solution have to be sprayed into the nose in order to induce sedation and anxiolysis. Subsequently midazolam is only partly absorbed from the nasal cavity and partly from the gastrointestinal tract after swallowing. The midazolam bioavailability after i.n. administration of the i.v. solution is frequently about 50% (Burstein et al., 1997). To reduce spilling and swallowing of the i.v. solution after i.n. administration, and to improve the bioavailability, the dosage has to be sprayed in small aliquots into the nasal cavity (Björkman et al., 1997). However, i.n. administration of the acidic i.v. solution can cause severe irritation in the nasal cavity.

The purpose of the present study was to investigate the effects of the reversible ring-opening of the diazepine ring and ionization on the cyclodextrin complexation of benzodiazepines, as well as

formulation and testing of physiologically acceptable aqueous midazolam nasal spray solution.

## 2. Materials and methods

### 2.1. Materials

Midazolam base was purchased from Sifa (Shannon, Ireland), and alprazolam and triazolam from Sigma (St Louis, MO). Sulfobutylether- $\beta$ -cyclodextrin sodium salt with molar substitution of 6.2 (Captisol<sup>®</sup>, SBE $\beta$ CD) was kindly donated by CyDex (Kansas City, KS). Randomly methylated  $\beta$ -cyclodextrin with degree of substitution (DS) of 1.8 (RM $\beta$ CD) and 2-hydroxypropyl- $\beta$ -cyclodextrin with DS of 0.6 (HP $\beta$ CD) were kindly donated by Wacker-Chemie (Burghausen, Germany). Hydroxypropyl methylcellulose 4000 (HPMC) was purchased from Mecobenzon (Denmark). All other chemicals used were of pharmaceutical or special analytical grade.

### 2.2. Solubility studies

An excess amount of the drug to be tested was added to water or aqueous Teorell–Stenhagen buffer system (Bates and Paabo, 1989), or the aqueous nasal formulation, containing various amounts of the different cyclodextrins with or without a polymer. The suspension formed was heated in an autoclave in a sealed container to 130°C for at least 30 min. After cooling to room temperature (22–23°C) a small amount of solid drug was added to the container to promote precipitation. Then the suspension was allowed to equilibrate for at least 3 days at room temperature, protected from light. After equilibration was attained, an aliquot of the suspension was filtered through a 0.45- $\mu$ m membrane filter (cellulose acetate from Schleicher & Schuell, Germany), diluted with the HPLC mobile phase and analyzed by HPLC. The pH values reported were determined at room temperature at the end of the equilibration period.

The effect of pH on the stability constant ( $K_c$ ) of the drug/cyclodextrin (1:1) complex was determined as previously described (Loftsson and Pe-

tersen, 1998). Briefly the drug solubility was determined in aqueous nasal formulation containing from 0 to 14% (w/v) cyclodextrin. The composition of the nasal formulation was as follows: benzalkonium chloride (0.02% w/v), EDTA (sodium edetate) (0.1% w/v), HPMC (0.1% w/v), phosphoric acid (0.43% v/v) and aqueous sodium hydroxide solution (for pH adjustment) in water. As before, the exact pH of each solution was determined at the end of the equilibration period. Differences in pH were corrected by drawing the pH-solubility profiles at each cyclodextrin concentration and determining the solubilities of the drug from these profiles at selected pH values. The values obtained were used to draw the phase-solubility diagrams, all of which were linear. Finally,  $K_c$  was calculated from the equation (Higuchi and Connors, 1965):

$$K_c = \frac{\text{Slope}}{S_o(1 - \text{Slope})}$$

where  $K_c$  is the stability constant of the drug–cyclodextrin (1:1) complex, slope is the calculated slope of the linear phase-solubility diagram and  $S_o$  is the apparent intrinsic solubility of the free drug determined in the aqueous complexation media, at appropriate pH, when no cyclodextrin or polymer was present.

