KINETIC AND EQUILIBRIUM STUDIES OF CYCLOMALTO-OCTAOSE (γ -CYCLODEXTRIN)-METHYL ORANGE INCLUSION COMPLEXES

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ABSTRACT

Measurements of the equilibrium and temperature-jump u.v., visible, and induced c.d. spectra of Methyl Orange (MO) in the presence of cyclomalto-octaose (γ -cyclodextrin, γ -CD) have been carried out. Three mechanistic steps were detected through the temperature-jump data (25.0°):

 $MO + \gamma$ - $CD \rightleftharpoons MO \cdot \gamma$ -CD fast (K_1) ,

MO + MO
$$\cdot \gamma$$
-CD $\stackrel{k_2}{\underset{k_{-2}}{\rightleftharpoons}}$ (MO)₂ $\cdot \gamma$ -CD slow (K₂), and

$$\gamma$$
-CD + (MO)₂ · γ -CD \rightleftharpoons (MO)₂ · (γ -CD)₂ fast (K₃),

where K_1 , K_2 , and K_3 are 45 (±7), 2.0 (±1.1) × 10⁶, and 6.1 (±2.5) × 10³ dm³.mol⁻¹, respectively, $k_2 = 9.4$ (±5.1) × 10⁹ dm³.mol⁻¹.s⁻¹, and $k_{-2} = 4.8$ (±0.8) ×10³ s⁻¹. The equilibrium u.v./visible data are also consistent with this reaction scheme. The high stability of the dimer inclusion complex (MO)₂ · γ -CD compared to that of the monomer inclusion complex MO · γ -CD appears to be related to the annular diameter of γ -CD and demonstrates a degree of selectivity in cyclodextrin inclusion complexes. The (MO)₂ · (γ -CD)₂ complex also contains a dimer, included by both γ -CD molecules.

INTRODUCTION

Recently, interest in inclusion complexes has grown because of their relevance to enzyme-substrate and drug-receptor interactions and their potential importance in synthesis^{1,2}. Amongst the simpler inclusion complexes are those formed from cycloamyloses (cyclodextrins), which are cyclic $(1\rightarrow 4)$ -linked α -D-

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glucosaccharides containing six³ (α -CD), seven⁴ (β -CD), and eight⁵ (γ -CD) D-glucosyl residues. The space within the annulus increases as the number of D-gluco-pyranosyl residues increases. This variation in annulus size should produce selectivity towards the included species, and some evidence has been presented^{6,7}.

The cyclodextrins form inclusion complexes with aromatic molecules, particularly those containing the azobenzene moiety, and the inclusion of Methyl Orange (MO) has been the subject of several spectroscopic studies^{3,5,8}. We now report on the MO/ γ -CD system.

EXPERIMENTAL

 γ -Cyclodextrin (cyclomalto-octaose) (Sigma), Methyl Orange (B.D.H. LR grade), Na₂HPO₄, and NaOH (B.D.H. Analar) were used without further purification.

Temperature-jump experiments were carried out using equipment of design similar to that described elsewhere⁹. Traces were obtained by photographing the screen of a Tektronix 549 storage oscilloscope and were hand-digitised. A minimum of three traces was recorded for each concentration at 465 and 430 nm.

Visible spectra were recorded with a Zeiss DMR10 double-beam spectrophotometer having a thermostatted cell-holder. Temperature control was $\pm 0.1^{\circ}$. Absorbances were measured at intervals of 2 nm; each scan was repeated and only duplicates which agreed within 0.003 absorbance unit at each wavelength measured were accepted. The data were stored on punched tape and were analysed by using a Cyber 173 computer.

C.d. spectra were recorded with a JASCO J40-CS spectropolarimeter equipped with a microprocessor for averaging repeated measurements at each wavelength.

Transition moment directions for MO were determined by using stretched poly(vinyl alcohol) films¹⁰.

