The effect of protonation on the stability of cyclodextrin inclusion complexes

Ph.D. Thesis

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SUMMARY

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In the present work, the effect of protonation on the stability of cyclodextrin inclusion complexes was investigated. For this purpose, the complex formation of various organic acid and base protonation forms and cyclodextrins was studied to assess their stability. The behaviour of protonation forms of the following acids has been investigated in the presence of β–cyclodextrin: homologous series of aliphatic α,ω–dicarboxylic acids from oxalic acid up to adipic acid, diethylmalonic acid, maleic acid and fumaric acid. The formation constants were determined by pH-potentiometry combined with competitive UV-Vis spectrophotometric measurements. Based on the measured constants some very interesting conclusions could be drawn on the role of intra- and intermolecular H-bonds in stabilization of inclusion complexes. Although β–cyclodextrin generally forms more stable complexes with undissociated acids than with their strongly hydrated, deprotonated derivatives, a relatively high and unexpected increase of inclusion complex stability could be detected in some half-dissociated species with intramolecular H-bonds. This is probably due to the better space filling of the compact structure caused by the intramolecular H-bonds.

When investigating organic bases stability of cyclodextrin complexes of some alkaloid salts used as medicaments was determined in different protonation forms. The poor water solubility of the free base and the high dissociation constant (K_A) often hinders the assay of alkaloid salts. Different cyclodextrin derivatives form complexes of appropriate stability to keep the base in solution and at the same time to shift favourably the protonation equilibrium. Based on these findings we have elaborated a new method of alkaloid titration that can be carried out in aqueous media by choosing the most appropriate CDs depending on the solubility and the basicity of the free base and the size of the molecules.

Therefore the use of cyclodextrins can provide an alternative, environment friendly assay for many salts of week bases in aqueous media beside the pharmacopoeial methods.

Introduction

One of the most important of supramolecular systems in industry as well as in fundamental research is the family of different cyclodextrins and their inclusion complexes.

Due to the unique nature of their structure, cyclodextrins (CD) are able to form host-guest complexes with a great variety of molecules. Host-guest complexes are supramolecular structures composed of two or more molecules held together without covalent bonds. The host (e.g. CD) forms a cavity in which molecules of a second compound (the guest) are included, partly or entirely. The high electron density inside the cavity can modify the electrochemical and spectral properties of the guest molecule. As a result of complexation, the guest is stabilized and protected against outside influences (e.g. oxidation or photochemical decay) inside the cavity. Inclusion complexes dissociate easily under suitable circumstances and the guest regains its original physico-chemical properties. This unique property of CDs allows very interesting applications in pharmaceutical and food industry or in analytics.

A guest of appropriate size, shape and polarity is essential to the formation of specific interactions between the guest and the cavity of the host (H-bonding, hydrophobic and van der Waals interactions). The protonation state of guest molecules with acid-base properties is also of main importance in the stability of complexes. Since the cavity of cyclodextrins is rather hydrophobic, the inclusion of uncharged, apolar species is highly preferred against the more hydrated polar or charged ones. The stability of the inclusion complexes with an undissociated acid or its conjugated anion can differ significantly, since the carboxylate ion is always more hydrated than its undissociated form; therefore, its inclusion is less favoured. Hydrogen bonds between the host and the guest also play a significant role and may cause an increase in complex stability.

Research Objectives

Our main goal was to study the effect of the protonation of acidic and basic guest molecules on the stability of cyclodextrin inclusion complexes.

For this purpose the behaviour of aliphatic α,ω–dicarbocylic acids from oxalic acid up to adipic acid were investigated and compared to unsaturated (maleic and fumaric acid) and substituted relatives (diethylmalonic acid). We aimed at studying the complexation of these molecules and β–cyclodextrin, the speciation and the stability constants in aqueous solution.

Investigations were made with base-protonated base pairs, too. Six alkaloid salts, used as medicaments were chosen to study their complexation ability to native and modified cyclodextrins in function of pH. The K_A values were also determined, if it was possible. The effect of the size and the substituents of host on complex formation were also studied

The results were applied for the elaboration of a new assay of alkaloid salts in aqueous media. The most appropriate CDs were chosen (wherever possible) depending on the size of the molecules and substituents.

