Native and substituted cyclodextrins as chiral selectors for capillary electrophoresis enantioseparations: structures, features, application, and molecular modeling

Paola Peluso[1] and Bezhan Chankvetadze[2]

[1] Istituto di Chimica Biomolecolare ICB, CNR, Sede secondaria di Sassari, Traversa La Crucca 3, Li Punti, 07100 Sassari, Italy.

[2] Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 0179 Tbilisi, Georgia.

*Correspondence should be addressed to the following authors:

Dr. Paola Peluso
Istituto di Chimica Biomolecolare
Consiglio Nazionale delle Ricerche
Traversa La Crucca 3, Li Punti, 07100 Sassari, Italy
E-mail: paola.peluso@cnr.it

Prof. Bezhan Chankvetadze
Institute of Physical and Analytical Chemistry
School of Exact and Natural Sciences, Tbilisi State University
Chavchavadze Ave 1, 0179 Tbilisi, Georgia.
E-mail: jpba_bezhan@yahoo.com

Keywords: Capillary electrophoresis / Computational methods / Cyclodextrins / Enantioseparation / Molecular modeling

Abbreviations: AGT, aminoglutethimide; **AM1**, Austin model 1; **AMBER**, assisted model building with energy refinement; **B3LYP**, Becke, 3-parameter, Lee-Yang-Par; **CEKC**, capillary electrokinetic chromatography; **CM-β-CD**, carboxymethyl-β-CD; **CMPA**, chiral mobile phase additive; **DFT**, density functional theory; **2,6-DM-β-CD**, heptakis(2,6-di-O-methyl)-β-CD**; EMO**, enantiomer migration order; **GBSA**, generalized Born/surface area; **HB**, hydrogen bond; **HDA-β-CD**, heptakis(2,3-di-O-acetyl)-β-CD; **HDAS-β-CD**, heptakis(2,3-di-O-acetyl-6-O-sulfo)-β-CD; **HDMS-β-CD**, heptakis(2,3-di-O-methyl-6-O-sulfo)-β-CD; **HF**, Hartree-Fock; **HMDS-β-CD**, heptakis(2-O-methyl-3,6-di-O-sulfo)-β-CD; **HP-β-CD**, hydroxypropy-β-CD; **ITC**, isothermal titration calorimetry, **MC**, Monte Carlo; **MD**, molecular dynamics; **MM**, molecular mechanics; **MMFF**, Merck molecular force field; **NOE**, nuclear Overhauser effect; **ONIOM**, own N-layer integrated molecular orbital molecular mechanics; **PCM**, polarizable continuum model; **PM3**, parametric method 3; **QM**, quantum mechanics; **SFC**, supercritical fluid chromatography; **TIP3P**, three-site transferrable intermolecular potential; **TM-β-CD**, heptakis(2,3,6-tri-O-methyl)-β-CD

Abstract

Cyclodextrins (CD) are cyclic oligosaccharides consisting of α-D-glucopyranosyl units linked through 1,4-linkages, which are obtained from enzymatic degradation of starch. The co-existence of hydrophilic and hydrophobic regions in the same structure makes these macrocycles extremely versatile as complexing host with application in food, cosmetics, environmental, agriculture, textile, pharmaceutical and chemical industries. Due to their inherent chirality, CDs have been also successfully used as chiral selectors in enantioseparation science, in particular for capillary electrophoresis (CE) enantioseparations. In the last decades, multidisciplinary approaches based on CE, NMR spectroscopy, X-ray crystallography, microcalorimetry, and molecular modeling have shed light on some aspects of recognition mechanisms underlying enantiodiscrimination. With the ever growing improvement of computer facilities, hardware and software, computational techniques have become a useful tool to model at molecular level the dynamics of diastereomeric associate formation to sample low-energy conformations, the binding energies between the enantiomer and the CD, and to profile noncovalent interactions contributing to the stability of CD/enantiomer association. On this basis, the aim of this review is to provide the reader with a critical overview on the applications of CDs in CE. In particular, the contemporary theory of the electrophoretic technique and the main structural features of CDs are described, with a specific focus on techniques, methods and approaches to model CE enantioseparations promoted by native and substituted CDs. A systematic compilation of all published literature has not been attempted.

1 Introduction

Application areas of cyclodextrins (CDs) include pharmaceutical, food, and chemical industry and to the widest extent analytical chemistry [1]. Here, CDs are used in UV-Vis, luminescence and NMR-spectroscopy, in electrochemical analysis. The ability of CDs to form inclusion complexes stereoselectively is used in the most efficient way in chiral separation techniques such as thin layer chromatography [2], gas chromatography [3], high-performance liquid chromatography (HPLC) [4-6], supercritical fluid chromatography (SFC) [7,8], capillary electrochromatography (CEC) [9-11] and, especially in capillary electrophoresis (CE) [12-18].

Over the last 30 years CDs and their derivatives have been established as major chiral selectors in CE. Principal requirements to a material to be used as a chiral selector in CE are the following: a) to interact with chiral compounds stereoselectively via intermolecular forces and b) the complexes formed should possess different mobility from the uncomplexed analyte. Other important properties are solubility in a background electrolyte, inertness (no response) to the detector used (for instance UV transparency), commercial availability, low costs, stability and environmental compatibility (nonhazardous, environmentally friendly). CDs meet these requirements better than any other chiral selector available at present time for chiral CE.

CDs offer certain advantages over other chiral selectors also from the viewpoint of molecular modeling studies. These are relatively small (especially compared to chiral polymers), well characterized chiral selectors, crystallographic data are available for quite many CDs and for some of their complexes, noncovalent interactions can be fine-tuned by selective modification of CDs. On the other hand, computed results can be correlated, corrected and double-checked based on instrumental techniques, such as CE and NMR spectroscopy.

Given this context, this review aims to cover the application of CDs as chiral selectors in CE, discussing the matter with a new approach by addressing experimental and theoretical issues in an integrated way, and focusing on understanding of chiral recognition mechanisms based on state-of-the-art computation techniques and methods. The intention of this review is not to cover comprehensively all the molecular modeling studies on CD-promoted CE enantioseparations. Rather than that, we aim to provide the reader with a modern and critical overview of the field, describing fundamentals of application of CDs as chiral selectors for CE enantioseparation, features of the most common CDs and related binding mecanisms, approaches to model CDs and their inclusion complexes, and representative applications of computational techniques in the field of CE enantiomer separation. In particular, we attempt to answer the following questions: a) What are current problems in modeling CDs, CD complexes with chiral guest molecules, and enantioselective recognition from the viewpoint of methodology and reliability of the results? b) How does CE challenge computation techniques and how can it contribute to the improvement of computation methods?

1.1 Brief historical tour about cyclodextrins

CDs were discovered in 1890s by the French scientist Villiers who obtained this material from the potato starch digest of *Bacillus Amylobacter* and named it "cellulosine" because of its similarity in some aspects to cellulose [19]. Later Schardinger found that one of the heat resistant bacteria was able to form crystalline "dextrins" from starch [20]. He distinguished two types of these dextrins, **A** and **B** and found later that form B was identical to the cellulosine of Villiers. Although Schardinger did not propose a structure for his crystalline dextrins, he made several observations regarding to their cyclic structure. One of the important finding by Schardinger was the complex-formation ability of CDs. He noticed: "With various substances, the crystalline dextrins form loose complexes" [21].

Freudenberg and Jacobi first succeeded to isolate pure α- and β-dextrins, as well as an additional crystalline dextrin that they named γ-dextrin [22]. In 1936, by the same research group, the ring structure of α-, β- and γ-dextrins was tentatively proposed [23], and soon experimentally confirmed that Schardinger dextrins are cyclic oligosaccharides composed solely of D-glucopyranosyl residues bonded by α-(1,4)-glycosidic linkages [24].

Freudenberg and co-workers initially assumed that the number of D-glucopyranosyl residues in α- and β-dextrin rings to be five and six, respectively. The correct values of six and seven D-glucopyranosyl residues per molecule, respectively, were determined by French who also proposed the names "cyclohexaamylose" and "cycloheptaamylose" for α- and β-dextrins, respectively [25]. Freudenberg later came to the same results on the basis of experimental data by his group and also proposed that the γ-dextrin consisted of eight D-glucopyranosyl residues connected via α-1,4 linkage in a cyclic structure, as in the case of α- and β- dextrins.

Another interesting aspect of CD history is the mechanism of their formation from starch. Initially, Freudenberg and co-workers [26] assumed that CDs are pre-formed in starch and are produced by the cleavage of the side branches by *Bacillus macerans* (Fig. 1A) [27]. However, the proposed structure of starch was soon abandoned by the same group [28], because it did not agree with usual conceptions regarding the linkage of the D-glucopyranose units. For example, it required certain D-glucopyranose units to be linked to three other glucopyranose moieties. Instead, the helical model of starch first proposed by Hanes, was adopted [29]. This model represents starch as α-linked

D-glucopyranose units in a helical arrangement. On the basis of this model Freudenberg interpreted the formation of CDs by *Bacillus macerans amylase* as a transglucosylation; that is, he suggested that a winding of the helix is cleaved by the enzyme (Fig. 1B) [30] and, because of the helical arrangement, the first and fifth or sixth D-glucopyranosyl residues are situated close to one other and are able to unite to form five- or six-member rings. Thus, it was concluded that the CDs are not pre-formed in starch, but their formation is made possible by the helicity of the starch chain. This mechanism of CD formation from starch was experimentally confirmed later and is currently generally accepted. The structures and the most important properties of α-, β- and γ-CDs are summarized in Figure 2 and Table 1 [1,31].

As mentioned above, the ability of CDs to form intermolecular complexes with other organic and inorganic molecules was already known to Schardinger who used the complexation ability of CDs with chloroform and alcohols for their precipitation and, moreover, the complexation with molecular iodine for the distinction between two dextrins which he named α and β [22]. Freudenberg was the first who assumed that these complexes are of the inclusion type [27,28].

The first direct evidence for molecular inclusions by CDs in the solid state came from X-ray crystallography. Hybl et al. determined the structure of the complex of α-CD with potassium acetate by using three-dimensional X-ray diffraction data. In the solid state, the acetate anions are included into the α-CD cavity [32].

An important advantage of CDs over some other host compounds is the ability to form inclusion complexes in solution. This property of CDs is especially important for their use in CE. In the case of many other hosts such as for example, urea, the guest molecule is included in the cavity which is formed by the crystal lattice of the host [33]. Thus, these inclusion complexes disintegrate upon dissolution. The cavity of CDs, however, is a property of the molecule (on the molecular level) and hence persists in solution.

^{1}H-NMR-spectroscopy provided the first direct evidence for an inclusion in the CD cavity in solution. Using aromatic guest molecules, Demarco and Thakkar found that, upon addition of a guest, the resonances of the hydrogen atoms of the CD situated inside the cavity were shifted significantly upfield due to the shielding by the aromatic guests. They noted little effect on the hydrogen atoms on the exterior of the CD annulus [34].

The most remarkable property of CDs, i.e. their ability to act stereoselectively in complex-formation, was discovered by Cramer in 1952. He noted: “Cyclodextrins distinguish not only molecules with different shape but optical antipodes too”. This statement was confirmed by the enantiomeric enrichment of mandelic acid, chlorophenylacetic acid and bromophenylacetic acid [35].

The very first example of CE-enantioseparations using CDs was reported in 1988 in capillary isotachophoretic mode [12].

1.2 General features of CD structure and related noncovalent interactions

The main intermolecular forces involved in complex formation between CDs and guest molecules are hydrogen bonding (HB), hydrophobic, dipole-dipole, and van der Waals interactions. As shown in Figure 2, α-, β- and γ-CDs possess 18, 21 and 24 hydroxy groups, respectively, which can be involved in HBs. Additionally, the hydroxy groups on the CD rims can be easily derivatized. This offers additional possibilities for the introduction of further HB, ionic or hydrophobic interaction sites (amino, carboxy, etc.) with desirable acidity and complexing abilities into the structure of CDs.

The inner cavity of CDs which is lined with hydrogen atoms and glycosidic oxygen bridges is hydrophobic which favors hydrophobic interactions between a guest and the CD host. In addition, even neutral CDs display an unusually high dipole moment. This means that CDs possess the ability to bind other molecules via dipole-dipole interactions.

Several studies document also significant contribution of van der Waals interactions in the complex formation between CDs and the guests. Thus, CDs offer multiple forces for efficient interaction with guest molecules. This multiplicity combined with different cavity dimensions of α-, β- and γ-CDs and their derivatives contribute strongly to a widespread application of CD hosts as selectors in CE.

The outer CD rims are formed by the secondary 2- and 3-, and the primary 6-hydroxyl groups. The location of the hydrophilic hydroxyl groups on the outer rim of CDs is responsible for the solubility of these materials in aqueous buffers. CD derivatives which are not soluble in aqueous buffers can be used in non-aqueous CE [36-38].

The HB between the secondary C(2) and C(3) hydroxyl groups of the adjacent D-glucopyranosyl residues stabilize the shape and the structure of the CD macrocycle

[1,31] and simultaneously cause the difference in the acidity of these hydroxyl groups. The former is very important for CDs as supramolecular hosts and the latter enables a regio- and site-specific derivatization of the CDs.

In CE the chiral recognition in selector/selectand interactions does not *a priori* mean that a chiral separation will be observed. Such a transportation mechanism must be realized through the separation capillary which can effectively differentiate between a complexed and free analyte.

In CE, the mobility of charged compounds depends on the effective charge density. The molecular mass of chiral analytes varies usually between 100 and 400 mass units. This means that a selectand/CD complex possesses a significantly higher mass and, thus, as a rule, a lower self-mobility than the free selectand (for exception see ref. 39). Alternative mechanisms can also be involved in CE-separation process. For instance, the self-mobility of complexed analyte in certain cases can exceed the mobility of free analyte. This can be used for a reversal of the enantiomer migration order (EMO) [40,41]. On the other hand, normally, no difference exists between the mobilities of complexed and free neutral analytes in the case of neutral chiral selectors. Therefore, no enantioseparation can be observed in this system in CE regardless of the binding selectivity. However, these are special cases which will be discussed in the appropriate sections in this review. With the last two paragraphs we want to stress that a mobility of a chiral selector is a very important property and it can be attached to CDs by derivatizing the hydroxyl groups with ionic substituents.

The important conclusions which can be drawn is that CDs are able to form stereoselective intermolecular complexes involving hydrophobic, dipole-dipole, dipole-induced dipole, van der Waals, HB and other interactions. These complexes may exhibit different mobility properties compared to the uncomplexed selectands. These are the conceptual reasons, together with easy availability, water solubility, UV-transparency and low cost of various CDs, for their successful applications as chiral selectors in CE.

1.3 Capillary electrophoresis

The major goal of this subsection is to give a short introduction to capillary electrophoresis (CE), about its advantages and bottlenecks, on the application of this technique for analytical purposes, as well as for better detecting of (enantioselective) intermolecular interactions.

Most related to contemporary CE technique seems to be the introduction of capillary zone electrophoresis (CZE) by Hjerten [42,43]. Although the instrumental set-up was relatively complex in these studies, it is important that for the first time an electrophoretic experiment was performed without supporting stabilizing media. The latter were used in previous experiments to prevent substantial zone dispersion due to hydrodynamic flow which was caused by Joule heating.

The most important breakthrough in development of CE technologies seems to be the work by Jorgenson and Lukacs published in 1981-1982 in which they used 75 μm open glass capillaries and an electric field as high as 30 kV/m [44,45]. Spectacular resolutions of various analytes achieved in these works attracted wider attention and played a key role in the further development of this technique.

The next important achievement was the introduction of micellar electrokinetic chromatography by Terabe and co-workers in 1984-1985 [46,47]. This technique owes its migration principle to electrophoresis and its separation principle to chromatography. The application range of capillary electrophoretic techniques were expanded to neutral compounds by this outstanding innovation.

The first automated CE instrument was introduced on the market under the name Microphoretic 1000 by Microphoretic Systems, Inc., Sunnyvale, CA, USA in 1987.

The first separation of enantiomers in CE was reported by Zare and coworkers in 1985 on the example of enantiomers of native amino acids resolved based on the ligand-exchange principle [48]. The first application of CDs as chiral selectors in various formats of CE were reported between 1988-1992 [12,49-51] and this was followed by a steep increase of the activity in this field.

What are the major advantages of CE for separation of enantiomers? Alternative separation mechanism to other separation techniques, high efficiency, high separation power, high flexibility, low consumption of materials, low costs. Below each of these aspects are discussed very shortly.

