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Review

Analysis and characterisation of cyclodextrins and their inclusion complexes by affinity capillary electrophoresis

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Abstract

α-, β- and γ-cyclodextrins, which are composed of 6, 7 and 8 (1→4)-linked α-D-glucose units, are capable of forming complexes with molecules ranging from gases to proteins and other biopolymers. The physico-chemical properties of the guest molecules, such as their solubility, stability and reactivity can thereby be modified. For this reason they have found numerous applications in the agricultural, food, chemical and pharmaceutical industries. Cyclodextrins (CDs) have also been shown to be valuable as selectivity reagents for the resolution of structural, positional and stereo isomers in analytical chemistry and are important as media modifiers in the capillary electrophoretic separation of chiral biomolecules. CDs are produced from starch together with linear oligosaccharides by the extracellular microbial enzyme cyclodextrin glycosyltransferase (E.C. 2.4.1.19). Cyclodextrin glycosyltransferases are capable of producing cyclodextrins with six or more glucose units, mainly α-, β- and γ-CDs, as the major product. The analysis of the enzymatic reaction products is difficult due to the formation of a variety of both linear and cyclic oligosaccharides by cyclodextrin glycosyltransferases. Capillary electrophoresis provides a versatile and selective tool for their analysis in complex samples. Furthermore, capillary electrophoresis enables the characterisation of the inclusion complex forming properties of cyclodextrins using only minute amounts of sample. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Affinity capillary electrophoresis; Inclusion complexes; Complexation; Cyclodextrins

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1. Introduction

1.1. Cyclodextrins

Enzymatic degradation of starch by the extracellular microbial enzyme cyclodextrin glycosyltransferase (CGTase) (E.C 2.4.1.19) yields a mixture of cyclic and linear oligosaccharides. The cyclic oligosaccharides, denoted cyclodextrins (CDs), are composed of a number of (1→4)-linked α-D-glucose units of which CDs with six, seven and eight glucose units are well known as α-, β- and γ-CDs, respectively. CGTases predominantly produce α-, β- and γ-CDs and only a small fraction of large CDs are formed [1–2]. Larger CDs with more than eight glucose units were first described by French and co-workers [3–5]. However, due to low yields and difficulties in their purification, they were not fully characterised. Only recently, CDs with 9 to 19 α-(1→4)-linked D-glucose units, denoted δ-, ϵ-, ζ-, η-, θ-, ι-, κ-, λ-, μ-, ν- and ξ-CDs have been purified and characterised [6–13]. The ability of CGTases to produce CDs with potentially several hundred α-(1→4)-linked D-glucose units has been reported by Terada et al. [14]. Furthermore, recent studies have shown that CGTases are not the only enzymes capable of producing CDs from starch. Small amounts of large CDs have been reported to be produced by a saccharifying α-amylase (E.C. 3.2.1.1) from *Bacillus subtilis* X-23 and a liquefying α-amylase (E.C. 3.2.1.1) from *Bacillus amyloliquefaciens* [15]. The production of very large CDs by 4-α-glycosyltransferase (EC 2.4.1.25) from potato, which, in contrast to the CGTases, was incapable of producing CDs with less than 17 α-(1→4)-linked α-D-glucose units, has also been reported [16–18]. A cyclodextrin containing 21 α-(1→4)-linked α-D-glucose units has been recently isolated and characterised [19]. CDs with less than six glucose units have not been found to be produced enzymatically but have been obtained by chemical synthesis [20]. Due to the low solubility and undesirable side effects of the native α-, β- and γ-CDs, a high number of derivatives of these molecules have been produced [21–23]. The CDs derivatives usually show altered characteristics with regard to solubility, biocompatibility and complex formation.