RESULTS AND DISCUSSION

Equilibrium and kinetic aspects. — The visible spectrum of MO ($\sim 4 \times 10^{-5}$ M) alone and in the presence of γ -CD concentrations ranging from 2×10^{-5} to 3×10^{-2} M in 0.10M Na₂HPO₄ adjusted to pH 9.00 at 25.0° is shown in Figs. 1 and 2; there are no fixed isosbestic points, which implies the existence of more than two environments for MO. A similar spectral variation was observed for MO in the presence of β -CD, but the change in the molar absorbance was much less and, in general terms, was similar to that observed for the α -CD system by Cramer *et al.*³, who concluded that more than two environments existed for MO in the presence of α -CD.

Temperature-jump studies of solutions of MO and γ -CD (in aqueous 0.10M Na₂HPO₄ adjusted to pH 9) monitored at 465 nm revealed two relaxation proces-



Fig. 1. Molar absorbance of MO (4×10^{-5} M) as a function of wavelength at pH 9 alone (dashed line) and in the presence of increasing concentrations of γ -CD ranging from 2.0×10^{-5} to 3.8×10^{-4} M (full lines).



Fig. 2. Molar absorbance of MO $(4 \times 10^{-5} \text{M})$ as a function of wavelength at pH 9 alone (dashed line) and in the presence of increasing concentrations of γ -CD ranging from 5.0×10^{-4} to $3.0 \times 10^{-2} \text{M}$ (full lines).

ses. The faster occurred within the heating time of the apparatus ($\sim 5 \ \mu s$) and was characterised by a decrease in absorbance, whereas the slower, single exponential relaxation was characterised by an increase in absorbance. The reciprocal relaxation-times ($1/\tau$) of the slower process are given in Table I and appear in Fig. 3 plotted against total γ -CD concentration. A similar variation of relaxation time for the slower process was observed at 430 nm, but the faster relaxation was not observed at this wavelength. It appears that the faster relaxation observed at 465 nm arises solely from MO and does not characterise an inclusion reaction, and accordingly is

[MO] (M × 10 ⁵)	[γ-CD] (М)	$\frac{1}{\tau}$	
		(S × 10 -)	
4.14 (±0.03)	$2.036 (\pm 0.028) \times 10^{-5}$	6.18 (±0.27)	
$4.10(\pm 0.03)$	$6.077 (\pm 0.073) \times 10^{-5}$	6.87 (±0.34)	
$4.09(\pm 0.03)$	$1.211 (\pm 0.018) \times 10^{-4}$	$7.16(\pm 0.22)$	
$4.07(\pm 0.03)$	$1.811 (\pm 0.024) \times 10^{-4}$	7.46 (±0.26)	
$4.06(\pm 0.03)$	$2.553 (\pm 0.028) \times 10^{-4}$	8.81 (±0.28)	
$4.04(\pm 0.03)$	$3.597(\pm 0.040) \times 10^{-4}$	9.24 (±0.34)	
$3.55(\pm 0.02)$	$8.491(\pm 0.062) \times 10^{-4}$	8.95 (±0.33)	
$3.54(\pm 0.02)$	$1.952 (\pm 0.014) \times 10^{-3}$	8.80 (±0.41)	
$3.54(\pm 0.02)$	$3.938(\pm 0.027) \times 10^{-3}$	$8.07(\pm 0.45)$	
$3.51(\pm 0.02)$	$6.663 (\pm 0.029) \times 10^{-3}$	7.35 (±0.22)	
$3.52(\pm 0.02)$	$8.598(\pm 0.037) \times 10^{-3}$	$6.51(\pm 0.26)$	
$3.52(\pm 0.02)$	$1.240(\pm 0.005) \times 10^{-2}$	$6.07(\pm 0.27)$	
$3.50(\pm 0.02)$	$1.762 (\pm 0.007) \times 10^{-2}$	$5.34(\pm 0.21)$	
$3.51(\pm 0.02)$	$2.243 (\pm 0.009) \times 10^{-2}$	$5.00(\pm 0.24)$	
3.50 (±0.02)	$2.820 (\pm 0.010) \times 10^{-2}$	4.79 (±0.28)	

