Evaluation of Molecularly Imprinted and Non-Imprinted Nanoparticles for Removal of Endocrine Disrupting Compounds from Surface Water and Wastewater

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ABSTRACT

Endocrine disruptors are trace contaminants of growing concern. Improved analytical procedures as well as increasing industrialization and use of pharmaceuticals have resulted in the measurement of trace levels of endocrine disruptors in many surface water bodies and drinking water resources. Endocrine disruptors are partially removed during conventional drinking water and wastewater treatment processes primarily during biological treatment, activated carbon treatment, disinfection, and membrane treatment. Biological treatment and disinfection function by degrading the endocrine disruptors into potentially harmful daughter products; the efficiency of activated carbon treatment is reduced by fouling, and membrane treatment is not economically feasible. Molecularly imprinted polymer (MIP) nanoparticles have the potential to completely remove endocrine disruptors from water without leaving daughter products behind since they function through adsorption but have a greater attraction for compounds similar to the imprinted template.

This study investigated the use of molecularly imprinted (MIP) and non-imprinted nanoparticles (NIP) for preconcentration of endocrine disruptors for analytical measurements and for water treatment applications. In the first phase, results indicated that the preconcentration of 17 β-estradiol (E2) onto MIP was not efficient enough for analytical preconcentration purposes and requires significant development work. E2 is a common endocrine disruptor found in wastewater and drinking water. In the second phase of experiments, the non-imprinted polymers (NIP) were shown to be effective for the removal of 17 β-estradiol (E2) 17α-ethinylestradiol (EE2) bisphenol-A, atrazine, and diethylstilbestrol. NIP particles were also shown to effectively remove unknown trace organic contaminants and disinfection by products from surface and secondary wastewater effluent, as well as ammonia and total organic carbon (TOC). In the third

phase of experiments, several chemical and physical removal schemes were investigated for the final removal of the particles from the water following treatment and centrifugation was shown to be effective.

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LIST OF ACRONYMS AND NOMENCLATURE

ACN	Acetonitrile
AIBN	2-isoburyonitrile
BPA	Bisphenol-A
COD	Chemical oxygen demand
DDW	Distilled-deionized water
DEHP	Bis(2-ethylhexyl)phthalate
DES	Diethylstilbestrol
DOC	Dissolved organic carbon
E1	Estrone
E2	17β-estradiol
EDC	Endocrine disrupting chemical
EE2	17α-ethynylestradiol
EGDMA	Ethylene glycol dimethacrylate
FD	Fluorescence detector
GAC	Granular activated carbon
HPLC	High-performance liquid chromatography
LC-MS	Liquid chromatography with mass spectrometry
MAA	Methacrylic acid
MeOH	Methanol
MIP	Molecularly imprinted polymers
NIP	Non-imprinted polymers
NP	Nonylphenol
PAC	Powdered activated carbon
PAH	Polyaromatic hydrocarbons
PES	Polyethersulfone

PVC	Polyvinylchloride
RPM	Revolutions per minute
SEM	Scanning electron microscope
SPE	Solid phase extraction
TEA	Triethylamine
TOC	Total organic carbon
UV	Ultraviolet also ultraviolet detector

1 INTRODUCTION

Molecularly imprinted polymers (MIP) are polymers in which the polymerization takes place in the presence of a template molecule. Following polymerization, the template can be removed from the polymer, leaving behind a cavity. The cavity is specific to the template or molecules with similar structures and MIP's can selectively remove molecules matching the template from solution. Non-imprinted polymers (NIP) are created in the same way as MIP except that they are not created with a specific template and function by non-specific surface adsorption.

In this study, MIP nanoparticles with a 17-β estradiol (E2) template and an average diameter of 580 nm were evaluated for two potential applications: preconcentration of E2 for analytical purposes, and treatment of endocrine disrupting compounds (EDCs) for water and wastewater treatment applications. Because MIP particles are specific to the template, preconcentration using MIP has the advantage of being capable of simultaneously pre-concentrating the analyte and eliminating sample interferences. Improved analytical analysis of E2 would then provide an advantage for the remainder of the study by enabling the measurement of very low concentrations of E2 in environmental samples. In addition, MIP particles which are specifically designed to remove E2, would have a high efficiency for removal of EDCs from water and wastewater by specifically targeting molecules with similar properties to the template estrogen, E2.

In the third phase of experiments, removal of the particles from water following treatment was investigated. For implementation of MIP particles for water or wastewater treatment applications, removal of the particles following treatment is imperative.

The endocrine system is a system of hormone producing glands acting as chemical messengers to control reproduction, growth, and maintenance activities. EDCs interfere with the endocrine system to produce disruptions affecting the health, growth, and reproduction of organisms (Birkett, 2003). Endocrine disruptors can enter wastewater through human waste, use of hormone treatments on livestock, or industrial sources. Consequently, complete removal of EDCs from both wastewater and drinking water is crucial.

Generally speaking, biological treatment, as well as oxidative treatment during disinfection, is efficient at removing EDCs from water. Nevertheless, for both of these treatments the EDCs are not completely removed, but rather are broken into smaller compounds with the potential to be even more harmful than the parent compounds in some cases. Adsorption is one treatment mechanism capable of completely removing EDCs from solution. Activated carbon adsorption is typically used. However, with activated carbon adsorption, fouling of the carbon with other interfering compounds within the wastewater can be problematic, and reduces the efficiency of adsorption (Meng et al, 2005). MIP particles are capable of completely removing EDCs from solution, but because of their specific binding sites, are inaccessible to interfering species. MIP particles can also be regenerated up to 30 times making them a cost-effective treatment method for EDCs.