### 2.3. Quantitative determinations

The quantitative determination of drugs was carried out on a high performance liquid chromatographic (HPLC) component system consisting of ConstaMetric 3200 isocratic solvent delivery system operated at 1.50 ml/min, a Merck-Hitachi AS4000 autosampler, a Luna C<sub>18</sub> 5  $\mu$ m (4.6  $\times$  150 mm) column, a Spectro Monitor 3200 UV/VIS variable-wavelength detector and a Merck-Hitachi D-2500 Chromato-Integrator. The mobile phase for alprazolam and triazolam consisted of methanol and water (68:32). The pH of the mobile phase was adjusted to 2.7 by addition of trifluoroacetic acid. The flow rate was 1.0 ml/min and the detector was operated at 254 nm. For alprazolam, the retention was 2.8 min for the ring-open form and 4.7 min for the ring-closed form. For triazolam the retention was 2.3 min for

the ring-open form and 3.9 min for the ring-closed form. The mobile phase for midazolam consisted of pH 7.2 aqueous 0.004 M phosphate buffer, acetonitrile and triethylamine (55:45:0.1). The flow rate was 1.5 ml/min and the detector was operated at 240 nm. The retention time was 2.6 min for the ring-open form and 4.2 min for the ring-closed form.

When the fraction of ring-open form was determined the concentration of the closed form was determined right after dissolving the benzodiazepine in the aqueous buffer solution, containing either no cyclodextrin or 10% (w/v) cyclodextrin, and again 24 h later (i.e. after equilibration at 23°C). Preliminary experiments had shown that equilibrium between the closed and the open form was attained within 3 h at 23°C and that no degradation of either the ring-open or the ring-closed form occurred during the 24-h experiment.

#### 2.4. Kinetic studies in aqueous buffer solutions

A stock solution ( $1.0 \times 10^{-3}$  M) of the drug to be tested was prepared in a 0.1 M aqueous hydrochloric acid solution (pH 1). This solution was equilibrated in a 37°C water bath for 3 h. This was to ensure that only the ring-open form was present in the stock solution. Cyclodextrin, ethanol or dimethyl sulfoxide (DMSO) was dissolved in, or mixed with, pH 7.5 aqueous 0.5 M tris(hydroxymethyl)aminomethane (Tris) buffer solution and the solution equilibrated at 37°C. At time zero, 30 µl of the stock solution was added to 1.5 ml of the buffer solution, mixed for a couple of seconds on a vortex mixer, and placed again in the 37°C water bath. At various time points samples were withdrawn from the reaction media and injected into a HPLC system (see Section 2.3). Both the ring-open and the ring-closed forms could be detected by HPLC and the disappearance of the ring-open form was proportional to the appearance of the ring-closed form. The first-order rate constants ( $k_{\text{obs}}$ ) for the disappearance of the ring-open form was calculated by linear regression of the natural logarithm of the peak height versus time plots.

#### 2.5. Kinetic studies in human serum

The rate constant for the ring-closing reaction was determined in serum. The previously described (Section 2.4) stock solution of the drug (15 µl) was added to 1485 µl of serum which had previously been equilibrated at 37°C. After thorough mixing on a vortex mixer for a couple of seconds the solution was placed in a 37°C water bath. Sample (100 µl) was withdrawn from the solution at various time points and mixed with 900 µl of ice cold methanol and the solution sonicated for 1 min. Then the solution was centrifuged and the clear supernatant analyzed by HPLC.

#### 2.6. Formulation of the aqueous nasal spray solution

The phase solubility of midazolam was determined in a medium which closely resembled the aqueous nasal spray vehicle, i.e. 7–13% (w/v) SBEβCD, 0.10% (w/v) HPMC, 0.02% (w/v) benzalkonium chloride, 0.10% (w/v) EDTA and 0.43% (v/v) concentrated phosphoric acid. Excess midazolam was added to this medium and the pH adjusted to 4.35 with concentrated aqueous sodium hydroxide solution, both before and after heating in an autoclave (121°C for 40 min). Then the samples were allowed to equilibrate for at least 4 days at room temperature and analyzed as before (Section 2.2). The exact composition of the nasal spray was based on this study. The viscosity of the nasal spray was determined with a Brookfield viscometer (UK) fitted with a ULADIN spindle and an UL sample holder with water-circulation jacket (25°C). The osmolarity of the nasal spray was measured by the freezing point depression method using a Knauer Osmometer Automatic (Netherlands). The buffer capacity of the nasal spray was estimated by the titration method using an aqueous 0.1 N sodium hydroxide solution. The preliminary evaluation of the chemical stability of midazolam in the nasal formulation was performed by determining the midazolam concentration after successive heating cycles in an autoclave (Midmark M7 SpeedClave). Each heating cycle consisted of heating to 121°C,

maintaining this temperature for 20 min, and cooling to room temperature. The midazolam concentration was determined after each heating cycle. The total number of heating cycles was six. Finally the midazolam nasal spray was stored at room temperature (22–23°C) and samples collected at 0, 3, 4 and 12 months and analyzed.

