Host–guest complexes of cucurbituril with 4-amino-4′-nitroazobenzene and 4,4′-diaminoazobenzene in acidic aqueous solutions

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The thermodynamics and kinetics of the reaction of cucurbituril (Cuc) with 4-amino-4′-nitroazobenzene (DO3) and 4,4′-diaminoazobenzene (Diam) have been studied in aqueous HCl, and it is shown that DO3 is well suited as an indicator for uncomplexed Cuc (actual concentration of Cuc) and Diam is well suited as an indicator for the total concentration of Cuc (sequestering agent) in aqueous solutions. The formation of 1 : 1 guest–host complexes is observed. For proton concentrations up to 1 mol dm^{-3}, DO3 reacts according to eqn. (*a*), with the values for the dissociation constant of DO3H$^+$ log(K_a/dm^3 mol^{-1}) =

$$\mathrm{CucH^+ + DO3H_2^{2+} \rightleftharpoons CucH^+ + DO3H^+ + H^+ \rightleftharpoons CucH^+ + DO3 + 2H^+ \rightleftharpoons CucDO3H^+ + 2H^+} \quad (a)$$

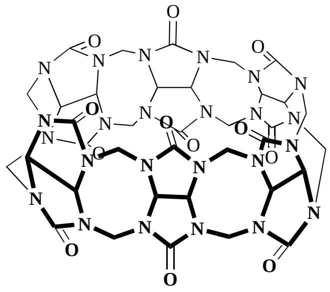
−2.42, DO3H$_2^{2+}$ log(K_b/dm^3 mol^{-1}) = 0.53, for the stability constant of CucDO3H$^+$ log (K_c/mol dm^3) = 8.05 and for the rate constant of complex formation log (k_f/dm^3 mol^{-1} s^{-1}) = 5.25. Reaction enthalpies and activation energies have also been determined. The complexation of Cuc with Diam has been studied in 0.1–1 mol dm^{-3} HCl where a very strong complexation has been observed.

Host–guest complexes of cucurbituril **1** with substituted amines and alkali metal ions have been studied by several authors using 48% aqueous formic acid as solvent,[1,2] and most of the results have been reviewed recently.[1,3] But it is known that cucurbituril is also sparingly soluble in dilute aqueous solutions of HCl, and a study of its complexation at different proton concentrations should give further insight into the mechanism of complex formation, especially concerning the degree of protonation of reactants and complex. Thus we report in this contribution results on the thermodynamics and kinetics of the binding of some ligands to cucurbituril in aqueous solutions of 0.05–5 mol dm^{-3} hydrochloric acid. Complexation is monitored by optical absorbance, and therefore the guest molecules should absorb light in the visible region where cucurbituril does not absorb light, and complexation should be accompanied by a large change in absorbance. Furthermore, ligands have been chosen which are suitable as indicators for the investigation of the binding of optically non-absorbing guests.

The following guests have been chosen: (*i*) 4-amino-4′-nitroazobenzene **2** forming relatively strong complexes with cucurbituril, so it may be used as an indicator for competing complex formation with non-absorbing guests, *e.g.* alkali metal ions, (*ii*) 4,4′-diaminoazobenzene **3** forming very strong complexes with cucurbituril, so it may be used as an indicator for the titration of total concentration of cucurbituril, (*iii*) 4-methylbenzylamine **4**, in order to be able to compare our results with those obtained previously in 48% formic acid.[1,2] The two carbonyl-fringed portals at the upper and lower side of the cucurbituril molecule have a diameter of 4 Å. The internal cavity has a diameter of *ca.* 5.5 Å, and the separation of the portals is 6 Å.[1]

Experimental

4-Methylbenzylamine **4** was obtained from Aldrich (97%) and used without further purification. 4-Amino-4′-nitroazobenzene **2** was obtained from Aldrich. Chromatography showed that the sample consisted of three major substances, which could be separated into *ca.* 90% 4-amino-4′-nitroazobenzene, 10% 4,4′-diaminoazobenzene and <1% insoluble white substance.



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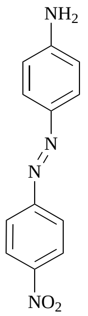

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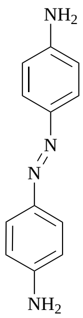

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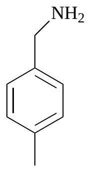

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Cucurbituril **1** was obtained from Merck. When it was suspended in water, an acidic suspension was obtained. Therefore cucurbituril was washed by suspending it several times in twice distilled water, until the pH of the suspension remained higher than 5.6. All other chemicals were commercially available with grade p.a., and thrice distilled water was used for preparing the solutions.

