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Modulation of pK_a by cyclodextrins; subtle structural changes induce spectacularly different behaviors

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Tuning of acidity-related properties by host-guest complexation is one of the most promising concepts in current supramolecular chemistry. However, still little is known about structural effects which determine direction and magnitude of supramolecular pK_a shifts. Here we present the first systematic comparison of cyclodextrin-induced pK_a shifts with a focus on minor structural differences between guests – phenolic drug warfarin and its six isomeric derivatives, and hosts – various structurally similar cyclodextrins. Warfarin and five hydroxywarfarins exert upward pK_a shifts upon complexation with β-cyclodextrin and its neutral derivatives. However, the magnitude of these shifts depends on cyclodextrin substituent, and even, on average substitution degree. The strongest shifts are observed for methyl-β-cyclodextrin, they are among the greatest cyclodextrin-induced pK_a shifts noted so far. By contrast, 10-hydroxywarfarin exhibits only minor shifts whose direction is, surprisingly, dependent on temperature. Furthermore, the temperature variations of pK_a show that endothermic dissociation is observed for 2-hydroxypropyl-β-cyclodextrin, similarly as in the host-free state, while it becomes exothermic for methyl-β-cyclodextrin. In other words, acid dissociation of two structurally similar host-guest complexes is characterized by dramatically different enthalpic contribution. Finally, some enantioselective effects are also observed. We infer that intramolecular hydrogen bonds and enantioselective interactions with portal cyclodextrin groups are likely crucial for these phenomena. Our work may open up new horizons in understanding of structural effects in the supramolecular pK_a tuning.

Introduction

Strategic modulation of acid-base properties of weakly acidic or basic compounds by complexation with macrocyclic hosts like cyclodextrins, curcubiturils or calixarenes, is one of the most promising concepts in current supramolecular chemistry.[1,2] Such controllable pK_a shifts can be employed in modern drug delivery systems to enhance bioavailability and therapeutic activity of drugs,[1-4] in host-assisted catalysis dependent on protonation state of substrate,[5,6] in sophisticated separation methods exploiting different ionization state of analytes, for example stereoisomers,[7,8] in modification of physicochemical properties of dyes,[2,9,10] or in other pH-responsive systems like in a molecular self-assembly.[2] In spite of many experimental evidences on the complexation-induced pK_a shifts obtained with various hosts,[1] especially for weakly basic nitrogen-containing guests, there is relatively little understanding of molecular structure-related mechanisms determining such phenomena.[9-13] It is still not clear what are decisive factors determining absolute magnitude of these shifts, how much this mechanism depends on chemical nature of guests and hosts, how much these effects vary for structurally-related guests and hosts, and in particular, what is the link between pK_a shifts and the enthalpic or entropic effects driving them. Filling the gap in knowledge in this area is a promising idea in the light of further applications based on, ultimately, fully predictable pK_a shifts.

Cyclodextrins (CDs), the only macrocycles which for now are in a common use in drug delivery, food industry, and separation methodology,[14,15] are known to induce relatively weak pK_a shifts, mainly below one pH unit.[7-9,16-22] However, in our recent work we postulated that for a phenolic drug warfarin (WAR) the shift induced by methyl-β-CD (Me-β-CD) reaches far over +1 pH unit, while 10-hydroxywarfarin (W10) which is one of the major in vivo metabolites of WAR possessing one additional oxygen atom, remains somehow uninvolved in such inducement.[7]

In the present study those very interesting effects observed initially in the arbitrary chosen experimental conditions have been thoroughly examined in the context of other hydroxywarfarins and CDs. It is to be highlighted that this is the first such broad comparison of pK_a shifts induced by closely related hosts and guests. We have also determined thermal variations of pK_a values for the selected systems, and

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Electronic Supplementary Information (ESI) available: [detailed information about composition of buffering solutions]. See DOI: 10.1039/x0xx00000x

calculated enthalpic and entropic factors that contribute to dissociation. Moreover, some stereoselective effects have also been identified. The capillary electrophoresis has been used as an experimental technique.[23]

It is noticeable that to date none systematic work has been done to investigate how much host-induced pK_a shifts may differ between various structural isomers of guests. Therefore, as guests molecules we have used warfarin and its six derivatives which are regio-isomers: 3'-hydroxywarfarin (W3), 4'-hydroxywarfarin (W4), 6-hydroxywarfarin (W6), 7-hydroxywarfarin (W7), 8-hydroxywarfarin (W8), and 10-hydroxywarfarin (W10). The reference thermodynamic pK_a values in the host-free medium of all these compounds except W10 fall in the range between 4.97-5.16, while for W10 it is 5.95.[24] These values correspond to the more acidic phenolic hydroxyl group, common for all molecules. In order to compare the effects caused by different structural variants of the same type of host, several CDs have been used: β-CD, 2-hydroxypropyl-β-CD (2HP-β-CD, 0.5-1.3 substitution degree per glucose unit), methyl-β-CD (Me-β-CD, 1.6-2.0 substitution degree), heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD), and two different sulphated-β-CDs (12 or 12-15 sulfate groups per CD unit). These CDs have been selected to check whether differently substituted CDs may exert different magnitude of pK_a shifts. Additionally, α-CD and sulphated-α-CD (11 sulfate groups per CD unit) have been used to check the relevance of CD cavity size. We have determined apparent pK_a shifts induced by each CD at 10 mM final concentration, added to aqueous buffers of constant 25 mM ionic strength.