There have been many previous studies which have looked at the use of MIP for analytical preconcentration of EDCs from solution. The preparation methods and thus efficiency of the MIP varied for each of these studies, but all showed that MIP have good potential to be used for analytical preconcentration (Alexander et al, 2006; Amalric et al, 2008; Bravo et al, 2007; Pap et al, 2002; Peng-Ju et al, 2007; Masque et al, 2001; Matsui et al, 1995; San Vicente et al, 2004; Sellergren, 1999; Sanbe and Haginaka, 2002; Randhawa et al, 2007; Watabe et al,

2005). However, there have been relatively few studies investigating the use of MIP for water treatment applications. (Meng et al, 2005; Le Noir et al, 2007; Lin et al, 2008; Fernandez-Alvarez et al, 2009; Randhawa et al, 2007). Meng et al (2005) looked at the removal of EDCs from spiked lake water, whereas studies by other researchers used deionized water, in some cases accounting for humic acid concentrations or pH changes. None of the previous studies looked at the use of NIP with treated wastewater or the use of NIP particles for general removal of a variety of EDCs.

Furthermore, none of these studies investigated the use of the particles following disinfection. Unlike MIP particles, NIP function through non-specific surface adsorption and thus have the ability to remove a wide-variety of hydrophobic contaminants from water. This poses an advantage if they are applied following chlorination because they have the potential to remove not only EDCs but also disinfection by-products. In addition, application following chlorination has the potential to increase the efficiency of the particles because most of the parent EDCs are oxidized during disinfection and the particles would mainly be applied to remove the disinfection by-products or daughter compounds. Although the application of MIP and NIP to drinking and wastewater treatment is still in its developmental stages, the additional factors investigated in this study can help to further that development and make it a viable technology for pilot or full-scale testing.

Overall, the results of this work indicated that the preconcentration of 17 β-estradiol (E2) onto MIP was not efficient enough for analytical preconcentration purposes and requires significant development work. In the second phase of experiments, the non-imprinted polymers (NIP) were shown to be effective for the removal of 17 β-estradiol (E2) 17α-ethinylestradiol (EE2) bisphenol-A, atrazine, and diethylstilbestrol from deionized water. NIP particles

effectively removed unknown contaminants from surface and secondary wastewater effluent and spiked E2 from secondary wastewater effluent. The application of NIP particles following disinfection was also successful for removal of disinfection by-products. In addition, NIP were tested for their ability to remove several common wastewater contaminants such as ammonia and total organic carbon (TOC). In the third phase of experiments, several chemical and physical removal schemes were investigated for the final removal of the particles from the water following treatment and centrifugation was shown to be effective.

The use of NIP particles for removal of EDCs will be useful for advanced treatment of wastewater for water re-use applications or for treatment of heavily contaminated wastewater prior to release to the environment. The treatment of EDCs through a combination of chlorination and treatment with NIP is also important for the future application of NIP.

2 LITERATURE REVIEW

There are several fundamental concepts which must be understood prior to an investigation of the applications of molecularly imprinted polymers (MIP) for analytical preconcentration and removal of EDCs for water and wastewater applications. The following literature review will provide this basic background by introducing the endocrine system and endocrine disrupting compounds, describing how these compounds are currently measured in the environment as well as the current removal efficiency of commonly applied water and wastewater treatment methods, and introducing molecularly imprinted polymers for analytical and water clean-up applications.

2.1 The Endocrine System

The endocrine system and the nervous system work together to integrate the functions of different cells in a multicellular organism. The endocrine system is responsible for growth, reproduction, maintenance, homeostasis, and metabolism. Endocrine glands include the hypothalamus, pituitary, thyroid, parathyroid, and adrenal glands, the pineal body, and the gonads. Each of these glands generates hormones that are transported to target organs through the bloodstream. Target cells contain a binding site or receptor as well as an effector site, which is affected by the action of the hormone. Hormones attach to the receptor and alter the effector site, producing a result. Some of the hormone molecules never reach the target cells because they are inactivated through metabolic clearance processes before excretion by the kidneys and liver. Due to metabolic clearance, hormones are short-lived in the body. However, EDCs, can interfere with clearance mechanisms, leading to bioaccumulation of hormone chemicals (Birkett, 2003). Since receptor sites typically possess a high affinity for specific hormones, trace amounts of a hormone can cause a response. Yet the receptor sites are capable of accepting other compounds.

Therefore, some EDCs other than the intended hormone can bind with receptor sites causing responses at very low concentrations (Birkett, 2003).

EDCs (endocrine disrupting chemicals) may bind to receptor sites causing one of two responses. The first occurs when the EDC binds to the receptor site causing a response at the effector site. This is called an agonistic effect, and mimics the effects of the hormone. Additionally, the EDC may bind to the receptor site without creating a response at the effector site. EDCs are typically small compounds and mimic smaller hormones such as steroid or thyroid hormones. Although there is no response to the EDC, the EDC occupies the receptor site so that natural hormones are not capable of binding to the receptor site. This is called an antagonistic effect (Birkett, 2003).

Furthermore, EDCs can stimulate cells causing the formation of additional receptor sites. The presence of additional receptor sites amplifies the effect of hormones. As well, EDCs can be endocrine flushers, increasing the natural hormone digestion processes within the body, leading to decreased levels of hormones. They can interfere with the enzymes within the body that breakdown hormones, leading to a longer hormone life in the body and higher hormone concentrations. EDCs are capable of destroying hormones or interfering with the hormone by altering its structure and making it incapable of acting on a receptor site (Birkett, 2003).

2.2 Endocrine Disrupting Chemicals

Endocrine disrupting chemicals are not limited to hormones or steroid compounds. There are a wide variety of endocrine disrupting compounds. These include alkylphenols, alkylphenol polyethoxylates, polyaromatic hydrocarbons, polychlorinated biphenols, phthalates, bisphenol-A, polybrominated flame retardants, dioxins, furans, herbicides, pesticides, and steroid hormones. Most endocrine disrupting compounds are typically found in pesticides. A European Union study

identified 118 endocrine disrupting compounds (Fraunhofer-Institut für Umweltchemie und Ökotoxikologie, 1995).

