

CHAPTER 1

INTRODUCTION

1.1. Background and rationale

Trichloroacetic acid (TCAA) is a harmful by-product from disinfection chlorination process in drinking water (Tetlow and Hayes, 1988; Simpson and Hayes, 1998). Its structure is shown in Fig. 1.1. It is considered to be a potential animal and human carcinogen. TCAA and its analogs, such as dichloroacetic acid (DCAA), monochloroacetic acid (MCAA), tribromoacetic acid (TBAA), dibromoacetic acid (DBAA), and monobromoacetic acid (MBAA), belong to the haloacetic acids (HAAs) disinfection by-product group. Since they are attributed as potential animal carcinogens as well, various countries have set regulations on the maximum permissible levels of TCAA and the related haloacetic acid (HAAs) in both domestic and commercial drinking water (USEPA, 1993).

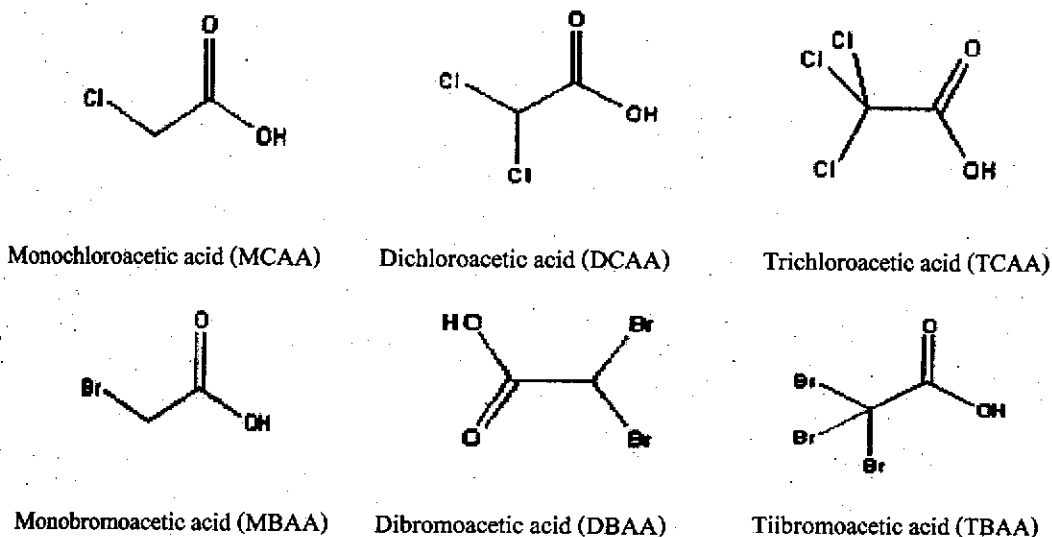


Fig. 1.1. Trichloroacetic acid (TCAA) and its analogs

The formation of haloacetic acids (HAAs) from the chlorination of individual and binary mixtures of organic fractions obtained from the intake of Bangkok Water Treatment Plant in

Bangkok, Thailand was investigated (Kanokkantapong *et al.*, 2006). Experimental results revealed that, as an individual fraction, hydrophobic base (HPOB) was the most active in forming HAAs (approx. $200 \mu\text{g mg}^{-1}$) whereas hydrophilic acid (HPIA) was the least (approx. $40 \mu\text{g mg}^{-1}$). In binary mixtures, acid fractions exhibited stronger inhibitory effect in forming HAAs than base fractions. Among the various individual HAA species obtained from the chlorination of each individual organic fraction, dichloroacetic acid (DCAA) was found to be predominant. On the other hand, the chlorination of binary organic fraction mixtures often led to the formation of monochloroacetic acid (MCAA) as the predominant HAA species. Bangkok Water Treatment Plant is one of the three major sources for supplying potable water to more than four million people in Bangkok, Thailand. The formation of HAAs as a result of the disinfectant reaction with the organic matter in the water, therefore, could have an impact on consumers who drink the water.

The traditionally practiced techniques used for direct determination of these compounds are electrochemical methods, however they give poor specificity. In addition, more sophisticated means for specific determination of HAAs, such as LC-MS, GC-MS and CE, are time consuming procedures and expensive. The bulky equipment demands high power and well-trained operators for the instrumentation (Urbansky, 2000). It is clear that there is a need for such simple and fast screening tool, capable of complementing the established methods. For such prospect, the use of sensors seems most promising in giving a fast, inexpensive, simple and continuous measurement (Ramanaviciene and Ramanavicius, 2002; Liang *et al.*, 2005).

In recent years, biosensors have been used extensively in the fields of diagnostics, environmental monitoring and food processes (Thevenot *et al.*, 1999; Stetter *et al.*, 2003). They utilize various biological molecules, such as microbes, antibodies or enzymes capable of recognizing a specific target molecule. However, difficulties such as instability against high temperature, pH and organic solvents, prohibit their practical use. In general it is hard to find and purify such candidate which possesses the desired properties. One recent technique, called molecularly imprinting polymers (MIPs), shows a promising alternative. MIPs are synthetic polymers, which are obtained by polymerizing a monomer with a cross-linker around a template (the analyte) molecule. After polymerization, the template is removed by washing, and the sites capable of selectively rebinding the target analyte are left (Fig. 1.2) (Sergeyeva *et al.*, 1999;

Piletsky *et al.*, 1995). It is well known that the stability of MIPs is superior to that of biological recognition materials. Also a sensor modified with a MIP is easily stored, operated and has a longer lifetime. At present, acrylate (Lehmann *et al.*, 2002; Sergeyeva *et al.*, 1999), styrene, or silane-based polymeric materials are frequently selected as the MIP. These polymers have a great stability in various conditions and therefore can keep the recognition property in a good length of time. Beside these types of polymeric materials, conducting polymers have also been investigated as recognition layers (Ramanaviciene and Ramanavicius, 2004; Shiigi *et al.*, 2003; Shiigi *et al.*, 2002; Okuno *et al.*, 2002). It could become versatile molecular recognition systems by being doped with anionic recognition elements. The remarkable advantage of this type of polymer over the cross-linked ones is in its ease of preparation as thin film onto the electrode surface via the electrodeposition process. Among the various types of conducting polymers, polypyrrole has many attractive features including a molecular recognition system. It can be used in a neutral pH region and its stable film can conveniently be polymerized on various substrate materials. These certainly rank polypyrrole among the most attractive sensor materials especially for the construction of all solid-state electrodes such as inter-digitated conductometric, voltammetric electrode and piezoelectric quartz crystal microbalance.