## 2.7. Evaluation in humans

The study was approved by both the ethics committee of the National University Hospital and the State Committee on Pharmaceuticals in Iceland. Six healthy volunteers (two females and four men) were recruited in a non-blind, crossover study. After obtaining informed consent and 8-h overnight fast, each participant received either intranasal (i.n.) or intravenous (i.v.) application of midazolam. The other application was carried out 7 days later. The participants continued to fast until 2 h after administration of the study formulation. For i.n. administration, the participants received 0.06 mg of midazolam per kg body weight ( $D_{in}$ ), or 200–300  $\mu$ l, of the aqueous nasal solution (Unit Dose closed spray system from Pfeiffer). For i.v. administration the participants received 2 mg of midazolam ( $D_{iv}$ ) in an i.v. solution (Dormicum® from Hoffmann–La Roche). Blood samples (5 ml) were collected from an intravenous catheter at 5, 10, 15, 20, 30, 60, 120, 180, 240 and 360 min. Samples were centrifuged and serum collected and kept frozen until analyzed by reversed phase HPLC method (performed by Medicinsk Laboratorium A/S, Denmark). The serum concentration of midazolam after i.n. and

i.v. administration was compared in each participant and the maximum serum concentration ( $C_{max}$ ) and time to reach  $C_{max}$  ( $t_{max}$ ) determined. In each participant the area under the serum–time curve from 0 to 6 h (AUC) was calculated after both i.n. and i.v. administration using the linear trapezoidal method, and the absolute availability determined from the  $AUC_{in}/D_{in}$  over  $AUC_{iv}/D_{iv}$  ratio.

## 3. Theoretical background

All the benzodiazepine drugs studied, i.e. alprazolam, midazolam and triazolam, contained 2,3-dihydro-1*H*-1,4-benzodiazepine structure (Fig. 1). Alprazolam and triazolam have a 1*H*-1,2,4-triazole ring fused on the 1,2-carbon–nitrogen bond of the diazepine nucleus (i.e. a triazolo [4,3-*a*] [1,4]benzodiazepine structure), where as midazolam has a imidazole ring fused on the 1,2-carbon–nitrogen bond (i.e. an imidazo [1,5-*a*] [1,4]benzodiazepine structure). Imidazole is relatively basic ( $pK_a$  6.9) compared to 1*H*-1,2,4-triazole. Thus, in midazolam the protonated nitrogen in position 2 on the imidazole ring (i.e. N-2a) has a  $pK_a$  of 6.15 whereas in alprazolam and triazolam the protonated N-2a on the triazole ring has  $pK_a \leq 1.5$  (Walser et al., 1978). In the diazepine nucleus the protonated nitrogen in position 4 (i.e. N-4) has been estimated to be about 2.4 (Cho et al., 1983). In aqueous solutions the benzodiazepines undergo a reversible and pH-dependent ring-opening reaction (Fig. 2) (Han et al., 1976, 1977a,b; Cho et al., 1983). The  $pK_a$  of the primary nitrogen formed has been estimated to be about 7.0 (Cho et al., 1983). There are some indications that the ring-opening should be pH-independent (Cho et al., 1983) in which case the ring-opening rate constant ( $k_1$ ) can be described by

$$k_1 = k_{H_2O} f_{HB^+} \quad (1)$$

where  $k_{H_2O}$  is the pH-independent rate constant and  $f_{HB^+}$  is the fraction of benzodiazepine which is protonated in position N-4. However, Eq. (1) is kinetically equivalent to Eq. (2)

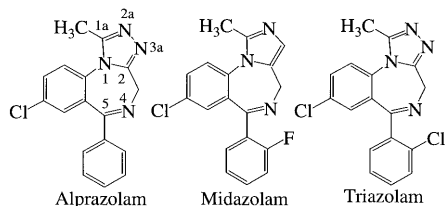


Fig. 1. The chemical structures of the benzodiazepine bases studied.

