Evidence for complexes of different stoichiometries between organic solvents and cyclodextrins†

L. García-Río,*[a] P. Hervés,[b] J. R. Leis,[a] J. C. Mejuto,[c] J. Pérez-Juste[b] and P. Rodríguez-Dafonte[a]

Received 19th September 2005, Accepted 1st January 2006
First published as an Advance Article on the web 30th January 2006
DOI: 10.1039/b513214b

The influence of the organic solvent on the acid and basic hydrolysis of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) in the presence of α- and β-cyclodextrins has been studied. The observed rate constant was found to decrease through the formation of an unreactive complex between MNTS and the cyclodextrins. In the presence of dioxane, acetonitrile or DMSO, the inhibitory effect of β-CD decreased on increasing the proportion of organic cosolvent as a result of a competitive reaction involving the formation of an inclusion complex between β-CD and the cosolvent. The disparate size of the organic solvent molecules resulted in stoichiometric differences between the complexes; the β-CD–dioxane and β-CD–DMSO complexes were 1 : 1 whereas the β-CD–acetonitrile complex was 1 : 2. The basic and acid hydrolysis of MNTS in the presence of α-CD showed a different behavior; thus, the reaction gave both 1 : 1 and 2 : 1 α-CD–MNTS complexes, of which only the former was reactive. This result was due to the smaller cavity size of α-CD and the consequent decreased penetration of MNTS into the cavity in comparison to β-CD. The acid hydrolysis of MNTS in the presence of α-CD also revealed decreased penetration of MNTS into the cyclodextrin cavity, as evidenced by the bound substrate undergoing acid hydrolysis. In addition, the acid hydrolysis of MNTS in the presence of acetonitrile containing α-CD gave 1 : 1 α-CD–acetonitrile inclusion complexes, which is consistent with a both a reduced cavity size and previously reported data.

Introduction

Cyclodextrins are cyclic oligosaccharides[1] and α-, β- and γ-cyclodextrins consist of 6, 7 and 8 α-amylose units, respectively. These macrocycles can act as hosts to form inclusion complexes with guest molecules in the solid state or in solution. The shape of a cyclodextrin molecule is a hollow, truncated cone (a toroid) of height 7.8 Å, which represents the width of the amylose unit.[2] The diameter of the cavity depends on the number of amylose units present.[3] All hydroxyl groups in a cyclodextrin are projected outward, so the cavity is hydrophobic and nonpolar. Thus, despite the prevalence of hydroxyl groups, cyclodextrins possess a hydrophobic surface to which organic molecules in aqueous solutions can bind hydrophobically. The primary requirement for a guest to be included in a cyclodextrin cavity is obviously that its size should be smaller than the cavity itself. If the guest molecule is of an appropriate size, then the hydrogen bonding network can be extended within the cavity through the open ends of the toroid and a hydration sheath can be formed around the guest molecule. A supramolecule containing a cyclodextrin molecule as the host is perhaps the model that best describes an enzyme–substrate complex.

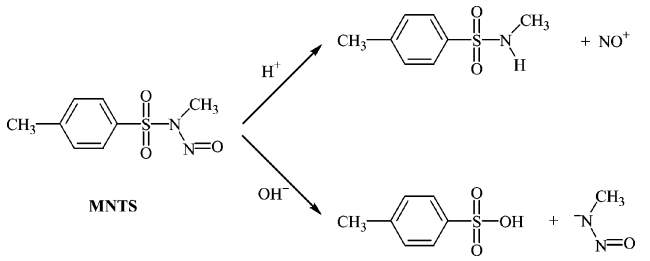
[a]*Departamento de Química Física, Facultad de Química, Universidad de Santiago, 15782, Santiago, Spain*
[b]*Departamento de Química Física, Facultad de Ciencias, Universidad de Vigo, Vigo, Spain*
[c]*Departamento de Química Física, Facultad de Ciencias, Universidad de Vigo, Ourense, Spain*

† Electronic supplementary information (ESI) available: Kinetic data, derivation of equations. See DOI: 10.1039/b513214b

It is clear that most studies concerning the stability of cyclodextrin complexes have been conducted in pure aqueous solutions; however, a number of authors have examined the formation of cyclodextrin complexes in aqueous–organic binary solvent mixtures or in pure organic solvents. There is evidence that the nature of the solvent can influence or even determine the structure of the complex.[4] Indeed, some cyclodextrin complexes have been found to be more stable in mixed solvents and pure organic media than in aqueous solutions[5] or more stable in D_2O than H_2O.[6] However, the solvent effect usually reduces the stability of the complex relative to water.[7] Most studies in this context have used aqueous mixtures with common water-miscible solvents such as alcohols, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dioxane or acetonitrile; however, complexes have also been found to form in pure solvents of these types[8] as well as in hydrocarbons and similar nonpolar solvents.[5d,9]

The effects of solvents on the stability of cyclodextrin complexes have been examined in light of various hypotheses.[10] One approach is based on the assumption that organic cosolvents act as competitive substrates; as their concentration is raised, their ability to compete in displacing the primary substrate from the guest cavity increases.[11] This hypothesis allows one to evaluate binding constants for the postulated cosolvent–cyclodextrin complex formation reactions, the results of which are usually chemically reasonable. Another hypothesis involves the assumption that the organic cosolvent undergoes inclusion alongside the primary substrate to form a complex in a 1 : 1 : 1—or higher—proportion.[5a,5b,5e,12] This hypothesis accounts for the increased complex stability observed when an organic cosolvent is added to an aqueous system.

In the work described here, we used a kinetic method to study the complexation of dioxane, DMSO and acetonitrile by α- and β-cyclodextrin. We examined the influence of the dioxane, DMSO and acetonitrile concentrations on the acid and basic hydrolysis of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) (Scheme 1) in the presence of α- and β-CD. Both reactions were previously examined in an aqueous medium.[13]



Scheme 1

The results allowed us to determine the binding constants for the complexes formed between the organic solvents and the cyclodextrins and also gave the stoichiometries of the complexes. The most salient finding is that, while dioxane and DMSO form 1 : 1 complexes with α- and β-CD, acetonitrile forms host–guest complexes with a 1 : 1 stoichiometry with α-CD and a 1 : 2 stoichiometry with β-CD. It should be noted that these organic molecules are frequently used as solvating agents in studies on the catalytic efficiency of cyclodextrins.

Experimental

MNTS, spectroscopic grade solvents (dioxane, DMSO and acetonitrile) and the cyclodextrins were purchased from Sigma or Fluka and used as received. Cyclodextrin solutions were prepared taking into account the amount of water contained in commercial α- and β-CD.

The kinetic procedures largely conformed to well-established practices.[13] Reactions were monitored through the first-order acid or basic hydrolysis of MNTS at 250 nm, using a Hewlett-Packard 8453 spectrophotometer with the observation cell thermostated at (25.0 ± 0.1) °C. All kinetic tests were conducted under *pseudo* first-order conditions ([MNTS] = 1.56×10^{-5} M ≪ [OH^-] or [H^+]). The integrated first-order rate law was fitted to the absorbance–time data by linear regression ($r > 0.999$) in all cases. The observed rate constants, k_{obs}, were thus reproduced to within ±3%. In the tests that did not involve organic solvent, MNTS was dissolved in water containing CD and a small volume of the resulting solution was subsequently added to the reaction mixture; in experiments involving an organic cosolvent, MNTS was first dissolved in dioxane, DMSO or acetonitrile and further solvent was subsequently added to the reaction medium in the required proportion.

Results and discussion

In this section the results obtained in the acid and basic hydrolysis of MNTS in the presence of α- or β-CD in dioxane, acetonitrile or DMSO as organic cosolvent are presented and discussed.

1. β-Cyclodextrin

1a. Acid and basic hydrolysis of MNTS in the presence of dioxane and DMSO. The influence of the β-CD concentration on k_{obs} for the acid hydrolysis of MNTS in dioxane over a range of concentrations as an organic cosolvent is illustrated in Fig. 1a. As can be seen, k_{obs} decreased on increasing the concentration of β-CD. These results are consistent with a mechanism involving the formation of an unreactive complex between MNTS and the cyclodextrin. In the absence of dioxane, the observed rate constant decreased by a factor of about 10 on increasing the cyclodextrin concentration from 4.00×10^{-4} M to 8.00×10^{-3} M. In the presence of dioxane, the inhibitory effect decreased from about 7 to 2.3 times on increasing the cosolvent concentration from 1.95×10^{-2} to 0.234 M.

