Recent advances in mass spectrometry studies of non-covalent complexes of macrocycles - A review.

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21 **Abstract**

22 Non-covalent molecular interactions are crucial for the formation of supramolecular systems
23 whose applications in the transport and release of drugs, the development of nanoreactors or the
24 design of molecular sensors, among others, make them very hot topic of investigation. The use of
25 mass spectrometry (MS) and soft ionization methods as electrospray ionization (ESI) and matrix
26 assisted laser desorption ionization (MALDI) has allowed the characterization of supramolecular
27 systems. This comprises the characterization of non-covalent complexes formed by synthetic
28 molecules and biomolecules and the study of these host-guest systems with different molecular
29 interactions and thermodynamic aspects. Mass spectrometry is capable of answering many
30 questions regarding analytical characterization of these systems including composition,
31 stoichiometry, and structural aspects as e.g., connectivities and building blocks in the
32 supramolecular complex. These soft ionization techniques together with MS have become the
33 technique of choice for characterizing molecular interactions allowing the design of new molecules
34 capable of self-assembly in supramolecular systems. In this review, the synthetic macromolecules
35 cucurbiturils, calixarenes, crown ethers, catenanes, rotaxanes and cyclodextrins are reviewed due
36 to the wide range of non-covalent interactions that they are able to form in their supramolecular
37 assemblies. Both the non-covalent interactions and the supramolecular structures of these
38 synthetic molecules have been studied by mass spectrometry to characterize their structures, thus
39 showing the power of the technique as a tool for supramolecular analysis.

40

41 **Abbreviations**

42 APCI: Atmospheric Chemical Ionization; APPI: Atmospheric Pressure Photoionization; CB[n]:
43 Cucurbiturils; CCS: Collision Cross Section; CE: Capillary Electrophoresis; CDs: Cyclodextrins; CID:

44 Collision Induced Dissociation; DESI: Desorption Electrospray Ionization; DTIM: Drift-Tube Ion
45 Mobility; DOSY: Diffusion-Ordered Spectroscopy; ECD: Electron Capture Dissociation; ECID:
46 Electron Capture-Induced Dissociation; ESI: Electrospray Ionization; FT-ICR: Fourier Transform-Ion
47 Cyclotron Resonance; HCD: Higher energy Collision Dissociation; HRMS: High Resolution Mass
48 Spectrometry; IM: Ion-Mobility; ISCID: In-Source Collision Induced Dissociation; ITC: Isothermal
49 Titration Calorimetry; LQTOF: Linear Quadrupole Time of Fly; LTQ: Linear Trap Quadrupole; MALDI:
50 Matrix Assisted Laser Desorption Ionization; MOFs: Metal-Organic Frameworks; MONC's: Metal-
51 Organic Nano-Capsules; MS: Mass Spectrometry; NMR: Nuclear Magnetic Resonance; QTOF:
52 Quadrupole Time of Fly; QIT: Quadrupole Ion-Trap; QqQ: triple quadrupole; SORI: Sustained Off-
53 Resonance Irradiation; TWIM-MS: Traveling-Wave Ion Mobility Mass Spectrometry.

54

1. Introduction

The discovery of the key-role of non-covalent interactions in molecular macrostructures of biological importance, sparked the enthusiasm for creating synthetic macromolecules able to emulate such assembled macrostructures through a wide variety of non-covalent molecular interactions. Supramolecular chemistry has used diverse synthetic macromolecules for applications and developments in different disciplines, such as transport and release of drugs [1], extraction of metal pollutants in wastewater [2–4], design of molecular sensors [5–7], new materials for analytical separations [8] or molecular machines [9,10], among others. Understanding of supramolecular systems and their applications are subjected to the knowledge of the non-covalent molecular interactions that have place inside such systems.

Non-covalent interactions are a variety of weak, reversible, inter or intramolecular attractive forces (see Figure 1). Their energies range from only a few kJ/mol for Van der Waals forces to several hundreds of kJ/mol (2 kJ/mol-20 kJ/mol in solution and up to 160 kJ/mol in gas phase) for coordination bonds. Ion-ion interactions are strongest forces with bond energies around 100-350 kJ/mol and the interaction between ions and dipoles are somewhat weaker (50-200 kJ/mol) [11]. Their force and stretch depend on the distance between charges and the delocalization over a part of the molecule. Interactions between two dipoles (5-50 kJ/mol) are weaker than ion-dipole forces.

Hydrogen bonds are among the most employed in artificial supramolecular structures. Hydrogen bonds are important because of their directionality. They allow the chemist to control the geometry of the complexes and in this way, a precise complementary host can be designed for a given guest. The hydrogen bond has a pivotal role in self-assemblies of molecular capsules from synthetic molecules such as calix[4]arenes, resorcin[4]arenes and pyrogallol[4]arenes[12].

π -systems can bind with cation, dipoles and other π -systems. The cation- π interaction is a nonbonding electrostatic attraction resulting of the favorable association between a cation or atom group and an aromatic- π system. Binding energies can reach 167 kJ/mol and 21 kJ/mol in gas phase or aqueous phase, respectively. Cation- π is a strong interaction that is present for instance in many inclusion complexes between metal or organic cations and calixarenes family.

The direct interaction between parallel aromatic rings is energetically unfavored due to the repulsion of the quadrupole moment of the aromatic rings. However, depending on the nature of the rings, two π -systems can interact between them in several geometries, what is called π -stacking. This attractive interaction could exist when the aromatic rings are placed face-to-face with respect to their neighboring aromatic ring or the two most frequently found in nature edge-to-face and parallel offset-stacked[13]. Edge-to-face geometry is observed between protein residues[14] and takes place when the negative π -cloud of one ring and the positive σ -scaffold of another ring interacts[15]. The offset-stacked geometry occurs when the electron density on the face of one or both rings is reduced. Particularly parallel offset plays an important role in DNA structure [16].

Lastly, the hydrophobic effects are important in macrocycles. These effects are based on the minimization of the energetically unfavorable surface between polar-protic and nonpolar-protic molecules [17]. For instance, hydrophobic effect plays an important role in guest binding of cucurbiturils and cyclodextrins, in which a nonpolar cavity is formed. Here, water and other polar molecules inside the nonpolar cavity cannot interact with the cavity wall strongly.

For a correct study of the different types of non-covalent interactions, macrocycles properties must be well known in order to use the most suitable ionization source. High-molecular mass species and thermally unstable compounds are typically not amenable to atmospheric chemical

ionization (APCI) or atmospheric pressure photoionization (APPI) since the ionization process could lead to possible thermal degradation effects. With the appearance in the late 1980s of soft ionization techniques, matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI), it was possible to analyze macrostructures consisting of several units[18,19]. The power of these two techniques lies in the ability to transfer molecules and complexes from solution to the gas-phase maintaining their native structure. In MALDI, the analyte is embedded into a matrix and irradiated with a laser pulse which leads to charged ions in gas phase [20]. Nevertheless, successful analysis depends upon several experimental factors, such as solution pH, matrix selection and number of laser shots [21]. MALDI has been applied successfully to preserve non-covalent complexes and its main advantage versus ESI is its tolerance to high salt concentrations. However, possible interactions with the matrix may also have an effect on the complex stability during the ionization process. For this reason, ESI is still the preferred ionization technique in the field. ESI takes place at atmospheric pressure. The analyte solution is infused into a metal capillary to which an electrical potential is applied [22]. This potential helps to form small charged droplets that can undergo rapid solvent evaporation to form multiply charged ions in gas phase. The basic operation principles of MALDI and ESI sources have been discussed in several excellent reviews [23–25]. During the first years of development of soft ionization methods, many reviews about the potential of these techniques for characterization of intermolecular interactions were written[26–29]. Furthermore, these techniques have been used to detect and understand complex architectures[30]. Thus, interactions between biomolecules as proteins, glycan, lipids, DNA, RNA, etc. have been widely investigated [31–34]. In addition, MS has the ability to discern between enantiomers coupled to chiral selectors by forming transit diastereomeric complexes[35]. Among a large number of chiral selectors, cyclodextrins and crown ethers are widely used in chiral capillary electrophoresis (CE) coupled to mass spectrometry (MS) analysis for drug development.

The use of MS together with different soft ionization methods to investigate supramolecular structures in gas-phase is now very common because these techniques can provide information about non-covalent gaseous ion, structural determinations, reactivity in gas-phase and gas-phase thermochemical data[36–38]. Recently, these techniques have been used to study supramolecular assemblies cage-like shape and capsules using compounds as cucurbiturils, calixarenes and, rotaxanes, among others (see Table 1).

Not only the introduction of soft ionization methods but also major developments in mass analyzers have led to an increasing application of mass spectrometry for the analysis of biomolecules and synthetic macromolecules. In mass spectrometry, one of the most common combinations of mass analyzers is quadrupole time-of-flight (QTOF) due to its high sensitivity and high resolution on a fast time scale. QTOF has been used with great success to characterize many complexes with molecular weights as high as million Daltons. Alternative mass analyzers with high sensitivity and high resolution are the Fourier transform-ion cyclotron resonance (FT-ICR) and Orbitrap mass analyzer. In order to generate structure information about the architecture of the complexes, gas phase ion dissociation methods can be applied (Table 2). These methods have the ability to induce fragmentation with little disruption of noncovalent interactions providing different information depending on the dissociation used. The most widely applied fragmentation method is collision-induced dissociation (CID) mainly because is compatible with a wide range of ionization techniques[39]. Under CID, the molecular ion collide with neutral gas molecules, contributing to the redistribution of the energy over the complex and subsequently bond breakage and fragmentation. The combination of tandem mass spectrometry with CID or ion mobility (IM-MS)[40] make possible the study of some thermodynamic aspects of supramolecular structures such as stoichiometry, binding energies and separation by size and shape[41,42].

The knowledge of the non-covalent molecular interactions that occur in supramolecular systems are the basis of their applications. In this point is where the analytical techniques of this review, MS together with soft ionization techniques such as ESI and MALDI, have been used to access to the macrostructure, in order to determine aspects of form, size, interaction energy between units that comprise it, as well as its composition[43]. The purpose of this review is to provide updated information about the study of non-covalent interactions between synthetic compounds, ionization/MS methods used, and the data obtained. We will focus on the analytical characterization of both the non-covalent interactions and the supramolecular structures of cucurbit[n]urils, calixarenes, crown ethers, catenanes, rotaxanes and cyclodextrins due to the wide range of non-covalent interactions that they are able to form in their supramolecular assemblies. This characterization includes composition, stoichiometry and other structural aspects.