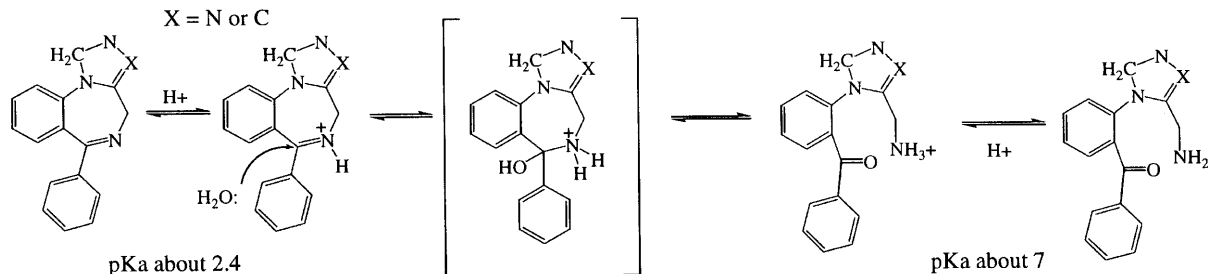


Fig. 2. The ring-opening reaction of benzodiazepines.

Table 1

The apparent equilibrium constant between the closed and open forms of the benzodiazepines:

$$K_{\text{eq}} = \frac{[\text{open}]_{\text{Total}}}{[\text{closed}]_{\text{Total}}}$$

where  $[\text{open}]_{\text{Total}}$  is the total concentration of benzodiazepine which is in the ring-open form and  $[\text{closed}]_{\text{Total}}$  is the total concentration of benzodiazepine which is in the ring-closed form at 37°C

Cyclodextrin	pH	$K_{\text{eq}}$		
		Alprazolam	Midazolam	Triazolam
No cyclodextrin	1	20	50	20
	2	6	3	2
	4	0.3	0.1	<0.1
10% (w/v) HP $\beta$ CD	1	100	100	20
	2	15	2	2
	4	0.3	<0.1	<0.1
10% (w/v) SBE $\beta$ CD	1	100	100	50
	2	25	6	3
	4	0.8	0.1	<0.1
10% (w/v) RM $\beta$ CD	1	100	50	20
	2	10	0.8	0.8
	4	0.1	0.1	<0.1

$$k_1 = k_{\text{H}}[\text{H}^+]f_{\text{B}} \quad (2)$$

$$f_{\text{HB}^+} = 1 - f_{\text{B}} = \frac{[\text{H}^+]}{[\text{H}^+] + K_{\text{a}}} \quad (3)$$

where  $k_{\text{H}}$  is the specific acid catalysis rate constant for the ring-opening reaction,  $[\text{H}^+]$  is the hydronium concentration and  $f_{\text{B}}$  is the fraction of benzodiazepine which is not protonated in position N-4. Comparable equations can be obtained for the ring-closing rate constant ( $k_{-1}$ ). Under normal conditions the ring-open forms of alprazolam, midazolam and triazolam are chemically stable in aqueous solutions.

## 4. Results and discussion

### 4.1. Solubilization

The aqueous solubility of benzodiazepines is a function of both the ionization of the drug molecule and the ring-opening of the diazepine ring. The ring-opening of the benzodiazepine ring is pH-dependent and fully reversible (Fig. 2). The observed equilibrium constant ( $K_{\text{eq}}$ ) between the total concentration of the open and closed forms is pH-dependent, strongly favoring the closed form at pH above 4, but the open form at pH below 2 (Table 1). In general, the cyclodextrins