The large interest in CDs and their derivatives lies in their ability to form inclusion complexes with a wide range of molecules, mediated by a hydrophobic cavity surrounded by a hydrophilic outer surface. The inclusion complexes thus formed can have a marked effect on the physico-chemical properties of the guest molecule. Examples of effects obtained by encapsulation with CDs are an altered solubility, reduced volatility, modified chemical reactivity, protection against degradation and improved bioavailability of the included molecule. CDs and their derivatives have found numerous applications in the pharmaceutical industry, mainly to improve the solubility of sparingly soluble drugs. Further beneficial effects include masking of unpleasant taste or odours, stabilisation of compounds sensitive to degradation caused by e.g., temperature, oxidation, hydrolysis or light and avoidance of incompatibility with other drugs or ingredients in a formulation, resulting in an overall enhanced bioavailability (for reviews on the use of CDs in the pharmaceutical industry see Refs. [21–29]). Similar encapsulation effects are also applicable in the food, cosmetic and agrochemical industries [21,30–33]. CDs and their derivatives have also obtained high attention in analytical chemistry, especially for the separation of structural, positional and stereo isomers of compounds. CDs have been used as media modifiers in a diverse range of analytical techniques such as nuclear magnetic resonance (NMR), circular dichroism, high-performance liquid chromatography (HPLC), gas chromatography, thin-layer chromatography and capillary electrophoresis (CE) [21,34–37]. In the field of CE, CDs are considered the first choice as media modifiers for the separation of chiral compounds [38–39].

CDs have the well known ability to stabilise compounds against degradation [21,22]. However, CDs can also show a catalytic ability by accelerating chemical reactions, e.g., hydrolysis reactions and have achieved considerable attention as components of "synzymes" [21,40–41]. Moreover, CDs are considered as some of the most important building blocks for supramolecular structures and functional units in supramolecular chemistry and nanotechnologies [42,43].

Larger CDs with more than eight glucose units in the macrocycle are currently not available at an

industrial scale. These CDs have potential applications in areas where α-, β- and γ-CDs and their derivatives are currently applied, thus exploiting their specific inclusion complex forming properties. δ-CDs has in one study proven to be capable of increasing the solubility of some drugs and caused, in contrast to α-, β- and γ-CDs, no haemolytic reactions [8]. The very large CDs produced by the action of a potato 4-α-glycosyltransferase are also, due to their high solubility, low viscosity and inability to retrograde, promising new ‘‘starch-like’’ modified oligosaccharides with applications especially in the food industry [16].

1.2. Affinity capillary electrophoresis

CE can be described as a method for the high-efficiency separation of molecules in narrow bore (I.D. 10–100 μm) capillary tubes filled with a electrolyte solution under the influence of an electric field. The separations can be based on the differences in the mobility of ionic species or differences in the affinity of charged or neutral molecules to charged electrolytes. Highly specific separations and analysis of molecules and intermolecular interactions based on affinity effects (e.g., hydrogen binding, electrostatic interaction, hydrophobicity and Van der Waals forces) have been termed affinity capillary electrophoresis (ACE). ACE has been widely used for the study and determination of physico-chemical parameters, such as the binding constants in the study of metal–protein complexes, drug–protein interactions, protein–protein and protein–carbohydrate interactions, DNA–peptide interactions, enzyme–substrate/inhibitor interactions, antigen–antibody interactions, oligonucleotide–oligonucleotide interactions and carbohydrate–drug interactions (for reviews see Refs. [44–47]). The application of CDs as chiral and achiral discriminators in CE has been reviewed previously [35–37].

In this review we provide an overview on the analysis of CDs and their complexes by ACE.

2. Analysis of cyclodextrins

CDs produced from starch by the action of CGTases occur in mixtures of cyclic and linear oligosaccharides. They are uncharged below pH 12 and optically inert molecules. Due to the difficulties in the separation and analysis of these molecules, a wide variety of methods for the detection, separation and analysis of CDs has been developed.

2.1. Detection and separation methods

The earliest method for the detection of CDs in starch digests of CGTases is based on the microscopic examination of the formation of iodine-α-CD crystals after addition of an iodine solution to the starch digest [48–49]. Since then, a wide range of methods have been developed to analyse CDs and their derivatives. These include absorbance and fluorescence spectroscopy [50], polarography [51], thin-layer chromatography [52–55], HPLC [52,56–61], mass spectrometry [62,63] and electrophoresis [63–70]. Methods based on immunoassays have also been developed [71].