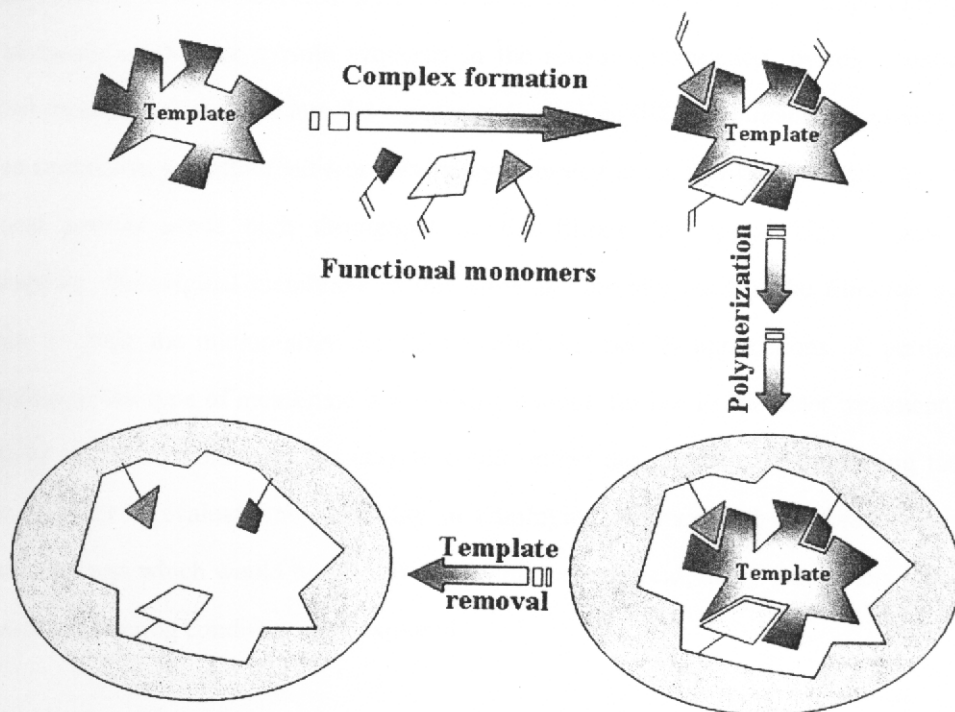


Fig. 1.2. Scheme of the imprinting polymerization

Therefore, in search of the haloacetic acid determination method in drinking water with high efficiency in terms of measurement time, result consistency and simple operation, two types of polymers had been selected and integrated with several frequently used transducers in recent years. For this, poly(ethyleneglycoldimethacrylate-co-vinylpyridine) was chosen as a candidate from the cross-linked polymer group to prepare a sensing layer onto the surface of three types of electrodes. Polypyrrole was the non-cross-linked polymer, which can be easily prepared onto the surface of interested electrodes by electropolymerization. The transducer studied was composed of a conductometric electrode, an inter-digitated conductometric and a voltammetric electrode, and a microgravimetric electrode, which was already called quartz crystal microbalance electrode. It was expected that their differences in electrode construction, sensing element integration and signal transduction when dealing with both polymers, could yield the most promising sensor in detecting the haloacetic acid in drinking water.

The other aim of this work was to find a suitable method of separating haloacetic acid from drinking water using the trichloroacetic acid imprinted polypyrrole modified microfiltration regenerated membrane. In the membrane composition, the imprinted polypyrrole was electrochemically synthesized as a recognition material using trichloroacetic acid as a template, and the microfiltration regenerated cellulose membrane was selected as a supporting layer. To take advantage of the polypyrrole synthesis in the sensor construction, pyrrole monomer was electrochemically polymerized into the regenerated cellulose (RC) membrane via a redox reaction with an ammonium persulfate initiator previously saturated in the membrane. Typically, the water treatment process needs high throughput of the filtrate to support high domestic water consumption. The original membrane in this work is normally used in the filtration system to separate or retain the micron-sized particles in the commercial applications. A permeation of water through this type of membrane is promising enough for use in the water treatment, and the selectivity of the membrane to the analyte could utilize the molecularly imprinting technique. Hence, in order to evaluate the possibility of employing the modified membrane in the water treatment process which would be selective to trichloroacetic acid, many parameters related to its synthesis and filtering condition were explored.

1.2. General introduction

1.2.1. Molecularly imprinted polymers (MIPs)

During the last decades molecular imprinting has received considerable attention as an approach for introducing binding sites mimicking those of biological receptors in synthetic polymers. Molecularly imprinted polymers (MIPs) can be prepared by the copolymerisation of functional and cross-linker monomers in the presence of a template molecule, provided a complex formed between the template and the functional monomers. After removal of the template, the 'imprints' retain the position of the functional groups and a shape complementary to the template (Wu and Syu, 2006). The specific interactions between the functional monomer and the template can be either reversible covalent or non-covalent in nature. According to the covalent approach, a polymerizable derivative of the analyte should be synthesized and used as template. The subsequent extraction of such template requires cleavage of covalent bonds. Since the choice of reversible covalent interactions and the number of potential templates are substantially limited, the attention is then very much focused on using non-covalent interactions. In addition to better versatility of this more general approach, it allows fast and reversible binding of the template (Caroia *et al.*, 2004).

In order to obtain MIPs which have reliable recognition sites, certain criteria must be considered with respect to the preparation formulas and conditions. They are listed as follow (Piletsky *et al.*, 1999).

1. Molecules with more rigid structures and those with few conformations in solution, produce better recognition site populations; i.e. are better templates.
2. Higher number and strength in the interactions between complementary functionalities in the template and the polymer enhances MIP affinity.
3. Polymerization and evaluation of MIPs should proceed under thermodynamic control to avoid adverse van der Waals interactions and conformational strain.
4. The polymer should be highly crosslinked to maximize the cavity integrity, but a certain residual flexibility is necessary to facilitate the rebinding of the template.

5. Polymerization and evaluation should be performed in the same solvent to minimize the loss of template-polymer conformational adaptation.

6. The bond between the template and functional groups should be strong but reversible (not permanent). Coulombic, hydrogen bonding, van der Waals forces as well as covalent bond, have also been explored.

