



We have recently shown that polarimetry can be profitably used as an experimental tool in order to study the formation of inclusion complexes with CDs.<sup>17</sup> Determinations of the binding constants are fast, accurate and reliable. Moreover, provided that a careful data analysis is carried out, polarimetric data can give interesting information also on the structure and the dynamic behaviour of the inclusion complex. So, to progress our previous work, we performed a polarimetric study on the effect of methanol, as organic co-solvent, on the inclusion equilibria between native  $\beta$ -cyclodextrin ( $\beta$ -CD) and a set of suitably selected *p*-nitro-aniline derivatives **1–8** (Fig. 1).

It is worth noting that *p*-nitro-aniline derivatives constitute for several reasons a class of ideal model substrates. As a matter of fact, the *p*-nitro-aniline moiety unambiguously directs the penetration of the guest into the host cavity<sup>18</sup> (the nitro group is always directed towards the primary hydroxyl rim). Then, it is an effective chromophore, whose behaviour can be comfortably investigated by means of UV–vis spectrophotometry. Furthermore, large variations in molecular properties (volume and steric hindrance, hydrophobicity, polarity, electric charge, and so on) can be easily achieved by changing the groups linked to the amino nitrogen atom (the ‘ancillary chain’). Consequently, large variations can be observed in the thermodynamic parameters for the inclusion process, as well as different enthalpy–entropy compensation behaviours.<sup>4</sup> Nonetheless, guests **1–8** were selected in view of their satisfactory water solubility, and in order to span a range of inclusion  $\Delta H^0$  and  $T\Delta S^0$  values as large as possible. On the other hand, methanol was chosen as co-solvent for its close similarity to water, in such a way as to achieve a smooth modulation in the intrinsic properties of the solvent system.

## 2. Results and discussion

The complete results of polarimetric determinations are reported as [Supplementary data](#), namely the values of the conditional binding constants  $K_{\text{cond}}$  measured at 298 K, the relevant conditional inclusion free energies  $\Delta G_{\text{cond}}^0$  and the molar differential optical rotations  $\Delta\theta$  (the difference between the molar optical rotations of the inclusion complex and of the free host), for guests **1–8** as a function of co-solvent molar fraction  $\chi_{\text{MeOH}}$ . Measurements were performed in non-buffered aqueous systems containing increasing amounts of methanol, at molar fractions ( $\chi_{\text{MeOH}}$ ) usually ranging up to 0.16 (30% v/v), with few exceptions. In particular, for the *N*-methyl-ethanolamine derivative **4** we were able to increase the amount of co-solvent up to a molar fraction of 0.31 (50% v/v). Plots

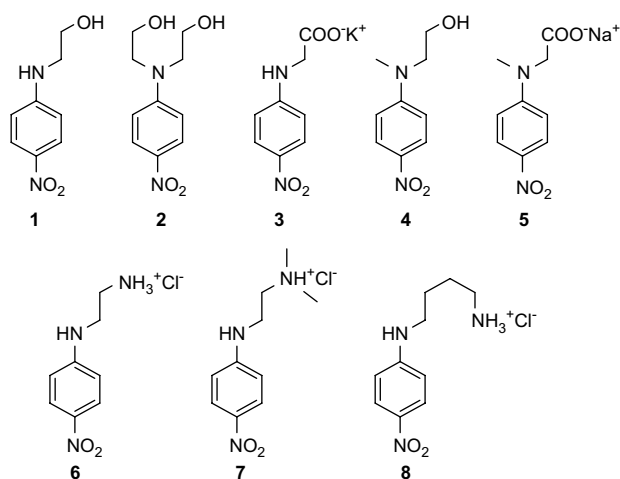


Figure 1. *p*-Nitro-aniline derivatives **1–8**.

of  $K_{\text{cond}}$ ,  $\Delta G_{\text{cond}}^0$  and  $\Delta\theta$  as a function of  $\chi_{\text{MeOH}}$  for guest **4** are shown in [Figures 2–4](#), respectively, and typically illustrate the effect of the increasing presence of the co-solvent on these parameters. In general, the values of  $K_{\text{cond}}$  decrease exponentially on increasing the amount of methanol ([Fig. 2](#)), with the exception of guests **3** and