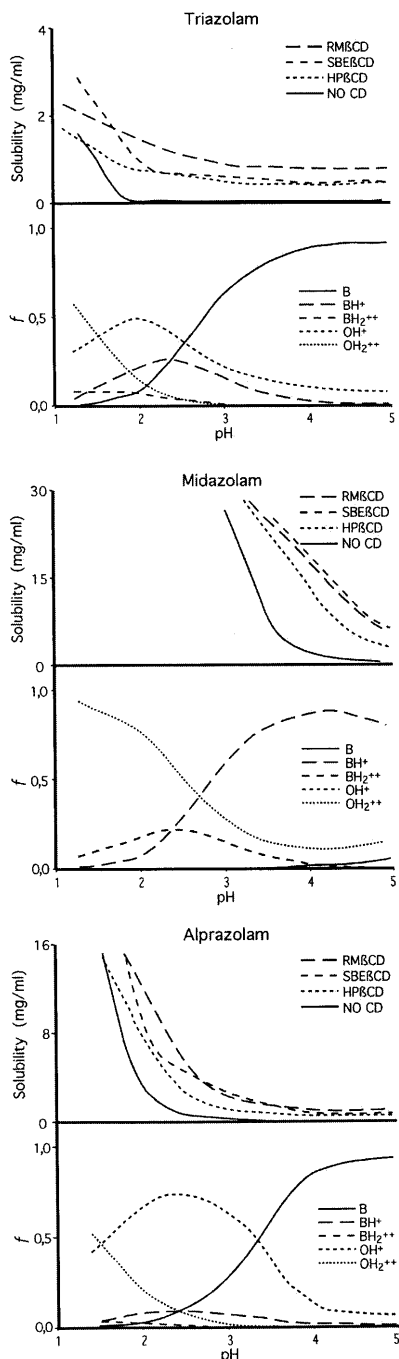


Fig. 3. The effects of cyclodextrins, ionization and ring-opening on the aqueous solubility of benzodiazepines at room temperature (22–23°C). The cyclodextrin concentration was 10% (w/v). *f*, mol fraction; B, benzodiazepine base (ring-closed form); BH<sup>+</sup>, monoprotionised benzodiazepine; BH<sub>2</sub><sup>2+</sup>, diprotonised benzodiazepine; OH<sup>+</sup>, monoprotionised ring-open form; OH<sub>2</sub><sup>2+</sup>, diprotonised ring-open form.

appear to stabilize the ring-open forms (i.e. OH<sup>+</sup> and OH<sub>2</sub><sup>2+</sup>) resulting in an increased  $K_{eq}$  value at low pH. The data presented in Fig. 3 are based on solubility studies, quantitative determination of the total amounts of the ring-open and ring-closed benzodiazepine forms, and the observed  $pK_a$  values of the different benzodiazepine forms in pure aqueous solution. From Fig. 3, it is possible to estimate the contribution of each species (i.e. different ionization forms of both the ring-open and ring-closed forms) to the overall benzodiazepine solubility in aqueous solutions. For example, it is clear that the monoprotionised (BH<sup>+</sup>) and diprotonised (BH<sub>2</sub><sup>2+</sup>) ring-closed forms, as well as the monoprotionised ring-open forms (OH<sup>+</sup>), have an insignificant effect on the overall aqueous solubility of the three benzodiazepines studied. Only when the diprotonised ring-open forms (OH<sub>2</sub><sup>2+</sup>) emerge do we observe a notable increase in aqueous solubility. Furthermore, it is apparent that the uncharged cyclodextrins (i.e. RMβCD and HPβCD) interact less strongly with OH<sup>+</sup> and OH<sub>2</sub><sup>2+</sup> than with the uncharged ring-closed form B, BH<sup>+</sup> or BH<sub>2</sub><sup>2+</sup> (Fig. 3). However, the negatively charged SBEβCD interacts somewhat more strongly than the uncharged cyclodextrins with OH<sub>2</sub><sup>2+</sup> resulting in enhanced solubilization at low pH. The  $pK_a$  values of midazolam are about 2.4 (N-4) and 6.15 (N-2a) while those of alprazolam and triazolam are about 1.5 (N-2a) and 2.4 (N-4). Thus, the main reason for greater aqueous solubility of midazolam with decreasing pH, compared to the other two benzodiazepines studied, is the early appearance of the protonized forms, especially the diprotonised OH<sub>2</sub><sup>2+</sup> form.

Cyclodextrins are able to form 1:1 complexes with the protonized forms and, thus, they are able to solubilize the positively charged ring-open and ring-closed forms (Figs. 3 and 4). However, the stability constants of these complexes are somewhat lower than those of comparable uncharged species. It is possible to increase the complexation efficacy by adding a small amount of a water-soluble polymer to the aqueous complexation media and heating (Loftsson, 1998; Loftsson et al., 1999). For midazolam, SBEβCD was the best solubilizer of the three cyclodextrins tested and

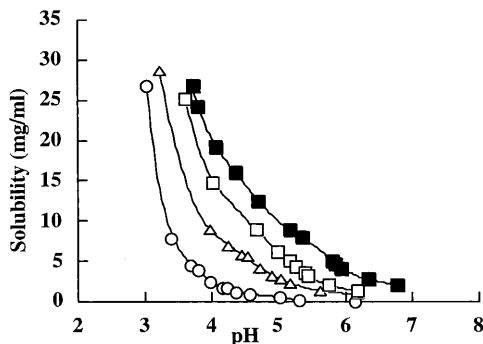


Fig. 4. The effects of pH and cyclodextrins on the solubility of midazolam in aqueous Teorell–Stenhagen buffer system. No cyclodextrin present (○); 10% (w/v) HPβCD (△); 10% (w/v) SBEβCD (□); 10% (w/v) SBEβCD and 0.10% (w/v) HPMC (■).

addition of 0.10% (w/v) hydroxypropyl methylcellulose (HPMC) and heating in an autoclave at 121°C for 20–40 min enhanced its solubilizing effect (Fig. 4). The value of the stability constant of the midazolam/SBEβCD (1:1) complex was determined to be 700 M<sup>-1</sup> at pH 4.8 but 425 M<sup>-1</sup> at pH 4.0.