The results outlined above rule out the formation of mixed inclusion complexes with MNTS and the organic cosolvent. Thus, ethanol forms inclusion complexes with β-CD and the small size of this system may allow the co-inclusion of ethanol and the substrate.[14] The inclusion of the organic cosolvent in the β-CD cavity must increase its hydrophobicity through the displacement of water molecules from within. The increased hydrophobicity that results should lead to an increase in the binding constant of MNTS

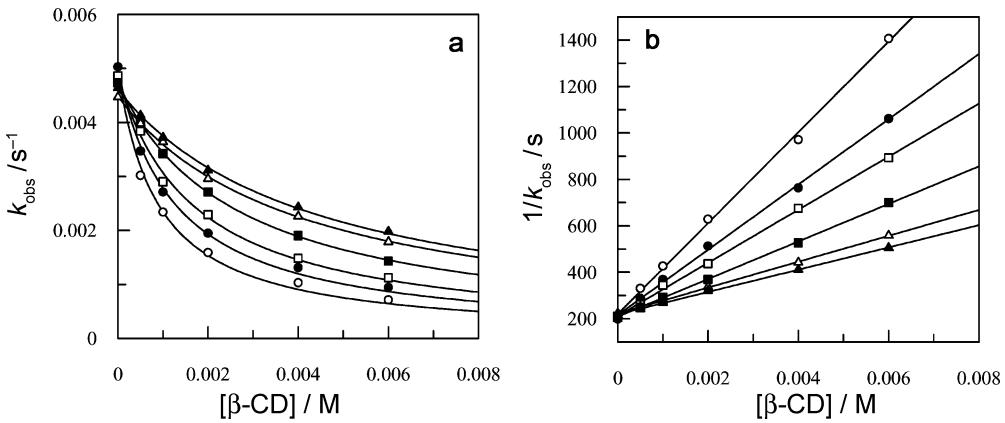
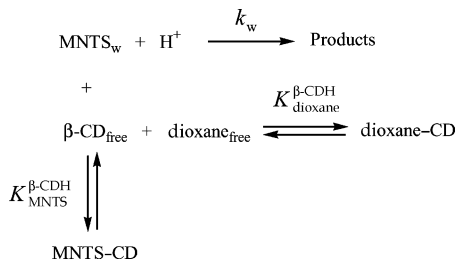


Fig. 1 (a) Influence of the concentration of β-CD on k_{obs} for the acid hydrolysis of MNTS in the presence of 0.160 M and variable concentrations of dioxane at 25.0 °C. (○) [Dioxane] = 1.95×10^{-2} M; (●) [dioxane] = 3.90×10^{-2} M; (□) [dioxane] = 7.03×10^{-2} M; (■) [dioxane] = 0.117 M; (▲) [dioxane] = 0.172 M; and (△) [dioxane] = 0.234 M. (b) Fitting of the data to eqn (2).

to β-CD as the proportion of organic solvent is raised. However, our results for dioxane, DMSO and acetonitrile contradict this assumption and do not support the formation of mixed inclusion complexes in this situation.

For a long time it was widely believed that cyclodextrins formed inclusion complexes only in pure aqueous solutions, mainly because attempts to induce precipitation of CD adducts from organic solvents failed. The first successful study concerning the influence of the solvent on the formation of inclusion complexes with CD was described by Siegel and Breslow,[15] who determined the stability constants for the complexes of various organic species with β-CD in DMF, DMSO and water–DMSO mixtures. Their results led to the following conclusions: (1) the solvent plays a key role in the binding process; (2) the complexes formed in nonaqueous and water–organic mixed solvents are weaker than those obtained in pure aqueous solutions. However, Nelson *et al.*[16] determined the stability constants of the complexes formed by pyrene with β- and γ-CD in the presence of 10% *tert*-butyl alcohol as being one order of magnitude greater than those obtained in pure aqueous solutions. Furthermore, other studies[5e] have shown β-CD and hydroxypropyl-β-CD to form ternary complexes in the presence of alcohols.

The effect of cyclodextrins on the acid hydrolysis of MNTS is due to the formation of an inclusion complex between MNTS and the cyclodextrin. Our kinetic results in the presence of a cosolvent are consistent with a competitive process involving the binding of dioxane molecules to the cyclodextrin. Raising the dioxane concentration increased the amount of β-CD that binds the organic cosolvent and, as a result, decreased the quantity of β-CD available for binding MNTS (see Scheme 2). As a consequence, the inhibitory effect of β-CD on the acid hydrolysis of MNTS decreases on increasing the concentration of organic solvent.



Scheme 2

In the reaction mechanism depicted in Scheme 2, k_w is the bimolecular rate constant for the acid hydrolysis of MNTS in an aqueous medium, $K^{\beta\text{-CDH}}_{\text{MNTS}}$ the equilibrium binding constant of MNTS to unionized cyclodextrin and $K^{\beta\text{-CDH}}_{\text{dioxane}}$ that for the formation of the inclusion complex between dioxane and the neutral form of the cyclodextrin. From Scheme 2 it is possible to derive the following expressions:[17]

$$k_{obs} = \frac{k_w[H^+]}{1 + K^{\beta\text{-CDH}}_{\text{MNTS}}[CD]_{free}} \quad K_{app} = \frac{K^{\beta\text{-CDH}}_{\text{MNTS}}}{1 + K^{\beta\text{-CDH}}_{\text{dioxane}}[\text{dioxane}]_T} \qquad (1)$$

The *pseudo* first-order rate constant can be written in linear form as:

$$\frac{1}{k_{obs}} = \frac{1}{k_w[H^+]} + \frac{K_{app}}{k_w[H^+]}[CD]_T \qquad (2)$$

The good fit between the experimental results obtained at variable dioxane concentrations and eqn (2) is shown in Fig. 1b. The K_{app} and k_w values obtained at each organic cosolvent concentration studied are shown in Table 1. It can be seen that both constants decrease on increasing the proportion of dioxane in the reaction medium. The variation of k_w with the dioxane concentration is consistent with the influence of the solvent polarity on the reaction rate in the absence of cyclodextrin.[18] On the other hand, the variation of K_{app} with the proportion of organic cosolvent conforms to the following equation:

$$\frac{1}{K_{app}} = \frac{1}{K^{\beta\text{-CDH}}_{\text{MNTS}}} + \frac{K^{\beta\text{-CDH}}_{\text{dioxane}}}{K^{\beta\text{-CDH}}_{\text{MNTS}}}[\text{dioxane}]_T \qquad (3)$$

As can be seen from Fig. 2a, the acid hydrolysis of MNTS in the presence of β-CD and dioxane fits eqn (3) quite closely. This equation allowed us to determine the binding constant of MNTS to unionized cyclodextrin, $K^{\beta\text{-CDH}}_{\text{MNTS}} = (1380 \pm 220)\ M^{-1}$, which is consistent with the value obtained in the absence of organic cosolvent: $K^{\beta\text{-CDH}}_{\text{MNTS}} = (1530 \pm 90)\ M^{-1}$. Similarly, we were able to obtain the binding constant of dioxane to the unionized form of β-CD$^-$ $K^{\beta\text{-CDH}}_{\text{dioxane}} = (23.8 \pm 3.7)\ M^{-1}$ (see Tables 2 and 3).

The experimental results for the basic hydrolysis of MNTS in the presence of variable concentrations of dioxane exhibited a similar trend to those for the acid hydrolysis (refer to the ESI). Thus, k_{obs} decreased on increasing the proportion of CD in the reaction medium. This was a result of the formation of an unreactive inclusion complex between MNTS and the ionized form of the cyclodextrin, with $K^{\beta\text{-CD}^-}_{\text{MNTS}}$. As the proportion of dioxane in the reaction medium was raised, a competitive equilibrium between the complexation of MNTS and dioxane by the anionic form of cyclodextrin was established. Consequently, the experimental results can be interpreted in terms of a mechanism similar to that in Scheme 2 and with rate equations similar to (2) and (3). This

Table 1 Kinetic constants obtained by fitting eqn (2) to the experimental results for the acid and basic hydrolysis of MNTS in the presence of β-CD and variable concentrations of dioxane at 25.0 °C

[NaOH] = 0.175 M			[HCl] = 0.160 M		
[Dioxane]/M	$K^{\beta\text{-CD}^-}_{app}/M^{-1}$	$10^2\ k_w/M^{-1}\ s^{-1}$	[Dioxane]/M	$K^{\beta\text{-CDH}}_{app}/M^{-1}$	$10^2\ k_w/M^{-1}\ s^{-1}$
			0	1690 ± 280	3.21 ± 0.54
0.0156	275 ± 11	7.51 ± 0.30	0.0195	886 ± 39	2.83 ± 0.12
0.039	212 ± 3	9.24 ± 0.15	0.039	650 ± 20	2.89 ± 0.09
0.117	103 ± 2	9.27 ± 0.16	0.0703	539 ± 14	2.95 ± 0.08
0.234	64 ± 1	9.19 ± 0.18	0.117	386 ± 4	2.99 ± 0.03
0.586	30 ± 1	9.65 ± 0.29	0.172	250 ± 3	2.81 ± 0.03
0.937	7 ± 1	9.59 ± 0.59	0.232	219 ± 3	2.85 ± 0.04

