*International Journal of Pharmaceutics*, 99 (1993) 85–92



IJP 03092

# Stabilization of aqueous thymoxamine using dimethyl-β-cyclodextrin

Donald G. Musson, David P. Evitts, Alison M. Bidgood and Orest Olejnik

*IOLAB Corporation (a Johnson & Johnson Company), Claremont, CA (USA)*

(Received 22 August 1991)
(Modified version received 25 June 1992)
(Accepted 28 October 1992)

*Key words*: Stability; Hydrolysis; Thymoxamine; Methylated β-Cyclodextrin; Dimethyl-β-Cyclodextrin; Hydroxypropyl-β-Cyclodextrin; Trimethyl-β-Cyclodextrin; HPLC-UV

**Summary**

Moxisylyte (or thymoxamine) is an α-adrenoceptor blocking agent used to reverse mydriasis. The drug molecule has an ester moiety which is vulnerable to acid and base catalyzed hydrolysis. Long-term storage of the drug at room temperature has not been possible because of this hydrolysis. An accelerated, stability-indicating assay was developed to study the hydrolysis and modify the formulation for room temperature storage (instead of refrigeration). Stability studies with pH variation and with β-cyclodextrins resulted in a formulation with dimethyl-β-cyclodextrin at pH 5.0. All other available β-cyclodextrins either accelerated the hydrolysis or had little effect.

## Introduction

Moxisylyte, thymoxamine (4-[2-(dimethylamino)ethoxy]-2-methyl-5-(1-methylethyl)phenol acetate) (Fig. 1) is an α-adrenoceptor blocking agent used to reverse mydriasis caused by epinephrine or ephedrine (Turner and Sneddon, 1968). The drug contains a phenyl acetate (ester) moiety which is vulnerable to base-catalyzed hydrolysis to a phenol and to acid-catalyzed hydrolysis with subsequent oxidation to a quinone (Fig. 1) (Mizutani, 1982). Formulated solutions of thymoxamine are not stable at ambient temperature unless refrigerated (Giovanni, 1988). Modifications of the formulation by adjusting the pH have not been successful.

Cyclodextrins can be described as having a doughnut shape with a hydrophobic center and a hydrophilic exterior. In an aqueous matrix the hydrophobic cavity may complex with an alcohol, an oil, an organic molecule or a functional group thereof; the exterior is hydrated. The cyclodextrins, β-cyclodextrin (β-CD) in particular, have been used to enhance solubility or stability of drugs (Pagington, 1987). Analogs of β-CD have been investigated in order to modify thymoxamine's formulation for long-term stability at room temperature.

The rate of hydrolysis of thymoxamine generally takes many days at pH 2–7. The rate can be

*Correspondence to:* D.G. Musson, IOLAB Corporation (a Johnson & Johnson Company), Claremont, CA, U.S.A.

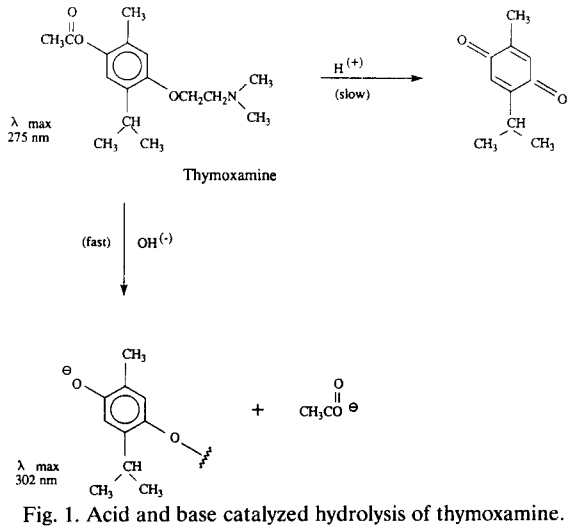


Fig. 1. Acid and base catalyzed hydrolysis of thymoxamine.

accelerated to less than 6 min with the addition of base (about pH 12). In order to study the stabilizing effects of the β-CDs on thymoxamine, a rapid, non-specific assay using a UV spectrophotometer and a slower, specific assay using HPLC-UV were developed to monitor the formation of the phenolate anion (Fig. 1).