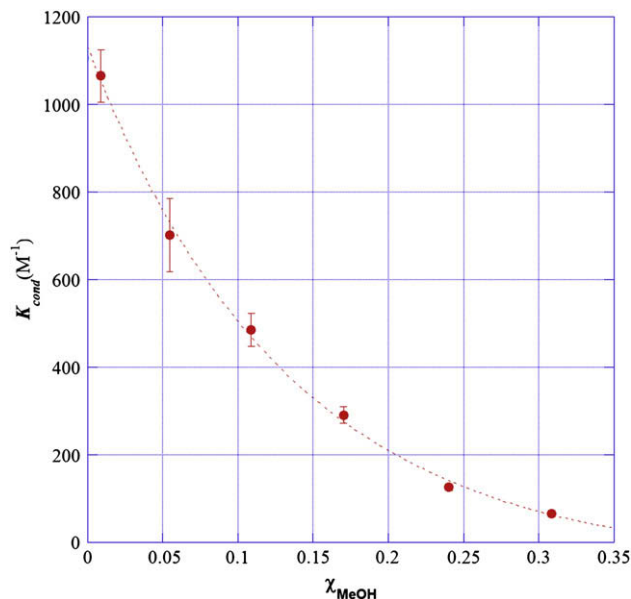


Figure 2.  $K_{\text{cond}}$  versus MeOH plot for guest **4**.

**6**, for which almost no effect is detected. As a consequence, trends of  $\Delta G_{\text{cond}}^0$  versus  $\chi_{\text{MeOH}}$  are linear ([Fig. 3](#)). Thus, a suitable regression data analysis allows us to obtain the values, extrapolated for pure water, of the inclusion free energy  $\Delta G_{\text{w}}^0$  and its derivative  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$ . The trends for  $\Delta\theta$  appear more complex. As a matter of fact, data plots for **4** ([Fig. 4](#)) and **5** are slightly curvilinear, whereas linear plots are found in all other cases. Furthermore,  $\Delta\theta$  usually decreases on increasing  $\chi_{\text{MeOH}}$ , but negligible variations are found for

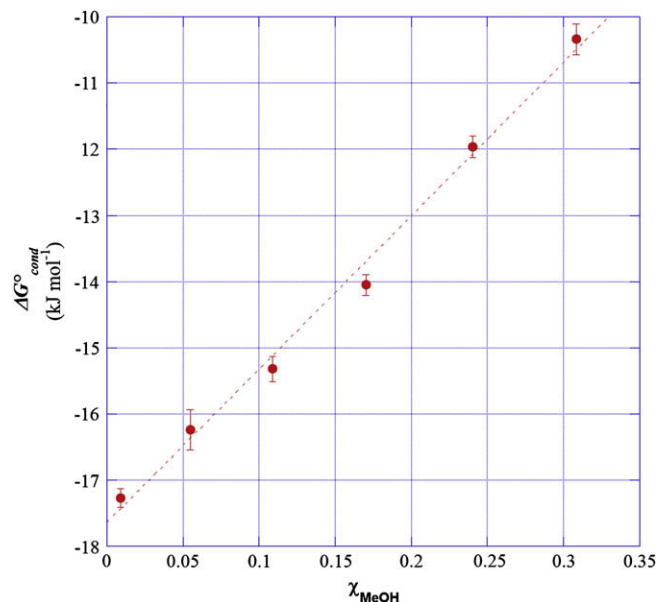
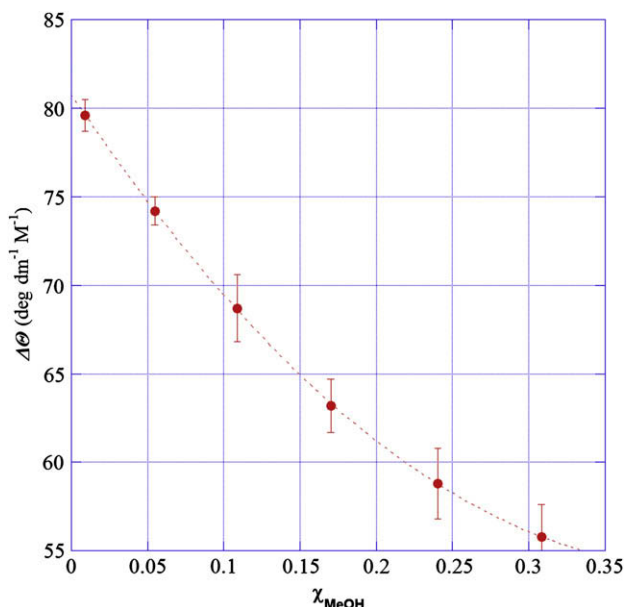


