Medium Effect (Transfer Activity Coefficient) of Methanol and Acetonitrile on β-Cyclodextrin/Benzoate Complexation in Capillary Zone Electrophoresis

ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 2003

Impact Factor: 5.64 · DOI: 10.1021/ac026407z

4 AUTHORS, INCLUDING:

Salvatore Fanali
Italian National Research Council
301 PUBLICATIONS **7,896** CITATIONS
SEE PROFILE

Ernst Kenndler
University of Vienna
258 PUBLICATIONS **6,364** CITATIONS
SEE PROFILE

Anal. Chem. **2003,** *75,* 1645−1651

Medium Effect (Transfer Activity Coefficient) of Methanol and Acetonitrile on β-Cyclodextrin/Benzoate Complexation in Capillary Zone Electrophoresis

Simo P. Porras,[†] Karim Sarmini,[†] Salvatore Fanali,[‡] and Ernst Kenndler*,[†]

Institute for Analytical Chemistry, University of Vienna, Vienna, Austria, and Istituto di Metodologie Chimiche del C.N.R., Rome, Italy

Association constants, K_c, were derived from the electrophoretic mobilities of the anionic solutes (seven benzoates with hydroxy or chloro substituents) by capillary zone electrophoresis in different solvent systems, consisting of binary mixtures of water with up to 20% (v/v) methanol or acetonitrile, respectively. The association constants expectedly are found to decrease with increasing organic solvent concentration. The effect of organic solvents on the K_c of the benzoates with β-cyclodextrin was analyzed applying the concept of the transfer activity coefficient (or the medium effect). This concept enables the evaluation of the significance of the contributions of the individual species involved in the complexation equilibrium in the different solvents: the benzoate ion, β-cyclodextrin, and the anionic benzoate−β-cyclodextrin complex. The medium effect on benzoate was calculated from the change in acidity constant of benzoic acid in the different mixed solvents and the corresponding transfer activity coefficients of the proton and the molecular acid. The transfer activity coefficients for β-cyclodextrin results from its solubility at saturation in the different solvents. In this way, an estimation of the standard free energy of transfer, ΔG_t^0, of each species involved in the complexation equilibrium was possible for the transfer from water into the respective mixed solvent. It was found that the organic solvents do not significantly affect ΔG_t^0 for the benzoate anion. However, the organic solvents play a different role concerning the stabilization of β-cyclodextrin and the complex anion: whereas the addition of acetonitrile has nearly no influence on ΔG_t^0 of the anionic complex, the reduction in K_c is caused by the enhanced stabilization of β-cyclodextrin (reflected by its better solubility). Addition of methanol, on the other hand, lowers the solubility of β-cyclodextrin, thus giving positive values for ΔG_t^0. Thus, the overall effect on K_c in methanolic solutions must be related to the pronounced destabilization of the benzoate−β-cyclodextrin complex.

Cyclodextrins are oligosaccharides with a truncated cone shape and a cavity, able to undergo complexation with a wide number of compounds. Cyclodextrins are advantageously used in different fields, for example, in pharmaceutical technology and the cosmetic and food industries, etc. In separation methods, they are applied as complexing agent to increase selectivity, especially for chiral resolution. During the past years, cyclodextrins have been well-established in capillary electrophoresis, documented by hundreds of papers describing their application as additives to the background electrolyte (see, e.g., some relevant reviews[1−8]). For the purpose of a rational selection of the appropriate experimental conditions for separation, the knowledge of complexation constants is of importance.

The present paper deals with the influence of the solvent on the complexation or association constants, K_c, of analytes with cyclodextrin in aqueous and in mixed aqueous−organic solvents, which are derived by capillary zone electrophoresis (CZE). As solutes, several benzoic acid derivatives are used, and the additive is β- cyclodextrin. Electrophoretic mobilities of the analytes are measured in water and in binary aqueous−organic solvent mixtures with methanol; MeOH; or acetonitrile, ACN (between 0 and 20% v/v), and the constants are calculated as usual by curve-fitting to the appropriate binding equation. The observed association constants as a function of the content of organic solvent are discussed on the basis of the standard free energy of transfer of the individual species in the complexation equilibrium upon changing the solvent, demonstrated on one solute. This interpretation will be based on the concept of the medium effect and the transfer activity coefficient, describing how the stabilization of the individual species is responsible for the shift in the complexation constants. This concept is of general applicability, and although it was introduced into the field of capillary electrophoresis more than a decade ago for interpreting the change of acid−base

* Corresponding author address. Institute for Analytical Chemistry, University of Vienna, Währingerstr. 38, A 1090 Vienna, Austria. Tel: +43 1 4277 523 05. Fax: +43 1 4277 9523. E-mail ernst.kenndler@univie.ac.at.

[†] University of Vienna.

[‡] Istituto di Metodologie Chimiche del C.N.R..

(1) Fanali, S. *J. Chromatogr., A* **2000**, *875*, 89−122.
(2) Vigh, G.; Sokolowski, A. D. *Electrophoresis* **1997**, *18*, 2305−2310.
(3) Schneiderman, E.; Stalcup, A. M. *J. Chromatogr., B* **2000**, *745*, 83−102.
(4) Amini, A. *Electrophoresis* **2001**, *22*, 3107−3130.
(5) Chankvetadze, B.; Blaschke, G. *Electrophoresis* **2000**, *21*, 4159−4178.
(6) Rizzi, A. *Electrophoresis* **2001**, *22*, 3079−3106.
(7) Gübitz, G.; Schmid, M. G. *Electrophoresis* **2000**, *21*, 4112−4135.
(8) Mosinger, J.; Tomankova, V.; Nemcova, I.; Zyka, J. *Anal. Lett.* **2001**, *34*, 1979−2004.