In this section, some traditional methods for the production of MIPs are briefly described according to their applications. Most of the methods mentioned involve radical polymerization, which is initiated either thermolytically or photolytically. Both covalent and non-covalent imprinting technologies have been used to mould the polymer. Use of an electropolymerization technique for the preparation of MIPs is another possibility, allowing the formation of ultra-thin polymer films on the electrode surface. This method is generally used for in-situ preparation of MIPs and presents one of the most interesting methods in developing electrochemical sensors.

1.2.1.1. MIP preparation method (Merkoci and Alegret, 2002)

A. In-block imprinted polymers

At present, one of the most popular methods of producing MIPs is bulk polymerization, in which functional monomers are bound either covalently or non-covalently to a printed molecule or template. The correct position of these functional groups allows them to converge on the template molecule in a reciprocal fashion. The resulting pre-polymer complex is copolymerised with an excess of cross-linked monomer in the presence of an equal volume of inert solvent and a free radical initiator. Polymerization of this mixture results in a highly cross-linked insoluble polymer. Removal of the template, in most cases by extraction or hydrolysis, leaves sites complementary in size and shape to the template molecule, resembling the “lock and key” model of enzymes. The block of polymer is then ground and sieved in order to produce imprinted receptor particles of appropriate size. This method, usually applied to prepare stationary phases for high-performance liquid chromatography (HPLC) (Fu *et al.*, 2003), can be used to

prepare imprinted polymeric membranes (Yoshikawa and Yonctanib, 2002) or polymer-coated electrodes for electrochemical sensing (Lopez *et al.*, 2004).

B. In-situ imprinted polymers

In-situ molecular imprinting is a technique for preparation of imprinted polymers where they are subsequently utilized, *i.e.* onto the surface of a transducer, such as an electrochemical sensor (Gong *et al.*, 2004). As a result, imprinted polymers prepared by an in-situ technique do not require any subsequent treatment of the resultant material, except washing to extract the template, and can be used directly in their applications.

The first reported case of this in-situ technique involved an electrosynthesized poly(o-phenylenediamine) (PPD) molecularly imprinted by a neutral template (glucose) (Malitesta *et al.*, 1999). PPD was employed as the recognition element of a quartz crystal microbalance (QCM) biomimetic sensor for glucose. The MIP was generated in situ onto a platinum electrode of the piezoelectric quartz crystal used as a working electrode during the polymerisation stage. The in-situ method has been the most reported method for the potentiometric sensors preparation.

C. Polymer-imprinted beads

Although the polymer-bead technique was introduced a few years ago, there is still room for improvement. However, as it becomes more apparent that MIPs need to be produced more cheaply, efficiently and reproducibly, it is evident that future developments will allow the application of imprinted beads in the electroanalytical systems.

To date, there is a wide range of techniques for preparing imprinted polymer beads, from the use of pre-formed beads (Kempe and Kempe, 2006) to the suspension polymerisation in water (Yavuza *et al.*, 2005) or organic solvents (Moral and Mayes, 2006) or dispersion polymerization. Both inorganic (silica) and organic (trimethyl propane trimethacrylate (TRIM)) have been used as bead cores on which MIPs are coated. In this procedure, bead

formation is separated from the imprinting process. The materials described are being developed mainly for use in packed columns for chromatography.

Polymer-imprinted beads, although not reported yet in electrochemical sensing systems, would be of potential interest for various analytical applications.

D. Applications of MIPs

Since the initial work by Wulff's group in the early 1970s on the development of MIPs for sugar and amino acid derivatives, MIPs of over 20 classes of compounds have been reported. The list includes sugars (Parnpi and Kofinas, 2004; Sineriz *et al.*, 2007), amino acids (Shim *et al.*, 2004; Peng *et al.*, 2001), peptides (Theodoridis *et al.*, 2004; Hart and Shea, 2001), proteins (Bossi *et al.*, 2001; Bossi *et al.*, 2007), therapeutic drugs (Chapuis *et al.*, 2006; Suedee *et al.*, 2006; Ansell, 2005), steroids (Davidson and Hayes, 2002; Hishiya, 1999), metal ions (Matsui, 1996), aromatic hydrocarbons (Kirsch, 2001), dyes, phosphorous esters (Hall *et al.*, 2005), and pesticides (Zhu *et al.*, 2005, Jenkins *et al.*, 2001, Baggiani *et al.*, 1999).

The major portion of applications of MIPs is in their use as solid-phase adsorbents for HPLC (Mullett *et al.*, 2001; Kubo *et al.*, 2004; Xiong *et al.*, 2006; Hu *et al.*, 2005; Moller *et al.*, 2004). In the past few years, the variety of compounds to which MIPs have been developed has increased dramatically. In addition to MIPs include the development for analytical applications and sensors (Fuchiwaki *et al.*, 2007; Huang *et al.*, 2007; Yan *et al.*, 2007; Syu *et al.*, 2006), membranes for purification of waste (Silvestri *et al.*, 2006; Takeda and Kobayashi, 2006) or drinking water (Suedee *et al.*, 2004; Trotta *et al.*, 2005), capillary electrophoresis (CE) (Takeuchi and Haginaka, 1999), and the production of polymers with special functions, such as drug-release matrices (Bodhibukkana *et al.*, 2006).

All these reported MIPs could potentially be used as recognition materials in electrochemical sensing systems.

1.2.1.2. Immobilisation of MIPs on transducer surfaces

The immobilisation of MIPs on the transducer surface is an important aspect of the design of MIP-based electrochemical sensors and can be categorised as follows.

A. In-situ polymerisation

In-situ polymerisation is the best immobilisation procedure and consists of in-situ electrosynthesis of MIPs at the transducer surface (Cheng *et al.*, 2001; Panasyuk *et al.*, 1999). In-situ polymerisation has the potential advantage of integrating the immobilisation step into an automatic mass-production process.

B. Surface coating

Surface coating (spin and spray coating) is another method of applying and immobilising MIPs onto a transducer surface (Suedee *et al.*, 2006; Ebarvia and Sevilla, 2005). Although normally used when MIPs are integrated with optical transducer, it is also possible to apply this surface-coating method with electrochemical transducers (Kroger *et al.*, 1999). Use of the surface-coating techniques allows production of a thin film of MIPs (previously dissolved in a proper solvent).

Manual deposition of a MIP layer is also used on various occasions to design electrochemical sensors described in the following sections.

C. Entrapment

Entrapment of MIP particles into gels or membranes has often been used for electrochemical transducers (Lopez *et al.*, 2004). These prepared materials are applied onto the transducer surface. An inert soluble polymer, such as PVC, entraps the MIPs particles, this technique has been also applied to acoustic transducer surfaces.