2.3 Measurements of EDCs

Measurement of EDCs can either be done using biological assays (or tests) or chemical analysis. Due to the wide range of EDCs, and the growing number being identified, assays are convenient because they can be used to screen for any endocrine activity. Chemical analysis alternately is only capable of measuring specific, identified EDCs. Assays may be either in vitro or in vivo. In vitro assays are the most appropriate for screening yet they frequently produce unreliable results, and should be checked with in vivo assays or chemical quantification. In vivo assays are the most accurate of the assays, and use both receptor and non-receptor mediated mechanisms to evaluate endocrine activity. To identify which compounds are causing the endocrine activity, and to quantify the amounts of these chemicals, further chemical analysis is required (Voulvoulis and Scrimshaw, 2003).

2.3.1 In vitro assays for measurement of EDCs

- **Competitive ligand binding:** Competitive ligand binding is based on the mode of action of estrogens, binding to the estrogen receptor. This then causes the related biological effect. Competitive ligand binding uses radio-labelled 17β-estradiol, which are used to confirm the presence of the estrogen receptor and to measure the level of response assuming no competitive binding. The sample is then introduced and the change in the amount of the radio-labelled hormone bound to the estrogen receptors is used to measure the degree to which EDCs in the sample are capable of competing for estrogen receptor sites (Voulvoulis and Scrimshaw, 2003).

- **Cell proliferation techniques:** These use human-derived cell lines. Cell proliferation techniques issue from the idea of increased growth of estrogen responsive cells with exposure to estrogen. Cell proliferation techniques are capable of differentiating between agonists, partial agonists, and inactive compounds (Voulvoulis and Scrimshaw, 2003).
- **Recombinant receptor-reporter assays:** This is the method of choice for first pass screening. However it has a low throughput, which can be a problem for large sample sizes. Recombinant receptor-reporter assays use genetically engineered mammalian cells or strains of yeast with the cells transformed by introducing vectors containing DNA sequences for the receptor, and response elements for the promoter regions of a reporter gene (Voulvoulis and Scrimshaw, 2003).

2.3.2 In Vivo Assays for Measurement of EDCs

In vivo assays measure the effect of an EDC on an animal. This is necessary to evaluate the effects of the EDC on the entire endocrine system and the entire organism. Multi-generational studies can be used to give a clearer picture of the effects of endocrine disrupting chemicals. The compounds may not have an effect on the animal itself, but may have an effect on the offspring. The most widely used in vivo assay is the rodent uterotrophic test, based on the actions of EDCs to stimulate uterine growth in mice or rats. An increase in uterine weight is considered to be a clear indication of estrogenicity (Voulvoulis and Scrimshaw, 2003).

2.3.3 Chemical Analysis for Measurement of EDCs

Both liquid and gas chromatography are well-suited to simultaneous analysis of a wide variety of compounds. Yet, generally, the clean up and analysis procedures vary depending on the EDC being analyzed for (Voulvoulis and Scrimshaw, 2003).

2.4 Sources of EDCs in the Environment

2.4.1 Sources of Steroids in the Environment

Estradiol, progesterone, and testosterone all produce growth effects. The aforementioned hormones have been used in meat animals for 50 years along with synthetic chemicals. Natural and synthetic steroid estrogens are major contributors to estrogenic activity and are typically found in sewage effluent. Such compounds are not completely removed during wastewater treatment, and are potent at low concentrations. Concentrations of EE2 (17-α ethinylestradiol) as low as 0.2 ng/L have been shown to cause male fish to produce vitellogenin, a female yolk protein (Birkett, 2003).

Wastewater treatment effluents contain steroid estrogens due to excretion. Women excrete 10 to 100 mg of estrogen per day and pregnant woman can excrete as much as 30 mg per day. Most of these estrogen compounds are excreted in urine in a biologically inactive and conjugated form as glucuronides and sulfates. Nevertheless, free estrogen has been observed in wastewater effluent, meaning that they are deconjugated during the wastewater treatment process.

Concentrations of estradiol, estrone, and estriol in wastewater sludge have been shown to range from 0.01 to 0.08 ng/L in sludge compared to 1.21 to 0.81 ng/L in wastewater effluent. Agricultural land that has had sewage sludge applied to it acts as an area source for these hormones (Birkett, 2003).

Synthetic estrogen compounds from birth control pills and hormone replacements can also be problematic. Contraceptives typically contain a combination of estrogen and progestin, with ethinyl estradiol (EE2) or mestranol acting as the active ingredient. Concentrations of EE2 in contraceptives range from 20-50 mg. Hormone replacement drugs use conjugated estrogens from equine sources. Conjugated estrogens are typically taken in a daily dose of 0.625 mg. These

pharmaceuticals, based on sales in the United States, create estimated concentrations in aquatic environments of 2.16 ng/L (Birkett, 2003).

2.4.2 Sources of Organo Oxygen Compounds in the Environment

2.4.2.1 Sources of Bisphenol-A in the Environment

Bisphenol-A is used in the plastics industry for polycarbonate and epoxy resins, unsaturated polyester-styrene resins, and flame retardants. It is frequently used in food and drink packaging. It can be expected to be present in wastewater influent and effluent as well as wastewater sludge. Concentrations in wastewater effluent have been measured at 0.018 to 0.702 mg/L and in wastewater sludge as 0.004 to 1.363 mg/kg (Birkett, 2003).

2.4.2.2 Sources of phthalate in the environment

Phthalates are used in PVC (polyvinyl chloride) and other resins for building materials, furniture, transportation, clothing, food packaging, and medical products. They are not chemically bonded to the polymeric matrix and are capable of leaching. Not highly soluble, they and adsorb onto sediments and suspended solids. Concentrations of Bis(2-ethylhexyl)phthalate, which is a phthalate commonly found in wastewater effluent, have been measured as 1.74 to 182 mg/L and 27.9 to 154 mg/kg in wastewater sludge (Birkett, 2003).

