DEVELOPMENT OF POLY(β-CYCLODEXTRIN FUNCTIONALIZED IONIC LIQUID) CONJUGATED MAGNETIC NANOPARTICLES AS AN ADSORBENT FOR MICROEXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM FOOD SAMPLES

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by

BOON YIH HUI

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To My Parents and Family Members

With love

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

[C4min] PF_6	1-butyl-3-methylimidazolium hexafluorophosphate
$[Fe(CN)_6]^{3-/4-}$	Potassium ferricyanide
[Hmim]BF_4	1-hexyl-3-methylimidazolium tetrafluoroborate
Ace	Acenapthalene
ACN	Acetonitrile
APTES	(3-aminopropyl)triethoxysilane
BaA	Benzo(a) anthracene
BaP	Benzo(a) pyrene
BBD	Box-Behnken design
BET	Brunauer Emmelt Teller
BGE	Background electrolyte
$BMIMBF_4$	1-butyl-3-methyl-imidazolium tetrafluoroborate
C	Carbon
Cd(II)	Cadmium (II)
CE	Capillary electrophoresis
CPE	Cloud point extraction
Cr^{6+}	Chromium (VI) ion
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
EA	Elemental analysis
EC	European Community
FAAS	Flame atomic absorption spectroscopy
Fe_3O_4	Iron(II, III) oxide
FF-DLPME	Ferrofluid-based dispersive liquid phase microextraction

Fla	Fluoranthene
Flu	Fluorene
FSQD	Food Safety and Quality Division
FTIR	Fourier transform infrared
GC-μECD	Gas chromatography-micro electron capture detector
GCE	Glass carbon electrode
GC-FID	Gas chromatography- flame ionization detector
GC-MS	Gas chromatography–mass spectrometry
H	Hydrogen
H_3PO_4	Phosphoric acid
HDI	Hexamethylene diisocyanate
HPLC-FLD	High-performance liquid chromatography-fluorescence detector
HPLC-UV	High-performance liquid chromatography-ultraviolet detector
HP-β-CD	(2-hydroxypropyl)-β-cyclodextrin
ICH	International Conference on Harmonization
IL	Ionic liquid
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid-phase microextraction
Magnetic μ-SPE	Magnetic micro-solid phase extraction
ME	Microextraction
Me-β-CD	Methylated-β-cyclodextrin
MeOH	Methanol

MgAl-LDH	Magnesium–aluminum layered double hydroxide
Mn(II)/Mn(VII)	Manganese (II) and Manganese (VII)
MNPs	Magnetic nanoparticles
MSPE	Magnetic solid phase extraction
N	Nitrogen
NaCl	Sodium chloride
NaH_2PO_4	Monosodium phosphate
NaOAc	Sodium acetate
NaOH	Sodium hydroxide
Nap	Naphthalene
ND/ n.d	Not detected
NH_4Cl	Ammonium chloride
NMR	Nuclear magnetic resonance
NTf_2	Bis(trifluoromethylsulfonyl)amide
OVAT	One-variable-at-a-time
PAHs	Polycyclic aromatic hydrocarbons
PAN	Polyacrylonitrile
PBS	Phosphate buffer solution
PF	Preconcentration factor
Phe	Phenanthrene
$PM_{2.5}$	Fine particulate matter
Pyr	Pyrene
RhB	Rhodamine B
RSD	Relative standard deviation
RSM	Response surface design

SCF	Scientific Community on Food
SEM	Scanning electron microscopy
SiO_2	Silicon dioxide or silica
SPE	Solid phase extraction
SPME	Solid phase microextraction
SUPRAS	Supramolecular solvents
TDI	Toluene diisocyanate
TEM	Transmission electron microscope
TEQ	Toxic Equivalent Factor
TGA	Thermogravimetric analysis
TiO_2	Titanium dioxide
TLC	Thin layer chromatography
TM-β-CD	Trimethylated-β-cyclodextrin
UiO-67	(University of Oslo) series-67
US EPA	United States Environmental Protection Agency
VSM	Vibrating sample magnetometer
WCA	Water contact angle
WHO	World Health Organization
XRD	X-ray powder diffraction
α-CD	Alpha-cyclodextrin
β-CD	Beta-cyclodextrin
βCD-IL	Beta-cyclodextrin functionalized ionic liquid
γ-CD	Gamma-cyclodextrin

LIST OF SYMBOLS

%	Percent
µg kg^{-1}	Microgram per kilogram
µg L^{-1}	Microgram per litre
µL	Microlitre
µm	Micrometre
A’	Absorbance of guest in the presence of host
A_0	Absorbance of guest in the absence of host
℃	Degree Celsius
Co	Centre points
D_c	Average crystalline diameter
DT	Degree of tolylation
g L^{-1}	Gram per litre
g mol^{-1}	Gram per mol
k	Number of factors
K	Apparent formation constant
kV	Kilovolt
M	Molarity
mA	Milliampere
mg L^{-1}	Milligram per litre
mM	Millimol
mmol	Millimol
mmol L^{-1}	Millimol per litre
mol L^{-1}	Mol per litre
MΩ	Milliohm

N	Number of experiment
ng L^{-1}	Nanogram per litre
ng mL^{-1}	Nanogram per millimetre
nm	Nanometre
ppm	Part per million
R^2	Coefficient of determination
rpm	Rotation per minute
V	Voltage
v/v	Volume per volume
w/v	Weight per volume
K	Particle shape factor
μA	Microampere

PEMBANGUNAN POLI(β-SIKLODEKSTRIN BERFUNGSIKAN CECAIR IONIK) BERKONJUGAT NANOPARTIKEL MAGNET SEBAGAI PENJERAP UNTUK PENGEKSTRAKAN MIKRO POLISIKLIK AROMATIK HIDROKARBON DARIPADA SAMPEL MAKANAN

ABSTRAK

Pencemaran makanan merupakan isu keselamatan makanan global yang boleh menimbulkan masalah kesihatan. Analisis terhadap pencemar adalah penting untuk memastikan keselamatan pengguna dan pematuhan terhadap had peraturan. Oleh itu, pembangunan teknik penyediaan sampel yang mudah, berskala kecil, dan sensitif sentiasa menjadi pilihan yang digemari. Kajian ini bertujuan untuk membangun dan menyiasat bahan nano yang baru, iaitu poli(βCD-IL)@Fe_3O_4 dan aplikasinya dalam pengekstrakan mikro polisiklik aromatik hidrokarbon (PAHs) yang mempunyai berat molekul rendah dan tinggi yang disenaraikan sebagai bahan pencemar utama oleh Komuniti Eropah (EC) dan Agensi Perlindungan Alam Sekitar (EPA). Dalam bahagian pertama kajian, poli(βCD-IL)@Fe_3O_4 telah berjaya disintesis dan dicirikan oleh pelbagai teknik termasuk FT-IR, analisis elemen CHN, XRD, TEM, SEM, VSM, BET, TGA, DSC, potensi zeta dan ukuran kebolehbasahan. Seterusnya, poli(βCD-IL)@Fe_3O_4 digunakan sebagai penjerap dalam kaedah magnetik μ-SPE bersama gas kromatografi pengesan pengionan nyala (magnetik μ-SPE-GC-FID) untuk penentuan lima PAHs terpilih yang mempunyai berat molekul rendah dalam sampel beras. Dalam keadaan optimum, lekuk penentukuran telah menunjukkan kelinearan dalam kepekatan antara 0.1 dan 500 μg kg^{-1} dengan pekali penentuan (R^2) di antara 0.9970 dan 0.9982 untuk kesemua analit. Had pengesanan (LODs) dalam matriks sebenar berada dalam julat 0.01-0.18 μg kg^{-1}. Nilai sisihan piawai relatif (RSD) berada pada 2.95%-5.34% (intra-hari, n=6) dan 4.37%-7.05% (antara hari, n=6). Penjerap