Materials and methods

Materials

Warfarin (WAR), the racemic mixture, was supplied by Sigma-Aldrich (St. Louis, MO, USA). 3'-hydroxywarfarin (W3), 4'-hydroxywarfarin (W4), 6-hydroxywarfarin (W6), 7-hydroxywarfarin (W7), 8-hydroxywarfarin (W8), and 10-hydroxywarfarin (W10), all the racemic mixtures, were supplied by LGC Standards (Teddington, UK). Cyclodextrins (CDs): α-CD, β-CD, 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD, 0.5–1.3 substituted groups per glucose unit), Me-β-CD, 1.6–2.0 substituted groups per glucose unit), heptakis(2,3,6-*O*-trimethyl)-β-cyclodextrin (TM-β-CD), and β-cyclodextrin sulfated sodium salt (12-15 substituted sulfate groups per CD unit) were supplied by Sigma–Aldrich (St. Louis, MO, USA), whereas highly sulfated-β-CD (12 substituted sulfate groups per CD unit) and highly sulfated-α-CD (11 substituted sulfate groups per CD unit) by Beckman Coulter (Beckman Coulter, Brea, CA, USA). All other chemicals were supplied by Avantor Performance Materials Poland. S. A. (Gliwice, Poland). All standard solutions were prepared in the deionized water (MilliQ, Merck-Millipore Billerica, MA, USA) and filtered through the 0.45 µm regenerated cellulose membrane, then degassed by centrifugation. The standard concentration of analytes in injected samples was 0.2 mg/mL. All analytes were dissolved in water/methanol (1:1 v/v) mixture to prepare the stock solution of concentration 1 mg/mL, then further dilution was made by mixing of the proper stock solution volumes with deionized water and electroosmotic flow (EOF) marker. Dimethyl sulfoxide (DMSO) was used as the EOF marker in 0.2% (v/v) concentration.

Instrumentation

Experiments were performed on the P/ACE MDQ Capillary Electrophoresis (CE) System (Beckman Coulter, Brea, CA, USA) equipped with a diode array detector (DAD). The bare fused-silica capillaries of 60 cm total length, 50 cm effective length and 75 µm internal diameter were used. Sample injection was conducted using forward pressure 0.5 psi for 5 s. During separations, 30 kV voltage (normal polarity) and the additional forward pressure of 0.3 psi were applied. The capillary was conditioned at given temperatures (15, 20, 25, 30 and 40°C) using the liquid cooling system. Every time by using DAD detector the whole absorbance spectra were collected between 200-600 nm. Signal recorded at 282 nm and 306 nm was used for the further analysis.

The capillary rinsing between runs was conducted applying pressure of 137.9 kPa (20 psi). The procedure included the step of 0.1 M NaOH for 1 min, and background electrolyte (BGE) for 2 min. During the first use of the capillary at a working day: methanol for 5 min, 0.1 M HCl for 2 min, deionized water for 2 min, 0.1 M NaOH for 10 min, and BGE for 10 min were applied. For the fresh capillary conditioning, the latter sequence was used but the duration of each individual step was doubled.

