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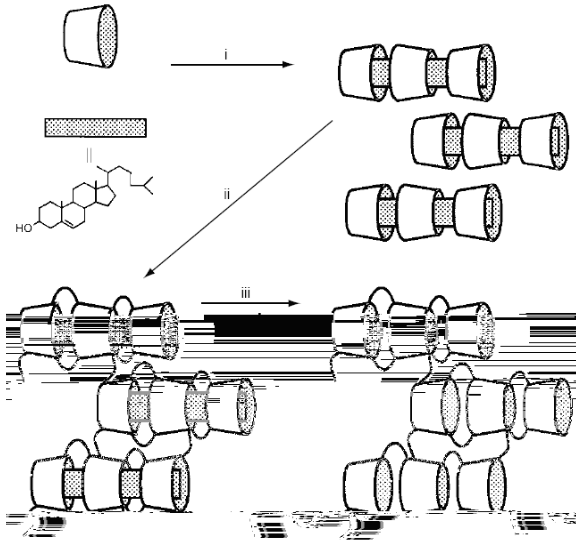

Fig. (1). Reagents and conditions of preparing MIP based on β-CD for recognition of cholesterol: i, hexane–H_2O; ii, diisocyanate in DMSO; iii, acetone, H_2O, THF, EtOH. Taken from Ref. [55].

important molecules [64]. MIP based on β-cyclodextrin (β-CD-MIP) (Fig. (**1**)) has been prepared for efficient recognition of cholesterol in dimethyl sulfoxide (DMSO) [55,56]. The crosslinking agents used are hexamethylene diisocyanate (HMDI) and toluene 2,4-diisocyanate (TDI). However, the imprinted polymer with TDI demonstrates greater absorbing ability to cholesterol than that with HMDI. It suggests that the molecular rigidity of TDI is more adequate to regulate the positions of the β-cyclodextrin (β-CD) residues strictly.

Tailor-made receptors for hydrophobic guest molecules are realized by imprinting of CDs (Fig. (**2**)) [65]. Among the templates of MIPs, those having rigid molecular structures are effectively imprinted, whereas flexible compounds with two aromatic rings connected by polymethylene chain do not exhibit imprinting effect. Imprinting effect of these guest molecules can be attributed to inclusion complexation, not to hydrogen bonding. Tailor-made receptors of CD-MIPs have been studied extensively [66] and are reviewed [67]. The CD-MIPs are prepared in DMSO and cannot be used for molecules having carboxylate or amino groups. Molecular imprinting is successfully carried out in bulk water by use of the vinyl monomer of CD (Fig. (**2A**)) [68]. This method is advantageous for the templates which are large enough and sparingly soluble in water. Between the two imprinting methods in Fig. (**2**), an appropriate one can be selected depending on the purpose and the target molecule. Sometimes, 6-O-α-D-glucosyl-β-CD (G1-β-CD) is used instead of β-CD in order to improve the solubility in water and facilitate the column chromatography [58].

There are some other CD-MIPs applied to separate cholesterol [69]. Zhong *et al*. uses cholesteryl acrylate (CA) and acryloyl-6-amino-6-deoxy-β (or γ)-cyclodextrin as monomers to prepare MIP [70]. Separation can be achieved in the solvents containing water since binding of guest molecules is based on inclusion complexation with CDs. Usually, MIPs ground with mortar and pestle are irregular in size and shape, thus they cannot be applied in pharmaceutical field. Instead, the uniformed molecularly imprinted microspheres (MIMs) by cyclodextrin have been prepared in a DMSO/poly(dimethylsiloxane) (PDMS) emulsion using cholesterol as the template [71]. Herein, PDMS is a suitable dispersing medium for the preparation of MIMs and the size of MIMs can also be changed by the viscosity of PDMS. Temperature also influences the MIMs. MIMs prepared at 65 ^{o}C are in an aggregated form, while uniform MIMs are obtained at 95 ^{o}C. The binding sites exist both on the surface of MIMs and within them. Similar to conventional MIPs, CD-MIPs can also be synthesized by the photochemical approach [72]. β-CD is not only a host molecule for cholesterol, but also a carrier of photoactive functionalities. A polymeric material which is obtained by crosslinking of β-CD substituted with cinnamoyl chromophores can be used as a matrix for photochemical reversible molecular imprinting. The condition is mild and provides a better kinetic control over imprinting process compared with conventional imprinting technology.