Despite the fact that enantioseparations in most cases in CE and chromatographic techniques rely on the same phenomenon, i.e. on enatioselective noncovalent interactions between the analyte and the chiral selector (for this reason all chiral CE separations belong actually to capillary electrokinetic chromatography (CEKC)) [52], there are significant differences between these techniques. Responsible for all differences between chromatographic and electrophoretic enantioseparations is the ability of the electrophoretic mobility to be selective for species residing in the same

phase. First of all due to this reason in CEKC it is possible to perform separations in monophase while in chromatographic techniques two phases are conceptually required [52]. Another important point is that in chromatographic techniques, except for the application of a chiral mobile phase additive (CMPA), the analyte is virtually immobile when associated with the chiral selector. In CEKC the analyte selector complex is usually mobile [52].

Many principal differences between chromatographic and electrophoretic enantioseparations can be derived analyzing the equation for the electrophoretic mobility difference Δμ between enantiomers [53,54]:

$$\Delta\mu = \mu_1 - \mu_2 = \frac{\mu_f + \mu_{C_1} K_1[C]}{1 + K_1[C]} - \frac{\mu_f + \mu_{C_2} K_2[C]}{1 + K_2[C]} \quad (1)$$

where μ_1 and μ_2 are the mobilities of the first and the second migrating enantiomer, respectively. K_1 and K_2 are the binding constants between enantiomer 1 and 2 and the chiral selector, respectively, μ_f and μ_c are the mobilities of the free and complexed analyte, respectively, and [C] is the concentration of a chiral selector.

One important point obvious from equation (1) is the crucial role of the mobilities in enantioseparations in CE. This parameter is absent in the major chromatographic techniques. The contribution of the mobilities in chiral CE separations results in the following distinguished effects:

1) it is feasible in chiral CE but not in chromatographic techniques that the apparent selectivity of enantioseparation exceeds the thermodynamic selectivity of the chiral recognition [55];

2) it is possible in chiral CE to adjust EMO without reversal of the affinity pattern between the enantiomers of the analyte and a chiral selector. This is impossible in chromatographic techniques at least in the mode when the chiral selector is immobilized and not used as a CMPA [40,41,52,56];

3) the most striking difference between these two techniques seems to be the fact that CE allows, in principle, the enantioseparation in the absence of the binding constant difference between the two enantiomers with a chiral selector [52,57-59].

Below, these differences between CE and chromatographic enantioseparations are illustrated using selected examples from the literature.

As already mentioned above, in chromatographic techniques the selectivity of enantioseparations is entirely defined by the chiral recognition, i.e. by the difference between the affinity of the enantiomers towards the chiral selector. Therefore, the selectivity of enantioseparations in common chromatographic techniques may in the best case approach the thermodynamic selectivity of the chiral recognition but will never exceed it. One major consequence of the mobility contribution in separations in CEKC is that the apparent separation selectivity may exceed the thermodynamic selectivity of the recognition. This is experimentally illustrated in Fig. 3 [55]. In all separations of the chlorpheniramine enantiomers with carboxymethyl (CM)-β-CD shown here, the components involved in chiral recognition on the molecular level are invariant. This means that chiral recognition itself does not change significantly. However, an enormous (in principle unlimited) enhancement of the apparent separation selectivity becomes possible in the step of transforming the chiral recognition into a chiral separation. In this particular example, this was achieved by applying a counterbalancing pressure to the separation capillary in the opposite direction to the analyte migration according to the scheme shown in Fig. 4 [55]. As shown schematically in Fig. 4, this concept may allow designing a separation system in a way that two enantiomers certainly possessing the electric charge of the same sign will migrate towards opposite electrodes, which means that the apparent enantioseparation factor becomes infinitely large [55].

Another difference between enantioseparations in CE and HPLC is the fact that an enantioseparation even in the absence of a binding constant difference for the enantiomers with a chiral selector is, in principle, feasible in CE [52,57-59]. This conclusion can be derived from equation (1) [52]. According to this equation, for the generation of a mobility difference between the enantiomers, e. g. enantioseparation in CE, the following is required:

a) formation of transient diastereomeric complexes between the analyte and chiral selector. This means that the enantioseparation is impossible in CE without chiral selector;

b) effective mobilities must be different for the free and complexed analyte.

If both prerequisites apply, enantiomers may be resolved with equal success by following two alternative mechanisms:

1) the residence time in the free and complexed forms is not equal for both enantiomers. The time which the enantiomers reside in the free and complexed form is defined by the binding constants, e. g. in this case a difference in binding constants is required. This means that the enantioseparation will be based on the same principle as in chromatographic techniques. If one assumes in such case that the diastereomeric associates of both enantiomers with a chiral selector have the same mobility (i.e. $\mu_{c1} = \mu_{c2}$ in equation (1)) then equation (1) simplifies as:

$$\Delta\mu = \frac{C(\mu_f - \mu_c)(K_1 - K_2)}{1 + C\left[K_1 + K_2\right] + C^2 K_1 K_2} \qquad (2)$$

2) alternatively, both enantiomers may reside the same time period in a free and complexed form, e. g. $K_1 = K_2 = K$. The enantioselective binding with the chiral selector, but not necessarily chiral separation is absent in this case. Under these conditions equation (1) can be rewritten in following form [52]:

$$\Delta\mu = \mu_1 - \mu_2 = \frac{K\left[C\right](\mu_{C1} - \mu_{C2})}{1 + K\left[C\right]} \qquad (3)$$

From equation (3) it is clear that the prerequisite for the enantioseparation in this case is a formation of the temporary diastereomeric complexes between both enantiomers and a chiral selector and these complexes must possess different mobilities μ_{c1} and μ_{c2}.

Thus, both principles, either the binding constants difference (chiral recognition) or a mobility difference of the corresponding diastereomeric complexes, may result in enantioseparations in CE. Rather common is the first case or a combination of both.

Thus, as summarized in this section, there are significant differences between enantioseparations in pressure-driven and electrically-driven systems. On one hand, these differences make the techniques complementary. This is an advantage. On the other hand, the rules and dependencies observed in one technique should be applied to the other with some care in order to avoid mistakes in the interpretations of the experimental results.

From the viewpoint of this review it has to be stressed that correlations between chiral recognition that can be computed on the molecular level with separation of enantiomers is more straightforward in HPLC compared to CEKC. Thus, chiral recognition in selector-selectand complex is a precondition and, at the same time, can

be sufficient for separation of enantiomers in HPLC, while in CEKC chiral recognition in selector-selectand complex is neither a prerequisite nor *a priori* sufficient for separation of enantiomers.

1.4 Advantages and disadvantages of CEKC for studying chiral recognition

In subsection 1.3 some advantages of CEKC over chromatographic techniques from the viewpoint of enantioseparations were mentioned. This subsection extends these advantages and stresses the limitation of CEKC for studies related not only to enantioseparations but to enantioselective recognition and related intermolecular interactions in general:

1) in CE highest peak performance can be achieved among all separation techniques. Due to high peak performance the required value of thermodynamic selectivity of recognition in order to observe baseline resolved peak is significantly lower in CE (ca. 1.01) compared to gas chromatography (ca. 1.05) and HPLC (ca. 1.10). Together with separation techniques there is also no separation technique (at least to the best of our knowledge) that can detect weaker intermolecular interactions than CE;

2) as mentioned in subsection 1.3 in CEKC high separation selectivity can be generated based on low thermodynamic selectivity [55]. Such kind of “amplification” of recognition is also advantageous for detection of weak intermolecular interactions;

3) a change of chiral selector is easy and equilibration time is short in CEKC compared to chromatographic techniques;

4) in CEKC the concentration of a chiral selector can be varied much easier than in chromatographic techniques. This is another tool for amplification of weak intermolecular interactions;

5) combination of chiral selectors is easier in CEKC than column coupling in chromatographic techniques;

6) CEKC as a miniaturized technique requires minute amounts of selectors, solvents (which in addition are mostly aqueous), and other consumables and are thus, less expensive and environmentally friendlier technique.

What are the bottlenecks of CEKC? From the separation science point of view the major problem is that CEKC cannot be used for preparative separations in commonly accepted scale. The infancy problems of CE, such as low detection sensitivity and

reproducibility of results have been successfully resolved by developing various sample preconcentration and detection tools. As the miniaturized technique, CE offers many advantages but at the same time a void volume becomes more critical in this technique. Thus, CE is less standardized technique and requires in general more know-how rather than performing chromatographic experiments.

From the viewpoint of molecular recognition studies, the major disadvantage of CEKC, similar with other separation techniques, is that it does not provide direct structural information about selector-selectand (host-guest) complexes. The information about host-guest association constants, as well as, indirectly, about the stoichiometry of complexes can be recovered but the structure of complexes commonly remains beyond the reach of CEKC.

2 Cyclodextrins as chiral selectors in capillary electrokinetic chromatography

2.1 Native cyclodextrins

As the experience of last three decades shows, native CDs are useful chiral selectors for analytical scale separation of enantiomers of charged chiral analytes. Based on the geometric considerations one may assume that the organic molecules with medium size will better fit to the cavity of β-CD, while α-CD can be somehow small for a complex formation, and γ-CD may form loose complexes. This assumption is supported by experimental results from CE studies where β-CD became most popular chiral selector of three native CDs. In the case when together with β-CD one or both of other native CDs also separate the enantiomers of a given chiral selector, the α- and γ-CDs are commonly required in higher concentration compared to β-CD (Table 2) [60-67]. Another interesting issue is the type of complexes and the affinity pattern of enantiomers of given chiral guest towards native CDs. After Freudenberg`s initial conclusion [27,28] and later experimental proofs that CD complexes in the solid state [32] and in solution [34] are of inclusion type, there is a tendency to believe that all complexes of CDs are of inclusion type. This does not seem to be true and there is at least one example described in the literature when successful chiral recognition/separation proved based on CEKC study while the α-CD/guest complex was of external type (Fig. 5) [64]. Since all CDs are built of D-glucopyranose units in

a single stereochemical configuration and there are no CDs available which are built of L-glucopyranose units, the initial belief was that the enantiomer affinity pattern toward at least all 3 native CDs (with only difference in the number of glucopyranose units in the macrocycle) would be the same. As CEKC studies show there are quite many exceptions from this assumption [60-67]. For instance, the enantiomers of aminoglutethimide (AGT) exhibit the same affinity pattern toward α- and γ-CDs, while the affinity pattern towards β-CD is opposite to that (Fig. 6) [60]. Based on rotating frame nuclear Overhauser effect (ROESY) experiments in NMR spectroscopy it was found that the enantiomers of AGT enter the cavity of β-CD and γ-CD from the opposite, secondary and primary sides, respectively (Fig. 6). However, if this difference in the structure of complexes is the reason of opposite affinity of the enantiomers towards these CDs has still to be proven by calculation of forces involved in binding and enantioselective recognition. The affinity pattern of the terbutaline enantiomers towards α-CD and β-CD was also opposite in the abovementioned case with external and inclusion complexes, respectively (Fig. 5) [64]. In quite many cases the opposite affinity pattern of enantiomers can be proved based on CEKC experiments, however there is only minor difference between the structure of CD-guest complexes deduced from NMR spectroscopy. The examples for ephedrine [61] and norephedrine [63] complexes are shown as examples in Figure 7. These are the cases when CEKC, due to its extremely high sensitivity for detection of intermolecular recognitions, challenges other instrumental and currently available computation tools. At the same time the results of CE study, as direct experimental evidence can be successfully used to refine the experimental and computation tools currently available for studies of intermolecular recognition [17,18].

2.2 Substituted cyclodextrins

One of the important advantages of using CDs as chiral CE selectors as mentioned above is the possibility of their derivatization by introducing various noncharged and charged groups randomly or selectively on the CD rims. The chemistry of CDs is independent research field as such and reviewing it even superficially in this review paper is impossible. Randomly substituted CD derivatives are useful chiral selector for CEKC. They can also be well characterized by state-of-the-art techniques [68,69], as well as can be produced in quite reproducible way. However, the randomly derivatized CDs do not represent ideal objects for mechanistic studies addressed in this paper.

The problem is that firstly, randomly substituted CDs represent multicomponent mixtures not having one defined molecular mass and thus, all molar properties (molar Gibbs energy, entropy and enthalpy), as well as the characteristics such as binding constants, selectivity, stoichiometry, etc. cannot be applied to such derivatives. In addition, the resonance signals in NMR spectra of randomly derivatized CDs are severely overlapped and not easy to be assigned and corresponding hydrogen atoms selectively irradiated in nuclear Overhauser effect (NOE)-based experiments for deducing the structure of complexes in solution. We will focus below on selectively substituted CD derivatives, although randomly substituted derivatives of CDs, especially hydroxypropyl-β-CD, methyl-β-CD, sulfobutyl-β-CD, sulfated CDs, CM-β-CD and several others are widely used chiral selectors in CEKC [14-18,52].

Different reactivity of the hydroxyl groups on the CD rims makes it possible to selectively protect, activate and finally derivatize these groups. Based on this strategy, nonionic and ionic CD derivatives can be synthesized carrying different functionalities on the primary or secondary rim or even more selectively, in positions 2, 3 and 6. Such kind of CD derivatives are better known, some of them commercialized and successfully used as chiral CEKC selectors. Based on more precise (fine) activation and protection strategy it is possible to derivatize a single glucopyranose unit in a CD, or make derivatives having various combination of derivatized glucopyranose moieties in the CD macrocycle, so called capped CDs [70,71]. These latter derivatives of CDs are commercially not available and actually not studied in CEKC, as well as perhaps in other techniques, from the viewpoint of (enantioselective) recognition ability in intermolecular interactions, although they have been systematically evaluated as artificial mimics of enzymes [70,71].

2.3 Alkylated and acylated cyclodextrins

Of neutral CD derivatives alkylated and acylated/acetylated derivatives are quite well studied as chiral selectors in CEKC. Methylation of the CD rim affects the size and the structure of the cavity, as well as ability of CD to get involved in intermolecular interactions. Multivariate scenarios observed due to selective methylation of hydroxyl groups from the viewpoint of stoichiometry, association constants, structure of complexes and chiral recognition ability of CDs were observed with various techniques, such as X-ray crystallography, ultraviolet-visible and circular dichroism spectroscopy, NMR spectroscopy, molecular modeling and among them also with

CEKC. These earlier studies are summarized in ref. [72]. For instance, the enantiomers of chlorpheniramine [73], verapamil [74], dimethindene [75] and brompheniramine [76] exhibit opposite affinity pattern towards the native β-CD and its permethylated derivative heptakis-(2,3,6-tri-*O*-methy)-β-CD (TM-β-CD) (Table 3) [17]. Multidisciplinary attempts were made to explain these differences in molecular recognition and some interesting results were obtained [77]. For instance, based on X-ray analysis of (+)-brompheniramine maleate and brompheniramine co-crystals with β-CD and TM-β-CD, different stoichiometry and structure of complexes were evidenced (Fig. 8) [76].

Another interesting group of neutral derivatives of CDs is acylated/(mostly acetylated) CDs. Among these, best studied is heptakis (2,3-di-*O*-acetyl)-β-CD (HDA-β-CD) [78-84]. Early detailed studies on the chiral recognition ability of HDA-β-CD and the structure of its complexes were performed by Holzgrabe and co-workers [78-82]. This group also paid attention to the opposite affinity of enantiomers of some chiral compounds towards native β-CD and HDA-β-CD [80]. It is obvious that HDA-β-CD possesses quite different recognition mechanism of enantiomers because the enantiomers of chiral compounds commonly exhibit opposite affinity pattern towards native β-CD and HDA-β-CD. Some examples of this kind are summarized in Table 4. Significantly different chiral recognition ability of HDA-β-CD may be related to self-association of acetyl substituents into the cavity of β-CD significantly hindering inclusion of guest molecules into the cavity from the same secondary side [85].

2.4 Charged cyclodextrins

The chemical modifications of CDs discussed in the previous subsection affect the chiral recognition ability of CDs on the molecular level but do not change their mobility in CE. As it has been stressed in subsection 1.3, in CEKC the mobility of selector (mostly responsible for the mobility of the selector-selectand complex) is as important, as its chiral recognition ability for obtaining separation of enantiomers. Thus, introduction of charged CDs was an important milestone in development of chiral CEKC and is shortly discussed below [51,52,57,86-90].