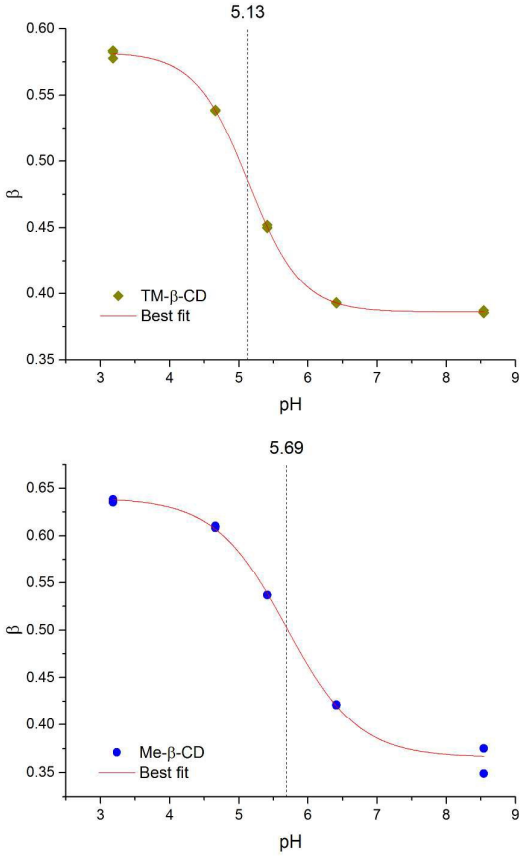
Buffering solutions

The BGEs of the same ionic strength 100 mM were prepared according to the receipts given in Table S-1 (Electronic Supplementary Information). For separations, BGEs were diluted to 25 mM ionic strength by mixing with deionized water. In the particular cases the buffers were enriched by addition of CDs at final 10 or 30 mM concentration. Every time the pH value was measured after mixing of all constituents, prior CE analysis. In measurements conducted at 10 mM concentration of CD, 5 BGEs of different pH were used, while in measurements at 30 mM concentration, 8 BGEs of different pH were applied. It was done to improve accuracy of pK_a determination when thermodynamic analysis was conducted.

Methods of pK_a determination

For our experimental investigation we have applied capillary electrophoresis (CE), a non-conventional method for pK_a determination, offering however many advantages and getting more and more popularity in recent years.[23] First of all, CE hyphenated with DAD detection system enables an accurate determination of pK_a values in the two independent ways, based on the changes in electrophoretic mobilities related to the changes in ionization state, and based on the changes in absorbance spectra, similarly as in the spectrophotometric titration method. Secondly, CE is fully automated, it uses only minute amounts of samples, and

enables the measurements to be conducted in precisely controlled temperature. Finally, the most prominent feature is



that pK_a values could be, in CD-containing media, determined separately for stereoisomers.

Figure 1. The relation between β values and pH obtained for W8 in the media containing Me-β-CD and TM-β-CD respectively.

In this work the values of pK_a were determined by using both accessible methods: one based on effective electrophoretic mobilities (μ_{eff}), and another one based on absorbance spectra. In the former method the plots of μ_{eff} values versus pH were used to function fitting (by Origin software) and finding the inflection point indicating the value of pK_a, according to the equation:

$$\mu_{eff} = \left[\frac{\alpha \cdot 10^{-pK_a}}{10^{-pK_a} + 10^{-pH}}\right] \quad (1)$$

where α is a fitting parameter.

The values of μ_{eff} were obtained from the following equation:

$$\mu_{eff} = \mu_{obs} - \mu_{eof} = \frac{L_{tot} \cdot L_{eff}}{V} \cdot \left(\frac{1}{t_{obs}} - \frac{1}{t_{eof}}\right) \quad (2)$$

where μ_{eff} and μ_{obs} are the effective and observed electrophoretic mobilities of analyte ($m^2\ V^{-1}\ s^{-1}$), respectively; μ_{eof} is the mobility of electroosmotic flow ($m^2\ V^{-1}\ s^{-1}$); L_{tot} and L_{eff} are the total and effective capillary lengths (m), 0.60 m and 0.50 m, respectively; V is the separation voltage (V); t_{obs} is the measured migration time of analyte (s), while t_{eof} is the time measured for neutral marker of EOF – DMSO (s).

In the latter method the values of absorbance recorded at the maxima of electrophoretic peaks were used in calculation of the β parameter, defined by equation:

$$\beta = \frac{A_{282}}{A_{282} + A_{306}} \quad (3)$$

where A_{282} and A_{306} are the values of absorbance at the given wavelength (nm).

Due to different shapes of spectra of the neutral and ionized forms of WAR and hydroxywarfarins, the value of β changes with the growing ionization of analyte (growing pH) similarly as the value of μ_{eff}. Therefore, the values of β were processed analogously to μ_{eff} including their plotting versus pH, function fitting of the same type and finding of inflection point indicating pK_a. Fig.1 shows the examples of pK_a calculation using this method. Both μ_{eff} and β were calculated for the three analytical runs conducted in the same conditions. In our recent work we proved that both methods are mutually consistent and give very similar pK_a values,[24] and that they can be used interchangeably according to the current needs.

In all cases we tried to calculate pK_a from both methods simultaneously. The spectrophotometric method gave better precision (lesser errors during function fitting). Importantly, this method is sensitive only on the first dissociation of hydroxywarfarins, related to the hydroxyl group common with WAR.[24] For that reason the potential errors deriving from interference with the second dissociation could be avoided. All pKa values presented in this manuscript have been obtained using the spectrophotometric method, unless stated otherwise.

Calculation of ΔH° and ΔS°

The values of ΔH° and ΔS° were calculated from the Van't Hoff model describing the relation between pK_a and temperature:

$$pK_a = \frac{\Delta H^\circ}{2.303RT} - \frac{\Delta S^\circ}{2.303R} \quad (4)$$

where ΔH° is a standard dissociation (deprotonation) enthalpy ($J \cdot mol^{-1}$), R is the gas constant (8.3145 $J \cdot mol^{-1} \cdot K^{-1}$), and ΔS° is a standard dissociation (deprotonation) entropy ($J \cdot mol^{-1} \cdot K^{-1}$).