Methods

Potentiometric method. pH-potentiometric measurements were carried out with a Radelkis OP 208/1 pH-meter fitted with a Radelkis OP 0808P combined glass electrode. The system was calibrated with buffers at known pH. Carbonate free NaOH solution of known concentration was prepared by the Sörensen method diluting 50 m/m % NaOH solution. The titrations were carried out with a Schott-Geräte automatic burette under stirring with nitrogen. The ionic strength was 0.2 M (NaCl), and the temperature was kept at 25±0.1 °C. The concentrations of guest molecules varied from $5 \cdot 10^{-3}$ to 10^{-2} mol·dm^{-3} while the host/guest ratio was between 1:2 and 25:1. The equipment and the titration was computer controlled. At least three parallel measurements were carried out.

Spectrophotometric methods. Camspec M330 and Spectromom 195D spectrophotometers were used with 1.000 cm cells. The temperature was kept at 25±1°C.

At pH = 10.5 phenolphthalein was used in $3 \cdot 10^{-5}$ mol·dm^{-3} concentration (freshly prepared), and the pH was adjusted with $2 \cdot 10^{-2}$ mol·dm^{-3} sodium carbonate. For determining the stability of the phenolphthalein - β–CD inclusion complex, the CD concentrations varied from zero to $2.5 \cdot 10^{-4}$ mol·dm^{-3}. The absorbances were measured at λ=550 nm.

For competitive measurements dicarboxylate stock solution was prepared from weighed amounts of acids with the addition of calculated amount of NaOH solution. The concentrations of the phenolphthalein and the cyclodextrin were as described above, and those of carboxylate salts varied from $3 \cdot 10^{-5}$ to 10^{-1} mol·dm^{-3} (but were constant within a series). Measurements were carried out at 4-5 different concentrations of dicarboxylate anion.

At pH=1, the azo-type acid-base indicator, methyl orange was utilized in $2.0 \cdot 10^{-5}$ mol·dm^{-3} concentration. Measurements were carried out in 0.1 mol·dm^{-3} HCl solution.

Preparation of the solutions were the same except the addition of NaOH and the concentrations of CDs (that varied from zero to $6.5 \cdot 10^{-3}$ mol·dm^{-3}). The absorbances were measured at 506 and 319 nm.

Results

1. α,ω–dicarboxylic acids

The equilibrium constants for the β–CD complexes obtained are summarized in Table 1.

Table 1. Stability constants of inclusion complexes of dicarboxylic acids (H_2A) and their deprotonated derivatives (HA^-, A^{2-}) with β–CD (D).

Acid	K_{211} [a]	K_{111} [b]	K_{011} [c]
Oxalic acid	4.2±0.1	[n]	[n]
Maleic acid	8.1±0.1	[n]	[n]
Succinic acid	17.5±0.2	6.6±0.2	[n]
Glutaric acids	54.2±0.5	10.5±0.2	[n]
Adipic acid	113.2±1.3	33.1±0.8	9.6±0.7
Diethylmalonic acid	324.3±2.7	127.0±1.4	5.5±1.8
Fumaric acid	53.6±0.6	12.3±0.2	4.2±0.5
Maleic acid	18.2±0.1	31.5±0.7	7.1±0.5

[n]: uncertain; $^{a}K_{211} = \frac{[H_2A \cdot D]}{[H_2A][D]}$; $^{b}K_{111} = \frac{[HA^- \cdot D]}{[HA^-][D]}$; $^{c}K_{011} = \frac{[A^{2-} \cdot D]}{[A^{2-}][D]}$

1.1 We found in the present work that 1:1 acid:CD supramolecules are formed. A continuous increase in stability constants is noticed in the homologous series with increasing chain length.

1.2 In the series investigated, β–CD generally form more stable inclusion complexes with the undissociated acids than with their deprotonated derivatives, suggesting the role of van der Waals and hydrophobic interactions between the guest molecule and the inner cavity of CD.

1.3 Hydrogen-maleate forms a more stable complex with β–CD than the undissociated acid. Hydrogen maleate forms an unusually strong intramolecular hydrogen bond stabilizing

by the *cis* structure, and the resulting ring provides a better fit into the cavity of β–CD than either the linear HA^- (hydrogen succinate) or the uncharged H_2A species.