menunjukkan kebolehulangan yang memuaskan dalam julat 2.9% ke 9.9% dan nilai perolehan semula yang boleh diterima pada 80.4% – 112.4% untuk sampel sebenar. Kaedah optimum magnetik μ-SPE-GC-FID kemudian berjaya diaplikasikan untuk mengakses kandungan keselamatan PAHs dalam 24 jenis beras komersial yang terdapat di Malaysia. Prestasi analitikal poli(βCD-IL)@Fe_3O_4 yang cemerlang dalam kaedah magnetik μ-SPE-GC-FID seterusnya menjadi dorongan untuk mengubahsuainya ke bentuk bendalir ferro dalam membangunkan teknik FF-DLPME di bahagian kedua kajian ini. Bendalir ferro ini telah digunakan untuk pengekstrakan mikro tujuh wakil PAHs yang mempunyai berat molekul rendah dan tinggi daripada makanan dan minuman. Dalam keadaan optimum, lekuk penentukuran telah menunjukkan kelinearan dalam kepekatan antara 0.1 dan 150 ng mL^{-1} dalam matrik air dengan R^2 daripada 0.9970 ke 0.9982 untuk kesemua analit. LODs kaedah adalah dalam julat 0.02 dan 0.07 ng mL^{-1}. RSD, % untuk intra-hari dan antara hari berada dalam julat 1.80%-7.56% dan 2.97%-8.23% dengan nilai kebolehulangan di antara 1.72% dan 5.90%. Manakala, nilai perolehan semula adalah pada 84%-110% untuk sampel sebenar. Parameter yang sama turut disahkan dalam matrik beras dan minuman teh. Kaedah optimum FF-DLPME-GC-FID akhirnya diaplikasikan untuk menilai kandungan keselamatan PAHs dalam pelbagai jenis makanan dan minuman komersial di Malaysia. Perbandingan antara kedua-dua teknik pengekstratan mikro yang dibangunkan juga telah dibentang. Akhir sekali, mekanisma penjerapan berdasarkan kajian kompleks telah dijalankan untuk menyiasat kehadiran interaksi supramolecular host-guest yang menyumbang kepada prestasi cemerlang pengekstrakan mikro bagi kedua-dua teknik. Pembentukan kompleks pepejal telah berjaya dipantau oleh pengukuran teknik FTIR, TGA, DSC, 1D 1H NMR dan 2D NOESY NMR, manakala kompleks cecair berjaya dipantau oleh pengukuran spektroskopi UV-vis. Keputusan

menunjukkan bahawa Phenanthrene membentuk nisbah stoikiometri 1:1. Skema daripada proses pembentukan kompleks yang dicadangkan oleh kajian doking molekul menyerupai keputusan eksperimen.

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ABSTRACT

Food contamination is a global food safety issue which pose a health concern. Analysis of food contaminants is essential to ensure consumer safety and compliance with regulatory limits. Thus, development of simple, miniaturize, and sensitive sample preparation techniques are always a choice of interest. This study aims to develop and investigate new nanomaterial, namely poly(βCD-IL)@Fe_3O_4 and their applications in the microextraction of low and high molecular weight polycyclic aromatic hydrocarbons (PAHs) that have been listed as priority pollutants by European Community (EC) and Environmental Protection Agency (EPA). In the first part of study, poly(βCD-IL)@Fe_3O_4 was successfully synthesized and characterized by various techniques including FT-IR, CHN elemental analysis, XRD, TEM, SEM, VSM, BET, TGA, DSC, zeta potential and wettability measurement. Thereafter, poly(βCD-IL)@Fe_3O_4 was applied in magnetic μ-SPE technique as an adsorbent coupled with gas chromatographic-flame ionization detection (magnetic μSPE-GC-FID) for the determination of five selected low molecular weight PAHs in rice samples. Under optimized conditions, the calibration curves were linear for the concentration ranging between 0.1 and 500 μg kg^{-1} with coefficient of determination (R^2) from 0.9970 to 0.9982 for all analytes. Detection limits (LODs) ranged at 0.01–0.18 μg kg^{-1} in real matrix. The RSD values ranged at 2.95%–5.34% (intra-day, n=6) and 4.37%–7.05% (inter-day, n=6). The sorbent showed satisfactory reproducibility in 2.9% to 9.9% range and acceptable recovery values at 80.4%–112.4% for the real samples. The

optimized magnetic μ-SPE-GC-FID method was then successfully applied to access content safety of selected PAHs for 24 kinds of commercial rice available in Malaysia. The excellent analytical performance of poly(βCD-IL)@Fe_3O_4 in magnetic μ-SPE technique further became a driven force to modify it into ferrofluid turning out with ferrofluid based dispersive liquid phase microextraction (FF-DLPME) technique, which was covered in the second part of this study. The ferrofluid was applied for the microextraction of seven representative PAHs including low and high molecular weight PAHs from food and beverages. Under optimized conditions, the calibration curves were found to be linear in water matrix from range of 0.1-150 ng mL^{-1} with R^2 between 0.9944 and 0.9986. The LODs of the proposed method were between 0.02 and 0.07 ng mL^{-1}. The intra and inter-day precision (RSD, %) were in the range of 1.80%-7.56% and 2.97%-8.23% with sorbent reproducibility between 1.72% and 5.90% and acceptable recovery values at 84% -110% for real sample. Same validation parameters also have been conducted in rice and tea beverages matrices. The optimized FF-DLPME-GC-FID method was finally applied to evaluate the content safety of studied PAHs in variety of commercial foods and beverage in Malaysia. Comparison between both developed microextraction techniques is been presented. Lastly, adsorption mechanism based on inclusion complex study was conducted to investigate the presence of supramolecular host-guest interaction that contribute to the excellent extraction performance for both developed microextraction techniques. The formation of solid kneaded inclusion complex was successfully monitored by FTIR spectroscopy, TGA, DSC, 1D ^1H NMR and 2D NOESY NMR measurement whereas liquid inclusion complex was monitored by UV-vis spectroscopy. Results revealed that Phenanthrene formed 1:1 stoichiometry ratio. A schematic representation of the energetically

favorable inclusion process is proposed by molecular docking studies which is in good agreement with the experimental results.