At equilibrium absorbances were determined spectrophotometrically with an accuracy of $\delta A = \pm 0.001$ for $A < 1.5$. Kinetic measurements were started by mixing equal volumes of solutions of cucurbituril and of guest, both containing the same concentration of HCl. The experiments were performed under pseudo-first-order conditions, *i.e.* (*i*) the concentration of HCl was large compared to those of cucurbituril and guest and (*ii*)

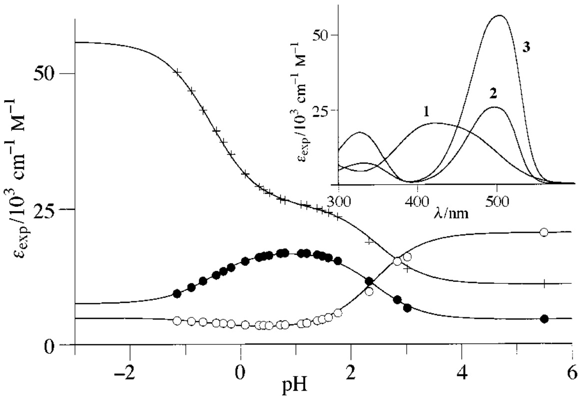

Fig. 1 ε_{exp} *vs.* pH for solutions of DO3 at different wavelengths, $T = 25$ °C: (●) $\lambda = 328$ nm; (+) $\lambda = 422$ nm; (○) $\lambda = 498$ nm. The curves are calculated with the dissociation constants given in Table 1. Insert: spectra of 1 DO3, 2 $DO3H^+$ and 3 $DO3H_2^{2+}$.

Table 1 Dissociation constants, absorption coefficients ($\lambda = 526$ nm), rate constants, and thermodynamic constants relating to species in eqns. (19)–(21), at $T = 25$ °C

$\varepsilon_{DO3}/M^{-1}\ cm^{-1}$	5 100 ± 200
$\varepsilon_{DO3H^+}/M^{-1}\ cm^{-1}$	14 200 ± 200
$\varepsilon_{DO3H2^{2+}}/M^{-1}\ cm^{-1}$	39 750 ± 200
$\varepsilon_{CucDO3H^+}/M^{-1}\ cm^{-1}$	54 500 ± 1000
$pK_{DO3H,1}$	2.42 ± 0.02
$pK_{DO3H,2}$	−0.53 ± 0.05
pK_{CucDO3}	8.05 ± 0.05
$pK_{CucH,2}$	−0.25 ± 0.08
$\Delta_r H°_{DO3H,1}/kJ\ mol^{-1}$	20 ± 2
$\Delta_r H°_{DO3H,2}/kJ\ mol^{-1}$	12 ± 2
$\Delta_r H°_{CucDO3}/kJ\ mol^{-1}$	79 ± 4
$\Delta_r S°_{DO3H,1}/J\ mol^{-1}\ K^{-1}$	21 ± 4
$\Delta_r S°_{DO2H,2}/J\ mol^{-1}\ K^{-1}$	51 ± 5
$\Delta_r S°_{CucDO3}/J\ mol^{-1}\ K^{-1}$	112 ± 10
$\log(k_f/s^{-1}\ M^{-1})$	5.25 ± 0.05
$\log(k_b/s^{-1})$	−2.8 ± 0.1
$E_{a,f}/kJ\ mol^{-1}$	18 ± 2
$E_{a,b}/kJ\ mol^{-1}$	97 ± 4

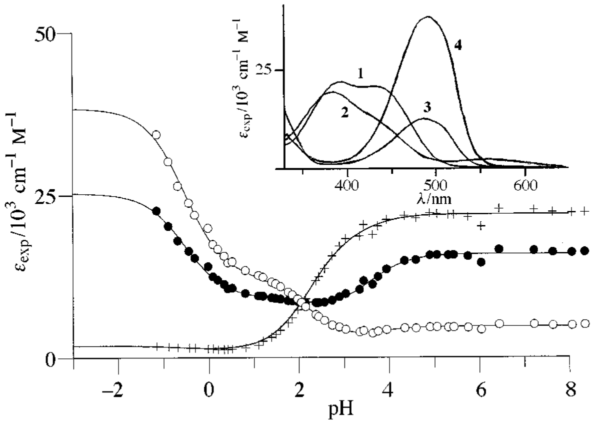

Fig. 2 ε_{exp} *vs.* pH for solutions of Diam at different wavelengths, $T = 25$ °C: (●) $\lambda = 393$ nm; (○) $\lambda = 460$ nm; (+) $\lambda = 490$ nm. The curves are calculated with the dissociation constants given in the text. Insert: spectra of 1 Diam, 2 $DiamH^+$, 3 $DiamH_2^{2+}$ and 4 $DiamH_3^{3+}$.

the concentration of cucurbituril was large compared to that of the guest molecule or *vice versa*. In all experiments an exponentially decaying curve was observed, *i.e.* the time dependence of the absorbance is described by eqn. (1), where A_0 and A_e are

$$A = (A_0 - A_e)\exp\left(\frac{-t}{\tau}\right) + A_e \qquad (1)$$

the absorbances immediately after mixing the solutions and after the reaction, when equilibrium is reached, respectively. $(A_0 - A_e)$ and τ are referred to as relaxation amplitude and relaxation time, respectively. pH values were measured with an accuracy of ±0.02 units.

Results

Protonation equilibria

The host–guest complexes were studied at different proton concentrations and therefore the protonation equilibria of the guests have to be known. For the guests 4-amino-4′-nitroazobenzene **2** (common name: Disperse Orange 3, and therefore abbreviated as DO3) and 4,4′-diaminoazobenzene **3** (abbreviated as Diam) the visible spectra were measured at varying proton concentrations. For selected wavelengths the results are shown in Figs. 1 and 2.