Spectroscopy has been the most widely used method for the analysis of the small α-, β- and γ-CDs, since it is inexpensive and requires only standard laboratory equipment. Several absorbance- and fluorescence-based spectrophotometric methods for the analysis of CDs have been reviewed by Mäkelä et al. [50]. However, these methods fail to fully discriminate between the individual CDs and are highly sensitive to changes in the pH and to the presence of other complex-forming molecules. Furthermore, three assays are necessary to measure the concentration of α-, β- and γ-CDs in one sample. Other non-selective non-chromatographic methods include polarography, which is based on the ability of CDs to prevent the oxidation of linoleic acid by lipoxygenase (E.C. 1.13.11.12) [51]. Furthermore, methods based on the increased rate of degradation of phenyl acetates in the presence of CDs [72] and on the glucoamylase resistance of CDs [73–75] have been reported.

Only liquid chromatography (e.g., HPLC), mass spectroscopy and CE are able to provide both qualitative and quantitative data when analysing complex CD mixtures. For the separation of CDs and their derivatives by HPLC, several stationary phases have been described, e.g., resins modified with specific absorbents [56,76] and reversed-phase media

used in combination with either refractive index detection [52,56–57,77–79], indirect photometric detection [58], post-column complexation with phenolphthalein [80], polarimetric detection [81–82] or pulsed amperometric detection [59,77,83,84]. The chromatographic methods may require extensive sample preparation when analysing complex samples such as CGTase products from starch containing CDs, oligosaccharides, residual starch, as well as proteins, salts and other substances. Using chromatographic techniques, the CDs often coelute together with linear oligosaccharides also present in the samples. In order to remove the oligosaccharides, the samples have to be incubated with exo-acting amylolytic enzymes (e.g., β-amylases and glucoamylases) for several hours prior to analysis. Furthermore, the chromatographic methods often suffer from poor sensitivity and resolution in combination with long separation times.

The number of reports on the analysis of large CDs is very limited. Those available include the work by French and co-workers, who employed the techniques of paper chromatography [3,4] and cellulose column chromatography [5]. Recently, Penninga et al. [60] reported the separation and detection of α- to η-CDs by the use of an amine reversed-phase HPLC column using acetonitrile–water (60:40, v/v) as eluent. However, the small amounts of large CDs present in the samples originating from CGTase reactions with starch were barely detectable. Furthermore, pre-treatment of the samples with β-amylase was necessary to remove co-eluting linear oligosaccharides. In the studies presented on large CDs containing up to hundreds of glucose molecules, anion-exchange chromatography has been employed [14,16–18]. The technique enabled the detection of large CDs after removal of linear oligosaccharides by glucoamylase treatment. However, α- to θ-CDs are co-eluting using this technique (own unpublished results).

In contrast to HPLC, CE techniques have only been introduced recently and are currently in rapid development. For the separation of chiral molecules, CDs have played a central role in the development of a wide variety of analytical methods based on CE. CE also provides a powerful analytical tool for the analysis of CDs and their derivatives.

2.2. Theory of cyclodextrin analysis by affinity capillary electrophoresis

In the capillary electrophoretic separation of CDs, the neutral CDs are carried towards the cathode and the detector by the electroosmotic flow (EOF) with the mobility μ_{EOF} (Fig. 1). The EOF is caused by cations attracted to the negatively charged silanol groups on the inside of the fused silica capillary. These cations move towards the cathode, dragging the solute in the capillary in the same direction. The aromatic anions in the background electrolyte (BGE) move towards the anode against the EOF and away from the detector with the mobility μ_{A^-}. An equilibrium is formed between free CDs being dragged towards the cathode by the EOF and CD–aromatic anion complexes migrating towards the anode with the mobility μ_{CDA^-}. This results in the CDs being separated from other neutral species not able to form complexes with the aromatic anions. When applying aromatic cations as BGE, the CD–aromatic cation complexes will cause the CDs to move towards the detector ahead of the EOF with the mobility μ_{CDC^+} (Fig. 1).