CHAPTER 1

INTRODUCTION

1.1 General background

In recent years, there has been a growing concern among the general public over the issue of food contamination by toxic compounds, particularly polycyclic aromatic hydrocarbons (PAHs). Generally, PAHs tend to accumulate in food products such as dairy, vegetables, fruits, oils, rice, cereals, grilled meat, smoked food, coffee, and tea (Bansal & Kim, 2015; Boon et al., 2019; Chung et al., 2011; Escarrone et al., 2014; Farhadian et al., 2010; Iwegbue et al., 2016; Jimenez et al., 2014; Lochan et al., 2016; Pincemaille et al., 2014; Plaza-Bolaños et al., 2010; Smoker et al., 2010; Yun et al., 2017; Zachara et al., 2018; Zelinkova & Wenzl, 2015; Zheng et al., 2016) due to their hydrophobic and lipophilic characteristics (Pensado et al., 2005). Many works have identified food as major contributor to human direct exposure to PAHs and therefore, their presence in food is a matter of concern.

PAHs are compounds that contain two or more fused benzene rings in a linear, angular or cluster arrangement (Shi et al., 2016). They are formed from the incomplete combustion (engine exhaust, industrial outlet, crude oil) or pyrolysis of organic matter (Chen & Chen, 2001; Zhao et al., 2011) and are widespread in the environment as organic pollutants (Jiao et al., 2007; Xu et al., 2014). Sixteen priority PAHs have been listed as persistent organic pollutants (POPs) by the U.S. Environmental Protection Agency (US EPA) due to their substantial toxicity and possible carcinogenic properties (Aguinaga et al., 2007; Fei et al., 2011; Jimenez et al., 2014). Furthermore, PAHs are

also highly resistant to natural degradation due to their stable molecular structures (Lau et al., 2014).

The occurrence of PAHs in food can be ascribed to diverse pathways that include both natural (as environmental) and synthetic sources (e.g. cooking practices and industrial food processing). Upon ingestion, PAHs will activate the mammalian cells to produce diolepoxides that will react with the DNA in tissue cells to cause mutation (Bansal & Kim, 2015).

Numerous efforts have been directed to address how precisely and accurately one can qualify and quantify the level of PAHs present in food products and this is non-trivial since food is typically non-homogenous and complex that make it hard to isolate and determine the analyte of interest.

"Direct and shoot" concepts using chromatography or spectroscopy method may experience some sensitivity issues which can lead to poor analysis result besides increasing the instrument maintenance. Subsequently, by considering the food matrix effects, many analytical methods that have been developed for monitoring and determining the food contaminations generally involve a pre-treatment step prior to instrumental analyses. Sample pre-treatment or sample preparation is regarded to be an important step in a chemical analysis as it aims to extract, pre-concentrate, and clean-up from interference apart from the advances in analytical instrumentation for analyte separation and detection. Sample preparations also improve the analysis results by enhancing the sensitivity, precision and instrument detection limits which could not be achieved by direct determination at a very low concentration. Thus, a good sample preparation can be economically valuable as well as analytically important.

1.2 Problem Statements

The level of PAHs in food have been regulated by the World Health Organization (WHO) due to their toxicity to human (Registry, 2008). For instance, 4 high molecular weight PAHs namely PAH4 (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) have been selected as food markers by the Commission Regulation (EU) No 835/2011 with their maximum limits between 1 and 30 μg kg^{-1} (Food Safety Authority of Ireland, 2015). However, there's still a need for reliable data on other PAHs, especially those with low molecular weight as they are more commonly found compared to high molecular weight PAHs that listed in food legislation (Escarrone et al., 2014; Matin et al., 2014; Plaza-Bolaños et al., 2010). Indeed, the Scientific Committee on Food (SCF) has urged the collection of data based on other types of PAHs in assessing food contamination and to monitor the changes of PAHs profile in food (Zelinkova & Wenzl, 2015).

By narrowing the scope of PAHs investigation in Malaysia, surprisingly, number of study regarding to the PAHs determination in food and beverage samples are far less compared to environmental samples. This reveals that inadequate attention has been paid, likely due to the absence of legislation concerning the safety levels of food PAHs in our country. Since PAHs can accumulate in the human body through food consumption, their analysis is essential to ensure the consumers' safety.

Different sample preparation techniques and separation methods have been developed and reported for PAHs determination. Among the sample preparations techniques, sorbent-phase based extraction technique stands out the most popular one. Various types of adsorbents have been designed to satisfy the applications. However, most of these adsorbents are tuned with the focus on their affinity for target analytes,

but with little consideration on their compatibility towards the sample matrices. Furthermore, PAHs usually exhibit low solubility and bioavailability (Ren et al., 2015), which limits their removal or extraction from the contaminated food.

Herein, supramolecular cyclodextrins (CDs) appear to be particularly promising agent through host-guest interaction as reported in previous works (Choi et al., 2017; Topuz & Uyar, 2017b, 2017a). This could be contributed by the merit of CDs which are able to reduce the toxicity, enhance the solubility and catalyse the degradation of contaminants (Bardi et al., 2000; Boving & Brusseau, 2000; Kamiya et al., 2001; Manolikar & Sawant, 2003). Moreover, CDs prevail other adsorbents due to their biocompatibility, non-toxic and environmentally friendly properties (Choi et al., 2017).

Recently, microextraction techniques have been developed as an alternative method for the determination of trace level compounds and are gaining much interests since these techniques greatly reduce the volume of organic solvent, sorbent dosage, and extraction times coupled with inexpensive instruments. Nonetheless, there is still a need to develop microextraction techniques that comply with the principles of green chemistry.

In the attempt to comprehensively address the above-mentioned issues, this study is systematically conducted.

We hope the findings in this study could be a preliminary benchmark or added value to the existing data for the establishment of the maximum permissible limits of food PAHs to Food Safety and Quality Division (FSQD), Ministry of Health Malaysia. In addition, we anticipate that the study outcomes provide insight and awareness into the rational diet suggestions to maintain or improve public health.

1.3 Scope of research

This study involves the synthesis of a nanocomposite, namely poly(βCD-IL) conjugated magnetic nanoparticles which combines the merits of polymeric β-CD, IL, and magnetic nanoparticles. It is important to highlight the rationality for such a design and fabrication of the nanocomposite since different organic moieties possess their own unique properties that contribute to the extraction efficacy. For this, β-CD is chosen instead of α- and γ- cyclodextrin due to its low cost, stability and is commercially available. Besides, it is well known that β-CD are guest friendly that is able to interact with various kinds of organic molecules by forming inclusion complex. In addition, the odd number of glucose rings present in its structure decreases the symmetry of the cavity, hence, increasing the resolving power of β-CD. This unique property has received great attention and is fully exploited by researchers in the field of separation sciences. On the other hand, IL is employed as modifier to increase the active sites of the nanocomposite. Due to the presence of double bonds, it can be used to extract the analytes through π to π adsorption mechanism. Herein, 1-vinylimidazole was selected in the modification process to give rise to 1-vinylimidazolium-β-CD as the unique functional monomer. However, the chemically modified cyclodextrins are soluble, which limit their applications in water, therefore, conversion of modified CD to polymeric materials is of great values. To attain this property, cross-linker agent (toluene-2,4-diisocyanate) was utilized to produce water insoluble IL modified cyclodextrin polymers. This polymer host networks are further grafted on the surface of magnetic nanoparticles in order to promote the developed sample preparation techniques.