In general, kinetic studies show that β-CD, hydroxypropyl-β-CD (HP-β-CD) and similar analogs appear to accelerate the hydrolysis of thymoxamine under alkaline conditions when compared to thymoxamine alone. Dimethyl-β-CD (DM-β-CD) slows the hydrolysis, and trimethyl-β-CD (TM-β-CD) seems to have little effect.

## Results

*Thymoxamine / phenol (drug / degradate) analytical analysis*

Thymoxamine has a phenyl acetate moiety which absorbs at 275 nm. In the presence of base catalysis, the ester will hydrolyze to the phenol which absorbs at 300 nm (Hizutani et al., 1982). This chromophoric change has been adapted to a non-specific assay using a UV spectrophotometer. The bathochromic shift is great enough to determine the formation of the phenol over time (Fig. 2). The absorbance of the phenol band is measured to calculate half-lives and rate constants of thymoxamine using the methylated cyclodextrins. The overlap of the absorbance bands is minimal and does not affect measurements.

A specific assay was developed using HPLC with a diode array. The drug and degradate can be separated on a reversed-phase column using 25% methanol and 75% sodium phosphate buffer, pH adjusted to 6.0. The degradate elutes before thymoxamine and is separated by about 4 min (Fig. 3). An aliquot (10 μl) is removed from the reaction and injected into the HPLC without any prior preparation.

The non-specific assay is used for rapid analysis with a turnaround time of approx. 3 min. The HPLC assay is appropriately used when there is not a clear distinction between the UV bands of thymoxamine and degradate on the spectrophotometer. This occurred when a complex was formed between HP-β-CD and thymoxamine (Fig. 4). The run-time for the HPLC-UV assay is about 10 min. The assays are linear ($r^2 > 0.998$) over the concentration range 0.025–0.4 mg/ml with reproducibility of less than 5.83%, coefficient of variation.

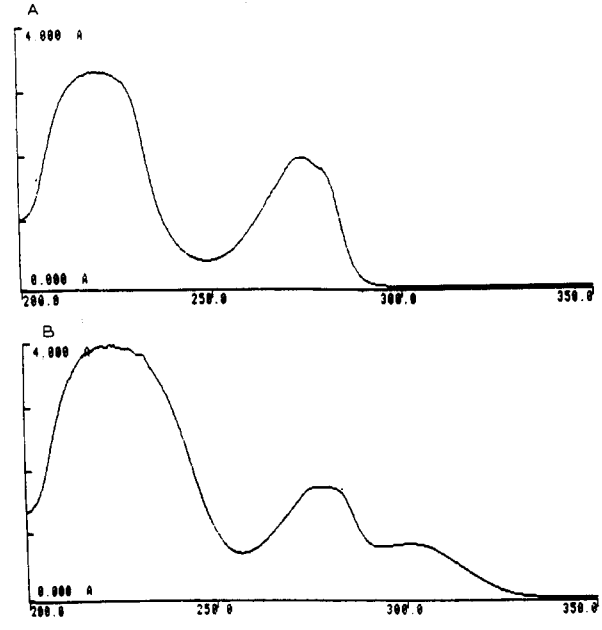


Fig. 2. UV spectra: (A) thymoxamine alone; (B) thymoxamine with base added (approx. 5 mM NaOH).

*Long-term stability study (shelf-life) of thymoxamine with respect to pH*

The degradation of thymoxamine is pseudo first-order and can be catalyzed by acid and base (Mizutani et al., 1982). The log *K*-pH profile gives a curve with a nadir at pH 5. The product has been determined to be the phenol in base and the quinone in acid (Mizutani et al., 1982). The hydrolysis of thymoxamine also gives a linear Arrhenius plot in water from 40 to 60°C. The formulation specifications for refrigerated thymoxamine has a pH range of 4.4–6.4. An accelerated stability study was set up with three formulations containing disodium edetate, benzalkonium chloride, sodium chloride and a sodium phosphate buffer. The formulations were prepared at pH 6.49, 5.07 and 4.21. The samples were analyzed at room temperature and 45°C over 3 months. Representative data are summarized in Table 1 and show a loss of thymoxamine to less than 80% of the initial concentration for the three formulations at both temperatures. The pH for all the formulations changed towards the acidic range.