#### 4.2. Kinetic studies

Equilibrium between the ring-open and ring-closed forms is reached within a few minutes upon dissolution of the benzodiazepine in aqueous media. The equilibrium constants are pH-dependent favoring the ring-open forms at low pH and the ring-closed forms at physiological pH (Table 1). It is believed that only the ring-closed forms of the benzodiazepines are pharmacologically active. Thus, it is important to determine how fast the ring closes under physiological conditions. The half-life of the first-order rate constant was determined in aqueous 0.5 M Tris buffer solution at pH 7.5 and 37°C. For alprazolam the half-life in pure aqueous buffer solution was determined to be 5.3 min, 3.9 min for midazolam and 53 min for triazolam. Addition of cyclodextrins to the aqueous reaction medium increased the half-life of the ring-closing reaction (Table 2). This effect of cyclodextrins on the half-life is in agreement with the observation that cyclodextrins stabilize the ring-open forms (i.e. OH<sup>+</sup> and OH<sub>2</sub><sup>+</sup>). Or-

ganic solvents such as ethanol and dimethylsulfoxide reduce the complexation by competing with the benzodiazepines for a space in the cyclodextrin cavity and, thus, reducing the effects of cyclodextrins. However, when no cyclodextrin was present in the reaction medium both ethanol and dimethylsulfoxide increased the half-life for the ring-closure of alprazolam and midazolam. In the case of triazolam the effects were much less pronounced.

In freshly collected human serum the half-life of the first-order rate constant for the ring-closing reaction was estimated to be less than 2 min for both alprazolam and midazolam (in vitro at 37°C). For triazolam the half-life was somewhat higher but still very short. Thus, it can be assumed that ring-open forms of the benzodiazepines close very rapidly upon absorption into the systemic circulation.

In the nasal cavity lipophilic molecules will compete with the drug molecules for a space in the cyclodextrin cavity in much the same way as ethanol and DMSO molecules do in our in vitro study. Thus, administration of the ring-open form of the benzodiazepines in a cyclodextrin-containing nasal spray solution should not have any effect on their pharmacological effect. That is beside enhancing aqueous solubility and delivery of the drug molecule through the biological membrane. However, excess cyclodextrin can decrease the drug bioavailability in the nasal spray solution (Masson et al., 1999). It is therefore important to use just enough cyclodextrin to solubilize the drug in the aqueous nasal spray solution.

#### 4.3. Formulation of a midazolam nasal spray

The phase solubility of midazolam in the aqueous nasal spray vehicle shows that 12.33% (w/v) SBEβCD is required to dissolve 17 mg of midazolam in 1 ml of the vehicle (Fig. 5). To ensure that no precipitation will be formed during storage, a small excess of SBEβCD is needed. Thus, the final formulation contained 14% (w/v) SBEβCD. The composition of the aqueous nasal formulation was as follows: midazolam (1.7% w/v), SBEβCD (14% w/v), HPMC (0.1% w/v), benzalkonium chloride (0.02% w/v), EDTA (0.1%



Table 2

The effects of cyclodextrins and organic cosolvents on the half-life for the rate of ring-closure in aqueous 0.5 M Tris buffer solution at pH 7.5 and 37.0°C

Cyclodextrin 10% (w/v)	Organic cosolvent <sup>a</sup> % (v/v)	Half-life ratio <sup>b</sup>		
		Alprazolam <sup>c</sup>	Midazolam <sup>c</sup>	Triazolam <sup>c</sup>
No cyclodextrin	No cosolvent	1.0	1.0	1.0
	10% EtOH	1.1	1.4	1.0
	50% EtOH	1.8	2.1	0.7
	10% DMSO	1.3	1.3	1.0
	50% DMSO	1.2	1.6	1.0
HP $\beta$ CD	No cosolvent	4.2	6.5	2.0
	10% EtOH	2.5	4.6	1.4
	50% EtOH	1.8	2.7	1.3
	10% DMSO	2.9	5.3	1.7
	50% DMSO	1.5	2.1	1.2
SBE $\beta$ CD	No cosolvent	4.2	13.7	2.3
	10% EtOH	2.5	5.4	1.6
	50% EtOH	2.2	2.8	1.4
	10% DMSO	2.7	8.0	1.8
	50% DMSO	1.4	2.1	1.2
RM $\beta$ CD	No cosolvent	5.2	6.0	2.1
	10% EtOH	2.6	4.2	1.4
	50% EtOH	1.9	2.6	1.3
	10% DMSO	3.3	3.6	1.7
	50% DMSO	1.6	2.1	1.2