In order to understand the mechanism for recognition directly, the processes of MIP based on β-CD with cholesterol and stigmasterol (cross-linking agent: TDI and HMDI) are studied by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) and NMR spectroscopy [73]. Dimers and trimers of β-CD are formed only in the presence of templates, while the formation becomes inefficient in the absence of templates. These ordered assemblies contain two or three β-CD molecules, which cooperate to bind large steroids. When β-CD is replaced with 2,6-di-*O*-methyl-β-cyclodextrin (2,6-DM-β-CD), ordered assemblies are also formed, which indicates the significant roles of the secondary OHs in the molecularly imprinting of β-CD. The proposed mechanism is as follows (Fig. (**3**)). First, one of the two isocyanate groups of cross-linking agent reacts with β-CD, which mainly occurs at the primary OH groups (either with the template or without it). When the other isocyanate groups react with β-CD under imprinting conditions, the reaction preferentially takes place at the secondary OH. Importantly, the cholesterol penetrates into the

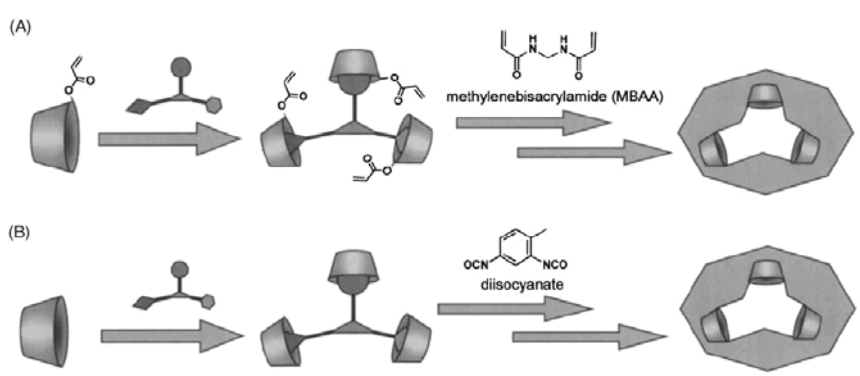

Fig. (2). Molecular imprinting of β-CD in water (**A**) and in DMSO (**B**). Taken from Ref. [68].

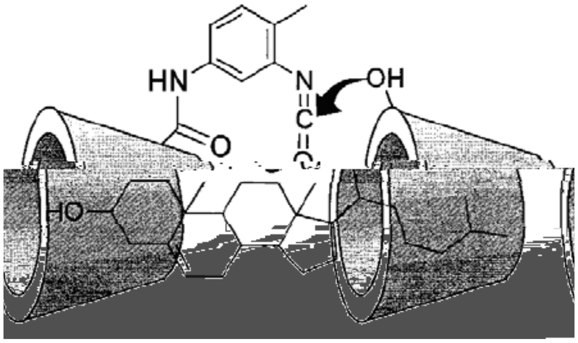

Fig. (3). Proposed mechanism for the molecular imprinting of β-CD with cholesterol (cross-linking agent: TDI). Taken from Ref. [73].

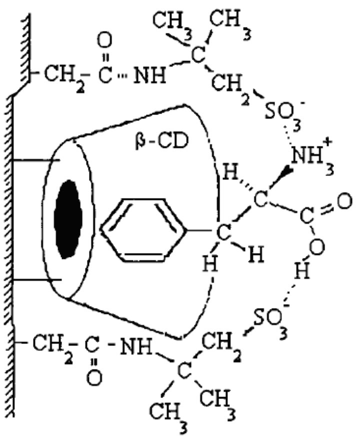

Fig. (4). Schematic illustration of the binding of D-phenylalanine in an imprinted polymer composed of polymerizable β-CD, 2-acryloylamido-2,2',dimethylpropane sulfonic acid (AMPSA), and *N,N*',diacryloylpiperazine. Taken from Ref. [74].

cavity of the second β-CD from its secondary hydroxyl side, rather than from the primary hydroxyl side.