2.4.2.3 Sources of Surfactants in the Environment

Surfactants are commonly used in detergents. Endocrine disrupting surfactants include alkylphenols and ethoxylates. Many of these products are used in domestic detergents. Most, used for domestic as well as commercial and industrial purposes, are disposed of through sewers. Wastewater treatment leads to degradation and a shortening of the ethoxylate chain. Ethoxylate degradation then produces nonylphenol, which is considered to be more toxic and more

persistent than its parent compounds. High freshwater concentrations of surfactants are found near wastewater treatment plants, mills, or heavily industrialized areas. Wastewater effluent concentrations of phonylphenols in the United Kingdom have been measured between 0.02 and 330 mg/L. High concentrations of nonylphenol are also found in wastewater sludges, and leaching from sludge disposal sites or agricultural land where fertilizers with sludge are used can cause contamination (Birkett, 2003).

2.4.2.4 Sources of Polyaromatic Hydrocarbons (PAH) in the Environment

PAH compounds are formed during incomplete combustion of organics. Wastewater effluents can be a major source of PAH's in freshwater. PAH's in wastewater come from industrial and domestic effluent, urban runoff, and atmospheric pollution. Concentrations of individual PAH's vary from 1 to 3520 ng/L during dry weather and 1840 to 16,350 ng/L in wet weather with urban runoff being a major source. PAH concentrations in sludge can be between 1.6 to 6 mg/kg. High concentrations of PAHs in wastewater sludge are found in areas where wastewater treatment plants serve industrial areas (Birkett, 2003).

2.4.2.5 Sources of Brominated Flame Retardants in the Environment

Flame retardants are used for fire prevention in plastics, textiles, electronics, and other products. They are persistent and bioaccumulate in animal fat. Some additives leach from manufactured products because they do not bind chemically. Concentrations of all brominated flame retardants have been found in wastewater sludge. Exact concentrations vary depending on local usage of brominated flame retardants. Concentrations have been measured between non-detectable to 450 μg/kg for polybrominated diphenylethers and between non-detectable and 220 μg/kg for tetrabromobisphenol-A (Birkett, 2003).

2.5 Removal of EDCs during Water and Wastewater Treatment

Treatment of EDCs in both wastewater and drinking water is an emerging research area and the use of MIP particles is only one potential treatment method. Thus, prior to discussing the use of MIP for water or wastewater clean-up, treatment during conventional wastewater and drinking water treatment will be discussed.

2.5.1 Removal of EDCs during coagulation, flocculation, and sedimentation

Coagulation, flocculation, and sedimentation are the first steps in drinking water treatment. Variations of this treatment method also occur during primary clarification of wastewater. Organic compounds, including EDCs, can be removed during this process either through co-precipitation with solid particles, or by adsorption onto the surface of particles during sedimentation or onto the surfaces of already sedimented particles (American Waterworks Association Research Foundation, 2007).

2.5.1.1 Removal of EDCs during Coagulation, Flocculation, and Sedimentation for Drinking Water Treatment

An American Water Works Association study completed in 2007, tested coagulation, flocculation, and sedimentation for removal of EDCs in both bench-scale and full-scale tests. The bench-scale tests found less than 15% removal for 34 of the 36 EDCs tested for coagulation with aluminum sulfate and ferric chloride. The full-scale tests did not provide highly accurate results because the concentrations were not spiked and in some cases were very close to the detection limits of analytical equipment. Yet several of the EDCs tested showed much higher removal than for the bench-scale tests. It is possible that treatment was more efficient due to differences in mixing regimes and contact between sedimented particles and the EDCs (American Waterworks Association Research Foundation, 2007).

Choi et al (2006) studied the removal of bisphenol-A and nonylphenol using 5 different coagulants. Although the turbidity and DOC (dissolved organic carbon) decreased substantially following coagulation and flocculation, the removal efficiencies of nonylphenol and bisphenol-A were only 4-7% and 0-3%. The study concluded that the 30 minute time allowed for coagulation and flocculation was not long enough for significant amounts of the EDCs to adsorb onto particles, and thus they were not removed from the water with the particles. The coagulant used had little effect on removal. Changing the coagulant changed the efficiency of particle removal, but the particles did not contain significant concentrations of EDCs (Choi et al, 2006).

Since the main mechanism for removal of EDCs during coagulation, flocculation, and sedimentation is the removal of the endocrine disrupting compounds sorbed onto particles, mechanisms for increasing removal during water treatment focus primarily on increasing the sorption. Rebhun et al (1998) increased the efficiency of flocculation for removal of hydrophobic contaminants with log Kow values less than 4.5 through the addition of humic acid prior to flocculation. Humic acid adsorbed onto the particles and increased the organic content of the particles, allowing for greater sorption of hydrophobic contaminants (Rebhun et al, 1998).

Modeling is important to understand the factors contributing to the removal of EDCs during coagulation and flocculation. Ballard and Mackay (2005) developed a model for removal of organic compounds (mainly pesticides) from water during water treatment and verified the model with experimental results. Some pesticides are EDCs, and the characteristics of typical EDCs are similar. This model assumed that all organic matter had the same potential for adsorption of the pesticides and ferric hydroxide flocs themselves had no adsorption potential. The aforementioned assumption was made because it is relatively easy to measure the TOC (total organic carbon) of the water, but not to measure varying organic content of different particles.

Overall, the model fit reasonably well with experimental results, which indicated removal values of 1-24%, indicating that coagulation and flocculation alone are not sufficient for removal of EDCs (Ballard and Mackay, 2005).

Results from studies of coagulation, flocculation, and sedimentation processes in typical drinking water treatment plants are not promising for the removal of EDCs. Although endocrine disrupting chemicals are hydrophobic, and do have a high capacity for adsorption, removal via removal of suspended solids from the drinking water does not seem to be an effective removal mechanism, and the efficiency was never more than 25% for any of the studies investigated. It may be that these drinking water treatment processes do not allow adequate time for adsorption to occur, or it may be that the organic content of the suspended materials in surface water is not high enough to promote adsorption. Regardless, this is not an adequate removal mechanism on its own, and further treatment of EDCs is required.