<sup>a</sup> EtOH, absolute ethanol, DMSO, dimethylsulfoxide.

<sup>b</sup> The half-life divided by the half-life in pure aqueous buffer solution (i.e. buffer solution containing neither cosolvent nor cyclodextrin).

<sup>c</sup> The half-life for formation of alprazolam, midazolam and triazolam in aqueous pH 7.5 buffer solution at 37.0°C was determined to be 5.3, 3.9 and 53 min, respectively.

w/v), concentrated phosphoric acid (0.43% v/v) and water (to 100% v/v). A concentrated aqueous sodium hydroxide solution was used to adjust the pH to 4.3. The nasal spray was prepared as follows. The solid components were weighed into a 100-ml volumetric flask. Phosphoric acid and most of the water was added and the solution stirred until all solid material had dissolved. Then the pH was adjusted to 4.35 with a concentrated sodium hydroxide solution under stirring. Water was added to the mark and the aqueous solution heated in a sealed container in an autoclave (121°C for 40 min). After cooling, the solution was filtered through a sterile 0.45- $\mu$ m membrane filter into amber glass vials under aseptic conditions.

The stability of midazolam in the nasal spray upon heating in an autoclave was investigated.

The aqueous nasal spray solution was heated in sealed containers for up to six successive heating cycles. Each heating cycle consisting of heating to 121°C for 20 min and cooling to room temperature. The midazolam concentration in the solution

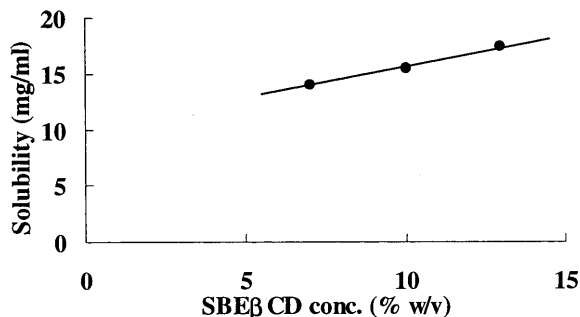


Fig. 5. The phase solubility of midazolam in the nasal spray vehicle at room temperature (22–23°C).

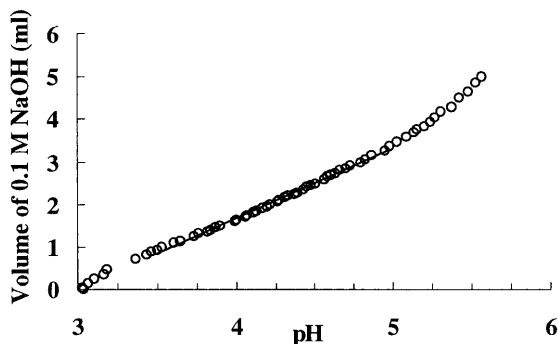


Fig. 6. Titration curve of the midazolam nasal spray. The line represents linear fit of data between pH 3.5 and 5.0.

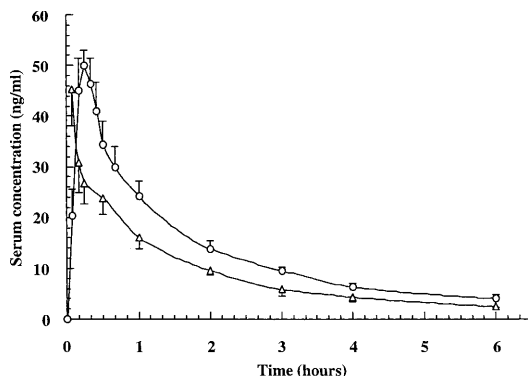


Fig. 7. Serum concentration–time profiles in healthy volunteers after i.n. administration of a 0.06 mg/kg dose ( $\circ$ ) or i.v. administration of a 2 mg fixed dose ( $\triangle$ ) of midazolam. Each point represents the mean value ( $n=6$ ) and the error bars represent SEM.