Accordingly, the pK_a values determined at various temperature were plotted against the inverse absolute temperature (1/T) and fitted by the linear function. Subsequently the ΔH° and ΔS° terms were calculated from the slope and intercept, respectively.

Results and discussion

Overall comparison

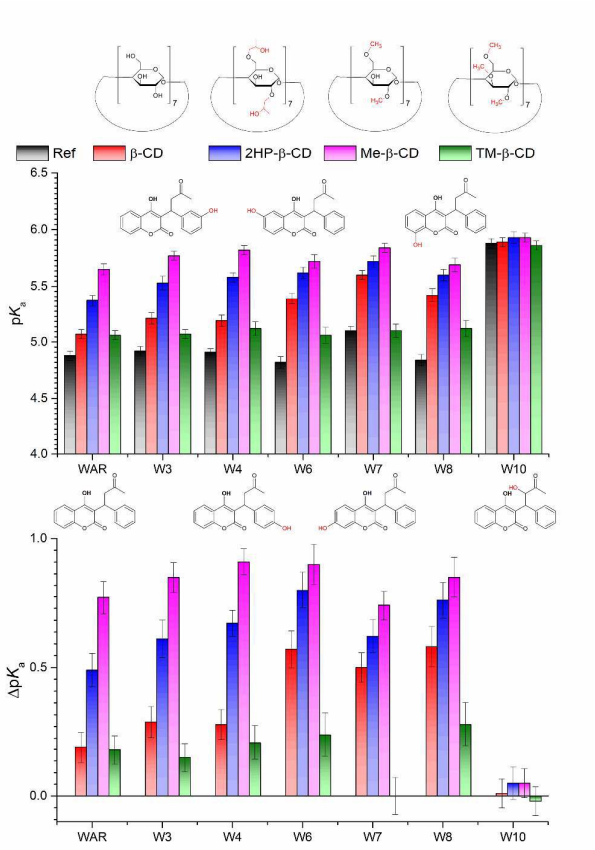


Figure 2. The pK_a values and related pK_a shifts obtained for the various guests and hosts.

At the first point, the apparent pK_a values have been determined at 10 mM concentration of hosts, at temperature of 298 K. All possible host-guest combinations have been tested. None or very small downward pK_a shifts below 0.2 pH unit have been obtained for α-CD and all sulphated CDs, respectively (data not shown). The lack of pK_a change for α-CD can be explained by its smaller cavity size, and consequently, none or only marginal complexation degree. The sulphated CDs, in turn, are the only non-neutral hosts studied in this research. Owing to their large negative charge, they are able to influence ionic strength of buffer to such extent that dissociation of a proton is noticeably facilitated by increased concentration of ionized species. The observed pK_a shifts are fully consistent with the changes which can be readily predicted by using Debye-Hückel theory.[25] Due to this fact one can assume that no notable complexation-related pK_a shifts take place in these systems.

By contrast, significant upward shifts have been noticed for the other CDs. They have been illustrated in Fig.2. As it can be seen, the magnitude of pK_a shifts rise in the order TM-β-CD < β-CD < 2HP-β-CD < Me-β-CD for each molecule. The relative changes between given CDs are very large, till now unobserved. Interestingly, partial methylation of β-CD (Me-β-CD) increases pK_a shifts even several times comparing to unsubstituted β-CD, while total methylation (TM-β-CD) decreases or even entirely suspends them (W7). It is also intriguing that W10 as the only one exhibits very small, barely measurable pK_a changes. One may assume at this point, that for this molecule there is none mechanism causing host-induced pK_a shifts. Differences in pK_a shifts between the other guests are noticeable virtually for each CD type. In the case of β-CD, for instance, the shifts noted for WAR, W3 and W4 are notably smaller than those observed for W6, W7 and W8. Remarkably, the results obtained for WAR complexed with the native β-CD are consistent with the shifts observed for these molecules by Datta and co-workers.[21,22] These outcomes imply that in each case the neutral form of all guests except W10 exerts significantly stronger affinity of binding than the ionized form, therefore the upward pK_a shifts take place. The type and number of CD substituents play significant role in this mechanism. Owing to the magnitude of possible host-guest interactions, its comprehensive investigation is difficult and it is not the current purpose of this work.

Thermodynamic analysis

To shed some ligth on mechanistic aspects of pK_a shifts, the second experiment has been carried out, in which thermal dependencies of pK_a values in the selected systems have been studied. We have decided to use the two CDs for which the greatest shifts have been obtained: Me-β-CD and 2HP-β-CD, and the two guests for which the explicitly opposite behaviors have been noted: WAR and W10. This time CDs have been applied at increased concentration to 30 mM, in order to keep complexation degree constant through the range of pH and temperature, and as high as possible (this issue is discussed more deeply in Section "Complexation degree"). Hence, one may assume that at this concentration given pK_a indicates "true" parameter valid for a host-guest complex, not only for a mixed population of the free and complexed forms. The obtained pK_a values have been afterwards plotted against inverse absolute temperature (Van't Hoff plots), what has enabled calculation of additional thermodynamic parameters: standard dissociation enthalpy – ΔH° and standard dissociation entropy – ΔS°. The Van't Hoff plots have been presented in Fig.3, while the values of thermodynamic parameters have been gathered in Table 1.