2.3. Methods for the analysis of cyclodextrins by capillary electrophoresis

Separation and analysis of α-, β- and γ-CDs by electrophoresis have been carried out in polyacrylamide gels [64] and more recently using CE [63,65–69]. An electrophoretic separation of CDs is not possible without their modification since CDs are only charged at a very high pH. However, Nardi et al. [65] demonstrated that CDs could be separated by the formation of inclusion complexes with benzoate using CE. It was proposed that the presence of benzoate also facilitated the detection of the CDs by indirect UV absorbance measurement due to a reduction in the absorbance of benzoate following complexation with the CDs [85]. Although a baseline separation between α-, β- and γ-CDs could be obtained using this method, only the detection of β-CDs was possible in complex samples [65]. Similarly, fluorescent 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS) has been used for the separation and detection of CDs in a CE system [66]. The sepa-

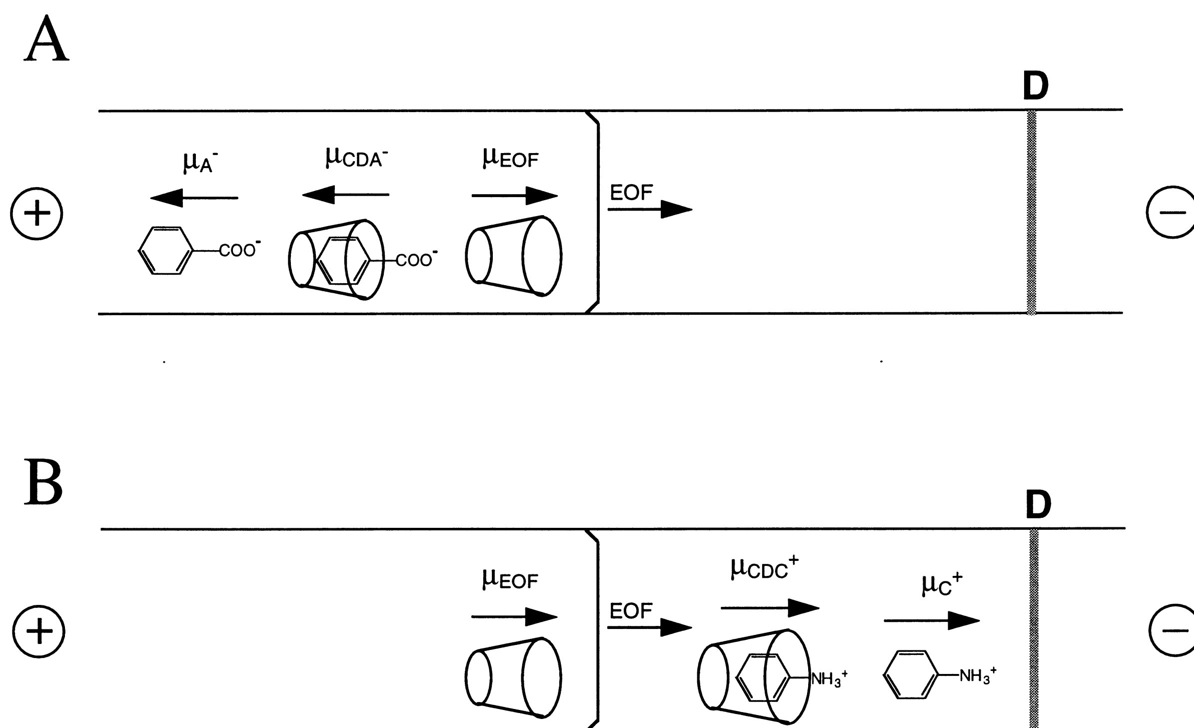

Fig. 1. Principle of separation of CDs by ACE by complex formation with aromatic anions e.g., benzoate (A) and aromatic cations e.g., benzylamine (B). (μ_{A^-}), (μ_{CDA^-}), (μ_{C^-}), (μ_{CDC^+}) and (μ_{EOF}) denote the mobilities of the aromatic anion, the cyclodextrin–aromatic anion complex, the aromatic cation, the cyclodextrin–aromatic cation complex and the electroosmotic flow, respectively. EOF = Electroosmotic flow; D = detector.

