Competitive CE-UV Binding Tests For Selective Recognition Of
Bisphenol A By Molecularly Imprinted Polymer Particles

By

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Abstract

Capillary electrophoresis with ultraviolet detection (CE-UV) was used to perform competitive binding tests to demonstrate the selective recognition of bisphenol A (BPA) by molecularly imprinted polymer (MIP) particles. Cross-linking polymerization of methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) in the presence of BPA yielded MIP particles with an average diameter of 164 ± 15 nm. Their ability to recognize BPA in the presence of non-ionic, anionic and cationic water contaminants was investigated. Binding efficiency was rapidly determined, after sequential injection of particles first and compounds next into the fused silica capillary provided a short overlapping time during their electrophoretic migrations. The MIP particles exhibited high binding efficiency ($99 \pm 1\%$) for BPA, which was not kinetically influenced by the applied voltage. Neither diclofenac nor metformin affected BPA binding, and 2-hydroxy-4-methoxybenzophenone was even displaced from the particles by BPA. These results verified the high selectivity of MIP towards its target compound. Rapid CE-UV desorption test was successfully demonstrated using 5 % TEA in methanol. Reusability of MIP particles over several adsorption cycles without regeneration of the particles by desorbing the already bound analytes was confirmed as MIP particles can still remove a fairly high percent of BPA in the next two injections (2nd and 3rd) even in the presence of large quantities of HMB.

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In the Name of God, All- merciful, All- compassionate

“Almighty God, the uninterrupted flow of Thy graciousness has distracted me from thanking Thee. The flood of Thy bounty has rendered me incapable of counting Thy praises. The succession of Thy kind acts has diverted me from mentioning Thee in laudation. My God, my thanking is diminutive in front of Thy great boons, and my praise shrinks beside Thy generosity toward me. Your favors are many, and my understanding falls short of grasping them, not to speak of exhausting them! So how can I achieve thanksgiving! Praise belongs to God; praise whose bound has no utmost end, whose number has no reckoning, whose period cannot be cut off”

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List of Symbols And Abbreviations

AIBN	2,2'-azobisisobutyronitrile
BPA	bisphenol A
CE	capillary electrophoresis
DDW	deionized distilled water
DFC	diclofenac
DLS	dynamic light scattering
EDCs	endocrine disrupting compounds
EGDMA	ethylene glycol dimethacrylate
HMB	2-hydroxy-4-methoxybenzophenone
LOD	limit of detection
LOQ	limit of quantification
MAA	methacrylic acid
MF	metformin
MIPs	molecularly imprinted polymers
MO	mesityl oxide
NIPs	non-imprinted polymers
PPCPs	pharmaceutical and personal care products
SEM	scanning electron microscopy
SPE	solid phase extraction
TEA	triethylamine
TFA	trifluoroacetic acid
UV	ultra violet

Chapter 1

Introduction

1.1 Introduction

Access to safe drinking water is essential to sustain life and maintain good health. As a consequence of the increased contamination of fresh water resources, demands for water-purifying treatments have been rising steadily over the past decade. Endocrine disrupting compounds (EDCs), along with pharmaceutical and personal care products (PPCPs), are toxic hazardous substances that have been detected in environmental waters at trace concentrations [1, 2]. These contaminants enter the aquatic environment due to incomplete removal during wastewater reclamation processes [3]. Bisphenol A (BPA) (2,2-bis(4-hydroxyphenyl)propane) is widely used as the building block in plastics, epoxy, and fire retardant supplies [4]. It has been at the center of media attention due to scientific paper claims against its potent estrogenic activity [5, 6]. It is a toxic compound according to Section 64 of the *Canadian Environmental Protection Act*, 1999 [7] and reported to have a hazard quotient of 2.24 at a concentration of 1.6 mg/L in river water [8]. The National Toxicology Program expressed several concerns regarding BPA effect on the brain, behavior, and prostate gland in fetuses, infants, and children [9]. 2-hydroxy-4-methoxybenzophenone (HMB) is a UV filter that is commonly found in cosmetic products such as creams, sunscreen lotions, moisturizers, shampoos, hair sprays, hair dyes and lipsticks [10]. It can cause cholestatic liver injury and renal damage in rats, as declared by the US National Toxicology Program [11]. Diclofenac (DFC) (2-[(2,6-Dichlorophenyl)amino] benzoic acid) and metformin (MF) (1,1-dimethylbiguanide) are non-steroidal anti-inflammatory and anti-diabetic drugs, respectively. Due to their extensive use, DFC is frequently detected in aquatic environments at concentrations as high as 15 µg/L and MF is detected at concentrations higher than 150 ng/L [12]. All these compounds can be released into

aquatic environments during production, use or disposal of consumer products [13]. Hence, it is crucial to develop a treatment technology that can efficiently remove these contaminants from water to alleviate potential threats to human health [14].

Solid phase extraction (SPE) is an effective water treatment technology that allows adsorption of EDCs and PPCPs from environmental waters onto solid polymeric materials [15]. SPE may analytically be performed on-line, coupling to gas chromatography, liquid chromatography [16] or capillary electrophoresis [17] systems. Other analytical techniques have utilized MIPs as selective sorbents for target analytes in environmental and biological samples [18], including immunoassays [19], quartz crystal microbalance [20], surface plasmon resonance [21], and fluorescent probes [22].

MIP synthesis is based on thermal or photo-induced polymerization between a functional monomer and a cross-linker in the presence of a template. Washing the polymer with a proper solvent will create binding cavities with high affinity towards the target analyte. MIPs possess great chemical stability, reusability, versatility in the choice of functional monomers and cross-linkers, and simplicity in preparation. In comparison to synthetic aptamers and natural antibodies, MIPs are considered to be of low cost and to have long shelf life [23].

Numerous techniques have been reported in the literature for the characterization of MIP particles. The morphology of MIP particles can be examined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) [24]. The verification of functional groups on MIP particles is typically done using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR). Their specific surface area (m^2/g), specific pore volume (cm^3/g) and pore size distribution are measured using the Brunauer Emmett Teller

(BET) method and nitrogen adsorption [25]. MIP particles can be characterized using gel permeation chromatography [26], dynamic light scattering (DLS) and capillary electrophoresis (CE) [27].

CE offers various advantages in comparison to other analytical techniques. These comprise of simplicity in operation, minute sample volumes, minimal consumption of environmentally unfriendly organic solvents, various separation modes, shorter analysis time, and most importantly impressive resolution of complex mixtures [28]. Characterization of MIPs and magnetic nano-particles had been successfully developed in our research group using CE [29]. The objective of this M.Sc. research was employing capillary electrophoresis with ultraviolet detection (CE-UV) to rapidly perform competitive binding tests in order to demonstrate the selective recognition of bisphenol A (BPA) by MIP particles. The rapid binding test requires only nano-liter volumes of particles and compounds for sequential injection. In this work, BPA was chosen as the template molecule for MIP preparation. As a target compound, its selective recognition by the MIP particles was evaluated using HMB as a structurally related compound. In addition, DFC and MF were chosen as potential interfering compounds that are negatively and positively charged in water, including the background electrolyte used for CE-UV analysis. Chemical structures and pK_a values of the target compounds and reagents used are shown in Table 1.1

Analyte (abbreviation)	Chemical Structure	pK _a
Bisphenol A (BPA)		9.6 [30]
2-hydroxy-4-methoxybenzophenone (HMB)		7.5 [31]
Diclofenac (DFC)		4.1 [32]
Metformin (MF)		12.4 [33]
Triethylamine (TEA)		10.7 [34]
Trifluoroacetic Acid (TFA)		0.6 [35]

Table 1.1 Chemical structures and pK_a values of the target compounds and reagents used.

1.2 Molecular Imprinting

Molecular imprinting is a highly selective recognition system that relies upon the formation of a complex between a molecular template and functional monomers, which are subsequently fixed via a polymerization reaction with cross-linker monomers. Removal of the template molecules reveals imprinted cavities complementary in shape and functionality to the template molecule [36]. In aqueous and non-aqueous environments, ideal MIP particles are the outcome of good spatial orientation of functional groups for molecular interactions [37]. MIP particles are commonly prepared via the non-covalent approach that was first developed by Mosbach [38]. In this approach, interaction between the template molecule and functional monomers takes place via hydrogen bonds, ion pairs, and hydrophobic interactions as well as van der Waals forces. This results in the formation of complexes with high stabilities [39]. Figure 1.1 presents a schematic of the non-covalent approach in MIP synthesis.