CONCENTRATIONS AND OBSERVED RECIPROCAL RELAXATION-TIMES FOR THE INCLUSION OF MO BY γ -CD at 25.0°^a

^aThe errors in the concentrations were estimated from the known uncertainties in weighing. The errors in $1/\tau$ were estimated from the linear regressions of ln (volts) *versus* time data derived from oscilloscope photographs.

not further considered^{*}. The slower relaxations observed at 465 and 430 nm are consistent with temperature variation of the equilibrium spectra of MO/γ -CD solutions over the range 15.0–35.0°.

The dependence of $1/\tau$ on concentration (Fig. 3) indicates that the observed relaxation is coupled to other faster processes occurring within the heating time of the apparatus and which are characterised by small absorbance changes at the monitored wavelengths. A variety of reaction schemes was considered^{**} in explanation

- (a) $2 \text{ MO} \rightleftharpoons (\text{MO})_2$ fast equilibrium
- $(MO)_2 + \gamma \cdot CD \rightleftharpoons (MO)_2 \cdot \gamma \cdot CD \qquad \text{slow inclusion}$ (b) MO + $\gamma \cdot CD \rightleftharpoons MO \cdot \gamma \cdot CD \qquad \text{fast equilibrium}$ MO + $\gamma \cdot CD \rightleftharpoons MO \cdot \gamma \cdot CD^* \qquad \text{slow isomerisation}$ MO + MO $\cdot \gamma \cdot CD^* \rightleftharpoons (MO)_2 \cdot \gamma \cdot CD^* \qquad \text{fast equilibrium}$ (c) MO + $\gamma \cdot CD \rightleftharpoons MO \cdot \gamma \cdot CD \qquad \text{fast equilibrium}$ MO + MO $\cdot \gamma \cdot CD \rightleftharpoons (MO)_2 \cdot \gamma \cdot CD \qquad \text{slow inclusion.}$

^{*}Temperature-jump studies of MO under identical conditions, but in the absence of γ -CD, were carried out at 465 and 430 nm. At 465 nm, a relaxation occurring within the heating time of the apparatus and characterised by a decrease in molar absorbance, similar to that observed for the faster relaxation in the presence of γ -CD, was observed. No relaxation was observed at 430 nm. Under the same experimental conditions, the equilibrium spectra of MO at 15.0, 25.0, and 35.0° exhibited a temperature variation with an isosbestic region encompassing 430 nm, but not 465 nm, consistent with literature data¹¹. Thus, the faster relaxation at 465 nm in the MO/ γ -CD solutions is considered to arise from MO alone.

^{**}Other reaction schemes considered, but rejected because they were unable to fit the data satisfactorily, were:



Fig. 3. Reciprocal relaxation-times for MO/ γ -CD mixtures as a function of γ -CD concentration at pH 9. Left-hand graph, [MO] $\sim 4.9 \times 10^{-5}$ M; right-hand graph, [MO] $\sim 3.5 \times 10^{-5}$ M. Errors in the reciprocal relaxation-times were estimated from linear regressions of ln (volts) *versus* time obtained from oscilloscope photographs. The full vertical lines represent a non-linear, least-squares regression fit to the data.

of the kinetic (Fig. 3) and equilibrium spectrophotometric results. The scheme below (Eq. 1) gave the best fit to the $1/\tau$ data and yielded equilibrium constants independently calculated from the equilibrium spectrophotometric data which were consistent with those calculated from the $1/\tau$ data. In Eq. 1, MO · γ -CD is an inclusion complex of MO within γ -CD, (MO)₂ · γ -CD is an inclusion

$$MO + \gamma - CD \stackrel{K_1}{\rightleftharpoons} MO \cdot \gamma - CD \quad \text{fast}$$
(1)
$$MO + MO \cdot \gamma - CD \stackrel{K_2}{\rightleftharpoons} (MO)_2 \cdot \gamma - CD \quad \text{slow}$$