Figure 3.  $\Delta G_{\text{cond}}^0$  versus MeOH plot for guest **4**.

Figure 4.  $\Delta\theta$  versus  $\chi_{\text{MeOH}}$  plot for guest 4.

3, and even a slightly increasing trend is found for 8. Anyway, a suitable regression data analysis for each guest provides the estimates of the values, extrapolated for pure water, of the differential optical rotation  $\Delta\theta_w$  and its derivative  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$ .<sup>19</sup>

Data for  $\Delta G_w^0$ ,  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$ ,  $\Delta\theta_w$  and  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  are collected in Table 1, together with the relevant literature values of the inclusion enthalpies  $\Delta H_i^0$  measured (UV) in buffered aqueous solution. It is worth stressing here that the derivatives  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  and  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  provide a quantitative estimation of the effect exerted by the co-solvent methanol upon  $\Delta G^0$  and  $\Delta\theta$ , respectively. We compared these derivatives with  $\Delta H_i^0$  values (Figs. 5 and 6), obtaining very interesting results.

We already mentioned that *p*-nitro-aniline guests show different enthalpy–entropy compensation behaviours, depending on the features of the ancillary chain.<sup>4</sup> In particular, we found that guests 1–3 belong to a subset of substrates the inclusion of which shows entropy variations that overwhelm enthalpy ones on a relative scale, according to the relationship:

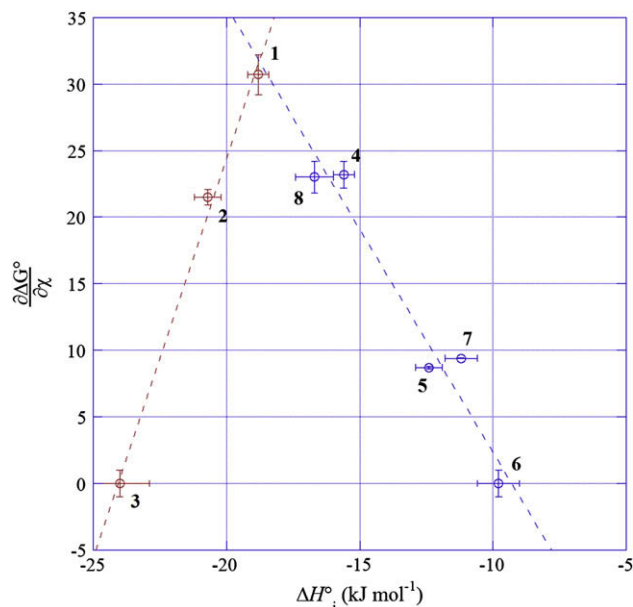
$$T\Delta S_i^0 = 20.9(\pm 0.1) + 1.27(\pm 0.04)\Delta H_i^0 \text{ kJ mol}^{-1} \quad (1)$$

This particular behaviour accounts for the peculiar rigidity of the inclusion complex, due to the occurrence of multiple host–guest hydrogen bond interactions. As a consequence, binding constants regularly decrease on increasing  $-\Delta H_i^0$ , according to the relationship:

**Table 1**  
Polarimetric and thermodynamic data

Guest	$\Delta C_w^0$ (kJ mol <sup>-1</sup> )	$\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$ (kJ mol <sup>-1</sup> )	$\Delta\theta_w$ (deg dm <sup>-1</sup> M <sup>-1</sup> )	$\partial\Delta\theta/\partial\chi_{\text{MeOH}}$ (deg dm <sup>-1</sup> M <sup>-1</sup> )	$\Delta H_i^{0a}$ (kJ mol <sup>-1</sup> )
1	-16.23±0.15	30.7±1.5	75.3±0.1	-75.5±0.7	-18.8±0.4
2	-15.56±0.15	21.5±0.6	89.1±0.5	-44.8±5.0	-20.7±0.5
3	-14.32±0.17	0±1.0	59.8±1.0	-5.0±5.0	-24.0±1.1
4	-17.64±0.18	23.2±1.0	80.8±0.1	-128.7±1.7	-15.6±0.4
5	-14.67±0.18	8.7±0.1	87.7±0.1	-168.2±1.3	-12.4±0.5
6	-14.14±0.25	0±1.0	55.9±0.1	-87.1±0.5	-9.8±0.8
7	-16.00±0.22	9.4±0.1	67.7±0.3	-63.7±3.2	-11.2±0.6
8	-17.31±0.11	23.0±1.2	80.1±1.0	18.8±0.6	-16.7±0.7

<sup>a</sup> From Ref. 4.

Figure 5.  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  plot.

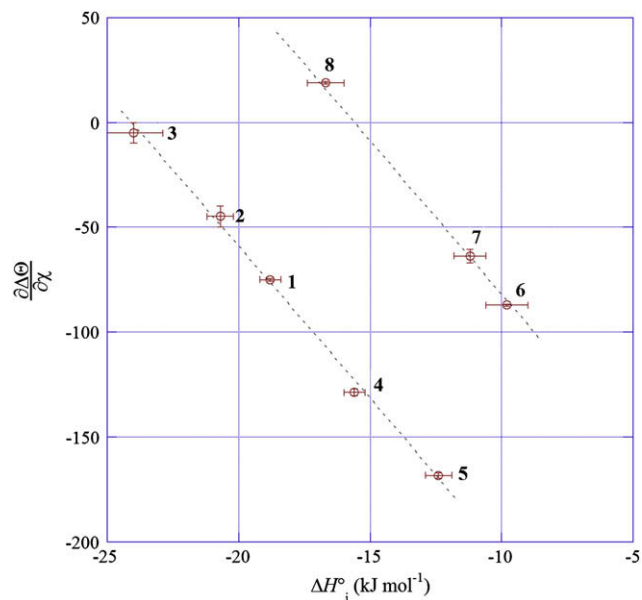
$$\Delta G_i^0 = -20.9(\pm 0.1) - 0.27(\pm 0.04)\Delta H_i^0 \text{ kJ mol}^{-1} \quad (2)$$

By contrast, guests 4–8 belong to a subset of substrates the inclusion complexes of which are mainly stabilized by non-specific (hydrophobic and/or dipolar) interactions,<sup>20</sup> so that enthalpy variations outweigh entropy ones, according to the relationship:

$$T\Delta S_i^0 = 9.8(\pm 0.2) + 0.60(\pm 0.08)\Delta H_i^0 \text{ kJ mol}^{-1} \quad (3)$$

Consequently, binding constants increase on increasing  $-\Delta H_i^0$  according to the relationship:

$$\Delta G_i^0 = -9.8(\pm 0.1) + 0.40(\pm 0.08)\Delta H_i^0 \text{ kJ mol}^{-1} \quad (4)$$

Figure 6.  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  plot.

Now, considering the  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  plot (Fig. 5), we can easily notice two distinct linear correlations, having opposite slopes, for the two guest subsets **1–3** and **4–8**, respectively. In particular, for the first subset the disfavouring effect of methanol addition on  $K_{\text{cond}}$  appears to become stronger on decreasing  $-\Delta H_i^0$ , whereas for the second subset the opposite is observed, according to the relationships:

$$\frac{\partial\Delta G_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} = 144(\pm 9) + 6.0(\pm 0.4)\Delta H_i^0 \quad (r = 0.997, n = 3) \text{ for guests } \mathbf{1-3} \quad (5)$$

$$\frac{\partial\Delta G_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} = -31(\pm 6) - 3.3(\pm 0.4)\Delta H_i^0 \quad (r = 0.972, n = 5) \text{ for guests } \mathbf{4-8} \quad (6)$$