Figure 1.1 Schematic of the non-covalent approach in MIP preparation [40].

Highly cross-linked MIP particles designed for the selective extraction of trace analytes are becoming more and more commercially available. AFFINIMIP® SPE, made and marketed by POLYINTELL, are solid phase extraction sorbents prepared by the molecular imprinting technique for sample cleanup and analyte pre-concentration prior to chromatographic analysis. POLYINTELL has developed MIPs specific to various analytes of interest including patulin, zearalenone, ochratoxin A, fumonisins, BPA, estrogens, phenols, chloramphenicol, amphetamines, metanephries, catecholamines, tamoxifen and carboxylic acids. AFFINIMIP® SPE bisphenol A cartridge has various advantages including high selectivity for BPA, chemical and thermal stability, good compatibility with all organic solvents and cost-effective. Selective extraction of bisphenol A from milk samples was carried out with a high recovery (> 80%). Quantification of BPA was demonstrated at 1 µg/L with fluorescence detection. The SPE protocol is summarized in Figure 1.2. It took almost 50 min to complete the SPE procedure as designed by the company.

Figure 1.2 SPE protocol for sample pre-concentration using AFFINIMIP® SPE Bisphenol A cartridges [41].

1.2.1 Functional Monomers

Functional monomers are selected to complement the template's functional groups. They should be available in large proportion to form binding cavities with the template molecules. Extra molecules of functional monomers remain throughout the MIP providing additional non-specific binding sites [42]. Basic groups on the template interact with acidic groups on the monomers, and vice versa, to form the complex [43]. The most common monomer used is methacrylic acid (MAA) since it contains a carboxylic group that functions as a hydrogen bond donor and acceptor.

Figure 1.3 Chemical structure of MAA.

Complex formation between BPA and MAA has been investigated by Pan *et al* using ^1H -NMR. They found that the chemical shift of $-\text{OH}$ protons of BPA and MAA were at 9.21 and 12.41, respectively. Upon the addition of BPA to MAA in a molar ratio of 1: 2, the chemical shift of $-\text{OH}$ protons of BPA and MAA decreased to 9.13 and 12.25, respectively, indicating that $-\text{OH}$ group of BPA was involved in the formation of hydrogen bonds with carbonyl groups of MAA as illustrated in Figure 1.4 [44].

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Figure 1.5 Molecular interactions between BPA and MAA in a molar ratio of 1:2 [44].

1.2.2 Cross-linker monomers

The rigidity, morphology, binding affinity and selectivity of MIP are strongly dependent on the proportion of cross-linker, which is typically 60-70% [45]. Also, cross-linker monomers join the functional monomers to create the binding cavities [39]. Carbonyl groups of the cross-linker could also appear on the surface of the polymer providing additional non-specific binding sites [42]. Ethyleneglycol dimethacrylate (EGDMA) is the most commonly used cross-linker in MIP synthesis as it provides sufficient wettability in most rebinding media, for rapid mass transfer [46], enough flexibility to allow for diffusion of the template, and optimum hardness to maintain the cavity shape after removal of the template [47].

Figure 1.6 Chemical structure of EGDMA.

1.2.3 Porogen

Selectivity of MIP particles is greatly influenced by the interaction energy between the template and functional monomers. When the porogen has poor hydrogen bonding ability, the interaction energy is mainly affected by the dielectric constant of the porogen. On the contrary, when the porogen has a strong ability to form hydrogen bonds, both the porogen's dielectric constant and hydrogen bonding ability will influence the interaction energy. Aprotic solvents (such as acetone, acetonitrile and chloroform) are commonly used in MIP synthesis since they have small dielectric constants, thereby increasing the probability of complexation between the template and functional monomers [37]. Larger volume of the porogen is more favorable to increase the porosity and total surface area of the polymer [23].

1.2.4 Initiators

Free radical polymerization is commonly used in MIP preparation due to its electrical neutrality. The relatively non-specific nature of free radical interactions makes the free radical polymerization the most useful. When the polymerization temperature is increased, the initiator starts to decompose forming free radicals with high reactivity. Such reactivity should be controlled by temperature to allow enough time for the radicals to react with the monomers. If the rate of the initiator decomposition is too high, then a large concentration of reactive radicals would be generated leading to early termination of the polymerization process and production of a polymer of low molecular weight. On the contrary, if the rate is too low, then the polymerization will not reach completion within a reasonable time frame [48]. 2,2'-azobisisobutyronitrile (AIBN) is the most commonly used initiator for methacrylate-based MIP preparation. It decomposes thermally at 60°C or via UV radiation as

shown in Figure 1.8.

Figure 1.7 Chemical structure of AIBN.

Figure 1.8 Formation of free radicals after decomposition of AIBN

1.2.5 Influence of Template –Monomer Complex Rigidity

The shape of the template itself is of high importance to create the steric complementarity for efficient recognition in the presence of other molecules [49]. It has been found that templates with branched structures produce MIPs with higher selectivity than their counterparts with straight-chain structures [50]. Selectivity of MIP particles can be further enhanced with increasing stability of the formed template-monomer complex [51]. As stated by Nichollas, the more conformationally defined the template and monomers, the more defined the recognition sites will be. Thus, upon rebinding process, the better the fit between the sites and the target

analyte, the less entropy will be lost due to conformational changes in the recognition sites and the analyte, creating MIPs of great affinity and selectivity [52].

1.3 Principles of Capillary Electrophoresis

Electrophoresis is the migration of ions in solution under the influence of an electric field. Cations are attracted to the negative electrode (cathode) whereas anions are attracted to the positive electrode (anode). In capillary electrophoresis, different ions migrate through the capillary at different speeds as they have different electrophoretic mobilities. Capillary electrophoresis provides unparalleled resolution in comparison to chromatography. An open tubular column eliminates multiple paths and thus reduces the plate height and improves resolution. There is no stationary phase in capillary electrophoresis, thereby eliminating the mass transfer term in the Van Deemter equation (1-1), which comes from the time needed for the analyte to equilibrate between the mobile and stationary phases. Longitudinal diffusion is the only source of peak broadening in capillary electrophoresis.

$$H = \underbrace{A}_{\text{Multiple paths}} + \underbrace{B/u}_{\text{Longitudinal diffusion}} + \underbrace{C u}_{\text{Equilibration Time}} \quad (1-1)$$

where H is the plate height (which is proportional to the variance of a peak), u is the linear flow rate, and A, B and C are constants for a given column/ capillary and stationary phase.

Capillary electrophoresis generates 50000- 500000 theoretical plates, which is an order of magnitude better performance than chromatography.

1.3.1 Electrophoresis

When placing an ion with charge q (coulombs) in an electric field E (V/m), the force on it is qE (newtons). The retarding frictional force $f u_{ep}$ is also influencing the movement of the ion in solution. When the frictional force equals the accelerating force, the ion rapidly attains a constant velocity ($f u_{ep} = qE$).

$$\text{Electrophoretic velocity: } u_{ep} = (q/f) E = \mu_{ep} E \quad (1-2)$$

where μ_{ep} is the electrophoretic mobility, which is the constant of proportionality between the ion velocity and the electric field strength. It is proportional to the charge of the ion and inversely proportional to the friction coefficient, f .

$$f = 6 \pi \eta r \quad (1-3)$$

where η is the viscosity of the solution, and r is the hydrodynamic radius. Hence, mobility is inversely proportional to the hydrodynamic radius. The greater the radius, the lower the mobility.

1.3.2 Electroosmosis

Above pH 2, the inside wall of a fused silica capillary is covered with silanol groups (Si-OH) that are negatively charged (SiO⁻). Figure 1.9 shows the electric double layer at the wall of the capillary, which composes of a fixed negative layer and excess cations nearby. The tightly adsorbed cations partially neutralize the negative charge on the wall. The mobile cations in the diffuse part of the double layer neutralize the remaining negative charges. When an electric field is applied, cations move toward the cathode and anions move toward the anode. A net momentum toward the cathode is produced by the excess cations in the diffuse part of the double layer. These cations drive a pumping action called electroosmosis and eventually

create a uniform electroosmotic flow (EOF) of the entire solution toward the cathode as shown in Figure 1.10.