$$(MO)_2 \cdot \gamma - CD + \gamma - CD \rightleftharpoons (MO)_2 \cdot (\gamma - CD)_2$$
 fast

complex comprising a MO dimer within γ -CD, and (MO)₂ · (γ -CD)₂ is an inclusion complex of the MO dimer (which is considered to be in a parallel-plane head-to-tail arrangement¹²) axially within two γ -CD molecules. Provided that the first and last steps are sufficiently fast to be considered in equilibrium throughout the relaxation of the second step, Eq. *1* predicts a single exponential relaxation characterised by τ . Using the substitution method of Czerlinski¹³, Eq. 2 may be derived for the variation of τ with concentration.

TABLE II rate and equilibrium constants for the inclusion of MO by γ -CD at 25.0° from relaxation spectra^a

 $k_2 = 9.4 (\pm 5.1) \times 10^9 \text{ dm}^3.\text{mol}^{-1}.\text{s}^{-1}$ $k_{-2} = 4.8 (\pm 0.8) \times 10^3 \text{s}^{-1}$ $K_1 = 45 (\pm 7) \text{ dm}^3.\text{mol}^{-1}$ $K_2 = 2.0 (\pm 1.1) \times 10^6 \text{ dm}^3.\text{mol}^{-1}$ $K_3 = 6.1 (\pm 2.5) \times 10^3 \text{ dm}^3.\text{mol}^{-1}$

^aThe errors in the rate and equilibrium constants were derived by the non-linear, least-squares fitting procedure and represent one standard deviation.

$$1/\tau = k_{2} \left\{ \frac{[MO]([MO] + [MO \cdot \gamma - CD] + 4[\gamma - CD])}{[MO] + [\gamma - CD] + 1/K_{1}} \right\}$$
$$+ k_{-2} \left\{ \frac{[(MO)_{2} \cdot \gamma - CD] + 1/K_{3}}{[\gamma - CD] + [(MO)_{2} \cdot \gamma - CD] + 1/K_{3}} \right\}$$
(2)

(where all concentrations are equilibrium values).

The $1/\tau$ data were fitted to Eq. 2 by using DATAFIT¹⁴, a non-linear, leastsquares fitting programme which incorporates a Newton-Raphson method subroutine for the calculation of equilibrium concentrations of all species shown in Eq. 1. The best-fit line is shown in Fig. 3, and the derived parameters are given in Table II.

A similar strategy was used to determine the equilibrium constants and molar absorbances (ϵ) which relate the spectra in Figs. 1 and 2 to Eq. 1. At any wavelength, the observed absorbance (A) was assumed to be given by Eq. 3.

$$A = \varepsilon_{MO}[MO] + \varepsilon_{MO + \gamma-CD}[MO \cdot \gamma-CD] + 2\varepsilon_{(MO)_2 + \gamma-CD}[(MO)_2 \cdot \gamma-CD] + 2\varepsilon_{(MO)_2 + (\gamma-CD)_2}[(MO)_2 \cdot (\gamma-CD)_2]$$
(3)

(where all concentrations are equilibrium values).

The equilibrium spectra of Figs. 1 and 2 were fitted to Eq. 3 by using programme DATAFIT at all monitored wavelengths, except those in the regions of the pseudo-isosbestic points where the small changes in absorbance precluded DATAFIT from converging to a best-fit value. It was found that the best fit to the equilibrium spectra was obtained if $[MO \cdot \gamma$ -CD] was taken to be negligible; in consequence, Eq. 1 simplifies to Eq. 4, in which $K_{12} = K_1 k_2 / k_{-2}$ of Eq. 1.

$$2 \operatorname{MO} + \gamma \operatorname{CD} \stackrel{\kappa_{12}}{\rightleftharpoons} (\operatorname{MO})_2 \cdot \gamma \operatorname{CD}$$

$$(4)$$

$$(\operatorname{MO})_2 \cdot \gamma \operatorname{CD} + \gamma \operatorname{CD} \stackrel{\kappa_3}{\rightleftharpoons} (\operatorname{MO})_2 \cdot (\gamma \operatorname{CD})_2$$



Fig. 4. Calculated spectra of $(MO)_2 \cdot (\gamma - CD)_2$ (A) and $(MO)_2 \cdot \gamma - CD$ (B) compared with the spectrum of MO alone (C) derived from spectra measured at pH 9 (see text).