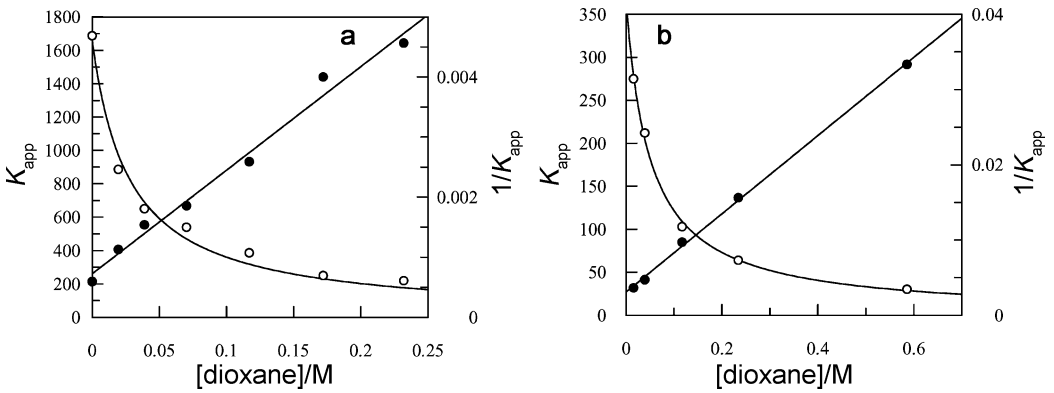


Fig. 2 (○) Variation of K_{app} for the acid (a) and basic (b) hydrolysis of MNTS in the presence of β-CD and dioxane as a function of the organic solvent concentration. (●) Fitting of the data to eqn (3).

binding model allowed us to determine K_{app} and k_w at different dioxane concentrations (see Table 1). As predicted by eqn (3), both parameters decrease on increasing the proportion of dioxane. The good fitting of eqn (3) (in Fig. 2b) allowed us to determine the binding constants of MNTS and dioxane to the anionic form of cyclodextrin, *viz.* $K_{MNTS}^{\beta\text{-CD}^-} = (325 \pm 30)\ M^{-1}$ and $K_{dioxane}^{\beta\text{-CD}^-} = (16.8 \pm 1.6)\ M^{-1}$, respectively (see Table 3).

The difference between the binding constant for the anionic and neutral forms of cyclodextrin is consistent with previously reported results: the binding constant of the substrate to the cyclodextrin anion is lower than that to the neutral cyclodextrin.[19]

Quantitative analysis of the solvent effect. Our results show that the addition of dioxane to a β-CD solution leads to the formation of a 1 : 1 complex between the solvent and the cyclodextrin. This reduces the amount of β-CD available for binding to MNTS and results in the inhibitory effect of the addition of CD decreasing on increasing the concentration of organic cosolvent. Providing a quantitative explanation for the experimental results entails considering the twofold effect of the addition of dioxane to a β-CD solution, namely: (a) the binding of dioxane to the cyclodextrin reduces the concentration of free β-CD; and (b) because the concentration of dioxane will normally be much higher than that of β-CD, the properties of the aqueous medium may be altered by the presence of the organic cosolvent.

The evaluation of $[\beta\text{-CD}]_{free}$ entails solving the following equation (refer to the ESI for details):

$$([\beta\text{-CD}]_{free})^2 + [\beta\text{-CD}]_{free}\,(1 + K_{dioxane}^{\beta\text{-CDH}}\,[dioxane]_T - K_{dioxane}^{\beta\text{-CDH}}\,[\beta\text{-CD}]_{total}) - [\beta\text{-CD}]_T = 0 \quad (4)$$

A simulated value for the formation constant of the inclusion complex between dioxane and cyclodextrin, $K_{dioxane}^{\beta\text{-CDH}}$, was used to calculate $[\beta\text{-CD}]_{free}$ at each $[\beta\text{-CD}]_T$ value, with the resulting data

Table 2 Kinetic constants obtained by fitting eqn (2) and (3) to the experimental results for the acid and basic hydrolysis of MNTS in the presence of β-CD and variable concentrations of acetonitrile at 25 °C

[NaOH] = 0.175 M			[HCl] = 0.160 M		
$[CH_3CN]$/M	$K_{app}^{\beta\text{-CD}^-}/M^{-1}$	$10^2\,k_w/M^{-1}\,s^{-1}$	$[CH_3CN]$/M	$K_{app}^{\beta\text{-CDH}}/M^{-1}$	$10^2\,k_w/M^{-1}\,s^{-1}$
			0	1690 ± 280	3.21 ± 0.54
0.0957	292 ± 10	7.28 ± 0. 26	0.0638	1467 ± 9	3.06 ± 0.02
0.638	252 ± 7	7.59 ± 0.22	0.510	1196 ± 33	2.79 ± 0.08
0.957	248 ± 3	8.24 ± 0.11	0.957	1009 ± 25	2.77 ± 0.07
1.910	142 ± 2	8.76 ± 0.12	1.468	813 ± 24	2.80 ± 0.08
2.870	53 ± 1	6.79 ± 0.16	1.978	476 ± 7	2.03 ± 0.03
3.830	29 ± 1	5.46 ± 0.15	2.872	244 ± 4	1.83 ± 0.03

Table 3 Thermodynamic parameters for the acid and basic hydrolysis of MNTS in the presence of α- or β-CD and dioxane, DMSO or acetonitrile as organic cosolvent. The solvent–cyclodextrin and cyclodextrin–MNTS complexes were both 1 : 1

Cyclodextrin	Cosolvent	Eqn	K_{MNTS}^{CDH}/M^{-1}	K_{MNTS}^{CD}/M^{-1}	$K_{solvent}^{CDH}/M^{-1}$	$K_{solvent}^{CD}/M^{-1}$
β-CD	None	(1)	1530 ± 90	400 ± 20	—	—
β-CD	Dioxane	(3)	1380 ± 220	325 ± 30	23.8 ± 3.7	16.8 ± 1.6
		(5)	1180 ± 14	363 ± 4	20.0 ± 2.5	20.0 ± 2.5
β-CD	DMSO	(3)	1600 ± 270	480 ± 200	2.8 ± 0.5	3.1 ± 0.4
		(5)	1780 ± 50	430 ± 13	4.0 ± 0.5	2.5 ± 0.5
α-CD	None	(9)	47 ± 4	—	—	—
α-CD	Acetronitrile	(3)	45 ± 3	—	9.0 ± 0.6	—
		(10)	51 ± 2	—	7.0 ± 1.0	—

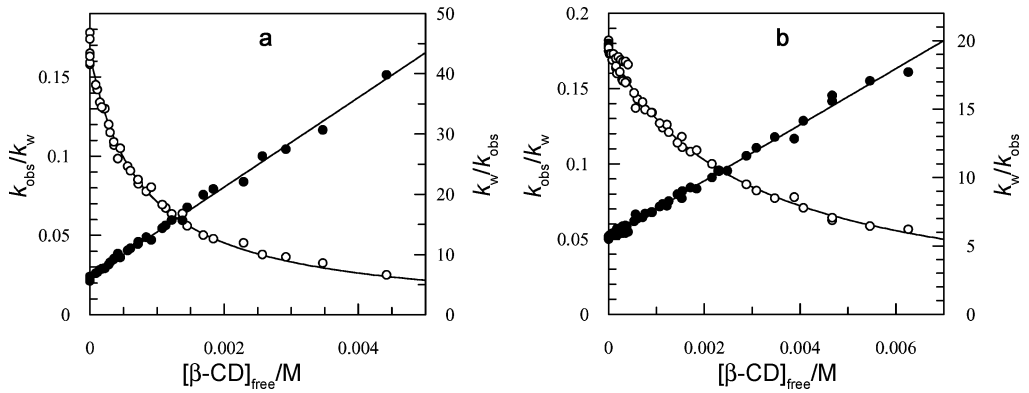


Fig. 3 (a) (○) Influence of the concentration of free β-CD on k_{obs}/k_w for the acid hydrolysis of MNTS in the presence of variable concentrations of dioxane (data from Fig. 1). (●) Fitting of the data to eqn (5). (b) Analysis of the experimental results for the basic hydrolysis of MNTS (data from the ESI).

employed to solve the rate equation

$$\frac{k_w}{k_{obs}} = \frac{1}{[H^+]} + \frac{K^{\beta\text{-CDH}}_{MNTS}}{[H^+]}[\beta\text{-CD}]_{free} \quad (5)$$

The simulated $K^{\beta\text{-CDH}}_{dioxane}$ values that best fit eqn (5) were adopted. The linearity criterion used was the correlation coefficient and the $K^{\beta\text{-CDH}}_{dioxane}$ value resulting in the highest coefficient in each case was adopted. The variation of the correlation coefficient used to fit eqn (5) to the experimental data as a function of $K^{\beta\text{-CDH}}_{dioxane}$ and $K^{\beta\text{-CD}^-}_{dioxane}$ for the acid and basic hydrolysis of MNTS, respectively, is given in the ESI. The values obtained, *viz.* $K^{\beta\text{-CDH}}_{dioxane} = (20.0 \pm 2.5)\ M^{-1}$ and $K^{\beta\text{-CD}^-}_{dioxane} = (20.0 \pm 2.5)\ M^{-1}$, are consistent with the previous ones. The good fitting of eqn (5) to the results for the acid (Fig. 3a) and basic (Fig. 3b) hydrolysis of MNTS is shown in Fig. 3. It should be noted that Fig. 3 includes the results obtained at variable dioxane concentrations. As can be seen, all data fitted a single equation and the solvent effect can therefore be assumed to be twofold, namely: (a) an alteration of the properties of the medium that can be corrected using the k_{obs}/k_w ratio; and (b) a competitive binding equilibrium involving the organic solvent and β-CD.