2. MS Techniques to investigate non-covalent interactions between synthetic molecules

2.1. Cucurbit[n]urils (CB[n])

Cucurbit[n]urils are a family of synthetic macrocyclic compounds synthesized from an acid catalyzed condensation reaction between glycoluril and formaldehyde. The first member of the family (cucurbit[6]uril) was synthesized by Behrend et al., in 1905 [44]. Behrend et al., were able to obtain a water-insoluble crystalline solid and demonstrated its ability to form crystal complexes with a variety of substances including KMnO_4 , AgNO_3 , H_2PtCl_6 , NaAuCl_4 . However, the structure of this substance was fully elucidated nearly 80 years later [45]. It was characterized as a cyclic-toroid structure composed of six units derived from glycoluril bonded by twelve methylene bridges [45], with a 0.55 nm hydrophobic cavity. Its rigid structure and ability to form stable complex with molecules and ions also make cucurbiturils attractive as a building blocks for supramolecular

assemblies. After 1990, other research groups were interested in developing applications using cucurbiturils in many areas such as science-chemistry, material science, fluorescence spectroscopy and nanotechnology, and for that reason, the CB[n] family has increased notoriously in the current millennium following the preparation of new CB[n] homologues ([CB[5], CB[6], CB[7], CB[8] and some dimers as CB[10]·CB[5] and CB[5]·CB[8] [46,47].

A combination of hydrophobic interactions with the interior of the cavity and hydrogen-bonding and/or electrostatic ion-dipole interactions with the carbonyl groups facilitates very strong bindings (see Figure 2). According to the binding properties, the cucurbit[n]urils are able to form inclusion complexes, with a large number of chemical species (amines, polyamides, peptides, surfactants, thiols, gases, hydrocarbons aromatics and aliphatic among others) as dyes and drugs.

Research on cucurbit[n]urils complexes has been focused on molecular recognition in aqueous and organic solutions. Molecular recognition has been studied using different spectroscopic techniques as NMR, UV-Vis, fluorescence and X-ray crystallography. During at least one-decade after 1981, mass spectrometry was not used in the characterization of cucurbit[n]urils complexes. First and foremost, the researchers in mass spectrometry had to answer the following question, can the supramolecular assemblies and host-guest system formed in solution survive the ionization process? That question led to the use to soft ionization techniques capable of generating intact complexes in gas phase[48].

Many cucurbit[n]uril complexes are formed through ion-dipole interactions. One of the first works that reported the use of mass spectrometry for demonstrating that cucurbit[6]uril pseudorotaxanes survive in gas phase was published by Dearden *et al.* in 2003 [49]. In their work several evidences indicated that CB[6] and a doubly protonated amine 1,4-butanediamine (DAB) cation form pseudorotaxanes. These rotaxanes survive to electrospray process resulting in strong

signals of positive ions consistent with complexes of ammonia ions with CB[6] such as $(\text{CB}[6] \cdot (\text{DAB} + \text{H})_2)^{2+}$ ion [49]. In addition, stoichiometry studies using ESI-FTICR-MS and sustained off-resonance irradiation collision-induced dissociation (SORI-CID) were carried out in this work. SORI-CID is a 'slow-heating' CID technique, which takes place in an ICR cell. Ions undergo acceleration-deceleration cycles and consequently ion activation is in competition with deactivation, allowing multiple collisions at low transitional energy (<10 eV). Thus, SORI-CID fragmentation of the precursor ion produces small increments of internal energy and softer fragmentation than classic CID. The results showed a doubly charged complex CB[6]:DAB with stoichiometry 1:2.

Arakawa *et al.* [50] presented a very interesting work in which they studied the ESI-MS identification of host-guest complex formation of CB[n] ($n = 6, 7$) with several guests as cations of alkali metals, alkylammonium ions, and some neutral organic compounds. The results of binding affinity for alkali metals ions were in the same order as their ionic radius at the same experimental conditions for CB[6] as well as CB[7]. Nevertheless, the selectivity changed when a 50% formic acid solution was used. In this case, Na^+ showed the largest binding with CB[6] among the alkali metal ions. The complexes of CB[6] were detected as doubly-charged ions $[\text{CB}[6] + 2\text{Na}^+]^{2+}$, and the singly charged ions could also be detected. The experiments with monoamines hydrochloric acid salts (propylamine, butylamine, hexylamine, and octylamine) showed that CB[6] can most comfortably accommodate butylamine. On the other hand, in the case of CB[7], hexylamine with a longer carbon chain was most favorable to the complex formation. When the complexation experiments were performed with diamines hydrochloric salts (1,2-ethyldiamine, 1,4-butyldiamine, 1,6-hexyldiamine) the results were in agreement with the cavity height favoring complex formation with the guest having longer aliphatic chain between the ammonium groups. Moreover, the increase of the number of carbon atoms in the aliphatic chain also favors the hydrophobic interaction inside the cavity. ESI-MS interaction between CB[6] and CB[7] with neutral molecules

(cyclopentanol, cyclopentanone, n-pentanol, and 3-pentanone) demonstrated that CB[6] formed complexes with cyclopentanol and cyclopentanone while CB[7] which has a larger cavity cannot form an inclusion complex with any of the above neutral molecules because of insufficient hydrophobic interaction. In conclusion, the results showed that selectivity of the complex formation is dependent both on: the dipolar interaction between the opening portal of the CB[n] host and the guest cations; and the hydrophobic interaction at the inner cavity of CB[n].

Using ESI-MS, Scherman *et al.* [51] described an approach for the formation of multi-component host-guest systems where the ion-dipole interactions and hydrophobic interactions were crucial in the supramolecular assembly. They reported the preparation of modular multi-component great size self-assembled structures based on CB[8] and a viologen dimer (MVdimer). This MVdimer is composed of two methyl-4,4'-bipyridinium units connected by a flexible triethylene glycol (TEG) linker [51]. Mass spectrometry revealed that a complex 1:1 CB[8]-MVdimer was obtained, when a single equivalent of CB[8] is added to MVdimer in water. When a second equivalent of CB[8] was added to the previous solution, the 2:1 host-guest complex was formed. Major peaks in the MS spectra at 447.0 and 779.3 m/z were detected respectively, corresponding to the 4⁺ charge state of these species. Additionally, ¹H-NMR measurements indicated that the CB[8] is mainly centered around the linker of MVdimer in solution. Furthermore, using titration experiments of 1:1 CB[8]-MVdimer with different ratios of CB[8] and naphthol derivatives, such as 2,6-dihydroxynaphthalene (DNp), 2-naphthol (Np) and tri(ethylene glycol)-2-naphthyl ether (NpTEG), a variety of predetermined self-assembled architectures can be accessed with different stoichiometries. For instance, 2:1:1 [CB[8]-MVdimer-DNp]⁴⁺ and [2:1:2 [CB[8]-MVdimer-DNp]⁴⁺. These results suggest that the assemblies could be used to construct supramolecular ABA triblock

241 copolymer structures and ESI-MS was corroborated as a useful tool for studying the polymeric
242 distribution of the assemblies and its stoichiometry.

243
244 Pseudorotaxanes are common assemblies of cucurbit[n]urils formed through hydrophobic and ion-
245 dipole interactions of the macrostructures. Schmitzer *et al.* [52] used high resolution mass
246 spectrometry (HRMS) for determining the binding interactions between CB[7] and different
247 polyimidazolium cations containing aromatic binding sites (1,4-xylylene units) [52]. CB[7] was
248 found to form [2] and [3]pseudorotaxanes by the inclusion of 1,4-xylylene unit acting as
249 hydrophobic linkers inside the cavity of cucurbit[n]uril.

250
251 Taking into account the high solubility of CB[7] in water, Macartney *et al.* [53] studied its
252 molecular recognition versus cations of biological interest such as choline, phosphocholine
253 ($R_3NCH_2CH_2OR'^+$), their phosphonium analogues ($R_3PCH_2CH_2ORC^+$) ($R_3 = Me_3, Et_3, \text{ or } Me_2Bz, \text{ or } R_3N$
254 $= \text{quinuclidinium, and } R' = H, COCH_3, CO(CH_2)_2CH_3, \text{ or } PO_3H$), and ($\pm$)-carnitine [53]. Stables
255 complexes with 1:1 ratio between neutral CB[7] and these cations were reported. The host CB[7]
256 was able to recognize choline in water via ion-dipole and hydrophobic interactions without the use
257 of aromatic or anionic components, which usually provide non-covalent cation- π and ion-dipole
258 interactions (for example with the $N(CH_3)_2^+$ head groups). ESI-MS was used to confirm the
259 formation of complexes CB[7]-choline in the process of recognition according with the result
260 previously obtained by 1H and ^{31}P -NMR.

261
262 Another noteworthy application of ESI-MS is the determination of binding constants that
263 quantitatively measure non-covalent interactions. Scherman *et al.* [54] established the
264 relationship between solution and gas-phase binding equilibria of cucurbit[8]uril ternary

complexes using binding affinities of 32 aromatic guests. To estimate the potential of ESI-MS in characterizing aqueous binding equilibria, guest-exchange experiments were conducted adding these 32 aromatic guests with known binding constant (K_a) measured by isothermal titration calorimetry (ITC). An excellent agreement between ITC and ESI constants was found. As has been mentioned before in this review, not only ITC is one of the most commonly used methods for measuring binding constants but also NMR titrations or UV-Vis spectroscopy, fluorescence spectroscopy and the use of nanopores[11,55,56]. Nonetheless, working with ESI-MS is a convenient selection due to time saving, sensitivity (NMR and calorimetric experiments usually need milligram quantities) and specificity. Moreover, above all, the main advantages of this technique are: 1) the fact that non-covalent complexes are directly observed providing stoichiometry information and 2) ESI-MS enables the multiple component detection simultaneously[57]. Due to ESI nature, it could be thought that the analyte would concentrate when the droplets evaporate and a displacement in the equilibrium would occur. Notwithstanding, in general, the off-rate for the binding is slower than the sub-millisecond droplet desolvation process, so there is not enough time for equilibrium shifts[58]. In other words, complexes are kinetically stable at the time-scale of the process. Nevertheless not everything is perfect; we must keep in mind some crucial point when using ESI-MS: the stability of the precursor and product ions.

The cucurbit[n]urils also present aggregation phenomena, Ramamurthy *et al.* [59] used ESI-MS to observe and identify aggregates of cucurbit[n]urils ($n = 7$ and 8) complexes with several guests. Thanks to ESI-tandem mass spectrometry (MS^n) the formation of dimers, trimers, and tetramers was demonstrated. All the studied nitroxides form 1:1 complex, leaving one of the CB[n] portals free for interaction and thus aggregation. On the other hand, with the dicationic guest, as diamines, both CB[n] portals are occupied, so no aggregates are possible.