TABLE III

EQUILIBRIUM CONSTANTS, ENTHALPIES, AND ENTROPIES FOR THE INCLUSION OF MO in γ -CD from Equilibrium spectra^{*a,b*}

Temperature (degrees)	$\frac{K_{12}}{(dm^6 \cdot mol^{-2})}$	$\frac{K_3}{(dm^3 \cdot mol^{-1})}$	
15.0 25.0 35.0	$5.5 (\pm 0.8) \times 10^{7}$ $3.8 (\pm 0.2) \times 10^{7}$ $1.2 (\pm 0.4) \times 10^{7}$ $AH_{12}^{0} = -56 (\pm 6) \text{ kJ.mol}^{-1}$ $AS_{12}^{0} = -47 (\pm 61) \text{ J.K}^{-1} \text{ .mol}^{-1}$ $AH_{3}^{0} = -32 (\pm 4) \text{ kJ.mol}^{-1}$ $AS_{3}^{0} = -58 (\pm 42) \text{ J.K}^{-1} \text{ .mol}^{-1}$	5.5 $(\pm 1.7) \times 10^2$ 4.7 $(\pm 0.9) \times 10^2$ 2.3 $(\pm 0.1) \times 10^2$	

^aThe errors in the equilibrium constants and thermodynamic quantities were derived by the non-linear, least-squares fitting procedure and represent one standard deviation.^bData averaged over 540 to 460 nm, and 440 to 412 nm.

The K_{12} and K_3 values calculated at 2-nm intervals in the ranges 412–440 nm and 460–540 nm were weighted according to their estimated uncertainties, and averaged to give the values shown in Table III together with the corresponding ΔH^0 and ΔS^0 values. These K_{12} and K_3 values, together with the directly determined molar absorbances of MO^{*}, were then used to derive the spectra of (MO)₂ · γ -CD and (MO)₂ · (γ -CD)₂ shown in Fig. 4, using DATAFIT.

The agreement between K_{12} (25.0°) = 3.8 ±0.2 × 10⁷ dm⁶.mol⁻² (obtained

^{*}The reported spectra of MO exhibit substantial variations despite being determined under similar conditions. Our MO spectra (Figs. 1-3) show molar absorbances calculated on the basis that our sample of MO contained 4 H_2O per mol of dyestuff.

from the equilibrium spectra) and $K_1k_2/k_{-2} = K_{12}$ (25.0°) = 9.0 ±5.1 × 10⁷ dm⁶.mol⁻² (obtained from the τ data) is reasonable. The K_3 (25.0°) values 4.7 ±0.9 × 10² dm³.mol⁻¹ and 6.1 ±2.5 × 10³ dm³.mol⁻¹ derived from the equilibrium spectra and τ data, respectively, agree less well, but, as the proportion of $(MO)_2 \cdot (\gamma$ -CD)₂ is very small except at the higher concentrations of γ -CD, this is not unexpected.