Figure 1.9 Electric double layer composed of negatively charged silica surface and nearby cations [53].

Figure 1.10 Net electroosmotic flow (EOF) toward the cathode driven by the cations in the diffuse part of the double layer [54].

Electroosmotic mobility is the constant of proportionality between the electroosmotic velocity (u_{eo}) and the strength of the applied electric field (E).

$$u_{eo} = \mu_{eo} E \quad (1-4)$$

where

μ_{eo} is the electroosmotic mobility

$$u_{eo} = (L_d / t_{neutral}) \quad (1-5)$$

where L_d is the effective length of the capillary (to the detector) and $t_{neutral}$ is the migration time of a neutral marker

$$\text{Electroosmotic mobility } \mu_{eo} = u_{eo} / E = (L_d / t_{neutral}) / (V/L_t) \quad (1-6)$$

where L_t is the total length of the capillary.

The high resolution of capillary electrophoresis results from having uniform EOF, which if disturbed, can cause peak broadening. Joule heating produced by the flow of ions in the capillary causes the solution viscosity to decrease, hence disturbing the flat profile of the EOF. Therefore, the capillary inner diameter should be sufficiently small ranging from 20 to 200 μm to rapidly dissipate the generated heat [55].

1.3.3 Mobility

The apparent (observed) mobility, μ_{app} , of an ion is the sum of the electroosmotic mobility of the solution and the electrophoretic mobility of the ion.

$$\text{Apparent mobility} \quad \mu_{app} = \mu_{eo} + \mu_{ep} \quad (1-7)$$

Cations move along with the EOF direction. Therefore, μ_{ep} and μ_{eo} have the same sign, contributing a greater value to μ_{app} than μ_{ep} ($\mu_{app} = \mu_{eo} + \mu_{ep}$). On the contrary, anions move in the opposite direction of the EOF. Thus μ_{ep} and μ_{eo} have opposite signs, contributing a lesser value to μ_{app} than μ_{ep} ($\mu_{app} = \mu_{eo} - \mu_{ep}$). For a neutral analyte, its apparent mobility equals the electroosmotic mobility of the solution ($\mu_{app} = \mu_{eo}$).

The apparent mobility of an ion is the net velocity of the ion, u_{net} , divided by the electric field, E .

$$\mu_{app} = u_{net}/E = (L_d/t)/(V/L_c) \quad (1-8)$$

where t is the migration time of the ion.

The electrophoretic mobility, μ_{ep} , is the difference $\mu_{app} - \mu_{eo}$. Variance from run to run should not have an effect on the electrophoretic mobility of an analyte as it is measured in relation to a neutral marker, unless there are time dependent (non equilibrium) interactions of the analyte with the capillary wall [56].

1.4 Research Goals

The aim of this research was to develop a new CE-UV technique for rapid binding of organic contaminants onto MIP particles, and to explore their desorption. This new technique would facilitate the design and development of new MIP particles that were better capable of targeting individual pollutants in water treatment processes. Development of an MIP tailored for BPA using other widely available monomers was investigated to expand the scope of MIPs capable of extracting BPA from environmental waters. Reusability of these MIP particles over several adsorption cycles was examined in the treatment of different water samples. The objective was to explore their efficiency in removing BPA without desorption of the already non-specifically bound analytes. This would demonstrate the major advantage of MIP particles over other SPE sorbents. Our goal was to build a technology platform toward large-scale applications in water and wastewater treatment.

Chapter 2

Experimental

2. Experimental

2.1 Materials

Bisphenol A (BPA), 2-hydroxy-4-methoxy benzophenone (HMB), diclofenac sodium salt (DFC), metformin hydrochloride (MF), ethylene glycol dimethacrylate (EGDMA), and methacrylic acid (MAA) were all obtained from Sigma-Aldrich (Oakville, ON, Canada). 2,2'-azobis(2-isobutyronitrile) (AIBN) was bought from Pfaltz & Bauer (Waterbury, CT, USA). Triethylamine (TEA) was purchased from Fluka (Buchs, Switzerland). Trifluoroacetic Acid (TFA) was bought from Aldrich Chemical Company, Inc. (Milwaukee WI, USA). Sodium phosphate dibasic was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All chemicals were used as received without any further purification. HPLC grade methanol and acetonitrile, and spectro grade acetone, were all purchased from Caledon (Georgetown, ON, Canada).

2.2 Apparatus and Analytical Method

CE-UV analysis was performed on a laboratory- built system including a Spellman CZE1000R high voltage power supply (Hauppauge, New York, USA). Fused-silica capillary (51 mm i.d., 356 mm o.d.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary for CE was 53.5 cm in total length, and 46.1 cm in effective length to the detector. The background electrolyte (BGE) was composed of 10 or 20 mM Na_2HPO_4 in deionized distilled water (DDW) to attain pH 7.5 ± 0.2 or 9.5 ± 0.2 , respectively. Prior to initial use of a capillary, a typical conditioning proceduar includes 1 M NaOH, followed by 0.1 M NaOH and then the run buffer. A preparative conditioning that is performed either dialy or after extended use was done by flushing the capillary with pure methanol, 1.0 M HCl, 1.0 M NaOH,

deionized distilled water (DDW), and the run buffer. Prior to each use, the capillary was equilibrated with the run buffer at an applied voltage of 17 kV for 10 min. A Bischoff Lambda 1010 (Leonberg, Germany) UV detector was employed, at a wavelength of 200 nm, to detect the analytes and monitor their migration. This 200 nm of wavelength was selected based upon the absorption of BPA in the UV and visible regions. The detector output signal was acquired through a PeakSimple Chromatography Data System (SRI model 203, Torrance, CA, USA). The capillary was re-equilibrated by running the buffer at 17 kV for 1 min in between sample analyses to eliminate the possibility of inter-analysis carryover. The capillary inlet and outlet buffers were changed after every ten CE analyses to maintain the purity and level of BGE. The capillary inlet should be 2 mm away and below the electrode as shown in Figure 2.1 to improve both precision and baseline stability.

Figure 2.1 Schematic of the positioning of the capillary inlet and electrode in buffer vial [57].

2.3 Preparation of MIP and NIP Particles

BPA-imprinted polymer (MIP) and non-imprinted polymer (NIP) particles were prepared following a procedure developed by DeMaleki *et al* [27]. In a free radical cross-linking polymerization, BPA the template, methacrylic acid (MAA) the functional monomer and ethylene glycol dimethacrylate (EGDMA) the cross-linker (at a molar ratio of 1:8:7) were dissolved in the porogen acetone/acetonitrile (1:3 v/v). 2,2'-azobis-2-isobutyronitrile (AIBN), the initiator, was added to the pre-polymerization mixture (at 2% by weight of the total monomers) followed by sonication for 5 min to obtain a homogenous solution. Since oxygen annihilates free radicals generated from the thermal decomposition of the initiator that starts the polymerization process, deoxygenation by pure nitrogen gas for another 5 min is imperative [42]. The solution vial was then sealed and placed in a 60°C thermostatted water bath for 24 h without further mixing or shaking. NIP particles were similarly prepared only in the absence of BPA the template molecule. The polymerization mixtures turned to be milky white in color, yielding submicron particles.

2.4 Template Removal

Centrifugation of polymerization products was carried out at 10,000 rpm for 10-20 min and the supernatants were discarded. Both MIP and NIP particles were rinsed 3 times with an aliquot of 5 mL of acetonitrile (ACN) and then were dried overnight at 50°C. It is of high importance to carefully choose the proper eluent in order to achieve the highest extraction efficiency. It has been found that methanol cannot completely elute BPA, thereby it is necessary to add organic modifiers with the ability to disrupt the hydrogen-bond network formed between BPA, the template, and MAA, the functional monomer [58]. To reveal the binding cavities in MIP

particles, an aliquot of 5 mL of 5% TEA in methanol was used to wash out the template molecules and any residual monomers. After sonication for 10 min, the homogenous suspension was centrifuged for 10 min and then the supernatant was discarded. This washing step was repeated 6-8 times to ensure the complete removal of the template. Subsequently, the particles were rinsed three times with ACN to remove any TEA remained in the pores and imprinted cavities. Finally, the particles were dried overnight to be ready for pre-concentration applications.