289

290 The development of practical applications of mass spectrometry for examining protein structure
291 remains an active field of research [60]. In this area, Kim *et al.* [61] investigated the gas phase
292 host-guest chemistry between CB[6] and lysine residue in peptides along with its potential utility
293 to probe the structure of small proteins, such as ubiquitin in the solution-phase using MSⁿ.
294 Complex formation of CB[6] and Lys residue was checked thoroughly using six isobaric model
295 peptides (A_nKA_{9-n}, n = 0, 1, 3, 5, 7, 9). They observed a huge increase in the complex abundance
296 when lysine residue is located at the N-terminus (KA₉). Considering the relative abundance of KA₉
297 as 100%, a reduction up to 75% is observed when Lys residue is far from N-terminal site (A₉K). The
298 reason is that the protonated ammonium groups of the lysine side chain N-terminus interact with
299 ureido carbonyl-located portals at both sides while the alkyl chains of lysine thread through the
300 hydrophobic interior of CB[6]. Besides, MSⁿ spectra of CB[6] complex fragment peptides revealed
301 details of the CB[6] binding sites suggesting that the proposed MS methodology has potential to
302 deduce small protein structures in solution phase.

303

304 Self-assembly is a strategy used by supramolecular chemists to reduce the effort required for the
305 generation of complex structures and architectures. The control of self-assembly is possible if the
306 location where non-covalent interactions may occur is known. A study about the chemistry that
307 occurs on the surface of the CB[n] was presented for the first time by Kim *et al.* [62] using ESI-MS
308 operating in negative ion-mode. In that work, they described non-covalent interactions of CB[6]
309 with anionic species on its exterior especially the equatorial region which is highly positive. The
310 study was carried out mixing haloacetate anions such as monoiodoacetate (MIA),
311 monobromoacetate (MBA) and monochloroacetate (MCA) with CB[6] and the results showed that
312 the CB[6]-haloacetate complexes are found as dominant peaks in their corresponding ESI-MS

spectrum. The confirmation of this interaction was performed isolating each CB[6]-haloacetate complex in the quadrupole followed by assigning collision energy in the ion trap. Remarkably CB[6]-haloacetate yielded haloacetate anions as the dominating product from CID. Ion-mobility (IM) mass spectrometry confirmed CB[6]-anion complex structures which interact exteriorly. The strong binding energy between haloacetate anion and CB[6] could be used for the design of self-organized supramolecular structures.

Another example of the use of negative ESI-MS was given by Rodriguez *et al.* [63], who reported the formation of host-guest complexes of CB[n] (n=7, 8) with adamantyl amine (AdA), 1,5-pentanediamine, styryl pyridine and styryl pyridine dimer cations. All styryl pyridines compounds form stable gas-phase 1:1 complexes doubly charged with CB[7] and CB[8] under negative ESI through formation of halide (Br, Cl, Br) adducts as $[CB[7]+AdA+H+3.Cl]^{2-}$. In this case, the MSⁿ spectra of CB[7] and CB[8] anion confirmed the ion-dipole interaction between halide and the outer surface hydrogens of host, similar to other cases mentioned above, with the advantage that negative ionization mode gave simpler spectra than positive ESI.

The combination of hydrophobic and dipole interactions in the complexation between CB[n] and amine dications has a potential use to complexation, recognition and delivery of guest of biological and medicinal interest. For instance, the biguanide functional group is found in drug molecules used to treat hypoglycemia and type 2 diabetes. MacGillivray and Macartney [64] studied the host-guest complexation of metformin, phenformin, chlorhexidine, and alexidine with CB[7] employing ESI-MS. The species observed in the ESI-MS spectra corresponded to 1:2 and 1:1 host-guest complexes for biguanidinium cation metformin, only 1:1 for biguanidinium cation

phenformin and 1:1, 2:1 and 3:1 for both dications (chlorhexidine and alexidine). These results were in agreement with previous studies of CB[7] [65].

In 2013 Kim *et al.* [66] studied how the water solvation shell contributes to the host-guest chemistry of CB[6] and CB[7] with a series of α , ω -alkyldiamines ($\text{NH}_2\text{-(CH}_2)_n\text{-NH}_2$, $n = 4, 6, 8, 10$ and 12). The ESI-MS spectra of the 1:1 stoichiometry of CB[6 or 7]- α , ω -alkyldiamines revealed the doubly charged complex ions as the principal species in their corresponding mass spectra. The relative abundances of CB[n] complex ions from ESI-MS spectra were in good agreement compared to their K_a values obtained by ITC in aqueous solution. However, this values were not identical. As has been commented before, ESI-MS has the ability to transfer intact complex ions into gas-phase; therefore, the observed minor changes were caused by the absence of the water solvent. It is well known that electrostatic interactions are consolidated with solvent evaporation as the competitive effect of the solvent is eliminated[67]. Thus, comparisons of the intrinsic gas-phase stability of the studied complexes with the information collected in solution is mandatory. For this reason, CID experiment are usually carried out. In this case, low-energy CID of each complex was used to examine the stability of CB[6 or 7]-alkyldiammonium. Each spectrum obtained from the QTOF revealed that the major dissociation products were the singly protonated diammonium and its ammonia-loss fragment. Furthermore, this work demonstrated that the binding energy of the CB[n]-alkyldiammonium ($\text{NH}_2\text{-(CH}_2)_n\text{-NH}_2$) are different from gas and solution phases. In absence of water, ion-dipole interactions determine the stabilities of the bindings because both ammonium groups of the guest interact with both carbonyl groups of the CB[n]. Nevertheless, in the solution phase, the surrounding water molecules formed hydrogen bonds with guest molecules affecting the complex ion-dipole interactions but maintaining the stability of the complex.

360

361 Continuing with the study of the role of direct interactions with water for high-affinity binding of
362 CB[n], in a second work Kim and co-workers [68] studied the hydration of CB[6] in gas phase using
363 ESI traveling wave ion mobility mass spectrometry (ESI-TWIM-MS). ESI confirmed a proton-bond
364 water dimer complex with CB[6], in which hydrogen bonds are formed with the carbonyl groups of
365 the CB[6]. Comparison of the results obtained by low-energy CID and TWIM-MS indicates that the
366 water molecules are placed inside the CB[6] portal. They also stated that the hydration pattern is
367 dependent on the CB[6] portal size. When a guest molecule is bound to a CB[6] portal, hydration
368 of the portal is not detected, with the exception of small alkali cations, such as Li^+ and Na^+ .

369

370 However, many research studies are still carried out in gas phase to avoid solvent influence.
371 Recently Dearden *et al.* [69] investigated the complexes of a large group of CB[n] ($n = 5, 6, 7, 8$) and
372 α, ω -alkyldiammonium $\text{H}_3\text{N}^+(\text{CH}_2)_x\text{NH}_3^+$ ($x = 2-10$), using SORI-CID. The dissociation of 1:1 singly
373 charged and 2:1 doubly charged complexes of CB[6], CB[7] and CB[8] needed more energy than
374 their homologous complex of CB[5]. This suggests an external bonding for CB[5] while larger CB[n]
375 ($n > 5$) form pseudorotaxanes structures. As the alkyldiamine chain length increases, changes in the
376 relative dissociation energies occurred suggesting that the complex stability depends on chain
377 length. In addition, the optimal α, ω -alkyldiamine chain length for large CB[n] was found to be $x =$
378 4-5 for CB[7], and $x = 5-6$ for CB[8], where x is the number of carbon atoms in the n -alkyl chain.

379

380 Host-guest chemistry combined with MS can also be used for solving analytical problems such as
381 isomers analysis of carbohydrates. Lee and Kim [70] studied the complexes formed between CB[7]
382 and monosaccharide derivatives as hexosamines, N-acetylhexosamine, deoxyhexoses and uronic
383 acids using MSⁿ. Thanks to the suitable cavity size of CB[7], monosaccharide derivatives were

encapsulated inside CB[7] and distinct fragmentation patterns were observed by ion mobility. Thereby, this methodology was able to discern between the different isomers. Essentially, in IM-MS analytes must be able to be ionized as in the other MS techniques, but the main advantage of IM is that 3D structure measurements are possible in dynamic motion[40]. Nonetheless, IM-MS is not recommended when the sample is very valuable or unique, since is a destructive technique. In these cases, is better to choose NMR spectroscopy, X-ray crystallography or circular dichroism for determining the structural information of the complex.

2.2. Calixarenes and analogues

A calixarene is a macrocyclic compound obtained by the condensation of formaldehyde and p-alkylphenols under alkaline conditions. Besides, other phenols with two or three hydroxyl groups and diverse aldehydes have been used for the synthesis of some analogs, known as resorcinarenes and pyrogallolarenes [71,72]. Calixarenes and their analogs form diverse oligomers that differ in the number of phenolic units that range from 4 to 14 (see Figure 3). The most studied oligomers consist of 4, 6 or 8 phenol units. These phenolic units are linked in 2- and 6- or 4- and 6- position via methylene bridges for calixarenes and analogs respectively. These macrocycles are bowl-shaped tridimensional structures with interesting receptor properties. Calixarenes and analogs are versatile bases as building blocks for large synthetic supramolecular assemblies and inclusion complexes [73].

Calixarenes offer several possibilities for non-covalent interactions. The first possibility is due to their bowl-shaped which provides a π -electron rich cavity[74]. The binding interactions that take place in this cavity could be, π -system type with inorganic and organic cations or polar- π and CH- π with neutral molecules. Hydrogen bond is the second possibility; these oligomers have a polar zone with four, eight or twelve hydroxyl groups on the upper rim of calixarenes, resorcinarenes

and pyrogallolarenes respectively. These hydroxyl groups play a key role in the formation of many supramolecular assemblies, such as 1:1 open inclusion complex, dimeric and hexameric capsules, as well as tubular assemblies. Another possibility is placed on the lower rim; aliphatic chains of the macrocycle can interact with organic molecules through hydrophobic effects. The remarkable structural properties of calixarenes and their possibilities of functionalization have permitted the development of diverse applications as chemosensors, drug delivery, analytic separations, biomedical and catalysis applications. Structurally, calixarenes may exist in a number of different conformations known as cone, partial cone, 1,3 alternate and 1,2 alternate[75]. Several researches in the area of supramolecular chemistry, analytical chemistry and material science have studied the encapsulation phenomenon, host-guest systems, aggregation phenomenon and molecular recognition in solution and solid state of these macrocycles[76,77] using many analytical tools including mass spectrometry. This technique is not only used to characterize each compound and its possible molecular interactions but also, help to understand the interactions due to different conformations.