In short, the application of β-CD and IL are to provide multiple binding sites to the nanocomposite while the employment of toluene-2,4-diisocyanate is to

polymerize the soluble entities. Meanwhile, magnetic nanoparticles are decorated to make the nanocomposites separate easily by means of external magnetic field and to improve the adsorption capacity by its large specific surface area. The synthesized nanocomposite is expected to exhibit few unique properties such as high surface area, high supramolecular recognition capability, superparamagnetism and large delocalized π-electron system.

The successful synthesis of this nanocomposite was confirmed by various characterization techniques such as Fourier transform infrared (FTIR), elemental analysis, Brunauer-Emmett-Teller (BET), vibrating sample magnetometer (VSM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), x-ray powder diffraction (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), zeta potential and wettability analysis. Thereafter, it was being applied in magnetic μ-SPE for the determination of selected low molecular weight PAHs from rice samples coupled with GC-FID. The excellent analytical performance of the developed nanocomposite in magnetic μ-SPE technique was encouraging and further became a driven force to modify the nanocomposite into ferrofluid turning out with FF-DLPME technique. Due to the merits of ferrofluid, it was successfully applied for the analysis of selected low and high molecular weight PAHs in various food and beverage samples. Subsequently, the extraction performances of both developed magnetic μ-SPE and FF-DLPME methods were then compared. Investigation on the adsorption mechanism by means of inclusion complex study was also conducted. A schematic representation of the energetically favorable inclusion process is proposed by molecular docking studies using PatchDock server.

1.4 Research Objectives

The objectives of this work are summarized as follows:

(i) To synthesize and characterize Ts_2O, βCD-OTS, βCD-Vinyl-OTS, Fe_3O_4, poly(βCD)@Fe_3O_4, and poly(βCD-IL)@Fe_3O_4

(ii) To develop, optimize, and validate poly(βCD-IL)@Fe_3O_4 based magnetic μ-SPE techniques coupled with GC-FID analysis for the determination of low molecular weight PAHs in rice samples

(iii) To develop, optimize, and validate poly(βCD-IL)@Fe_3O_4 based FF-DLPME techniques coupled with GC-FID analysis for the determination of low and high molecular weight PAHs in food and beverage samples

(iv) To compare the analytical performance of poly(βCD-IL)@Fe_3O_4 based magnetic μ-SPE and FF-DLPME techniques

(v) To investigate the adsorption mechanism of Phenanthrene via inclusion complex study between the native β-CD and ionic liquid modified β-CD

1.5 Organization of thesis

The present thesis is organized into five chapters. Chapter 1 outlines a brief introduction on the research background, problem statement, scope of research, research objective, and a structural organization of the whole thesis. A review of related literature is summarized in Chapter 2. Chapter 3 discusses the experimental procedures for the whole study and subdivided into five parts. Part one to part three describe about the synthesis methods and characterization techniques of adsorbents whereas part four reports the method development, validation and applications of the adsorbents in magnetic μ-SPE and FF-DLPME techniques, respectively. While investigation on the adsorption mechanism by means of inclusion complex and molecular docking are presented in final part. Chapter 4 consists of six parts that discuss the findings and outcomes of the study accordingly. The overall conclusions and further recommendations are presented in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Magnetic nanoparticles (MNPs)

2.1.1 Synthesis of MNPs

Researches and development of nano-based materials have gained a lot of attractions within the scientific community. The focus is primarily on designing particles that have uniform shape, arrangement, and nano-meter size. For this, metallic and metal oxides NPs have been widely exploited in the field of analytical chemistry due to their tuneable properties which are able to offer promising applications in terms of sensitivity, selectivity and reliability.

Magnetic nanoparticles (MNPs) are a type of metal oxides NPs, which can be handled by using an external magnetic field. Their large surface areas to volume ratio, high dispersibility (Amirhassan et al., 2015), quantum size effects, supraparamagnetic behaviour, and easiness of surface modification allow them to be used in a wide range of applications, including magnetic drug target (Pecina, 1978), nano-sorbent in environmental engineering (Awwad & Salem, 2013), magnetic resonance imaging (Anbarasu & Balamurugan, 2014; Yugandhar et al., 2013), biomedicine (Barrera et al., 2009), and magnetic sensing (Jun-Hua et al., 2007)

Maghemite (Fe_2O_3) and magnetite Fe_3O_4 are the most common iron oxides used to synthesize MNPs due to their biological compatibility, high magnetic moment, and easy to prepare. For this, ferrite oxide magnetite (Fe_3O_4) that possess large surface

area with nano-size dimension, excellent sorption capability, and strong response under an applied external magnetic field have been the focus of many research works.

Fe_3O_4 nanoparticles can be prepared using different ways such as laser ablation, mechanical grinding, chemical precipitation, high temperature decomposition of organic precursor, and microelmusion (Sun et al., 2004). The most employed method is chemical precipitation due to their low cost and simplicity preparation. This method involves mixing ferrous (II) and ferric (III) ions in molar ratio of 1:2 in highly basic solution at elevated or room temperature (Cabrera et al., 2008). **Equation 2.1** explains the synthesis route of magnetite nanoparticles through co-precipitation process.

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O \quad \textbf{(2.1)}$$

2.1.2 Surface modification of MNPs

Basically, MNPs tend to agglomerate due to their high surface energies resulting from their large surface area and volume ratio. Besides, unmodified MNPs also easily undergo oxidation which can cause the loss in their magnetic and dispersibility property, as well as difficulty to control their shape, size, and stability.

To address this problem, surface modification has been introduced to the design and fabrication of Fe_3O_4 with specific functionality and activity. Surface modifications are not only able to improve the stability of MNPs and their dispersion, but also enhance their compatibility in solid, liquid or biological matrices. In addition, surface modification also provides channel for further immobilizing, grafting and coating of additional organic or inorganic moiety (R ós & Zougagh, 2016). Surface modification generally involves three steps, including the synthesis of core (magnetite or maghemite), the coating of the magnetic core and the modification of the resultant

core-shell structure. **Figure 2.1** shows different types of shells and functional groups or final coatings that can be anchored/coated onto the MNPs surfaces.