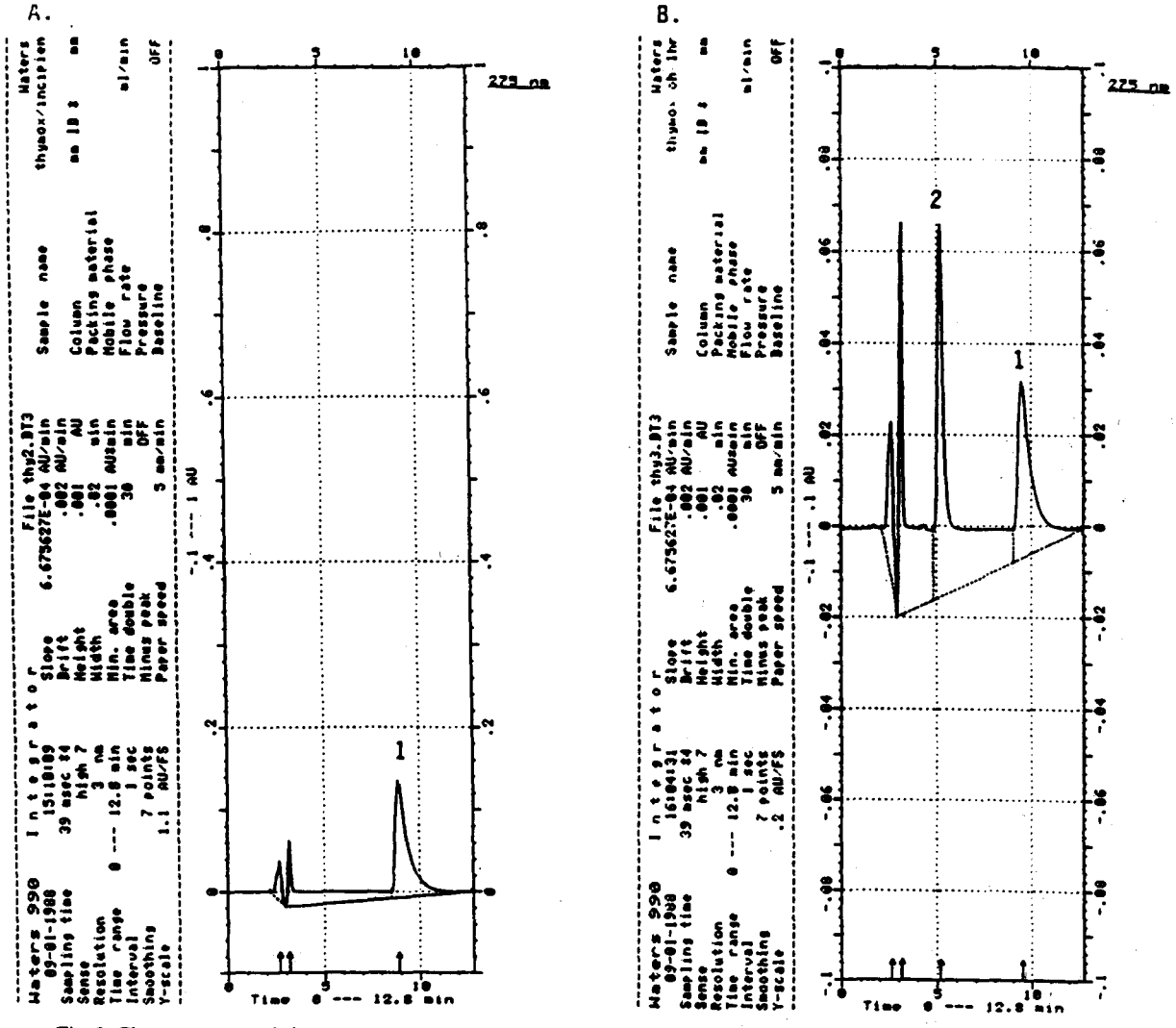


Fig. 3. Chromatograms: (A) thymoxamine only; (B) thymoxamine after the addition of base: thymoxamine, 1; degradate, 2.