2.2.1. Calix[n]arenes

The studies of non-covalent interactions of calix[n]arenes were focused in molecular recognition of ammonium cations [78], metal cations and some organic cations through the strong interaction cation- π inside the cavity [79]. More recently studies have been focused on the calix[n]arenes functionalization with different groups trying to find selectivity for some cations [80]. From the beginning mass spectrometry has been used for the evaluation of some aspects as the mass determination of the complex, stoichiometry evaluation and stability constants estimations. For instance, Felinto *et al.* [81] studied complexes of *p*-*tert*-butylcalix[6]arene with ammonium

hydroxide and ammonium and sodium ions. The singly charged species that appear in the mass spectra correspond to $[xM[6]+NH_4^+]^+$ and $[xM[6]+NH_4OH+Na^+]^+$ where M[6] represents the calixarene molecule ($x = 1, 2, 3, 6$). The doubly hexamer corresponding to $[6M[6]+NH_4OH+NH_4^++Na^+]^{2+}$ was also observed. In conclusion, the ESI-MS/MS experiments used to determine the stoichiometry showed monomers, dimers, trimers and hexamers complexes suggesting that *p-tert-butylcalix[6]arene* complexes could be present in the liquid phase. Furthermore, by increasing the collision energy in CID experiments, relative stability information of each complex was given.

A different example of functionalized calixarene was given by Sharma *et al.* [82] They used mass spectrometry to study the ability to complex calix[4]arene with N-protected α -amino acids. Better complexation was observed when methylene bridges were formed between the calix[4]arene and the urea motif employing a mixture of N-Fmoc- α -amino acids. The mass spectra results showed a considerable change in Fmoc amino acid selectivity.

It is worth mentioning the work published by Galić *et al.* in 2017 [83] in the field of functionalized calixarenes. They used ESI-MS/MS to study the coordination properties of three esters and one amide derivatives of calix[4]arenes towards some lanthanide cations as La^{3+} , Eu^{3+} and Yb^{3+} . The stability studies carried out by applying different collision energies showed that amide derivative complex of 1:1 stoichiometry was most stable in gas phase than the ester derivative complexes. Therefore, the amide derivative could be a promising extraction agent for these lanthanide cations.

The increasing interest in finding specific complexes structures to selective capture some cation has arrived at waste water treatments. In this regard ESI-TOF-MS achieve a rapid identification of calix[4]arene-bis-crown-6(W-BisC₆) to trap Cs^+ from wastewater containing competitive alkali metal ions[84]. In the mass spectrum $[W-BisC_6 + Na]^+$ ions and $[W-BisC_6 + Cs]^+$ were observed

however, the stability of $[W-BisC_6 + Na]^+$ complex is much more lower than the one of $[W-BisC_6 + Cs]$.

With respect to conformation studies, Jänis *et al.* [85] reported the recognition of mono and dicarboxylic acid anions by a conformationally flexible tetraglucosyl derivate and other two calixarenes with *di* and *tetra* functionalized receptors blocked in the cone conformation. The 1:1 stoichiometry complexes between calixarenes and mono and dicarboxylic acids were observed in the negative ion mass spectra obtained by ESI-FTICR. The diglicosyl calixarene formed singly charged ions while tetraglucosyl calixarenes were observed as doubly charged complex ions, the latter more abundant than the corresponding singly charged ions. Jänis *et al.* concluded that the selectivity increased with the number of glucosyl substituents and the rigidity of the calixarenes.

The study of interfacial environment gives the opportunity to accelerate the development of novel functional materials and molecular sensing techniques due to the potential to drive unexpected events of supramolecular recognition. Martinez-Haya *et al.* [86] introduced a new mass spectrometry method searching for enhancement on Cs^+ selectivity when competitive binding of alkali cations take place at the air-water interface in comparison to bulk solution. For this purpose, the interfacial complex of 4-tert-butylcalix[6]arene-hexaacetic acid hexaethyl ester (or cesium ionophore II, henceforth CI) were transferred as Langmuir-Blodgett (LB) films for MALDI-MS analysis (LB-MALDI-MS). In each experiment, a pair of cations were presented (Li^+/K^+ , Li^+/Rb^+ , Na^+/K^+ , Na^+/Rb^+ , K^+/Rb^+ , K^+/Cs^+ , and Rb^+/Cs^+) and the LB-MALDI-MS spectra showed $[CI-M]^+$ ions of both cations binding competitively with the calixarene. In general terms, the heavier of the two cations interacts more favorably within interfacial calixarene. Nevertheless, when the experiments were performed under solvent-less conditions, the gas-phase affinities of the CI calixarenes follow

a decreasing trend with cation size, thus there was a pronounced preference of the calixarene host for Li^+ in the absence of solvent.

2.2.2. Resorcin[4]arenes and pyrogallol[4]arenes

These macrocycles have different possibilities of functionalization at the second position of the aromatic ring or on the hydroxyl groups providing many opportunities for non-covalent interactions in a huge variety of supramolecular structures. Accordingly, determination of complex stoichiometry and mass of supramolecular structures have been carried out by applying mass spectrometry. Atwood *et al.* [87] carried out studies focused on the assembly of metal-organic nano-capsules (MONC's) based on C-alkylpyrogallol[4]arenes (PgC_n where n corresponds to alkyl chain length from C_2 to C_{12}) with copper. These assemblies are soluble in a number of common organic solvents and allow for study using MALDI-TOF mass spectrometry. In this case, using dithranol as a matrix, the spectra of the assemblies demonstrated the formation of Cu-MONC's capsules with PgC_3 , PgC_6 , PgC_8 , PgC_{12} , and PgC_{13} . They also studied the formation of hetero- PgC_n Cu-MONC's between PgC_6 and PgC_{11} . In this experiment, MALDI-TOF analysis showed a series of maxima in a broad overlapping spectrum corresponding to each possible permutation of $\text{PgC}_6/\text{PgC}_{11}$. The signals on the spectra indicated that the self-assembly process was driven by a huge propensity for copper to bind the PgC_n .

Another usual application of ESI-FTICR was the study of the displacement process of several guests incorporated in a resorcin[4]arene host system [88]. The exchange rates were determined following the displacement of the guest molecule. Complexes of resorcin[4]arene host with ammonium and sec-butyl ammonium guests were isolated in an ICR-cell and then, the displacement reaction was performed with methylamine, ethylamine or sec-butylamine. In the case of NH_4^+ complexes the reaction rate decreased with increasing steric hindrance of the host

while the inverse exchange of the sec-butylammonium guest by methylamine and ethylamine was significantly slower.

Other interesting fact dealing with cone resorcinarene supramolecular structures is the well-known accommodation of guest cations inside their cavities, whereas for anion binding, more elaborate cavitand receptor are needed and more investigation about anion binding is required [89]. For this reason, Schalley *et al.* [90] used ESI-FTICR-MS to assess the anion-binding behavior of a group of resorcin[4]arenes in cone conformation substituted with polar groups on position 2 of the aromatic ring. Non-bridged resorcinarenes formed complex with cations. On the other hand, methylene-bridged cavitands prefer anions such as chloride, bromide, iodide, and nitrate. These results were extended to other anions as sulfate showing an abundant formation of 2:1 complexes. These results provided evidences for a cavitand-anion interaction mediated by C-H...anion hydrogen bond that involves the acetal protons pointing into the cavitand bowl.

As it was mentioned before, calixarenes and their analogs are used for molecular recognition. Selective recognition of saccharides is vitally important for medical diagnostics through perception of alterations caused by diseases, or for monitoring of industrial processes. In this regard Vainiotalo *et al.* [91] reported the effect of configuration of the sugar ring and the size of the sugar on resorcinarene-sugar complexation with negative polarization electrospray ionization quadrupole ion-trap (ESI-QIT) and ESI-FT-ICR mass spectrometry. They studied tetraethyl-resorcin[4]arene and tetraphenyl-resorcin[4]arene versus *mono*-, *di*-, and oligosaccharides. The results showed that the resorcinarenes formed complexes singly charged with every monosaccharide, $[M - H + \text{monosaccharide}]^-$. In addition, the CID experiments allowed comparison of the relative kinetic stabilities of the complexes. The most stable monosaccharides complexes were formed with hexoses as a result of a maximum of three hydrogen bonds between the host and the hexose. Cellobiose and triose complexes were even more stable than the binding of

527 monosaccharide due to a maximum of four hydrogen bonds formed between saccharide and
528 resorcinarene.

529 In a different study of molecular recognition carried out by ESI-FT-ICR, certain open chain
530 dicarboxylic acids ($\text{HOOC}(\text{CH}_2)_n\text{COOH}$ $n=0-4$,) and some isomeric dicarboxylic acids were found to
531 form complexes with tetrahexylammonium resorcin[4]arenes[92]. The interaction between
532 saturated dicarboxylic acids, henceforth DCA and resorcinarene was poor. However, $[\text{R} + \text{H} + \text{DCA}]^+$
533 and $[\text{R} + 2\text{H} + \text{DCA}]^{2+}$ species were observed in the mass spectra with abundance smaller than $[\text{R} +$
534 $\text{H}]^+$ and $[\text{R} + 2\text{H}]^{2+}$ respectively. The competitive complexation experiments showed that the
535 complex formation was clearly stereospecific. The complexation of *cis* acid was more favored than
536 *trans* acid. The stereospecificity was also observed for aromatic acids; *ortho* and *meta* isomers
537 were more favored than *para* isomer.

538 In the search for selectivity reactions, a study with adducts of a racemic mixture of ethanolamine
539 neurotransmitters and chiral amidoresorcin[4]arenes receptors was carried out by Zappia *et al.*
540 [93] using a FT-ICR cell. In this work, the selectivity reaction was checked by determining the rate
541 of the displacement with a pure enantiomer of 2-aminobutane (R o S) finding that the stability and
542 the reactivity of the adducts depend on: 1) the relative position and the orientation of the
543 hydroxyl and amino functionalities on the ethanolamine neurotransmitter side chain, 2) the
544 presence of the methyl substituent on its N center and 3) the specific amidocarbonyl center of
545 resorcinarenes proton-bonded to the amino group of ethanolamine.

546 A nice example of enantiorecognition processes was given by Rozhenko *et al.* [94] This time, nano-
547 ESI-FT-ICR-MS was the selected technique for studying the effect of cyclochirality of *rrcc*-2,8,14,20-
548 tetra-*n*-decyl-4,10,16,22-tetra-O-methylresorcin[4]arene (C) receptor and the intrinsic factors
549 governing enantiodiscrimination of chiral bidental and tridentate aromatic and aliphatic

biomolecules guest (G). The formation of diastereomeric proton-bond $[C\cdot H\cdot G]^+$ complexes and the subsequent measurement of the rate of the guest substitution by the attack of several achiral and chiral amines was carried out. The interaction of cyclochiral with bidentate and tridentate biomolecules showed that the heterochiral adducts react faster than their homochiral counterparts. Cyclochiral resorcin[4]arene and the aromatic aminodiol formed an enantioselective system suggesting that the two hydroxyl groups interact with the upper region and the protonated amino group interact with the π -system inside the cavity. These results were supported by CID of $[C_{M,R}^X\cdot C_{P,S}^Y\cdot H\cdot N_1]^+$ (M, P = axial chirality; X, Y = H, D or D, H; N = aminoalcohol) and diastereomeric $[C_{M,R}^X\cdot H\cdot N_1]^+$, $[C_{P,S}^Y\cdot H\cdot N_1]^+$ complexes in which the adducts of $[C_{M,R}^X\cdot C_{P,S}^Y\cdot H\cdot N_1]^+$ indicated that FT-ICR selectivity was possible due to the combination of thermodynamic and kinetic factors of complexes.