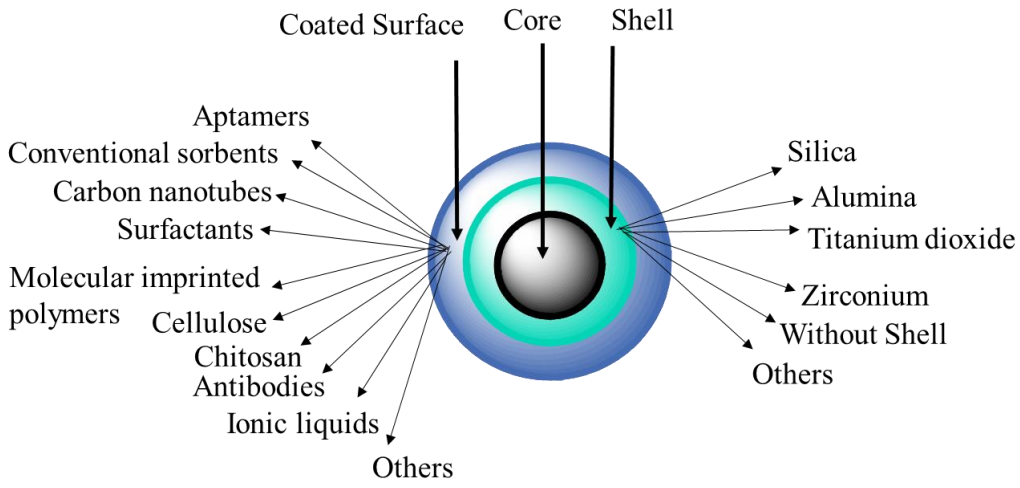

Figure 2.1: Structure of the core, shell of various composition, and the coating with different functional groups (R ós & Zougagh, 2016)

2.1.3 MNPs in analytical chemistry

In the context of analytical chemistry, modified MNPs have been widely applied in sample preparation, analyte separation and electrochemical sensing.

2.1.3(a) Modified MNPs in sample preparation

Lately, magnetic solid phase extraction (MSPE) has become a favorable technique among researchers because it simplifies the overall extraction procedures. Instead of packing the sorbent into SPE cartridge, the sorbent are being dispersed in the sample solution. This greatly facilitate the extraction rate due to better surface area of contact between analyte and sorbent. Furthermore, MSPE also avoid laborious steps such as filtration or centrifugation as the sorbents can be easily collected by using an external magnet. The applications of modified MNPs in MSPE are tabulated in **Table 2.1**.

Table 2.1: Selected examples of modified MNPs used in MSPE

Core/Shell	Functional group/ Coating	Analyte	Sample	Reference
Fe_3O_4/SiO_2	2-mercapto-4-methyl-5-thiazoleacetic acid bonding Au (MMTA-Au)	polycyclic aromatic hydrocarbons	river water and rainwater	(Xiupei et al., 2019)
Fe_3O_4	ferric sulfate hexahydrate and ferric chloride hexahydrate	polycyclic aromatic hydrocarbons	produced water from wells	(De Barros Caetano et al., 2019)
Fe_3O_4	-	rivastigmine	bulk and capsules	(Mohamed et al., 2019)
Fe_3O_4	curcumin loaded magnetic graphene oxide	parabens	toothpaste and mouthwash samples	(Razavi & Es'haghi, 2019)
Fe_3O_4@APTES	silicone-ethylene-oxide copolymer (DC193C)	parabens	river water, seawater and lake water	(Marinah et al., 2019)
Fe_3O_4	polydopamine	human genomic DNA	human whole blood	(Min et al., 2019)
Fe_3O_4@APTES	polyamidoamie dendrimer	tetrabromo- bisphenol A and 4-nonylphenol	environmental water	(Yalin et al., 2019)
Fe_3O_4/SiO_2	mesoporous silica nanoparticles (MCM-41)	biogenic amines	traditional dairy product	(Molaei et al., 2019)
Fe_3O_4	reduced graphene oxide (rGO)	phenolic compounds	oilseeds	(Lang et al., 2019)

2.1.3(b) Modified MNPs in separation science

Modified MNPs have also been studied in improving the resolution of electrophoretic and chromatographic separations. MNPs are typically being modified into stationary or pseudostationary phases which can achieve better outcomes compared to traditional phases. This can be attributed to their high adsorption capacity, longer lifetime and excellent surface chemistry. **Table 2.2** presents the application of MNPs in analyte separation.

Table 2.2: Selected examples of modified MNPs used in separation science

Material	Functional group/ Coating	Detection	Analyte	Reference
Molecularly imprinted magnetic nanoparticles	methacrylic acid and ethylene glycol dimethacrylate on 3-(methacryloyloxy)propyltrimethoxysilane	CE	racemic ofloxacin	(Qu et al., 2010)
Carboxyl functionalized magnetic cores	UiO-67 metal–organic frameworks	HPLC	phenol	(Weiwei et al., 2015)
Magnetic nanoparticles	β-cyclodextrin and mono-6-deoxy-6-(1-methylimidazolium)-β-cyclodextrin tosylate	CE	dansylated amino acids	(Xuan et al., 2019)

2.1.3(c) Modified MNPs in electrochemical sensing

Another important application of MNPs is in electrochemistry to enhance the electrochemical detection of analytes. Low electronic transmission resistance, large surface area, and ability to detect biological and chemical compounds are among the advantages offered by MNPs-modified electrodes. Moreover, the application of MNPs in the electrode composite also helps to reduce the surface deterioration, to increase the electrocatalytic activity and to simplify the immobilization process of functional molecules. **Table 2.3** summarizes some of the applications of MNPs in electrochemical sensors.

Table 2.3: Selected examples of modified MNPs used in electrochemical sensor

Magnetic Material	Detection	Analyte	Reference
Enzyme modified magnetic nanoparticles	amperometric	glucose	(Sheng et al., 2012)
Ferromagnetic carbon encapsulated iron nanoparticles	linear scan voltammetry and electrochemical quartz microbalance	haemoglobin	(Matysiak et al., 2015)
Chitosan coated Fe_3O_4 magnetic nanoparticle	amperometric	morphine	(Dehdashtian et al., 2016)
Magnetic nanoparticles decorated with monoclonal antibodies	linear scan voltammetry	transferrin	(Matysiak et al., 2017)
Magnetic nanoparticle decorated graphene	cyclic voltammetry	hydrogen peroxide	(Waifalkar et al., 2018)
Silica embedded magnetic nanoparticles	amperometric	nitrite	(Ispas et al., 2018)
Zinc iron oxide modified screen printed electrode	amperometric	sertraline	(Tajik et al., 2019)
Haemoglobin immobilized with magnetic molecularly imprinted nanoparticles	cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscope	3-chloro-1, 2-propandiol	(Yuan et al., 2019)

2.2 Cyclodextrin

2.2.1 Properties of cyclodextrin

Supramolecular chemistry is a discipline in chemistry that has recently received a great deal of attention. It provides a thorough understanding that there are no covalent bonds between the interacting species as most interactions involve host-guests interaction. Among various hosts, cyclodextrins (CDs) have been recognised as the most popular host as it can fit various types of guest molecules with suitable polarity and dimensions in their cavities (Ain et al., 2015; Malefetse et al., 2009; Raoov et al., 2013, 2014; Sambasevam et al., 2013; Sharifah et al., 2011; Singh & Bharti, 2010; Surikumaran et al., 2014). Example of guests molecules include straight or branched chain of aliphatics, aldehydes, ketones, alcohols, ionic liquid, organic acids or fatty acids, and polar compounds such as amines, oxyacids, and halogens (Charoensakdi et al., 2007). Due to their versatile nature, CDs are readily modified or functionalized in order to improve their complexation capability (Song & Purdy, 1992), leading to remarkable advances in analytical applications.