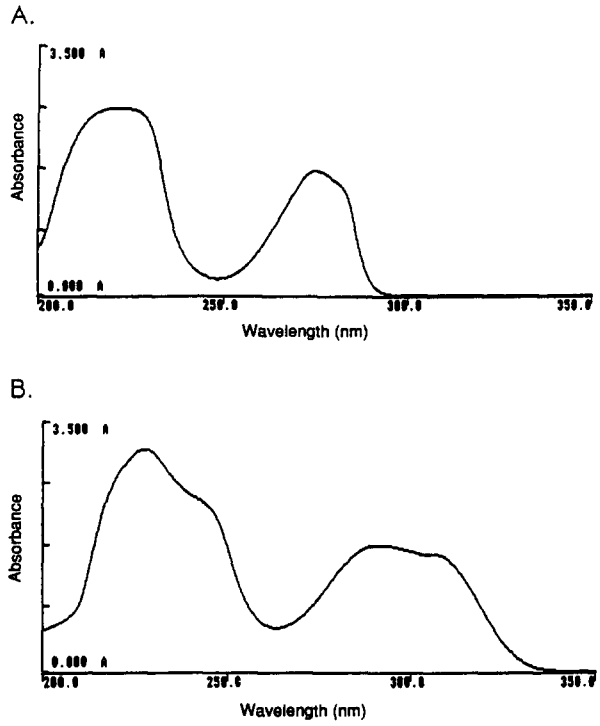


Fig. 4. UV spectra: (A) thymoxamine in the presence of 10% HP-β-CD; (B) complex of HP-β-CD and the phenol after the addition of NaOH. The new chromophore in spectrum B remains unchanged for over 2 h. It appears that the hydrolysis of thymoxamine happens quickly upon complexation with base and a new chromophore is formed. In contrast, DM-β-CD, which slows the hydrolysis of thymoxamine, does not effect the chromophoric changes that are observed during the hydrolysis of thymoxamine in the absence of a β-CD (Fig. 2).

*Stability-indicating assay for thymoxamine*

The instability of thymoxamine in aqueous base allowed for a quick, accelerated, stability-indicating test to be developed. The hydrolysis of thymoxamine and formation of its degradate could be monitored from beginning to end within 3–5 min. The two analytical assays described previously provide results within a reasonable period of time and/or specificity. Data generated on the hydrolysis of thymoxamine by HPLC-UV and by the UV spectrophotometer are depicted in Fig. 5. The kinetic half-life ($t_{1/2}$) of degradation is 5.11 min by HPLC and 4.92 min by UV.

TABLE 1

*Long-term degradation of thymoxamine [a] at different pH values*

| Week | Concentration (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Room temperature | | | 45°C | | |
| | pH6.49 | pH 5.07 | pH 4.21 | pH 6.49 | pH 5.07 | pH 4.21 |
| 1 | 0.903 | 0.960 | 0.949 | 0.759 | 0.942 | 0.933 |
| 12 | 0.770 | 0.780 | – | 0.233 | 0.777 | 0.737 |

[a] Formulation contained 1.0 mg/ml thymoxamine, 0.3 mg/ml $Na_2EDTA$, 0.1 mg/ml benzalkonium chloride, 0.1 mg/ml sodium phosphate monobasic, 2.5 mg/ml sodium phosphate dibasic, 7.0 mg/ml sodium chloride, diluted with water.

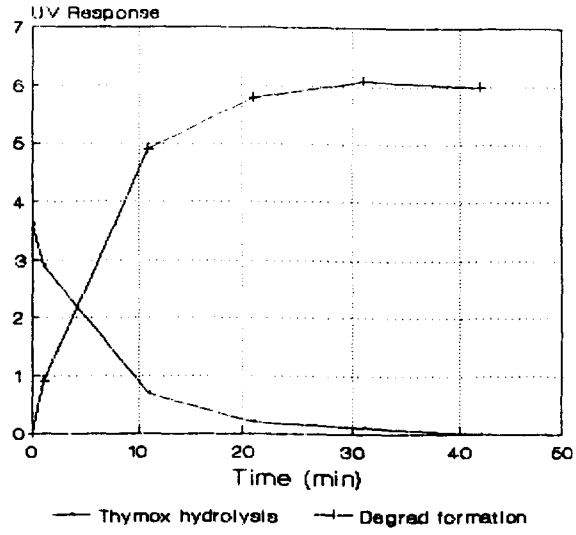


Fig. 5. Plot of the loss of thymoxamine and formation of its degradate. The data were generated by HPLC-UV.

*Stabilizing effects of β-cyclodextrin (β-CD) analogs on thymoxamine in aqueous solutions*

Analogs of β-CD have been demonstrated to stabilize aqueous solutions of compounds (e.g., prostaglandin $E_2$ and $A_2$ using methylated β-CDs) (Hirayama et al., 1984). Similar studies (summarized below) were performed with thymoxamine using the stability-indicating assay described above.