It is seen that the strong upward pK_a shifts are noted for WAR in the whole range of temperature, for Me-β-CD they reach even over one pH unit. Remarkably, they are among the greatest shifts noted hitherto for CDs.[1] W10, contrary to WAR, exerts appreciably lower shifts, and their direction is, surprisingly, dependent on temperature. This is, to the best of our knowledge, the first demonstration that direction of supramolecular pK_a shifts upon host-guest complexation is controlled directly by temperature. It shows also, that W10 is involved in some mechanism that alters its acid dissociation dynamics, although in the previously experiment such effect was not clearly visible. In addition, an opposite change of pK_a values across temperature has been noted for 2HP-β-CD and Me-β-CD, i.e. drop or rise of values with increase in temperature, respectively. It implies that deprotonation in the complexed state is either endothermic (2HP-β-CD), analogously to the host-free state, or exothermic (Me-β-CD), depending on the CD substituent. Finally, significant differences are observed for the WAR enantiomers complexed with Me-β-CD, including the magnitude of shifts and enthalpic contributions. However, absolute configuration of the enantiomers is unknown.

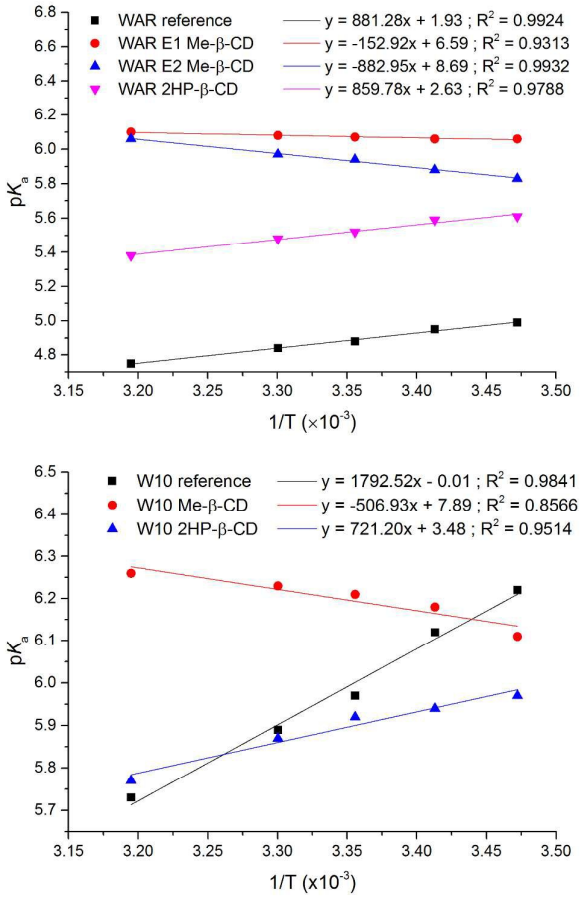


Figure 3. Van't Hoff plots obtained for WAR and W10 in the host-free and host-containing media, between 288 K and 313 K, E1 and E2 denote two enantiomers. The reference plots corresponding to CD-free medium have been taken from our different work (unpublished results).

Table 1. The values of thermodynamic parameters describing dissociation in the free and CD-bounded state, together with the selected pK_a shifts obtained in the particular systems.

guest	host	ΔH° (kJ·mol^{-1})	ΔS° (J·mol^{-1}·K^{-1})	ΔpK_a at 288 K	ΔpK_a at 313 K
WAR	2HP-β-CD	+16.5	-6.1	+0.62	+0.63
	Me-β-CD[a]	-15.8; -2.9	-20.0; -15.2	+1.07; +0.84	+1.35; +1.31
	none[b]	+16.9	-4.4	-	-
W10	2HP-β-CD	+13.8	-8.0	-0.25	+0.04
	Me-β-CD	-9.7	-18.8	-0.11	+0.53
	none[b]	+34.3	0.0	-	-

[a] Double values refer to the enantiomers. [b] The values corresponding to the host-free state have been taken from our different work (unpublished results).

Similar enthalpic factors noted for WAR in the host-free and 2HP-β-CD-containing systems and consequently almost constant pK_a shifts across temperature indicate, that entropic factors play dominant role in these shifts (see Table 1). In Me-β-CD-containing system, however, the situation changes dramatically. The enthalpic terms change their sign, while the entropic contributions become far more negative. Therefore the strong upward pK_a shifts are also determined by dissociation entropy, which upon complexation becomes energetically more unfavorable and thus increases pK_a, but a strong enthalpy-entropy compensation accounts for magnitude of the final pK_a shifts. Interestingly, the parameters obtained for complexed W10 are quite similar to WAR for both CDs, and thereby differ considerably from the reference values corresponding to the CD-free state. It indicates that for W10 the changes in thermodynamics and potential supramolecular effects are probably stronger than for WAR, despite lower pK_a shifts.