The first application of a charged CD, mono-(6-β-aminoethylamino-6-deoxy)-β-CD for separation of enantiomers in CEKC was reported by Terabe in 1989 [51]. The author also noted the possibility of the application of a charged chiral selector as a carrier for (neutral) analytes. Later, it was emphasized that the enantiomers of neutral chiral

analytes which were conceptually unresolvable with the neutral chiral selectors could be resolved with the charged ones [86,87]. There are many other advantages of charged chiral selectors related to their mobility [51,52,57,86-90]. Actually, the charge together with providing a self-mobility to a chiral selector can amplify the electrostatic interaction with oppositely charged guests and apparently positively affect its recognition ability. This can be seen on the comparison of chiral recognition ability of HDA-β-CD and its charged analogue heptakis(2,3-di-O-acetyl-6-O-sulfo)-β-CD (HDAS-β-CD). It has been highlighted in several studies that HDAS-β-CD exhibits significantly higher chiral resolving ability of enantiomers compared to HDA-β-CD [79, 91]. One recent example is shown in Fig. 9 [91]. It has to be noticed, that the chiral selector with the opposite charge compared to the chiral analyte will *a priori* enable higher separation selectivity compared to its neutral counterpart with the same thermodynamic selectivity of recognition [87]. This is a mobility contribution of a chiral selector in enantioseparation. However, the differences observed for these two chiral selectors in some cases are too large to be ascribed only to the countercurrent mobility of a chiral selector compared to an analyte.

There is one member in the family of single component sulfated derivatives of β-CD, namely heptakis(2-O-methyl-3,6-di-O-sulfo)-β-CD (HMDS-β-CD) which deserves a special attention from the viewpoint of enantioselective recognition. The intermolecular complexes of HMDS-β-CD with chiral guest is mostly not of inclusion but of external type. In addition, it exhibits quite strong enantiomer resolving ability and the enantiomer affinity towards native β-CD and HMDS-β-CD are frequently opposite to each other (Table 5) [92-94]. As these examples show, the introduction of charged groups into the structure of CDs not only attaches to CD derivative a mobility in CEKC experiment that *per se* is very important, but also significantly alters its chiral recognition ability (thermodynamic enantioselectivity).

3 Molecular modeling of cyclodextrins and their inclusion complexes: structures, techniques, and methods

Structural features of CDs and their complexes have been mostly explored using X-ray crystallographic analysis [95] and NMR spectroscopy [96,97] in the solid state and in solution, respectively. In particular, X-ray structures provides a direct evidence of the inclusion of the guest molecule in the CD cavity. For this reason, some of the

earliest attempts to understand structures of CDs through computational methods were performed in the frame of X-ray crystallography studies of this class of oligosaccharides starting in the early 1970s. In those years, French and Murphy analyzed existing structural data to define a suitable geometry for the glucose residue, and then a screw operator was used to build the model of the oligosaccharide chain [98]. This study represented a rough approach to modeling of CDs, however, it laid the bases for computing structures of completely circular amyloses, and the authors derived a set of structural parameters of α-, β- and γ-CDs just using that approach [99]. The first real computational study of CDs was published in 1970 by Sundararajan and Rao [100]. At that time, the X-ray structure of α-CD alone being known [32], a simplified molecular mechanics (MM) model was used by the authors to understand the conformations of other CD systems, and to determine whether the inability of *Bacillus macerans* to form smaller CD systems was due to enzyme specificity or to the instability of smaller macrocycle rings. The calculations showed that CDs with fewer than six glucopyranose units were too strained to exist. However, later Nakagawa and co-authors synthesized a cyclomaltopentaose derivative [101]. On this basis, the model proposed by Sundararajan and Rao proved to be inadequate for this predictive purpose. However, the comparative analysis of these first MM models of α-, β-, and γ-CDs allowed to predict a decrease of the macrocycle stability ranging from γ- to β-, and α-CDs, these calculations being in agreement with the flexibility observed for α-CD through spectroscopic and X-ray crystallographic studies [102,103]. Tabushi and co-authors developed a more elaborated model of the inclusion process of α-CD in water [104], calculating the free energy change, including enthalpy and entropy terms, as an apolar guest is included in the α-CD. Later, Tabushi's group tried to analyze the impact of guest polarity in the stabilization of the CD complexes by means of a MM model [105]. Despite the low level of refinement of these first models, they represent the first attempts to model CDs and their complexes, paving the way to application of atomistic modeling in this field.

In the following subsections, after a brief description of the structural features of the most common CDs determined by X-ray diffraction analysis, and of the main issues concerning the binding mechanisms of CD inclusion complexes, a general overview of techniques, methods and open questions concerning modeling of CDs and their complexes will be presented. This short overview covers examples of CD modeling

which are not strictly related to CE enantioseparation, but that can provide interesting pieces of information also in this field.

3.1 Solid state structures of common cyclodextrins and related inclusion complexes

In 1942, the crystallographic method was first applied to determine molecular weight of α- and β-CDs [25]. The crystal structure first solved by X-ray analysis in 1965 was that of the α-CD complex with potassium acetate [32]. The structures of β-CD [106] and γ-CD [103,107] were determined in 1976 and 1980, respectively. α-CD crystallizes from water in three hydrate forms, hexahydrate (form I and form II) and 7.57 hydrate (Form III). The α-CD ring in the form I crystal, including two water molecules, was found to be less symmetrical than the macrocycle in the form III crystal which contains 2.57 water molecules in the cavity of the round macrocyclic ring. β-CD crystallizes in two round forms which differ in the arrangement of water molecules in the cavity. γ-CD has the most symmetrical structure compared to α- and β-CDs [103,107,108]. In native CDs, the pyranose ring of each glucose unit is relatively rigid and assumes the $^4C_1$ chair conformation (Fig. 10). HBs are formed between an O(3)H hydroxyl group and the O(2)H hydroxyl group of an adjacent glucose unit. The effect of increasing glucose units on the stability (flexibility) of the macrocycle was assessed by comparing the crystal structures of α-, β-, and γ-CDs, and determining the length of the O(3)H···O(2)H HBs between the adjacent glucose units [103]. This distance was found to increase following the order γ-CD (2.81 Å) < β-CD (2.86 Å) < α-CD (3.00 Å).

X-ray crystallographic analysis has provided essential pieces of information on the impact of substitution on the conformation of the macrocyclic ring and host-guest interactions in the solid state. The hexakis(2,6-di-O-methyl)-α-CD (2,6-DM-α-CD) was found round as the native macrocycle [109]. Several studies reported the crystal structures of 2,6-DM-β-CD complexes [110], and showed that also this macrocyclic ring is almost round as β-cyclodextrin (Fig. 11A,B) [111]. In this case, the round shape is maintained by intramolecular O(3)H···O(2)Me. Otherwise, in crystal structures of permethylated α-CD, the macrocyclic ring was found to be distorted by the steric hindrance exerted by the methyl group introduced to secondary hydroxyl groups [112]. Harata and co-authors reported the first structural determination of TM-β-CD in the shape of an elliptically distorted and truncated cone [113]. In this case, due to the methylation of the O(3)H hydroxyl groups, the distance between O(2) and the O(3) of

the adjacent residue increased from 2.9 to 3.5 Å. Therefore, the methylation of the O(2)H hydroxyl groups does not affect the formation of intramolecular HBs sustaining the round shape of the macrocycle, whereas the further methylation at the O(3) position impacts the formation of the intramolecular HB network underlying the round structure. Moreover, it was found that the full methylation affects not only the macrocyclic conformation but also the pyranose conformation of the glucose residues. Guest-induced conformational change in the CD macrocycle was found in the TM-β-CD / *m*-iodophenol complex, with six glucosyl units in the $^{4}C_{1}$ chair conformation and one $^{0}S_{2}$-twist boat glucosyl unit (Figs. 10 and 11C) [114]. The cyclic CD structure with a fully inverted glucose ring to the $^{1}C_{4}$ chair conformation (Figs. 10 and 11D) was crystallized from hot water in the monohydrate TM-β-CD [115,116]. In agreement with X-ray derived findings, Chao and co-authors confirmed by molecular dynamics (MD) simulations how different degrees of methylation have an effect on the overall CD structural features, and that changing the conformation of one of the glucosyl rings within the CD can drastically alter the overall macrocyclic structure [117]. It is worth mentioning that the structural differences observed in the solid states between β- and TM-β-CD also may impact the complexation properties of the macrocycles in solution. As mentioned above, the enantiomers of chlorpheniramine, verapamil, dimethindene and brompheniramine showed opposite affinity pattern towards the native β-CD and its permethylated derivative TM-β-CD in capillary electrophoresis analyses (Table 3) [73-76]. Liu and co-authors investigated the binding behaviour of β- and TM-β-CD upon complexation with azobenzenes by X-ray crystallography, circular dichroism, 2D NMR spectroscopy, and isothermal titration calorimetry (ITC) [118]. The two CDs showed different binding modes toward the guests with a different spatial arrangement both in solution and in the solid state. Moreover, ITC investigations indicated that TM-β-CD formed more stable complexes with the azobenzene guests than β-CD due to the more favorable entropy change associated to the complexation process involving TM-β-CD. The opposite affinities were found by Bethanis and co-authors in the complexation of β-citronellol with β- and TM-β-CD [119]. In this case, MD simulations based on crystal structures showed that in a simulated aqueous medium the guest maintains the inclusion mode observed crystallographically. Moreover, the comparison of the binding affinity of the two CDs toward the guest based on MM-generalized Born/surface area (MM/GBSA) calculations indicated that the inclusion of β-citronellol in TM-β-CD is less favorable than in β-CD and DM-β-CD. On the other

hand, in the crystal structure of the complex β-citronellol / TM-β-CD the guest was partially encapsulated in the TM-β-CD due to the steric hindrance exerted by the methoxyl groups at the narrow rim (primary hydroxyl side).

Distorted macrocycles were also found in the crystal structures of complexes of peracylated CDs [85]. In particular, in heptakis(2,3,6-tri-O-butanoyl)-β-CD, all glucosyl units adopt the common $^4C_1$-chair conformation, and one butanoyl chain intramolecularly penetrates the cavity, whereas in heptakis(2,3,6-tri-O-acetyl)-β-CD and heptakis(2,3,6-tri-O-propanoyl)-β-CD, one glucosyl unit occurs in $^0S_2$-skew-boat conformation and one acyl chain closes the O_6 side (narrow rim) like a lid.

As highlighted in the previous discussion, native CDs containing from 6 to 8 glucopyranosyl units are rather rigid molecules, also maintaining a round shape after complexation of the guest. Otherwise, X-ray crystallographic analyses as well as computational studies evidenced a certain non-rigidity of some substituted CD rings. This observed flexibility is mostly ascribed to the rotational freedom of each glucose unit around the α-1,4-glycosidic linkage, and to small changes in the endocyclic torsion angles of the pyranose ring. Consequently, upon complex formation, CDs may change their macrocyclic structure and adjust the structure of the cavity to accommodate the guest molecule. In some cases, the flexibility of the glucosyl units is still restrained by the intramolecular HB network, the macrocycle maintaining its roundness. Otherwise, when the introduction of specific substituents weakens or disrupts intramolecular HBs, glucosyl units gain high flexibility around the glycosidic linkage, as it occurs in the structures of permethylated and peracetylated CDs.

3.2 Noncovalent interactions and binding mechanisms

One of the most important features of CDs concerns the presence of both hydrophilic and hydrophobic groups which co-exist in the same structure. The arrangement of these functional groups profiles hydrophilic and hydrophobic regions in the outer and in the inner part of the macrocycle, respectively. In particular, the cavity presents a hydrophobic surface, the inner part of the torus being occupied by axial C-H bonds. Otherwise, the hydrophilic hydroxyl groups occupy both rims of the cone, which make CDs soluble in water, even if the solubility of β-CD is lower than the others (Table 1) [120]. The occurrence of dynamical flip-flop O(2)···O(3) HBs at the secondary hydroxyl rim of β-CD makes the macrocycle more rigid, contributing to its low solubility [121]. Neutron diffraction studies demonstrated that the hydrogen atoms of the secondary

hydroxyl groups of the β-CD are not statistically disordered and, at ambient temperature, the HBs were observed as the average of O(2)···HO3 and O(3)···HO(2). The dynamical flip-flop of the intramolecular HBs was also confirmed by quantum mechanics (QM) calculations performed at DFT level of theory [122], even if these calculations showed some discrepancies with respect to X-ray structures [123]. Moreover, in β-CD crystals the flip-flop disorder was also observed in HB chains involving water molecules. The direction of the O-H bonds changes cooperatively (O-H···O ⇌ O···H-O), and this type of flip-flop network is considered to be entropically more favored than a network with ordered HBs [95,121].

The identification of noncovalent interactions is of great importance for the understanding of the binding mechanisms. In CD complexes a multitude of simultaneously occurring interactions, including polar interactions, dispersive interactions and hydrophobic effect [124] may make the analysis of the binding mechanisms challenging. The interactions between CD and the guest are often related to different mechanisms. Indeed, as mentioned in the previous section, CDs are known to form inclusion complexes, and to also bind guests outside the cavity [125]. In CE enantioseparations, external complexes have been observed either with highly charged CDs [92,93] or in non-aqueous electrolytes [38,126,127]. Very recently, the first case of native CD exhibiting chiral recognition ability not through inclusion but rather by formation of an external complex has been reported [64]. Moreover, deviations from 1:1 complexes may occur with formation of self-associated aggregates, cooperativity-driven assemblies, and higher order 1:2 and 1:3 complexes [39,76,128,129].

Thus, several factors may underlie CD complex stability, solely or in combination, some of them still considered rather controversial:

1) *noncovalent interactions* contributing to complex formation such as electrostatic interactions, van der Waals and dispersion forces, and HBs can control complex formation depending on the peculiar structures of the interacting partners [124];

2) on the basis of their studies in the solid state, Saenger and co-authors hypothesized that the empty cavity of α-CD hexahydrate provides a conformation that is energetically less stable than the included structure [102]. Following this reasoning, unfavorable HBs of the glucose backbone with water or a tendency of some macrocycles to collapse, or to steric hindrance-induced distortion, may contribute to destabilize the empty macrocycle. Consequently, the deviation from hexagonal

symmetry of α-CD hexahydrate in the solid state could constitute a store of energy, whose relief upon complex formation is a major source of energy, driving the complexation. However, later the hypothesis that the *relief of conformational strain energy* could drive complex formation in the solid state was criticized when applied to complex formation in solvated environment [120,124,130]. The first argument concerns the fact that, in the solid state, a CD has a higher conformational energy than that in solution, consequently the thermodynamics of the CD complexation in solution in general does not involve the energy of a solid state CD. On the other hand, β- and γ-CDs maintain a regular structure in their complexes, even when very stable complexes are formed. On this basis, it is likely that conformational strain energy does not play a dominant role in overall energetics of binding. More acceptable is the idea of an "induced fit" in terms of a conformational adjustment which allows for optimizing complex geometry and improving interaction modes [131];

3) in the last three decades, several studies explored the origin of the *hydrophobic effect* in CD inclusion complexation. The role of the hydrophobic interaction in CD complexation is another controversial issue. In the "classical" hydrophobic interaction between two apolar molecules the structure of water in the vicinity of the solutes is the key feature of the phenomenon [120]. Traditionally, the Frank-Evans model [132] explains the hydrophobic forces by invoking the formation of a large cavity around two nonpolar surfaces, for which a smaller number of solvating water molecules is required than for complexation in two smaller separated cavities with an entropic advantage due to the liberation of water molecules. On this basis, hydrophobicity is considered to be entropically driven as the ordered water around the solute gains entropy upon relocating to the bulk medium. The enthalpy and entropy changes of the process are both positive, this fact being considered as a sign of the effect [124,133]. In a complementary model, the liberated water molecules are able to form more cohesive water-water interactions with an associated enthalpy gain [134]. However, Connors observed [120] that these models are unsuitable for CD on the basis of the "semipolar" nature of these macrocycles and the specific features of related hydrophobic contacts. On the other hand, the experimental observation is that in most CD complex formation processes, $\Delta H°$ and $\Delta S°$ are both negative and the association appears to be "enthalpy driven" [135]. More recently, both computational and experimental studies have confirmed the importance of the so-called "*high energy water*" to explain enthalpically driven hydrophobic contact in CDs [136]. The phenomenon originates

from the fact that, in aqueous solution, in the absence of a guest molecule, the CD cavity is typically filled by water molecules, as shown by the X-ray and neutron diffraction studies. These water molecules confined within the cavity may not be able to fully participate in the hydrogen bond network as in bulk medium and, consequently, would be energetically frustrated [124]. Liberation of this high-energy water from the cavity, as a guest enters inside, makes the cavity-guest complexation an enthalpically-driven process [136,137]. The idea was developed in early 1970s by Bender in terms of “enthalpy-rich” water [138]. Saenger and co-authors defined this kind of water molecules “activated” water [139]. In the beginning this idea was rather controversial, the main criticism related to the problem that high-energy water hypothesis appeared to be focused on the water, neglecting the role of CD and the energetics of the entire system [120]. However, later the release of the high-energy waters from the cavity was confirmed through ITC and MD simulations as an essential driving force for high affinity binding of neutral guest molecules with cucurbit[n]urils [140]. Very recently, atomistic MD simulation of native CDs in water revealed that a water molecule in CD cavity loses HBs, remaining energetically frustrated but with higher degree of freedom compared to bulk water [136,137,141].