2.5 Characterization of Particles by SEM and DLS

To investigate the size and shape of both MIP and NIP, dry particles were placed onto individual carbon tapes and coated with 6 nm of gold using an RF sputtering system (Hummer VII, Anatech, Union City, CA, USA). The particles were imaged at an accelerating voltage of 15 kV by scanning electron microscopy (SEM) using a Tescan Vega II microscope (Cranberry Twp., PA, USA). The average diameters of these particles were then confirmed by dynamic light scattering (DLS) using a Brookhaven Instruments nanoDLS particle size analyzer (Holtsville, NY, USA). For accuracy, particles' diameter was measured in 10 replicates, each of which lasts for 30 s.

2.6 Determination of LODs And LOQs of The Target Compounds

Analytical instrumentations have been used to detect, quantify and qualify compounds of interest. Detection involves a baseline reading (no analyte) and a signal generated by the analyte of interest. Baselines are not perfectly flat; they usually have mild deviations known as "noise." Limit of detection generally requires the analyte

signal to be three times greater than the "noise" whereas that of quantification should be 10 times higher. Thus, the limit of detection (LOD) is based on a signal-to-noise ratio of 3:1 and the limit of quantification (LOQ) is based on a signal-to-noise ratio of 10:1. Both were calculated for the target and interfering compounds after injection of their standard solutions at 15 kV for 3 s and detection at 200 nm of wavelength.

2.7 Effect of Particles' Injection Time on % Biding

10 mg/mL of MIP and NIP suspensions were prepared in 10 mM Na_2HPO_4 BGE (pH 7.5 ± 0.2), as well as the standard stock solution of 200 ppm (or mg/L) BPA. Working Standard solution of 100 ppm BPA was prepared when needed. To investigate the injected amount of MIP particles required to completely bind BPA at 3 s of injection, introduction of different injection times of MIP particles was carried out at 3, 12, 21, 30, 39, and 48 s. Same experiment was repeated for NIP particles.

2.8 Effect of Applied Voltage on % Binding

Binding of compounds with MIP (or NIP) is highly dependent on their relative mobilities inside the capillary, on top of their affinities to binding sites. CE-UV binding test was repeated under different applied voltages (15, 20, 25 and 26 kV) to study the mobility effect. NIP particles were injected for 48 s while BPA was injected for 3s. The time available for overlapping between particles and analytes during their electrophoretic migration, Joule heating, and BGE viscosity are strongly influenced by the applied voltage. Thus, these three aspects should be considered when studying the effect of applied voltage on % binding.

2.9 Effect of Current Flow on BPA% Binding

The effect of current flow on % binding was next investigated for a new capillary and an old capillary running at currents of 10 and 26 μA , respectively, studying the effect of particles and BPA mobilities on % binding. The new capillary was conditioned only once whereas the old one was conditioned several times. NIP particles were injected at 3, 12, 21, 30, and 48 s of injection time keeping the amount of injected BPA constant (3 s).

2.10 CE-UV Binding Tests

10 mg/mL of MIP and NIP suspensions were prepared in 10 mM Na_2HPO_4 BGE (pH 7.5 ± 0.2), as well as the standard stock solution of 200 ppm BPA. Working Standard solution of 100 ppm BPA was prepared when needed. Rapid in capillary binding tests were performed by sequential injection of particles first for 48 s and the target compound next for 3 s at 17 kV of applied voltage. An individual run of mesityl oxide (MO), the neutral marker, was carried out for ionic charge determination of the target compound. All binding tests were carried out in duplicates, and % binding was calculated as

$$\% \text{ binding} = [(\text{peak area before binding} - \text{peak area after binding}) / \text{peak area before binding}] \times 100. \quad (2-1)$$

2.11 Selectivity Evaluation of MIP Particles

To evaluate the selectivity of MIP particles toward BPA, the template molecule, competitive CE-UV binding tests were carried out. Standard stock solutions of 800 ppm of both DFC and MF were prepared in the BGE. Working standard solutions of

200 ppm DFC and MF were freshly prepared when needed. A standard stock solution of 5000 ppm of HMB was prepared in methanol because it is insoluble in water. A working standard solution of 200 ppm HMB was daily prepared by dilution with the BGE. Competitive in capillary binding tests were performed by sequential injection of particles first for 48 s and the target compounds next for 3 s. For ionic charge determination of the target compounds, an individual run of MO was carried out. MIP selectivity was tested using HMB as a structurally similar compound. DFC and MF were also tested as interfering compounds that carried negative and positive charges in the run buffer, respectively. All binding tests were carried out in duplicates. Since binding of compounds with MIP (or NIP) greatly depends on their relative mobilities inside the capillary, on top of their affinities to binding sites, CE-UV binding tests were repeated under different applied voltages (15, 17, 19 and 21 kV) to study the mobility effect.

2.12 CE-UV Desorption Test

To demonstrate the capability of CE in performing rapid desorption test, 10 mg/mL of BPA-MIP and 5 %TEA in methanol were used in this investigation. MIP particles were prepared exactly the same way except for the amount of BPA. This amount was increased from 1 mmol to 2 mmol to ensure the presence of plenty molecules of BPA in MIP particles. In capillary desorption tests were performed by sequential injection of BPA-MIP particles first and 5% TEA in methanol next for 3 s at 17 kV of applied voltage. 10 % trifluoroacetic acid (TFA) in methanol was next investigated to test its ability to elute BPA out of MIP cavities. An individual run of MO was carried out for ionic charge determination of TEA and TFA.

2.13 External Desorption Test

In capillary desorption test was then confirmed by performing an external desorption test. It was done by mixing 10 mg/mL of BPA-MIP particles with 5 % TEA in methanol or 10 % TFA in methanol externally in the same vial. After sonication for 5 min, the mixture was analysed by CE-UV. The mixture was injected for 3 s at 17 kV of applied voltage. MO run was carried out for ionic charge determination of the eluted peak.

2.14 Reusability of MIP Particles

Reusability of MIP particles is an important feature that warrants further research to demonstrate the major advantage of these particles over other SPE sorbents. The reusability of these particles is a key factor in cost- saving processes as it could open the opportunity for large-scale applications including water and wastewater treatments. MIP reusability was demonstrated by injecting MIP particles for 21 s followed by multiple injections of BPA, each for 3 s. Based on MIP migration time, up to 5 injections of BPA were carried out following MIP injection. Peak overlapping between NIP particles and BPA restricted the multiple injection of BPA up to 3 injections following 21 s of NIP injection.

2.15 Chemical Interference

To study the effect of the presence of an interfering compound on BPA % binding, first injection of MIP particles for 21 s followed by second injection of HMB for 3 s, third injection of BPA for 3s, and finally fourth injection of BPA for 3 s were conducted. To further investigate the effect of HMB on BPA % binding, gradual

increase of HMB injection time was carried out using 6, 9, 12, and 15 s of injection time. Same experiment was repeated for NIP particles.

Chapter 3

Results and Discussion

3. Results and Discussion

3.1 Characterization of MIP and NIP Particles' Sizes

Methods reported in the literature for MIP preparation did not always involve purification of all chemicals, but used the chemicals as supplied [42]. Monomer purification prior to use is intended to remove the polymerization inhibitor, which is typically present at ppm levels. Removal of the polymerization inhibitor is not always required because the use of an appropriate polymerization temperature often overwhelms any retardation by the inhibitor. In our work, the amount of initiator was optimally increased to obtain a satisfactory polymerization rate. Any residual initiator was washed out by rinsing the polymer with ACN.

Figure 3.1 SEM images of MIP and NIP particles.

The average diameter (or size) of MIP particles was first determined by SEM and then confirmed by DLS. The SEM images in Figure 3.1 illustrate the morphology, size and distribution of the prepared MIP and NIP particles. Their average diameters were 164 ± 15 nm and 187 ± 7 nm, respectively. The larger diameter

of NIP particles suggests that BPA, as the template molecule, had negative impact on particle growth during polymerization. DLS analysis obtained an effective diameter of 168 ± 4 nm for MIP particles and 184 ± 3 nm for NIP particles, as shown in Figure 3.2.

Figure 3.2 DLS analysis of MIP and NIP particle sizes.