Compared with CD-MIPs in DMSO, imprinting in water is much more important. However, MIP prepared in aqueous medium is difficult because water generally destroys the polar interaction between the functional monomer and the template molecule. The hydrogen bonding between CDs and analytes will be also destroyed by the addition of water. As a result, if the hydrophobic effect is the main interaction, imprinting can be realized in aqueous medium. The first rational use of the hydrophobic effect in conjunction with molecular imprinting in aqueous solution is reported for D- and L-phenylalanine, where bisacryloyl β-CD and 2-acryloylamido-2,2'-dimethylpropane sulfonic acid (AMPSA) are functional monomers [74,75]. The imprinting effect is strong enough to reverse the inherent chiral selectivity of CD molecule for the L-form. This contributes to the combination of binding of the template phenyl moiety into the hydrophobic cavity of the CD, ion pairing and hydrogen bonding (Fig. (**4**)). The recognition utilizes a combination of the entropy-driven hydrophobic effect and enthalpy-motivated electrostatic interactions. Similar studies are reported for selective adsorption of norfloxacin [76] and enantioselective recognition of phenylalanine in aqueous media [77].

Many other molecules can be separated by CD-MIPs in aqueous medium. MIPs for 4,4'-(1,4-phenylenediisopropylidene)bisphenol (BPP) are prepared by bulk imprinting where β-CD is the functional monomer. The obtained MIPs can bind the template selectively in aqueous medium [78]. By the capping method of hydroxyl groups on β-CD molecule, the fact that hydrophobic effects play an important role in the recognition process is discovered.

CD-MIPs can recognize oligopeptides, antibiotics and so on. Creatinine-imprinted poly (β-CD) is synthesized for the specific binding of creatinine [79,80]. As creatinine molecule is hydrophilic, it has less chance to enter the hydrophobic central core of β-CD. By comparing the binding effect of MIP and chlorotrimethylsilane (CTMS)-capped MIP, it is found that hydrogen bonding and stereo-shape effects are important factors for the efficient binding of creatinine.

MIP using β-CD as functional monomer recognizes bilirubin (BR) specifically and reversibly [81]. The specificity may be due to the cooperative effects of inclusion complexation and hydrogen bonding between BR and β-CD. For the BR-imprinted polymer, a self-assembled process between β-CD and BR exists prior to polymerization. β-CDs are arranged in order in the presence of BR. Then, following the polymerization and subsequent removal of BR, this kind of orderly arrangement is reserved. And the formed cavities that are complementary in size and shape to BR are capable of recognizing BR with a high specificity. Whereas, for the non-imprinted polymer, due to the absence of a self-assembled process prior to polymerization, β-CDs are organized randomly. Therefore, the formed β-CD cavity arrangement in the non-imprinted polymer has no size-fit effects with BR. This interpretation is in accordance with the report before [73].

CD-MIPs can be applied in other fields, such as protein refolding [82], that is, molecularly imprinted poly (β-CD) polymer strips detergent molecules from the detergent-protein complexes and results in successful protein refolding processes. Also, CD-MIPs can be used to improve adsorption capacity [62] and as an adsorbent matrices applied in chromatography, especially as stationary phases of high performance liquid chromatography (HPLC) [65,69,70,83].

2.2. Surface Imprinting

Surface imprinting, or surface molecular imprinting technology is a method which generates cavities on the surface or close to the surface of materials facilitating the mass transfer of template [84]. The solid support can be silica-gel, alumina and polymers [83]. Fig. (**5**) demonstrates the process of CD-MIPs on the surface of silica-gel, which is one of the most effective methods for the immobilization of imprinted polymers [85].

Conventionally, the redox initiator is directly added to the mixture of the β-CD-template complex, crosslinker, and surface-modified silica-gel. A new polymerization process is developed [85]. Firstly, the redox initiator is mixed with the surface-modified silica-gel. Then, vinylated β-CD, crosslinker, and the template are added. This modification promotes the immobilization of β-CD copolymer to the silica-gel, resulting in still lower pump pressure when it is used as stationary phase of HPLC. Consequently, the imprinting efficiency is increased. A thin layer of CD-MIPs on a porous silica-gel support is prepared for the recognition of L-Phe-L-Phe and D-Phe-D-Phe [83]. By this method, CD-MIPs can be stiff enough for the stationary phase for HPLC.

