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Abstract. The full volume and entropy profiles of the inclusion reaction between α-cyclodextrin and the guest molecules, ethylorange (**1**) and mordant yellow 7 (**2**), have been constructed from variable-pressure and -temperature stopped-flow kinetic experiments.

Introduction

Cyclodextrins are well known as molecular hosts capable of binding a range of guest molecules *via* noncovalent interactions with their hydrophobic cavity. Recently, some progress has been made in the understanding of the complex mechanisms involved in the molecular recognition by α-cyclodextrin (α-CD) [1]. The success of high-pressure studies is related to the fact that it is comparatively simple to interpret volume differences in molecular terms even in the case of complex chemical systems, whereas it is often very difficult to interpret entropy differences on a similar level [2].

Description of the HPSF Apparatus

The third generation of our high-pressure stopped-flow instrument (*Fig. 1*) is able to operate over a temperature range of –40 to +100° and up to 200 MPa [3]. The system has been designed so that it can perform measurements in absorbance or in fluorescence mode or both. The observation cell has path lengths of 10 and 2 mm for absorbance and fluorescence measurements, respectively. The stopped-flow unit can easily be combined with an optical system of a conventional ambient pres-

Table. *Kinetic Parameters for the Inclusion Reaction Between α-CD and a Selection of Azo Dyes, in Aqueous Solution*

Parameters	1	2	3	4
$k_{1,f}^{298}$/M^{-1} s^{-1}	(1.22±0.02) 10^4	(1.52±0.03) 10^4	2.0 10^4	1.2 10^4
$k_{1,r}^{298}$/s^{-1}	1.8 ± 0.1	25.4 ± 0.5	6.0	9.4
K_1^{298}/M^{-1}	6660 ± 470	600 ± 20	3300	1280
$H_{1,f}^{\ddagger}$/kJ mol^{-1}	+20.1 ± 1	+27.3 ± 1	+22.5	–
$H_{1,r}^{\ddagger}$/kJ mol^{-1}	+54.8 ± 3	+38.6 ± 1	+49.8	–
$S_{1,f}^{\ddagger}$/J K^{-1} mol^{-1}	–99.3 ± 3	–73.3 ± 4	–87	–
$S_{1,r}^{\ddagger}$/J K^{-1} mol^{-1}	–55.9 ± 8	–88.6 ± 4	–65	–
$k_{2,f}^{298}$/s^{-1}	0.20 ± 0.02	1.83 ± 0.04	0.87	0.58
$k_{2,r}^{298}$/s^{-1}	(9.3 ± 1.2) 10^{-2}	0.17 ± 0.02	0.55	0.26
K_2^{298}	2.1 ± 0.5	11 ± 1	1.6	2.2
$H_{2,f}^{\ddagger}$/kJ mol^{-1}	+72.0 ± 3	+50.8 ± 1	+54.5	–
$H_{2,r}^{\ddagger}$/kJ mol^{-1}	+43.8 ± 3	+39.5 ± 5	+63.3	–
$S_{2,f}^{\ddagger}$/J K^{-1} mol^{-1}	–16.8 ± 9	–69.4 ± 4	–63	–
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References	this work	this work	[1b]	[1b]

1: R_3=R_5=H, R_4=N(CH_2CH_3)$_2$; **2**: R_3=COO^-, R_4=OH, R_5=CH_3; **3**: R_3=$CH_2CH_2CH_3$, R_4=OH, R_5=H; **4**: R_3=$CH(CH_3)_2$, R_4=OH, R_5=H.

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sure set-up using inexpensive light guides. Optimum optical performance and a wide operating wavelength range (220–850 nm) are achieved as the light is not passing through the pressurizing fluid. The dead time of the system is found to be less than 2 ms at 298 K, and is pressure-independent up to 200 MPa.