*Testing of hydroxypropyl-β-CD (HP-β-CD) and dimethyl-β-CD (DM-β-CD)* At 10% concentration DM-β-CD significantly stabilized thymoxamine over HP-β-CD (Table 2). The $t_{1/2}$ was 4.1 min for thymoxamine alone, 44.1 min for DM-β-CD (relative rate to thymoxamine alone, 0.093) and less than 1 min for HP-β-CD. The concentration of thymoxamine was 0.011 mg/ml. HP-β-CD

TABLE 2

*Thymoxamine [a] stability in the presence of HP-β-CD and DM-βCD [b]*

| β-CD | $t_{1/2}$ (min) | Rate $K$ ($min^{-1}$) | Relative rates |
|---|---|---|---|
| None | 4.20 | 0.1690 | 1.000 |
| DM-β-CD | 44.10 | 0.0157 | 0.093 |
| HP-β-CD | < 1.00 | > 0.6930 | > 4.100 |

[a] Thymoxamine concentration: 0.01 mg/ml.
[b] β-CD concentrations: 10% w/w.

actually accelerated the hydrolysis of thymoxamine when compared to a neat solution.

*Comparison of DM-β-CD with trimethyl-β-CD (TM-β-CD)* Several literature references (Hirayama et al., 1984, 1987) have compared the stabilizing properties of methylated β-CDs and have shown DM-β-CD to be superior to TM-β-CD. A stability study, similar to that described above, was performed for thymoxamine using the two methylated β-CDs. The relative rates of hydrolysis were 1.0 for thymoxamine alone, 0.899 with TM-β-CD and 0.0929 with DM-β-CD.

*Relationship of DM-β-CD concentration (molar ratio) to the rate of hydrolysis of thymoxamine* It has been shown that the stabilizing effects of the methylated β-CDs increase with concentration or molar ratio of the βCD to the unstable drug (Hirayama et al., 1984). A similar relationship exists for DM-β-CD with thymoxamine (Table 3).

TABLE 3

*Thymoxamine [a] stability in the presence of DM-β-CD at different molar ratios [b]*

| Molar ratios (DM-β-CD/ thymoxamine) | $t_{1/2}$ (min) | Rate $K$ ($min^{-1}$) | Relative rate |
|---|---|---|---|
| Thymoxamine | 1.85 | 0.3750 | 1.0000 |
| 1.3:1 | 4.60 | 0.1510 | 0.4030 |
| 3.4:1 | 6.48 | 0.1070 | 0.2850 |
| 6.7:1 | 12.22 | 0.0567 | 0.1512 |
| 13:1 | 20.19 | 0.0343 | 0.0915 |

[a] Thymoxamine HCl concentration: 0.275 g/ml.
[b] Molar ratio based on a DM-β-CD molecular weight of 1330.

TABLE 4

*Hydrolysis rate of thymoxamine [a] with various β-CD analogs [b]*

| β-CD | $t_{1/2}$ (min) | Rate $K$ ($min^{-1}$) | Relative rate |
|---|---|---|---|
| No β-CD | 4.10 | 0.169 | 1.0000 |
| β-CD | < 1.00 [c] | | |
| DM-β-CD | 44.10 | 0.0157 | 0.0929 |
| TM-β-CD | 4.61 | 0.1500 | 0.8980 |
| HP-β-CD | < 1.00 [c] | | |
| Methyliertis-β-CD | < 1.00 [c] | | |
| Methyl-HP-β-CD | < 1.00 [c] | | |
| Methyl-HE-β-CD | < 1.00 [c] | | |
| Poly-β-CD | < 1.00 [c] | | |

[a] Thymoxamine HCl concentration: 0.011 mg/ml (aq).
[b] Concentrations of β-CD analogs were prepared at 10% w/v. However, β-CD was prepared as a saturated solution (< 5%).
[c] The reaction was faster than could be measured by HPLC. Chromophoric changes observed on the UV spectrophotomer with HP-β-CD and methyl-HP-β-CD were instantaneous from one run to the next.

As the ratio of DM-β-CD to thymoxamine increases, the rate constant decreases.