According to molecular recognition of ammonium, Märk *et al.* [95] studied the behavior of a triphosphonate cavitand (3iPO) in solution. Using ESI-TOF they elucidated the ability of triphosphonate cavitand to complex choline (Ch) and acetylcholine (Ach) cations. The titration experiments showed that acetylcholine ion have selective binding by phosphonatocavitand. In addition, Ch^+ to ACh^+ and Ach^+ to Ch^+ ion exchange constants were directly measured to be $7.1 \times 10^4 M^{-1}$ and $5.8 \times 10^3 M^{-1}$ respectively.

Studies of resorcinarenes specifically substituted are carried out in order to improve not only the interaction but also the selectivity. In this area of research, Rissanen *et al.* [96,97] have studied the influence of substituents on the upper rim of resorcinarenes and their influence on self-assembly and host-guest complexes with small guest and their behavior in gas phase. Complexes of N-alkyl ammonium resorcinarenes with N-alkyl acetamides and diacetamides were investigated using ESI-MS. N-alkyl amides formed 1:1 complexes $[acetamide\cdot M\cdot 4HCl + H]^+$ where a progressive loss of hydrogen was observed. Similarly the diamides formed 1:1 host-guest complexes and

furthermore higher assemblies such as $[M + M@(\text{diamide-4HCl} + 2H)]^{2+}$ resulting from the diacetamides aggregation was observed in some cases. The resorcinarenes substituted on the upper rim with halides as Cl, Br, and I formed complexes with ammonium salts, such as Me₂DABCO and tetramethyl ammonium salt. The determination of small differences in binding strength was possible by applying tandem mass spectrometry with an infrared multiphoton dissociation (IRMPD) laser[98]. For instance, the diquatery salt Me₂DABCO was encapsulated as a heterodimeric capsule $[Me_2DABCO@M-Br.M-Cl]^{2+}$ and its fragmentation resulted in the monomeric complexes $[Me_2DABCO@M-Cl]^{2+}$ and $[Me_2DABCO@M-Br]^{2+}$. In this case, the cation... π interactions are mainly responsible for the binding of the cation in monomeric assemblies. The resorcinarenes substituted on the upper rim with Cl, Br, and I halides also form capsular assemblies solely based in halogen bonding [99]. These capsular assemblies were observed through ESI as heterodimeric capsules (XB) with N-methylpyridinium guest (G) resulting in $[G@XB]^+$. No homodimeric complexes were also observed indicating that the heterodimeric capsule was indeed formed due to specific XB interactions of the different cavitand.

Finally, in one of the most latest works of Rissanen *et al.* [100], ESI-MS was used to corroborate the high-affinity binding of pyrophosphate (PPi) in water by N-alkyl ammonium resorcinarenes chloride receptors (NARCl). In all cases the 1:1 binding stoichiometry NARCl-PPi complex were observed in negative mode. Surprisingly, the receptor with the terminal propyl hydroxyl groups OH-C₃-NARCl at the upper rim gave an extremely high binding for PPi.

2.3. Crown ethers

The crown ethers are macroheterocyclic compounds composed of several repeating units of the type $(-Y-CH_2-CH_2-)_n$, where Y can be the hetero-atom as O, S or P, with macrocyclic polyethers ($-$

CH₂-CH₂-O-)_n, being the most common form. Since their discovery in 1967, the crown ethers have exhibited enormously versatile range of interactions with metal ions, ammonium cation and organic species such as protonated amines through a Lewis acid-base phenomenon (see figure 4). Crown ethers are frequently used as potential chiral selectors, dyes when contain chromophores or to improve metal selectivity and complex stability in analytical methods [101]. The flexibility of crown ethers allows them to accommodate to a wide variety of coordination requirements, providing the molecules with some attractive properties such as solubility in aqueous and lipophilic solvents, which makes them appropriated to applications as ionophores[102].

The behavior of crown ethers as ionophore of tetramethylammonium(-phosphonium) cations was studied by ESI-MS [103]. The inclusion complexes between the crown ethers 24C8, 18C6, DB30C10, DB24C8, DB18C6, DC18C6 with tetramethylammonium and tetramethylphosphonium bromides were observed. The full scan ESI mass spectra obtained for the methanolic solution showed signals corresponding to the complexes [24C8+(CH₃)₄N]⁺, [24C8+(CH₃)₄P]⁺, [DB24C8+(CH₃)₄N]⁺, and [DB24C8+(CH₃)₄P]⁺. Similar abundances of these complexes were obtained but 24C8 was more prone to complexing (CH₃)₄N⁺ than (CH₃)₄P⁺ because (CH₃)₄N⁺ can form hydrogen bonds easier than (CH₃)₄P⁺. Contrarily, DB24C8 was more prone to complexing (CH₃)₄P⁺ because cation- π interactions also exist due to the presence of aromatic benzene rings.

In this area of research, Frański *et al.* [104,105] studied the formation of organometallic complexes between fluorosubstituted N-nitrophenylaza-18C6 conjugates with metals influenced by the presence of a nitro group at the *ortho* or *para* position. In a first work, ESI-CID-MS/MS experiments demonstrated that the fluorosubstituted N-nitrophenylaza-18C6 with the nitro group at *ortho* position coordinated the alkaline cations by a crown ether ring and the nitro group in the scorpionate mode [104]. On the other hand, for complexes with metal as lead, the species [M-

621 $\text{H+Pb}]^+$ were observed for conjugates containing hydrogen atom at the *ortho* position to the crown
622 moiety[104,105]. In this case, the nitro group affects the formation of $[\text{M-H+Pb}]^+$, however, the
623 metallic cation was also complexed by crown ether conjugates aza-18C6 moiety as a scorpionate.
624 In these type of studies, solvent changes affected to the relative peak intensities obtained, not
625 only due to the polarity of the solvent, but also due to the type of interactions the solvent can
626 provide. For instance, the presence of acetonitrile as solvent, decreases the ratio of
627 $[\text{DB24C8}+(\text{CH}_3)_4\text{N}]^+ / [\text{DB24C8}+(\text{CH}_3)_4\text{P}]^+$, compared to methanol, because acetonitrile affects
628 directly the cation- π interactions.

629 The crown ethers have been frequently used as chemosensors of metals with good results. For
630 instance, Schröder *et al.* [106] studied the coordination properties of maleonitrile tetra- and
631 pentathiacrown ethers complexed with bivalent metals (Ni^{2+} , Cu^{2+} , Co^{2+} and Zn^{2+}) as ligands (L)
632 through ESI-MS. Although the most abundant species was $[(\text{L})\text{Cu}]^+$, other singly charged complexes
633 $[(\text{L})\text{CoCl}]^+$, $[(\text{L})\text{ZnCl}]^+$ and $[(\text{L})\text{NiCl}]^+$ were formed. In the case of copper, the complex formation was
634 accompanied by the reduction from Cu^{2+} to Cu^+ in solution. CID was used for the determination of
635 18-crown-6 complexes equilibrium constants. These constants showed good correlations with the
636 ones obtained by other techniques, as has been mention in other sections. They showed that the
637 magnitude of the constant increase when large thiacycrown ether were used and confirm the
638 selectivity of thiacycrown ethers to Cu^{2+} .

639 The crown ethers also have been successfully applied as chiral selectors in diverse analytical
640 techniques like NMR and chromatographic techniques for the enantiomeric distinction of primary
641 amines and amino acids. More direct approaches can be carried out using ESI-MS and ESI-MS/MS,
642 for instance, direct chiral recognition based on differences in relative abundance of
643 diastereoisomeric complexes[107], and measurements of kinetic constants for guest exchange
644 reactions[108]. A nice example is the study of the molecular mechanisms that drive through chiral

recognition between (-)-[18]crown-6-2,3,11,12-tetracarboxylic acid and phenylglycine (PG), threonine (Thr) and proline (Pro)[109]. The enantiomer-label guest method developed by Sawada [110,111], was follow in this case. Herein, a solution with an isotopically labeled and unlabeled pair of enantiomers is prepared in the presence of a deficit of crown ether. Thus, separately peaks from each enantiomer can be observed by $\Delta m/z$ units. The relative peak intensity ratio ($IRIS = I_R/I_S$) from the ESI-MS spectrum is used to estimate the ability of chiral recognition. ESI-TOF gave IRIS ratios of 0.61, 1.64 and 1.01 for PG, Thr and Pro respectively, showing that the L-PG and D-Thr interact stronger with the crown ether than the other enantiomers, while for D and L-Pro there is not chiral distinction. Ligand-displacement reactions with ammonia indicated that D-PG and L-Thr exchanges are faster than the ones of their enantiomers. In addition, the exchange was more efficient for Pro than PG and Thr, suggesting different noncovalent interaction for this proline complex compared with the other amino acids.

Cryptands (figure 4c) are three-dimensional bicycles crown ethers widely studied due to their relevant properties for host-guest molecular recognition. Zhou *et al.* [112] reported inclusion complexes based on the recognition of crown ether-based cryptands to tropylium cations which were characterized by ESI-MS. The spectra of singly charged species of cis-dibenzo-24-crown-8, bis(*m*-phenylene)-26-crown-8 and bis(*m*-phenylene)-32-crown-10 cryptands with tropylium hexafluorophosphate ($C_7H_7-PF_6$) indicated the formation of 1:1 inclusion complex in solution. The association constants of cryptands are 27-427 times higher than those of their simple analogue crown ether due to their structures and additional binding sites, making them wonderful hosts for encapsulating $C_7H_7-PF_6$.

The Lariat ethers are crown ethers with an exocyclic electron-donating sidearm which provides additional coordination sites (figure 4d). A study of these derived 15-crown-5 with sodium salts was performed using MALDI-MS by Bradikhin *et al.* [113] They investigated the interaction and the

669 effect of the exocyclic methoxyphenoxymethyl substituent placed in *ortho* (ether 1), *para* (ether 2)
670 and the unsubstituted crown (ether 3) in gas phase. Ethers 2 and 3 generated cluster ions of the
671 composition $[2M + 2Na + An]^+$ (where M is the crown ether molecule and An is a monobasic anion)
672 whereas ether 1 formed this type of clusters in residual amounts. The lariat effect caused by the
673 effective shielding of the cation by the host molecule was intense for ether 1, weaker for ether 2
674 and absent for ether 3. In the MALDI experiments, crown ethers and salts were placed separately
675 by the matrix layer. Thus, if the spectra showed clusters, it would be clear that the clusterization
676 takes place in the gas phase, what is the most interesting from an analytical point of view. Another
677 study about lariat ethers, specially about PNP-lariat ethers was carried out by Kwiecień *et al.* [114]
678 In a PNP-lariat ether, the -P-N=P- subunit of the sidearm belongs to the macrocyclic skeleton. In
679 that work, the complexation behavior of PNP-lariat ethers towards different types of ions (Ag^+ ,
680 Ca^{2+} , Cd^{2+} , Cu^{2+} , and Pb^{2+}) was examined using ESI-MS. The PNP-lariat ether ligand formed 1:1
681 stoichiometry complex with all of the investigated metal ions. Furthermore the formation of
682 species as $[AgL_2]^+$, $[CdL_2]^{2+}$, $[Cu^{(1+)}L_2]^+$ indicated a sandwich-like 1:2 stoichiometry.