D. MIP-based composites

Another interesting technique is the integration of MIPs with transducer component, such as graphite (Lopez *et al.*, 2004), in the MIP matrix. In this way, the binding sites and the conducting particles are in a close contact. A simple mechanical polishing can renew the sensor surface, hence offering the advantages in the mass production of MIPs sensors.

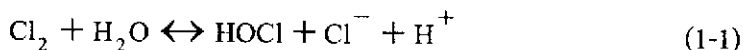
1.2.2. Chlorinated disinfection by-product

1.2.2.1. Disinfectants

Chlorine is the most widely used disinfectant. It is common known that finished drinking water leaves the treatment plant with a residual disinfectant. When surface water is used as the source for drinking water, residual disinfectant is required by regulation. Therefore, chlorine is often added to finished water, even if a different oxidant is used for primary disinfection. Chlorine is added to water in a various forms, usually as gas or in the solid hypochlorite form.

A. Chlorine Gas

Chlorine gas, properly referred to as dichlorine (Cl_2) is a greenish yellow gas that has a familiar and pungent smell. Chlorine (oxidation state: 0) is modestly soluble in water. When added to water, chlorine hydrolyzes, producing hypochlorous and hydrochloric acids:



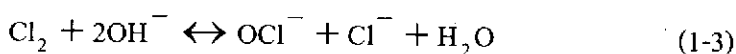
Hydrochloric acid is a strong acid and is completely dissociated into hydrogen and chloride ions. Hypochlorous acid (HOCl, chlorine oxidation state: +I) is a weak acid with a $\text{p}K_a$ of about 7.5, and it dissociates into hydrogen and hypochlorite (OCl^-) ions:



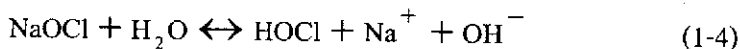
It is believed that chlorine(0) and chlorine(I) compounds work primarily by denaturing enzymes or proteins, thereby inactivating microorganisms. In some cases, physical disruption of cell membranes may also contribute. HOCl is thought to be the more active species.

B. Hypochlorite

The equilibrium in Equation 1-2 can be driven forward using a strong base to deprotonate the hypochlorous acid and to neutralize the hydrogen ion:

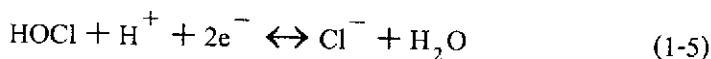


When sodium hydroxide is used as base, the familiar sodium hypochlorite, found in household bleach, is formed, which in turn undergoes the following reaction:

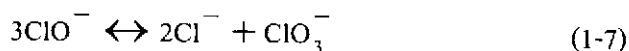


Thus, the same active species, HOCl, is produced from both the reaction of chlorine gas and solid hypochlorite.

Hypochlorous acid may also be produced by addition of solid calcium hypochlorite salt to water. The choice of using chlorine gas or hypochlorite salts is a matter of preference by water utilities and is often dictated by cost, safety concerns, and the availability of raw materials. The chemistry of chlorine has practical considerations in this regard. In the chlorine(I)-cation transfer step, chlorine and hypochlorous acid both undergo 2-electron reductions. If a reducing agent cannot offer 2 electrons, reactions are generally slow or difficult. The 2-electron reduction can be expressed as follows:



Chlorine(I) is unstable and disproportionates; thus, hypochlorite solutions are slowly converted to chlorate and chloride, which are not disinfection by-products, in the sense that no other reactant is required:



Given enough time, solutions of sodium hypochlorite (e.g., chlorine laundry bleach) will be more than 99% converted to chlorate and chloride. Equilibrium is achieved faster at higher temperatures. However, chlorate is not a good disinfectant. Although the central chlorine atom has a high oxidation state (+V), chlorate reacts much more slowly than hypochlorite and only in acidic conditions, which, in turn, reacts more slowly than hypochlorous acid. This kinetic barrier clearly precludes its use as an oxidizing disinfectant. Unlike hypochlorous acid, which reacts primarily by chlorine(I) cation transfers, chlorate must react either by a reductant attacking the central chlorine atom or an oxygen atom transfer. Hypochlorite loss via Equation 1-7 requires that a fresh supply of sodium hypochlorite solutions be available. As a rule, most chlorination plants dissolve chlorine in a small amount of water just before adding it to the main stream, or they add the chlorine gas directly to the stream. Nonetheless, chlorate has been found in these disinfection solutions (Zhu *et al.*, 2006; Dabeka *et al.*, 2002). In contrast, Cl₂ gas is stable indefinitely if stored properly.

1.2.2.2. Disinfection By-Product (DPB) Formation Source Material

The source material for DBPs is important in understanding the chemistry and mechanism of DBP formation, once the disinfectant reacts with the source material.

A. Inorganic Sources

Source material for the formation of DBPs is inorganic and organic in nature. Inorganic components are traced to various minerals and other substances in water derived from nonbiological sources. These substances occur naturally in water or may be anthropogenic

in nature. One such naturally occurring substance is anion known as bromide, which is implicated in by-product formation, particularly when used with ozone. Bromide in water can also contribute, through a series of reactions, to brominated products when chlorine is used. Bromide contamination in chlorine solutions is another route through which bromide enters drinking water.

B. Natural Organic Matter (NOM)

Natural waters used as sources for drinking water supplies contain variety of organic matters. Some of these organic matters come from natural sources. When organisms die, a mixture of biological and chemical processes take place. These processes produce a mixture of compounds that are collectively referred to as NOM. NOM can be highly variable, depending on its source and extent of degradation. Many factors beside native flora and fauna influence NOM composition. These include temperature, rainfall/humidity, light, microbial populations, and geography. There is a complex interplay among the native flora and fauna as well as the climate and season. There is much interest in understanding the makeup of this NOM material.

Various schemes have been used to classify NOM. These categories are by no means mutually exclusive. One of the oldest and most respected (albeit generalized) methods is based on the solubility under different pH conditions. Humic acid, one fraction of NOM in water, is not soluble at $\text{pH} < 2$, but soluble at higher pH. Fulvic acid is soluble at all pHs. Humin is not soluble at any pH. When describing the conjugate bases (e.g., the sodium salts), the terms humate and fulvate are used, respectively.