ration relied on the complex formation of CDs with the anionic 2,6-ANS. These complexes could be detected by fluorescence spectroscopy due to an enhanced fluorescence of 2,6-ANS following complexation with CDs. Although this method had a good selectivity for β-CDs allowing the separation and detection of 2,6-di-*O*-methyl β-CDs in a heterogeneous sample, α- and γ-CDs were barely detectable. A further development of this technique was presented by Lee and Lin [67], who improved the versatility of the method by showing that both anionic (e.g., salicylic acid) and cationic (e.g., benzylamine) electrolytes could be employed for the separation of α-, β- and γ-CDs and some derivatives. CE has also been successfully used for the separation of charged sulphoalkyl ether β-CDs derivatives [63,70,85].

A method for the separation and analysis of δ-CDs by CE has been developed by Larsen and Zimmermann [69]. The separation of CD mixtures containing 2 m*M* of α-, β-, γ- and δ-CDs was achieved using a range of aromatic anions as BGE. Although the different BGE solutions gave good separations of α-, β-, γ- and δ-CDs, their detection limit was rather high, especially for δ-CDs, which is produced in much smaller amounts compared to the other three types of CDs. To improve the sensitivity of the CE system for the separation and analysis of the four CDs, an acid–base indicator which changes its absorbance upon inclusion with CDs was added to the BGE. Methyl orange {4-[*p*-(dimethylamino)-phenylazo]benzenesulphonic acid, sodium salt, MO} was chosen as a media modifier and combinations of different aromatic anions with MO were tested for their ability to separate α-, β-, γ- and δ-CDs, while maintaining the improved sensitivity provided by the addition of MO. However, the addition of the aromatic anions to MO can result in the formation of

precipitates thus limiting the concentration range in which MO can be used in combination with other aromatic anions [69]. The separation of α-, β-, γ- and δ-CDs by the combination of MO and another aromatic anion was optimised and a BGE consisting of 40 m*M* 4-*tert*.-butylbenzoic acid and 3.5 m*M* MO in 2 m*M* phosphate buffer, pH 8.0 was suitable for the separation and sensitive detection of α-, β-, γ- and δ-CDs. This method allowed a quantitative analysis of the production of δ-CDs by a range of CGTases [86–87].

A large range of aromatic ions has been shown to be able to facilitate the analysis of CDs and their derivatives using CE [65–69]. The separation of several CDs and CD derivatives is shown in Fig. 2. It is evident that the underivatised CDs, as well as the glucosylated and the maltosylated β-CDs have distinct mobilities and thus binding characteristics. On the other hand, the separation of the methylated and hydroxypropylated CDs result in relatively broad peaks due to the polydispersity of the samples and probably also due to the different binding characteristics of the components (Fig. 2).

It has been proposed that the detection of CDs in CE is based on a change in the absorption of the aromatic ion due to the formation of an inclusion complex with the CDs [85]. In this case, the change of absorbance should be dependent on the type of CDs, since the complex stability and type of CDs will influence the absorbance of the included molecule. Penn et al. [66] observed that the change of fluorescence of the fluorescent BGE 2,6-ANS was dependent on the type of CDs. However, with the exception of the separation of CDs using MO–aromatic anion mixtures [69], all aromatic anions used for the separation of CDs by CE showed similar detector responses on a molar basis for the different CDs analysed (Fig. 2). This indicates that the absorbance difference caused by CDs is due to a displacement of BGE in the sample region and not to a specific absorption changes caused by inclusion complex formation. This assumption is also supported by the negative absorbance differences observed. However, this type of detection is different from the indirect detection mode where highly absorbent or fluorescent charged BGE are displaced by a optically inert charged analyte [88–90]. Of all the aromatic anions tested, only the MO–aromatic anion mixtures resulted in a positive absorbance difference for α-, γ- and δ-CDs [69] (Fig. 2). The difference in the affinity of the CDs for the two electrolytes used in the MO–aromatic anion mixtures results in a different degree of displacement of the electrolytes in the sample zone and thereby in different absorbance. It has been shown that this ACE technique can be extended to include the analysis of δ- to θ-CDs as well, since these larger CDs gave also distinct signals comparable to α-, β-, γ- and δ-CDs [91]. However, the analysis of very large CDs, such as those produced by the 4-α-glycosyltransferase from potato, will probably not be possible with ACE, since the inclusion complex forming properties of these CDs may be very similar in contrast to the distinct inclusion complex forming properties of the smaller CDs.