3.3 Computational modeling of cyclodextrins and their complexes

Given this molecular context, the inclusion of guest molecules into CDs is a complex phenomenon involving a dynamic network of noncovalent interactions as well as conformational and solvation/desolvation factors which may impact the overall process. On one hand, several experimental techniques such as X-ray crystallography and NMR spectroscopy, among others, have provided relevant information on CDs and their complexes. On the other hand, the experimental techniques are somehow limited to provide details at microscopic level.

A microscopic (atomistic) model representing a real event can be built for predictive purposes or to explain the experimental reality at molecular level. With the ever growing improvement of computer facilities, hardware and software, molecular modeling has become a basic tool to model medium and large molecular systems such as CDs and their complexes. With the aim of getting a better understanding of the binding event and affinity of CDs towards the guest(s), theoretical techniques such as MM, semiempirical, DFT and ab *initio* calculations, molecular docking, Monte Carlo (MC) and MD simulations can be used. In this regards, very good reviews have been

reported periodically [142-145]. However, as computation chemistry is used in the field of CDs, some questions may still arise, which have to be carefully considered, depending on the features of the real experimental system:

a) CDs are large molecules, in some cases showing a certain degree of conformational flexibility depending on rotatable bonds. Thus complete conformational search serves to locate all populated states at ambient temperature. Moreover, the conformations of the constituent α-glucopyranose units are found to differ significantly from a free monomeric α-glucopyranose units;

b) CDs often are studied in aqueous environment where, as discussed in the previous subsection, the solvent has a pivotal role in determining thermodynamics of complexation. In principle, there are two ways to model the solvation effect [146]. Explicit-solvent methods introduce solvent molecules by computing interactions involving solvent atoms, whereas implicit-solvent methods speed up calculations by approximating the discrete solvent as a continuum, thus drastically reducing the number of particles in the system. Nowadays, the possibility to perform explicit solvent simulations for inclusion complexes allows the evaluation of the critical role of water molecules in the complexation process. Not only the interaction of the included molecules can be modeled, but also the thermodynamics associated with the inclusion process that occurs when the guest molecule moves from the bulk into the interior of the CD cavity. It is true that the implicit-solvent-based simulations can speed up the sampling of conformational space relative to explicit-solvent simulations, but the speed-up comes at the cost of making additional approximations to reality. Indeed, if implicit-solvent calculations can sample conformational space faster, they may also alter the free-energy landscapes [146]. A fruitful approach is to perform comparatively calculations in the vacuum, and with both explicit and implicit treatment of solvent in order to evaluate the capability of each model to represent the reality, also evaluating the actual impact of solvent in the complexation process under investigation. On the other hand, the implicit treatment of solvent by selecting the proper dielectric constant with values ranging from 1 (vacuum) to 80 (water) can be fruitfully used to screen the impact of different solvents on the studied system. In the last year, Alvira reported several studies on the influence of solvent in enantiodiscrimination processes promoted by β-CDs [147-150];

c) in some cases, the size of CDs and their complexes may make applications of QM calculations difficult due to too longer computational times, in these cases

semiempirical methods or the use of two-level hybrid semiempirical/DFT methods allow for faster calculations compared to methods at higher level of theory;

d) nowadays, some techniques and methods still remain really time-consuming for modeling large systems. In these cases, coupling molecular docking, MC or MD simulations for sampling low-energy conformations with semiempirical, hybrid, DFT or *ab initio* calculations, for single-point energy refinement of the lowest-energy structures, may be a useful approach to obtain reliable and adequate results to describe the reality. In this regard, it is worth mentioning that an important aspect of modelling enantioselection concerns the concept of molecular potential energy surface which determines shape and dynamic features of the molecular system. In this regard, two main questions have to be tackled, namely where to locate the guest, in or around the CD [151], and how many host-guest complexes must be computed to make the calculation really representative of the experimental system [152]. As response to the questions, docking, MC and MD simulations are exploited to reduce the number of sampling on the potential energy surface and define initial and equilibrium mutual positioning of selector and selectand [153];

e) on the other hand, the theoretical data should be always verified by confronting them with the experimental outcomes. So far, the predictivity power of the most theoretical models remains rather weak. Moreover, the modeling of a single molecule or of a complex with a single guest, instead of a large series, looking at a single "absolute value" may have low scientific significance [144]. Rather, a well designed series of experimental results is a better benchmark to identify a trend and to evaluate the inherent consistence and reliability of a virtual model which is able to explain more than one single result.

Although theoretical details on computational methods are beyond the scope of this review, in the next lines a brief description of working basis of the main computational techniques available for studying CDs and related complexes at an atomistic level is provided:

a) QM is the most well founded theory of molecular structure. In contrast to MM where electrons are implicitly treated, in QM the electrons are explicitly treated. The objective of QM is to describe the spatial position of all electrons and nuclei [142]. Electrons are allowed to flow around fixed nuclei (Born-Oppenheimer approximation) until they reach a self-consistent field (SCF), where the attractive and repulsive forces of all electrons

with themselves and the stationary nuclei are in a steady state. The nuclei are then moved iteratively until the energy of the entire system can go no lower. This process is called energy minimization or geometry optimization and allows for predicting structural and electronic features of molecules. The QM methods include *ab initio*, DFT and semiempirical methods. In general, high-level QM methods can be successfully applied only to small systems and the reliability for modeling CDs has to be carefully evaluated on a case-by-case basis. Moreover, this type of calculation provides a partial view on the CD-based system, the major disadvantages being a) the problem of finding the absolute energy minimum of the complex shape of the CD's energy hypersurface, and b) QM calculations consider the molecular system as isolated, neglecting the system's dynamics. In several cases analysis of the proximity of the interacting molecules on the basis of less time-expensive MM calculations may be the most rational approach. Also semiempirical approaches, in particular PM3, and more recent PM6 and PM7, have shown a good level of reliability in modeling CDs and related complexes as well as two levels hybrid methods. These methods are based on the partition of a large system in a QM region and a MM, or a lower-level QM region. The ONIOM (our Own N-layer Integrated molecular Orbital molecular Mechanics) method is one of the most popular and easily-to-implement hybrid quantum mechanics/molecular mechanics (QM/MM) methods to treat complex molecular systems. Hybrid QM/MM methods take advantage of the high accuracy of QM methods and the low computational cost of MM methods [154]. Although ONIOM can be used as a two-layer QM/MM method, it can also combine different QM methods, and can easily be extended to multiple layers [155];

b) MM is a nonquantum mechanical method of computing structures, energies, and some properties of molecules. This method uses an empirical force to reproduce a molecule's potential energy surface. The conceptual basis underling MM is to view a molecule as a collection of particles (nuclei) held together by some type of elastic forces (electrons). These forces are defined in terms of potential energy functions of internal coordinates such as bond lengths, bond angles, and torsion angles. Once all the potential functions and associated force constants have been determined, the internal energy is minimized by moving the particles toward their equilibrium positions (*geometry optimization*). In contrast to QM where electrons are explicitly treated, in MM the electrons are implicitly treated [142]. On this basis, the MM energy of a

molecule is described in terms of a sum of contributions arising from distortions from ideal bond distances, bond angles, and torsion angles, together with contributions due to non-bonded (van der Waals and Coulombic) interactions;

c) MC is a technique to randomly sample conformational space, usually considered a form of simulation. This method uses the same empirical force field as in MM. The calculation starts with a particle system, computing the system's energy, E_1, for that initial state. One or more of the particles is then randomly selected and moved to create a second state. The energy of this state, E_2, is computed, and that new state is considered acceptable if $E_2 < E_1$ or if $E_2 > E_1$ with some probability, $p = \exp[(E_2 - E_1)/kT]$. On this basis, a large number of random moves are made, and a large number of energetically acceptable states are obtained, providing averaged energies and properties of the system using statistical mechanics [142];

d) molecular docking is generally used to simulate the interaction between the enantiomer pairs and the active site of the CD as selector in order to predict both energy and geometry of host-guest binding. A docking process consists of two general steps, namely conformational search through various algorithms, and scoring or ranking of the docked conformations (host-guest mutual orientations) [153]. In the preliminary preparation step to docking, three dimensional grid boxes are created and, in the computational space profiled by the grid box, each atom type of the guest is positioned and its interaction energy with each atom of the CD will be computed and assigned to a grid point. All grid points collected for a particular atom-type constitute a map, and during docking the maps are used for extracting interaction energies of the enantiomers with the CD. At the end of docking calculations, several conformers of the enantiomers are obtained and clustered in several sets. The results are given in terms of the mean binding energy of the clusters or the mean energy of the most populated cluster, and their consistency with the experimental data is a basic requirement to develop a reliable predictive model;

e) MD is a simulation that shows how molecules move, vibrate, diffuse, and interact over time [153]. The MD protocol normally consists of six phases: initial assignment, system minimization, heating, cooling, equilibration, and dynamics production [156]. On the basis of this sequence, the molecular system is free to run for a period of time and the process is iterated for thousands of steps in order to bring the system to an equilibrium state, saving all the information about the atomic positions, velocities, and other variables as a function of time. The set of data emerging from the MD experiment

is called trajectory that profiles positions and velocities of the chiral partners in the system and their variation with time. All the equilibrium and dynamic properties of the system can be calculated from trajectory data set. Interestingly, the root mean square deviation of all atoms in a molecule can be plotted against time to summarize the degree of fluctuation for the entire structure. It is worth mentioning that in the last decades the increasing performances of computer facilities have allowed for a substantial increase of the production time from few to more than one hundred nanoseconds. Numerous MD simulation studies have investigated the conformational dynamics and hydration of native and substituted CDs using various force fields [117,141]. An interesting discussion on the features of the available force fields is reported in ref. 141. In the last few years, Gilson's group developed MD strategies with explicit solvent for carrying out high-precision calculations of binding free energy and binding enthalpy in CD complexes. The self-consistency of the approaches was established by using experimental binding enthalpy determined by ITC [157,158].

4 Molecular modeling of capillary electrophoresis enantioseparations promoted by cyclodextrins: applications

In the last three decades intense research has been performed aiming to understanding of chiral recognition mechanisms of CDs. It has to be noticed that based on multidisciplinary approach involving separation science, spectroscopic techniques, X-ray crystallography, and molecular modeling significant advancements were achieved. At the same time, despite the fact that the first studies to model CD / chiral analyte complexes formed in CE environment date back to the 1990s [82,159], there is still a long way to go in order to reach the state when it will be possible, based on the structure of analyte and CD, to predict the binding strength and affinity patter in CD complexes with chiral guests. Further advancement in this field requires combined application of the most advanced separation, spectroscopic and molecular modeling tools.

What can be modeled related to enantioseparations in CEKC?

1) The dynamics of separation based on the mobilities of free guests and diastereomeric associates, as well as binding constants between the guest (selectand)

and host (selector). Based on such models, separation results can be computed without paying attention to the fine mechanisms of intermolecular host-guest (selector-selectand) interactions on the molecular level [17,160-166]. These are *macroscopic models* related to the selectivity of separation.

2) Selector-selectand (host-guest) interactions can be computed on the *microscopic, molecular level*. The outcome of this modeling is of course also related to a selectivity of separation. However, since it does not consider mobilities *a priori*, correlations between the computed selectivity of recognition and observed selectivity of separation in CE will be poor in contrast to chromatographic techniques. Indeed, in CEKC host-guest complex is mobile while it is immobile in chromatographic techniques.

3) The binding energy ($E_{binding}$) between enantiomer and CD can be calculated on the basis of the energies of the enantiomer / CD complex, CD and enantiomer (eq. 4)

$$E_{binding} = E_{complex} - E_{enantiomer} - E_{CD} \quad (4)$$

where the $E_{binding}$ term derived from the contributions of the van der Waals (vdW) and the electrostatic (es) interaction terms (eq. 5). The term E_{vdW} in turn is composed of repulsive (rep) and dispersive (disp) energy (eq. 6)

$$E_{binding} = E_{es} + E_{vdW} \quad (5)$$

$$E_{vdW} = E_{rep} + E_{disp} \quad (6)$$

4) The thermodynamics of the complexation can be determined in terms of free-energy, enthalpy and entropy, even if the calculation of the entropy contribution to free-energy requires focused choices and boundary conditions, in particular concerning the selection of the proper solvation model.

In the following subsections, representative modeling studies are discussed for the most popular CDs used in CE enantioseparation as chiral selectors (Table 6). It is worth mentioning that in the last few years two reviews were published dealing with molecular modeling application in CE enantioseparation [167,168]. Moreover, focused examples have been reported in some reviews on chiral recognition in separation science [169,170], on molecular modeling in liquid phase enantioseparation [153], and on capillary electrophoresis in pharmaceutical analysis [171].

Modeling studies of inclusion complexes involving new CDs with specific functionalization and used as chiral selectors have been also performed in the field of CE enantioseparation. In this regard, Guo's group studied the inclusion complexes of

brompheniramine, chlorpheniramine, and pheniramine enantiomers with the single isomer derivative heptakis{2,6-di-O-[3-(1,3-dicarboxylpropylamino)-2-hydroxypropyl]}-β-CD (glutamic acid-β-cyclodextrin) by using the two layered hybrid ONIOM method (ONIOM2, B3LYP/6-31G(d):PM3) [172]. Li and co-authors modeled the complexes of terbutaline with the heptakis{2,6-di-O-[2-hydroxy-3-(sulfoammino)propoxy]}-β-CD using a hybrid ONIOM method [173]. Moreover, in this study the molecular electrostatic potential was calculated for the isolated guest and host molecules. Later, molecular docking was also used to visualize the inclusion complexes of dansyl amino acids and naproxen with mono-6-deoxy-6-(4-amino-1,2,4-triazolium)-β-CD chloride as ionic liquid functionalized CD [174], and of a series of 13 chiral drugs with carboxymethyl-6-(4-methoxybenzylamino)-β-CD [175].

4.1 Native cyclodextrins

One of the first MM studies applied to a CE enantioseparation was reported in 2002 to explore at microscopic level the opposite EMO of the enantiomers of ketamine when native α-CD (*S*>*R*) and β-CD (*R*>*S*) were used as chiral selectors [62]. In this study, the possible mechanisms of the affinity reversal were investigated by employing electrospray ionization mass spectrometry (ESI-MS), ^{1}H-NMR and 1D-ROESY spectroscopies, and molecular modeling techniques. In agreement with the results observed in the 1D-ROESY experiments, the optimized structures of *S*- and *R*-ketamine complexes with α- and β-CD showed a deeper inclusion of the enantiomers of ketamine into the cavity of β-CD compared to α-CD (Fig. 12). These results appeared consistent with the fact that a slightly better enantioselectivity was observed with β-CD in CE ($t_2/t_{1\ \beta\text{-CD}}$ = 1.04, $t_2/t_{1\ \alpha\text{-CD}}$ = 1.03). The force-field energies of diastereomeric complexes (Table 7) involving the periodic water box were in reasonable correlation with the observed migration order of all three native CDs. Otherwise, the values calculated in vacuum correlated with the experimental EMO with α-CD but not with β- and γ-CD.

This study highlighted important aspects of modeling enantioseparation in CE:

1) the inherent high separation efficiency of CE allows for observation of selective effects of intermolecular interactions with very low free-energy differences;

2) the model calculations being based on several assumptions and simplifications, multidisciplinary studies involving molecular modeling along with experimental

techniques allows for the fine tuning of assumptions, approximations, and parameters of the calculation methods;

3) modeling CE enantioseparation in vacuum, without explicit or implicit consideration of the medium may reduce computational time. Nevertheless, this choice may impact the reliability of the calculated results in particular taking into account the pivotal role of water in CD binding mechanisms;

4) more than one complex has to be evaluated in order to check the inherent sensitivity of the methods, accounting for the impact of variations of structural and experimental conditions on enantioseparation.

Along with MM and MD with various force field, semi-empirical methods such as AM1 and PM3 can be used for structure optimization of medium and large systems. It is worth mentioning that PM3 showed high computational efficiency for modeling of large systems which are beyond the capacity of *ab initio* methods. Moreover, PM3 describes noncovalent interactions and steric effects better than AM1 [176,177]. In some cases, hybrid methods are used by applying two different levels of calculation to model guest and CD, respectively. In this regard, Huang and co-authors performed a theoretical study on the inclusion complexes of β-CD with salsolinol, N-methylsalsolinol and 1-benzyltetrahydroisoquinoline enantiomers by using comparatively PM3 semi-empirical and ONIOM hybrid (B3LYP/6-31G*:PM3) as computational methods [178]. In all cases, the calculated stabilization energies correlated very well with the EMO (*S*>*R*) observed in CE enantioseparation by using β-CD as chiral selector.