CDs belong to the series of macro cyclic torus-shaped oligosaccharides which are compose of α (6), β (7), and γ (8) -CD (**Figure 2.2**) connected by α-1,4 glucopyranose units) (**Figure 2.3**). Due to the presence of carbon and hydrogen atoms, they have a hydrophobic interior cavity (**Figure 2.4**) and this feature enables them to host various hydrophobic compounds in their cavities without having to form chemical bonds (**Figure 2.5**). Whereas the presence of hydroxyl groups on the outer part of the torus surface cause the exterior to be hydrophilic. β-CD is less soluble in water compared to to α-CD and γ-CD. The is because in α-cyclodextrins, the hydrogen belt is incomplete since one of the glucopyranose unit is distorted and only four out of six possible H-bonds can be formed. Meanwhile, γ-CDs have a non-coplanar and flexible

structure causing it to be the most soluble among the three CDs. Several characteristics of the three different CDs are summarize in **Table 2.4**.

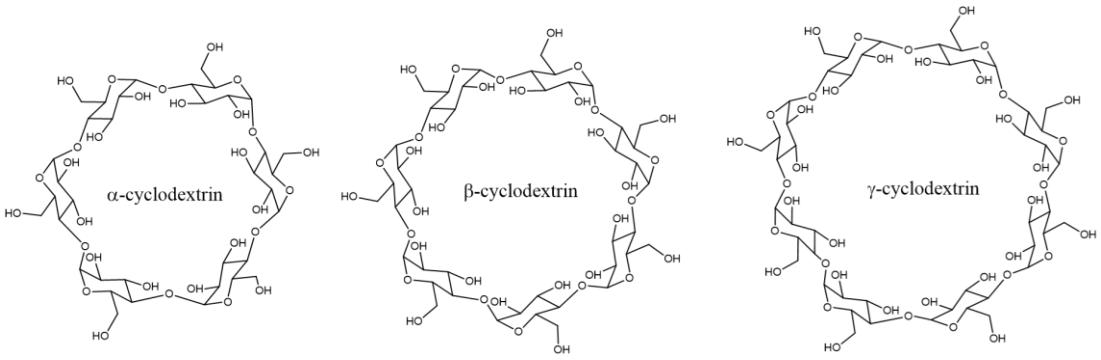

Figure 2.2: Chemical structure of α-CDs, β-CDs and γ-CDs

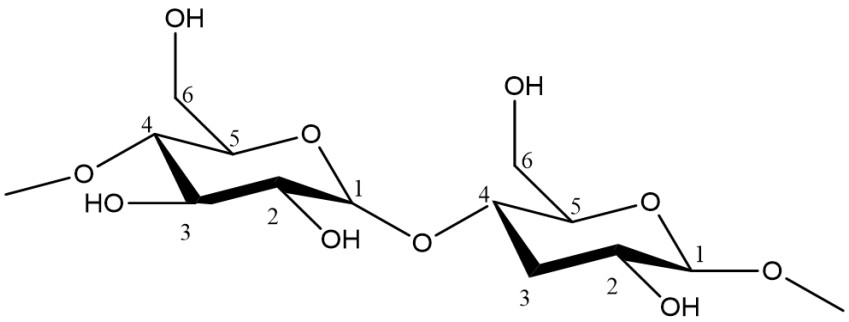

Figure 2.3: α-1,4 glucopyranose units linkages and the numbering system in a CD molecule

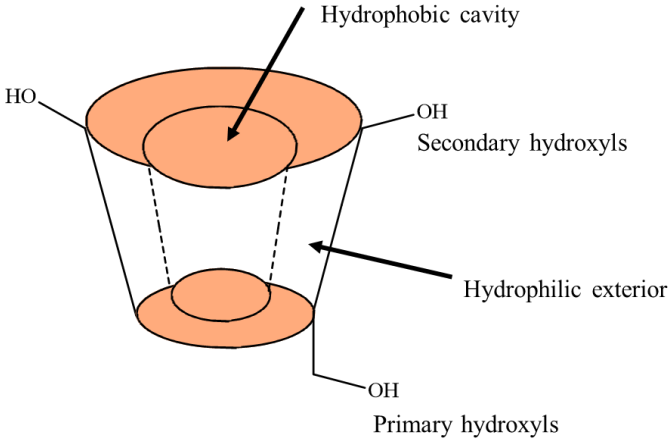

Figure 2.4: Hydrophobic cavity and hydrophilic exterior of a CD molecule

Table 2.4: Characteristics of α, β, γ- cyclodextrins (Szejtli, 1988)

Cyclodextrin	Molecular weight ($g\ mol^{-1}$)	Solubility in water at 25°C (g/100 mL)	Volume of cavity ($Å^3$)	Cavity diameter (Å)	Outer diameter (Å)	Torous height (Å)
α	972	14.5	174	4.7-5.3	14.6	7.9
β	1135	1.85	262	6.0-6.5	15.4	7.9
γ	1297	23.2	427	7.5-8.3	17.5	7.9

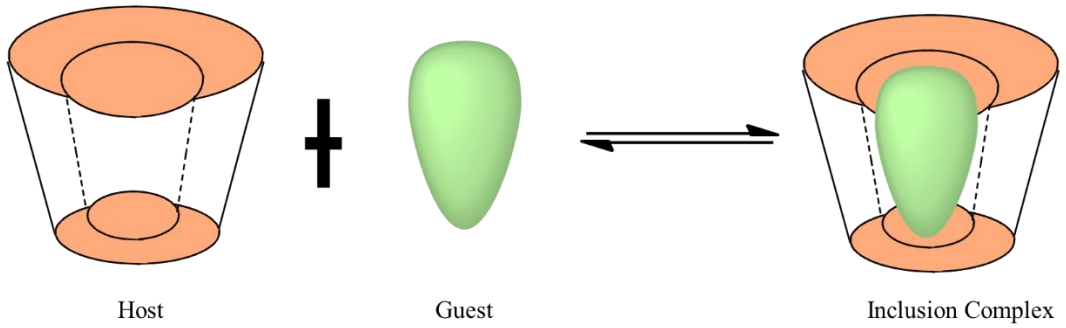

Figure 2.5: Schematic illustration of the association of free host (cyclodextrin) and guest molecules to form inclusion complex

2.2.2 Toxicological considerations

Generally, natural CDs and their hydrophilic derivatives can hardly permeate through biological membranes which are lipophilic such as the eye cornea (Del Valle, 2004). Besides, toxicity tests have shown that orally ingested CDs is essentially harmless because absorption through gastrointestinal tract is unlikely to occur (Irie & Uekama, 1997).