Spectroscopic aspects. — The perturbation of the visible spectrum of MO by γ -CD, which is greater than that produced by either α -CD or β -CD, is largely due to the formation of $(MO)_2 \cdot \gamma$ -CD. Molecular models suggest that the included (MO)₂ dimer consists of two MO molecules (in parallel planar arrangement) parallel with the annular axis of γ -CD (it is probable that a head-to-tail arrangement is adopted in the dimer, to minimise repulsion between the negatively charged sulphonate groups). Exciton theory¹⁵ predicts a blue shift for dimers of such "relatively weakly" absorbing dyes as MO, and, in Fig. 4, the spectra attributed to $(MO)_2 \cdot \gamma$ -CD and $(MO)_2 \cdot (\gamma$ -CD)₂ exhibit a blue shift in comparison with that of MO. The spectrum of $(MO)_2$ has not been determined, but the spectrum of a 1.66 $\times 10^{-2}$ M solution exhibits a blue shift compared to that of a 4.16×10^{-5} M solution, and the more concentrated solution has a lower molar absorbance at the maximum¹⁶. It may be inferred from these data that dimerisation of MO produces a blue shift and a decrease in molar absorbance. The $(MO)_2 \cdot \gamma$ -CD and $(MO)_2 \cdot (\gamma - CD)_2$ spectra in Fig. 4 do not exhibit a decrease in molar absorbance, which suggests an environmental effect on the $(MO)_2$ spectrum. The observation¹¹ that the spectra of ethanolic solutions of MO are blue-shifted compared to those of aqueous solutions and that the former also exhibit increased molar absorbances suggests that the environment of $(MO)_2$ included in γ -CD is more akin to that in ethanol than in water. Solid-state X-ray studies¹⁷ show γ -CD to approximate to a



Fig. 5. Induced c.d. shown by MO (4 \times 10⁻⁵M) in the presence of γ -CD (0.01M) as a function of wavelength.

truncated cone in which the D-glucosyl residues form the wall of the cone, and HO-6 and HO-2,3 are situated at the narrow and broad ends of the cone, respectively. Thus, in solution, it is anticipated that one end of the MO dimer in $(MO)_2 \cdot \gamma$ -CD will experience an aqueous environment, whereas, in $(MO)_2 \cdot (\gamma$ -CD)₂, both ends will be included in γ -CD, and $(MO)_2$ will experience little if any interaction with water. On the basis of this discussion, it is anticipated that $(MO)_2 \cdot (\gamma$ -CD)₂ will exhibit a greater molar absorbance at its absorption maximum than will $(MO)_2 \cdot \gamma$ -CD.

The induced c.d. spectrum of MO in a two hundred-fold excess of γ -CD (Fig. 5) differs markedly from that of MO in a similar excess of β -CD, as measured in this study and reported elsewhere^{18,19}. This finding suggests that the environments of the MO chromophore in γ -CD and β -CD are very different. In the latter environment, MO exhibits a single, symmetrical, broad, positive peak (350–510 nm) with a maximum at 455 nm. This spectrum is characteristic of the occurrence of a π - π^* transition in the MO monomer, whereas that arising from the MO/ γ -CD system (Fig. 5) is characteristic of dimeric MO in which the π - π^* transition moments are parallel²⁰. Dimeric MO had previously been observed in association with poly-L-lysine, in which system both a blue shift in the absorbance spectrum and two peaks of the opposite sign in the induced c.d. spectrum occurred²¹.

Our linear dichroism studies of MO in stretched films and SCFMO calculations on the MO π -system show a single high-intensity transition polarised approximately on the line interconnecting the MO phenyl groups consistent with the results of Popov and Smirnov²² (the work of these authors also supports our assignment of the MO spectrum to a π - π * transition). These results support the deduction made from the kinetic and equilibrium spectroscopy that (MO)₂ is the preponderant MO species included by γ -CD. It has been reported that γ -CD can include simultaneously pairs of other aromatic systems, as exemplified by pyrenes²³, sodium naphthylacetates²⁴, and sodium napthylacetate and picric acid²⁵.