*Testing DM-β-CD in the thymoxamine formulation* The effectiveness of DM-β-CD in stabilizing thymoxamine in its formulation (containing polyvinyl alcohol, dextrose, $Na_2EDTA$, benzalkonium chloride) was tested under above the criteria, initially. The relative rate of hydrolysis of thymoxamine's formulation with DM-β-CD as compared to the formulation without is 0.1134.

*Stabilizing effects of other β-CDs on thymoxamine* Other available β-CDs tested were primarily analogs of β-CD. All of the additional β-CDs (Table 4) accelerated the rate of hydrolysis, including β-CD and poly-β-CD.

*Long-term shelf-life of thymoxamine's formulation with and without DM-β-CD* Based on the results of the stability studies with β-CDs, formulations were prepared with and without DM-β-CD (2.5% w/v). The formulations also contained $Na_2$edetate, benzalkonium chloride, polyvinyl alcohol and dextrose. A phosphate buffer was not used and the pH was adjusted to 5.0 with HCl (from 5.5). The formulations were stored at room temperature and 45°C and analyzed over time for thymoxamine. A plot of thymoxamine's concen-

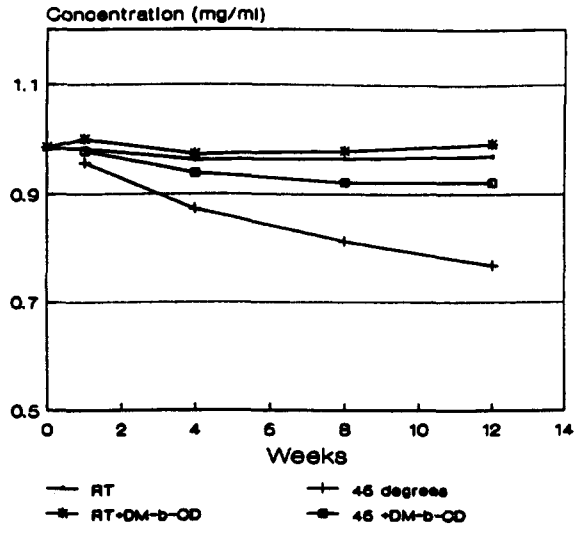


Fig. 6. Long-term degradation of thymoxamine in its formulation with and without DM-β-CD. RT, room temperature.

trations vs time (Fig. 6) of the stored samples shows only the 45°C sample without DM-β-CD falling below 90% (or 0.9 mg/ml) of its initial concentration within 1 month. The pH steadily changed for most of the formulations towards the acidic range 4.57–5.06.

The long term shelf-life of formulated 0.5% thymoxamine with 2.5 and 5.0% DM-β-CD (1.186 and 2.372 M, respectively) was determined over 23 weeks at 45°C. The data show thymoxamine's level for the 2.5%, DM-β-CD preparation at 88.8% of initial concentration at 12 weeks.

## Discussion

Thymoxamine is vulnerable to chemical and metabolic hydrolysis forming its phenolate ion (Mizutani et al., 1982; Duchenc et al., 1988). The chemical hydrolysis of thymoxamine can be slowed by formation of a complex with DM-β-CD. The rate of hydrolysis appears dependent on the molar ratio of DM-β-CD to thymoxamine but not on the concentration of the complex (at a fixed ratio). TM-β-CD, however, appears to have very little stabilizing effect on thymoxamine at a molar ratio of 20.1 : 1, using the methylated β-CDs (based on a TM-β-CD molecular weight of 1428). In addition, the data available do not clearly suggest that a complex is forming or to what degree with thymoxamine. Similar results, however, concerning stabilization have been observed for other compounds (Hirayama et al., 1987; Glomot et al., 1988; Hoshino et al., 1989).

The difference between DM-β-CD and TM-β-CD has been attributed to their conformational differences in the hydrophobic cavity. The TM-β-CD cyclic ring is distorted from the 'regular' heptagonal symmetry of β-CD and DM-β-CD (Harata et al., 1981; Hirayama et al., 1987). Thus, there may exist greater steric hindrance to the penetration (the complexation) of Th-β-CD with the guest molecule or functional group.