Typical soluble NOM has a molecular mass range from 300 to 30,000 unified atomic mass units (or daltons, Da). Common moieties include aromatic rings, alkyl chains, carboxylates, phenols, and other alcohols. However, polynuclear (polycyclic) aromatic compounds are not a significant portion of NOM. A good amount of work has been dedicated to characterizing NOM (Kwon *et al.*, 2005; Moon *et al.*, 2006; Swietlik and Sikorska 2005; Kim and Yu, 2005; Delapp *et al.*, 2005).

Because NOM does not reflect a single compound or even a closely related group of compounds, it is very difficult to characterize. Therefore, NOM is sometimes

fractionated based on its physical properties, such as polarity, namely its relative retention on functionalized poly(styrene-divinylbenzene) resins (e.g., Rohm & Haas XAD[®]). Other physical properties, such as ionizability, are also used. The U.S. Geological Survey has developed elaborated techniques to fractionate NOM and characterize the individual fractions. EPA currently is involved in multiple cooperative efforts to relate NOM characteristics to DBP formation.

Aside from fractionation, another avenue of NOM characterization is via properties of bulk solution rather than individual chemical components. For the bulk source of organic carbons, NOM is often measured in raw and finished water using total organic carbon (TOC) analyzers (Urbansky, 2001). Modern TOC analyzers convert carbon in organic carbon compounds to carbon dioxide, which is then measured with an infrared detector. In addition to TOC, which includes suspended particulate matter, dissolved organic carbon (DOC) can also be reported. In practice, DOC is most often used, and most TOC analyzers are more effective at determining DOC than TOC.

Techniques commonly used for characterization mostly rely on the identification of individual functional groups, such as amines, thiols, alcohols, carboxylates, and halides. NOM can be subjected to traditional elemental analysis through combustion. Infrared spectroscopy is one instrumental technique that can assess some of the functional groups present via distinct infrared absorption bands that correspond to O-H stretching, C=O stretching, or other types of independent vibrations. Nuclear magnetic resonance (NMR) spectroscopy, another, is used to distinguish among aromatic, alkyl, and alkenyl compounds. Relative contributions of these different types of carbon-carbon bonds can be estimated from the NMR spectra. Pyrolysis-GC/MS can also fingerprint NOM in terms of four biopolymer groupings, namely, polysaccharides, proteins, aminosugars, and polyhydroxyaromatic compounds. It is evident the more complex the sample, the more difficult the fingerprinting and the interpretation.

1.2.2.3. Halogenation of NOM

In addition to the NOM composition, a few other factors determine the composition of DBPs. The choice of oxidizing disinfectant is one obvious factor. The presence of

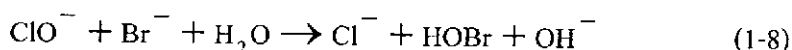
other ions, such as bromide, can have a profound impact on the nature and distribution of the DBPs formed during water treatment. Temperature, pH, and oxidant dosing rates all can affect DBP formation. By definition, NOM is a reducing agent. When an oxidant, such as chlorine or hypochlorous acid, is exposed to NOM, a number of oxidation-reduction reactions are likely. All natural water has an oxidant demand. For example, when chlorine is used, the chlorine demand is a measure of the ability of dissolved organic matter to react with chlorine. Until the chlorine demand is satisfied, disinfection is a compromise between the oxidant reacting with the NOM and the microorganism, therefore disinfection efficiency decreases. Once the chlorine demand is satisfied (essentially everything that can react with chlorine has), additional chlorine goes to disinfection. As far as DBP formation is concerned, the chlorine demand in and of itself is not a measure of the tendency to form DBPs. Much of the chlorine added to meet demand is reduced entirely to chloride rather than being incorporated into a halogenated by-product.

Halogenated (brominated and/or chlorinated) compounds are of greatest concern due to health effects observed in laboratory animals. Total organic halide (TOX), a concept largely developed/promoted by EPA is defined as the sum of the concentrations of all halogenated organic compounds. The true value of the TOX concentration cannot be determined; the number and identities of the individual halogenated compounds formed during disinfection are also unknown. Therefore, in practice, the TOX concentration is operationally defined with measurement by a TOX analyzer. TOX analyzers use activated carbon to capture halogenated organic matter. The carbon is then combusted at about 800-1000°C to convert all halogens to the hydrohalic acids (HX). The halide ion is then coulometrically titrated with silver(I) and expressed as chloride. Halogenated organic matter that is not readily or strongly adsorbed to activated carbon is routinely lost, negatively biasing the reported TOX value. Compounds other than trihalomethanes (THMs) and HAAs, such as 2,2,2-trichloroethanediol (chloral hydrate), haloacetonitriles, or trichloronitromethane (chloropicrin), can also be found in chlorinated portable water supplies. Together, the haloacetonitriles make up about 2% of the halogenated organic matter, and 2,2,2-trichloroethanediol also makes up about 2% of the halogenated organic matter after disinfection takes place. These DBP species are formed regardless of the NOM source. It is believed that the same types of structures are responsible for DBP formation on a molecular level. These structures are thought to be duplicated with all NOM molecules regardless

of the overall size of the molecule. This results in fairly uniform distribution of baseline DBPs, such as THMs and HAAs when water is chlorinated.

As noted earlier, a number of factors can influence the DBP formation (Wu *et al.*, 2003). EPA has funded and specifically worked on several of them. Comprehensive disinfectant decay and disinfection by-product formation (D/DBP) models in chlorination and ozonation were developed and applied to various types of raw and treated waters (Sohn *et al.*, 2004; Abdullaha *et al.*, 2003).

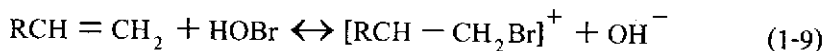
When waters contain bromide, chlorination produces a variety of brominated by-products. Bromide is oxidized by chlorine(I) to give bromine(I). At drinking water pH, most chlorine(I) is in the form of hypochlorite. However, hypobromite is a stronger base, and therefore the oxidation-reduction reaction is accompanied by hydrolysis:



HOBr is kinetically more labile than hypochlorous acid even though it is a weaker oxidant from a thermodynamic standpoint. Thus, bromination reactions abound during chlorination. In this fashion, a mixture of brominated, chlorinated, and bromochlorinated by-products are formed during disinfection. Studies have attempted to evaluate the effect of bromide on the formation of mutagenic by-products; for example, a study conducted with Jefferson Parish, LA, water considered the effect of bromide (Hua *et al.*, 2006). Chlorination of source water containing bromide results in the formation of not only chlorinated DBPs, but also brominated and bromochlorinated DBPs (Hua *et al.*, 2006). Other studies have identified some of these brominated, chlorinated, and bromochlorinated by-products (Yang and Shang, 2004; Richardson, 2003; Nobukawa and Sanukida, 2001).