The kinetic implications of the changes in the properties of the medium are significant; in fact, the acid and basic hydrolysis of MNTS are both sensitive to the amount of organic cosolvent present in the reaction medium.[13] The term corresponding to the competitive binding of dioxane arises from the solvent molecule being sufficiently large to fill most of the β-CD cavity; this excludes the possibility of a single cyclodextrin molecule simultaneously binding a dioxane molecule and an MNTS molecule.

The investigation into the influence of organic cosolvents on the acid and basic hydrolysis of MNTS in the presence of β-CD was extended to DMSO. The rate constants for the two reactions were found to decrease on increasing the concentration of cyclodextrin (see the ESI). As in the previous case, one can assume the formation of 1 : 1 MNTS–β-CD and DMSO–β-CD inclusion complexes. The reaction mechanism must be similar to that shown in Scheme 2 and the resulting equations similar to those previously formulated for dioxane, with $K^{\beta\text{-CDH}}_{DMSO}$ instead of $K^{\beta\text{-CDH}}_{dioxane}$ as the equilibrium constant. The use of eqn (3) enabled us to determine the binding constant of MNTS to the ionized and unionized forms of cyclodextrin in the acid and basic hydrolysis reaction. The values found were $K^{\beta\text{-CDH}}_{MNTS} = (1600 \pm 270)\ M^{-1}$ and $K^{\beta\text{-CD}^-}_{MNTS} = (480 \pm 200)\ M^{-1}$, respectively. These values are similar to those obtained in water containing dioxane (*vide supra*). We also obtained $K^{\beta\text{-CDH}}_{DMSO} = (2.8 \pm 0.5)\ M^{-1}$ and $K^{\beta\text{-CD}^-}_{DMSO} = (3.1 \pm 0.4)\ M^{-1}$.

The quantitative analysis of the solvent effect on the acid and basic hydrolysis of MNTS in the presence of β-CD was based on the same mathematical treatment previously used for dioxane. The optimization procedure provided the following values: $K^{\beta\text{-CDH}}_{DMSO} = (4.0 \pm 0.5)\ M^{-1}$ and $K^{\beta\text{-CDH}}_{MNTS} = (1780 \pm 50)\ M^{-1}$ for the acid hydrolysis, and $K^{\beta\text{-CD}^-}_{DMSO} = (2.5 \pm 0.5)\ M^{-1}$ and $K^{\beta\text{-CD}^-}_{MNTS} = (430 \pm 15)\ M^{-1}$ for the basic hydrolysis of MNTS, respectively.

1b. Acid and basic hydrolysis of MNTS in the presence of acetonitrile. We also examined the influence of the β-CD concentration on the acid and basic hydrolysis of MNTS in media containing variable concentrations of acetonitrile. The addition of β-CD was found to decrease k_{obs} in all cases. The inhibition due to the addition of cyclodextrin becomes less marked on increasing the proportion of acetonitrile in the reaction medium (see Fig. 4 for the acid hydrolysis). The addition of high proportions of acetonitrile caused the k_{obs} value at a zero concentration of β-CD to decrease on increasing the content of organic cosolvent. This observation is consistent with the effect of organic solvents on the rate of acid and basic hydrolysis of MNTS (see the results for the basic hydrolysis in the ESI).

It can be seen from Fig. 4 that eqn (3) fits the experimental results quite well. The $K^{\beta\text{-CDH}}_{app}$ and $K^{\beta\text{-CD}}_{app}$ values obtained are given in Table 2 along with the rate constants for the reactions in the absence of cyclodextrin and the presence of variable concentrations of acetonitrile. In contrast to the previous results—and the predictions of eqn (3)—a linear relationship between $1/K^{\beta\text{-CDH}}_{app}$ or $1/K^{\beta\text{-CDH}}_{app}$ and the acetonitrile concentration was not observed; rather, the plot (not shown) was clearly curved, which suggests that the stoichiometry of the complex between acetonitrile and β-CD is higher than 1 : 1. The formation of a 2 : 1 acetonitrile–β-CD complex is illustrated in Scheme 3.

Bearing in mind that the concentration of cyclodextrin bound to MNTS was negligible relative to the combination of free and acetonitrile-bound cyclodextrin, if one assumes that the concentration of acetonitrile bound to cyclodextrin is negligible

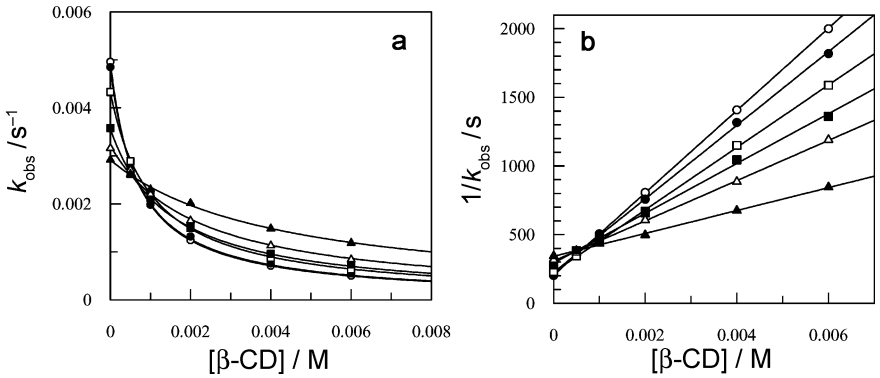
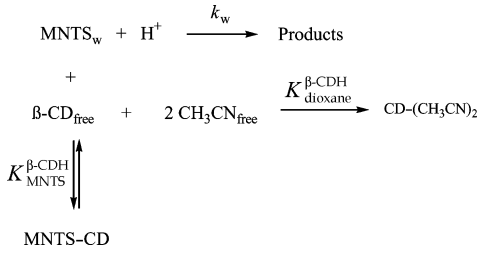


Fig. 4 (a) Influence of the concentration of β-CD on k_{obs} for the acid hydrolysis of MNTS in the presence of variable concentrations of acetonitrile. (○) $[CH_3CN] = 6.38 \times 10^{-2}$ M; (●) $[CH_3CN] = 0.510$ M; (□) $[CH_3CN] = 0.957$ M; (■) $[CH_3CN] = 1.468$ M; (△) $[CH_3CN] = 1.978$ M and (▲) $[CH_3CN] = 2.872$ M. [HCl] = 0.160 M; T = 25.0 °C. (b) Plot of $1/k_{obs}$ as a function of [β-CD] based on eqn (2).



Scheme 3

relative to the total concentration of the solvent, then $K_{app}^{\beta\text{-CDH}}$ can be expressed as follows:

$$K_{app}^{\beta\text{-CDH}} = \frac{K_{MNTS}^{\beta\text{-CDH}}}{1 + K_{CH_3CN}^{\beta\text{-CDH}}[CH_3CN]_T^2};$$
$$\frac{1}{K_{app}^{CDH}} = \frac{1}{K_{MNTS}^{\beta\text{-CDH}}} + \frac{K_{CH_3CN}^{\beta\text{-CDH}}}{K_{MNTS}^{\beta\text{-CDH}}}[CH_3CN]_T^2 \quad (6)$$

Eqn (6) predicts a linear relationship between $1/K_{app}^{\beta\text{-CDH}}$ or $1/K_{app}^{\beta\text{-CDH}}$ and $[CH_3CN]^2$. The results obtained for the acid and basic hydrolysis of MNTS (Fig. 5) are quite consistent with this prediction and hence with the presence of a 2 : 1 complex between acetonitrile and β-CD. Plots such as that shown in Fig. 5 were used

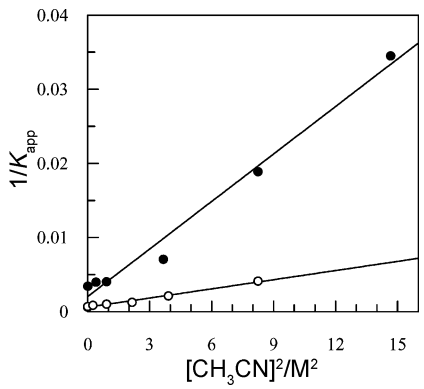


Fig. 5 Fitting of eqn (6) to the $1/K_{app}^{\beta\text{-CDH}}$ values obtained as a function of $[CH_3CN]^2$ in the acid (○) and basic (●) hydrolysis of MNTS in the presence of β-CD.

to determine the binding constants of MNTS to β-CD in acidic and basic media. These constants were found to be $K_{MNTS}^{\beta\text{-CDH}} = (1690 \pm 200)$ M^{-1} and $K_{MNTS}^{\beta\text{-CD}^-} = (495 \pm 240)$ M^{-1}, respectively. These values are consistent with the results previously obtained in the absence of an organic solvent. The relationship between the slope and the intercept allowed us to determine the equilibrium formation constants for the $(CH_3CN)_2$–β-CD complex in acidic and basic media: $K_{CH_3CN}^{\beta\text{-CDH}} = (0.70 \pm 0.08)$ M^{-1} and $K_{CH_3CN}^{\beta\text{-CD}^-} = (1.05 \pm 0.50)$ M^{-1}, respectively.