2.5.1.2 Removal of EDCs During Coagulation, Flocculation, and Sedimentation for Wastewater Treatment

EDCs are hydrophobic and hence typically adsorb onto suspended solids (Langford and Lester, 2003). EDCs adsorbed onto solids are then removed from the treatment stream either during primary treatment, when solids settle; or during secondary treatment, when biological solids are settled. Adsorbed EDCs are concentrated in the wastewater sludge (Auriol et al, 2006). The partitioning coefficients (K_{ow}) can typically be used to determine the degree of adsorption for a specific EDC. K_{ow} is a measure of the equilibrium partitioning of a compound between an organic liquid and water. It can be used as a measure of the hydrophobicity of the endocrine disrupter, which in turn can be used to predict the degree of adsorption. For a log(Kow) above 4, EDC removal is achieved predominantly by sorption to settled sludge and suspended solids.

Along with the hydrophobicity of the EDC, the organic content of the solids in the wastewater also influence the degree of adsorption (Langford and Lester, 2003). Wastewater contains fats, oils, greases, and surfactants. These compounds are typically dissolved, and can adsorb hydrophobic EDCs (Langford and Lester, 2003).

2.5.2 Removal of EDCs from Wastewater during Activated Sludge Treatment

Activated sludge treatment, like coagulation, flocculation, and sedimentation, has the capacity to remove EDCs on the surface of sludge particles via adsorption, but has the added advantage of being a biological treatment process. Thus both adsorption and biological degradation occur during activated sludge treatment.

Biodegradation pathways for estrogens are complex and have not been studied extensively enough to produce conclusive results (Lee and Liu, 2001). Typically, one form of estrogen is degraded into another estrogen prior to further degradation. Hence, measurements of influent and effluent concentrations can be complex. Contradictory results occur where some estrogens appear to have increasing concentrations between the inlet and outlet. Yet, this is due to the degradation of another estrogen and does not mean that the overall concentrations of estrogens are increasing.

Li et al (2008) studied the biodegradation of natural estrogens in activated sludge. 17β-estradiol (E2) and estrone were spiked into semi-continuous aerobic activated sludge reactors. Experiments were conducted with differing initial concentrations of glucose to determine the effect of readily biodegradable materials in wastewater on the degradation of estrogens. Results indicated that when the reactor was spiked with E2 alone, the E2 disappeared quickly. Measurable concentrations of E1 (estrone) then appeared, indicating that E1 is a degradation product of E2. The E1 then slowly began to degrade once the majority of the E2 had already

degraded to form E1. Whereas the degradation of E2 in a reactor spiked with E2 alone took approximately 2 hours, the degradation of E1 in a reactor spiked with E1 alone was much slower and took approximately 6 hours. The addition of glucose was found to decrease the degradation rates for both E1 and E2. At a dose of zero glucose, the average conversion ratio of E2 to E1 was 28.9% whereas at a glucose dose of 100 mg/L the average conversion ratio was 13.4%, meaning that less E1 was formed in the presence of glucose. This in turn indicates that the biodegradation of E2 is somewhat inhibited by the presence of glucose.

A study of activated sludge treatment plants in Rome found removals of estriol, 17β-estradiol, estrone, and ethinylestradiol to be 95, 87, 61, and 85%, respectively. Four of the 30 sites tested exhibited increased estrone from influent to effluent due to biological oxidation of 17β-estradiol to estrone (Langford and Lester, 2003).

Vader et al (2000) studied the degradation of EE2 in nitrifying activated sludge. The nitrifying capacity of activated sludge was a determining factor for EE2 removal. For the activated sludge displaying a significant nitrifying capacity, the EE2 was completely degraded within 6 days with no acclimatization period required for the microorganisms. The nitrifying sludge was also able to degrade EE2 in the absence of ammonium. The EE2 was oxidized by nitrifying bacteria, resulting in the formation of hydrophobic compounds. The hydrophobic compounds were then adsorbed by activated sludge solids. The addition of hydrazine as an electron donor further increased the degradation of EE2.

Lee and Liu (2001) conducted aerobic and anaerobic batch experiments to determine a reaction pathway for E2. E2 as well as its metabolites were not environmentally persistent in wastewater samples and could be degraded quickly by bacteria native to the sewage samples. Biodegradation occurred under both aerobic and anaerobic conditions although biodegradation

was faster under aerobic conditions. Under aerobic conditions, 88% of the initial E2 spike was degraded after 1day. Under anaerobic conditions, 50% of the spike was degraded after 7 days.

The results of the aforementioned studies reveal that the majority of EDC removal from wastewater occurs during activated sludge treatment. There are two important rationales. Firstly, the contact time for activated sludge treatment is longer than for primary treatment or for coagulation, flocculation, and sedimentation. This allows more time for adsorption to occur. Secondly, biodegradation of EDCs in solution or sorbed to the surface of activated sludge particles increases removal efficiency.

2.5.3 Removal of EDCs with Activated Carbon Treatment

Activated carbon treatment is typically reserved for drinking water applications and not wastewater treatment. Therefore, only drinking water treatment will be subsequently discussed. Activated carbon functions via adsorption processes similar to the molecularly imprinted nanoparticles tested in this study. Accordingly, results from activated carbon studies are important for three reasons. Firstly, if activated carbon can be applied with a high level of efficiency for removal of EDCs, there will be no need to use the molecularly imprinted polymers. Secondly, results from activated carbon studies can be used to gain a better understanding of the general adsorption processes occurring for treatment of EDCs, which will be useful to better understand adsorption of EDCs onto the nanoparticles of this study. Additionally, adsorption presents an alternative to oxidation for complete removal of EDCs rather than chemical degradation which yields daughter compounds.