1.4 Comparing the stability constants of maleic and succinic acids, they are similar, but that of the fumaric acid is surprisingly high. The straight *trans* structure of fumaric acid allows for intermolecular hydrogen bonding, keeping the carboxylic groups within a favourable distance for H-bonding with the OH-groups of the β–CD rims.

1.5 Formation of mixed ternary complexes has been detected in the course of spectrophotometric investigations. It appears as if the unsaturated acids prefer the ternary complex formation.

2. Investigation of alkaloid salts

2.1. In the series investigated 1:1 base:CD inclusion complexes are formed. Different cyclodextrins form more stable complexes with the unprotonated bases than with their protonated derivatives which results in an increase in the apparent acidic dissociation constants of the alkaloids The base is included into the hydrophobic cavity of the CD and its solubility in water is increased. These two effects of CDs make an alkalimetric titration possible even in aqueous media using potentiometric end-point detection (Fig. 1.).

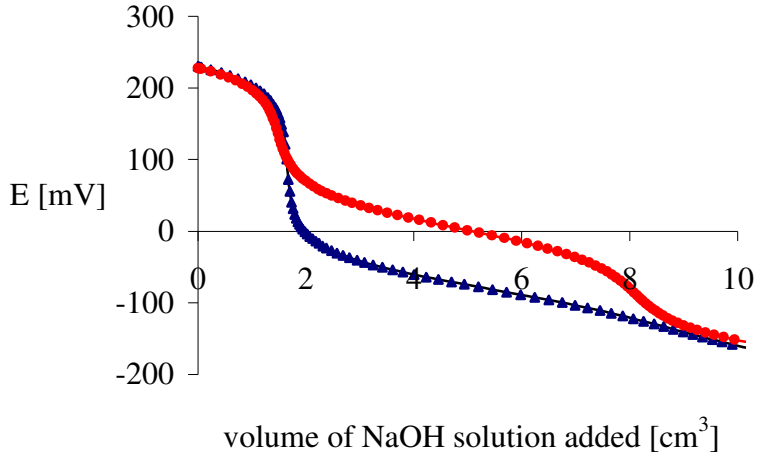

Fig. 1. Titration curve of $4 \cdot 10^{-3}$ mol·dm^{-3} codeine hydrochloridum and 10^{-3} mol·dm^{-3} HCl (V_0=20 cm^3) with $1.2 \cdot 10^{-2}$ mol·dm^{-3} NaOH solution in absence (▲), and in presence of $1.2 \cdot 10^{-2}$ mol·dm^{-3} γ–CD (•).

2.2.a. The larger cavity of γ–CD is suitable for **codeine** to form stable complexes. Threefold excess of the CD is enough to get well separated inflexion points on the titration curve

(see Fig. 1.). Among the alkaloids studied, **papaverine** has the highest K_A and this value is further increased by complex formation, but the increased solubility could help the separation of the first titration step. Dimethyl-β-CD (DIMEB) is better, even if its twentyfold excess is necessary. Among the alkaloids studied, **homatropine** has the lowest acidity and its assay is hindered by the flat (practically nonexistent) second inflexion. The potentiometric determination of homatropine is aided by DIMEB in twentyfold excess. **Quinine**– γ-CD supramolecule precipitates during titration providing a nice example of GES (when the guest enforces its poor solubility on the host). In contrast to γ-CD, both β-CD and DIMEB are suitable for the determination of quinine.

2.2.b. In case of **pilocarpine** no significant change was observed in the shape of the titration curves with different CDs since the difference is too small between the stability of the neutral and the protonated species. We have found that pilocarpine can be directly titrated in aqueous media (in 5 mM concentration) without any auxiliary material, because of its good water solubility and convenient pK_A value.

2.2.c. The determination of **ephedrine** failed with all of the investigated CDs. Ephedrine has a low acidity so the second inflexion is not observable. In agreement with the literature it was found that only complexes of very low stability can be formed.

Publications

Publications the dissertation is based on

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2. Csernák O, Buvári-Barcza Á, Barcza L. (2006) Cyclodextrin Assisted Nanophase Determination of Alkaloid Salts. Talanta, 69 (2): 425-429.

Publication connected with the dissertation

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