Because of the low solubility of unprotonated DO3 (1.8×10^{-6} M) the measurements were performed at different concentrations and different optical pathlengths (1–5 cm). M is used as an abbreviation for mol dm^{-3}. Correspondingly, in Figs. 1 and 2, ε_{exp}, the absorption per pathlength and per concentration [see eqn. (2)], is plotted. A, d and D_o are absorbance,

$$\varepsilon_{exp} = \frac{A}{d\,D_o} \qquad (2)$$

optical pathlength and total concentration of ligand, respectively. pH values were calculated from the concentration [H$^+$] and mean activity coefficient f_{HCl} of hydrochloric acid according to eqn. (3). f_{HCl} was calculated from literature data.[4–6]

$$pH = -\log([H^+]f_{HCl}/M) \qquad (3)$$

DO3 may be protonated both at the amino and at the azo group, and the equilibrium spectra are well described by the two dissociation constants given in eqns. (2) and (3), with $pK_{DO3H,1} = 2.42 \pm 0.03$ and $pK_{DO3H,2} = -0.53 \pm 0.05$.

$$K_{DO3H,1} = \frac{[DO3][H^+]}{[DO3H^+]} \qquad (4)$$

$$K_{DO3H,2} = \frac{[DO3H^+][H^+]f_{HCl}}{[DO3H_2^{2+}]} \qquad (5)$$

The first dissociation constant was determined at a relatively low ionic strength (pH < 1), where activity coefficients depend predominantly on the charge of the ions, and thus they cancel in eqn. (4). The second dissociation constant had to be determined at high ionic strength, where activity coefficients are difficult to determine or to estimate. Therefore $K_{DO3H,2}$ as described by eqn. (5) (*i.e.* assuming that activity coefficients for $DO3H^+$ and $DO3H_2^{2+}$ cancel) is a conditional and not a thermodynamic constant. This conditional constant is used for determining the protonation of DO3, since it describes well the pH-dependence of the absorption at high proton concentration, as can be seen in Fig. 1. The dissociation constants of DO3 were determined at different temperatures. It should be emphasised that for all three differently protonated species the absorption coefficients do not depend on temperature. The results (equilibrium constants, reaction enthalpies, reaction entropies and absorption coefficients at $\lambda = 526$ nm) are summarised in Table 1.

Analogously the spectra for Diam have been evaluated. Diam may be protonated at one or both amino groups and at the azo group. Thus three protonation steps have to be taken into account. The absorbance is described well by the constants defined in eqns. (6)–(8), with $pK_{DiamH,1} = 3.54 \pm 0.02$, $pK_{DiamH,2}$

$$K_{\mathrm{DiamH},1} = \frac{[\mathrm{Diam}][\mathrm{H}^+]}{[\mathrm{DiamH}^+]} \qquad (6)$$

$$K_{\mathrm{DiamH},2} = \frac{[\mathrm{DiamH}^+][\mathrm{H}^+]f_1^{\,2}}{[\mathrm{DiamH}_2^{\,2+}]f_2} \qquad (7)$$

$$K_{\mathrm{DiamH},3} = \frac{[\mathrm{DiamH}_2^{\,2+}][\mathrm{H}^+]f_{\mathrm{HCl}}}{[\mathrm{DiamH}_3^{\,3+}]} \qquad (8)$$

$= 2.11 \pm 0.02$ and $\mathrm{p}K_{\mathrm{DiamH},3} = -0.5 \pm 0.1$; the agreement may be seen in Fig. 2.

Again activity coefficients cancel in the determination of $K_{\mathrm{DiamH},1}$. $K_{\mathrm{DiamH},2}$ was determined at low ionic strength, where f_i (i = charge of ion) can be calculated from eqn. (7) from the ionic strength I.[6]

$$-\log(f_i) = 0.509\, z_i^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3I\right) \qquad (9)$$

$K_{\mathrm{Diam},3}$ was determined at high ionic strength, and again it is a conditional constant due to neglecting the activity coefficients for $\mathrm{DiamH_2}^{2+}$ and $\mathrm{DiamH_3}^{3+}$. For $\lambda = 481$ nm the following values were determined: $\varepsilon_{\mathrm{Diam}} = 7800 \pm 200$, $\varepsilon_{\mathrm{DiamH^+}} = 4100 \pm 200$, $\varepsilon_{\mathrm{DiamH2^{2+}}} = 12\,300 \pm 200$ and $\varepsilon_{\mathrm{DiamH3^{3+}}} = 37\,200 \pm 200$ $\mathrm{M^{-1}\ cm^{-1}}$. For 4-methylbenzylamine **4** (abbreviated as Met) the dissociation constant has been determined previously to be $\mathrm{p}K_{\mathrm{Met},1} = 9.62$.[7]