Activated carbon operates through the creation of a highly porous structure possessing a large surface area to volume ratio. The use of nanoparticles similarly increases the surface area to volume ratio to provide an ideal adsorption surface. Activated carbon, similar to the particles

studied in herein functions through hydrophobic interactions between the activated carbon surface and organic contaminants in the water.

The American Waterworks Association study (2007) found removal efficiencies of greater than 50% for bench scale tests. In pilot scale tests, efficiencies were greater than 50% treatment for powdered activated carbon (PAC) concentrations of 5 mg/L and greater than 80% for 25 out of 29 EDCs studied for concentrations of 35 mg/L. Estradiol presented a 40-45% removal and a 90-95% removal for 5 and 35 mg/L powdered activated carbon. Atrazine exhibited removal of 40-45% and 90-95% and for ethinylestradiol the activated carbon achieved removal efficiencies of 35-40% and 90-95% (American Waterworks Association Research Foundation, 2007).

Choi et al (2006) studied the removal of bisphenol-A and nonylphenol for powdered activated carbon concentrations ranging from 1-10 mg/L. Removal efficiencies increased with increasing initial concentrations of bisphenol A or nonylphenol. For example, the removal efficiency increased from 2-36% for an initial concentration of 497 ng/L to 15-39% for an initial concentration of 1,053 ng/L. They also found an increase in efficiency with increased PAC dose. Granular activated carbon tests using seven different granular activated carbons were also conducted. Tests using granular activated carbon which had been completely saturated with dissolved organic carbon and had reached exhaustion for treatment of dissolved organic carbon, bisphenol-A and nonylphenol could be completely removed from starting concentrations of 2.3 and 2.9 mg/L for bisphenol-A and nonylphenol, respectively. Breakthrough tests for EDCs were not completed (Choi et al, 2006).

2.5.4 Chlorine Oxidation for Removal of EDCs

Oxidation of EDCs during the disinfection stage of drinking water treatment is probably one of the most extensive removal options. However, like biological degradation, chemical oxidation does not completely remove the EDCs and creates potentially harmful daughter products which must be investigated.

Benotti et al, (2009) analyzed drinking water from 19 American drinking water treatment plants for 51 different EDCs. They found that the concentrations of these compounds in finished drinking water was primarily controlled by the type of disinfection method employed (Benotti et al, 2009). Therefore, the method of disinfection, as well as some of the controlling principles of the disinfection process such as contact time and concentration, is essential in determining the final fate of EDCs.

Deborde et al (2004) studied the potential for treatment of six EDCs using oxidation with chlorine. They researched the reaction rate kinetics for the reactions of progesterone, 4-n-nonylphenol, 17α-ethynylestradiol, β-estradiol, estrone, and estriol with chlorine. Of these six EDCs, only progesterone could not be oxidized with chlorine given chlorine concentrations of up to 1 mg/L of chlorine and half hour contact times. Pseudo- first-order reaction kinetics were observed for all other EDCs. Reaction half-lives at a chlorine concentration of 1 mg/L varied from 6.3-7.3 minutes for estrone, estriol, β-estradiol, and 17α-ethynylestradiol. The half-life for 4-n-nonylphenol was 65.1 minutes, indicating that a much longer contact time or a higher chlorine concentration would be required to effectively remove 4-n-nonylphenol. For a typical chlorine concentration of 2 mg/L and contact time of 15 minutes, 95% reduction of estrogen would occur, but only 27% reduction of 4-n-nonylphenol. A contact time of 140 minutes would be required to provide a 95% reduction of 4-n-nonylphenol (Deborde et al, 2004).

One important consideration for removal of EDCs with chlorination is the formation of disinfection by-products. When EDCs are oxidized, the initial form of the EDC may no longer be measurable, but this does not mean that there are no longer EDCs in the water or even that the water is safer. Korshin et al (2006) completed studies measuring the total organic halogen during chlorination of both bisphenol-A and diethylstilbestrol. Eventually, the concentrations of these byproducts decreased, but the time required for complete removal was much longer than the typical time provided for disinfection in a typical drinking water treatment plant. For bisphenol-A, the total organic halogen concentrations continued to increase for 4 hours following the initial introduction of chlorine. However, for diethylstilbestrol, the reaction time was only 5 minutes and was within the time typically provided for drinking water treatment (Korshin et al, 2006).

Since chlorination produces a variety of currently unknown disinfection byproducts, it is difficult to determine how the addition of chlorine would change the overall estrogenicity of water. Lee et al (2004) studied the estrogenicity following treatment of E2, nonylphenol, and bisphenol-A with chlorine. Overall, they found that the estrogenicity decreased with the addition of chlorine. However, for bisphenol-A an initial increase in the estrogenicity of the solution was observed after a 3 minute contact time. It should also be noted that the chlorine concentration used for this study was high (7.5 mg/L). Following this initial increase in estrogenicity, the estrogenicity decreased and reached the initial estrogenicity again after a ten minute reaction time. They also found that complete removal of the estrogenicity resulting from E2 did not occur until 36 hours following disinfection with a chlorine concentration of 1.5 mg/L which would not occur in typical drinking water treatment (Lee et al, 2004).

For bench test results, sodium hypochlorite was used as the chlorination agent and was quenched with ascorbic acid following a 24 hour reaction time. The chlorine dose, measured as

free chlorine, varied between samples from 2.8 mg/L to 6.75 mg/L (American Waterworks Association Research Foundation, 2007). A 24 hour reaction time is very long because chlorine contact times during treatment are more likely to be in the order of 15-30 minutes.