Orlandini, Furlanetto and co-authors studied the separation mechanism involved in CD-MEKC enantioseparation of ambrisentan enantiomers with γ-CD by means of a combined CE/NMR/MD approach [179]. The study provided information on the aggregates, inclusion complexes and noncovalent interactions underlying the separation system. In particular, γ-CD was shown to have a great tendency of forming mixed 1:1:1 and 1:2:1 complexes with one or two SDS molecules and the analytes, and the existence of ternary complexes was demonstrated by NMR spectroscopy. Moreover, within 1:1:1 complexes with different CDs, the highest difference of potential energy between the complexes with the enantiomers was calculated for γ-CD.

Very recently, Suliman and co-authors observed that the addition of 18-crown-6 can improve CE enantioseparation of phenylalanine and tyrosine with native β-CD [180], whereas tryptophan enantiomers were not separated with β-CD alone, and with the

dual additive system of β-CD / 18-crown-6. For the three amino acids a slight improvement of the enantioseparation was observed by adding the crown ether to α-CD. In this study, electrospray ionization mass spectrometry (ESI-MS) proved that the amino acids formed stable complexes with the individual host and ternary complexes involving both CD and crown ether. Binary and ternary complexes were visualized by molecular modeling. The structures of both α- and β-CDs were refined subjected to energy minimization using the semiempirical PM6 method. The diastereomeric complexes between the CD and the analytes were obtained by docking each enantiomer into the respective cavity. In both systems, the cluster with the maximum number of conformations was the one with aromatic moiety inserted into the nanocavity of the host via the wider opening of the CD (Fig. 13A). Six binary and six ternary complexes were individually placed in the center of an orthorhombic box containing TIP3P water molecules. The simulations were run for 15 and 20 ns for the β-CD and α-CD complexes, respectively. These calculations confirmed the existence of extensive HB interactions, which may contribute significantly to the stability of these complexes together with hydrophobic effects and van der Waals interactions. The calculations also showed that in all binary complexes the aromatic ring is inserted into the cavity of the CD, while the polar end of the amino acid remains outside the cavity and solvated by the water molecules (Fig. 13A). In ternary complexes, the ammonium group remained interacting with the crown ether through strong HBs with the electron rich ether groups of 18-crown-6 (Fig. 13B). The presence of the carboxyl group in the pseudocavity between the two hosts resulted in extensive HB network justifying the stability of these complexes.

4.2 Methylated β-cyclodextrins

In 1997, Liu and co-authors reported CE enantioseparation of a series of water soluble melatonergic drugs with β-CD and 2,6-DM-β-CD as chiral selectors [159], indicating higher affinity of the CDs towards the *S*-enantiomer. Using BMS-191435 (Fig. 14) as a model, molecular modelling studies were carried out to gain insights into the chiral discrimination in the complexation of 2,6-DM-β-CD with the two *R*- and *S*-enantiomers. Calculations were performed using the AMBER force field, treating the solvent implicitly. The basic amine group of both enantiomers was assumed to be protonated according to the experimental conditions, where an acidic buffer (pH = 2.58) was used. Simple energy minimization indicated a differentiation in the complexation of 2,6-DM-

β-CD with the two enantiomers. The predicted $\Delta\Delta E_{binding}$ resulted of 1 kcal/mol in favor of the *S*-enantiomer, indicating its tighter binding with the CD.

Later, a molecular modeling study was conducted by Aboul-Enein and co-authors to explore the interaction between aminoglutethimide enantiomers and methyl-β-CD [181]. Computational calculations for the inclusion complexes for aminoglutethimide enantiomers and M-β-CD were performed by using molecular docking and the PM3 semiempirical method. The results of these calculations showed that the difference in the stability of these complexes leading to different migration times of the enantiomers under CE conditions.

Chai and co-authors reported the enantioseparation of triadimenol antifungal compounds by CEKC with TM-β-CD, which showed higher enantiorecognition ability compared to other CDs such as α-CDs, HP-α-CD, HP-β-CD, and 2,6-DM-β-CD [182]. In this study, molecular docking was used to visualized the structures of low-energy guest / TM-β-CD complexes and the involved noncovalent interactions. The binding energies were calculated from the most stable conformations of the most populated clusters. For each chiral compound, the authors correlated the experimental separation parameters, α and R_s, to the differences between the binding energies of the two diastereomeric associates ($\Delta\Delta E_{binding}$). The correlation failed for some compounds. In principle, two main factors could contribute to the observed results: a) a misinterpretation of the statistical clustering in molecular docking, b) the fact that the modeling was performed in the vacuum, neglecting the role of solvent. On the other hand, the other CDs involved in this study as chiral selectors were not modeled, thus the sensitivity of the docking procedure towards different CD structures was not verified. Another question concerns the choice to have built TM-β-CD starting from the coordinates of β-CD crystal structure, while several TM-β-CD crystal structures are available in the Cambridge Crystallographic Data Center [114,115,183]. Otherwise, later Ibrahim and co-authors modelled TM-β-CD starting from the crystal structure of the CD as released from the CCDC (entry XAQJII) [183,184]. The authors performed a molecular docking study using MM calculations and semiempirical PM3 calculations to explore at microscopic level the enantiodiscrimination of TM-β-CD toward ketoconazole. The binding energies were calculated by using the PM3 semiempirical and the B3LYP/6-311G (d,p) level. The solvent was treated implicitly by means of the polarizable continuum model (PCM). On this basis, the calculated EMO for the four

stereoisomers of ketoconazole was determined, but no comparison with the experimental EMO was reported in the paper to verify the reliability of the results.

4.3 Heptakis(2,3-di-O-acetyl)-β-cyclodextrin (HDA-β-CD)

As reported [84], MD simulations in explicit water have shown that the experimentally observed hydrophilic-hydrophobic characteristics of β-CD molecules can be accurately reproduced in the absence of experimental restraints [185], assessing the magnitude of the different interactions that can stabilize a bound analyte within the cavity of a given β-CD [186]. The sampling of the conformational space is performed by MD, which has to be sufficiently intensive to make the results meaningful for correlation with the experiment (reality). MD allows for explore the different steps of the recognition process between CD and the guest [84]: a) approach of the two binding partners, (b) displacement of loosely bound, enthalpically frustrated, [136] water molecules from the β-CD cavity and partial desolvation of the ligand inside the cavity, c) assimilation of the displaced water molecules by the surrounding bulk solvent, which results in an entropy gain, (d) intermolecular interactions involving van der Waals and electrostatic forces, possibly leading to the formation of direct and/or water-bridged HBs, and (e) reconstitution of the hydrated structure around the finished complex.

On this basis, Salgado and co-authors explored by NMR spectroscopy experiments and MD simulations the structural and energetic determinants of the distinct binding of the clenpenterol enantiomers to β-CD and HDA-β-CD, and the migration order reversal of their respective inclusion complexes in CE [84]. After 100 ns MD, the glucosyl units of both β-CD and HDA-β-CD retained the $^4C_1$ chair conformation throughout the whole simulation. In both β-CD / clenpenterol inclusion complexes (Fig. 15A,B), each enantiomer was bound with the dichloroanilino part protruding out of the bottom of the cavity, and with the hydroxyl and amino groups on the opposite side engaged in HB with the surrounding water molecules, some of which bridge interactions with the O(2) and O(3) hydroxyls in the upper rim. The isopentyl group of clenpenterol resulted fully exposed to the solvent. Otherwise, the orientation of clenpenterol appeared to be reversed in the HDA-β-CD / clenpenterol complexes (Fig. 15C,D), and in this case the isopentyl group was found within the cavity. Therefore, the clenpenterol inclusion complexes were shown to be different depending on whether the β-CD is diacetylated or not. Importantly, some of the intermolecular HBs were shown to be mediated by bridging water molecules. The computed interaction

energies allowed for gaining insight into the nature of the forces that drive association and their ranking order could account for the EMO reversal detected upon replacing β-CD with HDA-β-CD as the chiral selector in CE experiments.

4.4 Hydroxypropyl substituted β-cyclodextrins (HP-β-CDs)

The hydroxyalkylation of native CD results in mixtures of positional isomers, therefore molecular interaction with host possessing an undefined substitution pattern may be difficult to study experimentally. Even more difficult is modeling randomly substituted CDs due to the presence of a number of isomers. On the other hand, this issue should be carefully considered as modelling of randomly substituted CDs is approached. Representative studies are reported below.

Chai and co-authors used NMR spectroscopy (2D-ROESY), molecular docking, and binding energy calculations to explore the chiral recognition mechanism involved in the CE enantioseparation of iodiconazole (Fig. 16A) and structurally related triadimenol analogues with hyroxypropyl-γ-CD [187]. The HB between iodiconazole enantiomers and the hydroxyl groups on the HP-γ-CD rim, and face to face π–π interactions were found to highly contribute to the enantiorecognition process. In accord with this results, 2D-ROESY experiments indicated that the two phenyl rings of iodiconazole are inserted in the cavity of the CD. The authors correlated the calculated binding energies difference $\Delta\Delta E_{S\text{-}R}$ (-22.47 kcal/mol) of *R*- (-62.94 kcal/mol) and *S*-iodiconazole (-40.47 kcal/mol) with the good enantioresolution obtained under CE experimental conditions (α = 1.02, R_s = 1.26). Unfortunately, no details about the correlation between calculated and experimental EMO is reported in this paper. On the other hand, the authors proposed a new mathematical equation, based on the results of MM calculations, which proved to be able to predict the theoretical resolution of enantioseparation for the triadimenol analogues (Fig. 16B). The question of modeling a randomly substituted CD was not addressed in this paper.

Otherwise, Tóth and co-authors modeled the complexes of ofloxacin (Fig. 17A) with HP-β-CDs DS4, with different substitution pattern (Fig. 17B-D) by using MMFF94 force field in order to explore the impact of substitution pattern on complex stability [188]. In this study, ofloxacin was placed into the CD cavity via its wider rim in two orientations, either with the carboxyl group or the N-methyl-piperazine group inside. Each structure was subject to energy minimization, simulating implicitly aqueous environment (ε = 78.3). Then, MD calculations were performed, and the resulting 100 structures/guest

orientation/charge state/CD were re-optimized and, according to the energy values of the optimized structures, the lowest energy ones were taken into account for the interaction energy determination. The complex formation among the HP-β-CDs was most favorable energetically in the case of HP-β-CDs DS4c (Fig. 17D). It is likely that in this CD the cavity is less overcrowded because the CD substituents are close to each other, making the cavity more accessible to the guest.

Suliman and co-authors performed an extensive theoretical study to unravel the mechanism of the separation of the enantiomers of ofloxacin [189]. Using Autodock as software platform, the authors generated the most stable conformers of the *S*-ofloxacin / HP-β-CD (Fig. 18A) and *R*-ofloxacin / HP-β-CD (Fig. 18B) by MM calculations. The optimum conformations generated by this technique were further optimized by the PM7 semiempirical method. *R*-ofloxacin / HP-β-CDs complex was found more stable ($\Delta E_{binding}$ = -29.5 kcal/mol) compared to the *S*-ofloxacin / HP-β-CDs complex ($\Delta E_{binding}$ = -14-5 kcal/mol), and therefore migrates at a slower velocity towards the detector, these theoretical results corroborating the experimental findings obtained by CE enantioseparation (EMO_{exp} = *S*>*R*). In this study, the structure of HP-β-CDs was built from the β-CD structure by substitutions of 2-hydroxypropyl moieties randomly at O(2) and O(6) positions as a representation for the CD mixture. Moreover, each system consisting of a guest and host molecule was solvated in a sphere of TIP3P water molecules using periodic boundary conditions, and the monoprotonated cation was used in the modeling study, which was considered dominant in the experimental pH range (2 ≤ pH ≤ 4).The nature of bonding between the guest and host molecules was investigated using 5 ns MD simulations in aqueous media, and the obtained results indicated that the complexes were stabilized by weak HBs between ofloxacin enantiomers and CD.

Raoov and co-authors reported CE enantioseparation of miconazole and ketoconazole with β-CD and HP-β-CD by a multidisciplinary study involving molecular docking. Unfortunately, in this paper essential details about the 3D structure of HP-β-CD, and the absolute configuration of ketoconazole enantiomers used in the modeling were not provided [190]. Indeed, two enantiomers of ketoconazole were modelled, neglecting the presence of two chiral centers, and consequently the need to specify their absolute configuration.

Recently, Du and co-authors reported the modeling by molecular docking of ternary complexes of five chiral drugs with HP-β-CD and chiral ionic liquid derived from L-

valinol, L-prolinol, and L-phenylalaninol used as additives of a synergistic system in CE enantioseparation [191].

4.5 Carboxymethyl-β-cyclodextrins (CM-β-CDs)

Nascimento and co-authors modeled the inclusion process of 4-hydroxypropranolol in CM-β-CD, which was used as chiral selector in CE enantioseparation, by means of MD simulation, and hybrid PM3/DFT calculations [192]. In this study, the geometries for the isolated enantiomers of 4-hydroxypropranolol and CM-β-CD were fully optimized in the gas phase without any geometrical or symmetry constraints at the BLYP/6-31G(d,p) level of theory. Considering the inclusion process between host and guest molecules in 1 : 1 ratio, four orientations were assumed for the CM-β-CD / 4-OH-Prop complexes (Fig. 19). Consequently eight distinct spatial 4-hydroxypropranolol / CM-β-CD arrangements were generated, and eight 10 ns MD simulations under vacuum were performed in order to provide detailed information on the fluctuations and conformational changes of the complexes, obtaining the global minimum geometries on the equilibrium for each complex after the MD simulations. The equilibrium complexes geometries were optimized with PM3 semiempirical calculations, and binding energies and Gibbs free energy calculated by BLYP/6-31G(d,p) // PM3 level of theory. The solvent effect was considered implicitly using the PCM. The energies calculated for the inclusion complexes were in good agreement with the experimental results (EMO_{exp} = $EMO_{calculated}$ = *S*-*R*). Moreover, a systematic structural analysis indicated that form A inclusion mode was the most stable for both enantiomes, and that the HBs formed between host and guests played a major role in the complex stabilization.

Very recently, Hancu and co-authors modeled the inclusion complexes of citalopram in CM-β-CD which was used as chiral selector in CE enantioseparation of the chiral drug (EMO = *S*>*R*) [193]. All structures were fully optimized using the semiempirical method RM1 using the Maestro software. By combining the individual enantiomers of citalopram with the CD, complexes of various energy and stability were prepared. The created structures were minimized at every step, keeping the CD restricted. The calculations for the electron energy of the created complexes were made at M06-2X-D3/6-31G** level of theory and SM8 as solvation model. Following the calculations, it was shown that the complex CM-β-CD / *R*-citalopram is more stable ($E_{binding}$ = -67.75 kJ7mol) than the complex CM-β-CD / *S*-citalopram those with S-CIT ($E_{binding}$ = -48.32

kJ/mol), in accord with the faster migration of the *S*-enantiomer observed experimentally.

You described the enantioseparation of 1-(4-methoxyphenyl)-1-(methylamine) ethanol, salbutamol sulfate, sotalol hydrochloride, and 2-amino-1-phenylethanol using β-CD and CM-β-CD as chiral selectors. Better enantioseparation was achieved for all compounds with charged CM-β-CD compared to β-CD. In this regard, energy information calculated from ITC and molecular docking confirmed that more stable inclusion complexes were formed between analytes and CM-β-CD according to the experimental results [194]. Recently, molecular docking has also been used to model inclusion complex of chiral drugs with the dual system based on chondroitin sulfate D/CM-β-CD [195] and CM-β-CD-based chiral ionic liquid [196] used in CE enantioseparation as chiral selectors.

It is worth noting that, despite the fact that carboxymethylation can be performed also in a selective way [90,197,198], the carboxymethylation of native CD may results in mixtures of isomers. In this case, molecular interaction between the analyte and the host possessing an undefined substitution pattern may be challenging to model. Surprisingly, the studies mentioned in this subsection did not address the question, which remained rather overlooked in terms of molecular modeling.