By comparing Eqs. 5 and 6 with Eqs. 2 and 4, respectively, the following expressions can be derived algebraically:

$$\frac{\partial\Delta G_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} = -320(\pm 75) - 22(\pm 4)\Delta G_i^0 \quad \text{for guests } \mathbf{1-3} \quad (7)$$

$$\frac{\partial\Delta G_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} = -112(\pm 20) - 8.3(\pm 1.9)\Delta G_i^0 \quad \text{for guests } \mathbf{4-8} \quad (8)$$

Eqs. 7 and 8 show that  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  values are linearly correlated to the corresponding  $\Delta G_i^0$  for both guest subsets, with negative slopes. Thus, within each subset we find that  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  values become more positive on increasing  $-\Delta G_i^0$ . As a consequence, the co-solvent methanol increasingly tends to destabilize the inclusion complex on increasing the intrinsic stability of the complex itself. Contrariwise, on decreasing the intrinsic stability of the complex, the unfavourable effect of methanol addition on  $\Delta G_{\text{cond}}^0$  becomes weaker and weaker, until it fades almost completely for guests **3** (first subset) and **6** (second subset). Hence, we might also predict (although we did not actually observe the case) that methanol should even revert its effect and stabilize complexes having a particularly poor intrinsic stability.

The  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  plot (Fig. 6) is particularly intriguing. As a matter of fact, data points seem to draw two parallel straight lines. The first one is defined by the neutral and anionic guests **1–5**, whereas the second one is defined by the cationic derivatives **6–8**. Thus, the entire dataset can be suitably fitted by means of a unique biparametric equation:

$$\frac{\partial\Delta\theta}{\partial\chi_{\text{MeOH}}} = -353(\pm 7) + 124(\pm 4)\mathbf{Q} - 14.7(\pm 0.4)\Delta H_i^0 \quad (r = 0.998; n = 8) \quad (9)$$

where  $\mathbf{Q}$  is an ad hoc conventional parameter accounting for the electric charge status of the guest ( $\mathbf{Q}=0$  for neutral and anionic guests,  $\mathbf{Q}=1$  for cationic ones).

These correlations deserve a thorough analysis. Let us examine first  $\Delta\theta$  trends. We have already shown in previous works that  $\Delta\theta$  values for *p*-nitro-aniline derivatives depend on both electronic and structural/dynamic factors.<sup>18</sup> In particular, the lowest  $\Delta\theta$  values were found for the most rigid complexes, having the guest forced to assume a tilted position within the host cavity. (By intuition,  $\Delta\theta$  depends on the time-averaged dot product between the dipole momenta of the cavity and the chromophore.) In principle, a variation in optical activity on varying the solvent medium could be ascribed to a change in the solvent refractive index  $n$ ,<sup>21</sup> depending linearly on  $(n^2+2)$ . However, on the grounds of literature data for water–methanol mixtures,<sup>22</sup> we can predict that such an effect should provide a small, reasonably negligible contribution to  $\Delta\theta$  variations. It is worth noting that we actually measured no

significant variation in the molar optical activity  $\Theta_{\beta\text{-CD}}$  of pure  $\beta\text{-CD}$  in water–methanol mixtures within the range  $0 \leq \chi_{\text{MeOH}} \leq 0.4$  (i.e., 0–60% v/v). Thus, considering the peculiar correlation found between  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  and  $\Delta H_i^0$ , the occurrence of variations on  $\Delta\theta$  seems rather to account for a specific effect exerted by the co-solvent methanol on the dynamic behaviour of the complex. It is worth recalling, indeed, that  $\Delta H_i^0$  values for this class of guests are positively controlled by the ‘nominal’ counterpart of the binding process.<sup>4</sup>