683 Regarding sandwich complexes of crown ethers with metal cations, diverse studies have been
684 carried out through ESI-MS/MS in the last ten years. In one of the most recent works, gas-phase
685 stabilities of the sandwich complexes formed by 12-crown-4, 15-crown-5 with different metal
686 cations were compared with the stability of sandwich complexes formed by their analogues
687 benzo-crowns[115], where additional cation- π interactions could exist. ESI-MS indicates the
688 formation of sandwich complexes between 12C4 and B12C4 with all the monovalent and divalent
689 cations studied. However, only for thallium, the CID-MS/MS experiments showed an increase in
690 the gas-phase stability of the $[(B12C4)_2]^+$ versus $[(12C4)_2 + Tl]^+$, mainly due to cation- π interactions
691 that accompany the ion-dipole interaction between metal cation and oxygen from the crown. It is
692 hard to understand why only thallium and no other cations such as Ag^+ or Rb^+ , the latter with

693 identical ionic radii than Tl^+ , seems to be favored by cation- π interactions. Moreover, the opposite
694 results for Tl^+ -crown and Rb^+ -crown ether were published by Frańska and Michalack[116]. In
695 addition, only the sandwich complexes of B15C5 with Sr^{2+} and Pb^{2+} were more stable in gas phase
696 than those of 15C5 due to cation- π interactions. These works together with another published
697 previously by Frański [117], where the formation of sandwich complex of benzo-crown ethers with
698 alkali cations is supposed to be enhanced by cation- π interactions leave many unanswered
699 questions.

700 Ion-dipole and dipole-dipole interactions are the most important non-covalent interactions for
701 crown ethers, nevertheless weak interactions are possible such as hydrophobic effects with alkyl
702 side chains of organic cations. Qi and Volmer [118] used tandem mass spectrometry technique
703 with electron-capture dissociation (ECD) to evaluate the binding interactions of host-guest
704 complexes between 18C6 and different peptides. Contrarily than in CID, in ECD the fragmentation
705 is induced by low energy electron addition (<0.2 eV) generating radical cations for a multiply
706 protonated complex. Due to the requirements of trap electrons, ECD is limited to FTIRC. Hence,
707 the cost implications are its main disadvantage, followed by the speed limitation. Nevertheless,
708 the charged reduced species formed provokes a soft and random cleavage, what is suitable for the
709 study of facile cleavage sites. In the example, ECD was able to locate 18C6 adducts while CID
710 dissociate them from the peptides. This work suggests that 18C6 could interact slightly not only
711 with Lys but also with other amino acids with different side-chain properties.

712 Complexes formed by 18C6 and 1,*n*-diaminoalkanes were used for the comparison of ECD with
713 electron capture-induced dissociation (ECID)[119]. In ECID, multiple charge cations are accelerated
714 and collide with neutral gas, Na or Cs. Then, an electron is transferred from the neutral specie to
715 the cation, producing radical cations and fragmentation after the electron capture. The loss of an
716 ammonium hydrogen was observed with both ECD and ECID. That was the main fragment in ECD

experiments while for ECID, fragments from C-C bond cleavages from the alkyl chain were observed. These differences were mainly due to the different intrinsic nature of the dissociation processes.

Non-covalent interactions between 18C6 with 20 protonated amino acids were studied by Ding *et al.* [120] using ESI-MS. Results showed non-covalent complexes in a stoichiometry ratio of 1:1 for 18C6 with L-amino and D-amino acids. In addition, through competitive ESI-CID-MS experiments, their binding constant ($\log K_a$) were determined. The lowest $\log K_a$ were for the complexes of 18C6 with D-pro and L-pro and; the largest $\log K_a$ values were for the complexes of 18C6 with the basic amino acids and amino acids with alkyl side chain.

A clear example of the study of the existence of functionalized crown ether aggregates in gas phase was published by Drewello *et al.* [121,122]. Their research was focus on the aggregation behavior of the tetra-[18]crown-6 ether functionalized zinc-pthalocyanine (ZnPcTetCr) in absence of coordinating metal cation using ESI-MS. In the first paper [121], it was necessary the use of aprotic solvents for suppressing the protonation and sodiation since the molecule contains four crown ether units which are known for their high affinity for alkali metal ions[123]. The fragmentation behavior using classical CID and ion-source collision-induced dissociation (ISCID) reveal that the clusters consisted of a combination of neutral ZnPcTetCr and radical cation ZnPcTetCr^{•+}. This fact suggests that electrostatic interactions are responsible for the clusters formation between neutral molecules and their radical cations without the need of an alkali ion inside the crown ether. In this work, an additional acceleration of the droplets during ISCID was needed to ensure that clusters with high mass-to-charge ratio were completely desolvated.

Given the crown ethers ability to form stable complexes with cations, they could be used for multiple applications, such as detection of improvised explosives. Yost *et al.* [124] developed a

method for detecting ammonium nitrate and urea nitrate with ion mobility MS. The method is based on the addition of 18C6 as modifier, what leads to a change in mobility and enhanced the detection of both nitrates. Positive mode ESI-MS showed the complex species $[18C6+NH_4]^+$ (m/z 282) and $[18C6+uronium]^+$ (m/z 325) in a cleaner way in comparison to their native clusters. In conclusion, the addition of 18C6 as modifier provided an economic alternative screening method for the sensitive and selective detection of ammonium nitrate and urea nitrate.

2.4. Catenanes and rotaxanes

Catenanes and rotaxanes are mechanically-interlocked molecular architectures. Catenanes consist of two or more interlocked macrocycles that cannot be separated unless the covalent bonds of the macrocycles are broken, while rotaxanes consist of at least a dumbbell-like axle threaded by macrocycles terminated by bulky stoppers. These architectures can be built using numerous non-covalent interactions such as hydrogen bonds, charge-transfer, hydrophobic and electrostatic interactions, π - π stacking, and metal-ligand coordination[125,126].

In this section we will justify why mass spectrometry is a very useful tool to establish the exact composition and the self-assembly synthesized catenanes and rotaxanes. For example, Tezuka *et al.* [127] used MALDI-TOF to demonstrate the formation of a polymer [2]catenane comprised of two different cyclic poly(tetrahydrofuran) components isophthaloylbenzylic amide group(I) and biphenyldicarboxylate group(II) respectively. Only the synthesis carried out through electrostatic self-assembly and covalent fixation led to a series of peak molar masses assignable to the adducts of the polymer [2]catenane I/II, and a series of minor signals peaks assignable to [2]catenane II/II. Furthermore, the molecular weight obtained was higher than either of the cyclic

762 poly(tetrahydrofuran)s, I and II, while it was smaller than twice of the cyclic polymer component
763 confirming the assembly by non-covalent interaction of polymer precursors.

764 Another example was given by Nitschke *et al.* [128], who reported the preparation of a new cyclic
765 [3]catenane, a chiral assembly of three mutually interpenetrating rings constructed by means an
766 intricate metal-templated self-assembly procedure. In this work, the solution measurements taken
767 by ESI-MS/MS and NMR revealed the self-assembly structure which was confirmed by X-ray in the
768 solid state. The three major components observed in MS/MS were the expected [3]catenane,
769 [2]catenane and the macrocycle formed from hydrolytic dethreading of the catenane during
770 reduction. The precise intertwining of the molecular subunits of cyclic [3]catenanes shows
771 topological chirality, in contrast to the achiral Borromean links[129].

772 Elucidation of metallomacrocycles and [2]catenanes based on leucine substituted naphthalene
773 diimide (LeuNDI) ligand was studied by Turner *et al.* [130]. In this work, theta capillary
774 nanoelectrospray ionization mass spectrometry was used to confirm the solution persistence of
775 $[\text{Cd}_2(\text{LeuNDI})_2-(1,10\text{-phen})_2(\text{OH}_2)_2]$ and $\{[\text{Cd}_2(\text{LeuNDI})_2(2,2'\text{-bipy})_2(\text{OH}_2)_2]\}_2$. This technique is
776 based on two nanoelectrosprays place in parallel for rapid mixing of two different solutions
777 immediately prior to the detection[131]. The short droplet lifetime (sub-microseconds) make this
778 technique suitable for monitoring different fast reactions using MS[132].

779 Mass spectrometry can be used to identify self-assembly intermediates, wrongly assembled
780 structures, and thermodynamic products to study self-sorting systems of rotaxanes. For instance,
781 Schalley and co-workers [133,134] provided information for understanding the kinetics formation
782 of self-sorting pseudorotaxanes and more complex assemblies by using ESI-MS, when a mixed-
783 flow microreactor is coupled to the ion source. For the first time self-sorting of rotaxanes between
784 ammonium salts as axles and crown ethers as wheels were detected. These self-sorting systems

include specific sequence of [3]pseudorotaxanes with a ditopic axle and two monomeric crowns ether, as well as [3]pseudorotaxanes consisting of a crown ether heterodimeric and two monotopic axles. These mass spectrometry experiments provided interesting information on the major assembly pathway, which leads to the thermodynamic product. Fragmentation pathways of different charge states of pseudorotaxanes can be described using a combination of ESI-TOF-HRMS and ESI-FTICR-MS. The effect of Coulomb interaction involved on the fragmentation pattern was shown, either attractive ones between the positive charged axles and counterions that have not been stripped away during the ESI process or the repulsive ones in multiply charged ions.