The major advantages of the analysis of CDs and their derivatives by ACE compared to other analysis methods are the short analysis times, high versatility and the possibility to customise the analysis system to meet specific needs, as well as a minimal sample size requirement. Furthermore, the ACE analysis method of CDs is only sensitive for molecules capable of inclusion complex formation, thus allowing the analysis of CDs and their derivatives in complex samples with only minimum sample preparation. The specificity of this methodology can therefore facilitate the discovery of new hosts for molecular encapsulations.

3. Analysis of cyclodextrin inclusion complexes

The cavity of the CDs enables the formation of inclusion complexes with a wide range of molecules. An inclusion complex consists of at least two entities of which one, the host, forms a cavity in which it can bind a guest molecule by non-covalent forces. CD inclusion complexes are attractive models for the study of non-covalent interactions between molecules. These studies are of paramount importance for a better understanding and application of CD inclusion complexes and provide insight in the non-covalent forces involved in e.g., enzyme–substrate or receptor–drug interactions.

Despite the simplicity of the CD–guest model system, the forces involved in the formation of such

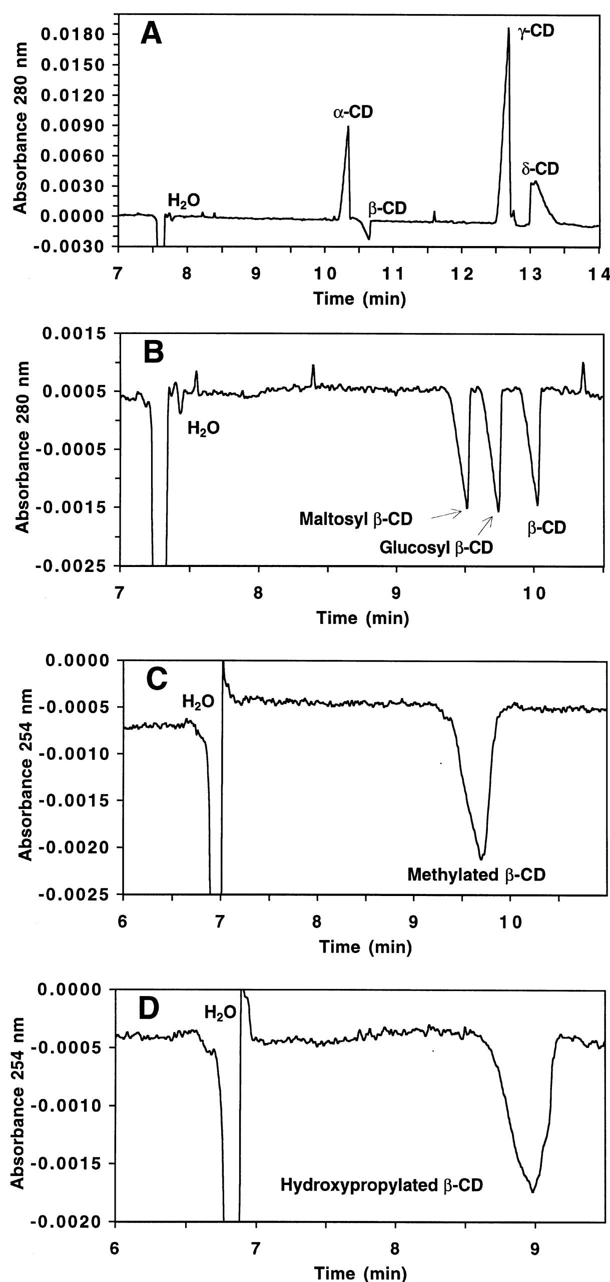

Fig. 2. Electropherograms of CDs and some derivatives using different methods. (A) 2 m*M* α-, β-, γ-, δ-CDs and (B) 2 m*M* of maltosyl-β-CD, glucosyl-β-CD and β-CD separated using 3.5 m*M* methyl orange {4-[*p*-(dimethylamino)-phenylazo]benzenesulphonic acid, sodium salt}, 40 m*M* 4-*tert*.-butyl benzoic acid in 2 m*M* phosphate buffer, pH 8.0. (C) Ten mg/ml methylated β-CD (degree of substitution, 1.8; Wacker-Chemie, Vallensbeak, Denmark) and (D) 10 mg/ml hydroxypropylated β-CD (Acros Organics, NJ, USA) separated using 75 m*M* 4-*tert*.-butyl benzoic acid in 2 m*M* phosphate buffer, pH 7.0. Separations were carried out according to Larsen and Zimmermann [69] using a Beckmann P/ACE MDQ (Beckmann, Fullerton, CA, USA) equipped with a 80 cm (70 cm to the detector)×50 μm I.D. fused silica capillary (Composite Metal, CA, USA). Samples loading was performed by pressure (3.4 kPa) and the separations was carried out at 30°C. α-, β- and γ-CDs were obtained from Wacker-Chemie, maltosyl-β-CD and glucosyl-β-CD from Sigma (St. Louis, MO, USA) and δ-CD was a gift from Dr. H. Ueda, Hoshi University, Tokyo, Japan.

complexes are not yet fully understood. Several hypothesis have been proposed to explain the formation of CD inclusion complexes. These include hydrophobic interaction, relief of conformational strain, release of high-energy cavity-bound water, dipole–dipole interaction, hydrogen-bonding interaction, induction and dispersion forces [21–22,92–96]. However, inclusion complex formation cannot be explained by one of these factors alone since the involvement of several types of forces in the inclusion complex formation is evident [22,96].