Quantitative analysis of the solvent effect. Once the stoichiometry of the inclusion complex formed by acetonitrile and β-CD had been determined, we sought to confirm whether the proposed reaction scheme would account for the body of experimental results. The proposed model assumes that the properties of the aqueous medium are altered by the addition of the organic cosolvent. The effect of this addition on the reactivity can be suppressed by using the k_w/k_{obs} ratio, where k_w is the rate constant in the absence of cyclodextrin. We studied aqueous media consisting of either water alone or water–organic cosolvent mixtures. We also assumed that the cyclodextrin established a competitive equilibrium where the binding of acetonitrile molecules decreased the amount of β-CD available for binding MNTS.

The mass balances for MNTS, acetonitrile and the cyclodextrin yield the equation

$$K_{CH_3CN}^{\beta\text{-CD}}[CH_3CN]_{free}^3 + (2K_{CH_3CN}^{\beta\text{-CD}}[\beta\text{-CD}]_T - K_{CH_3CN}^{\beta\text{-CD}} [CH_3CN]_T)[CH_3CN]_{free}^2 + [CH_3CN]_{free} - [CH_3CN]_T = 0 \quad (7)$$

This third-order equation was solved by using the method described previously: a simulated value for the formation constant of the complex between acetonitrile and β-CD was used to calculate the concentration of unbound acetonitrile [eqn (7)]. The relationship between the observed rate constant and the free cyclodextrin concentration, eqn (5), was then used to identify the $K_{CH_3CN}^{\beta\text{-CDH}}$ value that provided the free β-CD concentrations that best fitted eqn (5). In this way, the following values were obtained: $K_{CH_3CN}^{\beta\text{-CDH}} = (0.60 \pm 0.05)$ M^{-2} and $K_{CH_3CN}^{\beta\text{-CDH}} = (0.50 \pm 0.05)$ M^{-2}.

The results obtained in this way for the acid and basic hydrolysis of MNTS are shown in Table 4 and Fig. 6. As can be seen, all experimental data fitted a single curve—a fact that confirms the accuracy of the proposed method, which assumes the solvent effect to encompass two contributions: namely, the alteration of the

Table 4 Thermodynamic parameters for the acid and basic hydrolysis of MNTS in the presence of α- or β-CD. The complex formed by β-CD with acetonitrile as the cosolvent was 2 : 1 (see Scheme 3) and that formed by α-CD with MNTS in the basic hydrolysis reaction was 1 : 2 (see Scheme 4)

Cyclodextrin	Cosolvent	Eqn	K^{CDH}_{MNTS}/M^{-1}	K^{CD}_{MNTS}/M^{-1}	$K^{CDH}_{solvent}/M^{-2}$	$K^{CD}_{solvent}/M^{-2}$
β-CD	Acetonitrile	(6)	1690 ± 200	495 ± 240	0.70 ± 0.09	1.05 ± 0.50
		(5)	1490 ± 15	340 ± 4	0.60 ± 0.05	0.50 ± 0.05
α-CD	None	(8)	—	2.6 ± 0.3	—	—

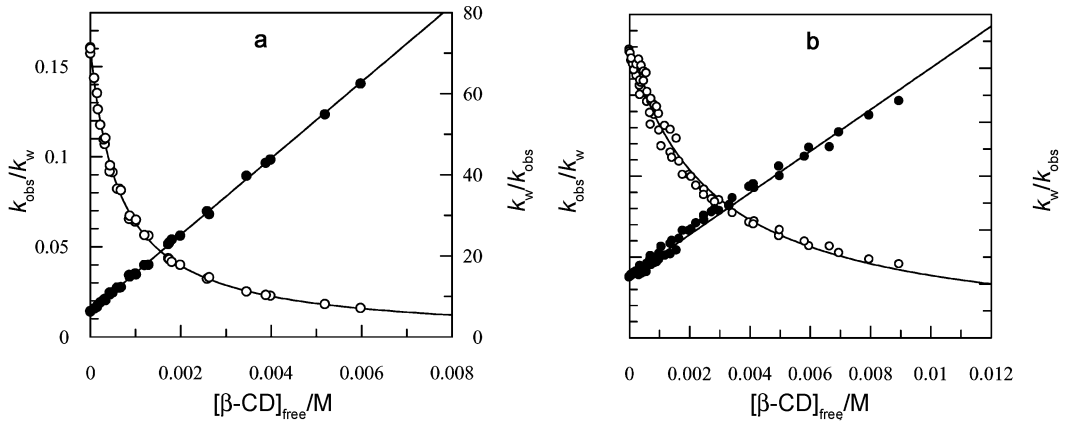


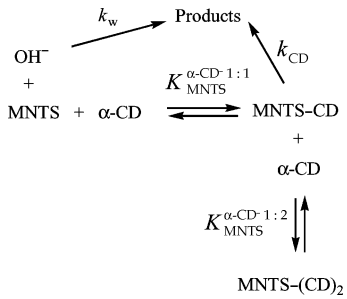
Fig. 6 (a) (○) Influence of the concentration of free β-CD on k_{obs}/k_w for the acid hydrolysis of MNTS in the presence of variable concentrations of acetonitrile (data from Fig. 4). (●) Fitting of eqn (5) to the data. (b) Analysis of the experimental results for the basic hydrolysis of MNTS (data from the ESI).

properties of water and the competitive binding of the organic solvent to the cyclodextrin.

Our results contradict those of Taraszewska,[20] who studied the binding of ethanol, DMSO, DMF, acetone and acetonitrile to β-CD and obtained constants similar to those found for α-CD and CD–solvent complexes of 1 : 1 stoichiometry, as well as a binding constant of $K^{\beta\text{-CD}}_{1:1} = 6.0\ M^{-1}$ for the β-CD–acetonitrile complex. It should be noted that the binding constants varied with the proportion of organic solvent. This is because the change in the concentration of free cyclodextrin on increasing the amount of the organic solvent in the mixture was ignored. Furthermore, Taraszewska found differences between the complexes of acetonitrile and acetone and those of the other solvents, and assumed that the zone inside the β-cavity where *o*-chloronitrobenzene could be accommodated in the presence of acetone and acetonitrile was more hydrophobic than in the presence of other solvents. Park[21] studied the complexes of various organic solvents, including acetonitrile, with β-CD by spectrophotometry. In that study the inhibitory effect of solutes on the binding of CD by phenolphthalein was investigated. All complexes were 1 : 1 and the resulting binding constant of acetonitrile to β-CD was $K^{\beta\text{-CD}}_{1:1} = 0.54\ M^{-1}$. Park's values for the binding constant of acetonitrile to β-CD are consistent with our own; however, the stoichiometry of the complex is different. We believe that Park's results may have been influenced by the fact that the acetonitrile concentrations considered only covered a small range (*viz.* 0.73–0.86 M). In the study described here, acetonitrile concentrations from *ca.* 6.00×10^{-2} M to 3.00 M were considered. The wider range used here may have allowed us to detect the presence of 2 : 1 complexes. Other authors have also studied the displacement of phenolphthalein from its complex with β-CD by the addition of tetrahydrofuran.[22]

2. α-Cyclodextrin

2A. Basic hydrolysis of MNTS. The basic hydrolysis of MNTS in the presence of β-CD is strongly inhibited by the formation of an unreactive complex between MNTS and the cyclodextrin. The presence of α-CD results in a distinct type of behavior: k_{obs} increases moderately to a maximum value on increasing the α-CD concentration but beyond this maximum the constant decreases on further increasing [α-CD] (see Fig. 7). The experimental results can be interpreted in terms of the mechanism shown in Scheme 4.