To evaluate the accuracy of our results, the pK_a shifts obtained using the two different CE-based methods have been thoroughly examined. They have been gathered in Table 2.

Table 2. The values of pK_a shifts and their thermal dependency obtained from the two different methods exploiting CE technique.

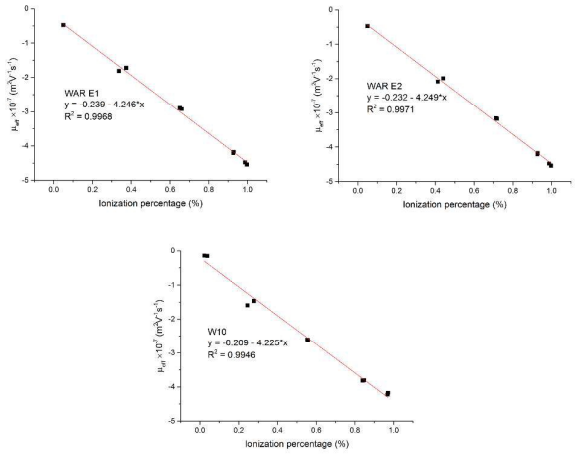
host:	Me-β-CD			2HP-β-CD	
Spectrophotometric (DAD) method					
temperature (°C)	WAR E1	WAR E2	W10	WAR	W10
15	+1.07 (0.03)	+0.84 (0.03)	-0.11 (0.04)	+0.62 (0.03)	-0.25 (0.03)
20	+1.11 (0.02)	+0.93 (0.03)	+0.06 (0.04)	+0.64 (0.02)	-0.18 (0.04)
25	+1.19 (0.04)	+1.06 (0.02)	+0.24 (0.05)	+0.64 (0.02)	-0.05 (0.03)
30	+1.24 (0.03)	+1.13 (0.04)	+0.34 (0.04)	+0.64 (0.04)	-0.02 (0.03)
40	+1.35 (0.05)	+1.31 (0.04)	+0.53 (0.04)	+0.63 (0.04)	+0.04 (0.03)
Electrophoretic mobility-based method					
temperature (°C)	WAR E1	WAR E2	W10	WAR	W10
15	+1.02 (0.06)	+0.82 (0.05)	-0.20 (0.05)	+0.63 (0.06)	-0.19 (0.05)
20	+1.07 (0.08)	+0.90 (0.04)	-0.10 (0.06)	+0.67 (0.05)	-0.13 (0.04)
25	+1.08 (0.07)	+0.93 (0.07)	+0.03 (0.05)	+0.64 (0.05)	-0.08 (0.07)
30	+1.10 (0.07)	+0.99 (0.06)	+0.17 (0.06)	+0.59 (0.07)	-0.03 (0.08)
40	+1.14 (0.08)	+1.08 (0.06)	+0.33 (0.06)	+0.59 (0.08)	+0.02 (0.05)

E1 and E2 denote two enantiomers. The standard errors are presented in the brackets.

It is to be highlighted that the overall magnitude of pK_a shifts and their behaviors along increase in temperature are evidently consistent. Particularly, the second method confirms that direction of changes for W10 is strictly temperature-dependent. In addition, the considerable role of enthalpic effects is visible from the rise of Me-β-CD-induced pK_a shifts of WAR with temperature, evidently different in magnitude for the two enantiomers. Nevertheless, this thermal effect appears to be weaker when the electrophoretic mobility-based method is considered. This may be explained by some undesirable deviations of electrophoretic mobility at higher temperatures related to insufficient capillary cooling, especially at the inlet and outlet sections.

The temperature-dependent direction of pK_a shifts noted for W10 originate most likely from the concurrent operation of two opposite mechanisms, favoring and disfavoring the protonated form of W10 in the complex. At higher temperature when direction of shifts is consistent between WAR and W10, the protonation-stabilizing effects dominate, while at lower temperature when pK_a shifts of W10 become negative, the opposite pK_a-lowering effects are stronger. The protonation-stabilizing effects, due to the similar stereoelectronic structure of both compounds, seem to be analogous to WAR. In contrast, the nature of pK_a-lowering effects must refer to the only distinctive feature of W10, such feature is the additional hydroxyl group enabling the specific type of intramolecular interactions – OH•••OH•••O.[7,24] It is very important observation that the values of ΔH° and ΔS° become similar for WAR and W10 in the presence of 2HP-β-CD and Me-β-CD, confirming that some phenomenon reduces the enthalpic and entropic effects distinguishing WAR and W10 in the host-free medium. It seems to be logical that such phenomenon would be the specific interaction with host leading to weakening of the strong double intramolecular hydrogen bonding, since such bonding causes probably the huge discrepancy of ΔH° and ΔS° observed between WAR and W10 in the host-free medium. It would entail destabilization of the proton and drop of pK_a.