4.6 Sulfated-β-cyclodextrins (S-β-CDs)

As substituted CDs are used as mixture of position and substitution isomers such as in the case of S-β-CD and analogue derivatives, an overview of the published literature reveals different approach to model this type of CD systems: a) in some cases the question is neglected, or the CD is treated as a single isomer due to the inherent difficulties to predict the substitution degree [199]; b) given the number of substituted hydroxyls, a structure representing one of the possible structure is randomly generated [200]; c) different isomeric forms are selected and modelled to improve the description of the overall system as the sum of all isomers [201]; d) finally, some authors argue that is not reliable to model mixtures of CDs [202]. In the lines below two representative examples are described.

Orlandini, Furlanetto and co-authors developed a method for the enantioseparation of sulpiride enantiomers by CE based on the addition of a dual CD system to the BGE, namely the negatively charged S-β-CD sodium salt and the neutral M-β-CD [200]. A multidisciplinary approach based on both NMR and MD was used by the authors to

investigate the recognition mechanism. MD was performed with 3 ns of production time, in implicit solvent. The results of MD simulations suggested, in agreement with CE experiments, a relationship between the gain in potential energy and migration time. NMR showed the inclusion of the benzene sulfonamide moiety of the analyte inside the hydrophobic cavity of the CDs. It is worth noting that, S-β-CD being used experimentally as a mixture containing a number of isomers with a degree of substitution ranging from 12 to 15, for MD calculations the number of sulfate groups attached to CD was fixed to 12, with all the sulfated groups in anionic form. In this paper, the S-β-CD was randomly generated, representing one of the possible structures, the authors considering that the behavior of the compounds in terms of docking average energies does not change significantly by modifying the positions of sulfate groups.

A different choice was made by Scriba and co-authors, and randomly substituted S-β-CD was not included in a molecular modeling study due to the fact that this CD is a mixture of positional and substitution isomers. In this regard, the authors argued that molecular modeling would only be possible for the individual CD isomers because, whereas modeling CDs used as isomeric mixture experimentally would not allow to deduce meaningful data of the overall complexation process [202]. On this basis, the influence of both cavity size and substitution pattern of other CDs used as selectors in CE environment on EMO of medetomidine (Fig. 20) was investigated [202]. In this study, both NMR and MD simulations (100 ns simulation time) contributed to rationalize the binding mechanism, showing that for native β-CD and γ-CD the phenyl moiety of medetomidine enters the cavity from the wider secondary rim of the CDs, while the protonated imidazole ring points toward the bulk solvent. Otherwise, in the complex with single component heptakis(6-O-sulfo)-β-CD (HS-β-CD), the protonated imidazolium moiety appeared to be positioned inside the CD cavity interacting with the sulfate groups in 6 position of the glucopyranose unit.

4.7 Heptakis(2,3-diacetyl-6-sulfo)-β-cyclodextrin (HDAS-β-CD) and Heptakis(2,3-dimethyl-6-sulfo)-β-cyclodextrin (HDMS-β-CD)

Given the successful CE enantioseparation of linezolid with HDAS-β-CD as chiral selector (EMO *S*>*R*) [203], Bednarek and co-authors used NMR and MD simulations for investigating the host-guest complexation of *R*- or *S*-linezolid with HDAS-β-CD, in particular to obtain information about the mode and strength of the linezolid

complexation into the hydrophobic cavity of the host [204]. The linezolid enantiomers were manually docked to the HDAS-β-CD in two ways (Fig. 21), immersing the oxazolidinone (A) and the morpholine (B) parts in the CD cavity. In this study, a 40 ns MD simulation was performed for each of the four complexes in periodic water box. NMR experiments showed that the linezolid interacts mainly with the inner region of the HDAS-β-CD cavity. However, the interaction of host-guest not involving cavity occupation was also shown possible. Both observed chemical shifts changes of proton of *S*- and *R*-linezolid and calculated binding energies for the four complexes evidenced that inclusion via the morpholine part was equally probable for both enantiomers. Otherwise, inclusion via the oxazolidinone parts was more probable for *R*-linezolid in accord with calculated binding energies. On this basis, the stereoselectivity appeared based on the inclusion orientation with the oxazolidinone tail immersed in HDAS-β-CD cavity.

Recently, Michalska and co-authors also studied the CE enantioseparation of sutezolid (Fig. 22) and its *R* enantiomer with HDAS-β-CD [205]. The features of the *R*/*S*-sutezolid / HDAS-β-CD inclusion complexes were studied by a multidisciplinary approach involving FT-IR and NMR spectroscopies and 500 ns MD. Taking into account the results obtained from FT-IR measurements, HBs were found to be the reason for complex formation and stereoselective recognition of sutezolid enantiomers by HDAS-β-CD. In particular, the analysis of the C=O stretching revealed the involvement of the oxazolidinone ring in the interaction with the HDAS-β-CD. Based on the NMR results, it could be concluded that the protonated sutezolid can form a complex with the CD, whereas molecular modeling calculations confirmed that sutezolid binds deeply into the CD cavity, as well as that the most stable conformations are those in which the thiomorpholine nitrogen atom of sutezolid close to the CD sulfate groups.

Molecular docking was used by Guo and co-authors to model CE enantioseparation of clenbuterol, oxybutynin, salbutamol, and penehyclidine by using HDAS-β-CS as chiral selector [206]. The authors built the HDAS-β-CD from the crystallographic coordinates of β-CD. In this study, docking simulation were performed to explore the interaction modes in host-guest inclusion complexes. The results differing by less than 2 Å in a positional root mean square deviation were clustered together, and in ech group the lowest binding energy conformation with the highest percentage frequency was selected as the group representative. On this basis, the authors derived the

binding energies of the complexes (Table 8), and the ΔE_{R-S} increasing in the order clenbuterol > oxybutynin > salbutamol > penehylidine. Despite the good correlation with the experimental results (Fig. 23), it is worth noting that very low energy difference (0.02, 0.26) may be not really meaningful due to the statistical nature of the docking clustering. Moreover, the experimental EMO was not reported, therefore the calculated reversal of EMO observed for oxybutynin compared to the other compounds could not be verified through a proper comparison with the experimental data. Observation of the inclusion complexes showed the presence of interactions including HBs and π–S interactions. For clenbuterol enantiomers, one of the O atoms of the glucoside on HDAS-β-CD formed HBs with the H atoms of hydroxyl and amino groups on *R*-clenbuterol. Moreover, for *S*-clenbuterol, in addition to the formation of HB of the analyte with the oxygen atom of the SO_3 group, a π–S contact between the phenyl ring of *S*-clenbuterol and one of the S atoms of HDAS-β-CD could simultaneously occur. The formation of HBs together with the π–S key contact made *S*-clenbuterol / HDAS- β-CD complex more stable than *R*-clenbuterol / HDAS-β-CD.

In some papers, the recognition properties of HDAS- and HDMS-β-CDs are examined and compared [91,207]. In order to have deep insights into the mechanisms of enantiomer affinity pattern in both aqueous and non-aqueous systems, Zhao and co-authors used an approach combining CE and molecular modeling. In this study, acebutolol (Fig. 24A) was enantioseparated in aqueous CE and non-aqueous CE using HDAS- and HDMS-β-CDs as chiral selectors. With HDAS-β-CD, the enantiomer affinity pattern of acebutolol was found to be opposite when an aqueous background electrolyte (*S*>*R*) was replaced with non-aqueous background electrolyte (*R*>*S*), but experimental EMO remained the same in the presence of HDMS-β-CD [207]. Molecular docking and MD simulations showed that both enantiomers of acebutolol were included with the amide moiety close to the 2,3-acetylated groups in HDAS-β-CD (B), while in HDMS-β-CD the amide moiety was found to be close to the sulfate groups (C). According with CE results, further calculations of the complex energy with implicit solvent effect indicated that HDAS-β-CD had higher affinity to *S*-acebutolol than *R*-acebutolol in non-aqueous CE, while it showed better binding to *R*-acebutolol in aqueous CE. However, the HDMS-β-CD bound better to *R*-acebutolol in both aqueous and non-aqueous CE. This trend confirmed that host-guest interaction played more important role in chiral separation of HDMS-β-CD, while the solvent effect had prevailing impact on HDAS-β-CD.

It is worth highlighting that in several cases, the 3D model of HDAS-β-CD have been built from the crystal coordinates of β-CD. However, the crystal structure of heptakis(2,3,6-tri-O-acetyl)-β-CD may be a more suitable benchmark structure to model HDAS-β-CD [90] as well as HDA-β-CD [84] by modifying the position O(6). On the other hand, as reported by Holzgrabe and co-authors [82] the comparison of β-CD and acetylated macrocycles shows that acetylation of either the primary or the secondary hydroxyl groups can give conformations with distortions of the torus. Steric interactions apparently expand the substituted rim and in the case of the 2,3-di-O-substituted CDs in which the effect would be expected to be more severe, distortion of the circular shape of the cavity occurs. This distortion could prevent a good fit or determine different inclusion modes compared to round CDs. Indeed, the distorted cavity may cause the binding of a ligand to depend more critically on the correct molecular geometry.

5 Concluding remarks

The inherent chirality as well as the bivalent hydrophilic/hydrophobic surface are some features which make CDs privileged selectors for enantiorecognition. Native and substituted CDs have also found wide application in enantioseparation science, becoming the most used and studied chiral additive for CE enantioseparation. The recognition mechanisms involving CD may be not easy to identify and decode. Indeed, guest molecules may interact with CD macrocycle through inclusion in their hydrophobic cavity, but CD-guest interaction may also involve the external surface of the torus. Several factors may impact CD conformation and recognition ability, which concern not only structural features of CD itself but also boundary conditions, such as guest structure and medium properties. In addition, the possible formation of higher order complexes and ordered aggregates make the chemistry of CDs rather intricate. X-ray derived structures of CDs have the merit to provide the exact geometries of CDs (in the solid state) and other relevant structural information. However, CD structures in solution can deviate substantially from X-ray determined crystal structures. Moreover, in the solid state weak interactions between the guest and the CD may become not detectable in presence of stronger interactions in the crystal packing. Solvation effect and related entropic contribution may be complex to quantify experimentally. Computational approaches represent a promising tool to tackle these

issues, for the identification of interaction mechanisms and related noncovalent interactions, for quantifying binding affinity. In this perspective, computational science needs reliable experimental benchmarks which may have the essential function to check the reliability of virtual methods and approaches [62,208].

A multidisciplinary approach based on the use of orthogonal techniques, involving also molecular modelling, usually enables researchers to obtain reliable mechanistic information. In this frame, NMR spectroscopy, ITC, ESI-MS when associated to molecular modeling proved to be the best choice to disclose the molecular bases undelying CE enantioseparation.

There is a tendency to develop computational software and platform increasingly friendly, and a continuous research for improving theoretical models and force fields for treatment of large molecules in chemical and biochemical context. Over time, the knowledge of CD chemistry growing more and more, and also in the field of CE enantioseparation important advancements for understanding function and mechanisms of CDs as chiral selectors occurred. However, some key factors appear to be crucial in modelling the spatial proximity of guest analyte and CD macrocycle in the enantiorecognition process, and some pitfalls still emerge from the published literature:

a) *force fields suitable for both analyte and CD*. In principle, the choice of an incorrect force field applied the same error to both enantiomers, thus calculation of the binding energy difference may be not affected by this factor. However, incorrect force field may neglect or damp the impact of specific factors, affecting the reliability of the calculations;

b) the theoretical environment needs to be consistent with the experimental conditions, for example in terms of *solvent composition*. A useful approach to evaluate the impact of medium on the stability of host-guest complexes is to calculate and compare binding energies in vacuum and solvent;

c) the design of host and guest molecules involved in the calculations should be made taking into account the responses expected by the theoretical study. Indeed, the comparison of the computational and experimental responses for structurally related series of analytes and CDs can provide useful information about the impact on recognition of focused frameworks and structural variations. Moreover, the *design of benchmark experiments* may allow for checking the sensitivity of the computational methods towards variations of structures and boundary conditions;

d) all choices should always emerge from a balanced compromise between the need to obtain *theoretical results as reliable as possible* to describe reality, and *approximations*, which are dependent on computational time and performances, and complexity of the modelled chromatographic system;

e) in some studies, essential details of calculation methods, modeling of structures, description of the adopted protocols are missing or superficially discussed with a negative impact on the reliability and repeatability of the study;

f) in general, calculations performed to model CD inclusion complex confirmed the existence of extensive HB interactions, which may contribute significantly to the stability of these complexes together with hydrophobic effects, and van der Waals interactions. It seems still demanding to identify which forces are responsible for CD-guest association and which chiral recognition since these forces should not *a priori* be the same;

g) X-ray coordinates have to be carefully selected to build 3D starting structures of CDs which are not crystallized yet as pure macrocycle or complexed with a guest. Starting from the CD crystal coordinates close to the target CD allows to direct geometry optimization towards the global minimum of energy, avoiding the risk to build high-energy structures which require more steps of energy refinement;

h) modelling CDs such as M-, HP-, CM-, and S-β-CD, which are available and used experimentally as mixtures of positional and substitution isomers, as a unique structurally defined molecule may provide results which are not representative of the real molecular system, and the theoretical approach has to be carefully evaluated in these cases, in order to provide balanced description of the overall chiral system.

Acknowledgements

B.C. thanks Shota Rustaveli National Science Foundation (RNSF) of Georgia for financial support of his research in this field in last few years through the Project No. 217642.

Conflict of interest

The authors have declared no conflict of interest.

6 References

[1] Li, S., Purdy, W. C., *Chem. Rev.* 1992, *92*, 1457-1470.

[2] Del Bubba, M., Checchini, L., Cincinelli, A., Lepri, L., Enantioseparations by thin-layer chromatography. In: Scriba G. K. E. (ed) Chiral Separations. Methods in Molecular Biology, vol 970. Humana, New York, NY., 2013, 29-43.

[3] Schurig, V., Juza, M., *Adv. Chromatogr*. 2015, *52*, 117-168.

[4] Armstrong, D. W., DeMond, W., *J. Chromatogr. Sci.* 1984, *22*, 411-415.

[5] Li X., Wang Y. HPLC Enantioseparation on Cyclodextrin-Based Chiral Stationary Phases. In: Scriba G. K. E. (ed) Chiral Separations. Methods in Molecular Biology, vol 1985. Humana, New York, NY., 2019, 159-169.

[6] Guo, J., Lin, Y., Xiao, Y., Crommen, J., Jiang, Z., *J. Pharm. Biomed. Anal.* 2016, *130*, 110-125.

[7] Xiao, Y., Ng, S. -C., Tan, T. T. Y., Wang, Y., *J. Chromatogr. A.* 2012, *1269*, 52-68.

[8] West, C., *TrAC – Tr. Anal. Chem.* 2019, *120*, Article number 115648.

[9] Mayer, S., Schurig, V., *J. High Resol. Chromatogr.* 1992, *15*, 129-131.

[10] Li, S., Lloyd, D. K., *J. Chromatogr. A* 1994, *666*, 321-335.

[11] Fanali, S., Chankvetadze, B., *J. Chromatogr. A* 2021, *1637*, Article number 461832.

[12] Snopek, J., Jelínek, I., Smolková-Keulemansová, E. *J. Chromatogr.* 1988, *438*, 211-218.

[13] Fanali, S., *J. Chromatogr.* 1991, *545*, 437-444.

[14] Fanali, S., *J. Chromatogr. A* 1996, *735*, 77-121.

[15] Zhu, Q., Scriba, G. K. E., *Chromatographia* 2016, *79*, 1403-1435.

[16] Bernardo-Bermejo, S., Sánchez-López, E., Castro-Puyana, M., Marina, M. L., *TrAC-Tr. Anal. Chem.* 2020, *124*, Article number 115807.

[17] Chankvetadze, B., *J. Chromatogr. A* 2018, *1567*, 2-25.

[18] Fanali, S., Chankvetadze, B., *Electrophoresis* 2019, *40*, 2420-2437.

[19] Villiers, A., *C. R. Acad. Sci. Paris* 1891, *112*, 536-538.

[20] Schardinger, F., *Z. Unters. Nahr. Genusm.* 1903, *6*, 6865-6880.

[21] Schardinger, F., *Zentralbl. Bakteriol. Abt. II* 1911, *29*,188-197.

[22] Freudenberg, K., Jakobi, R., *Ann.* 1935, *518*, 102-108.

[23] Freudenberg, K., Blomquist, G., Ewald L., Soft, K., *Ber.* 1936, *69*,1258-1266.

[24] Freudenberg, K., Mayer-Delius, M., *Ber.* 1938, *71*, 1596-1600.

[25] French, D., Rundle, R. E., *J. Am. Chem. Soc.* 1942, *64*, 1651-1653.

[26] Freudenberg, K., Mayer-Delius, M., *Ber.* 1938, *71*, 1596-1600.