3.2 Characterization of MIP and NIP Particles' Charges

CE-UV was not used as a separation technique for analytical determination of BPA in this study. Rather, it was used as a simple and rapid technique for performing competitive binding tests to demonstrate selective recognition of BPA, thereby facilitating the research and development of new MIP particles. The wavelength of UV detection for BPA was first optimized. As reported by Rosenfeldt and Linden [59], the molar absorptivity (ϵ) of BPA is 3.4×10^4 at 200 nm, 3.8×10^4 at 202 nm ($\lambda_{\max}$) and 0.4×10^4 at 278 nm as shown in Fig. 3.3. In previous CE-UV literature,

BPA was detected at 192 nm by Hu *et al* [60]. A very sharp peak was obtained in our CE analysis of BPA using 200 nm for optimal UV detection.

Figure 3.3 Molar absorptivities (ϵ) of BPA at different wavelengths

CE-UV analyses were performed by electrokinetic injection of MIP or NIP suspensions prepared in the BGE. The electrophoretic mobility would indicate the amount of positive or negative charges carried by the particles or analytes. It was calculated after subtracting the electroosmotic mobility of the solution from the apparent mobility of the particles or analytes [61]. MIP and NIP particles migrated after the neutral marker, yielding negative electrophoretic mobility values as summarized in Table 3.1. MIP particles are smaller in size (as determined by SEM and DLS) and more negatively charged (as demonstrated by CE-UV), giving a higher charge-to-size ratio in comparison with NIP particles. This higher ratio dragged MIP particles toward the anode (near the capillary inlet) resulting in slower migration toward the UV detection point (near the capillary outlet). Table 3.1 summarizes the

electrophoretic mobility results observed for all the particles and analytes, which basically correspond to their charge-to-size ratio values.

	Net Migration Time (min)	Molecular Weight (g/mol)	Electrophoretic Mobility ($\text{m}^2\text{V}^{-1}\text{s}^{-1}$)
MIP particles	8.1	-----	-2.13×10^{-06}
NIP particles	7.1	-----	-1.88×10^{-06}
Diclofenac (DFC)	5.9	318.13	-1.46×10^{-06}
Bisphenol A (BPA)	3.75	228.29	-1.03×10^{-07}
2-Hydroxy-4 methoxybenzophenone (HMB)	3.8	228.24	-1.03×10^{-07}
Mesityl oxide	3.7	98.14	0.00
Metformin (MF)	2.7	165.62	1.45×10^{-06}
Triethylamine (TEA)	3.7	101.19	0.00
Trifluoroacetic Acid (TFA)	11.6	114.02	-2.67×10^{-06}

Table 3.1 Electrophoretic mobility values of particles and analytes at pH 7.5±0.2.

3.3 CE-UV Binding Tests

Based on the electrophoretic mobility results in Table 3.1, it can be concluded that both BPA and HMB are neutral compounds since they migrated at velocities similar to that for mesityl oxide (MO) (the neutral marker). On the other hand, late migration of DFC implies its negative ionic charge in the BGE. MF migrated at earlier migration time than MO owing to the positive ionic charge(s) it carried.

Both the limit of detection and the limit of quantification were calculated after injection of standard solutions at 15 kV for 3 s and detection at 200 nm, as summarized in Table 3.2.

Analyte	C_{LOD} (S/N=3) ($\mu\text{g/mL}$)	C_{LOQ} (S/N=10) ($\mu\text{g/mL}$)
Bisphenol A (BPA)	0.1	0.3
2-Hydroxy-4-methoxy-benzophenone (HMB)	0.5	1.6
Diclofenac (DFC)	0.5	1.6
Metformin (MF)	0.5	1.6

Table 3.2 LODs and LOQs for BPA and interfering compounds.

The absorbance of an analyte depends linearly on its concentration and thus it is necessary to correct the absorbance value when comparing the spectra of different analytes. This corrected absorption value is called "molar absorptivity". The strength of absorbance of a molecule depends on the chemical nature of its chromophores. Table 3.3 shows the wavelength of maximum absorbance (λ_{max}) and the maximum molar absorptivity of target analytes. BPA has a maximum molar absorptivity at 202 nm of wavelength, which is very close to the chosen value of detection wavelength.

Hence, both the LOD and LOQ of BPA are much lower than the other analytes of interest.

Analyte	Wavelength of Maximum Absorbance ($\lambda_{\max}$) (nm)	Maximum Molar Absorptivity ($M^{-1} \text{ cm}^{-1}$)	Molar Absorptivity at 200 nm. ($M^{-1} \text{ cm}^{-1}$)
BPA	202	3.80×10^4 [59]	3.4×10^4 [59]
HMB	287	1.53×10^4 [62]	-----
DFC	276	1.04×10^4 [63]	-----
MF	234	1.74×10^4 [64]	-----

Table 3.3 Maximum molar absorptivities of target compounds at the wavelength of maximum absorbance.

3.4 Effect of Particles' Injection Time on % Binding

Particles were injected at 3, 12, 21, 30, 39, and 48 s of injection time to investigate the effect of particles' injection time on BPA % binding. As shown in Figure 3.4, increasing the amount of particles improved BPA % binding with both MIP and NIP particles. 48 s of MIP injection was sufficient to completely bind BPA molecules at 3 s of injection.

Figure 3.4 CE-UV binding efficiencies of MIP and NIP particles with BPA (100 ppm) under different injection times.

3.5 Effect of Applied Voltage on BPA% Binding

The effect of applied voltage on BPA % binding with NIP particles was examined. As shown in Fig. 3.5, % binding of BPA with NIP particles decreased with increasing applied voltage above 20 kV. One plausible reason is that electrophoretic velocities of BPA and particles were directly proportional to the applied voltage. A high voltage would increase these velocities, resulting in less overlapping time and hence a low % binding between BPA and particles. Another reason is more Joule heating inside the capillary as a result of a high voltage. Since viscosity decreases with increasing temperature, both EOF and electrophoretic mobility will increase slightly to reduce the overlapping time further. Moreover, binding is an exothermic process and a high temperature environment would be less favorable to the binding

efficiency.

Figure 3.5 CE-UV binding efficiencies of NIP particles with BPA (100 ppm) under different applied voltages.

3.6 Effect of Current Flow on BPA% Binding

The effect of current flow on % binding was next investigated for a new capillary and an old capillary running at currents of 10 and 26 μA . Depending on the number of negative charges present on the capillary wall and the number of counter ions in the double layer, the electroosmotic flow of background electrolyte solution through the capillary could be affected. Both BPA and NIP migration times decreased at the high current due to fast electroosmotic flow. As shown in Fig. 3.6, a slight increase in % binding occurred for the new capillary running at 10 μA . Thus, current flow had no significant impact on % binding.

Figure 3.6 CE-UV binding efficiencies of NIP particles with BPA (100 ppm) under different current flows in two different capillaries.

3.7 Binding Assays with BPA and HMB

Competitive binding with neutral, positive, and negative compounds in the presence of BPA was conducted to test the selectivity of MIP particles. BPA and HMB have almost the same molecular weight and migration time. Thus, they were injected separately (in the second and third injections for 3 s) to attain separation of their peaks. Figure 3.7 presents a typical CE-UV analysis that showed the BPA and HMB peaks at different migration times.

Figure 3.7 CE-UV characterization of BPA (100 ppm) and HMB (200 ppm).

Electrokinetic injection at 15 kV: first injection of 10 mM Na_2HPO_4 BGE for 48 s, second injection of BPA for 3 s, third injection of HMB. CE analysis at 17 kV; UV detection at 200 nm.

MIP/NIP particles were then injected first for 21 or 48 s. BPA binding to 21 s of MIP injection was $51 \pm 2\%$, whereas HMB binding was $62 \pm 1\%$. Injection of NIP particles for 21 s had BPA bound by $24 \pm 1\%$ and HMB by $99 \pm 1\%$. When the NIP injection time was increased to 48 s, BPA binding became $42 \pm 1\%$ while HMB binding remained at $99 \pm 1\%$. These results suggest that HMB had more affinity to non-specific binding sites than to the imprinted cavities and could be completely bound. Having less affinity to non-specific binding sites, BPA reached only $42 \pm 1\%$ binding. Due to the strong organic nature of HMB, hydrophobic interactions play a

big role in enhancing % binding. Also, HMB has -OH, ketone as well as methoxy groups that can participate in hydrogen bonding with the MAA functional groups at the binding sites. On the contrary, BPA is less hydrophobic and has only -OH groups to play a part in hydrogen bonding. As for specific binding with MIP particles, increasing the injection time to 48 s had $99\pm1\%$ of BPA bound but only $60\pm1\%$ of HMB bound. Obviously, BPA could displace some HMB, especially those molecules bound to the imprinted cavities.