Percent removals for chlorination varied for different EDCs depending on their chemical structures and functional groups. The reaction mechanism is different for different EDCs and some are more easily oxidized than others. Estradiol showed greater than 97% removal for all of the river water samples tested, ethinyl estradiol showed greater than 98% removal, but atrazine showed less than 25% removal. Chloramine was also tested, but exhibited poor removal values of less than 10% for 20 out of the 34 contaminants after 24 hours. 168 hours were required to achieve similar removals to the sodium hypochlorite. Mixed oxidants and sodium hypochlorite were also tested to provide a mixture of free and combined chlorine. Generally, the results using the mixed oxidants were similar to those using sodium hypochlorite alone (American Waterworks Association Research Foundation, 2007).

Choi et al (2006) observed removal efficiencies of 58 and 5% for bisphenol-A and nonylphenol using chlorine concentrations of 1 mg/L and to undetectable levels at concentrations of greater than 5 mg/L. This shows that chlorine dose is incredibly important for removal of EDCs and that disinfection processes should be carefully optimized for removal of EDCs.

2.5.5 Ozone Oxidation for Removal of EDCs

Ozone reacts quickly and decays quickly in water systems leaving fewer byproducts than chlorine. It can lead to varied percent removals of EDCs due to differences in chemical structures. The percent removals were high for the majority of the EDCs tested in an AWWA study. Ozone alone as well as a combination of ozone and peroxide were both tested for 5 minute

reaction times. Ozone was applied to achieve a final residual concentration of 0.2 to 0.3 mg/L after 3 minutes and no residual for 10 minutes. A low dosage of hydrogen peroxide was added. Percent removal values varied between different water samples due to differences in the dissolved organic matter concentrations in the water samples; however, it was possible to gain approximate ideas of removal. Estradiol showed greater than 99% removal for all of the samples tested for both ozone alone and for ozone/hydrogen peroxide. Ethinyl estradiol showed greater than 98% removal. Atrazine, however, exhibited removal values as low as 17% for ozone alone for one water sample and 24% for the combination of ozone and hydrogen peroxide for the same sample.

Although ozone reacts more quickly to destroy EDCs and creates fewer byproducts, this does not mean that there are no byproducts associated with the ozone oxidation of EDCs. Deborde et al (2008) found five byproducts for the ozonation of bisphenol-A: catechol, orthoquinone, muconic acid, benzoquinone, and 2-(4-hydrophenyl)-propan-2-ol. The estrogenicities and general toxicities of each of these by-products must be determined to evaluate the environmental effects of each of these byproducts. Other intermediate byproducts were also identified, but these were quickly consumed in the reaction. They also found that a decrease in pH from 6.5 to 4.5 occurred during treatment due to the formation of carboxylic acid and that an ozone dose of 4-4.5 times the concentration of bisphenol-A existing in the water was required for treatment (Deborde et al, 2008).

Rivas et al (2009) measured minearalization of bisphenol-A by measuring CO_2 production during the reaction of bisphenol-A and ozone. Mineralization with ozone, although initially very fast, only reached 30% TOC conversion. This suggests significant concentrations of oxidized by-products. They then tested the use of both activated carbon and TiO_2 as catalysts to

encourage complete mineralization of bisphenol-A. The addition of the TiO_2 catalyst was capable of increasing the total mineralization to 60% and the use of activated carbon removed 90-95% of total TOC, representing better options for removal. Activated carbon was thought to work as a catalyst by catalyzing the formation of $HO^{\cdot}$ radicals from ozone (Rivas et al, 2009).

Additionally, pilot scale tests were conducted using spiked concentrations of the EDCs. The pilot scale tests were highly efficient with 55% of the EDCs showing greater than 95% removal for an ozone dose of 1.25 mg/L and still greater treatment for an ozone dose of 2.5 mg/L. Addition of 0.0625 mg/L hydrogen peroxide led to a 10% greater removal, with most of the removal occurring within a 2 minute reaction time. Tertiary treated wastewater was also tested for removal. Ozone decay rates were found to be much faster with the tertiary treated wastewater. Therefore higher ozone concentrations were used. 15 EDCs were tested in the tertiary wastewater since it was not spiked. Again, 53% of these were removed to below detectable limits.

Deborbe et al (2005) studied the ozone oxidation of several EDCs including BPA, NP, E_1, E2, E_3, and EE2. They found that the initial parent compounds were removed very quickly, but stressed the need to test the estrogenicity and other potential effects of oxidation byproducts. They proposed second-order reaction kinetics and suggested that low pH values (~pH 5) led to the highest efficiency while high pH values (pH~10) decreased the efficiency for all compounds.

Choi et al (2006) measured removal rates 60 and 89% of bisphenol-A and nonylphenol respectively for ozone removal at a concentration of 1mg/L and were able to remove both EDCs to below detection limits for a higher ozone concentration of 4 mg/L.

2.5.6 Ultraviolet Irradiation and Advanced Oxidation for Removal of EDCs:

UV light can either directly cleave bonds to oxidize organics or it can produce hydroxyl radicals which can oxidize the organics. UV advanced oxidation processes function primarily through the creation of hydroxyl radicals which is enhanced by the addition of hydrogen peroxide or other advanced oxidation agents (American Waterworks Association Research Foundation, 2007). In bench-scale tests, minimal treatment was observed at typical disinfection doses of 40 mJ/cm^2. Higher removal efficiencies were observed at doses of 1000 mJ/cm^2 but the further addition of hydrogen peroxide appeared to have little effect. In some cases, the addition of hydrogen peroxide actually decreased the level of treatment. This was attributed to the addition of methanol to the water samples which was said to act as a hydroxyl radical scavenger. Although the removals at the very high UV doses were promising, this is an inefficient treatment method at UV doses typically used at treatment plants for disinfection purposes (American Waterworks Association Research Foundation, 2007).