[27] Freudenberg, K., *Annu. Rev. Biochem.* 1939, *8*, 81-112.

[28] Freudenberg, K., Schaaf, E., Dumpert, G., Ploetz, T., *Naturwissenschaften* 1939, *7*, 850-853.

[29] Hanes, C. S., *New Phytol.* 1937, *36*, 189-239.

[30] Clarke, R. J., Coates, J. H., Lincoln, S. L., *Adv. Carbohydr. Chem. Biochem.* 1988, *46*, 205-249.

[31] Szejtli, J., *Chem. Rev.* 1998, *98*, 1743-1753.

[32] Hybl, A., Rundle R. E., Williams, D. E., *J. Am. Chem. Soc.* 1965, *87*, 2779-2788.

[33] Cramer, F., *Rev. Pure Appl. Chem.* 1955, *5*, 143-164.

[34] Demarco, P. V., Thakkar, A.L., *Chem. Commun.* 1970, 2-4.

[35] Cramer, F., *Angew. Chem.* 1952, *64*, 136.

[36] Sanchez-Vindas, S., Vigh, G., *J. Chromatogr. A* 2005, *1068*, 151-158.

[37] Lomsadze, K., Salgado, A., Calvo, E., López, J. A., Chankvetadze, B., *Electrophoresis* 2011, *32*, 1156-1163.

[38] Chankvetadze, L., Servais, A. -C., Fillet, M., Salgado, A., Crommen, J., Chankvetadze, B., *J. Chromatogr. A* 2012, *1267*, 206-216.

[39] Krait, S., Salgado, A., Villani, C., Naumann, L., Neusüß, C., Chankvetadze, B., Scriba, G. K. E., *J. Chromatogr. A* 2020, *1628*, Article number 461448.

[40] Chankvetadze, B., Schulte, G., Blaschke, G., *J. Chromatogr. A* 1996, *732*, 183-186.

[41] Chankvetadze, B., Schulte, G., Blaschke, G., *Enantiomer* 1997, *2*, 157-179.

[42] Hjerten, S., *Arkiv Kemi* 1958, *13*, 151-157.

[43] Hjerten, S., *Chromatogr. Rev.* 1967, *9*, 122-219.

[44] Jorgenson, J. W., Lukacs, K. D., *Anal. Chem.* 1981, *53*, 1298-1302.

[45] Jorgenson, J. W., Lukacs, K. D., *J. Chromatogr.* 1982, *218*, 209-216.

[46] Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., Ando, T., *Anal.Chem.* 1984, *56*, 111-113.

[47] Terabe, S., Otsuka, K., Ando, T., *Anal. Chem.* 1985, *57*, 834-841.

[48] Gassmann, E., Kuo, J. E., Zare, R., *Science* 1985, *230*, 813-815.

[49] Guttman, A., Paulus, A., Cohen, A. S., Grinberg, N., Karger, B. L., *J. Chromatogr.* 1988, *448*, 41-53.

[50] Fanali, S., *J. Chromatogr.* 1989, *474*, 441-446.

[51] Terabe, S., *Trends Anal. Chem.* 1989, *8*, 129-134.

[52] Chankvetadze, B., Blaschke, G., *J. Chromatogr. A* 2001, *906*, 309-363.

[53] Stepanova, N. D., Stepanov, A. V., *Zh. Prikl. Khimii* (Russ. J. Appl. Chem., Engl. Edn.) 1969, *42*, 1576-1578.

[54] Wren, S. A. C., Rowe, R. C., *J. Chromatogr.* 1992, *603*, 235-241.

[55] Chankvetadze, B., Burjanadze, N., Bergenthal, D., Blaschke, G., *Electrophoresis* 1999, *20*, 2680-2685.

[56] Chankvetadze, B., *Electrophoresis* 2002, *23*, 4022-4035.

[57] Chankvetadze, B., *J. Chromatogr. A* 1997, *792*, 269-295.

[58] Chankvetadze, B., Lindner, W., Scriba, G., *Anal. Chem.* 2004, *76*, 4256-4260.

[59] Lomsadze, K., Martinez-Giron, A. B., Castro-Puyana, M., Chankvetadze, L., Crego, A. L., Salgado, A., Marina, M. L., Chankvetadze, B., *Electrophoresis* 2009, *30*, 2803-2811.

[60] Chankvetadze, B., Fillet, M., Burjanadze, N., Bergenthal, D., Bergander, K., Luftmann, H., Crommen, J., Blaschke, G., *Enantiomer* 2000, *5*, 313-322.

[61] Domínguez Vega, E., Lomsadze, K., Chankvetadze, L., Salgado, A., Scriba, G., Calvo, E., López, J. A., Crego, A. L., Marina M. L., Chankvetadze, B., *Electrophoresis* 2011, *32*, 2640-2647.

[62] Chankvetadze, B., Burjanadze, N., Bergenthal, D., Breitkreutz, J., Bergander, K., Kataeva, O., Fröhlich, R., Blaschke, G., *J. Sep. Sci.* 2002, *25*, 1155-1166.

[63] Lomsadze, K., Domínguez Vega, E., Salgado, A., Crego, A. L., Scriba, G. K.E., Marina, M. L., Chankvetadze, B., *Electrophoresis* 2012, *33*, 1637-1647.

[64] Gogolashvili, A., Chankvetadze, L., Takaishvili, N., Salgado, A., Chankvetadze, B., *Electrophoresis* 2020, *41*, 1023-1030.

[65] Chankvetadze, B., Burjanadze, N., Santi, M., Massolini, G., Blaschke, G., *J. Sep. Sci.* 2002, *22*, 733-740.

[66] Sidamonidze, N., Süß, F., Poppitz, W., Scriba, G. K. E., *J. Sep. Sci.* 2001, *24*, 777-783.

[67] Konjaria, M. -L., Scriba, G. K. E., *J. Chromatogr. A* 2020, *1623*, Article number 461158.

[68] Chankvetadze, B., Endresz, G., Blaschke, G., Juza, M., Jakubetz, H., Schurig, V., *Carbohyd. Res.* 1996, *287*, 139-155.

[69] Sungthong, B., Iványi, R., Bunz, S. -C., Neusüß, C., Scriba, G. K. E., *Electrophoresis* 2010, *31*,1498-1505.

[70] Tabushi, I., Shimokawa, K., Shimizu, N., Shirakata, H., Fujita, K., *J. Am. Chem. Soc.* 1976, *98*, 7855-7856.

[71] Engeldinger, E., Armspach, D., Matt, D., *Chem. Rev.* 2003, *103*, 4147–4174.

[72] Chankvetadze, B. Capillary Electrophoresis in Chiral Analysis, Wiley & Sons, Chichester, 1997, 555 pp.

[73] Chankvetadze, B., Pintore, G., Burjanadze, N., Bergenthal, D., Strickmann, D., Cerri, R., Blaschke, G., *Electrophoresis* 1998, *19*, 2101-2108.

[74] Chankvetadze, B., Burjanadze, N., Pintore, G., Strickmann, D., Bergenthal, D., Blaschke, G., *Chirality* 1999, *11*, 635-644.

[75] Chankvetadze, B., Pintore, G., Burjanadze, N., Bergenthal, D., Bergander, K., Breitkreutz, J., Mühlenbrock, C., Blaschke, G., *J. Chromatogr. A* 2000, *875*, 455-469.

[76] Chankvetadze, B., Burjanadze, N., Pintore, G., Bergenthal, D., Bergander, K., Mühlenbrock, C., Breitkreutz, J., Blaschke, G., *J. Chromatogr. A* 2000, *875*, 471-484.

[77] Samakashvili, S., Salgado, A., Scriba, G., Chankvetadze, B., *Chirality* 2013, *25*, 79-88.

[78] Branch, S. K., Holzgrabe, U., Jefferies, T. M., Mallwitz, H., Matchett, M. W., *J. Pharm. Biomed. Anal.* 1994, *12*, 1507-1517.

[79] Wedig, M., Holzgrabe, U., *Electrophoresis* 1999, *20*, 2698-2704.

[80] Hellriegel, C., Händel, H., Wedig, M., Steinhauer, S., Sörgel, F., Albert, K., Holzgrabe, U., *J. Chromatogr. A* 2001, *914*, 315-324.

[81] Thunhorst, M., Otte, Y., Jefferies, T. M., Branch, S.K., Holzgrabe, U., *J. Chromatogr. A* 1998, *818*, 239-249.

[82] Branch, S. K., Holzgrabe, U., Jefferies, T. M., Mallwitz, H., Oxley, F. J. R., *J. Chromatogr. A* 1997, *758*, 277-292.

[83] Chankvetadze, B., Lomsadze, K., Burjanadze, N., Breitkreutz, J., Pintore, G., Chessa, M., Bergenthal, D., Bergander, K., Blaschke, G., *Electrophoresis* 2003, *24*, 1083-1091.

[84] Salgado, A., Tatunashvili, E., Gogolashvili, A., Chankvetadze, B., Gago, F., *Phys. Chem. Chem. Phys.* 2017, *19*, 27935-27939.

[85] Añibarro, M., Gessler, K., Usón, I., Sheldrick, G.M., Harata, K., Uekama, K., Hirayama, F., Abe, Y., Saenger, W., *J. Am. Chem. Soc.* 2001, *123*, 11854–11862.

[86] Schmitt, T., Engelhardt, H., *Chromatographia* 1993, *37*, 475-481.

[87] Chankvetadze, B., Endresz, G., Blaschke, G., *Electrophoresis* 1994, *15*, 804-807.

[88] Chankvetadze, B., Endresz, G., Blaschke, G., *Chem. Soc. Rev.* 1996, *25*, 141-153.

[89] Chankvetadze, B., *Electrophoresis* 2009, *30*, S211-S221.

[90] Fejős, I., Kalydi, E., Malanga, M., Benkovics, G., Béni, S., *J. Chromatogr. A* 2020, *1627*, Article number 461375.

[91] Gogolashvili, A., Lomsadze, K., Chankvetadze, L., Takaishvili, N., Peluso, P., Dallocchio, R., Salgado, A., Chankvetadze, B., *J. Chromatogr. A* 2021, *1643*, 462084.

[92] Chankvetadze, B., Burjanadze, N., Maynard, D. M., Bergander, K., Bergenthal, D., Blaschke, G., *Electrophoresis* 2002, *23*, 3027-3034.

[93] Gogolashvili, A., Tatunashvili, E., Chankvetadze, L., Sohajda, T., Szemann, J., Salgado, A., Chankvetadze, B., *Electrophoresis* 2017, *38*, 1851-1859.

[94] Gogolashvili, A., Tatunashvili, E., Chankvetadze, L., Sohajda, T., Szemann, Gumustas, M., Ozkan, S., Salgado, A., Chankvetadze, B., *J. Chromatogr. A* 2018, *1571*, 231-239.

[95] Harata, K., Crystallographic Study of Cyclodextrins and Their Inclusion Complexes. In: Dodziuk, H. (ed), Cyclodextrins and Their Complexes, Wiley-VCH, Weinheim, 2006, 147-198.

[96] Schneider, H.-J, Hacket, F., Rüdiger, V., Ikeda, H., *Chem. Rev.* 1998, *98*, 1755-1785.

[97] Salgado, A., Chankvetadze, B., *J. Chromatogr. A* 2016, *1467*, 95-144.

[98] French, A. D., Murphy, V. G., *Carbohydr. Res.* 1973, *27*, 391-406.

[99] French, A. D., Murphy, V. G., *Polymer* 1977, *18*, 489-494.

[100] Sundararajan, P. R., Rao, V. S. R., *Carbohydr. Res.* 1970, *13*, 351-358.

[101] Nakagawa, T., Koi, U., Kashiwa, M., Watanabe, J., *Tetrahedron Lett.* 1994, *35*, 1921-1924.

[102] Hingerty, B., Saenger, W., *J. Am. Chem. Soc.* 1976, *98*, 3357-3365.

[103] Lindner, K., Saenger, W., *Biochem. Biophys. Res. Commun.* 1980, *92*, 933-938.

[104] Tabushi, I., Kiyosuke, Y. -I.;, Sugimoto, T., Yamamura, K., *J. Am.Chem. Soc.* 1978, *100*, 916-919.

[105] Tabushi, I., Mizutani, T., *Tetrahedron* 1987, *43*, 1439-1447.

[106] Hamilton, J. A., Sabesan, M. N., Steinrauf, L. K., Geddes, A., *Biochem. Biophys. Res. Commun.* 1976, *73*, 659-664.

[107] Maclennan, J. M., Stezowski, J. J., *Biochem. Biophys. Res. Commun.* 1980, *92*, 926-932.

[108] Catenacci, L., Sorrenti, M., Bonferoni, M. C., Hunt, L., Caira, M. R., *Molecules* 2020, *25*, Article number 998.

[109] Harata, K. *Chem. Lett.* 1986, *15*, 2057-2060.

[110] Harata, K., *Bull. Chem. Soc. Jpn.* 1988, *61*, 1939-1944.

[111] Alexander, J. M., Clark, J. L., Brett, T. J., Stezowski, J. J., *Proc. Natl. Acad. Sci. USA* 2002, *99*, 5115-5120.

[112] Harata, K., Uekama, K., Otagiri, M., Hirayama, F., *Bull. Chem. Soc. Jpn.* 1982, *55*, 3904-3910.

[113] Harata, K., Uekama, K., Otagiri, M., Hirayama, F., *Bull. Chem. Soc. Jpn.* 1983, *56*, 1732-1736.

[114] Harata, K., Hirayama, F., Arima, H., Uekama, K., Miyaji, T., *J. Chem. Soc. Perkin Trans. 2* 1992, 1159-1166.

[115] Caira, M. R., Griffith, V. J., Nassimbeni, L. R., van Oudtshoorn, B., *J. Chem. Soc. Perkin Trans. 2* 1994, 2071-2072.

[116] Steiner, T., Saenger, W., *Angew. Chem. Int. Ed.* 1998, *37*, 3404-3407.

[117] Li, W. –S., Wang, S. –C., Hwang, T. –S., Chao, I., *J. Phys. Chem. B* 2012, *116*, 3477-3489.

[118] Shi, J., Guo, D.-S., Ding, F., Liu, Y., *Eur. J. Org. Chem.* 2009, 923-931.

[119] Fourtaka, K., Christoforides, E., Mentzafos, D., Bethanis, K., *J. Mol. Struct.* 2018, *1161*, 1-8.

[120] Connors, K. A. *Chem. Rev.* 1997, *97*, 1325-1358.

[121] Betzel, C., Saenger, W., Hingerty, B. E., Brown, G. M. *J. Am. Chem. Soc.* 1984, *106*, 7545-7557.

[122] Stachowicz, A., Styrcz, A., Korchowiec, J., Modaressi, A., Rogalski, M., *Theor. Chem. Acc.* 2011, *130*, 939-953.

[123] Lindner, K., Saenger, W., *Carbohydr. Res.* 1982, *99*, 103-115.

[124] Liu, L., Guo, Q. –X., *J. Incl. Phenom. Macrocycl. Chem.* 2002, *42*, 1-14.

[125] Loftsson, M. M. T., Brewster, M. E., *J. Pharm. Sci.* 2004, *93*, 1091-1099.

[126] Servais, A.-C., Rousseau, A., Fillet, M., Lomsadze, K., Salgado, A., Crommen, J., Chankvetadze, B., *Electrophoresis* 2010, *31*, 1467-1474.

[127] Lomsadze, K., Salgado, A., Calvo, E., Lopez J. A., Chankvetadze, B., *Electrophoresis* 2011, *32*, 1156-1163.

[128] Kfoury, M., Landy, D., Fourmentin, S., *Molecules* 2018, *23*, Article number 1204.

[129] Ménand, M., Adam de Beaumais, S., Chamoreau, L. –M., Derat, E., Blanchard, S., Zhang, Y., Bouteiller, L., Sollogoub, M., *Angew. Chem. Int. Ed.* 2014, *53*, 7238-7242.

[130] Bergeron, R. J., Meeley, M. P., *Bioorg. Chem.* 1976, *5*, 197-202.

[131] Gelb, R. I., Schwartz, L. M., *J. Inclusion Phenom. Mol. Recognit. Chem.* 1989, 7, 465-476.

[132] Frank, H. S., Evans, M. W., *J. Phys. Chem.* 1945, *13*, 507-532.

[133] Nemethy, G., Scheraga, H. A., *J. Phys. Chem.* 1962, *36*, 3382-3400.

[134] Smithrud, D. B., Wyman, T. B., Diederich, F., *J. Am. Chem. Soc.* 1991, *113*, 5420-5426.

[135] Rekharsky, M. V., Inoue, Y., *Chem. Rev.* 1998, *98*, 1875-1917.