Scheme 4

Such a mechanism enables the following rate law to be derived:

$$k_{obs} = \frac{k_w[OH^-] + k_{CD}K^{\alpha\text{-CD}^-1:1}_{MNTS}[\alpha\text{-CD}]_T}{1 + K^{\alpha\text{-CD}^-1:1}_{MNTS}[\alpha\text{-CD}]_T + K^{\alpha\text{-CD}^-1:1}_{MNTS}K^{\alpha\text{-CD}^-1:2}_{MNTS}[\alpha\text{-CD}]^2_T} \quad (8)$$

where k_w and k_{CD} are the rate constants for hydrolysis by OH^- ions in water and by the alkoxide groups in the cyclodextrin, respectively; and $K^{\alpha\text{-CD}^-1:1}_{MNTS}$ and $K^{\alpha\text{-CD}^-1:2}_{MNTS}$ are the equilibrium binding constants for the 1 : 1 complex and 1 : 2 complex [MNTS-$(CD)_2$]

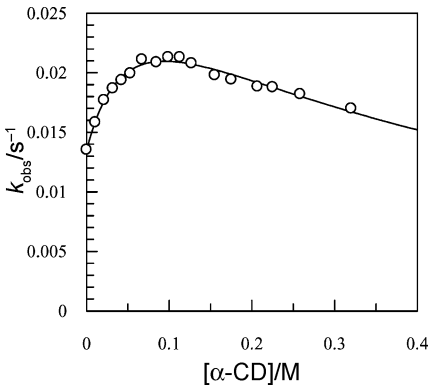
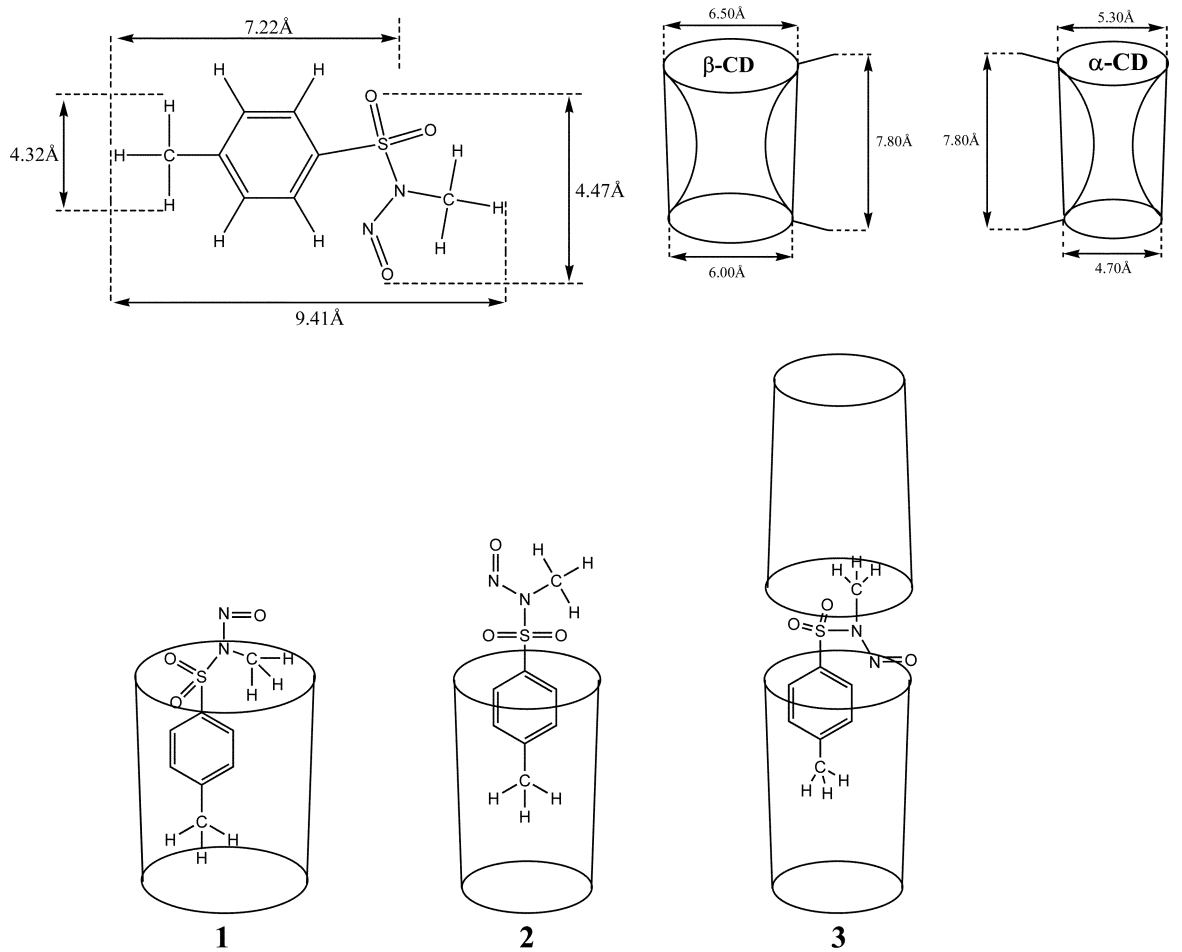


Fig. 7 Influence of the concentration of α-CD on k_{obs} for the basic hydrolysis of MNTS. $[OH^-] = 0.162$ M, $T = 25.0$ °C.

between MNTS and the anionic form of α-CD, respectively. By fitting eqn (8) to the experimental data, and keeping the k_w value constant ($k_w = 0.083\ M^{-1}\ s^{-1}$), we obtained $k_{CD} = (3.1 \pm 0.1) \times 10^{-2}\ s^{-1}$, $K_{MNTS}^{\alpha\text{-CD}^-1:1} = (15 \pm 2)\ M^{-1}$ and $K_{MNTS}^{\alpha\text{-CD}^-1:2} = (2.6 \pm 0.3)\ M^{-2}$.

Although k_{CD} (in s^{-1}) and k_w (in $M^{-1}\ s^{-1}$) cannot be compared directly, the fact that the latter exceeded the former is worthy of note. The hydrolysis reaction *via* the MNTS–CD complex is equivalent to nucleophilic attack of an alkoxide ion on MNTS. Such a reaction was studied in our laboratory[23] and its rate was found to exhibit a Brønsted-type relationship with the basicity of the starting alcohol for methanol, isopropanol, tetrafluoroethanol and hexafluoroisopropanol, with a slope $\beta_{nucl} = 0.49 \pm 0.01$. The reactivity of the OH^- ion was lower than expected from its basicity, probably due to the need for desolvation prior to nucleophilic attack, as in other hydroxyl ion- and thiolate-catalyzed reactions.[24] The observed rate constant for the reaction *via* the ionized hydroxyl group in the cyclodextrin was roughly half that expected for an alkoxide with a similar pK_a (*e.g.* $CF_3CH_2O^-$).

The results are consistent with the formation of 1 : 1 and 1 : 2 MNTS–α-CD complexes, as well as with transition states that involve a cyclodextrin molecule. These differences between the behavior of α-CD and β-CD must be a result of geometric factors. The binding of guests to cyclodextrins is determined largely by two factors: namely, the size of the included moiety and its hydrophobicity.[25] The disparate binding abilities of α- and β-CD must be ascribed to the widths of their cavities (4.5 and 7.0 Å, respectively) since their depths are identical (7.0 Å). The energy-minimized structures obtained using the MNTS dimensions and optimal geometry derived from molecular mechanics calculations are shown in Scheme 5. Based on the size of MNTS, the molecule can be fully accommodated inside the β-CD cavity (1), so the sulfonyl group will hardly be able to approach the ionized RO^- group in β-CD.

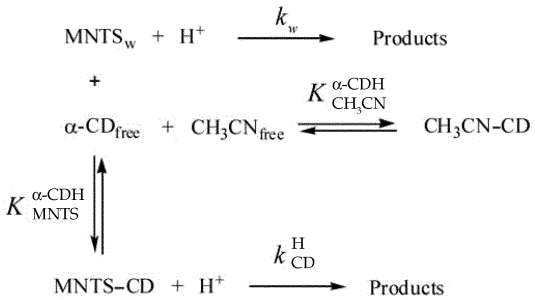


Scheme 5

In the presence of α-CD, the toluene group in MNTS is too large to be fully accommodated inside the α-CD cavity. Therefore, the binding constant of MNTS to both the neutral form (*vide infra*) and the anionic form of α-CD will be quite small relative to that for β-CD. The kinetics of the basic hydrolysis of MNTS with ionized α-CD clearly indicate that both 2 : 1 and 1 : 1 binding situations are substantial. The initial increase in the rate constant suggests that the former binding stoichiometry involves the toluene group (2), whereas the latter involves the nitroso group (3).

2B. Acid hydrolysis of MNTS in the presence of acetonitrile. We studied the influence of α-CD on the acid hydrolysis of MNTS in the presence of variable amounts of acetonitrile. As an illustrative example, the results obtained in the presence of variable concentrations of cosolvent ranging from 3.19×10^{-2} to 0.511 M are shown in Fig. 8. The resulting k_{obs} values differed markedly from those found in the presence of β-CD (see Fig. 4). As can be seen in Fig. 8b, the variation of the rate constant with the α-CD concentration (dashed line) deviated strongly from linearity.