In contrast to the methylated β-CDs, β-CD, HP-β-CD and similar analogs (e.g., hydroxyethyl-β-CD) will accelerate the hydrolysis of the ester. UV analysis of thymoxamine mixed with HP-β-CD will show an immediate chromophoric change. This change, which is different from that of the methylated β-CDs, suggests the immediate formation of the complex and a subsequent reaction. HPLC-UV analysis demonstrates the accelerated hydrolysis with formation of the phenol and loss of thymoxamine. The complexation of thymoxamine with HP-β-CD appears to accelerate or catalyze the ester hydrolysis. Possibly, the available hydroxyl groups on the cyclodextrin act as neighboring-group catalysts after complexation, facilitating the hydrolysis of the phenolate acetate moiety.

Accelerated stability studies of three thymoxamine formulations prepared at pH 6.49, 5.07 and 4.21 show degradation with less than 80% remaining. The studies were repeated with samples containing 2.5% DM-β-CD (molar ratio 5.94 : 1, DM-β-CD to thymoxamine) at pH 5.0. The formulations containing DM-β-CD gave acceptable thymoxamine concentrations over a 3 month study period. Only the formulation without DM-β-CD and stored at 45°C degraded below the acceptable 90% of the initial concentration (1.0 mg/ml). The formulation prepared in the former study without DM-β-CD at pH 5.0 and stored at room temperature showed faster thymoxamine degradation than the latter. The contradiction may be the result of the presence or absence of certain excipients – phosphate

buffer, dextrose, polyvinyl alcohol and/or sodium chloride.

## Materials and Methods

*Materials and reagents*

The β-CD analogs were obtained from Janssen Pharmaceutica, Inc.: DM-β-CD (lot no. 860507) (elemental analysis – Calc.: C, 50.53%; H, 7.37; Found: C, 48.77%; H, 7.29), methylhydroxyethyl-β-CD (DS 1.51, MS 0.22), methylhydroxypropyl-β-CD (DS 0.97, MS 0.43), L-poly-β-cyclodextrin (lot no. 81), β-CD (lot no. 850429), Methyliertes-β-CD (MS 1.8–1.9), TM-β-CD (lot no. 6), and HP-β-CD (lot no. 87a0056). DM-β-CD was also purchased from American Tokyo Kasel Inc. Thymoxamine was obtained from Goedecke; the formulation excipients consist of polyvinyl alcohol USP from Pharma Chem Inc., dextrose anhydrous USP from Pfanstiehl Labs, disodium edetate USP from Ciba-Geigy, and benzalkonium chloride 50% solution from Ruger Chemical. The water used was deionized and continuously distilled. The base used to accelerate the reaction was 1% NaOH (aq) w/w.

*Non-specific UV assay*

UV analysis was performed on a Perkin Elmer, Lambda 4B UV/Vis Spectrophotometer from 350 to 200 nm. The slits were set at 1 mm and the scan speed, at 200 nm/min. The cells used were purchased from Fisher Co., 10 mm, Cell Spec. UV Rectangular. During an experiment the reference cell would contain the same matrix as the sample cell except for the drug, thymoxamine. The stability studies were started by adding 1% NaOH directly into the sample and reference cells. The UV scan was repeated over time. The concentration range was 0–0.400 mg/ml. The chromophoric changes were measured in absorbance units only. A standard calibration curve was not used to follow the spectral changes.

*HPLC-UV assay*

The specific assay was performed by reversed-phase HPLC using a diode array (200–350 nm) or a UV/Vis absorbance detector (275 nm). The HPLC system consists of a Waters 600E Powerline Module (High Performance Delivery with PowerLine HPLC System Control), Waters 990 Photodiode Array Detector (or Applied BioSystems 783a Programmable Absorbance Detector, 275 nm) and a Waters 712 WISP. The column used was a μBondpak $C_{18}$ (10 μm particle size); the mobile phase contained 30% 2-propanol/70% sodium phosphate buffer (4 ml $H_3PO_4$ in 2 l of water), pH adjusted with 1 M NaOH to 6.0. The mobile phase was filtered through a 0.45 μm nylon filter under vacuum. The flow rate was 1.0 ml/min; the injection volume was 10 μl.

*Base-catalyzed hydrolysis of thymoxamine (accelerated-stability test)*

*UV analysis* The degradation of a 2 ml sample containing thymoxamine was initiated by the addition of 60 μl 1% NaOH; a 1 ml sample was initiated by the addition of 20 μl. The base was also added to the reference cell. Preparation of a sample involved mixing an aliquot (0.5–1 ml) of thymoxamine HCl from the stock solution with an equal volume of a β-CD solution. The sample might be diluted further with water, depending on the experiment. The reference cell contained the diluted βCD only. The spectrophotometer was scanned repeatedly until enough data had been obtained.