On the surface of different solid supports, *i.e.*, modified silica-gels, alumina and poly (hydroxyethyl methacaylate) (Poly-HEMA) (Fig. (**6**)), the MIPs recognizing tripeptide H-Phe-Lys-Phe-NH_2 have been compared [86]. All the imprinted β-CD polymers show notable imprinting effects, though there are differences in the surface charges and other physicochemical properties of these solid supports. This report validates that ordered assembly of β-CD is the origin of molecular imprinting.

The position of vinyl group on β-CD influences the imprinting effect greatly because it governs the distance between the template and polymerization site. Two kinds of vinyl monomers of β-CD, mono-3-(*N*-acrylamido)-3-deoxy-altro-β-cyclodextrin (3-AAm-CD) and mono-6-(*N*-acrylamido)-6-deoxy-β-cyclodextrin (6-AAm-CD or 6-AAm-β-CD), which tether a vinyl group on either wider rim of the truncated cone or its smaller rim, are synthesized [87]. The imprinting effects of 3-AAm-CD and 6-AAm-CD toward *N*-

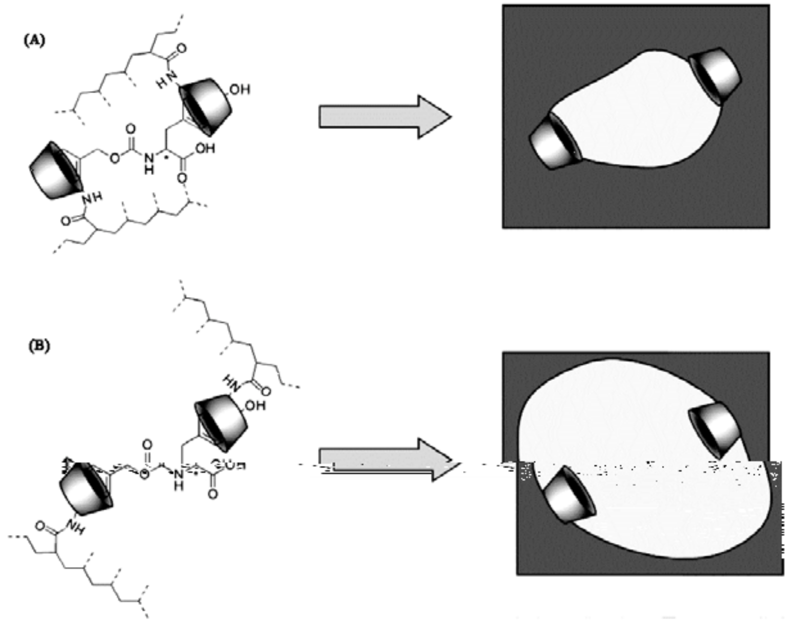

Fig. (7). Possible structure of the binding site prepared by the imprinting from (**A**) 3-AAm-CD and (**B**) 6-AAm-CD. Taken from Ref. [87].

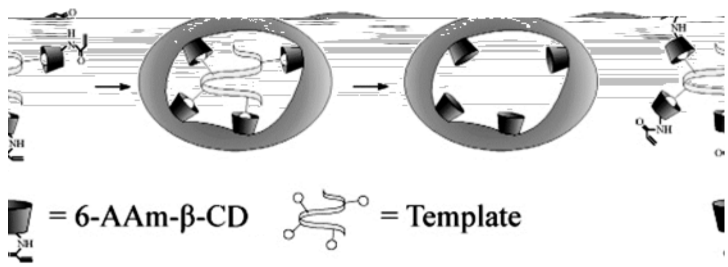

Fig. (8). Schematic view of molecular imprinting of β-CD toward oligopeptide template. Taken from Ref. [57].

A novel MIP prepared with vinyl-bonded β-CD and AA by surface molecular imprinting technique on functionalized silica gel can selectively recognize tryptophan (Trp) in aqueous medium [90]. This method is different from reports using acryloyl β-CD [74,87]. It provides more effective recognition sites than the polymer of acryloyl β-CD, which is just simply random grafted on the matrix. By HPLC column packed with this MIP, it can separate not only Trp from other aromatic amino acids, but also the template from its enantiomer in aqueous mobile phase. However, the absence of AA cannot reverse the inherent selectivity of the β-CD moiety for the L-form. The good property is mainly attributed to the combination interactions of hydrophobic effect between bonded β-CD and Trp and hydrogen bond between AA and Trp. It is a promising method for chiral amino acid separation and purification.