The precise quantities of the specific brominated, chlorinated, and bromochlorinated by-products requires further research. Some studies, however, have focused particularly on HAAs and THMs because they are known to make up much of the identifiable DBPs and are the subject of regulation. As pH goes down, the formation of brominated species increases (Myllykangas *et al.*, 2005). This occurs because most reactions involving hypohalous acids proceed through a halogen(I) cation transfer step (Equation 1-9). This elementary reaction proceeds faster in acidic solutions because a hydroxide leaving group is more favorable than an

oxide leaving group (which would have to be converted to hydroxide in water due to the leveling effect of the solvent).



The tendency to form brominated versus chlorinated species is also dependent on the DBP precursor material (NOM). For example, some types of NOM tend to form brominated HAA species, while some types of NOM tend to form chlorinated species (Li *et al.*, 2002).

In addition to more fundamental studies of chemical kinetics, attempts have been made to empirically model the DBP formation (Nokes *et al.*, 1999). Because NOM is an ill-defined material, it is not possible to elucidate rigorously detailed reaction mechanisms. To help water utilities comply with the surface water treatment rules and the disinfection by-products rules, the Office of Water has prepared a modeling program that can be used in conjunction with site-specific chemicals and engineering data (EPRI and the California Energy, 2004; Nikolaou *et al.*, 1999).

1.2.2.2. Regulations

Mass spectrometry allows the study of molecules by, to put it colloquially, weighing them. To be more precise, the mass/charge ratio of ions resulting from the fragmentation of a molecule, as well as the fragmentation pattern, is determined accurately. Mass spectrometry has long been the dominant means to identify DBPs regardless of the oxidizing agent. The quantification of DBPs through mass spectrometry as well as other detectors forms basis of many EPA methods in monitoring regulated DBPs.

Analytical method development has taken an important role in the EPA/ORD DBP strategy. In order to monitor, study, and regulate DBPs, a reliable method of analysis is needed. Mass spectrometry is often the recommended technique in identifying and/or quantifying DBPs, although other detectors are permissible. The use of mass spectrometry, for its definitive result, has gone far in ensuring the quality of data generated from the compliance monitoring and risk management studies. Ensuring the quality is essential if decisions are to be

based on those data. Several researchers have focused on defining practices in ensuring quality data (Zwiener and Richardson, 2005; Xie, 2001).

HAAAs are more difficult to determine than THMs. Their analytical chemistry has recently been reviewed elsewhere (Nikolaou, 2005). This is a result of the acidic nature of these contaminants, which causes them to not be amenable to direct GC analysis like the THMs. To solve this problem, EPA Method 552.0 (Hodgeson, 1990) provides for the analysis of 5 HAAAs using diazomethane to esterify the analytes after extraction into *tert*-butyl methyl ether. The methyl esters are then injected into a GC and detected by electron capture. Method 552.1 followed, replacing the diazomethane with acidified methanol. In Method 552.1, the analytes were extracted by running the tap water through a solid phase anion exchange resin. The current version of the method, Method 552.2 (USEPA, 1995), eliminates the use of explosive diazomethane, which is the most carcinogenic substance known to man (on a base pair methylation basis). Method 552.2 was designed with the preferred steps from both 552 and 552.1. Method 552.2 combines an MTBE extraction with acidified methanol esterification. Method 552.2 was verified for all 9 HAAAs. Although EPA promulgated Method 552.2 to monitor HAA9 under the Information Collection Rule, many laboratories have continued to use Method 552. More care is necessary with Method 552 because diazomethane used in Method 552 degrades the brominated trihaloacetic acids, especially in white light. Following the promulgation of the Information Collection Rule, EPA attempted to discern how well labs were doing using EPA-approved methods for DBP quantification. The performance of Method 552.2 is dependent on both the specific water used and the skill of the analyst, particularly for the brominated trihaloacetic acids. As an alternative, complexation electrospray mass spectrometry was recently used to determine HAA9 in drinking water. Because it does not have the acidic methanol step, problems with the brominated trihaloacetic acids are reduced (Paull and Barron, 2004).

There are nine haloacetic acid (HAA) congeners that contain chlorine or bromine. Five of the HAAAs (HAA5) are regulated under the Stage 1 D/DBP Rule, including monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA). The four unregulated congeners include bromochloroacetic acid (BCAA) and the brominated trihaloacetic acids, bromodichloroacetic acid (BDCAA), chlorodibromoacetic acid (CDBAA), and tribromoacetic

acid (TBAA) (Brophy *et al.*, 2000). Identification of disinfection byproducts and concern over the possible adverse health effects of these compounds has promoted considerable research activity in USA and Europe in order to minimize the risk of cancers. United State Environmental Protection Agency (USEPA), World Health Organization (WHO) and the European Union (EU) introduced regulations for HAAs in drinking water. In 1979 the United States EPA initiated a regulatory standard of $60 \mu\text{g l}^{-1}$ for HAAs under the safe drinking water act. A level of $10 \mu\text{g l}^{-1}$ has also been introduced for bromate (Nikolaou *et al.*, 2007).

1.2.3. Transducers

MIPs could be considered as appropriate alternatives to the biological receptors for use in sensors principally because of their high stability. MIPs can be synthesized for analytes for which no receptor or enzyme is available or when they are too expensive. Additionally, the polymerization step is fully compatible with microfabrication used in sensor technology. The transducer part of a sensor serves to transfer signal from the output domain of the recognition system to, mostly, the electrical domain. Because of the general significance of the word, a transducer provides a bidirectional signal transfer (*i.e.*, non-electrical to electrical and vice versa); the transducer part of a sensor is also called a detector, sensor or electrode, but the term transducer is preferred to avoid confusion. Recently, many transducers have been used concurrently with MIP as the sensors selective to the various analytes of interest. A piezoelectric quartz crystal microbalance (QCM) has been used as a transducer of the biosensors for measuring mass loading on the conducting electrode surface of a crystal. Piezoelectric crystals are well known to be quite sensitive to pressure and any mass change on their surfaces. The oscillating frequency of a piezoelectric crystal decreases with the adsorption of foreign substances on the surface. Hirayama *et al.* (2002) developed a quartz crystal microbalance (QCM) sensor selective to acetaldehyde, which was prepared by using a copolymer of methyl methacrylate and ethylene dimethacrylate as a sensitive layer. The polymerization reaction was initiated with lauroyl peroxide in chloroform containing acetaldehyde. The sensor showed good sensitivity and response towards acetaldehyde in chloroform, and also in acetone or trichloroethane aqueous solution. An artificial receptor for microcystin-LR was synthesized using the technique of