Inclusion Kinetics with α-Cyclodextrin in Aqueous Solution

The kinetics for the inclusion reaction between the dyes, S, and α-CD (*Reaction 1*) were investigated as a function of the α-CD concentration, ($C_{\alpha\text{-CD}}$), under pseudo-first-order conditions, and at pH of *ca.* 6.5. The protonation constants determined from spectrophotometric titrations, at 298 K, *I*/M = 0.15 (NaCl) for **1** (**2**) are: pK_{a1} 4.11 ± 0.01 (pK_{a1} 2.46 ± 0.02, pK_{a2} 11.88 ± 0.01). For both dyes, two distinct rate constants were observed (*Reaction 1*). The very fast $k_{1,obs}$ and the slower $k_{2,obs}$, can be expressed by *Eqns. 2* and *3*, where $K_1 = k_{1,f} / k_{1,r}$.

$$S + \alpha\text{-CD} \underset{k_{1,r}}{\overset{k_{1,f}}{\rightleftharpoons}} S.\alpha\text{-CD}^* \underset{k_{2,r}}{\overset{k_{2,f}}{\rightleftharpoons}} S.\alpha\text{-CD} \quad (1)$$

$$k_{1,obs(fast)} = k_{1,f} \cdot C_{\alpha-CD} + k_{1,r} \quad (2)$$

$$k_{2,obs(slow)} = k_{2,f} \frac{K_1 \cdot C_{\alpha-CD}}{(1 + K_1 \cdot C_{\alpha-CD})} + k_{2,r} \quad (3)$$

Temperature-dependent multiwavelength kinetic measurements were carried out between 278 and 318 K on a commercial stopped-flow spectrometer. Pressure-dependent kinetic measurements were carried out on our *HPSF* spectrometer [3], between 230 and 600 nm using a diode array detector for **2** [4], and with a mono-wavelength detection system for **1**. For both the temperature and pressure multiwavelength experiments, the observed rate constants were calculated from the multiwavelength data sets *via Global Analysis* software. Then the data were fitted globally according to *Eqns. 2* and *3*; the results are listed in the *Table* and *Fig. 2*.