A new surface imprinting technique is applied to synthesize uniformly sized MIMs using ursolic acid (UA) as the template [91]. Uniformly sized functionalized poly (glycidyl methacrylate) microspheres (F-P_{GMA}) are used as the support matrix. As shown in Fig. (**10**), bonded β-CD and AA can form complex with UA through the hydrophobic interaction and the hydrogen bonding simultaneously, which is in good agreement with the two-site binding model. As a result, MIMs can separate UA from herbs effectively. This method provides a new way of preparing uniformly sized spherical imprinted polymers with β-CD as functional monomer. This method is also used in solid-phase extraction and it is the first time for the molecularly imprinted solid-phase extraction (MISPE) to be applied to the extraction of UA from herb [92].

3. COOPERATIVE EFFECTS OF CYCLODEXTRINS AND IONIC LIQUIDS IN SEPARATION PROCESSES

CDs and ILs are used together mainly in the separation methods of capillary electrophoresis (CE) and gas chromatography (GC). In the processes, the interaction of ILs, CDs and analytes is important. ILs and CDs demonstrate cooperative effects for separation of analyte. Typical cations and anions of ILs are shown in Fig. (**11**).

3.1. Capillary Electrophoresis

CE is one of the most important methods in separation science. CDs are now widely used as run buffer additives for CE analyses and have been reviewed [93,94]. It has been shown that the selectivity of CE is enhanced by using CDs as chiral selectors due to their ability to include a wide variety of water-insoluble molecules into their hydrophobic cavity. ILs are also applied in CE extensively [95,96]. In general, the presence of ILs in running electrolytes make the ions coat the capillary walls thus engendering anodic electroosmotic flow (EOF).

ILs can be separated by CDs. The separation of 1-alkyl-3-methylimidazolium, including isomers and related imidazole derivatives is performed by α-cyclodextrin (α-CD) modified capillary zone electrophoresis [97]. This is the first report on separation of dialkylimidazolium and related imidazoles by CE, due to different interactions of ILs and α-CD. For example, the migration time of 1-butyl-3-methylimidazolium (C_4mim) increases more significantly than those of other ILs on the addition of α-CD. As for 1-iso-butyl-