10.1021/ac026407z CCC: $25.00
Published on Web 02/14/2003

equilibria in different solvent systems,[9−11] it is not common to evaluate the contributions of the species to complexation equilibria such as those under consideration. In the present paper, this concept is applied to gain insight into the cause of the change of the equilibrium constants of complexation between benzoate and cyclodextrin in aqueous and aqueous−organic solvents. For a detailed discussion of this concept, see for example, refs 12−17. Here, we will briefly describe the principles.

Medium Effect and Transfer Activity Coefficient. If a species, i, is transferred from one solvent to another (e.g., from water, W, to solvent, S), the difference of its chemical potential in the standard states in the two solvents can be expressed by the so-called transfer activity coefficients, ${}_{m}\gamma_i$, of the individual species, which is defined as

$$\ln {}_{m}\gamma_i = \Delta G^0_{t,i}/RT = ({}_{S}G^0_i - {}_{W}G^0_i)/RT \quad (1)$$

R is the gas constant; T, the absolute temperature; ${}_{m}\gamma_i$ is the ratio of the activity coefficients in the two solvents, ${}_{m}\gamma_i = {}_{W}\gamma_i/{}_{S}\gamma_i$, both at unit concentration. Its logarithm is named the medium effect. The medium effect reflects the stabilization of species i in the standard state in solvent S as compared to the standard state in water. If the species is more stable in water, the medium effect is positive, if negative, it is stabilized upon transfer into S. Knowledge of the medium effect for the individual species involved in an equilibrium gives a tool for the assessment of its contribution to the shift in equilibrium constants upon changing the solvent.

Accordingly, the standard free energy of transfer, ΔG^0_t, by transferring the equilibrium

$$dD + eE = uU + vV \quad (2)$$

with equilibrium constant

$$K = \frac{a_U^u a_V^v}{a_D^d a_E^e} \quad (3)$$

from water to solvent S is

$$\Delta G^0_t = {}_{S}G^0 - {}_{W}G^0 = (RT/2{,}3)\,(p_S K - p_W K) = (RT/2{,}3)\log \frac{{}_{m}\gamma_U^u\,{}_{m}\gamma_V^v}{{}_{m}\gamma_D^d\,{}_{m}\gamma_E^e} \quad (4)$$

a_i is the activity of the species. ${}_{S}G^0$ and ${}_{W}G^0$ represent the Gibbs free energy in the standard states in the particular solvent. It is the reversible work of transferring one mole of D and E from water to S, and to transfer one mole of U and V respectively, from S to water, all solutes at infinite dilution.

It can be seen that ΔG^0_t is related to the difference, $\Delta pK^{S-W} = p_S K - p_W K$, of the p$K$ values (the negative logarithm of the equilibrium constants) in the two solvents. Thus, ΔpK of the reaction depends on the transfer activity coefficients or medium effect of the individual species.

EXPERIMENTAL SECTION

Instrumentation. The CE instrument used was a programmable unit (P/ACE 2100 Beckman Instruments, Fullerton, CA) with a variable UV detector operated at 214 nm. An untreated fused-silica capillary (Polymicro Technologies Inc., Bloomfield, NJ.; i.d. 50 μm, o.d. 375 μm; total length 47.5 cm, length to detector window 40.8 cm) was applied in a thermostated cartridge at 25.0 °C during all measurements. A constant voltage of 10.0 kV with a linear voltage ramp-up set at 0.17 min was applied in the experiments. For data collection, Gold Software 3.0 (Beckman) was employed.

Capillaries were daily conditioned by rinsing 5 min with 100 mmol/L NaOH, 5 min with double-distilled water, and finally, with the running buffer for 5 min. Rinsing between consecutive runs for 1 min with double-distilled water and for 2 min with background electrolyte was performed with high pressure (140 kP). The capillary was stored empty overnight.

A 10 mmol/L phosphate (I = 20 mmol/L) buffer, made by mixing of 5 mmol/L Na_2HPO_4 and 5 mmol/L NaH_2PO_4 was used as the background electrolyte (BGE) throughout all measurements. Its pH was not measured further; according to the Henderson−Hasselbalch equation, it is 7 in water. Upon addition of the organic additive, it increases slightly, by only a few tenths of pK units under the experimental conditions. However, because the benzoic acids (with pK_a values smaller than 5 in water[18]) will exhibit about the same shift in pK_a as the phosphate, it can be assumed that the analytes are fully dissociated.

Prior to use, buffer and sample solutions were filtered through a filter with a 0.45-μm pore size (minisart-plus, Sartorius, Göttingen, Germany). Injections were performed in the low-pressure mode (3.5 kPa) for 1 s.

The viscosity was determined by pressurized mobilization (3.5 kPa) of a neutral marker (dimethyl sulfoxide) in the separation capillary; the viscosity of the fluid phase was related to its velocity according to the well-known Hagen−Poisseulle equation.