It is well-known that cyclodextrin-based polyrotaxanes are difficult to analyze by mass spectrometry. For this reason, mass spectrometry is commonly used as tool to complement other techniques such as NMR. However, Jarroux *et al.* [135] reported a successful analysis of a polydisperse sulphated α -cyclodextrin polyrotaxane by nano-ESI-LTQ hyphenated with orbitrap high-resolution analyzer. The results showed the power of high resolution to overcome the polydispersity restriction, allowing unambiguous assignment of peaks to structures as poly[2] to poly[6]rotaxanes. Moreover, the structures analyzed were found to be constituted of 16 to 35 ethylene oxide units, 1 to 5 α -cyclodextrins threaded and with 0 to 5 sulfo groups grafted. This mass spectrometry method demonstrated the potential to help analysis of polyrotaxanes even those not including cyclodextrins. In another work with the same class of polyrotaxanes MALDI-TOF was used to detect high molecular weight structures of cyclodextrin-based rotaxanes[136]. They evaluated eight matrices finding that 1,1,3,3-tetramethylguanidinium salts of 2-(4-hydroxyphenylazo) benzoic acid and 2',4',6'-trihydroxiacetophenona are the most efficient. The aforementioned matrices allow the detection of masses of 24804 and 26447 g. mol⁻¹ corresponding to poly[17]- and poly[18]rotaxanes respectively.

Identification of isobaric components and separation of oligomers from complex systems, can be reached using IM-MS. Scrivens *et al.* [137] used both MALDI-TWIM-MS and ESI, to study crown ethers threaded onto a polymeric backbone of dialkenyl ammonium salt that have been end-capped by bulky stopper groups (see Figure 5). Rotaxanes species with several repeat unit:macrocycle ratios of 1:1, 2:2, 3:3, and 4:4 were observed, suggesting the existence of each species in one stable conformation. In addition, the comparison of stimulated collision cross sections (CCS) for multiple polyrotaxanes species and relevant fragments ions revealed additional structural information. In general, the use of TWIM-MS can be suitable as separation and structural tool for ions generated by CID (as in the case) or ECD. Nonetheless, the estimation of CCS needs for a simple calibration using standards with known cross sections, what increase the analysis time. To overcome this situation drift- tube ion mobility – mass spectrometry (DTIM-MS) is able to measure molecular diameter values without the need of a calibration or comparison to structural methods such as DOSY NMR or single-crystal X-ray diffraction [138].

The last example of rotaxanes is the work published by Pischel *et al.* [139] where ESI-MS was used to monitor the supramolecular assemblies of hetero [5]pseudorotaxanes with self-assembly of a heteroditopic axle and different cucurbituril(CB) homologs as wheels(CB6, CB7, and CB8). Gas-phase studies confirmed the presence of different rotaxanes with CB8:[3]pseudorotaxane with the fully protonated axle, [2]pseudorotaxane with varying charge status and [5]pseudorotaxane composed of two molecules of the heteroditopic axle and three molecules of CB8. Other less abundant species observed were [2] and [3]pseudorotaxanes with CB6 which was consistent with solution studies by other techniques.

2.5. Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six or more D-glucopyranoside units linked in α -(1,4) [140]. Typically, native cyclodextrins contain six, seven or eight glucose units and are denoted as α , β , and γ -cyclodextrins respectively [141]. Due to the conformation of D-glucopyranoside units, cyclodextrins have a truncated cone tri-dimensional structure instead of a perfect cylinder shape. Its central cavity is hydrophobic, allowing low-polarity molecules to be encapsulate partially or entirely by host-guest interaction; meanwhile the external part is hydrophilic because of the presence of 21 hydroxyl groups making CDs soluble in water[142]. Therefore, CDs could be widely applied in the field of molecular recognition of drug delivery. Furthermore, CDs are commonly used as chiral selectors in methodologies to separate and detect enantiomers in drug development[143]. The physicochemical properties of the guest with which CDs complex are modified favorably and in a greater extent than using other supramolecular hosts. Some properties that can be improved by CD complexation are enhancement of solubility, control volatility, oxidation prevention or off-odors masking what makes their use essential in textile[144], cosmetics[145], environment protection [146,147], food[148] and biomedical industries[149,150]. The driving forces for complex formation include solvent effects, hydrophobic interactions, hydrogen bonds, Vander Waals interactions, and dipole-dipole interactions.

The inclusion complexes of several ligands binding to CDs and their dissociation constants can be studied by ESI-MS. Going further, in the last years efforts have been made in development of methodologies that save time, consume less amount of hosts and guests compounds and are more automated, similarly as it was discussed before in section 2.1. Schug *et al.* [151] presented a dynamic titration technique alternative to the classical titration methods in which dissociation constants must be calculated one by one. This new methodology goes further, providing the chromatographic separation of guests on a HPLC column with the simultaneous determination K_d by ESI-MS when using multiple host deliberately. The K_d values obtained for complexes of β and γ -

855 CDs with the non-steroidal anti-inflammatory drugs ibuprofen, naproxen, and flurbiprofen were in
856 agreement with the ones obtained by traditional static titration; thus, the ESI-MS method was
857 validated.

858 As already mentioned, CID is a mass spectrometry fragmentation technique for both the
859 determination of ion structures and the analytical identification of compounds with high
860 specificity. This technique can be applied for the study of cyclodextrins. For instance, Fang *et al.*
861 [152] studied the gas-phase fragmentation of host-guest complexes formed between β -CD and
862 small guest (G) 1-acetamidoadamantane (AA), 4-methylacetanilide (MA), 4-isopropylacetanilide
863 (IA), N,N-dimethylaminomethylferrocene (DMAMF), and 1,4-diazabicyclo[2,2,2]octane (DABCO)
864 by CID. The complex ions generated by ESI were isolated as singly charged species $[AA+\beta\text{-CD}+H]^+$,
865 $[IA+\beta\text{-CD}+H]^+$, $[DABCO+\beta\text{-CD}+H]^+$, $[DMAMF+\beta\text{-CD}+H]^+$. CID experiments for each complex ion
866 showed protonated guest molecules with a distribution depending on the affinity of the guest
867 molecule. It is worth mentioning that interactions always occur between intact β -CD and the
868 different guest, since $[\beta\text{-CD fragment}+G]^+$ were not observed.

869 Another example is the study of non-covalent interaction between imidazolium-based ionic liquids
870 (IL) with α - and β -cyclodextrins carried out by Coutinho *et al.* [153]. In this work the results
871 showed that anions (Cl^- , Br^- , $[BF_4]^-$, $[PF_6]^-$, $[CF_3SO_3]^-$, $[CH_3SO_3]^-$, $[N(CN)_2]^-$, HSO_3^- , $[(CF_3SO_2)_2N]^-$) and
872 imidazolium ($[C_nC_{1im}]^+$) cations formed 1:1 complexes with α - and β -CDs. CID experiments for $[CD-$
873 $ILanion]^-$ adducts revealed that stronger interactions with CDs were obtained when the basicity of
874 the anion increases. Similar results have been observed in other cases where the halides form 1:1
875 complex with native cyclodextrins[154]. On the other hand CID experiments with $[CD-ILcation]^+$
876 showed that the longer the alkyl chain in the imidazolium cation, the weaker the interaction with
877 CDs. The dissociation energy values (0.39-0.59 eV) for $[CD+cation]^+$ adducts were lower than those
878 of the anions (0.41-1.94 eV) but neither of them generated inclusion complexes in the gas phase.

879 Both, anions and cations, prefer to form interactions with the rim of the cyclodextrin, just the
880 opposite of the behavior in solution [155].

881 The non-covalent interactions, binding sites and the binding stretch of the complex between
882 native or derivatized cyclodextrins with alkali metal cations have been also studied employing CID
883 and ESI-MS. For instance, Ding *et al.* [156,157] studied the non-covalent interactions between
884 alkali cations(Li^+ , Na^+ , K^+ , Rb^+ , Cs^+) and α -CD, β -CD, γ -CD (see table 3), heptakis(2,6-Di-O-methyl)- β -
885 CD(DM- β -CD), heptakis(2,3,6-tri-O-methyl)- β -CD (TM- β -CD). The main results of this research
886 were the following: 1) Non-covalent complexes of 1:1 stoichiometry are formed between CDs and
887 all the studied cations; 2) The dissociation constant for the complex of lithium with native CDs was
888 smaller than derivative CDs due to its steric effect of the substituent $-\text{CH}_3$; 3) The stability of
889 $[\text{CD}+\text{Cs}]^+$ complexes follows the trend α -, β -, γ -CD respectively; 4) The binding constants of Li^+ , Na^+ ,
890 K^+ and Rb^+ complexes of α -, β -, or γ -CD decrease with the increasing size of CDs and with the
891 increasing radii of alkali cations.

892 Following the same line of research, Cézard *et al.* [158] investigated the relative affinity of
893 common alkali cations with native and methylated β -CD using ESI-MS/MS. CID and higher energy
894 collision dissociation (HCD) were applied for the calculation of the dissociation constants. In terms
895 of dissociation, complementary to CID, HCD is carried out with higher activation energies, which
896 changes the mass spectra of the complexes to a more fragmented pattern. CID activations
897 occurred in the LTQ and detection in the Orbitrap, while HCD activation occurred outside the ion
898 trap, in a separate octopole collision cell, and then fragment ions were transferred to the C-trap
899 and detected in the Orbitrap. CID can be the more versatile because can be performed with high
900 or low resolution and a wide range of collision energies can be applied. Nevertheless, it shows low
901 mass cutoff limitations. On the other hand, HCD has not low-mass cutoff limitations, however its
902 higher activation energy could lead to secondary fragmentation. As can be seen, each method

903 shows advantages and disadvantages, but unfortunately there is not a perfect single solution. That
904 is why these dissociation methods are considered and used as complementary. In the example,
905 both energy regimes led to the same conclusions. The β -CD selectivity order when only one or a
906 mixture of two cations are present was $\text{Na}^+ > \text{Li}^+ > \text{K}^+$ for mono and doubly charged species. The
907 same trend was found when the three cations are present for mono charge species and for doubly
908 charged Li^+ and Na^+ turn their order.

909 Inclusion complexes between CDs and hydrophobic drugs have been used to increase the drug
910 bioavailability, making CDs excellent pharmaceutical excipients[159]. Studies of non-covalent
911 interactions between α -, β -CDs with amino acids and peptides have been carried out using tandem
912 mass spectrometry to determinate the force of interaction and the exact location of the binding
913 site [160–162]. ESI-MS spectra of complexes of α -CD with L and D-amino acids showed 1:1
914 complex stoichiometry. The comparison of the binding constants ($\log K_a$) of aliphatic amino acids
915 suggests that the long aliphatic chain of lysine obstruct its approach to α -CD as its low value
916 indicates (see table 3). Moreover, the study of non-covalent interaction of β -CD with peptides was
917 carried out using ECD and FT-ICR tandem mass spectrometry. This technique leads to new insights
918 into the interactions involved in CD/peptides complexes, and is able to locate CD binding sites
919 unequivocally (table 4). The peptides angiotensin I, Angiotensin II, neurotensin (fragment 1-8),
920 substance P and Bombesin all formed stable inclusion complexes with β -CD regardless of whether
921 an amino acid includes an aromatic moiety.