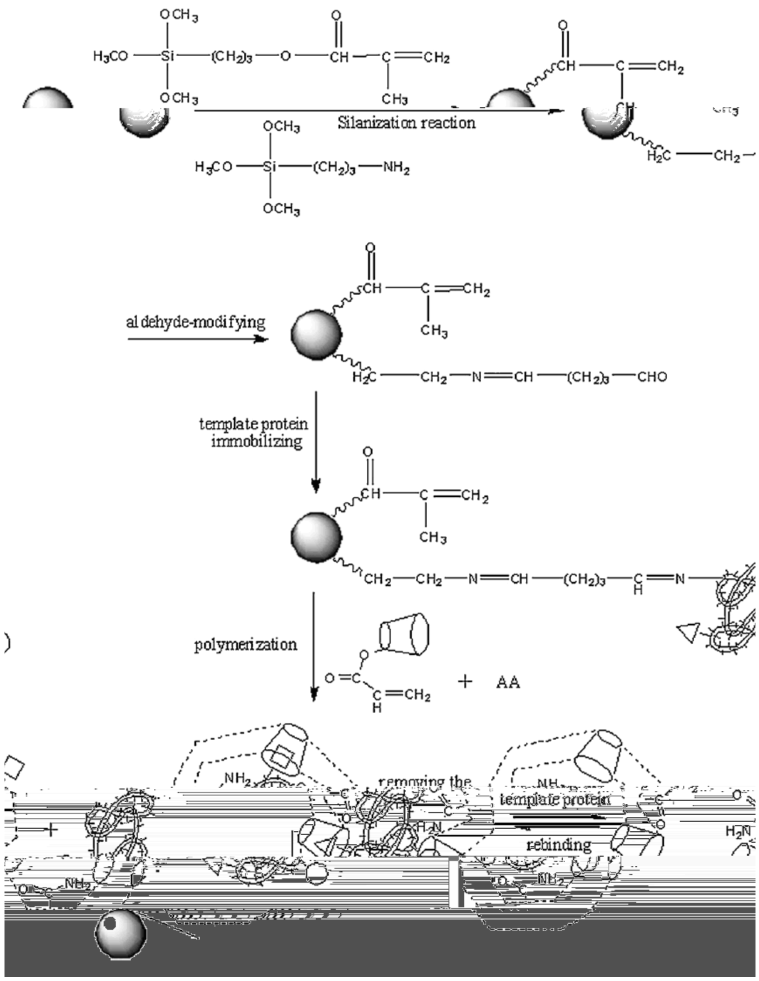

Fig. (9). The protocol for synthesis of the protein imprinted polymer. Taken from Ref. [89].

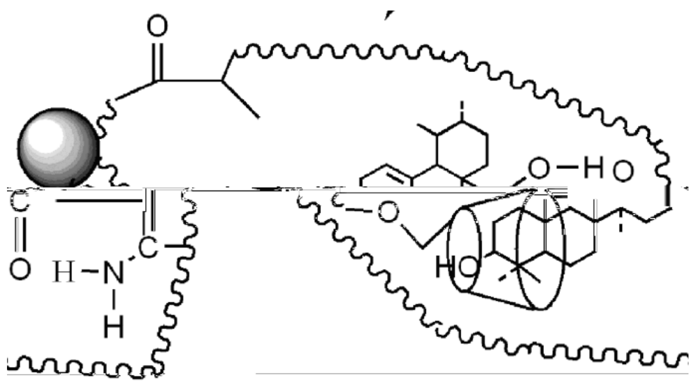

Fig. (10).

3-methylimidazolium (i-C_4mim), the methyl group on the isobutyl makes it not favor entering the α-CD cavity. Instead, C_4mim enters the cavity. This method can be employed to detect impurities in commercial chemicals because most of ILs originates from 1-alkyllimidazolium. Also, the method can be applied in process analysis during synthesis of ILs and provide information on the reaction mechanism.

When ILs are used as background electrolyte (BGE) and covalent coating reagent for determination of metal ions, CDs can be added to improve the properties and effects [98]. With the addition of α-CD into run buffer, the mobility of C_6mim^+ can be modified. The influence of α-CD on the detection sensitivity of ions is demonstrated in Fig. (**12**). Though the peak height ratio increases significantly with the concentration of α-CD lower than 12mM, introduction of α-CD does not bring obvious influence on the mobilities or migration order of the metal ions. This may be because the complexing ability of α-CD to 18-crown-6 or metal ion is very weak. As a result, we can choose proper concentration of α-CD to realize the separation.

Chiral ILs as additives to CDs for enantiomeric separations are evaluated by CE [23,24,99]. These chiral ILs (ethyl- and phenylcholine (EtChol and PhChol), of bis(trifluoromethylsulfonyl)imide)

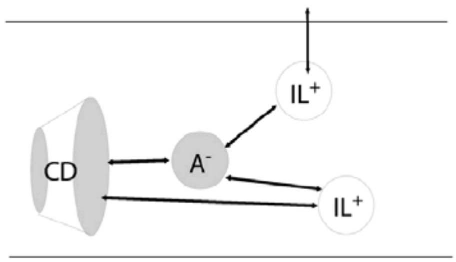

Fig. (13). Schematic description of the interaction system between anionic profen A⁻, chiral IL⁺ cation, free in the BGE or adsorbed onto the capillary wall, and β-CD derivatives. Taken from Ref. [24].

time of [C_4Py][PF_6] (Fig. (**14**)). The process may be conducted by hydrophobic, hydrogen bonding or ion-dipole/ion-induced-dipole interactions. The long chain of [C_8Py][PF_6] enters the cavity of β-CD, which competes with β-agonists enantiomers and results in poor chiral resolutions.