Chemicals. All aromatic acids used were purchased from EGA-Chemie (Steinheim, Germany) at the highest purity available. Na_2HPO_4, NaH_2PO_4, methanol, and acetonitrile (all analytical grade) were obtained from E. Merck (Darmstadt, Germany). β-Cyclodextrin was obtained from Fluka (Buchs, Switzerland). Water used in all experiments was distilled twice from a quartz apparatus. All samples had a concentration of 0.1 mmol/L, which is at least 25 times smaller than the lowest cyclodextrin concentration.

(9) Kenndler, E.; Jenner, P. *J. Chromatogr.* **1987**, *390*, 185−197.
(10) Kenndler, E.; Jenner, P. *J. Chromatogr.* **1987**, *390*, 169−184.
(11) Chiari, M.; Kenndler, E. *J. Chromatogr., A* **1995**, *716*, 303−309.
(12) Bates, R. G. *Determination of pH, Theory and Practice*; Wiley: New York, 1973; pp 211−253.
(13) King, E. J. *Acid−Base Equilibria*; Pergamon Press: Oxford, 1965.
(14) Kolthoff, I. M.; Chantooni, M. K. In *Treatise on Analytical Chemistry*; Kolthoff, I. M., Elving, P. J., Eds.; John Wiley & Sons: New York, 1979; Part I, Vol. 2, Sec. D, pp 239−302.
(15) Popovych, O. In *Treatise on Analytical Chemistry*, 2nd ed.; Kolthoff, I. M., Elving, P. J., Eds.; John Wiley & Sons: New York, 1979; Part I, Vol. 1, Sec. D, Chapter 12, pp 711−771.
(16) Šafařík, L.; Stránský, Z. *Titrimetric analysis in organic solvents*; Elsevier: Amsterdam, 1986.
(17) Izutsu, K. *Electrochemistry in Nonaqueous Solutions*; Wiley-VCH: Weinheim, 2002.
(18) Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants − A Laboratory Manual*; Chapman and Hall: London, 1984.

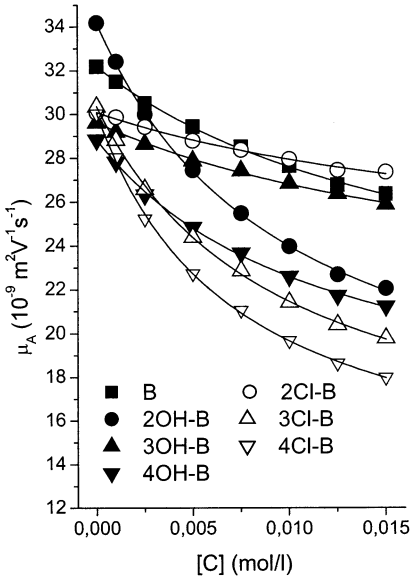

Figure 1. Mobility, μ_A, measured in the pure aqueous solvent as function of the concentration of β-cyclodextrin (C). Temperature of the capillary cartridge, 25 °C. The symbols of the benzoates give the position of the substituent (either hydroxy, OH, or chloro, Cl) in the aromatic ring. 4-Cl-B, for example, is 4-chlorobenzoate. The data were determined from measurements in duplicate, with a typical relative span in the percentage range. Fitted curves according to eq 5.

RESULTS AND DISCUSSION

Mobilities in Mixed Solvents. The mobilities of the solutes as a function of the concentration of MeOH and ACN are given in Figures 1−3 for different concentrations of β-cyclodextrin. In the symbols of the solutes, the number indicates the position of the substituent, either hydroxy, OH, or chloro, Cl, in the aromatic ring (e.g., 3Cl-B is 3-chlorobenzoate). The influence of the solvent on the mobilities in the presence of cyclodextrin will not be discussed here in detail. Briefly, complexation is based mainly on two types of interactions: lipophilic interactions of the solute with the inside of the cyclodextrin cavity, and hydrophilic interaction with the rim of the cavity. The present solutes, benzoates, are positioned such that the anionic group is outside the cavity of the cyclodextrin, and the aromatic ring is positioned inside. For uncharged benzoic acids, the situation can be the opposite, as shown by NMR measurements.[19,20]

Addition of MeOH (at zero cyclodextrin concentration) leads to a general decrease of the actual mobilities. Upon increase of the cyclodextrin concentration, the typical shape of the μ vs [C] curve is observed, with the steepest decay for 2OH-B and 4Cl-B for all mixed solvents. Compared to MeOH, a smaller reduction of the actual mobilities is found for ACN as cosolvent. This effect can in part be attributed to the smaller change in viscosity of the binary water−ACN mixture. Increase of the cyclodextrin concentration decreases the mobilities here as well, and the resulting dependence of the mobility on the cyclodextrin concentration is less pronounced than in the methanolic solvents (compare Figures 2 and 3). The steepest slopes in the ACN-containing systems are found for 4Cl-B. 3OH-B and 4OH-B, on the other hand, finally exhibit about constant mobilities between 5 and 20 mmol/L cyclodextrin at 20 vol % ACN.

Association Constants. There are several CE techniques to determine association constants of solute−ligand complexes from the mobilities of the particles involved. When the solutes are only partially charged (and partially neutral) the total mobility measured not only depends on the actual mobility of the free ion and the mobility of the ion−ligand complex, but also obviously on the effective mobility of the partially (de)protonated solutes, determined by the ionization constant, K_a, of the analyte and the pH of the BGE. However, under the pH conditions chosen, the solutes exist as fully ionized anions. Therefore, the mobility of free anion and of charged anion−cyclodextrin complex is relevant. It is clear that the association constant derived is that of the anion−cyclodextrin complex and not that of the complex of cyclodextrin with the uncharged molecular acid. It should be pointed out that 1:1 stoichiometry of complex formation is assumed and that the association constants are based on concentrations, not on activities. However, the difference between the thermodynamic constants and those based on concentrations seems negligible, because the activity coefficient of the neutral cyclodextrin is unit, and those of the ions in the equilibrium are similar; moreover, they cancel each other in the equation of the complex constant (see below).