Stability of the host–guest complexes

For the determination of the stability of the host–guest complexes solutions of the dyes were titrated with solutions of cucurbituril at different concentrations of hydrochloric acid. For constant concentration of hydrochloric acid the titration curves agree with the simple reaction in eqn. (10). That means

$$\mathrm{Guest} + \mathrm{Host} \underset{k_b^{\,Q}}{\overset{k_f^{\,Q}}{\rightleftharpoons}} \mathrm{Complex} \qquad (10)$$

eqns. (11)–(14) describe well the absorption values at constant wavelength.

$$[\mathrm{guest}] + [\mathrm{complex}] = D_0 \qquad (11)$$

$$[\mathrm{host}] + [\mathrm{complex}] = \mathrm{Cuc}_0 \qquad (12)$$

$$Q = \frac{[\mathrm{guest}][\mathrm{host}]}{[\mathrm{complex}]} \qquad (13)$$

$$\frac{A}{d} = \varepsilon_{\mathrm{guest}}[\mathrm{guest}] + \varepsilon_{\mathrm{complex}}[\mathrm{complex}] + \varepsilon_{\mathrm{host}}[\mathrm{host}] \qquad (14)$$

D_0 and Cuc_0 are the total concentrations of dye and cucurbituril, respectively, d is the optical path length and the values of Q, $\varepsilon_{\mathrm{guest}}$ and $\varepsilon_{\mathrm{complex}}$ were obtained by the fitting procedure. $\varepsilon_{\mathrm{host}}$ was measured separately. For DO3 and Diam the measurements were performed in the visible range, where cucurbituril does not absorb. Q (= quotient) is not a thermodynamic constant, since it depends on proton concentration, and furthermore activity coefficients are not taken into account. For three different proton concentration results are shown in Fig. 3. Due to the dependence of the solubility of DO3 on the proton concentration the measurements were performed at different concentrations. Therefore $\varepsilon_{\mathrm{exp}}$, as defined in eqn. (2), is plotted in Fig. 3. The absorption was evaluated at $\lambda = 526$ nm, where the complex absorbs most strongly. The results are summarised in Table 2. For $[\mathrm{H}^+] \leqslant 0.1$ the minor component is quantitatively bound in the complex (*i.e.* either [guest] or [host] equals zero); thus for Q only a lower limit can be obtained. The values of $\varepsilon_{\mathrm{guest}}$ may also be calculated from eqn. (15) from the absorption

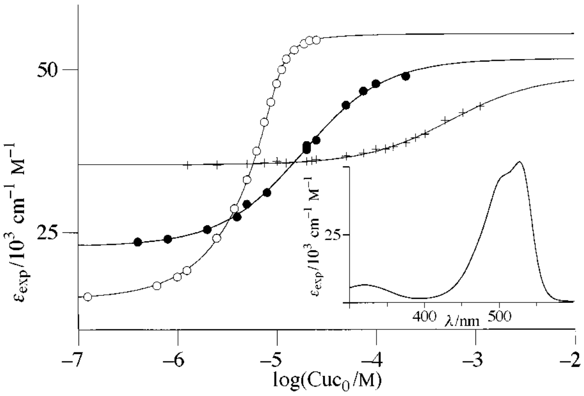

Fig. 3 Titration of solutions of DO3 with Cuc at $T = 25$ °C and $\lambda = 526$ nm: (○) $\mathrm{HCl_0} = 0.125$; (●) $\mathrm{HCl_0} = 2.0$ and (+) $\mathrm{HCl_0} = 5.0$ M. The curves are calculated with the absorption coefficients and dissociation constants given in Table 2. Insert: spectrum of the complex at $\mathrm{HCl_0} = 0.125$ M.

$$\varepsilon_{\mathrm{guest}} = \frac{\varepsilon_{\mathrm{DO3}}K_{\mathrm{DO3},1}K_{\mathrm{DO3},2} + \varepsilon_{\mathrm{DO3H^+}}K_{\mathrm{DO3},2}[\mathrm{H}^+] + \varepsilon_{\mathrm{DO3H_2^{2+}}}[\mathrm{H}^+]^2 f_{\mathrm{HCl}}}{K_{\mathrm{DO3},1}K_{\mathrm{DO3},2} + K_{\mathrm{DO3},2}[\mathrm{H}^+] + [\mathrm{H}^+]^2 f_{\mathrm{HCl}}} \qquad (15)$$

coefficients obtained as in the previous paragraph for DO3, $\mathrm{DO3H}^+$ and $\mathrm{DO3H_2}^{2+}$. The consistency of the evaluation is proved by the good agreement of the values in Table 2. The insert in Fig. 3 shows the spectrum of the complex, which may be compared with the spectra of the differently protonated dye shown in Fig. 1.

Analogously solutions of Diam were titrated with Cuc; see Fig. 4. The binding was studied at both absorption maxima of the complex, $\lambda = 481$ and 497 nm, see insert of Fig. 4. From the titration curve it is evident that Cuc binds much more strongly to Diam than to DO3. For $\lambda = 481$ nm the results are summarised in Table 2. Again the absorption coefficient of $\varepsilon_{\mathrm{guest}}$ can be calculated from the dissociation constants of Diam and the absorption coefficients of the differently protonated species, and comparison with fitted absorption coefficients shows good agreement; see Table 2.