*HPLC-UV analysis* The degradation of a 1 ml sample involved the addition of 20 μl of 1% NaOH. Preparation of the sample involved addition of 10 μl of thymoxamine stock solution to 1 ml of a 10% solution of a β-CD. The sample was repeatedly chromatographed until enough data had been obtained.

*Long-term stability studies of modified formulations*

Degradation of thymoxamine HCl with respect to the pH of the formulation was tested over 3 months at room temperature and 45°C. The formulations contained (in mg/ml): thymoxamine HCl, 1.0; $Na_2$edetate, 0.3; benzalkonium chloride, 0.1; sodium phosphate (monobasic), 0.1; sodium phosphate (dibasic), 2.5; and sodium chloride, 7.0.

The pH of the three formulations was adjusted with dilute HCl to 6.49, 5.07 and 4.21. Tonicity

was adjusted with sodium chloride to approx. 300 mOsm.

The degradation of thymoxamine HCl with respect to the presence of DM-β-CD in the formulation was studied for 3 months at room temperature and 45°C. Two formulations were prepared: one with and one without DM-β-CD. The formulations contained (in mg/ml): DM-β-CD, 25; thymoxamine HCl, 1.0; polyvinyl alcohol, 10.0; dextrose, 41.0; $Na_2$edetate, 0.3; and benzalkonium chloride, 0.1.

A third formulation contained the above excipients with thymoxamine at 5.0 mg/ml and HP-β-CD at 25.0 and 50.0 mg/ml.

The formulations were filtered through 0.2 μm Tuffryn. The tonicities of the formulations were adjusted with dextrose to 300 mOsm; the final pH was adjusted to 5.0 with HCl.

## Acknowledgement

The authors wish to thank Janssen Pharmaceutica, Inc., for the β-cyclodextrin analogs used in this study.

## References

Duchene, F., Bernouillet, C., Bromet-Petit, M., Mosser, J., Feniou, C., Gaudin, D. and Virelizier, H., Metabolism of $^{14}$C-thymoxamine in rat and man. *Xenobiotica,* 18 (1968) 919–928.

Glomot, F., Benkerrour, L., Duchene and Poelman, M.C., Improvement in availability and stability of a dermocorticoid by inclusion in β-cyclodextrin. *Int. J. Pharm.,* 46 (1988) 49–55.

Harata, K., Uekama, K., Otagiri, M. and Hirayama, F., Conformation of permethylated cyclodextrin and the host-guest geiometry of their inclusion complexes. *J. Incl. Phenom.,* 1 (1984) 270–293.

Hirayama, F., Kurihara, M. and Uekama, K., Improvement of chemical instability of prostacyclin in aqueous solution by complexation with methylated cyclodextrins. *Int. J. Pharm.,* 35 (1987) 193–199.

Hirayama, F., Kurihara, M. and Uekama, K., Improving the aqueous stability of prostaglandin E2 and prostaglandin A2 by utilizing inclusion complexation with methylated-β-cyclodextrin. *Chem. Pharm. Bull.,* 32 (1984) 4237–4240.

Hoshino, T., Hirayama, F., Uekama, K. and Yamasaki, M., Reduction of protriptyline-photosensitized hemolysis by β-cyclodextrin complexations. *Int. J. Pharm.,* 50 (1989) 45–52.

Mizutani, H., Ichida, S., Watababe, D., Kanai, T., Araki, Y. and Kawashima, T., Physico-chemical properties and stabilities of moxisylyte hydrochloride (M-101). *Iyakuhin Kenkyu,* 13 (1982) 1006–1016.

Padington, J.S., Beta-cyclodextrin: the success of molecular inclusion. *Chem. Br.,* May (1987) 455–458.

Prosdocimo, G. and De Marco, D., Intraocular dapiprazole to reverse mydriasis during extracapsular cataract extraction. *Am. J. Ophthalmol.,* 105 (1988) 321–322.

Turner, P. and Sneddon, J.M., Alpha receptor blockade by thymoxamine in the human eye. *Clin. Pharmacol. Ther.,* 9 (1968) 45–49.