The values of the association constants, K_c, were obtained according to Rundlett and Armstrong[21,22] from the measured mobilities as a function of the cyclodextrin concentration by nonlinear curve-fitting by the following equation,

$$\mu_A = \frac{\mu_{A^-} + \mu_{CA^-} K_C[C]}{1 + K_C[C]} \qquad (5)$$

where μ_A is the mobility measured at a certain ligand concentration, corrected by the viscosity of the solution containing the cosolvent and cyclodextrin as additive, as well. μ_{A^-} and μ_{CA^-} are the mobilities of the free anion, A^-, and the complexed solute, CA^-, respectively. Linear forms of eq 5[21,22] were also applied for the calculation. A rather good agreement with the constants derived from nonlinear fittings was found for the largest complexation constants. For the smallest constants, the respective agreement was, however, worse. This was probably due in part due to the use of unweighted mobility data for the linear fittings. The error in deriving the constants by nonlinear curve-fitting was in the range between 1 and 13 L/mol.

The resulting association constants, K_C, calculated from the mobilities by eq 5, are shown in Figure 4 and are dependent on the concentration of the organic solvent additive. 4Cl-B exhibits the highest constant, followed by 3Cl-B and 2OH-B, which is reasonable concerning the possibility for interaction of the solutes with cyclodextrin. The lipophilic interaction of the chloro groups with the alkyl moieties in the cavity seems to attenuate complex formation.

Addition of organic solvent leads to a nonlinear decrease of the equilibrium constants, steeper at lower and flatter at high solvent concentration. ACN has a more pronounced effect on the

(19) Simova, S.; Schneider, H.-J. *Perkin 2* **2000**, *8*, 1717−1722.
(20) Salvatierra, D.; Jaime, C.; Virgili, A.; Sanchez-Ferrando, F. *J. Org. Chem.* **1996**, *61*, 9578−9581.
(21) Rundlett, K. L.; Armstrong, D. W. *J. Chromatogr., A* **1996**, *721*, 173−186.
(22) Rundlett, K. L.; Armstrong, D. W. *Electrophoresis* **1997**, *18*, 2194−2202.

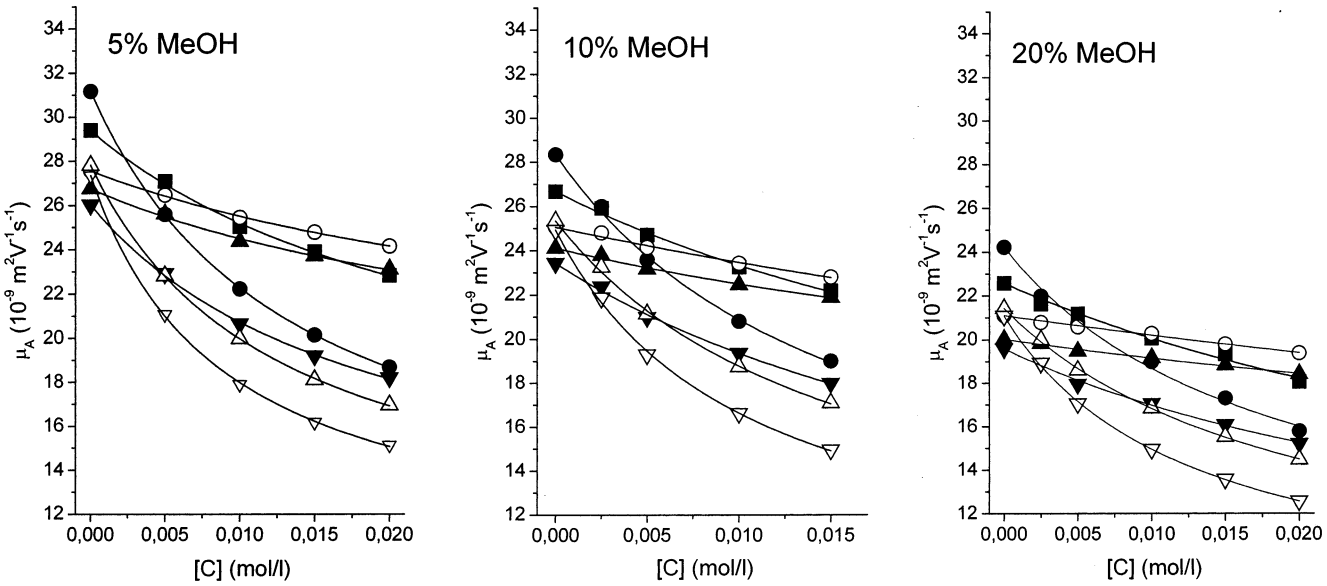

Figure 2. Mobility, μ_A, measured in mixed aqueous−organic solvents with 5, 10, and 20% (v/v) methanol as a function of the concentration of β-cyclodextrin (C). Temperature of the capillary cartridge, 25 °C. Symbols for the analytes and reproducibility of the data as in Figure 1. Fitted curves according to eq 5.