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molecular imprinting, which was reported by Chianella *et al.* (2003). The synthesized copolymer of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), urocanic acid ethyl ester (UAEE) and ethylene glycol dimethacrylate (EGDMA) was used both as a material for solid-phase extraction (SPE) and as a sensing element in a piezoelectric sensor. Using the combination of SPE followed by detection with a piezoelectric sensor, the minimum detectable amount of toxin was 0.35 nM. An acrylamide-methacrylate copolymer imprinting with a series of triazine herbicides was explored by Pogorelova *et al.* (2002). The polymer was deposited on the gate surfaces of ion-sensitive field-effect transistors (ISFETs) and piezoelectric Au-quartz crystals. Binding the substrates onto the imprinted polymer associated with the gate of the ISFET alters the electrical charge and potential of the gate interface, thus allowing the potentiometric transduction of the binding events. The association of the substrates with the imprinted membrane linked to the Au-quartz crystal results in the membrane swelling, thus enabling the microgravimetric quartz crystal microbalance assay of the substrate binding events. The specificity of the imprinted recognition sites is attributed to the complementary H-bond and electrostatic interactions between the substrates and the acrylamide-methacrylic acid copolymer. Overoxidized polypyrrole films templated with L-glutamate have been utilized for enantioselective detection of L- and D-glutamic acid, which were accomplished by Deore *et al.* (2000). The electrochemical quartz crystal microbalance technique, which can also be used to perform voltammetric experiments, was used as a signal transduction scheme. The L-glutamate was inserted into the film, about 30 times higher than D-glutamate. The sensor templated with L-glutamic acid also exhibited excellent selectivity over several other L- and D-amino acids. High enantioselectivity of the overoxidized polypyrrole film can be attributed to the potential-induced uptake/release of targeted molecules. A chemical sensor for caffeine through the combination of molecularly imprinted polypyrrole and a piezoelectric quartz transducer was proposed by Ebarvia *et al.* (2005). The caffeine-imprinted polymer was synthesized using galvanostatic electropolymerization of pyrrole monomer directly onto one of the gold electrodes of a 9 MHz AT-cut quartz crystal in the presence of caffeine. A steady-state response was achieved in about 10 min. The sensor exhibited a linear relationship between frequency shift and \ln of caffeine concentration in the range of 0.1–10 mg mL⁻¹. The sensitivity of the sensor was about 255 Hz/ \ln concentration (mg mL⁻¹). A good repeatability, R.S.D. = 9 ($n = 6$) for 0.5 mg mL⁻¹ caffeine solution was also observed. Feng *et al.*

(2004) investigated the biosensor for the determination of sorbitol based on molecularly imprinted electrosynthesized polymers. A relevant molecularly imprinted film was prepared by *o*-phenylenediamine (*o*-PD) using the electrochemical method. Quartz crystal microbalance was employed as a sensitive apparatus of biosensor for the determination of sorbitol. A linear relationship between the frequency shift and the concentration of analyte in the range of 1–15 mM was found. The detection limit was about 1 mM.

Voltammetric sensors generate sensing response from the redox peak current characteristic of the analyte under a sweep of the electrode voltage over a range of redox potentials associated with the target redox reaction. The voltammetric sensors offer an additional advantage with which redox signals for reference molecules added to the sample can be simultaneously measured for accuracy improvement. Voltammetric sensors are especially attractive for their remarkable detectability, experimental simplicity and low cost. A cholesterol-selective sensor based on poly(2-macaptobenzimidazole) (PMBI) layer prepared on gold electrode surface by electropolymerization using imprinting technology was also reported by Gong *et al.* (2003). This sensor was employed in conjunction with differential pulse voltammetry (DPV) and ferricyanide as a mediator. Maximum concentration of cholesterol of 100 μM could be detected with a linear determination range up to 20 μM and a detection limit of 0.7 μM . Lopez *et al.* (2003) studied a voltammetric sensor for vanillylmandelic acid (VMA) based on acrylic MIP-modified electrodes. Thin layers of MIPs for VMA have been prepared by spin coating the surface of a glassy carbon electrode with a mixture of monomers (template, methacrylic acid, a cross-linking agent and a solvent) followed by in situ photopolymerization. The peak current recorded with the imprinted sensor after rebinding has a linear relationship with VMA concentration in the range 19–350 $\mu\text{g ml}^{-1}$. The sensor was able to differentiate between VMA and other, closely related structurally, compounds, such as 3-methoxy-4-hydroxyphenylethylene glycol, or 3,4- and 2,5-dihydroxyphenylacetic acid. The voltammetric and quartz crystal microbalance sensor integrated with the molecularly imprinted overoxidized polypyrrole highly selective to glutamic acid was proposed by Deore *et al.* (1999). The sensor showed the chiral-recognition efficiency towards each chiral of glutamic acid, L-Glu or D-Glu, templated. In addition, Chen *et al.* (2000) continued to explore such an overoxidized polypyrrole as a

molecularly imprinted colloid selective to L-lactate, and it showed higher sensitivity to this template than the other structurally related amino acids.

One of the most compact and sensitive transducers, a miniaturized planar four-electrode or inter-digital conductometric (IDC) transducer, is an electronic transducer system. It has now become an important transducer in medical, biological and environmental diagnostics. Generally, an electronic transducer system leads to a compact design, which is easy to use, inexpensive and portable. Liao *et al.* (2004) studied the preparation of a MIP-based capacitive sensor specific for tegafur. It was constructed by electropolymerization of *m*-aminophenol onto the surface of a gold electrode and the Au-coated quartz crystal electrodes. These sensors were not treated with alkanethiol after electropolymerization and showed even more satisfactory performance. Suedee *et al.* (2004) revealed the use of a conductometric sensor based on MIP embedding in polyvinyl chloride membrane for the screening of a complex mixture of haloacetic acids (HAAs) in drinking water. The recognition of the HAAs was achieved by trichloroacetic acid (TCAA)-imprinted polymers synthesized from the copolymerization of 4-vinylpyridine (4-VPD) and ethylene glycol dimethacrylate (EDMA) in the presence of TCAA in acetonitrile. The sensors showed high selectivity and sensitivity to the response toward TCAA. The detection limit of each HAA in the range of 0.2-5.0 $\mu\text{g l}^{-1}$ was obtained. Yu and Lai (2005) developed a molecularly imprinted polypyrrole (MIPPy) film synthesized on the Spreeta sensor, a miniaturized surface plasmon resonance (SPR) device, for the detection of ochratoxin A (OTA). The MIPPy was electrochemically polymerized on the sensor surface from a mixture solution of pyrrole and OTA in ethanol/water. The SPR angle increased when binding of OTA molecules occurred, within a linear correlation range from 0.05 to 0.5 ppm.