[C_4mim][BF_4] and β-CD can be used in CE for the determination of anthraquinones in Chinese herb [25]. When there is no β-CD, analytes are only partially separated. After adding β-CD of proper concentration into the running buffer, clear separation of real sample can be achieved. The mechanism of the separation of anthraquinones is shown in Fig. (**15**). Anthraquinones can associate

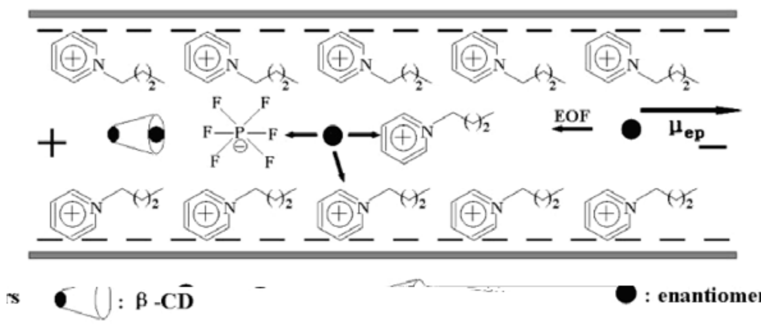

Fig. (14). Mechanism of enantioseparation of β-agonists enantiomers using [C_4Py][PF_6] with β-CD. Taken from Ref. [100].

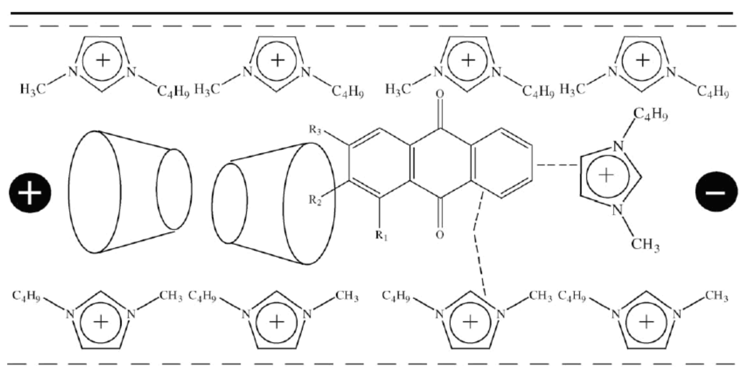

Fig. (15). Mechanism of the separation of anthraquinones using C_4mim^+ based ionic liquid and β-CD. Taken from Ref. [25].

inclusion complexation between β-agonists and ILs. TMAOH is the smallest one with the least tendency to be included in the cavity of β-CD, thus it is less competitive for the enantioselective sites of β-CD. There are many factors attributed to the length of the migration

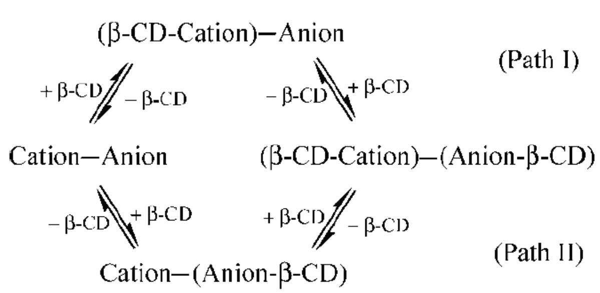

Fig. (16). Two-Step Equilibrium in an IL/β-CD System (Path I: the cation interacts with β-CD more strongly than the anion does. Path II: the anion interacts with β-CD more strongly than the cation does.). Taken from Ref. [115].

with the imidazolium ions or β-CD, respectively. In the presence of β-CD coexisting with IL, anthraquinones may be entirely or partly embedded the cavity of β-CD and the association of anthraquinones with free imidazolium ions is weaken. This association is partially driven by the hydrophobic, hydrogen bonding, or by the ion-dipole/ion-induced-dipole interactions between the anthraquinones and [C_4mim][BF_4]. And those analytes, which are not embedded the cavity of β-CD have rather stronger association with the imidazolium ions in the system. Therefore, the association between the analytes and the imidazolium ions is different. Consequently, this different association or embedding makes the mixture of anthraquinones to be separated excellently. Similar studies are used to simultaneous determination of bioactive flavone derivatives in Chinese herb [101]. [C_4mim][BF_4] and [C_2mim][BF_4] are appropriate to be used as running electrolytes in CE, especially in high ionic strength.