3.8 Effect of BGE pH

pH 9.5 was purposely selected to ionize the -OH group of HMB while keeping the two -OH groups of BPA neutral, based on the understanding that -OH ionization would not occur completely at only two pH units below the pK_a of BPA [65]. Conversion of HMB to be an anion could reduce its hydrophobic interaction with the polymeric particles. When employing 21 s for injection of MIP particles, HMB binding decreased from $62\pm1\%$ to $34\pm3\%$ as expected from changing the pH to 9.5. Table 3.4 summarizes the % binding results obtained using pH 7.5 ± 0.2 and pH 9.5 ± 0.2 . Apparently, pH 9.5 is more satisfactory than 7.5 (in terms of lower HMB % binding to MIPs). In any case, the pH of a water sample can be adjusted to match the background electrolyte to avoid problems with mismatch between sample and electrophoresis buffers for CE.

		pH 7.5 BGE	pH 9.5 BGE		pH 7.5 BGE	pH 9.5 BGE
BPA % binding To MIP	21s of MIP/NIP Injection Time	51± 2	48± 1	48 s of MIP/NIP Injection Time	99± 1	99± 1
BPA % binding To NIP		24± 1	24±1		42± 1	64±2
HMB % binding To MIP		62±1	34±3		60±1	61±2
HMB % binding To NIP		99± 1	99± 1		99± 1	99± 1

Table 3.4 % binding results for BPA and HMB at pH 7.5±0.2 and pH 9.5±0.2.

3.9 Binding Assays with BPA, DFC and MF

Figure 3.8 CE-UV characterization of MF (200 ppm), BPA (100 ppm) and DFC (200 ppm). Electrokinetic injection at 15 kV: first injection of 10 mM Na_2HPO_4 BGE for 48 s, second injection of BPA for 3 s, third injection of HMB. CE analysis at 17 kV; UV detection at 200 nm.

A mixture of BPA, DFC and MF, prepared in the pH 7.5 BGE, was successfully separated according to their charge to size ratios, as shown in Figure 3.8. BPA, DFC and MF bindings to MIP and NIP particles are summarized in Table 3.5. DFC, with a pK_a of 4.15, tends to be negatively charged at pH 7.5. A recent study by Dai *et al* demonstrated that DFC mostly interacted through electrostatic attractions with appropriate functional monomers, which gave rise to a high binding efficiency of

DFC [66]. In our present work, the anionic nature of DFC in the BGE resulted in electrostatic repulsion and hence low % binding with the negatively charged MIP and NIP particles. Also, due to the anionic nature of DFC, hydrophobic interactions with the polymeric particles were eliminated leading to attenuation of the non-specific interactions. MF has primary, secondary, and ternary amine groups that can form hydrogen bonds with the carboxyl groups of MAA, as previously reported by Katakya *et al* [67]. Moreover, MF is positively charged in the pH 7.5 BGE. Thus electrostatic attractions may take place as well as hydrogen bonding, resulting in higher binding affinity in comparison with DFC. Most significantly, BPA binding with MIP particles was not affected by the presence of DFC and MF. This is an excellent demonstration of the selectivity of MIP for its target compound, which corresponds to several determining factors such as the strength of interactions between the target compound and the binding sites, the distribution of the functional groups, and most importantly the shape and size of the binding cavities [68].

% Binding	MIP Particles	NIP Particles
BPA	99±1	56±3
DFC	6±2	2±1
MF	20±2	12±1

Table 3.5 % binding results for BPA, DFC and MF with MIP and NIP particles
(injection time = 48 s) at pH 7.5±0.2.

Figure 3.9 Competitive CE-UV binding test. Electrokinetic injection at 15 kV: first injection of MIP particles for 48 s, second injection of the mixture of MF (200 ppm), BPA (100 ppm) and DFC (200 ppm) for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

Figure 3.10 Competitive CE-UV binding test. Electrokinetic injection at 15 kV: first injection of NIP particles for 48 s, second injection of the mixture of MF (200 ppm), BPA (100 ppm) and DFC (200 ppm) for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

3.10 Effect of Running Voltage on % Binding

Binding of compounds with MIP (or NIP) is highly dependent on their relative velocities inside the capillary, on top of their affinities to binding sites. Sarafraz-Yazdi and Amiri confirmed that dynamic micro-extraction has superior extraction efficiency over the static mode within short extraction times. They attributed this to the improved kinetics brought about by the movement of both particles and analytes [69].

The CE-UV binding test was repeated under different applied voltages (15, 17, 19 and 21 kV) to study the mobility effect. BPA binding to MIP particles (injection time = 48 s) was not affected by the different voltages. However, DFC and MF bindings were influenced to some extent. It turned out that lower voltages (15 and 17 kV) slightly increased DFC and MF bindings to the MIP particles up to $9\pm1\%$ and $22\pm1\%$, respectively. Higher voltages (19 and 21 kV) decreased their bindings down to $0\pm1\%$ and $7\pm1\%$, respectively. Apparently, a low voltage of 15 kV decreased their electrophoretic mobilities (and hence velocities) and EOF. That would provide more time for DFC and MF to overlap with the MIP particles, resulting in higher % bindings. It worked similarly for binding of DFC and MF with NIP particles. The slow migration of anionic DFC and fast migration of cationic MF determined how much overlapping time they had with MIP/NIP particles. Generally, the use of low voltages could enhance their % bindings. As shown in Figure 3.11, fast binding of BPA to the specific cavities inside MIP particles was verified by attaining $99\pm1\%$ with all the applied voltages. Our viewpoint is that BPA can function as an organic base because it has two phenolic rings and a high pKa value of 9.6. Therefore, the choice of MAA as a functional monomer helps to bind BPA via hydrogen bonding. Moreover, the cross-linker (EGDMA) was used in a relatively high proportion (7 mmol) to help create more binding cavities. Our result apparently contradicts the result of a previous study, where BPA was treated as an acid [70]. Their MIPs prepared using the same monomer (MAA), yet with a different ratio, demonstrated very low binding to the target molecule. This was attributed to the poor affinity of MAA-based MIPs toward BPA due to the weak interactions between BPA and MAA prior to polymerization. Moreover, their use of acetic acid to wash out the template molecules could be the major cause for getting a low % binding of BPA to MAA-

based MIP particles. Acetic acid may not work properly to break up the binding interactions between BPA (the template) and MAA (the functional monomer) due to the weak interactions between BPA and acetic acid.

The presence of the template molecule in MIP particles could induce alterations in the morphology of the polymer, including porosity and surface area [71]. These imprinted cavities offer higher binding affinity than non-specific binding sites, resulting in a significantly higher binding efficiency as compared with NIP particles. Also, the ratio of BPA: MAA (1:8) used in this work was larger than required (each BPA molecule needs two MAA molecules to form a complex). Extra MAA molecules (which do not form a complex with template molecules) would remain throughout the porous polymer, providing additional non-specific binding sites.

BPA binding to the non-specific binding sites inside NIP particles varied significantly under different applied voltages. As shown in Figure 3.12, the best binding was $56\pm3\%$ as obtained when using 17 kV. Higher voltages up to 21 kV decreased BPA, DFC and MF bindings with NIP particles down to $37\pm1\%$, $0\pm1\%$, and $6\pm1\%$, respectively. Apparently, the velocities of analytes and particles would increase with a higher voltage, resulting in less overlapping time and hence low % bindings due to limitation by their diffusion rate. Buffer viscosity would be reduced as the voltage increases due to Joule heating inside the capillary, thereby increasing both the EOF and the electrophoretic mobility of analytes. Moreover, due to the exothermic nature of binding, efficiency would be reduced in a high temperature environment.

Figure 3.11 CE-UV binding efficiencies of MIP particles with BPA (100 ppm), MF (200 ppm), and DFC (200 ppm) under different applied voltages.

Figure 3.12 CE-UV binding efficiencies of NIP particles with BPA (100 ppm), MF (200 ppm), and DFC (200 ppm) under different applied voltages.