Finally, the complexation equilibrium of Met with Cuc was determined in aqueous 1 M HCl. This amine absorbs only in the UV region, where the absorption of Cuc also has to be taken into account, with $\varepsilon_{\mathrm{host}} = 280\ \mathrm{cm^{-1}\ M^{-1}}$ at $\lambda = 218$ nm. The spectra and titration curve are shown in Fig. 5 and the pQ-values were evaluated at three wavelengths: $\lambda = 216$, 218 and 220 nm; at all wavelengths the evaluation yields $\mathrm{p}Q = 5.0 \pm 0.1$, for $T = 25$ °C.

Kinetics

Assuming that the reaction proceeds according to the simple scheme in eqn. (10), the relaxation time should be given by eqn. (16), where the rate constants are related to Q according to

$$1/\tau = k_f^{\,Q}\,([\mathrm{host}] + [\mathrm{guest}]) + k_b^{\,Q} \qquad (16)$$

eqn. (17). The values of Q (see Table 2) indicate that for Diam

$$k_b^{\,Q} = k_f^{\,Q} Q \qquad (17)$$

and Met the contribution of $k_b^{\,Q}$ to the reaction rate is negligible in the concentration range of this investigation. The values of $k_f^{\,Q}$ were obtained by plotting the kinetic measurements according to eqn. (18), and $k_b^{\,Q}$ was calculated from $k_f^{\,Q}$ and Q.

$$\log\left(\frac{s}{\tau}\right) = \log(k_f^{\,Q}/s^{-1}\ \mathrm{M}^{-1}) + \log(\{[\mathrm{host}] + [\mathrm{guest}]\}) \qquad (18)$$

Table 2 Results obtained for Q, $\varepsilon_{\text{guest}}$, $\varepsilon_{\text{complex}}$, k_f^Q and k_b^Q by fitting eqns. (14) and (17) to the values of optical absorption and to relaxation times. The results refer to $T = 25$ °C if not indicated otherwise. The values in parentheses are calculated from eqn. (15) for $\varepsilon_{\text{guest}}$, eqn. (27) for pQ, eqn. (28) for k_f^Q and eqn. (17) for k_b^Q

Guest	HCl/M	$\varepsilon_{\text{guest}}$/M^{-1} cm^{-1}	$\varepsilon_{\text{complex}}$/M^{-1} cm^{-1}	pQ	log(k_f^Q/s^{-1} M^{-1})	log(k_b^Q/s^{-1})
DO3	0.050	13 500 ± 500 (13 800)	55 600 ± 500	>6.9 (6.89)	4.05 ± 0.05 (4.09)	— (< −2.9)
DO3	0.100	13 800 ± 500 (14 400)	55 600 ± 500	>6.6 (6.60)	3.80 ± 0.05 (3.80)	— (< −2.8)
DO3	0.125	14 700 ± 500 (14 650)	55 500 ± 500	6.53 ± 0.02 (6.51)	3.72 ± 0.05 (3.71)	— (−2.8)
DO3	0.250	15 350 ± 500 (15 400)	55 300 ± 500	6.19 ± 0.02 (6.20)	3.42 ± 0.05 (3.40)	— (−2.8)
DO3	0.50	16 750 ± 500 (16 700)	54 600 ± 500	5.88 ± 0.02 (5.88)	3.03 ± 0.05 (3.08)	— (−2.9)
DO3	1.00	19 550 ± 500 (19 100)	53 800 ± 500	5.46 ± 0.02 (5.54)	2.63 ± 0.05 (2.73)	−2.8 ± 0.1 (−2.8)
DO3	2.00	22 900 ± 500 (23 800)	52 900 ± 500	4.80 ± 0.02	2.00 ± 0.05	−2.8 ± 0.1 (−2.8)
DO3	5.0	35 900 ± 500 (34 800)	49 550 ± 500	3.25 ± 0.02	—	—
DO3[a]	1.00	18 900 ± 500 (18 500)	53 800 ± 500	5.17 ± 0.02	2.89 ± 0.05	−2.3 ± 0.1 (−2.3)
DO3[b]	1.00	18 700 ± 500 (18 150)	53 800 ± 500	5.00 ± 0.02	3.00 ± 0.05	−2.0 ± 0.1 (−2.0)
DO3[c]	1.00	18 300 ± 500 (17 900)	53 800 ± 500	4.87 ± 0.02	3.10 ± 0.05	−1.8 ± 0.1 (−1.8)
Diam	0.100	12 800 ± 500	58 000 ± 500	>7.7	—	—
Diam	0.200	13 500 ± 500	58 300 ± 500	7.2 ± 0.1	—	—
Diam	1.00	16 800 ± 500	58 900 ± 500	6.6 ± 0.1	2.17 ± 0.05	— (−4.4)
Met	1.00	8 025 ± 50	4 200 ± 100	5.0 ± 0.1	3.16 ± 0.05	— (−1.8)

[a] 35 °C. [b] 40 °C. [c] 45 °C.