Now, assuming  $\Delta\theta$  as an estimate of the time-averaged tilt angle for the *p*-nitro-aniline chromophore with respect to the ideal axis of the host cavity, data suggest that in general the immediate effects of the co-solvent addition consist in an increase of guest tilting and, consequently, in a diminished dynamism of the complex. It is important to stress that the existence of two parallel lines (i.e., having the same slope, but different intercepts) in the correlation plot indicates that these effects work in a somehow coherent way over the entire set of guests, regardless of the actual charge of the guest ancillary chain. So, the ‘distinct’ behaviour of cationic guests **6–8** towards co-solvent addition could be explained, in our opinion, considering the peculiar electrostatic interaction between the intrinsic dipole momentum of the **CD** cavity and the cationic ancillary chain. This interaction is probably able to offset the tilting effect, but does not significantly affect the possible modifications in the dynamic behaviour of the complex induced by the co-solvent. Anyway, the trends for  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  show that these effects increase on decreasing  $-\Delta H_i^0$ . Consequently, on the grounds of the enthalpy–entropy isoequilibrium correlations, we may infer that greater effects are exerted on intrinsically less rigid complexes.

The latter consideration leads us into the analysis of the  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  plot. We can initially notice that the experimental data cannot be simply explained on the grounds of the solvation energies of the guests, because there is no apparent relationship between the features of the ancillary chains of the guests and the relevant  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  values. For instance, **4** is undoubtedly less hydrophilic than both **1** and **3**, but it shows an intermediate  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  value. On the other hand, the existence of different linear correlations with  $\Delta H_i^0$ , having opposite slopes, for subsets of guests obeying different enthalpy–entropy compensation models, is particularly meaningful. As a matter of fact, the relative variations of conditional inclusion free energies are the output of the concomitant relative variations in both the conditional inclusion enthalpies ( $\Delta H_{\text{cond}}^0$ ) and the entropies ( $\Delta S_{\text{cond}}^0$ ). In other words, from the obvious  $\Delta G_{\text{cond}}^0 = \Delta H_{\text{cond}}^0 - T\Delta S_{\text{cond}}^0$ , follows:

$$\frac{\partial\Delta G_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} = \frac{\partial\Delta H_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} - \frac{\partial T\Delta S_{\text{cond}}^0}{\partial\chi_{\text{MeOH}}} \quad (10)$$

The derivatives  $\partial\Delta H_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  and  $\partial T\Delta S_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$ , of course, quantify the relative effect exerted by the co-solvent methanol upon the inclusion enthalpies and entropies, respectively. Unfortunately, our data do not provide us with the values of these two derivatives for each guest. However, it can be easily demonstrated (see [Supplementary data](#)) that the simultaneous occurrence of the enthalpy–entropy compensation and of a linear dependence of  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  on  $\Delta H_i^0$  implies that both  $\partial\Delta H_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  and  $\partial T\Delta S_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  must be linearly correlated with  $\Delta H_i^0$  too. It is at once evident that the latter statement is perfectly consistent with the considerations on  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  developed previously. From that standpoint, we may deduce that in general the addition of methanol decreases the inclusion entropies, because of the stiffening of the inclusion complex, with a stronger relative effect for intrinsically less rigid complexes. This effect should work coherently over the entire guests set, regardless of both the charge status of the ancillary chain and the actual enthalpy–entropy compensation model followed by the guest. Therefore, all the guests should

eventually define a unique linear  $\partial T\Delta S_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  correlation plot having a negative slope. On the other hand, the occurrence of distinct  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  correlations for the different subsets of guests suggests that distinct correlations must consequently exist for  $\partial\Delta H_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  values. This implies also that different types of effects, for the two guest subsets, are exerted by methanol on inclusion enthalpies.

In more detail, we might reason that for guest **6**, showing a null  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  and a large  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  value, the addition of methanol makes the binding process more exothermic, in order to counterbalance the stiffening effect of the complex (and in such a way that enthalpy and entropy variations compensate almost perfectly). However, if we extend our consideration to the entire guest subsets **4–8** (with **6** as a sort of anchor point), the occurrence of an overall negative slope for the  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  correlation implies that on increasing  $-\Delta H_i^0$  (i.e., the intrinsic strength of the complex) any possible favourable effect of methanol on  $\Delta H_{\text{cond}}^0$  vanishes very rapidly, and that the overall co-solvent effects on  $\Delta H_{\text{cond}}^0$  outweigh the concomitant effects on  $T\Delta S_{\text{cond}}^0$ . By contrast, for guests **1–3** the occurrence of a positive slope in the  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  versus  $\Delta H_i^0$  plot indicates a somehow opposite behaviour for this guest subset (further discussion on these points is reported in [Supplementary data](#)).