The original membrane in the "native form" was found unusable for applications like separation, pervaporation and reverse osmosis because of its poor selectivity and specificity reasons. It must be modified to have the required properties and characteristics. Kondo *et al.* (2000) prepared MIP chiral membranes from amorphous poly(hexamethylene terephthalamide/isophthalamide) imprinted with N- α -Benzyloxycarbonyl-D-glutamic acid (Z-D-Glu) or N- α -Benzyloxycarbonyl-L-glutamic acid (Z-L-Glu), using the dry phase inversion technique. The membrane imprinted by D-isomer recognized D-isomer in preference to the corresponding L-isomer, and vice versa. Electrodialysis of the racemic amino acid showed that its

permselectivity directly reflects its adsorption selectivity. MIP membranes containing artificial recognition sites for 4-vinylpyridine were studied by Guo *et al.* (2000). These membranes were prepared by photocopolymerization of methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDM), using 4-aminopyridine and 2,2'-azobisisobutyronitrile (AIBN) as a template and a photo-initiator, respectively. The membranes could bind higher amount of 4-aminopyridine than of 2-aminopyridine, whereas the latter could transport through the 4-aminopyridine imprinted polymer membrane faster than the former. Ramamoorthy and Ulbricht (2003) reported the molecular imprinting of cellulose acetate (CA)-sulfonated polysulfone (SPS) blend membranes selective to Rhodamine B dye by phase inversion precipitation technique. The CA/SPS imprinted membranes were found to have relatively higher binding capacities than the blank membranes. The terbumeton herbicide-selective MIP membranes, synthesized from surface graft copolymerization of 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS), methacrylic acid (MAA) or acrylic acid (AA) and a cross-linker *N,N'*-methylene-bis-acrylamide onto a hydrophilized polyvinylidene fluoride microfiltration membrane, using UV irradiation and benzophenone as a photoinitiator, was developed by Sergeyeva *et al.* (2001). Imprinting with AMPS gave higher affinities than with MAA and AA. The terbumeton-imprinted membranes showed a significantly higher sorption capacity to this herbicide than to similar compounds (atrazine, desmetryn, metribuzine). Yang *et al.* (2005) proposed molecularly imprinted polypyrrole nanowires with glutamic acid binding sites situated at the surface. In the imprinting step, the imprinted molecule is immobilized on the pore walls of a silane-treated nanoporous alumina membrane. The nanopores are filled with the pyrrole mixture, and the polymerization is then initiated. The alumina membrane is subsequently removed by chemical dissolution, leaving behind polypyrrole nanowires with glutamic acid binding located at, or close to, the surface. The imprinted nanowires indeed exhibit a higher capacity for glutamic acid than the control nanowires. The glutamic acid-imprinted nanowires also show a high selectivity for glutamic acid over the related compounds phenylalanine and arginine. Polypyrrole (PPy) modified microporous polyethylene (PE) membranes were prepared by oxidative polymerization in the presence of FeCl_3 , by Tishchenko *et al.* (2000). At low (< 2 h) and high (> 6 h) duration of pyrrole polymerization, the diffusion permeability of PPy/PE membranes is low. In the first case of short duration of polymerization, the PPy covers mainly the outer membrane surfaces; the surface of

pores remains hydrophobic as pyrrole has no time to penetrate into them. In the second case, PPy forms thick layers on both the membrane and pore surfaces, resulting in their blocking. The diffusion transport of acid and alkali was accompanied by removing Fe from the PPy/PE membranes. Zhou *et al.* (1996) studied the conducting polypyrrole composite membranes with a separation layer of a polypyrrole doped with hexafluorophosphate (PF_6^-) and *p*-toluenesulfonate ($\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$). Both membranes displayed preferential permeation of methanol. The highest selectivity to methanol over toluene, accompanying an acceptable methanol flux, was obtained with the membrane doped by PF_6^- . Cation-exchange membranes based on polymeric composites of a sulfonated poly(styrene-*co*-divinylbenzene) and a polypyrrole were reported by Scherer *et al.* (2001). The amount of polypyrrole in the composites depends on the oxidant solution concentration used in the synthesis. Comparing composite membranes, the highest electronic conductivity and the best thermal stability were obtained from the composite membranes with the greatest content of polypyrrole. The membranes were thermally stable up to 250°C . Polypyrrole is homogeneously distributed in the sulfonated poly(styrene-*co*-divinylbenzene) matrix. Consequently, composite membranes swell in water much less than base a membrane alone.

1.3. Objective

The aim of this work was to seek a specific and reliable method of direct detection of HAA disinfection by-products in drinking water by means of molecularly imprinted poly(ethyleneglycoldimethacrylate-*co*-vinylpyridine) and a polypyrrole recognition element. The sensing system was to be incorporated into a suitable transducing analysis system known as the interdigitated, microgravimetric and voltammetric transducers. Additionally, the micro-filtration regenerated cellulose (RC) membrane was used as a based membrane and merged with the imprinted polypyrrole for extension to separate of HAA disinfections by-product in drinking water. To generate a pre-defined HAA selectivity for cross-linked poly(ethyleneglycoldimethacrylate-*co*-vinylpyridine) and a non cross-linked polypyrrole using the molecular imprinting technique TCAA was used as template. Effects from various factors, including intrinsic polymer properties and technological parameters, were first explored. The TCAA-imprinted polymers were made as a thin film onto the surface of both a cyclic

voltammetric (CV), inter-digitated conductometric (IDC) transduction unit, and a piezoelectric quartz crystal microbalance (QCM) transduction unit, and then were embedded in the RC micro-filtration membrane. The recognition property and sensing performance of the TCAA-imprinted polymers integrated in all these transducer systems as well as the membrane have been assessed for the applications of these sensors and the modified membrane in the determination and separation of haloacetic acid in drinking water.