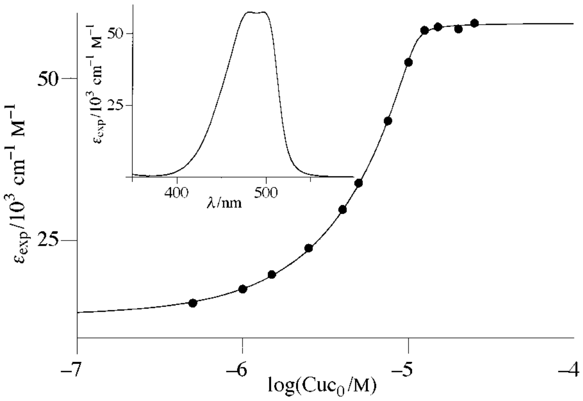

Fig. 4 Titration of solutions of Diam with Cuc at $T = 25$ °C, λ = 481 nm and (●) $HCl_0 = 1.0$ M. The curves are calculated with the absorption coefficients and dissociation constants given in Table 2. Insert: spectrum of the complex at $HCl_0 = 0.2$ M.

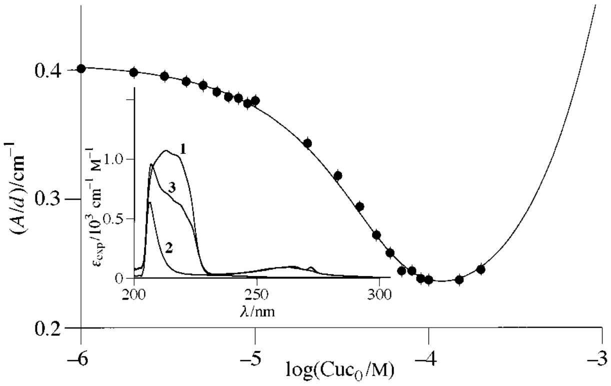

Fig. 5 Titration of solutions of Met with Cuc at $T = 25$ °C, λ = 218 nm and (●) $HCl_0 = 1.0$ M. The curves are calculated with the absorption coefficients and dissociation constants given in Table 2. Insert: spectra of solutions of 1 M HCl containing 1 $Met_0 = 1.25 \times 10^{-4}$, 2 $Cuc_0 = 1.25 \times 10^{-4}$ and 3 $Met_0 = 1.25 \times 10^{-4}$ and $Cuc = 1.25 \times 10^{-4}$ M.

For DO3 in 1 and 2 M HCl both values k_f^Q and k_b^Q may be obtained by fitting the relaxation times to eqn. (16), and Table 2 shows the good agreement between k_b^Q and $Q\,k_f^Q$. For those solutions the measurements are shown in Fig. 6.

Discussion

Table 2 shows that for DO3 the reaction quotient Q and the rate constant k_f^Q depend strongly on the hydrogen ion concentration, and these dependencies will be used to develop a mechanism for the reaction of cucurbituril with substituted amines. The strong dependence of $\varepsilon_{\text{guest}}$ on [H$^+$] has already been discussed in the previous section. $\varepsilon_{\text{complex}}$ depends weakly on [H$^+$] only for concentrations larger than 0.5 M.

The evaluation of the experiments is based on the following considerations. (*i*) At constant proton concentration the measurements agree with the simple mechanism in eqn. (10) indicating that Cuc and DO3 form a 1 : 1 complex. (*ii*) The protonation equilibria of DO3 are determined by the dissociation constants in eqns. (4) and (5). (*iii*) The amino group of DO3 remains protonated when forming a complex with Cuc due to the favourable electrostatic interaction between the cationic group and the carbonyl groups of Cuc. Moreover the azo group may also be protonated. Thus the complex may be of the stoichiometry $CucDO3H^+$ or $CucDO3H_2^{2+}$. (*iv*) The solubility of Cuc increases with proton concentration indicating that Cuc is protonated in solution, *i.e.* it is present as $CucH^+$ or $CucH_2^{2+}$. Thus the overall reaction should follow the system of reactions in eqns. (19)–(22).

$$DO3 + 2H^+ \xrightleftharpoons{K_{DO3H,1}} DO3H^+ + H^+ \xrightleftharpoons{K_{DO3H,2}} DO3H_2^{2+} \qquad (19)$$

$$CucH^+ + H^+ \xrightleftharpoons{K_{CucH,2}} CucH_2^{2+} \qquad (20)$$

$$DO3 + CucH^+ \xrightleftharpoons{K_{CucDO3,K}} CucDO3H^+ \qquad (21)$$

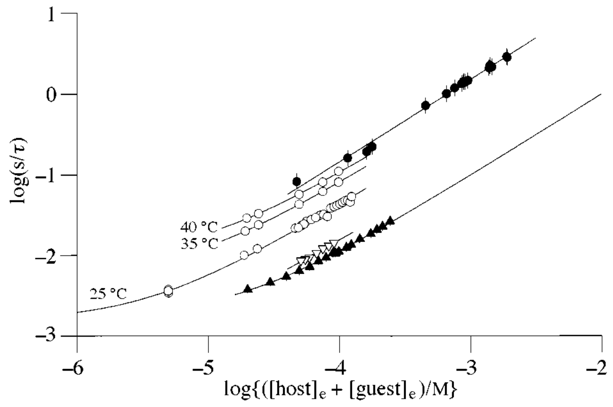

Fig. 6 Log(s/τ) *vs.* log({[host]$_e$ + [guest]$_e$}/M) in 1 M HCl for (○) DO3; (▽) Diam; (●) Met and in 2 M HCl for (▲) DO3. The curves are calculated with rate constants given in Table 2. $T = 25$ °C if not indicated otherwise.