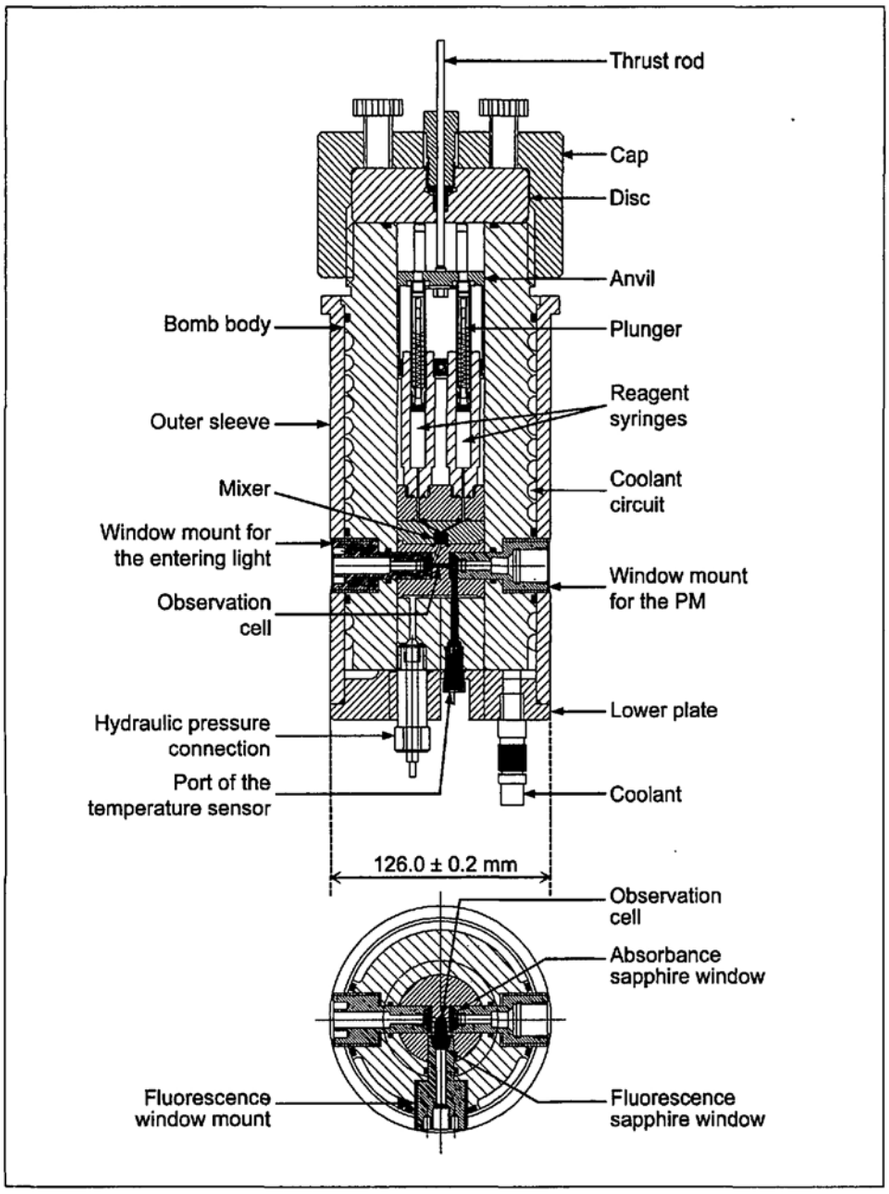


Fig. 1. *Schematic vertical and horizontal sections of the high-pressure stopped-flow autoclave*

Discussion

The inclusion of the guest molecules, **1** and **2**, into the α-CD cavity proceeds *via* the hydrophilic sulfonate moiety exclusively (*Scheme*), as previously referred [1][4].

The first step in the inclusion reaction of **1** and **2** may be seen to require the desolvation of the sulfonate group when the guest is initially included in the α-CD cavity. At the first transition state, strong interactions can exist between the sulfonate group and the two not fully coordinated 'activated' H_2O molecules which are included in the cavity [5]. This interaction should be the principal contribution to the significant contraction at the transition state, as shown by the large negative values of $V_{1,f}^{\ddagger}$ (*Fig. 2*). In the intermediate state, the sulfonate group is resolvated from the bulk water, as it was initially, resulting in a slowing of the reverse step and also hindering further access to the cavity. The two 'activated' H_2O molecules have left the cavity, thus enabling full H-bonding with the H_2O molecules from the bulk. This explains the relatively small negative volume of reaction ΔV_1° for both **1** and **2**.