Mechanistic aspects. — Azo dyes tend to form dimers and higher aggregates in solution, but this tendency is very weak for MO, to the extent that dimers are not normally detectable¹⁶ at MO concentrations <0.1mM. Thus, the stabilisation of (MO)₂ through inclusion by γ -CD and the greater stability of (MO)₂ · γ -CD in comparison with MO · γ -CD demonstrate the selectivity of the "host" γ -CD towards the "guests" MO and (MO)₂. This selectivity may be interpreted qualitatively in terms of the relative tightness of fit of MO and (MO)₂ into the γ -CD annulus. That α -CD and β -CD do not appear to include (MO)₂ suggests that the dimer is too large to be contained in the smaller annuli of these cyclodextrins without introducing a great distortion of α -CD and β -CD. The concept that higher stability constants characterise host-guest interactions in which the guest closely fits the host is widely applicable, particularly in the cryptates²⁶, and appears to have a general validity in inclusion complexes.

Solid-state X-ray structural studies¹⁷ show the annulus of γ -CD to be occupied by disordered water molecules and it is assumed that a similar situation pre-

vails in aqueous solution. Thus, the rapid formation of MO $\cdot \gamma$ -CD will result in expulsion of some of this water, but it appears that the included and loosely fitting MO will share the annulus with water. It is reasonable to assume that the rate constant for formation of MO $\cdot \gamma$ -CD, $k_1 > 10^{10} \text{ dm}^3 \text{.mol}^{-1} \text{.s}^{-1} [k_2 \text{ for entry of the sec-}$ ond MO to form $(MO)_2 \cdot \gamma$ -CD = 9.4 × 10⁹ dm³.mol⁻¹.s⁻¹], from which it may be calculated that $k_{-1} \ge 2 \times 10^8 \,\mathrm{s}^{-1}$ on the basis of $K_1 = 45 \,\mathrm{dm}^3 .\mathrm{mol}^{-1}$ (Table II). The high lability and low stability of MO $\cdot \gamma$ -CD is in marked contrast to the low lability and high stability of $(MO)_2 \cdot \gamma$ -CD. The large k_2 value which characterises the expulsion of most if not all of the water from MO $\cdot \gamma$ -CD and subsequent formation of a tightly fitting (MO)₂ dimer in (MO)₂ $\cdot \gamma$ -CD is not unexpected, as the MO $\cdot \gamma$ -CD with which the incoming MO reacts appears to be a rather loosely assembled inclusion-complex. In contrast, $(MO)_2 \cdot \gamma$ -CD appears to be an ordered and tightly assembled species, such that the dissociation of MO from $(MO)_2 \cdot \gamma$ -CD (characterised by k_{-2}) must overcome a greater energy barrier than its dissociation from MO $\cdot \gamma$ -CD, owing to the disruption of the secondary forces existing between the $(MO)_2$ components and also those existing between $(MO)_2$ and γ -CD. The interactions involved in the equilibrium characterising $(MO)_2 \cdot (\gamma - CD)_2$ formation are principally those of the partially included (MO)₂ dimer with a second γ -CD. The reaction scheme in Eq. 1 indicates that $(MO)_2 \cdot (\gamma - CD)_2$ is more labile than $(MO)_2 \cdot \gamma$ -CD, which requires $k_{-3} > k_{-2}$. This suggests that the barrier to removal of doubly included (MO)₂ from the annulus of one γ -CD is greater than that for dissociating a MO from $(MO)_2 \cdot \gamma$ -CD, but, in the absence of ΔH^{\ddagger} and ΔS^{\ddagger} data, the enthalpic and entropic origins of this difference cannot be debated. The ground-state enthalpy and entropy changes appear in Table III and, although these data are highly derived, it is apparent that the inclusion of MO by γ -CD is exothermic and produces a decrease in entropy. All three formation reactions involve the expulsion of disordered water from the γ -CD annulus and the incorporation of this water into the bulk water structure. These reactions also involve MO in forming secondary bonds with either γ -CD or MO, or both, and an increase in ordering. Although the enthalpy and entropy changes for the association of MO to form dimers in aqueous solution are not readily available, both are negative¹² in the formation of dimers by the azo dye" Orange II". Thus, on a qualitative basis, decreases in enthalpy and entropy are expected to accompany the formation reactions described here.

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