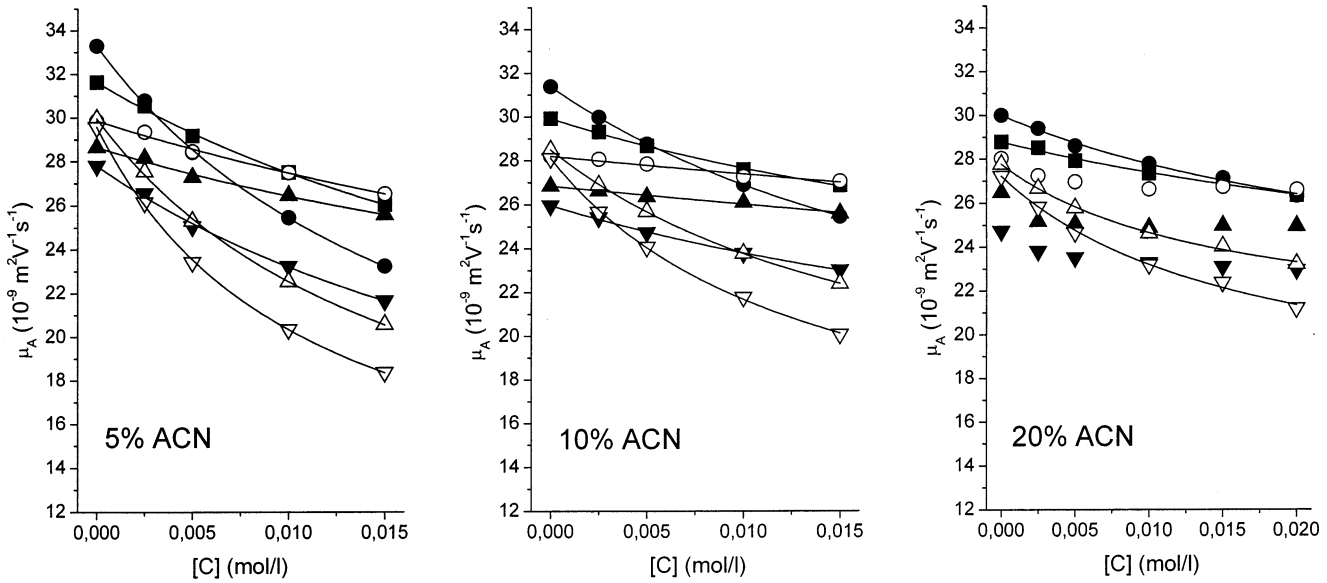

Figure 3. Mobilities, μ_A, measured in mixed aqueous−organic solvents with 5, 10, and 20% (v/v) acetonitrile as a function of the concentration of β-cyclodextrin (C). Temperature of the capillary cartridge, 25 °C. Symbols for the analytes and reproducibility of the data as in Figure 1. Fitted curves according to eq 5.

reduction of the constant than MeOH, especially at concentration below 10%. Here, the curves decrease much steeper for ACN. The curves indicate a certain saturation of the solvent effect, apparently approaching a constant value of the constants at high organic solvent concentration.

It should also be noted that the nonlinear fitting method leads to quite uncertain results for small association constants, especially at high organic solvent contents. This holds especially for 3OH-B and 2Cl-B at 20% ACN. For this reason, these data are not included.

Interpretation of the Change of the Complexation Constants. We use the concept of the medium effect here to get a more detailed view of the mutual contributions of the individual species that are involved in the complexation equilibrium in the mixed solvents, as compared to the pure aqueous one. In this way, we try to obtain information about the extent of stabilization of the individual species in the particular solvents. For this purpose, we formulate the transfer activity coefficients for the equilibrium given in general in eq 2 to the special complexation reaction between anion A^- and neutral cyclodextrin C to the complex CA^- according to

$$A^- + C = CA^- \tag{6}$$

This equilibrium is characterized by the association constant, K_c

$$K_c = \frac{a_{CA^-}}{a_{A^-} a_C} \tag{7}$$

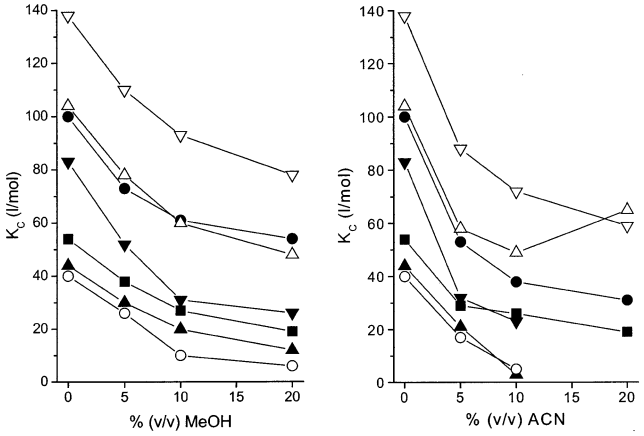

Figure 4. Complexation constants, K_c, of the substituted benzoates as a function of the concentration of MeOH or ACN, respectively, in the mixed aqueous−organic solvent. Temperature of the capillary cartridge, 25 °C. The symbols for the analytes are as in Figure 1.