This finding is inconsistent with both eqn (2) and the reaction mechanism shown in Scheme 2, but is consistent with the mechanism in Scheme 6.



Scheme 6

The acid denitrosation of MNTS can occur in the aqueous medium (k_w) or through the MNTS–CD complex (k^H_{CD}). The binding constants $K^{\alpha\text{-CDH}}_{CH_3CN}$ and $K^{\alpha\text{-CDH}}_{MNTS}$ correspond to the formation of the respective inclusion complexes between MNTS or CH_3CN and the neutral form of α-cyclodextrin. The corresponding observed rate constant can be expressed as

$$k_{obs} = \frac{k_w[H^+] + k^H_{CD}K^{\alpha\text{-CDH}}_{app}[H^+][\alpha\text{-CD}]_{free}}{1 + K^{\alpha\text{-CDH}}_{app}[\alpha\text{-CD}]_{free}} \quad (9)$$

where $K^{\alpha\text{-CDH}}_{app}$ is the apparent binding constant of MNTS to α-CD. As can be seen from Fig. 8, this equation closely fits the results obtained at variable concentrations of acetonitrile. The $K^{\alpha\text{-CDH}}_{app}$, k_w and k^H_{CD} values obtained for different concentrations of cosolvent are shown in Table 5. As the acetonitrile concentration in the reaction medium was increased, the polarity and the rate constants k_w and k^H_{CD} decreased. The error in the determination of k^H_{CD} increased with increasing concentration of acetonitrile in the reaction medium. This trend is a result of the fact that $K^{\alpha\text{-CDH}}_{app}$ was low at high acetonitrile concentrations, where $k_w \gg k^H_{CD}K^{\alpha\text{-CDH}}_{app}[\alpha\text{-CD}]_{free}$.

The ability of the α-CD-bound substrate to react with H^+ ions in the aqueous medium is due to the geometry of the inclusion complex. As shown in **1**–**3** (Scheme 5), the size of α-CD is sufficient to accommodate the toluene group in MNTS, but the nitroso group remains outside—a situation that facilitates attack by H^+ ions in the medium. This situation cannot occur in β-CD as its cavity is large enough to accommodate the nitroso group as well.

It can be seen from Table 5 that raising the acetonitrile concentration in the reaction medium led to a decrease in $K^{\alpha\text{-CDH}}_{app}$, a change due to the formation of an inclusion complex between CH_3CN and α-CD. Therefore, the variation of $K^{\alpha\text{-CDH}}_{app}$ with the acetonitrile concentration should fit an equation similar to (3). This assumption was confirmed. Fitting eqn (3) to the experimental results (Fig. 9) provided the following values:

Table 5 Kinetic constants obtained by fitting eqn (9) to the experimental results for the acid hydrolysis of MNTS in the presence of α-CD, 0.168 M HCl and variable concentrations of acetonitrile at 25.0 °C

$[CH_3CN]$/M	$K^{\alpha\text{-CDH}}_{app}$/M^{-1}	10^2k_w/M^{-1} s^{-1}	$10^3k^H_{CD}$/M^{-1} s^{-1}
0	47 ± 4	3.17 ± 0.03	8.9 ± 0.1
3.19×10^{-2}	30 ± 1	3.10 ± 0.02	5.5 ± 0.4
6.38×10^{-2}	30 ± 2	3.21 ± 0.02	5.9 ± 0.4
0.128	22 ± 3	3.11 ± 0.04	6 ± 1
0.195	17 ± 1	3.03 ± 0.01	4.5 ± 0.4
0.319	11 ± 2	2.97 ± 0.03	2 ± 2
0.511	8 ± 2	2.98 ± 0.04	3 ± 3

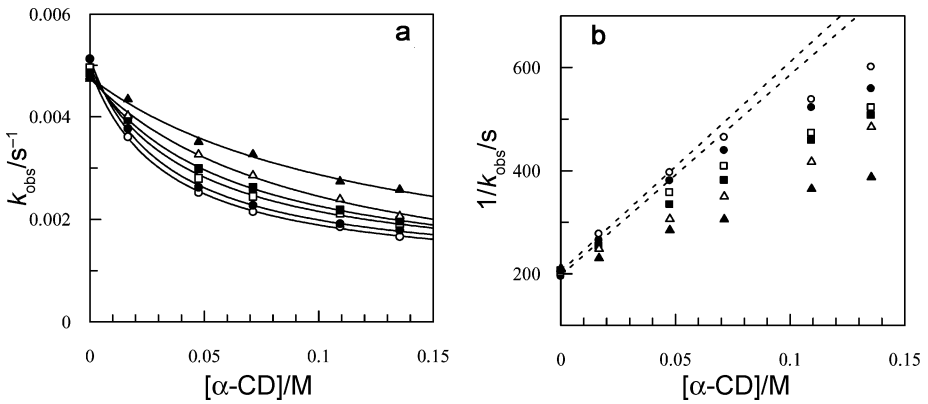


Fig. 8 (a) Influence of the concentration of α-CD on k_{obs} for the acid hydrolysis of MNTS in the presence of 0.168 M HCl and variable concentrations of acetonitrile at 25.0 °C. (○) $[CH_3CN] = 3.19 \times 10^{-2}$ M; (●) $[CH_3CN] = 6.38 \times 10^{-2}$ M; (□) $[CH_3CN] = 0.128$ M; (■) $[CH_3CN] = 0.195$ M; (△) $[CH_3CN] = 0.319$ M; and (▲) $[CH_3CN] = 0.511$ M. (b) Analysis of the data using eqn (9).

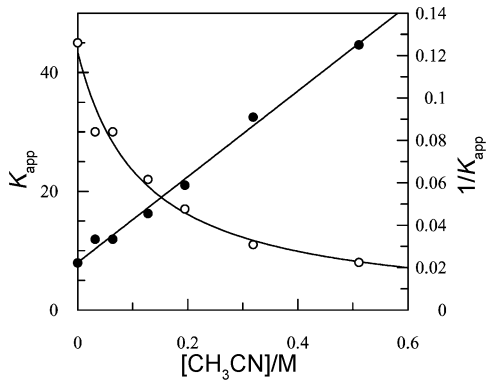


Fig. 9 (○) Variation of K_{app} for the acid hydrolysis of MNTS in the presence of α-CD and acetonitrile as a function of the concentration of organic cosolvent. (●) Fitting of the results to eqn (3).

$K_{MNTS}^{\alpha\text{-CDH}} = (45 \pm 3)\ M^{-1}$ and $K_{CH_3CN}^{\alpha\text{-CDH}} = (9.0 \pm 0.6)\ M^{-1}$, both of which are consistent with the $K_{MNTS}^{\alpha\text{-CDH}}$ value obtained in the absence of acetonitrile [$(47 \pm 4)\ M^{-1}$] (see Table 5).

The kinetic results reveal the formation of a 1 : 1 complex between acetonitrile and α-CD, which is consistent with previous results obtained from X-ray diffraction measurements.[26] Furthermore, the results for β-CD suggest the formation of a 2 : 1 complex. This disparate behavior is a result of the increased cavity size of β-CD relative to α-CD, which allows the former to accommodate two acetonitrile molecules rather than one. The larger size of dioxane relative to acetonitrile results in the formation of a 1 : 1 complex with β-CD.

A more rigorous quantitative interpretation of the experimental results can be provided by considering a reaction mechanism such as that shown in Scheme 6. On the basis of this mechanism the observed rate constant can be expressed in terms of the concentration of free α-CD as follows:

$$\frac{k_{obs}}{k_w} = \frac{[H^+] + \frac{k_{CD}^H}{k_w} K_{MNTS}^{\alpha\text{-CDH}}[H^+][\alpha\text{-CD}]_{free}}{1 + K_{MNTS}^{\alpha\text{-CDH}}[\alpha\text{-CD}]_{free}} \quad (10)$$

where $[\alpha\text{-CD}]_{free}$ can be obtained by using the simulation procedure previously employed with dioxane [eqn (4)]. The $K_{CH_3CN}^{\alpha\text{-CDH}}$ value obtained in this way was $(7 \pm 1)\ M^{-1}$ and provided the free α-CD concentrations shown in Fig. 10. Finally, fitting the data in the figure to eqn (10) provided values of $K_{MNTS}^{\alpha\text{-CDH}} = (51 \pm 2)\ M^{-1}$ and $k_{CD}^H/k_w = (0.23 \pm 0.02)\ M^{-1}$, both of which are quite consistent with values reported previously for this system.

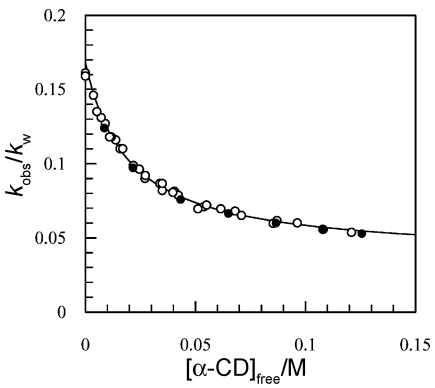


Fig. 10 (○) Influence of the concentration of free α-CD on k_{obs}/k_w for the acid hydrolysis of MNTS in the presence of variable concentrations of acetonitrile [data from Fig. 8 as fitted to eqn (10)]. (●) Results obtained in the absence of acetonitrile.