On the grounds of the previous discussion, it is clear that the role played by the co-solvent in the binding equilibrium cannot be limited to its effects on the ‘environmental’ process only. Data seem rather to account for an involvement of the solvent system in the ‘nominal’ process too, by means of specific solvent–complex interactions. This suggests the need to reconsider critically the overall contribution of the solvent in affecting the structure and the intimate features of the inclusion complex. The idea that a **CD** may have enough room to host some water molecules together with the guest is not new. If anything, it is a specific assumption of the theory of ternary complexes formation.<sup>23</sup> However, at the best of our knowledge this concept has been seldom exploited comprehensively in rationalizing the structure and the thermodynamics of formation for ‘ordinary’ 1:1 complexes.<sup>6</sup>

In our opinion, the experimental results may be suitably explained by admitting that solvent molecules can be ‘dynamically co-included’, up to a certain extent, into the **CD** cavity together with the guest. In other words, we may reasonably hypothesize the occurrence of a continuous swap of solvent molecules<sup>24</sup> between the solvent bulk and the residual space within the complex, without requiring the formation of any individual structure/stoichiometry-defined species. This ‘dynamic co-inclusion’ process is able to affect the time-averaged conformational dynamics of the complex, as well as the inner interplay of molecular interactions. In the presence of an organic co-solvent, namely methanol, this is probably able to compete successfully with water molecules for this dynamic process, because of the occurrence of more effective hydrophobic interactions within the host cavity. As a consequence, the complex tends to become on average more rigid. From a thermodynamic point of view, the increased extent of hydrophobic interactions tends to counterbalance the loss of conformational freedom for the complex, as well as the less favourable desolvation of the guest. However, when we consider guests **4–8**, we have to conclude that such a counterbalance mechanism works effectively only with the weakest complex of the subset, i.e.,  $\beta$ -**CD-6**. As a matter of fact, on increasing the intrinsic stability of the complex, the increasing strength of neat host–guest interactions rapidly vanquishes the possible favourable effects due to methanol co-inclusion, as accounted for by the overall negative slope of the  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  plot. On the other hand, things change dramatically when we consider the behaviour of guests **1–3**. Owing to the paramount role assumed by hydrogen bonding in these cases, now the possible increased occurrence of hydrophobic interactions due to the

co-solvent affects unfavourably the overall inclusion enthalpies. Consequently, the stiffening effect of methanol co-inclusion prevails, in a much more apparent way for the intrinsically least rigid (and most stable!) complexes of the group, as accounted for by the overall positive slope of the  $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  plot.

In order to gain further support for our hypotheses, we studied the effect of methanol addition on the inclusion of guest **4** in  $\alpha$ -**CD** (data in [Supplementary data](#)). We already knew that the narrowest  $\alpha$ -**CD** host is able to include very tightly only the aromatic moiety of the guest. We actually detected no effect on  $\Delta\theta$  (i.e., a nearly null value for  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$ ). On the other hand, the concomitant unfavourable effect on  $\Delta G_{\text{cond}}^0$  due to the presence of the co-solvent is much more significant for  $\alpha$ -**CD** ( $\partial\Delta G_{\text{cond}}^0/\partial\chi_{\text{MeOH}}=62\pm 4$  kJ mol<sup>-1</sup>, compared with the data reported in [Table 1](#)) than for  $\beta$ -**CD**. Clearly, both these findings are perfectly consistent with the lack of any inner solvent–complex interaction when the narrowest  $\alpha$ -**CD** host is involved.