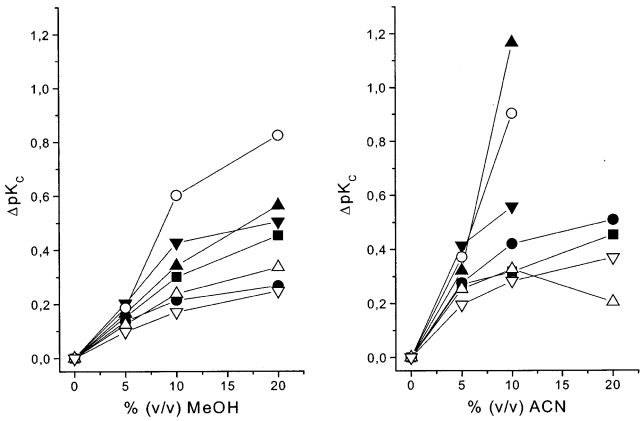

Figure 5. ΔpK_C values for complex formation vs organic solvent. The symbols for the analytes are as in Figure 1. Temperature, 25 °C.

Specifying the generally formulated equilibrium (eqs 2 and 3) to the complexation reaction (eq 6 and 7) allows expression of the change of the complexation constants upon transfer from water to the organic solvent by the transfer activity coefficient, ${}_m\gamma_i$, of the individual particle, i, as

$$\log {}_WK_C - \log {}_SK_C = \Delta pK_C^{S-W} = \log \frac{{}_m\gamma_{CA^-}}{{}_m\gamma_C\,{}_m\gamma_{A^-}} = \log {}_m\gamma_{CA^-} - \log {}_m\gamma_C - \log {}_m\gamma_{A^-} \quad (8)$$

It is seen that the medium effects on three particles play a role on the anion, A^-, on the neutral cyclodextrin, C, and on the anionic complex, CA^-.

In Figure 5 the ΔpK_C^{S-W} values are depicted, calculated from the data given in Figure 4. A nonlinear increase is found in both cases. The data of the ΔpK_C^{S-W} values for 3OH-B and 2Cl-B in 10% ACN are probably too high, because the estimation of K_c at the very low values tends to be erroneous. Even these values, however, do not contradict the overall tendency.

Because ΔpK_C^{S-W} expresses only the overall change of the complexation equilibrium, a better insight into the magnitude of the effects for the individual species can be obtained by separating the particular contributions.

Medium Effect on the Individual Species. It should be mentioned that the concept of the medium effect has been applied so far mainly to investigate the effect of the solvent on acidity constants. In the present work, we will try to use it for the evaluation of the extent of stabilization of the individual particles (A^-, AC^-, and C) in both solvent systems, W and S.

Whereas the determination of the medium effect on neutral species (like cyclodextrin) is less problematic, there is no possibility to derive medium effects of single ions with thermodynamic methods. Therefore, the derivation of the medium effect on the anion A^-, and the anionic complex, AC^-, will not be a straightforward task. However, we will derive these transfer activity coefficients indirectly. For simplicity (and because of the availability of data) we will concentrate the discussion on one typical solute, underivatized benzoic acid. This analyte seems typical because it has an average complexation constant and a mean change of the constant with organic solvent addition, as well (see Figure 4).

Medium Effect on *β*-Cyclodextrin. The medium effect on neutral cyclodextrin (suffix C) can be obtained from the solubilities at saturation in the particular solvents according to

$$\log {}_m\gamma_C = \log \frac{c_C^W}{c_C^S} \quad (9)$$

where c is the molar concentration at saturation in both solvents, W and S.

To our knowledge, there are no solubility data in the literature for *β*-cyclodextrin in water−ACN at 25 °C. A comparison of ΔG_t^0 values for *β*-cyclodextrin calculated from the available solubility data at 20,[23] 22,[24] and 25 °C[25] in water−MeOH, and at 20[23] and 22 °C[24] in water−ACN (data not given) indicate that temperature does not affect the ΔG_t^0 values to a considerable extent in the given solvent composition range. Thus, the solubility data by Chatjigakis et al.[24] for *β*-cyclodextrin at 22 °C was used for both water−MeOH and water−ACN systems. The data were converted from concentrations given in g/100 mL to mol/L. The results will be shown below (in Figure 7). Note that the positive sign of $\log {}_m\gamma_C$ would contribute to an increase in the complex constant upon addition of methanol; the opposite would be the case for ACN.

Medium Effect on the Anion, A^-. It has been pointed out that the medium effect on an individual ionic species cannot be determined by thermodynamic methods, in contrast to that for electrically neutral solutes. With such methods, the combined effect for both constituents of the electrolyte, the anion and the cation, is measured. However, we will derive the transfer activity coefficients for the individual anionic species using their acid−base equilibria, namely from the change of the ionization (or acidity) constant, K_a, of the respective acids, and from an approximation of the medium effect on the proton and on molecular acid HA.

For this purpose, we consider the protolysis equilibrium of a neutral acid, HA, according to $HA = H^+ + A^-$, which is

(23) Taghvaei, M.; Stewart, G. H. *Anal. Chem.* **1991**, *63*, 1902−1904.
(24) Chatjigakis, A. K.; Donzé, C.; Coleman, A. W.; Cardot, P. *Anal. Chem.* **1992**, *64*, 1632−1634.
(25) Okada, Y.; Kubota, Y.; Koizumi, K.; Hizukuri, S.; Ohfuji, T.; Ogata, K. *Chem. Pharm. Bull.* **1988**, *36*, 2176−2185.