The spectrophotometric results reported by Connors[27] are consistent with the formation of inclusion complexes between α-CD and methanol, DMSO, ethylene glycol, dioxane ($K_{1:1}^{\alpha\text{-CD}} = 30.6\ M^{-1}$), 2-propanol, acetonitrile ($K_{1:1}^{\alpha\text{-CD}} = 40.3\ M^{-1}$) and acetone, all with a 1 : 1 stoichiometry. The binding of organic molecules to α-cyclodextrin has also been studied by ^{1}H-NMR spectroscopy.[28] The results revealed the formation of a 1 : 1 α-CD–acetonitrile complex and the binding constant was found to be $K_{1:1}^{\alpha\text{-CD}} = 5.6\ M^{-1}$. A potentiometric study of the formation of weak inclusion complexes between α-cyclodextrin and small organic molecules—including ethanol, 2-propanol, 2-methyl-2-propanol, cyclohexanol, dioxane, DMSO and phenol—in an aqueous medium at 25.0 °C provided a $K_{1:1}^{\alpha\text{-CD}}$ value of 5.6 M^{-1} in acetonitrile and 4.5 M^{-1} in dioxane.[29] These results are quite consistent with our value [$K_{CH_3CN}^{\alpha\text{-CDH}} = (7 \pm 1)\ M^{-1}$], which provides further evidence of the accuracy of the proposed model.

Conclusions

The acid and basic hydrolysis reactions of MNTS in the presence of β-cyclodextrin have been studied and the formation of an unreactive β-CD–MNTS complex was observed in both cases. The results with β-CD were different as a consequence of the smaller cavity size of this cyclodextrin, which allows only partial penetration of MNTS. The basic hydrolysis process involved the formation of a reactive 1 : 1 α-CD–MNTS complex at low cyclodextrin concentrations but a 2 : 1 complex was formed as the α-CD concentration was raised.

Size differences in the host and guest molecules resulted in different stoichiometries for the complexes. For example, dioxane and DMSO were found to form 1 : 1 inclusion complexes with β- and α-CD, respectively. On the other hand, acetonitrile formed 1 : 2 inclusion complexes with β-CD. The competitive formation of such inclusion complexes accounts for the experimental behavior observed in the acid and basic hydrolysis of MNTS in the presence of α- or β-CD and variable amounts of organic cosolvent.

Acknowledgements

Funding from Xunta de Galicia (PGIDT03-PXIC2095PN and PGIDIT04TMT209003PR) and Spain's Ministry of Science and Technology (projects BQU2002-01184 and MAT2004-02991) is gratefully acknowledged.

References and notes

1 Special issue on Cyclodextrins, ed. V. T. D'Souza and K. B. Lipkowit, *Chem. Rev.*, 1998, **98**, 1741.
2 W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 1980, **21**, 300.
3 (*a*) M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, New York, 1978; (*b*) J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akademiai Kidao, Budapest, 1982.
4 (*a*) R. Fornasier, M. Parmagnani and U. Tonellato, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 1991, **11**, 225; (*b*) A. F. Danil de Namor, R. Traboulssi and D. F. V. Levis, *J. Chem. Soc., Chem. Commun.*, 1990, 751; (*c*) N. Kobayashi and T. Osa, *Carbohydr. Res.*, 1989, **192**, 147; (*d*) O. S. Tee, C. Mazza, R. Lozano-Hemmer and J. B. Giorgi, *J. Org. Chem.*, 1994, **59**, 7602.

5 (*a*) A. Ueno and T. Osa, *J. Inclusion Phenom.*, 1984, **2**, 555; (*b*) A. Nakajima, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 1143; (*c*) A. E. Aboutaleb, A. A. Abdel Rahmen and E. M. Samy, *J. Pharm. Belg.*, 1988, **43**, 437; (*d*) S. Hamai, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 2323; (*e*) Y. Liao and C. Bohne, *J. Phys. Chem.*, 1996, **100**, 734.
6 A. S. Wang and Y. Matsui, *Bull. Chem. Soc. Jpn.*, 1994, **67**, 2917.
7 R. L. Van Etten, G. A. Clowes, J. F. Sebastian and M. L. Bender, *J. Am. Chem. Soc.*, 1967, **89**, 3253.
8 (*a*) B. Siegel and R. Breslow, *J. Am. Chem. Soc.*, 1975, **97**, 6869; (*b*) A. Harada and S. Takahashi, *J. Inclusion Phenom.*, 1984, **2**, 791; (*c*) A. Harada and S. Takahashi, *Chem. Lett.*, 1984, 2089.
9 M. Komiyama, H. Yamamoto and H. Hirai, *Chem. Lett.*, 1984, 1081.
10 K. A. Connors, *Chem. Rev.*, 1997, **97**, 1325.
11 (*a*) R. I. Gelb, L. M. Schwartz, M. Radeos, R. B. Edmonds and D. A. Laufer, *J. Am. Chem. Soc.*, 1982, **104**, 6283; (*b*) J. Taraszewska, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 1991, **10**, 69; (*c*) J. Huang, G. C. Catena and F. V. Bright, *Appl. Spectrosc.*, 1992, **46**, 606; (*d*) J. M. Schuette, T. T. Ndou, A. Muñoz de la Peña, S. Mukunden and I. M. Warner, *J. Am. Chem. Soc.*, 1993, **115**, 292.
12 W. R. Bergmark, A. Davis, C. York, A. Macintosh and G. Jones, *J. Phys. Chem.*, 1990, **94**, 5020.
13 (*a*) D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1976, 691; (*b*) L. García-Río, J. R. Leis, J. A. Moreira and F. Norberto, *J. Chem. Soc., Perkin Trans. 2*, 1998, **11**, 756; (*c*) L. García-Río, J. R. Leis, J. A. Moreira and F. Norberto, *J. Phys. Org. Chem.*, 1998, **11**, 756.
14 (*a*) M. V. Rekharsky, F. P. Schwarz, Y. B. Tewari and R. N. Goldberg, *J. Phys. Chem.*, 1994, **98**, 10282; (*b*) O. S. Tee and M. Bozzi, *J. Am. Chem. Soc.*, 1990, **112**, 7815; (*c*) O. S. Tee, M. Bozzi, J. J. Hoeven and T. A. Gadosy, *J. Am. Chem. Soc.*, 1993, **115**, 8990.
15 D. Siegel and R. Breslow, *J. Am. Chem. Soc.*, 1975, **97**, 6869.
16 G. Nelson, G. Patonay and J. M. Warner, *J. Inclusion Phenom.*, 1988, **6**, 277.
17 The total concentration of MNTS was very low ([MNTS] $< 5 \times 10^{-5}$M), so [MNTS] $\ll [CD]_T$.
18 A. Castro, J. R. Leis and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1861.
19 (*a*) L. Viola and R. H. de Rossi, *Can. J. Chem.*, 1999, **77**, 860; (*b*) E. Iglesias, *J. Am. Chem. Soc.*, 1998, **120**, 13057.
20 J. J. Taraszewska, *Inclusion Phenom. Mol. Recognit. Chem.*, 1991, **10**, 69.
21 J. H. Park and T. H. Nah, *J. Chem. Soc., Perkin Trans. 2*, 1994, 1359.
22 P. K. Zarzycki and H. Lamparczyk, *J. Pharm. Biomed. Anal.*, 1998, **18**, 165.
23 L. García-Río, J. R. Leis and J. A. Moreira, unpublished results.
24 (*a*) W. P. Jencks, M. T. Haber, D. Herschlag and K. L. Nazaretian, *J. Am. Chem. Soc.*, 1986, **108**, 479; (*b*) J. P. Richard, *J. Chem. Soc., Chem. Commun.*, 1987, 1768; (*c*) R. A. McClelland, V. M. Kanagasabapathy, N. S. Banait and S. Steenken, *J. Am. Chem. Soc.*, 1992, **114**, 1816; (*d*) C. F. Bernasconi, K. W. Kittredge and F. X. Flores, *J. Am. Chem. Soc.*, 1999, **121**, 6630.
25 M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, 1998, **98**, 1875.
26 T. Aree, J. Jacob, W. Saenger and H. Hoier, *Carbohydr. Res.*, 1998, **307**, 191.
27 K. A. Connors, M. J. Mulski and A. Paulson, *J. Org. Chem.*, 1992, **57**, 1794.
28 Y. Matsui and S. Tokunaga, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2477.
29 R. I. Gelb, L. M. Schwartz, M. Radeos, R. Edmonds and D. A. Laufer, *J. Am. Chem. Soc.*, 1982, **104**, 6283.