[136] Biedermann, F., Nau, W. M., Schneider, H. –J., *Angew. Chem. Int. Ed.* 2014, 53, 11158-11171.

[137] Setny, P., Baron, R., McCammon, J. A., *J. Chem. Theory Comput.* 2010, *6*, 2866−2871.

[138] Griffiths, D. W., Bender, M. L., *Adv. Catal.* 1973, *23*, 209-261.

[139] Chacko, K. K.; Saenger, W. *J. Am. Chem. Soc.* 1981, *103*, 1708-1715.

[140] Biedermann, F., Uzunova, V. D., Scherman, O. A., Nau, W. M., De Simone, A., *J. Am. Chem. Soc.* 2012, *134*, 15318−15323.

[141] Sandilya, A. A., Natarajan, U., Priya, M. H. *ACS Omega* 2020, *5*, 25655-25667.

[142] Lipkowitz, K. B., *Chem. Rev.* 1998, *98*, 1829-1873.

[143] Liu, L., Guo, Q. –X., *J. Incl. Phenom. Macrocycl. Chem.* 2004, *50*, 95-103.

[144] Dodziuk, H., Modeling of CyD and their complexes. In: Dodziuk, H. (ed), Cyclodextrins and Their Complexes, Wiley-VCH, Weinheim, 2006, 333-355.

[145] Quevedo, M. A., Zoppi, A., *J. Incl. Phenom. Macrocycl. Chem.* 2018, *90*, 1-14.

[146] Anandakrishnan, R., Drozdetski, A., Walker, R. C., Onufriev, A. V., *Biophys. J.*, 2015, *108*, 1153-1164.

[147] Alvira, E., *Tetrahedron: Asymmetry* 2013, *24*, 1198-1206.

[148] Alvira, E., *Tetrahedron: Asymmetry* 2015, 26, 853-860.

[149] Alvira, E., *Chem. Phys. Lett.* 2017, 679, 31-37.

[150] Alvira, E., *Molecules* 2018, *23*, Article number 928.

[151] Lipkowitz, K. B., *J. Chromatogr. A* 2001, *906*, 417-442.

[152] Lämmerhofer, M., *J. Chromatogr. A* 2010, *1217*, 814-856.

[153] Peluso, P., Dessì, A., Dallocchio, R., Mamane, V., Cossu, S., *Electrophoresis* 2019, *40*, 1881-1896.

[154] Chung, L. W., Sameera, W. M. C., Ramozzi, R., Page, A. J., Hatanaka, M., Petrova, G. P., Harris, T. V., Li, X., Ke, Z., Liu, F., Li, H. –B., Ding, L., Morokuma, K., *Chem. Rev.* 2015, *115*, 5678-5796.

[155] Chung, L. W., Hirao, H., Li, X., Morokuma, K., *WIREs Comput. Mol. Sci.* 2012, *2*, 327-350.

[156] Patodia, S., Bagaria, A., Chopra, D., *J. Phys. Chem. Biophys.* 2014, *4*, 1-4.

[157] Henriksen, N. M., Fenley, A. T., Gilson, M. K., *J. Chem. Theory Comput.* 2015, *11*, 4377−4394.

[158] Henriksen, N. M., Gilson, M. K., *J. Chem. Theory Comput.* 2017, *13*, 4253−4269.

[159] Liu, J., Coffey, H., Detlefsen, D. J., Li, Y., Lee, M. S., *J. Chromatogr. A* 1997, *763*, 261-269.

[160] Rawjee, Y.Y., Staerk, D.U., Vigh, G, *J. Chromatogr. A* 1993, *635*, 291-306.

[161] Rawjee, Y.Y., Vigh, G., *Anal. Chem.* 1994, *66*, 619-627.

[162] Williams, B.A., Vigh, G., *J. Chromatogr. A* 1997, *777*, 295-309.

[163] Thormann, W., Chankvetadze, L., Gumustas, M., Chankvetadze, B., *Electrophoresis* 2014, *35*, 2833-2841.

[164] Caslavska, J., Thormann, W., *Electrophoresis* 2020, *41*, 502-513.

[165] Dubský, P., Svobodová, J., Gaš, B., *J. Chromatogr. B* 2008, *875*, 30-34.

[166] Müllerová, L., Dubský, P., Gaš, B., *Electrophoresis* 2014, *35*, 2688-2700.

[167] Elbashir, A. A., *J. Applied Solution Chem. Model.* 2012, *1,* 121-126.

[168] Elbashir, A. A., Aboul-Enein, H. Y., *Critical Rev. Anal. Chem.* 2013, *43*, 131-137.

[169] Scriba, G. K. E., *Chromatographia* 2012, *75*, 815-838.

[170] Scriba, G. K. E., *J. Chromatogr. A* 2016, *1467*, 56-78.

[171] Krait, S., Konjaria, M. –L., Scriba, G. K. E., *Electrophoresisis* 2021, DOI: 10.1002/elps.202000359.

[172] Liu, Y., Deng, M., Yu, J., Jiang, Z., Guo, X., *J. Sep. Sci.* 2016, *39*, 1766-1775.

[173] Li, L., Wu, C., Ma, Y., Zhou, S., Li, Z., Sun, T., *Analyst* 2017, *142*, 3699-3706.

[174] Li, J., Yu, T., Xu, G., Du, Y., Liu, Z., Feng, Z., Yang, X., Xi, Y., Liu, J., *J. Chromatogr. A* 2018, *1559*, 178-185.

[175] Zhao, Y., Wang, J., Liu, Y., Jiang, Z., Song, Y., Guo, X., *New J. Chem.* 2020, *44*, 958-972.

[176] Murrell, J. N., *J. Mol. Struct. (Theochem)* 1998, *424*, 93-99.

[177] Zheng, Y. –J., Merz, K. M. Jr, *J. Comput. Chem.* 1992, *13*, 1151-1169.

[178] Huang, M. -J., Quan, Z., Liu, Y. –M, *Int. J. Quantum Chem.* 2009, *109*, 81-90.

[179] Pasquini, B., Melani, F., Caprini, C., Del Bubba, M., Pinzauti, S.,Orlandini, S., Furlanetto, S., *J. Pharm. Biomed. Anal.* 2017, *144*, 220-229.

[180] Suliman, F. O., Burtomani, S. K. A., Elbashir, A. A., Schmitz, O. J., *Electrophoresis* 2021, DOI: 10.1002/elps.202000290.

[181] Elbashir, A. A., Suliman, F. E. O., Saad, B., Aboul-Enein, H. Y., *Talanta* 2009; *77*, 1388-1393.

[182] Li, W., Zhao, L., Chen, X., Chen, S., Zhu, Z., Hong, Z., Chai, Y., *Electrophoresis* 2014, *35*, 2855-2862.

[183] Rontoyianni, A., Mavrids, I. M., Israel, R., Beurskens, G., *J. Incl. Phenom. Mol. Recognit. Chem.* 1998, *32*, 415-428.

[184] Arsad, S. R., Maarof, H., Ibrahim, W. A. W., Aboul-Enein, H. Y., *Chirality* 2016, *28*, 209-214.

[185] L. Lawtrakul, H. Viernstein and P. Wolschann, *Int. J. Pharm.* 2003, *256*, 33-41.

[186] K. B. Lipkowitz, *Acc. Chem. Res.* 2000, *33*, 555-562.

[187] Li, W., Tan, G., Zhao, L., Chen, X., Zhang, X., Zhu, Z., Chai, Y., *Anal. Chim. Acta* 2012, *718*, 138-147.

[188] Tóth, G., Mohácsi, R., Rácz, Á., Rusu, A., Horváth, P., Szentem L., Béni, S., Noszál, B., *J. Incl. Phenom. Macrocycl. Chem.* 2013, *77*, 291-300.

[189] Suliman, F. O., Elbashir, A. A., Schmitz, O. J., *J. Incl. Phenom. Macrocycl. Chem.* 2015, *83*, 119-129.

[190] Azhari, N. R., Yahaya, N., Faiz Bukhari M. Mohd Suah, F. B. M., Prabu, S., Hui, B. Y., Shahriman, M. S., Zain, N. N. M., Raoov, M., *Chirality* 2021, *33*, 37-50.

[191] Ma, X., Du, Y., Sun, X., Liu, J., Guang, Z., *J. Chromatogr. A* 2019, *1601*, 340-349.

[192] Nascimento, C. S. Jr, Lopes, J. F., Guimarães, L., Borges, K. B., *Analyst* 2014, *139*, 3901-3910.

[193] Budău, M., Hancu, G., Muntean, D. L., Papp, L. A., Cârje, A. G., Garaj, V. *Chirality* 2020, *32*, 1119-1128.

[194] Li, L., Li, X., Luo, Q., You, T., *Talanta* 2015, *142*, 28-34.

[195] Yang, X., Yan, Z., Yu, T., Du, Y., Chen, J., Liu, Z., Xi, Y., *Anal. Bioanal. Chem.* 2018, *410*, 5889-5898.

[196] Zhu, X., Chen, C., Chen, J., Xu, G., Du, Y., Ma, X., Sun, X., Feng, Z., Huang, Z., *J. Pharm. Biomed. Anal.* 2020, *180*, Article number 113030.

[197] Gábor Benkovics G., Fejös, I., Darcsic, A., Varga, E., Malanga, M., Fenyvesi, E., Sohajda, T., Szente, L., Béni, S., Szemán, J., *J. Chromatogr. A* 2016, *1467*, 445-453.

[198] Fejös, I., Varga, E., Benkovics, G., Malanga, M., Sohajda, T., Szemán, J., Béni, S., *Electrophoresis* 2017, *38*, 1869-1877.

[199] Fonseca, M. C., Santos da Silva, R. C., Nascimento, C. S. Jr, Borges, K. B., *Electrophoresis* 2017, *38*, 1860-1868.

[200] Melani, F., Pasquini, B., Caprini, C., Gotti, R., Orlandini, S., Furlanetto, S., *J. Pharm. Biomed. Anal.* 2015, *114*, 265-271.

[201] Szabó, Z. –I., Ludmerczki, R., Fiser, B., Noszál, B., Tóth, G., *Electrophoresis* 2019, *40*, 1897-1903.

[202] Krait, S., Salgado, A., Chankvetadze, B., Gago, F., Scriba, G. K. E., *J. Chromatogr. A* 2018, *1567*, 198-210.

[203] Michalska, K., Pajchel, G., Tyski, S., *J. Chromatogr. A* 2008, 1180, 179-186.

[204] Bednarek, E., Bocian, W., Michalska, K., *J. Chromatogr. A* 2008, *1193*,164-171.

[205] Michalska, K., Bocian, W., Bednarek, E., Pałys, B., Cielecka-Piontek, J., *J. Chromatogr. A* 2019, *169*, 49-59.

[206] Yao, Y., Song, P., Wen, X., Deng, M., Wang, J., Guo, X., *J. Sep. Sci.* 2017*, 40*, 2999-3007.

[207] Guo, J., Wang, J., Lin, H., Feng, Y., Shen, H., Huang, R., Liu, L., Zhao, Z., *J. Sep. Sci.* 2019, *42*, 1077-1087.

[208] Schneider, H. –J., *New J. Chem.* 2019, *43*, 15498-15512.

FIGURE CAPTIONS

Fig. 1. (**A**) Freudenberg's initial model of formation of cyclodextrin, and **B**) model based on transglucosylation by *Bacillus macerans amylase* (adapted from ref. 30 with permission).

Fig. 2. Structures and dimensions of native CDs.

Fig. 3. Effect of counterpressure on the separation of (±)-chlorpheniramine in the presence of 2 mg/ml CM-β-CD (adapted from ref. 55 with permission).

Fig. 4. Schematic representation of flow-counterbalanced separation principle in CE: (**A**) without counterbalanced flow; (**B**) with counterbalanced flow; (**C**) resulting mobilities (adapted from ref. 55 with permission).

Fig. 5. Structure of the terbutaline complexes with β-CD (**A**), α-CD (**B**), and γ-CD (**C**) (adapted from ref. 64 with permission).

Fig. 6. Opposite affinity of AGT enantiomers towards β-and γ-CDs (adapted from ref. 60 with permission).

Fig. 7. Structures of the ephedrine / α-CD (**A**), ephedrine / β-CD (**B**) (adapted from ref. 61 with permission), norephedrine / α-CD (**C**), and norephedrine / β-CD (**D**) complexes (adapted from ref. 63 with permission).

Fig. 8. Structures of (+)-brompheniramine maleate / β-CD (**A**) and (+)-brompheniramine / TM-β-CD (**B**) complexes in the solid state. Capillary electrophoresis enantioseparation of brompheniramine with β-CD (**C**) and TM-β-CD (**D**) as chiral selectors (adapted from ref. 76 with permission).

Fig. 9. Capillary electrophoresis enantioseparation with HDA- (**A**) and HDAS-β-CD (**B**) as chiral selectors (adapted from ref. 91 with permission).

Fig. 10. Glucopyranose ring conformations observed in X-ray crystal structures of β-CDs and their inclusion complexes.

Fig. 11. X-ray structures of (**A**) β-CD, (**B**) 2,6-DM-β-CD, (**C**,**D**) TM-β-CD (structures released from the CSD entries AGAZOX [111], DEZMIE10 [110], GELKEN10 [114], and HEZWAK10 [115].

Fig. 12. MM+ optimized structure of *R*-ketamine / α-CD (**A**), *S*-ketamine / α-CD (**B**), *R*-ketamine / β-CD (**C**), and *S*-ketamine / β-CD (**D**) (adapted from ref. 62 with permission).

Fig. 13. Snapshots of D-phenylalanine / α-CD complex (**A**), and D-phenylalanine / α-CD / 18-crown-6 ternary (**B**) collected during MD simulation (adapted from ref. 180 with permission).

Fig. 14. Structure of the water-soluble melatonergic drug BMS-191435 [159].

Fig. 15. Representative geometry-optimized snapshots from the simulated MD trajectories of (**A**) β-CD / *S*-clenpenterol and (**B**) β-CD / *R*-clenpenterol complexes, showing the closest water molecules in the analyte's solvation shell (HBs connecting guest and β-CD through some water molecules that exchange with the bulk solvent

are indicated as yellow dashed lines); overlay of representative low-energy structures of (**C**) HDA-β-CD / *S*-clenpenterol and (**D**) HDA-β-CD / *R*-clenpenterol (the long-residence water molecule that bridges an interaction between the protonated amino group of clenpenterol and oxygen atoms in HDA-β-CD in each complex is displayed in sticks, with the O atom coloured in cyan) (adapted from ref. 84 with permission).

Fig. 16. Structure of iodiconazole (**A**), and correlation between predicted resolution and experimental resolution (**B**) for the CE enantioseparation of iodiconazole with HP-γ-CD (adapted from ref. 187 with permission).

Fig. 17. Structure of ofloxacin (**A**), and structures of the three HP-β-CD with different substitution pattern: HP-β-CD DS4a (**B**), HP-β-CD DS4b (**C**), and HP-β-CD DS4c (**D**) (adapted from ref. 188 with permission).

Fig. 18. Geometries of the inclusion complexes of *S*-ofloxacin / HP-β-CD (**A**) and *R*-ofloxacin / HP-β-CD (**B**) (adapted from ref.189 with permission).

Fig. 19. Structure of 4-hydroxypropranolol, and modes of inclusion (**A**) form A, naphthyl ring of 4-hydroxypropranolol is inserted in the hydrophobic cavity CM-β-CD by the wider rim, (**B**) form B, naphthyl ring of 4-hydroxypropranolol is included by the narrower rim of CM-β-CD, (**C**) form C, aliphatic part of 4-hydroxypropranolol is inserted in the hydrophobic cavity CM-β-CD by the wider rim, (**D**) form D, aliphatic part of 4-hydroxypropranolol is included by the narrower rim of CM-β-CD (R = carboxymethyl group) (adapted from ref. 192 with permission).

Fig. 20. Structure of medetomidine.

Fig. 21. Orientations of linezolid in the complex with HDAS-β-CD as derived from 40 ns MD calculations (adapted from ref. 204 with permission).

Fig. 22. Structure of sutezolid.

Fig. 23. Electropherograms of CE enantioseparation of clembuterol (**A**), oxybutynin (**B**), salbutamol (**C**), and penehyclidine (**D**) with HDAS-β-CD (adapted from ref. 206 with permission).

Fig. 24. Structure of acebutolol (**A**), and inclusion mode of acebutolol in HDAS- (**B**) and HDMS-β-CD (**C**) complexes (adapted from ref. 207 with permission).

TABLE CAPTIONS

Table 1. Characteristics of α-, β-, and γ-cyclodextrins.