3.11 CE-UV Desorption Test

Based on the electrophoretic mobility results in Table 3.1, TEA is neutral as it appeared at the same migration time as MO. BPA-MIP particles were injected first, and TEA next, for 3 s. The BPA-MIP peak shape changed (after TEA injection) as shown in Figures 3.13 and 3.14. A small peak, appearing around 5.4 min in front of the MIP peak, was suspected to be the eluted BPA. To further investigate the identity of this peak, the BPA-MIP suspension was spiked with 100 ppm BPA. An increase in peak area (and height) was observed at 5.4 min, thus proving the peak to be BPA.

Figure 3.13 CE-UV characterization of BPA-MIP particles. Electrokinetic injection at 15 kV: first injection of BPA-MIP particles for 3 s, second injection of BGE for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

Figure 3.14 CE-UV desorption test. Electrokinetic injection at 15 kV: first injection of BPA-MIP particles for 3 s, second injection of 5 % TEA in methanol for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

3.12 External Desorption Test

An external desorption test was next carried out to confirm the eluent ability of TEA. A mixture of BPA-MIP and TEA was sonicated for 5 min before injection (3 s) for CE-UV analysis. As shown in Figure 3.15, besides the appearance of TEA and BPA-MIP peaks, a new peak was observed around 4.9 min. This eluted peak was suspected to be BPA. For further investigation, the mixture was spiked with 100 ppm BPA. The area (and height) of this peak increased significantly, hence confirming it to be BPA.

Figure 3.15 External desorption test. Electrokinetic injection at 15 kV: first injection of a mixture of BPA-MIP particles and 5 % TEA in methanol for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

Absolute methanol cannot completely elute BPA, thereby it was necessary to add acidic/basic modifiers with the ability to disrupt the hydrogen-bond network formed between BPA, the template, and MAA, the functional monomer [58]. Hence, it is crucial to carefully select the appropriate eluent in order to achieve the highest extraction efficiency. Having two phenolic hydroxyl groups, BPA can act as a weak acid ($pK_a=9.6$). TEA is an organic base that can interfere with the binding equilibrium between BPA and carboxyl functional groups inside the MIP cavities. Martin *et al.* explored the ability of basic and acidic modifiers to overcome the electrostatic interactions between basic analytes and acidic MIPs. They reached a conclusion that only TEA achieved this with superior performance when compared

with TFA [72]. In addition, methanol contains an -OH group, which can form H-bonds with the MIP carboxyl groups and force BPA out of the cavities [73]. As characterized by CE, TEA is neutral in BGE because of the presence of methanol that works as an alcohol modifier preventing the protonation of TEA [74]. Hence, TEA can interfere with the binding equilibrium between BPA and carboxyl groups of MIP, releasing BPA out of the cavities. When injecting TEA first and BPA next for 3 s, a complex of TEA-BPA was observed. Based on their migration times before and after complexation, both peaks migrated at slightly longer migration times as shown in Figure 3.16. This can be attributed to the formation of H-bond between BPA and TEA. BPA works as a hydrogen bond donor while TEA is a hydrogen bond acceptor (since it contains a lone pair of electrons on the nitrogen atom). All in all, 5 % TEA in methanol is a proper elution reagent that could successfully desorb BPA out of MIP cavities.

Figure 3.16 CE-UV binding test. Electrokinetic injection at 15 kV: first injection of 5 %TEA in methanol for 3 s. Second injection of BPA (100 ppm) for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

In spite of the time required for the elution reagent to desorb all of the tightly bound BPA out of the cavities, in capillary desorption test was demonstrated to be a rapid method for evaluating the desorption ability of an eluent that can interfere with the binding equilibrium between the template molecules and MIP cavities.

The elution ability of TFA was next investigated. TFA is negatively charged in the BGE, based on its electrophoretic mobility value as summarized in Table 3.1. 10 % TFA in methanol was more than sufficient to interact quantitatively with BPA and still within the dynamic range of CE-UV detection. A mixture of TFA and 100 ppm BPA was injected for 3 s to examine the interaction of TFA with BPA. As shown in Fig. 3.17, one peak was observed at 10.9 min, which is believed to be the TFA-BPA complex peak. When injected individually, BPA appeared at a migration time of 3.7

min while TFA appeared at 11.6 min. Based on the new migration time for the complex peak, the electrostatic interactions between BPA and TFA turned BPA from being neutral to negatively charged (or deprotonated) whereas TFA became less negatively charged, with the formation of an overall negatively charged complex. Varying concentrations of BPA (10, 50, 100 and 200 ppm) were used to further investigate whether the obtained peak is the TFA-BPA complex. Figure 3.18 shows an increasing peak area of the TFA-BPA complex as BPA concentration is gradually increased. This is a convincing proof of the complex formation.

Figure 3.17 CE-UV binding test. Electrokinetic injection at 15 kV of a mixture of 10% TFA in methanol and 100 ppm BPA in BGE for 3 s. CE analysis at 17 kV; UV detection at 200 nm.

Figure 3.18 Effect of BPA concentration on the area of TFA-BPA complex peak.

10% acetic acid had previously been used to elute BPA out of MIP cavities. TFA had also been utilized as an organic modifier in HPLC mobile phases to elute BPA out of HPLC columns [75]. Although TFA could form a complex with BPA, it was apparently not able to break up the binding interactions between BPA and MAA inside the MIP binding cavities. Both in capillary and external desorption tests showed no impact of TFA in BPA desorption. It is believed that coulombic/electrostatic repulsion between TFA and MIP particles would not allow TFA to enter MIP cavities. Apparently, TFA could not be used as an eluent for BPA in methacrylate- based MIP particles. Table 3.6 compares TEA and TFA for use as elution reagents.

	5% TEA in MeOH	10 % TFA in MeOH
Chemical Structure		
pKa	10.7 (basic)	0.6 (strongly acidic)
Charge in 10 mM Na ₂ HPO ₄	Neutral	Negatively charged
Complexation with BPA	<p>It formed a complex via hydrogen bonding. TEA and BPA peaks were associated to each other and migrated at later migration times)</p> <p>TEA migration time changed from 3.5 (before) to 3.8 (after complexation with BPA)</p> <p>BPA migration time changed from 4.1 to 4.6 min</p>	<p>It formed a complex (via electrostatic interactions) peak in between the migration times of BPA and TFA</p>
Ability to interfere with the binding between MAA and BPA	<p>It was able to disrupt the binding between MAA and BPA and eventually elute BPA out of MIP cavities.</p>	<p>TFA was not able to break up the binding interactions between MAA and BPA to elute BPA out of MIP cavities.</p>

Table 3.6 Comparison between TEA and TFA in their capability to elute BPA out of MIP particles.

3.13 Reusability of MIP Particles

Reusability was demonstrated over several adsorption cycles without regeneration of the MIP particles (by desorbing the already bound analytes). Figure 3.19 shows a typical CE-UV analysis of five consecutive injections of 100 ppm BPA (each for 3s). These five consecutive injections were done following one injection of MIP particles (for 21 s). On the other hand, peak overlapping (between NIP particles and BPA) allowed up to only three injections of BPA following the injection of NIP particles. All BPA injections were observed to bind onto the MIP and NIP particles, with a gradual decrease in % binding as shown in Figure 3.20 (a) and (b), respectively and summarized in Table 3.7.

Figure 3.19 CE-UV characterization of multiple BPA (100 ppm) injections (for 3 s each). Electrokinetic injection at 15 kV. CE analysis at 17 kV; UV detection at 200 nm, on a full y-scale of 2 milli-absorbance units (mAU).

Figure 3.20 (a) CE-UV binding test. Electrokinetic injection at 15 kV: first injection of MIP particles for 21 s, followed by five injections of BPA (100 ppm) for 3 s each. CE analysis at 17 kV; UV detection at 200 nm, on a full y-scale of 2 milli-absorbance units (mAU).

Figure 3.20 (b) CE-UV binding test. Electrokinetic injection at 15 kV: first injection of NIP particles for 21 s, followed by three injections of BPA (100 ppm) for 3 s each. CE analysis at 17 kV; UV detection at 200 nm, on a full y-scale of 2 milli-absorbance units (mAU).

% Binding	First BPA Injection	Second BPA Injection	Third BPA Injection	Fourth BPA Injection	Fifth BPA Injection
MIP Particles	68±2	62±1	60±1	58±1	51±2
NIP Particles	26±1	23±1	22±1	-----	-----

Table 3.7 % binding results for multiple injections of BPA (injection time = 3 s each) with MIP and NIP particles (injection time = 21 s) at pH 7.5.