As far as surprising differences between Me-β-CD and 2HP-β-CD are concerned, they background becomes still unclear and enigmatic. Unarguably, appreciably more negative deprotonation enthalpies obtained for Me-β-CD should be ascribed to the presence of numerous methyl substituents, and specific enantioselective host-guest interactions. One may suppose that it is somehow related to release of "high energy water molecules" from CD cavity upon ionization of guest, that may decrease the system enthalpy due to reorganization of water molecules.[10] In order to verify above reasoning, however, further experimental and theoretical work is necessary.



Complexation degree

Figure 4. The plots of electrophoretic mobility versus approximated ionization percentage for WAR and W10 complexed with Me-β-CD at 288 K. E1 and E2 denote the two enantiomers of WAR.

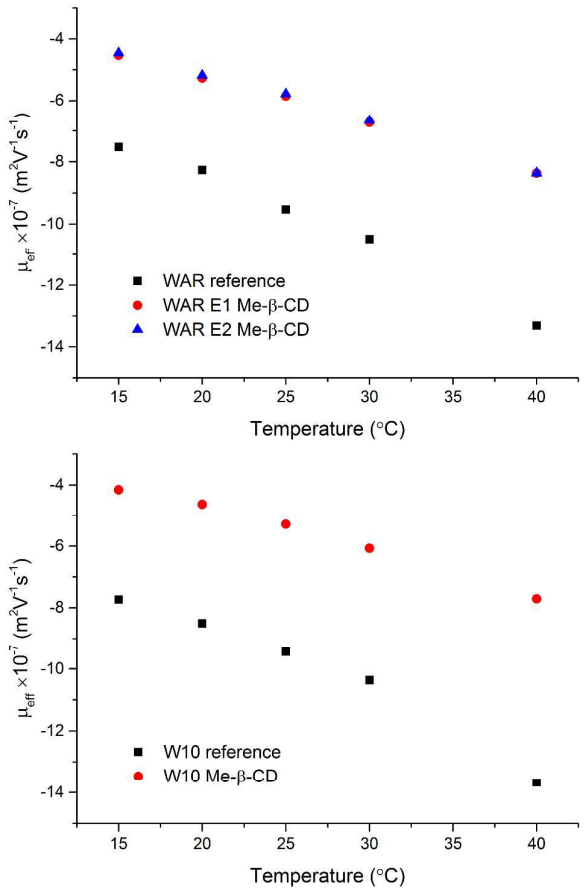


Figure 5. The relation between electrophoretic mobility of WAR (top) or W10 (bottom) and temperature assessed for the fully ionized forms of compound, in the free and complexed state with Me-β-CD. E1 and E2 denote the two enantiomers.

Application of the capillary electrophoresis technique for pK_a determination gave us possibility to monitor concurrently the absorbance spectra and the changes in electrophoretic mobility of the complexed species across the range of pH. The data obtained with the use of 30 mM Me-β-CD and 2HP-β-CD suggest that the complexation percentage of both WAR and W10 actually does not change across pH and temperature range owing to a large excess of the host with respect to WAR and W10 population. It is deducible from the following observations.

The value of θ corresponds to shape of absorbance spectrum thus also to ionization percentage, which can be easily calculated. Then, the plot of electrophoretic mobility of analyte in the presence of CD versus ionization percentage can be created. It points to the relationship between charge-to-size ratio (electrophoretic mobility) and charge (ionization percentage). If this relation is linear throughout the range of pH, charge-to-size ratio changes only with respect to a charge component, but a size component corresponding to a hydrodynamic radius is constant. As it can be seen from Fig.4, a good linearity has been obtained for WAR and W10, what implies that in these conditions the complexation percentage is approximately constant throughout pH. Moreover, similar slope of the fitted function is indicated by both WAR enantiomers and W10. This in turn shows, that at 30 mM concentration of CD two compounds exhibiting potentially different affinity are complexed in the similar degree. Hence, most probably, the saturation is actually almost total at this concentration of host (determined pK_a values equal to "true" values that characterize host-guest complex when all guest molecules are associated with CD molecules).

We have also studied electrophoretic mobility of the free and the complexed species at basic pH (9.0) when both species are totally ionized, in relation to temperature (Fig.5). As we see the ratio between two mobilities is approximately constant throughout temperate range. It confirms that the ratio of hydrodynamic radii of both forms is also constant, so that the same is true for the complexation degree.