3.1. Determination of inclusion complex stability constants

The determination of inclusion complex formation constants between CDs and various guest molecules is of high importance for the understanding and evaluation of inclusion complex formation. The enhancement of the solubility and stability of molecules, the controlled release of drugs and the selective separation of compounds from mixtures are a few examples of properties that can be obtained by complex formation. These effects all rely on the stability of the complexes formed and thereby on the inclusion complex formation constants.

A range of methods has been applied for the determination of inclusion complex formation constants [21]. Spectroscopic methods based on the change of absorbance [97–100], fluorescence [100–103], solubility [99] and NMR spectroscopy [104–106] have been reported. Electrochemical methods based on a change of the dissociation constant of the complexed guest molecule, measured by potentiometry or conductometry, have also been developed [107–109]. Other methods are based on isothermal titration calorimetry [110–113], chromatography [100,114–117], ultrafiltration [118], freezing point depression [119,120] or on the reaction rate of CDs catalysed reactions [121,122].

The inclusion of a guest molecule will not always give rise to easily measurable changes in the physico-chemical properties. In these cases, competition methods can be used. These are based on a competition between the CDs and two guest molecules of which one gives rise to a measurable change, e.g., in absorbance or fluorescence, upon complex formation [21,121,123,124]. From measurements of the absorbance change at different ratios between the two guest molecules, the complex formation constant of the guest can be calculated. To use these methods it has to be assumed that complex formation occurs in a 1:1 ratio, that no ternary complexes are formed, and that the two types of binary complexes do not interfere with each other.

CE has been used for the estimation of the binding constants between CDs and a range of molecules. In general, two methods have been applied with either an aromatic ion or the CD as analyte. With an aromatic ion as analyte, the inclusion complex formation constant between CDs and a charged guest molecule can be estimated by measuring the change of mobility of the guest molecule in buffers containing various concentrations of CDs [67,100,117,125–134]. This method has frequently been applied for the estimation of the binding constants of drug enantiomers. If CD is used as the analyte, its mobility can be measured as a function of the concentration of the charged guest molecule [67,91].