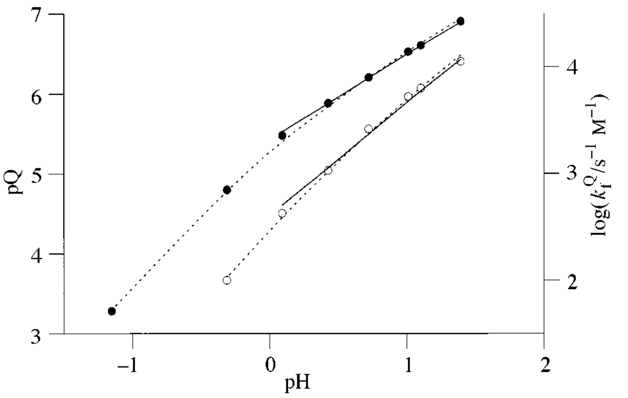

Fig. 7 Double logarithmic plots of Q (scale on the left) and k_f^Q (scale on the right) *vs.* pH. The solid curves are calculated according to eqns. (27) and (28); the dashed lines are calculated with consideration of the diprotonated $CucH_2^{2+}$.

$$CucDO3H^+ + H^+ \underset{}{\overset{K_{cucDO3H,2}}{\rightleftharpoons}} CucDO3H_2^{2+} \qquad (22)$$

The rate determining step of the overall reaction is given by eqn. (21); the other reaction steps proceed by protonation with diffusion controlled speed. The numerical evaluation of the experimental data show that for proton concentrations smaller than 1 M the species $CucH_2^{2+}$ and $CucDO3H_2^{2+}$ do not contribute to absorption and relaxation time [*i.e.* reactions (20) and (22) do not need to be taken into account]. In this range the equilibrium is determined by the dissociation constant in eqn. (23), and the

$$K_{CucDO3,K} = \frac{[DO3][CucH^+]}{[CucDO3H^+]} \qquad (23)$$

rate law is given by eqn. (24). In eqns. (23) and (24) it is assumed

$$\frac{d[CucDO3H^+]}{dt} = k_f[DO3][CucH^+] - k_b[CucDO3H^+] \qquad (24)$$

that the activity coefficients of the monovalent ions $CucH^+$ and $CucDO3H^+$ and of the activated complex cancel.

$$\frac{1}{\tau} = \frac{k_f}{a}(a[DO3] + [CucH^+]) + k_b \qquad (25)$$

$$\text{with } a = 1 + \frac{[H^+]}{K_{DO3H,1}} + \frac{[H^+]^2 f_{HCl}}{K_{DO3H,1}K_{DO3H,2}} \qquad (26)$$

Q and k_f^Q are related to equilibrium and rate constants by eqns. (26) and (27)

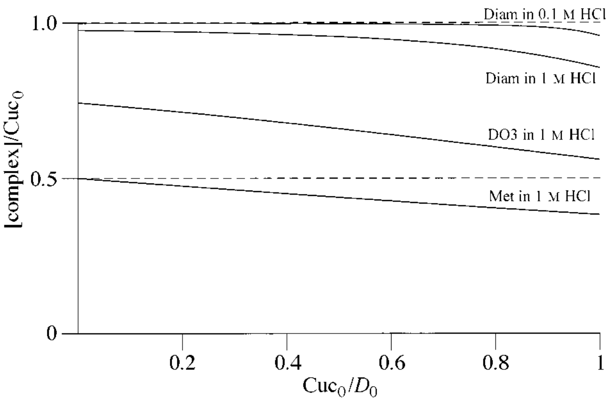

Fig. 8 Degree of association *vs.* total concentration of Cuc for different ligands at $D_0 = 10^{-5}$ M and $T = 25$ °C

$$Q = K_{CucDO3,K}a \qquad (27)$$

$$k_f^Q = \frac{k_f}{a} \qquad (28)$$

The fitting of eqns. (27) and (28) to the experimental data yields p$K = 8.05 \pm 0.05$ and $\log(k_f/s^{-1}\ M^{-1}) = 5.25 \pm 0.05$. The constants describe well the experiments up to 1 M HCl as can be seen in Fig. 7. From the temperature dependence of Q, k_f^Q, $K_{DO3H,1}$ and $K_{DO3H,2}$ the following parameters were determined: activation energies $E_{a,f} = 18 \pm 2$ kJ mol^{-1} and $E_{a,b} = 92 \pm 4$ kJ mol^{-1}, reaction enthalpy $\Delta_f H^0 = 79 \pm 4$ kJ mol^{-1} and reaction entropy $\Delta_f S^0 = 112 \pm 10$ J mol^{-1} K^{-1}.