A progressive decrease in BPA % binding to MIP and NIP particles implies that less and less binding sites were available. Binding is a physicochemical interaction dictated by an equilibrium constant:

$$K_{eq}$$

The 1st injection of BPA did not bind completely probably because the effective equilibrium constant did not have a very high value.

$$K_{eq} = [(C_i - C_e) / C_e] / V/m = [(\% \text{ Binding}) / (1 - \% \text{ Binding})] / V/m \quad [76] \quad (3-1)$$

where C_i is the initial concentration of analyte

C_e is the concentration of analyte at binding equilibrium,

V is the volume of solution (mL) and

m is the mass of particles (g).

An effective equilibrium constant can be calculated for the 1st injection of BPA binding with MIP particles to be $K_{eq} = 212$ (arbitrary units); for NIP particles, $K_{eq} = 35$.

3.14 Chemical Interference

The presence of other organic compounds in real-world samples can interfere with the % binding of BPA with MIP. A representative compound, HMB, was next employed for the investigation of chemical interference. It was a nice surprise to observe that 3 s of 200 ppm HMB injection had no significant effect on BPA % binding onto either MIP or NIP particles, in spite of the high % binding of HMB. Thus, the HMB injection time was increased to 6, 9, 12 and 15 s. Figure 3.21 presents HMB % binding results, for both MIP and NIP particles, obtained after different injection times of HMB.

Figure 3.21 CE-UV investigation of HMB (200 ppm) binding efficiencies with/for MIP and NIP particles (21 s of injection).

As discussed earlier, HMB has more affinity to non-specific binding sites as it demonstrated a complete binding to NIP particles. In spite of the large quantity of HMB bound to the (non-specific binding sites inside) MIP particles, BPA % binding to MIP particles was not significantly decreased in the next two injections. Figure 3.22 presents BPA % binding to MIP particles after injection of HMB for various times. Apparently, HMB had only slight effect (due to occupation of non-specific binding sites) on decreasing BPA % binding to MIP particles in the second injection. The notable decreases in BPA % binding to MIP particles in the third injection could be explained by a lower number of specific binding sites available. Figure 3.23 represents BPA % binding to NIP particles at different injection times of HMB.

Figure 3.22 CE-UV investigation of BPA (100 ppm) binding efficiencies with MIP particles (21 s of injection) after injection of HMB (200 ppm) for various times.

Figure 3.23 CE-UV investigation of BPA (100 ppm) binding efficiencies with NIP particles (21 s of injection) after injection of HMB (200 ppm) for various times.

The effective equilibrium constant for HMB binding to MIP particles (for 15 s of injection time) was calculated to be $K_{eq} = 4900$; for NIP particles, $K_{eq} = 9900$.

In summary, HMB exhibited almost insignificant effect in decreasing BPA % binding to MIP particles in the second and third injections, meaning that MIP particles are not affected by the non-specific binding of HMB despite its large quantities (3 – 15 s of injection of 200 ppm). MIP particles can still remove a reasonably high percent of BPA in the next two injections (2nd and 3rd). This is an excellent demonstration of MIP capability to recognize and bind BPA in the presence of interfering compounds. These results are in agreement with the results obtained by Watabe *et al.* They concluded that the removal of interferences from water by the surface modified MIP

particles through the non-specific binding did not hinder BPA binding to MIP cavities [77].

Chapter 4

Conclusion

4.1 Conclusion

Protecting water at its source (including lakes, rivers and aquifers) is the first step in ensuring that each individual has access to safe drinking water. Stopping contaminants from getting into drinking water sources provides a line of defense in the protection of the health of individuals. However, occurrence of environmental pollutants is rising as a consequence of more anthropogenic activities and elevated quantities of wastes released into the environment. Dangerous levels of BPA have been detected in fresh and seawaters due to urban, agricultural and industrial practices with abundant discharge of liquid and solid wastes. Lee and Peart performed a large-scale study in Canada and found that BPA concentration is 37 mg/kg in sewage sludges, 149 mg/L in industrial wastewaters and 5 mg/L in freshwater resources [78]. It has been reported that the estrogenic activity of BPA occurs at concentrations below 1 ng/L [77]. Hence, it is crucial to develop efficient technologies to reduce or eliminate the presence of BPA (and other pollutants) especially in aquatic environments. Conventional techniques for the removal of BPA include ultra-filtration membranes, membrane bioreactor, ozonation, solid phase extraction, reverse osmosis, ultrasound destruction, as well as microbial degradation [79]. Among these technologies, SPE using activated carbon is the most common for industrial applications.

In this research study, the molecular imprinting technique was used to target the pollutants individually. MIP particles with an average diameter of 164 ± 15 nm were prepared following a molar ratio of 1:8:7 for BPA, MAA and EGDMA. They were used successfully in CE-UV binding tests to demonstrate rapid removal of BPA from water. A high binding efficiency of $99 \pm 1\%$ was obtained for BPA when 48 s of MIP injection time was employed. Competitive binding experiments with non-ionic HMB,

anionic DFC and cationic MF verified the selectivity of MIP towards BPA. DFC and MF did not reduce the amount of BPA binding with MIP particles, while HMB had higher affinity to non-specific binding sites than imprinted cavities. It was further demonstrated that MIP particles have excellent capability to recognize BPA with a fairly high percent binding even in the presence of large quantities of HMB (3–15 s of injection of 200 ppm). This is an excellent demonstration of MIP specificity for its target compound in a rapid competitive binding test, which represents a major advantage of MIP particles over non-specific SPE sorbents in targeting water contaminants individually. Different applied voltages did not affect BPA binding to MIP particles, but BPA binding to NIP particles were significantly influenced. On the contrary, current flow had no significant impact on % binding. Rapid CE-UV desorption test was demonstrated using 5% TEA in methanol. The external desorption test confirmed the ability of TEA to elute BPA out of MIP cavities. 10% TFA in methanol could not work as an eluent though it can form a complex with BPA due to the electrostatic repulsion between the negatively charged TFA and MIP particles. Reasonably high % bindings of BPA were attained after one injection of MIP particles for 21 s followed by multiple injections of BPA (each for 3 s). This confirms the reusability of MIP particles over several adsorption cycles without regeneration of the particles by desorbing the already bound analyte until the binding capacity is reached (and all specific binding sites are occupied).

This M.Sc. research study focused on the development of MIP particles for use as a cost effective sorbant that is capable of treating water contaminated with BPA. Also, this study had expanded the use of CE-UV from being an analytical tool (used mainly to detect analytes based on their charge to size ratios) to performing rapid binding tests due to its ability to characterize solid polymeric particles suspended in a liquid.

All tests conducted had short analysis times. As such this research study had proven the fast binding kinetics between particles and the target compounds where equilibrium is reached within several seconds not after overnight incubation as previously practiced [80].

4.2 Future Work

Now that online extraction has been demonstrated in a CE-UV system, the technology of MIP particles will undergo further development in our research lab for rapid removal of BPA from larger volumes of water. A method is under intensive development for the rapid pre-concentration of BPA in ten to one hundred liters of water using MIP particles to open up new opportunities towards large-scale applications. One approach is to prepare MIP monoliths. Monolithic capillaries and columns can be obtained by *in situ* photo-initiated polymerization. A fused-silica capillary or glass column is filled with a pre-polymerization mixture of template, functional monomer, cross-linker, initiator and porogen. Placing the capillary, with ends sealed, under a UV light source at ambient temperature will start the polymerization. Flushing the remaining monomers and initiator out of the capillary stops the polymerization. Washing the capillary with a proper solvent creates the binding cavities. Equilibration with the background electrolyte makes the capillary ready for pre-concentration application. Figure 4.1 illustrates the preparation of a capillary filled with an MIP monolith. In-capillary regeneration of the MIP monolith that has fully bound the target molecules and reached its binding capacity could possibly be achieved by extraction of the bound molecules with a proper solvent.

Figure 4.1 *In situ* photo-initiated polymerization procedure to induce an MIP-filled capillary [48].

For engineering, encapsulation of magnetic nanoparticles inside the MIP particles will facilitate their magnetic separation from water in municipal treatment plants [81]. This is a powerful technology platform as it combines the specific adsorption property of the MIP particles and the magnetic separation provided by the magnetic core.

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