was determined after each heating cycle by HPLC. No loss of midazolam could be detected in the nasal spray during heating in an autoclave. Further evaluation of the chemical stability of midazolam in the nasal spray solution was performed at room temperature. The solution was stored in several sealed containers in the dark, three containers were removed at various time points for up to 12 months and the midazolam concentration determined by HPLC. The degradation rate constant was estimated by linear regression of the natural logarithm of the peak height versus time plots. This yielded a half-life of approx. 350 months and an estimated 95% expiration limit ( $t_{95}$ ) of over 2 years at room tempera-

ture. Thus, the ring-open form of midazolam has adequate chemical stability in the aqueous nasal spray solution. The aqueous nasal spray solution showed Newtonian flow characteristics and its viscosity was determined to be  $2.80 \pm 0.02$  mPa s. The osmolarity of this solution was determined to be  $541 \pm 14$  mOsm/kg. The buffer capacity of the aqueous nasal spray solution was determined from linear fit of the titration curve (Fig. 6) between pH 3.5 and 5.0. The buffer capacity was determined to be 0.016 M. These results show that the nasal spray solution is a low viscosity, somewhat hypertonic solution with adequate buffer capacity to maintain constant pH during storage.

#### 4.4. Evaluation in humans

Six healthy volunteers, two women and four men, were enrolled in this investigation. The mean ( $\pm$  S.D.) age and weight of the participants were 26.0 ( $\pm$  5.7) years and 74.5 ( $\pm$  10.8) kg, respectively. The participants only reported mild to moderate irritation within the nasal passage and/or throat area following administration of 200–300  $\mu$ l (based on body weight) of the nasal spray solution. Plots of midazolam serum concentration–time curves for i.n. and i.v. administration are shown in Fig. 7 and the main pharmacokinetic

Table 3

Mean ( $n=6$ ) pharmacokinetic parameters and SEM of midazolam in healthy volunteers following i.n. administration of a 0.06 mg/kg dose or i.v. administration of a 2 mg fixed dose<sup>a</sup>

Pharmacokinetic parameter	Intranasal		Intravenous	
	Mean	SEM	Mean	SEM
$C_{\max}$ (ng/ml)	54.3	5.0	–	–
$t_{\max}$ (h)	0.25	0.04	–	–
$t_{1/2}$ (h)	2.2	0.3	1.7	0.2
$k_a$ ( $h^{-1}$ )	16	3	–	–
$AUC/D_0$ 10 <sup>6</sup> (min/ml)	1278	68	1860	224
$F$ (%)	73	7	–	–

<sup>a</sup>  $C_{\max}$ , maximum serum concentration;  $t_{\max}$ , time to reach  $C_{\max}$ ;  $t_{1/2}$ , elimination half-life;  $k_a$ , absorption rate constant;  $AUC/D_0$ , area under the serum–time curve from 0 to 6 h divided by the dose;  $F$ , absolute availability.

parameters in Table 3. In both cases, the profiles followed a two-compartment open model. Midazolam was absorbed rapidly after i.n. administration, reaching maximum concentrations at a mean of 15 (range 10–20) min, which was first followed by a rapid decline, i.e. distribution phase, and then by a somewhat slower decline, i.e. elimination phase, with a mean elimination half-life of 2.2 (range 1.6–3.9) h. Maximum concentrations were variable with a mean value of 54.3 (range 40–72) ng/ml. The mean absolute availability was 73% (range 41–95%).

## 5. Conclusions

This study has shown that it is possible to enhance cyclodextrin solubilization of benzodiazepines through reversible ring-opening of the benzodiazepine ring and ionization. Additional solubilization was obtained through interaction of a water-soluble polymer with the drug/cyclodextrin complex. Through this multiple techniques it was possible to minimize the amount of cyclodextrin needed to solubilize the benzodiazepine drug in the aqueous nasal spray solution, keeping the drug/cyclodextrin ratio close to one. A physiologically acceptable aqueous nasal spray solution containing 17 mg of midazolam per 1 ml at pH 4.3 was tested in humans. After i.n. administration of this formulation, midazolam was rapidly absorbed from the nasal cavity into the systemic circulation without causing notable nasal irritation.

## Acknowledgements

This work was supported by the Icelandic Science Foundation, the Icelandic Research fund for Graduate Students and the University of Iceland Research Fund.

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