Similar results have been also obtained for 2HP-β-CD (data not shown). Based on these observations one can assume that apparent pK_a values determined at 30 mM concentration of CDs at variable temperature refer to approximately constant complexation percentage, and most importantly, deprotonation enthalpies and entropies calculated from these values refer only to the process of deprotonation and not to complexation/dissociation. This allows us to consider the host-guests complexes as stable during ionization. In other words, any structural changes reflected by ΔH° and ΔS° do not result from complexation/dissociation process, but rather from other ionization-related effects.

Conclusions

In conclusion, we have demonstrated for the first time that structurally very similar guests and hosts may exhibit unprecedentedly different behaviors in supramolecular pK_a-modulation systems. It occurs that minor structural changes are capable to affect appreciably the basic thermodynamic forces that govern acid dissociation process. It has been revealed that hydroxylation site of guest, substitution type of host, and even average substitution degree may have a great importance for direction and magnitude of supramolecular pK_a shifts. Involvement of dissociating group of W10 in the specific intramolecular interactions, and potential intermolecular interactions of guests with the portal methyl groups of Me-β-CD are the most probable reasons for spectacularly different behaviors observed in the experiment. Our work has opened up a novel direction of research, which will be further continued in the near future. We plan to: extent the spectrum of guests and hosts tested experimentally, combine complexation and acid dissociation processes in a one thermodynamic cycle, and finally, to verify our hypothesis experimentally and by molecular modelling.

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Notes and references

1 I. Ghosh and W.M. Nau, *Adv. Drug Deliver. Rev.*, 2012, **64**, 764;
2 A. C. Bhasikuttan, H. Pal and J. Mohanty, *Chem. Commun.*,2011, **47**, 9959;
3 N. Saleh, A.L. Koner and W.M. Nau, *Angew. Chem. Int. Ed.*, 2008, **47**, 5398;
4 N. Saleh, M.A. Meetani, L. Al-Kaabi, I. Ghosh and W.M. Nau, *Supramol. Chem.*, 2011, **23**, 654;
5 C. Klock, R.N. Dsouza and W.M. Nau, *Org. Lett.*, 2009, **11**, 2595;
6 M.D. Pluth, R.G. Bergman and K.N. Raymond, *Science*, 2007, **316**, 85;
7 P. Nowak, M. Garnysz, M.P. Mitoraj, F. Sagan, M. Woźniakiewicz and P. Kościelniak, *J. Chromatogr. A*, 2015, **1377**, 106;
8 A.M. Rizzi, and L. Kremser, *Electrophoresis*, 1999, **20**, 2715;
9 M. Shaikh, Y.M. Swamy and H. Pal, *J. Photoch. Photobio. A*, 2013, **258**, 41;
10 F. Biedermann, W.M. Nau, and H.J. Schneider, *Angew. Chem. Int. Ed.*, 2014, **53**, 11158;
11 N. Barooah, J. Mohanty, H. Pal and A.C. Bhasikuttan, *J. Phys. Chem. B*, 2012, **116**, 3683;
12 V. Khorwal, B. Sadhu, A. Dey, M. Sundararajan and A. Datta, *J. Phys. Chem. B*, 2013, **117**, 8603;
13 N. Barooah, M. Sundararajan, J. Mohanty and A. Bhasikuttan, *J. Phys. Chem. B*, 2014, **118**, 7136;
14 K. Uekama, F. Hirayama and T. Irie, *Chem. Rev*, 1998, **98**, 2045;
15 J. Li and X.J. Loh, *Adv. Drug Deliver. Rev.*, 2008, **60**, 1000;
16 X.Y. Zhang, G. Gramlich, X.J. Wang and W.M. Nau, *J. Am. Chem. Soc.*, 2002, **124**, 254;
17 M. Shaikh, J. Mohanty, P.K. Singh, W.M. Nau and H. Pal, *Photochem. Photobiol. Sci.*, 2008, **7**, 408;
18 J. Mohanty, A.C. Bhasikuttan, W.M. Nau and H. Pal, *J. Phys. Chem. B*, 2006, **110**, 5132;
19 I. Enoch and M. Swaminathan, *J. Fluoresc.*, 2006, **16**, 697;
20 M. Casolaro, C. Anselmi and G.Picciocchi, *Thermochim. Acta.*, 2005, **425**, 143;
21 S. Datta and M.Halder, *J. Photoch. Photobio. B*, 2014, **130**, 76;
22 S. Datta, S. Panja, and M. Halder, *J. Phys. Chem. B*, 2014, **118**, 12153;
23 P. Nowak, M. Woźniakiewicz and P. Kościelniak, *J. Chromatogr. A*, 2015, **1377**, 1;
24 P. Nowak, P. Olechowska, M. Mitoraj, M. Woźniakiewicz and P. Kościelniak, *J. Pharm. Biomed. Anal.*, 2015, **112**, 89;
25 P. Debye and P. Hückel, *Phys. Z.*, 1923, **24**, 305;
26 M.V. Rekharsky and Y. Inoue, *Chem. Rev.*, 1998, **98**, 1875.

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