The separation of CDs by CE relies on the formation of inclusion complexes between the CDs and a charged BGE. Differences in the formation constants between the various CDs and the BGE leads to their separation. When the pH of the solution is kept above the pK_a of the BGE, the anionic form of the BGE, $[A^-]$, predominates. Therefore, only the equilibrium expression for the formation of inclusion complexes between CDs and the anionic BGE needs to be considered, assuming that only 1:1 inclusion complexes are formed between CDs and the BGE.

$$K = \frac{[ACD^-]}{[A^-][CD^-]} \quad (1)$$

Assuming that the amount of complexed aromatic anion $[ACD^-]$ is negligible compared to the amount of free aromatic anion $[A^-]$, the following equation can be derived, where μ_{eff} is the observed mobility of the complex, μ_{ACD^-} is the maximum mobility of the complex, K is the stability constant and $[HA]_0$ is the concentration of aromatic anion in the BGE [67,91].

$$\mu_{eff} = \frac{K[HA]_0 \mu_{ACD^-}}{1 + K[HA]_0} \quad (2)$$

The reciprocal form of Eq. 2 multiplied with $[HA]_0$ results in Eq. 3.

$$\frac{[\mathrm{HA}]_0}{\mu_{\mathrm{eff}}} = \frac{[\mathrm{HA}]_0}{\mu_{\mathrm{ACD}^-}} + \frac{1}{K\mu_{\mathrm{ACD}^-}} \quad (3)$$

A plot of $[\mathrm{HA}]_0/\mu_{\mathrm{eff}}$ against $[\mathrm{HA}]_0$ will give a straight line where the intercept and the slope are equal to $1/K\mu_{\mathrm{ACD}^-}$ and $1/\mu_{\mathrm{ACD}^-}$, respectively. By dividing the slope by the intercept K is obtained. Several other plotting methods can be used for the extraction of the stability constant [67,135].

This method has been applied for the analysis of the complex stability constants between CDs and various anions and cations [67,91]. Table 1 shows that the results that can be obtained with ACE compare well with those obtained with other methods. The complex forming ability of the larger CDs with 9 to 13 glucose units in the macrocycle has been studied using this method (Table 1) [91].

4. Conclusions

ACE is a versatile analytical tool for the analysis of CDs and their derivatives and also allows the detection and analysis of other inclusion complex forming compounds such as CD derivatives, larger CDs and other polysaccharide derived CD-like com-

Table 1
Comparison of inclusion complex formation constants of 1:1 complexes measured at 25°C or room temperature using different methods

Compound/	Inclusion complex formation constants (M^{-1})								
detection method	α-CD	β-CD	γ-CD	δ-CD	ε-CD	ζ-CD	η-CD	θ-CD	Ref.
Benzoate									
Capillary electrophoresis[a]	16	23	3	3	3	5	4	5	[91]
Spectrophotometry	13±1								[138]
Microcalorimetry	10±1	15.9±1.2							[113]
Potentiometry	10.51±0.05								[139]
Potentiometry		60±10							[137]
Potentiometry	11.2±0.35								[107]
Microcalorimetry		10±1[b]							[140]
Microcalorimetry		20							[112]
4-Methyl benzoate									
Capillary electrophoresis[a]	36	66	8	3	4	5	7	6	[91]
Capillary electrophoresis[c]		100.3±1.0							[117]
HPLC		90							[117]
Potentiometry		110±1							[137]
Salicylate									
Capillary electrophoresis[a]	11	65	13	9	8	8	9	10	[91]
Capillary electrophoresis[c]	8±0.3	82±3							[67]
Capillary electrophoresis[a]	15±6	50±7							[67]
4-tert.-Butyl benzoate									
Capillary electrophoresis[a]	51	382	74	47	3	9	15	25	[91]
Potentiometry	64±19								[136]
Microcalorimetry		18 400							[112]
Ibuprofen anion									
Capillary electrophoresis[a]	56	>2500[d]	67	27	2	12	29	39	[91]
Spectrophotometry		2900±500							[98]
Capillary electrophoresis[c]		1280±5[e]							[125]

[a] CD run as analyte.
[b] Measured at 30°C.
[c] Aromatic ion run as analyte.
[d] Too high to be accurately determined.
[e] Measured at 37°C.

pounds with an ability to analyse complex mixtures and requiring only minute amounts of sample.

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