922 Multimode sources have demonstrated to extend the range of compounds that can be analyzed
923 by mass spectrometry. For instance, the combination of ESI with APPI, called ESPI (electrospray
924 photoionization), is capable of monitoring both polar (with ESI) and non-polar (with PI) compounds
925 simultaneously[163,164]. A nice example of the use of ESPI for investigating mixed-polarity
926 binding systems was reported by Syage *et al.*[165]. They will be able to measure low-polarity

pharmaceuticals, polar cyclodextrins and polar complexes at the same time, which is very helpful for the pharmaceutical industry. Specifically, the pharmaceutical-substrate non-covalent interaction between naproxen, flurbiprofen, nimesulide, progesterone, and cholesterol with β -cyclodextrin were studied. They demonstrated that the binding between CD and the compounds tested is significantly affected by the degree of molecular substitution of cyclodextrin. In addition, the gas-phase stability constant calculations showed that the cholesterol has the lowest bound-complex stability while the progesterone has the greatest stability. Finally, the comparison between multimode ESPI with single ESI and multimode ESI/chemical ionization (ESCI) highlighted considerable advantages over both sources, including an improvement in the detection of complexes and better efficiency at lower solvent flow rates. However, multimode sources also could show disadvantages. The interaction of the initially formed ions when both sources (ESI and APPI) work at the same time could lead to a suppression of the ESI multiply-charged signal.

MS has been proved as an effective technique for the investigation of chiral effects in the gas phase. Chiral recognition based on differences in kinetic constants of gas phase exchange reactions is based on a proton transfer process mediated by a host when a reaction with gaseous amine is carried out[166]. Many pharmacologically important products contain an amine in their skeletons what is required for this kind of measurements. For example, cyclodextrins are used for determining enantiomeric excess of different drugs such as 3,4-dihydroxyphenylalanine (DOPA), penicillamine, ephedrine and amphetamine only using mass spectrometry[167].

Due to the variety of possible applications in aqueous solution of inclusion complexes of cyclodextrins, the formation of these complexes in liquid phase has been widely studied. ESI and MALDI mass spectrometry correlated well with other techniques when characterizing CDs complexes, with the advantage of working directly in liquid-phase. Some examples of the characterization in aqueous solution are the complexes formed between native α -, β -, and γ -CDs

with folic acid[168], between 2-[(R-Phenyl)amine]-1,4-naphthalenediones(PAN) with β - and γ -CDs 1:1 and 1:2 stoichiometry [169], between corannulene with γ -CD[170], between ethyl 3-(7-hydroxy-2-oxo-2H-chromen-3-yl)-3-oxopropanoate with coumarin CD[171] and between morigin and α -CD can be found elsewhere[172].

In some cases[173], ESI form nonspecific artifacts which can be considered false positives of cyclodextrins inclusion complexes. In this wise, new tools for supramolecular mass spectrometry analysis, such as desorption electrospray ionization (DESI), have been compared to ESI, the accepted reference ionization technique for this type of work. This mass spectrometry technique is similar to an ESI but it takes place at ambient conditions. DESI also has the capability of integrating reagents into the spray solution for developing ionic reactions, what is called reactive DESI. A reactive DESI for a fast screening of guests for supramolecular inclusion complexes was carried out by Powell *et al.*[174]. Reactive DESI and ESI results were compared with the solution-phase NMR data. Results revealed that reactive DESI yielded data consistent with solution-phase behavior while ESI data suffer from artefact formation; another example of what was mentioned above. This work evidences one of the major advantages of DESI versus ESI, its ambient nature, which allows the study of the spray solution in a very easy way.

3. Conclusions

The use of soft ionization methods as MALDI and ESI together with mass spectrometry has allowed the characterization of the shape and size of non-covalent complexes, which is crucial for the design of supramolecular systems with different structural aspects such as connectivities or building blocks, interactions and assemblies. The advantages of MALDI-MS or ESI-MS for analysing host-guest complexes are mostly the following: ionization under relatively mild conditions; direct

974 ionization of samples from the solution phase under native conditions that enables preservation of
975 non-covalent interactions; and rapid response capability which enables monitoring of dynamics.

976 Combining MS with other methods, such as NMR, X-ray or even computational modelling gives the
977 opportunity of unravelling complexes architectures and fine-tune synthesis processes of new
978 molecules by manipulating the size and shape of the assembly in supramolecular systems.
979 Furthermore, exclusive MS approaches in this field are increasingly cross-validated with these
980 conventional methods, demonstrating that it is no longer just a complementary technique.

981 Synthetic chemists have provided a wide array of supramolecular assemblies able to encapsulate
982 guest molecules. Mass spectrometry analysis of these supramolecular host-guest complexes
983 opens a new window of opportunities for the enhanced or novel applications as transport of
984 medicaments, chemosensors, nanoreactors or removal of harmful substances from aqueous
985 environments, among others, due to the good correlation found between the complex formed in
986 solution phase and the ionization produced in gas-phase.

987 The progress made in developing new mass spectrometry methods based on techniques such as
988 Collision Induced Dissociation (CID) and Ion Mobility (IM) has allowed the determination of
989 thermodynamic aspects such as binding constants, solvation energies or host-guest interaction
990 energies using CID, meanwhile IM has emerged as a potential tool for isomer recognition. For the
991 moment, IM-MS is not only used for separate ions in complex mixtures, such as isomers, but also
992 is a technique that completes the structural chemistry investigations of a wide range of
993 supramolecular macrocycles. However, we believe that the continuous improvements in IM-MS
994 will make this technique become essential in a near future.

995 Future trends will focus on the development of mass spectrometry methods that give the
996 possibility to effectively discern enantiomers and to determine larger molecular assemblies. In

other words, host-guest systems that will be able to be discerned thanks to slight interaction variations should be studied. Considering the huge amount of supramolecular systems developed in the last years, it is easy to infer that this will be the future of clinical targeting accuracy and smart drug release applications.

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1525

1527 Figure 1. Important non-covalent interactions in synthetic supramolecular systems

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1529

Figure 2. a) Cucurbit[8]uril and electron-rich aromatic guest , b) Inclusion complex, c) Side view of the complex well-encapsulate. Reprinted from [54] with permission from American Chemical Society, Copyright 2010.

1535 Figure 3. Structure of calixarenes and analogs; a) calix[6]arenes, b) resorcin[4]arene, and c)
1536 pyrogallol[4]arene.

1537

1538

1539 Figure 4. Principal structures of crown ethers: a) 16C6 with a metal cation, b) B18C₆, c) criptad, d) larial
1540 ether.

Figure 5. MALDI-TWIM-MS spectrum of the polyrotaxane system. Reprinted from [137] with permission from American Chemical Society, Copyright 2012.

1547 Table 1. Main non-covalent interactions and mass spectrometry analysis for cucurbiturils, calixarenes, crown
 1548 ethers, catenanes, rotaxanes and cyclodextrins.

Compounds	Assemblies	Main non-covalent interactions	Ion-source/MS technique
Cucurbiturils			
	Host-guest systems Rotaxanes, pseudorotaxanes	Hydrophobic effects, Ion-dipole, Dipole-dipole	ESI-TOF, ESI-QTOF, ESI-FTICR, ESI-TWIM-MS
Calixarenes			
	Host-guest systems, Capsules, Carcerands, Hemi carcerands, aggregates, MONCs, MOFs	Cation- π , Hydrogen-bond, Ion-dipole, Dipole-dipole	ESI-TOF, ESI-FTICR, ESI-QIT, MALDI-TOF
Crown ethers			
	Host-guest systems, Aggregates, Rotaxanes	Ion-dipole, Dipole-dipole	ESI-TOF, ESI-QTOF, ESI-FTICR, MALDI-TOF, ESI-Q-Orbitrap
Catenanes and rotaxanes			
	Molecular machines, MOFs, MONCs	Hydrophobic effects, Ion-dipole	ESI-TOF, ESI-LTQ, MALDI-TOF
Cyclodextrins			
	Rotaxanes and polyrotaxanes, Host-guest systems, Aggregates, MOFs	Hydrophobic effects, Ion-dipole, Dipole-dipole	APCI-TOF, ESI-TOF, ESI-Q-Orbitrap, ESI-FTICR, DESI-TOF

1549

1550 Table 2. Common dissociation techniques used in mass spectrometry of non-covalent complexes of
 1551 macrocycles. Adapted from reference [39] with permission from John Wiley and Sons, Copyright 2018.

Dissociation technique	Fragmentation induced by	Polarity	Typical instrument(s)
CID	collisions	+ / -	QTOF / QqQ
HCD	collisions	+ / -	LTQ-Orbitrap
SORI-CID	collisions	+ / -	FTICR
ECD	electron addition	+	FTICR

1552

1553

1554 Table 3. Binding constants for the complexes of cyclodextrins with alkali metal cations and L-amino acids,
 1555 derived by competitive ESI-MS. Adapted from references [157, 161].

1556

log K_a	Li⁺	Na⁺	K⁺	Rb⁺	Cs⁺	L-Ala	L-Ile	L-Leu	L-Val	L-Lys
α-CD	4.44	4.32	4.25	4.08	3.94	4.49	4.48	4.48	4.45	4.11
β-CD	4.28	4.13	4.09	3.97	3.88					
γ-CD	4.05	3.95	3.91	3.87	3.80					

1557

1558

1559 Table 4. Peptide cleavage map of β -CD inclusion complexes (CD binding fragments are marked with #).
 1560 Reprinted from [160] with permission from Springer Nature, Copyright 2015.

Peptide	Cleavage map	Binding sites
Angiotensin I	<div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> <div>D</div><div>R</div><div>V</div><div>Y</div><div>I</div><div>H</div><div>P</div><div>F</div><div>H</div><div>L</div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> </div>	Asp1, Tyr4
Angiotensin II	<div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> <div>D</div><div>R</div><div>V</div><div>Y</div><div>I</div><div>H</div><div>P</div><div>F</div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> </div>	Arg2, Tyr4
[ASN ¹ , VAL ⁵ Angiotensin II	<div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> <div>N</div><div>R</div><div>V</div><div>Y</div><div>V</div><div>H</div><div>P</div><div>F</div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> </div>	Asn1, Tyr 4
Neurotensin Fragment 1-8	<div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> <div>p</div><div>E</div><div>L</div><div>Y</div><div>E</div><div>N</div><div>K</div><div>P</div><div>R</div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> </div>	Asn5, Lys6
Substance P	<div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> <div>R</div><div>P</div><div>K</div><div>P</div><div>Q</div><div>Q</div><div>F</div><div>F</div><div>G</div><div>L</div><div>M</div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> </div>	Arg1 or Pro2, Lys3
Bombesin	<div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> <div>p</div><div>E</div><div>Q</div><div>R</div><div>L</div><div>G</div><div>N</div><div>Q</div><div>W</div><div>A</div><div>V</div><div>G</div><div>H</div><div>L</div><div>M</div> <div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div><div>#</div> </div>	Lys4, Gln7

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