2.2.3 Cyclodextrin derivatives

2.2.3(a) Modification reactions of cyclodextrins

CDs can be chemically modified to enhance both their physical and chemical properties. They can be modified by one of these four methods (Szejtli, 1988):

(a) Substitution of one or more H-atom of the primary and/or secondary hydroxyls

(b) Substitution of one or more primary and/ or secondary hydroxyls

(c) Elimination of the hydrogen atom from the C5-CH_2OH group

(d) Splitting one or more C2 and C3 bonds by periodate oxidation

There are two important factors that need to be taken into consideration when modifying cyclodextrin: the nucleophilicity of the hydroxyl groups at the C2-, C3- and C6 carbon and the tendency of cyclodextrins to form complexes with the reagent used. Among the hydroxyl groups, C6-OH is the most basic and nucleophilic, whereas, C2-OH is the most acidic and C3-OH is the most inaccessible. Consequently, electrophilic reagents will initially and primarily attack the C6-OH groups. However, more reactive groups will attack C6-OH groups and also with C2-OH and C3-OH. Apart from that, the orientation, structure, and the strength of the intermediate complex is also affected by the size of the cyclodextrin cavity (Hattori & Ikeda, 2007).

2.2.3(b) Mono-modification at the C6-position

Mono-modification of CDs involves regioselective reaction of only one targeted hydroxyl group, while per-modification involves the whole set of hydroxyl groups (Sutyagin et al., 2002). In mono-modification, C6 carbon normally will be targeted. An example modification is the reaction of β-CD with tosyl chloride in an

aqueous alkaline medium to give mono-6-tosylated β-CD. During the modification, the electrophilic tosyl chloride attacks the primary side C6-OH groups of β-CD by substituting one H-atom to form C6-tosyl-β-CD as shown in **Figure 2.6**.

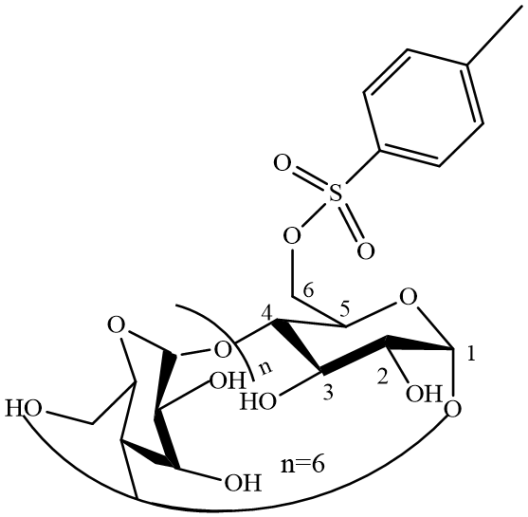

Figure 2.6: Tosylation of β-CD to form C6-tosyl- β-CD

It is interesting to point out that C6-tosyl-β-CD are important precursors for a variety of modified CDs because the tosylate group can be easily replaced by nucleophile (e.g. halogens, azides and acetates) (Mahlambi et al., 2011) that can contribute its corresponding functionality to the β-CD (Hattori & Ikeda, 2007).

2.3 Ionic liquid

Ionic liquids (ILs) are referred as liquefied organic salts at room temperature (RTIL) and are poorly coordinated. The first IL was synthesized in 1914 but its importance in the chemistry was not realised until the late 70s and 80s. Generally, ILs consists of organic cations and inorganic anions. They are designed to have a delocalized charge and one component is organic in order to prevent the formation of stable crystal lattice (Devi, 2015). ILs have been labelled as green recyclable alternatives to the conventional organic solvents and are developed to be environmentally friendly in line with the concept of sustainable chemistry (Devi, 2015; Hallett & Welton, 2011; Hernández-Fernández et al., 2010; Rahim et al., 2011; Zaijun et al., 2011). They are widely used in analytical chemistry due to their fascinating properties such as non-flammability, non-volatility, high polarity, low viscosity, and great electrochemical stability. In addition, ILs can be tuned to have desired properties by altering the combinations of cationic and anionic constituents. Because of this versatile nature, ILs are regarded as "designer solvents" and finds use as extraction solvents, catalysts, electrode modifiers (Anping et al., 2011; Mohamad et al., 2015; Mohd Rasdi et al., 2016; Sinniah et al., 2015; Yu et al., 2013), modified sorbents in sample pre-treatment and modified chiral stationary phases or additives in separation chemistry (Galán-cano et al., 2013; Huang et al., 2010; Jieping & Zhu, 2016; Jingjing et al., 2011; Miyi et al., 2016; Songqing et al., 2016; Tokalıoğlu et al., 2016; Zhiming et al., 2010).

Common examples of cations and anions that have been investigated with regard to ionic liquid phase formation are shown in **Figure 2.7**.

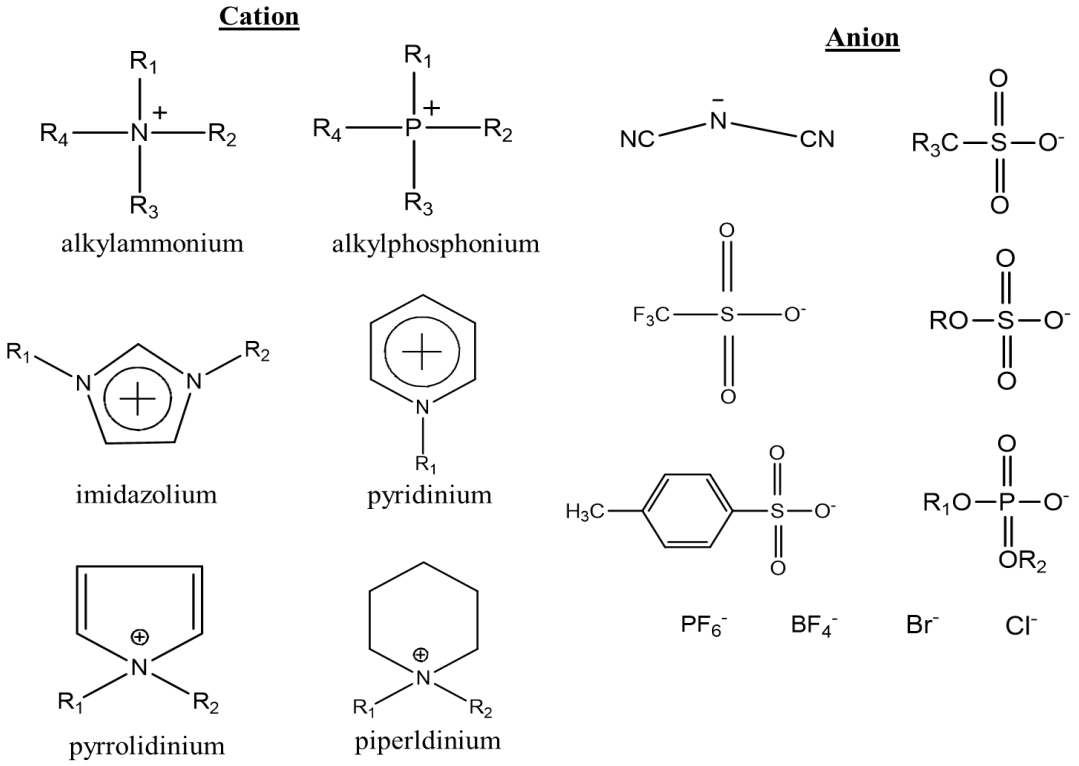

Figure 2.7: Common structures of cations and anions (Hua & Baker, 2013)