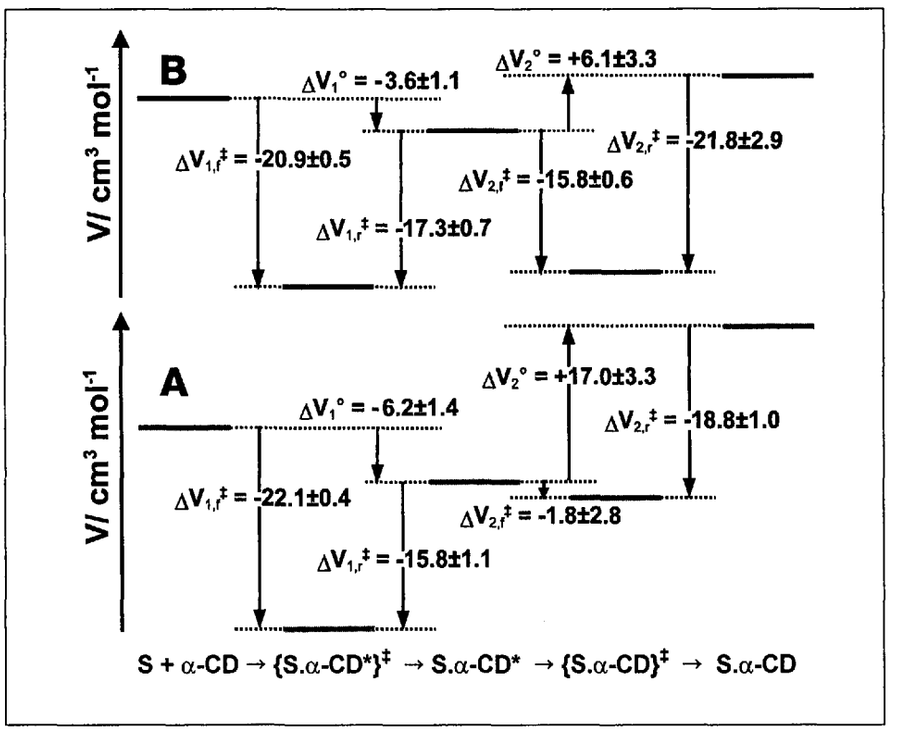
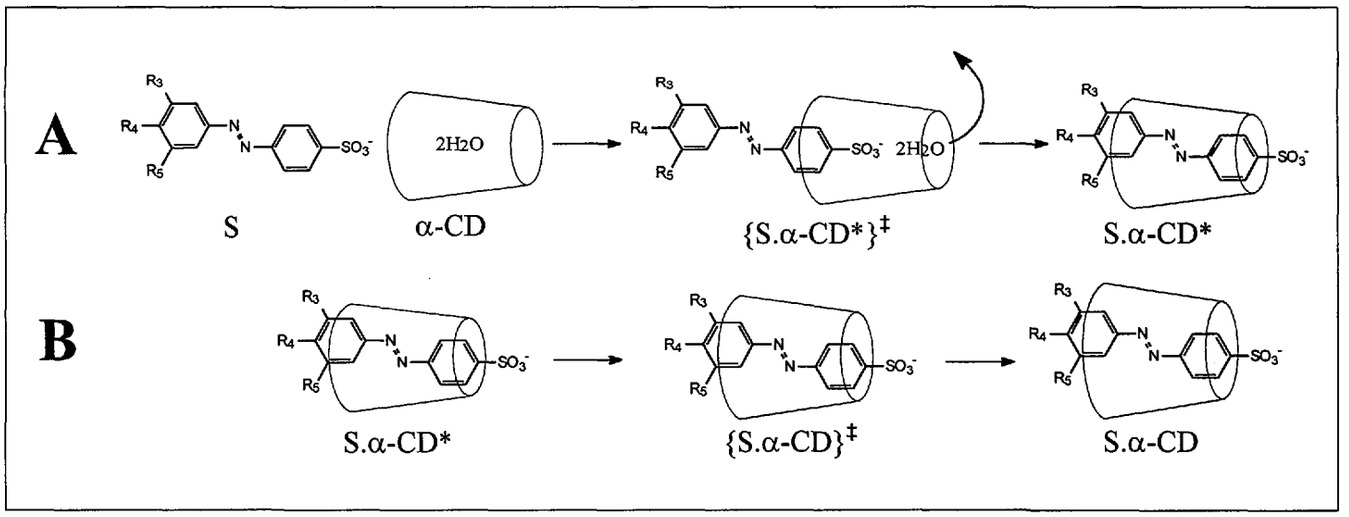


Fig. 2. *Volume reaction profiles for the inclusion formation and dissociation reactions between 1*(A) *and 2*(B) *with α-CD, in aqueous solution*

Scheme. *First* (A) *and Second* (B) *Steps of the Postulated Mechanism for the Inclusion Reaction between α-CD and the Dyes*



The second step is assumed to be a subsequent intramolecular rearrangement of the intermediate complex. This slow process is anticipated owing to the intramolecular motion in the small α-CD unit. The very negative $\Delta V_{2,f}^{\ddagger}$ value for **2** may be explained by the formation of H-bonds between the OH and carboxylate groups of the dye and α-CD. Such bonding is not possible with **1**, resulting in a very small $\Delta V_{2,f}^{\ddagger}$. The postulated stronger interaction for **2** is also represented by a higher value of K_2 compared to **1**. It has been suggested that an 'empty' α-CD in H_2O exists in a distorted conformation, which arises as a result of the rotation of one or more D-glucose rings around glycoside linkages [5]. For better overlap between α-CD and the dye, this distorted conformation is relieved to have a relaxed conformation of the α-CD in the final product.

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