The use of IL and CD in CE can also be applied to investigate the complexation between IL and CD. Affinity capillary electrophoresis (ACE) method is developed to quantitatively characterize the complexation between alkyl (methyl) methylimidazolium-based IL cations and neutral CDs in water [102]. ACE is based on the alteration of analyte effective mobility due to in situ complexation in ligand-containing electrolytes. The absolute mobilities of the free and bound forms of the analyte are different, then a shift in the position of the analyte peak is expected as the ligand concentration in the running buffer varies. Upon increasing β-CD concentration, an increase in the migration time of the IL cation is observed, indicating the formation of a complex between these species. Thus, by classical nonlinear and linear treatment, the complex stoichiometry and formation constant K are obtained. This method can keep the consumption of analytes and ligand to a minimum. It realizes the online detection, short analysis times and should be of interest for liquid chromatography (LC) and CE.

Beside the application in CE, [C_2mim][BF_4] as the working electrolyte is used in chiral separation for dipeptides in glass microchip electrophoresis [103]. In this report, different CDs are selected for chiral separation of Gly-D,L-Phe. β-CD and negtively charged carboxy-methyl-β-CD (CM-β-CD) cannot perform the chiral separation when [C_2mim][BF_4] is the working electrolyte. For CM-β-CD, the migration times of analytes are delayed. It is the result of the direction of CM-β-CD inclined to the anode while the analytes are driven toward the cathode by EOF in basic buffer, and more probabilities exist for analytes to touch with selector. It is suggested that outer groups of CD cavity in IL solution are related to the chiral separation.

3.2. Gas Chromatography

As early as 1999, the idea that ILs can be used as stationary phases for GC was promoted [104]. There are many reviews about the application [105,106]. Maybe it is because their wetting ability and viscosity allow them to be coated onto fused silica capillaries.

BF_4^-	=	tetrafluoroborate
BGE	=	background electrolyte
BPP	=	4,4'-(1,4-phenylenediiso-propylidene) bisphenol
BR	=	bilirubin
CA	=	cholesteryl acrylate
CD	=	cyclodextrin
CD-MIP	=	MIP based on cyclodextrins
CE	=	capillary electrophoresis
CM-β-CD	=	carboxy-methyl-β-CD
$(CN)_2N^-$	=	dicyanimide
C_nmim^+	=	1-alkyl-3-methylimida-zolium
$C_nOSO_3^-$	=	alkylsulfate
C_nPy^+	=	1-alkylpyridinium
C_nvim^+	=	1-alkyl-3-vinylimidazolium
CTMS	=	chlorotrimethylsilane
2,6-DM-β-CD	=	2,6-di-*O*-methyl-β-cyclodextrin
DM-β-CD	=	dimethyl-β-cyclodextrin
DMSO	=	dimethyl sulfoxide
EOF	=	electroosmotic flow
EtChol	=	ethylcholine
$F\text{-}P_{GMA}$	=	functionalized poly (gly-cidyl methacrylate) micro-spheres
G1-β-CD	=	6-O-α-D-glucosyl-β-CD
GC	=	gas chromatography
HMDI	=	hexamethylene diisocyanate
HPLC	=	high performance liquid chromatography
$i\text{-}C_4mim$	=	1-iso-butyl-3-methylimida-zolium
IL	=	ionic liquid
LC	=	liquid chromatography
Lyz	=	lysozyme
MALDI-TOF MS	=	matrix-assisted laser desorp-tion/ionization time-of-flight mass spectroscopy
MIM	=	molecularly imprinted mi-crosphere
MIP	=	molecularly imprinted polymer
MIT	=	molecular imprinting tech-nology
NfO^-	=	nonafluoro- butanesulfonate
$OTos^-$	=	tosylate
PDMS	=	poly(dimethylsiloxane)
PF_6^-	=	hexafluorophosphate
PhChol	=	phenylcholine
Poly-HEMA	=	poly(hydroxyethyl methacaylate)
TBAOH	=	tetrabutylammonium hy-droxide
TDI	=	2,4-diisocyanate
TEOS	=	tetraethoxysilane
Tf_2N^-	=	bis(trifluoro- methylsul-fonyl)imide
TfO^-	=	trifluoromethanesulfonate
TMAOH	=	tetramethylammonium hy-droxide
TM-β-CD	=	trimethyl-β-cyclodextrin
$TPhB^-$	=	tetraphenylborate
Trp	=	tryptophan
UA	=	ursolic acid
XRD	=	Powder X-ray diffraction
Z-Tyr	=	*N*-benzyloxycarbonyl-tyrosine

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