2.4 Cyclodextrins and ionic liquids-based material in analytical chemistry

Presently, the combination of CDs and ILs as advanced novel materials has received much interests as versatile tool in analytical applications. When CD-IL complexes are used, such as, in separation studies, the functional group of the ionic liquid is able to improve the extraction performance of cyclodextrin besides retaining the hydrophobic cavity of cyclodextrin molecule (Raoov et al., 2013). The behaviour of these materials are mainly dependant on the separation mechanism, which cover multiple interactions (electrostatic, hydrophobic, and $\pi - \pi$). Generally, there are two types of CD-IL complex formation processes known as functionalization and physical loading process as illustrated in **Figure 2.8**.

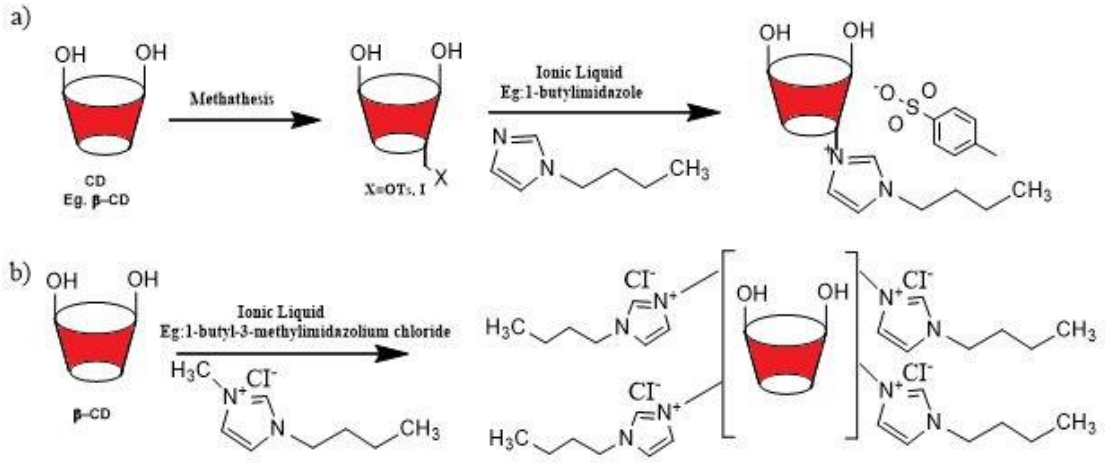

Figure 2.8: Formation of CD-IL complex through (a) functionalization process (b) physical loading process

The following part describes some applications of CD-IL materials in various separation techniques such as capillary electrophoresis (CE) (**Table 2.5**), high-performance liquid chromatography (HPLC) (**Table 2.6**), gas chromatography (GC) (**Table 2.7**), and sample preparation techniques including cloud point extraction (CPE) (**Table 2.8**), solid phase extraction (SPE) (**Table 2.9**), and microextraction (ME) (**Table 2.10**), as well as application in electrochemical sensor (**Table 2.11**). Furthermore, CD-IL modified magnetite materials also have been listed **Table 2.12**, accordingly.

Based on the reported literatures, it is interesting to point out that CD-IL based materials have combined the unique properties of cyclodextrin, ionic liquids, and magnetic nanoparticles. Therefore, they should deserves more considerations when developing excellent materials, particularly in analytical chemistry.

Table 2.5: CD-IL modified materials in CE

Type of IL	Type of CD	Analytes	Sample	BGE/Buffer	References
1-hexyl-3-methylimidazolium	α-CD	metal ions and ammonium	standard stock solution	7.5 mM lactic acid, 0.6 mM 18-crown-6, 12 mM α-CD at pH 4.0	(Qin & Li, 2004)
1-butyl-3-methylimidazolium tetrafluoroborate	β-CD	anthraquinones	chinese Herb (*Paedicalyx attopervensis Pierre ex Pitard)*	1B-3-MI-TFB [20-80 mM] and β-CD [1-6 mM]	(Qi et al., 2004)
Alkylimidazolium chlorides where alkyl= methyl, butyl, decyl, 1,2-dimethyl	β-CD	dansyl amino acid	standard solution	acid BGE at pH 6.0 and basic BGE at pH 9.6 phosphate/acetate buffer	(Ong et al., 2005)
Ethyl and phenylcholine of bis(trifluoromethylsulfonyl)imide	Di or trimethyl-β-CD	2-arylpropionic acids	anti-inflammatory drugs	acetic acid/sodium acetate (5 mM and 6 mM) at pH 5.0	(François et al., 2007)
3-methylimidazolium chloride	β-CD	dansyl amino acids	standard solution	50 mM of NaH_2PO_4 titrated with NaOH or H_3PO_4 at pH (6.5–9.6)	(Tang et al., 2007)

Table 2.5 (continued)

Alkylimidazolium chloride where alkyl= methyl, ethyl, propyl, butyl, and hexyl	β-CD	dansyl amino acid	standard solution	50 mM acetic acid in deionized water at pH 5.0 and 6.0	(Weihua et al., 2007)
N-undecenoxycarbonyl-L-leucinol bromide (L-UCLB)	2,3,6-tri-o-methyl-β-CD	ibuprofen, fenoprofen, indoprofen, suprofen, and ketoprofen	standard solution	35 mM TM-β-CD, 5 mM sodium acetate at pH 5.0	(Wang et al., 2009a)
N-undecenoxycarbonyl-L-leucinol bromide (L-UCLB)	2,3,6-tri-o-methyl-β-CD	ibuprofen, fenoprofen, indoprofen, suprofen, and ketoprofen	standard solution	5 mM NaOAc at pH 5.0 with TM-β-CD and without TM-β-CD	(Wang et al., 2009b)
6-O-2 hydroxpropyltrimethylammonium tetrafluoroborate	β-CD	bifonazole brompheniramine chlorpheniramine, liarozole pheniramine, promethazine, tropicamide, warfarin	eight racemic drugs	NaH_2PO_4 (pH 2.5-5.0)	(Jia et al., 2013)
L-alanine and L-valinetert butyl ester bis (trifluoromethane) sulfonimide	Me-β-CD, HP-β-CD, and Glu-β-CD	naproxen, pranoprofen,warfarin, carprofen, ibuprofen and ketoprofen	standard solution	30 mM sodium citrate/citric acid buffer containing organic modifier (20%v/v)	(Jinjing et al., 2013)