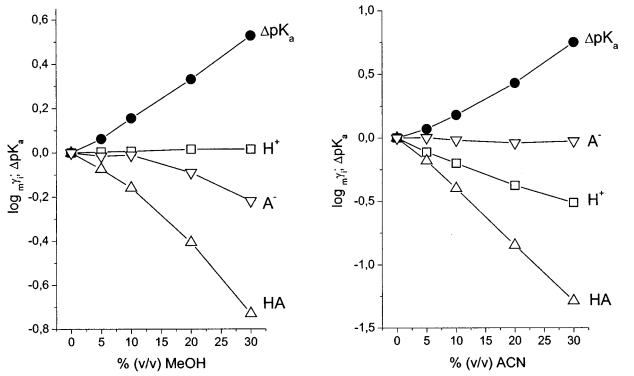

Figure 6. Medium effects (log $_m\gamma_i$) of individual species, i, taking part in the dissociation (according to HA = $H^+ + A^-$) and ΔpK_a of unsubstituted benzoic acid in mixed aqueous−methanolic (left) and aqueous−acetonitrilic (right) solvent. Values calculated by interpolation from the literature data after transformation to the molar scale (see Results and Discussion). Temperature, 25 °C.

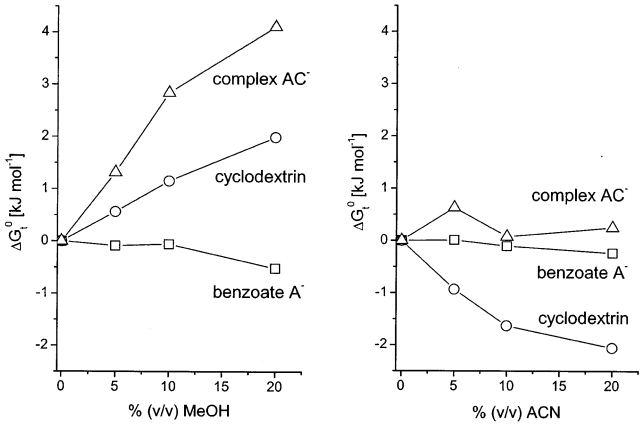

Figure 7. Standard free energy of transfer, $\Delta G^0_{t,i}$, for the individual species, i, depending on the composition of the mixed aqueous−methanolic (left) and aqueous−acetonitrilic (right) solvent. Data are for molar scale. For details, see text.

characterized by the well-known ionization constant

$$K_a = \frac{a_{H^+} a_{A^-}}{a_{HA}} \qquad (10)$$

In analogy to the equilibrium described above, we can express the medium effect on this equilibrium for the transfer from water to solvent S as

$$\log {}_W K_a - \log {}_S K_a = \Delta pK_a^{S-W} = \log \frac{{}_m\gamma_{H^+}\,{}_m\gamma_{A^-}}{{}_m\gamma_{HA}} = \log {}_m\gamma_{H^+} + \log {}_m\gamma_{A^-} - \log {}_m\gamma_{HA} \qquad (11)$$

It is composed from the medium effect on the proton, the anion, and the molecular acid. Thus, the medium effect on the anion (that is needed for further investigation of the complexation equilibrium with cyclodextrin) can be obtained when ΔpK_a and the medium effects on the proton and on HA are known.

(i) ΔpK_a values for benzoic acid were obtained from the ionization constants in water−MeOH and water−ACN published by Juillard and Simonet[26] and Azab et al.,[27] respectively. Former values were converted from molal scale to molar scale (solvent densities taken from ref 28). In both cases, the solvent compositions were converted to volume percents, and the pK_a values for the present solvent compositions were interpolated from fitted curves. They are given in Figure 6. It can be seen that for both solvent mixtures, upon addition of organic solvent, they increase by ∼0.5 and 0.7 pK units in 30% MeOH and ACN, respectively. This is only a small increase in pK_a when compared with the drastic change observed in the pure organic solvents. It is known, on the other hand, that there is a clear nonlinear dependence of pK_a on solvent composition, with a steep increase at high organic solvent contents.

(ii) Values for the neutral species can be obtained from solubility data as described above for cyclodextrin. The data for ΔG_t^0 of molecular benzoic acid from water to water−MeOH and from water to water−ACN were taken from Das et al.[29] and Datta and Kundu,[30] respectively. The ΔG_t^0 data given in mole fraction scale was converted to molar scale[31] using density values taken from ref 28. Solvent compositions were converted from weight percents to volume percents. The ΔG_t^0 values in the desired solvent composition were then derived from curve-fitting. As a result, the better solubility of the molecular benzoic acid observed in both solvent mixtures, as compared to water, leads to negative values for the medium effects, which are about −0.7 and −1.3 for 30% MeOH and ACN, respectively (Figure 6).

(iii) It should be noted that the medium effect on the proton reflects the basicity of the solvent. If it is negative, the proton is better stabilized in the solvent, which is therefore more basic than water. In contrast to data for ΔpK_a values and solubilities, which can be determined with high accuracy, there are many contradictory data available for the proton, because the latter can be obtained only by extrapolation methods (see, e.g., Marcus[32]). The data for Gibbs free energy of transfer for H^+ from water to solvent mixtures was taken from a recent critically evaluated compilation of Kalidas and co-workers.[31] Because the solvent compositions were given in mole fractions, they were converted to volume percents. The ΔG_t^0 values on the molar scale were then interpolated from fitted curves. The corresponding transfer activity coefficients are shown in Figure 6. It can be seen that the two solvent mixtures behave slightly differently. Whereas addition of MeOH in the given concentration range does not influence the basicity of the solvent, its basicity increases to some extent upon addition of ACN. This is an interesting result, because ACN is considered to be almost inert at the present low concentrations. It should be pointed out that the curves again strongly deviate from linearity at high contents of the organic cosolvent, with a drastic increase in pure organic solvents.