For proton concentrations higher than 1 M Fig. 7 shows strong deviations of the calculated curves from the experimental values of both absorbance and relaxation time. The deviations seem to be too large to be attributed to a faulty calculation of activity corrections, and therefore the deviations may be caused either by neglecting species $CucH_2^{2+}$ in eqn. (20) or by neglecting of species $CucDO3H_2^{2+}$ in eqn. (22). At high proton concentration the deviations are due to a weaker complex formation than expected from eqns. (19) and (21), whereas the presence of $CucDO3H_2^{2+}$ should lead to an increase in complex formation at high proton concentration. The independence of k_b^Q on pH indicates also that the product $CucDO3H^+$ is not further protonated, *i.e.* the concentration of $CucDO3H_2^{2+}$ is negligible. Thus $CucH_2^{2+}$ is included in the reaction scheme assuming that (*i*) the equilibrium is determined by the stability constant given in eqn. (29), where activity coefficients are included analogously to eqn. (5) and (*ii*) the protonation rate of $CucH^+$ is fast compared to the complex formation. Introducing species $CucH_2^{2+}$ with $K_{CucH,2} = -0.25 \pm 0.08$ [see eqn. (29)] into the

$$K_{CucH,2} = \frac{[CucH^+][H^+]f_{HCl}}{[CucH_2^{2+}]} \qquad (29)$$

evaluation of absorbance and relaxation time leads to excellent agreement between calculated curve and experimental values both for Q and $1/\tau$, see Fig. 7. (Details of the evaluation are available on request from the authors.)

In summary, for the reaction of Cuc with DO3 the mechanism is given by eqns. (19)–(21). All constants involved are given in Table 1.

Finally it should be discussed whether the guests studied can be used as indicators. For that purpose the degree of association is plotted *versus* the quotient of total concentration of Cuc divided by D_0 for the different ligands in Fig. 8.

In 0.1 M HCl Cuc reacts nearly quantitatively with Diam as long as $[Cuc]_0 < 0.95[Diam]_0$. (At higher proton concentrations the stability of the complex is reduced.) Thus the total concentration of Cuc in aqueous solution may be determined by adding HCl to obtain $[\mathrm{H^+}] = 0.1$ M and titrating the solution with Diam. This method works for $[\mathrm{Cuc}]_0 < [\mathrm{Diam}]_0$, where the latter value is restricted to $[\mathrm{Diam}]_0 = 3 \times 10^{-5}$ M. Thus the solution has to be diluted for higher concentrations of Cuc. For the determination of the total concentration of Cuc the conditions required above can always be established, and therefore the reaction of Diam with Cuc has not been investigated at other proton concentrations.

In Fig. 8 the curve for DO3 shows a degree of association slightly higher than 0.5 for total concentration of approximately 10^{-5} M both for Cuc and DO3. This means that DO3 is an optimal indicator for the concentration of free cucurbituril in this range, *i.e.* DO3 can act as an indicator for the study of the binding of non-absorbing ligands to Cuc. For those studies the concentrations of the reacting species cannot be changed, and correspondingly the reaction of Cuc with DO3 has been studied in detail over broad ranges of concentrations, pH and temperature.

Fig. 8 also shows that Met binds only weakly to Cuc, *i.e.* Met is not a suitable indicator (furthermore, it does not absorb light in the visible range). The aptitude of Diam and DO3 as indicators for total and actual concentrations is studied in further investigations measuring pH dependence of the solubility of Cuc and the binding of metal ions to Cuc, respectively.

Conclusions

In aqueous solutions of 0.05–1 M HCl DO3 may be used as indicator for the concentration of free Cuc in this range. The reaction of Cuc with DO3 is well described by the following mechanism in eqn. (30).

$$\begin{tikzcd}
\mathrm{CucH^+} + & \mathrm{DO3} + 2\mathrm{H^+} \arrow[r, rightleftharpoons, "K_{\mathrm{CucDO3,K}}"] \arrow[d, shift right] & \mathrm{CucDO3H^+} + 2\mathrm{H^+} \\
\mathrm{CucH^+} & \mathrm{DO3H^+}\ \mathrm{H^+} \arrow[u, shift right, "K_{\mathrm{DO3H,1}}"'] \arrow[d, shift right] & \\
\mathrm{CucH^+} & \mathrm{DO3H_2^{2+}} \arrow[u, shift right, "K_{\mathrm{DO3H,2}}"'] &
\end{tikzcd} \tag{30}$$

The constants involved are summarised in Table 1. In 2 M and 5 M HCl the presence of $\mathrm{CuCH_2}$ has to be taken into account.

For Diam the reaction with Cuc has been studied only in 0.1–1 M HCl, where a very strong binding is observed. Therefore Diam is a sequestering agent for Cuc, *i.e.* it is an indicator for the total concentration of Cuc in 0.1 M HCl. The absorption coefficients at $\lambda = 418$ nm are $\varepsilon_{\mathrm{guest}} = 12\,800 \pm 500$ and $\varepsilon_{\mathrm{complex}} = 58\,000 \pm 500$ $\mathrm{M^{-1}\ cm^{-1}}$. The results of the measurements at equilibrium are confirmed by the kinetic studies.

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Paper 7/08015H
Received 6*th November* 1997
Accepted 14*th November* 1997