### 3. Conclusions

The addition of methanol as organic co-solvent significantly affects the binding equilibria between native  $\beta$ -**CD** and *p*-nitro-aniline derivatives. In particular, general guest tilting and complex stiffening effects have been observed, which turn out in an unfavourable effect on the conditional inclusion entropies. On the other hand, the effects on the conditional inclusion enthalpies may be different, depending on the particular features of the guest ancillary chain (and, consequently, on the occurrence of different kinds of host–guest interactions). Nevertheless, all these effects can be suitably correlated with the intrinsic stabilities of the complexes in water, as accounted for by  $\Delta H_i^0$  values. A detailed analysis of experimental data led us to explain the observed behaviours in terms of a ‘dynamic co-inclusion’ of the solvent within the host–guest complex, with an effective competition of methanol versus water molecules.

As a final remark, it is important to stress, in our opinion, that the concept of a ‘dynamic co-inclusion’ of the solvent arises from an overall critical consideration of the role of the solvent system in the thermodynamic economy of the process. It seems evident, from the discussions developed previously, that a full participation of solvent molecules in the intimate dynamics of the inclusion complex, seriously questions a strict distinction between ‘nominal’ and ‘environmental’ processes. Although such a distinction has constituted up to now the implicit interpretative framework of binding phenomena, it appears somehow artificial under the light of our results. This implies, in turn, that current views on solvation effects are still too limited, indeed, and probably need to undergo a thorough reconsideration in the future.

## 4. Experimental

### 4.1. Materials

All reagents, solvents (HPLC grade) and materials needed were used as purchased, without further purification. Guests **1–8** were prepared, purified and characterized as described elsewhere.<sup>1e,4</sup> Cyclodextrins were dried before use in vacuo over P<sub>2</sub>O<sub>5</sub> at 90 °C for at least 24 h, and stored in the same apparatus at 40 °C.

### 4.2. Polarimetric measurements

A general protocol for performing the polarimetric determination of binding constants has been described in our previous papers.<sup>17</sup> The standard procedure provides the preparation of a set of sample solutions, by adding variable micro-amounts (up to 150  $\mu$ L) of a concentrated guest solution (usually ca. 0.3 M) to fixed volumes (5 mL) of a standard  $\beta$ -**CD** solution (usually 2.0 mM). For

the aims of this work, suitable standard  $\beta$ -CD solutions were prepared by dissolving a weighted amount of dry  $\beta$ -CD into the proper water–methanol mixture. Nevertheless, in order to achieve a more reliable estimate of low ( $\leq 200 \text{ M}^{-1}$ )  $K_{\text{cond}}$  values, in some cases we slightly modified the procedure for preparation of sample solutions, by directly dissolving weighed amounts of the appropriate guest into the  $\beta$ -CD solutions. Whatever the procedure chosen, polarimetric data were subjected to suitable fitting analysis as described elsewhere.<sup>17</sup>

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### Supplementary data

The complete polarimetric data and a discussion about the linear dependence of  $\partial\Delta H_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  and  $\partial T\Delta S_{\text{cond}}^0/\partial\chi_{\text{MeOH}}$  on  $\Delta H_{\text{f}}^0$  are reported. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.01.008.

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- Noticeably,  $\Delta\theta$  data for guests **4** and **5** are conveniently fitted by means of a second-order (parabolic) polynomial. Therefore, the value of  $\partial\Delta\theta/\partial\chi_{\text{MeOH}}$  (referred to pure water) is given by the coefficient of the first-order term.
- It is interesting to notice that all the substrates belonging to the first subset possess a poorly hydrophobic, conformationally flexible ancillary chain able to donate/accept two hydrogen bonds simultaneously. On the other hand, the substrates belonging to the second subset have ancillary chains unable to form multiple hydrogen bonds for various reasons: **4** and **5** possess only one donor/acceptor site, whereas the ammonium cationic head group of **6–8**, presumably, is too strongly solvated. Moreover, the occurrence of particularly effective hydrophobic interactions between the CD cavity the ancillary chains of **7** and **8** is likely.
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- Of course, the binding equilibrium is per se a dynamic process. Therefore, it is implicit that the solvent swap process is 'fast' in the lifetime scale of the inclusion complex.