Finally, the medium effect of the anion, A^-, can be calculated from eq 10. It can be seen (Figure 6) that it slightly decreases in

(26) Juillard, J.; Simonet, N. *Bull. Soc. Chim. Fr.* **1968**, 1883−1894.
(27) Azab, H. A.; Ahmed, I. T.; Mahmoud, M. R. *J. Chem. Eng. Data* **1995**, *40*, 523−525.
(28) Landolt-Börnstein *Numerical Data and Funcional Relationship in Science and Technology*; Springer-Verlag: Berlin, 1977.
(29) Das, K.; Das, A. K.; Bose, K.; Kundu, K. K. *J. Phys. Chem.* **1978**, *82*, 1242−1245.
(30) Datta, J.; Kundu, K. K. *J. Phys. Chem.* **1982**, *86*, 4055−4061.
(31) Kalidas, C.; Hefter, G.; Marcus, Y. *Chem. Rev.* **2000**, *100*, 819−852.
(32) Marcus, Y. *Pure Appl. Chem.* **1990**, *62*, 899−940.

methanolic solutions to a value of −0.2 at 30% MeOH, which means that stabilization occurs to some extent. In ACN solutions, this reduction, although present, is seemingly marginal, and ACN acts as an inert cosolvent.

Standard Free Energy of Transfer, $\Delta G^0_{t,i}$, of the Benzoate/β-Cyclodextrin System. Because the data for the complex constants and the transfer activity coefficients for the benzoate anion and for cyclodextrin are known, we are able now to derive the medium effect on the anionic complex from eq 8. From these data, we are going to discuss the individual contributions to the standard free energy of transfer, $\Delta G^0_{t,i}$ (eq 4). It should be mentioned that Penn et al.[33] have already discussed the stabilization of another complex, that of the tioconazole cation with β-cyclodextrin in solutions up to 25% MeOH, on the basis of their standard free energy of transfer.

The results are depicted in Figure 7 for both solvent mixtures. The standard free energy of transfer for the benzoate anion, A^-, changes only slightly when the solvent composition is modified. There is seemingly no significant difference in benzoate anion stabilization between the two organic cosolvents (see above). However, there is a striking difference concerning the effect on the two other species. Whereas cyclodextrin has a negative value for $\Delta G^0_{t,i}$ in ACN−water mixtures (up to −2 kJ mol^{-1}), positive values are found for methanolic solutions (up to +2 kJ mol^{-1}). The overall decrease of the complex constants in the mixture with the two solvents must originate from the opposite behavior directed to the benzoate−cyclodextrin complex. It is revealed that, although in both cases the complex is less stabilized in mixed solvent systems, there is a clear difference in the magnitude of this effect. In mixtures with ACN, the values for $\Delta G^0_{t,i}$ are only slightly positive, whereas a much steaper increase in $\Delta G^0_{t,i}$ is seen for mixtures of MeOH ($\Delta G^0_{t,i}$ reaches +4 kJ mol^{-1} in 20% v/v MeOH).

CONCLUSIONS

The overall effect of the organic cosolvents MeOH and ACN, respectively, for aqueous background electrolytes on the complex formation of benzoates with β-cyclodextrin was analyzed by the transfer activity coefficient or medium effect, which is related to the standard free energy of transfer of the individual species involved in complexation. These species are neutral β-cyclodextrin and the two anions, free benzoate and the benzoate−cyclodextrin complex. As expected, the complex constants decrease upon addition of organic solvent. However, the cause for the seemingly equal change in K_c after addition of MeOH or ACN differs. Upon addition of ACN, stabilization of the two anions, benzoate and the benzoate−cyclodextrin complex, is almost unaffected, as compared to water ($\Delta G^0_{t,i}$ for both species is smaller than 0.5 kJ mol^{-1}), and the reduction of the association constants is related to the increased solubility of cyclodextrin, reflecting its better stabilization in the solvent mixtures (shifting the complex equilibrium to the side of the reactants there). In contrast, upon addition of MeOH, the lower solubility of cyclodextrin in the mixed solvents (which would lead to an increase in complexation constant) is overcompensated for by the pronounced destabilization of the large complex anion in these solvents, which shifts the equilibrium to the side of the reactant, as compared to the pure aqueous solution. The concept of the medium effect enables one to relate the apparently similar overall variation of the complexation constants by addition of organic solvent to the individual contributions of the species and to clarify the different role the solvent plays concerning the stabilization of cyclodextrin and the complex anion.

ACKNOWLEDGMENT

The authors acknowledge donation of an Ernst Mach fellowship for S.P.P. from the Austrian Academic Exchange Service and financial support from the University of Vienna (project no. 89/2) for K.S.

Received for review December 10, 2002. Accepted January 14, 2003.

AC026407Z

(33) Penn, S. G.; Bergström, E. T.; Goodall, D. M.; Loran, J. S. *Anal. Chem.* **1994**, *66*, 2866−2873.