A second test was completed by Trojan Technologies in Ontario using chlorinated water samples from Lake Huron. The chlorinated samples were first exposed to UV doses of greater than 1000 mJ/cm^2 to destroy any remaining chlorine and to bring the chlorine concentrations below detectable limits. This test found that the contaminant decay occurred in a first-order pattern, which is common for trace contaminants. The first order decay is due to the fact that most of the UV energy was absorbed by the water rather than the EDCs themselves. Again, results for $40mJ/cm^2$ were poor for most of the contaminants tested and those for 1000 mJ/cm^2 were good, showing more than 80% removal for most contaminants. The results for EDCs such as estradiol, which are typically removed easily using chlorination, were high even at the lower UV dose. This was attributed to interferences with hydrogen peroxide in the samples, although

control samples with hydrogen peroxide did not show an effect. Pilot scale tests were similar and showed poor removal at typical UV doses, but good removal at very high doses 800 mJ/cm^2 (American Waterworks Association Research Foundation, 2007).

Rivas et al (2009) computed very low mineralization levels for bisphenol-A exposed to low-pressure UV light at 254 nm. Only 15% of the bisphenol-A was completely mineralized after 120 minutes of reaction, which is greater than typical reaction times. This was attributed to bisphenol-A's low capacity for adsorption of light at 254 nm. With the addition of a TiO_2 catalyst however, a mineralization rate of 80% was possible after 120 minutes of reaction (Rivas et al, 2009).

Chen et al (2007) examined the removal of a mixture of estradiol (E2) ethinyl estradiol (EE2) bisphenol-A and nonylphenol at concentrations ranging from ng/L to mg/L. To quantify the overall effect of the EDCs following oxidation with a combination of UV and hydrogen peroxide, they used both in vitro (yeast estrogen screen) and in vivo (Vitellogenin) tests. The use of these tests are important, but it should be noted that the effects of bisphenol-A and nonylphenol on the overall estrogenicity of the solution will be negligible compared to the contributions from E2 and EE2. This study was important in comparison to assays completed for a single EDC because the effects of a variety of endocrine disruptrors is important because it is possible that they may have synergistic effects. In fact, synergistic effects were observed in in-vitro assays in comparison with single-component samples. First order reaction kinetics were observed for decreases in both in vivo and in vitro estrogenicity for deionized and river water samples. Removal of in vitro estrogenic activity decreased significantly with UV/ hydrogen peroxide solution, but the removal of in vivo estrogenicity was less efficient for mixtures of

EDCs than for single-component systems, removal rates for both types of tests were also faster for deionized water in comparison to river water (Chen et al, 2007).

2.5.7 Nanofiltration and Membrane Removal of EDCs

Recently nanofiltration and reverse osmosis have received increased interest for the removal of EDCs, as well as other trace contaminants, from water for water treatment applications or wastewater for water reuse applications. Nanofiltration has the capacity to remove EDCs from water, but is not economically feasible in many cases. Work has been done to increase the efficiency of removal and to better understand factors influencing the removal such as the presence of natural organic matter.

Radjenovic et al (2008) studied the removal of several EDCs and pharmaceuticals from a full-scale drinking water treatment plant for treatment of groundwater employing both nanofiltration and reverse osmosis. High overall removal of greater than 85% was observed for both nanofiltration and reverse osmosis for all of the compounds studied. However, disposal of the brine following membrane filtration was raised as a potential environmental concern because concentrations of several of the EDCs and pharmaceuticals in the brine were in the order of hundreds of ng/L, posing a potential environmental risk if released to natural water bodies.

Retention by nanofiltration membranes is influenced primarily by the molecular size of the compounds to be removed. Neverless, Braeken et al (2009) found that the hydrophobicity of the compounds also plays an important role. They studied the removal of estrone, estradiol, and salicine using two different nanofiltration membranes and found that because salicine was less hydrophobic than either estrone or estradiol, it was capable of forming more hydrogen bonds

with water in solution. Salicine formed larger hydrated molecules which increased its effective size for filtration and therefore its level of removal from water during membrane treatment.

The presence of natural organic matter can also influence the retention of EDCs by nanofiltration membranes. Jermann et al (2009) concluded that the presence of natural organic matter increases the retention of hydrophobic pollutants while having no effect on the removal of hydrophilic pollutants. They also determined that this was not due to increased adsorption because the adsorption of hydrophobic pollutants onto the membrane actually decreased with addition of organic matter. Comerton et al (2009) also found that the presence of natural organic matter increased retention of hydrophobic EDCs, but not in the presence of cations. They suggested that the mechanism for increased adsorption was due to interactions between the EDCs and the natural organic matter within the water matrix to form larger molecules and not due to increased interactions between the membrane and the EDC.

Work has also been done to increase the efficiency of membranes for removal of EDCs by modification of the membranes. Kazner et al (2008) used activated carbon additions to increase the removal of two EDCs and two cytostatics from 35-70% to greater than 95%. Likewise, Kim et al (2008) achieved complete removal of bisphenol-A using a modified nanofiltration membrane including adsorption and an iron (III) tetrasulphthalocyanine catalyst in the presence of hydrogen peroxide for the oxidation of bisphenol-A on the surface of the membrane.

Despite modifications and improvements leading to nearly complete removal of EDCs from water using nanofiltraion or reverse osmosis membranes; the membranes remain economically unfeasible. Braeken et al (2009) studied several implementation strategies for

nanofiltration treatment of EDCs for a ten year return period and found all to be unfeasible. They suggested that perhaps the cost might be justifiable due to the high quality of water produced, and calculated the cost of treatment despite their conclusion that treatment with nanofiltration was not feasible. The cost of producing water using nanofiltration for EDC removal was shown to be twice that of treatment without nanofiltration, which would make it unfeasible in most cases despite the benefits of increased water quality.

2.6 Molecularly imprinted and non-imprinted particles

Preconcentration of environmental estrogen samples for analytical evaluation is a necessary step for quantifying concentrations in water and wastewater treatment as well as in natural systems. Solid phase extraction is a common preconcentration technique used for many applications of trace analysis, and molecularly imprinted polymers (MIP) were first developed as a medium for solid phase extraction for trace analysis purposes (Masque et al, 2001).

During most solid phase-extraction procedures, the wide range of contaminants contained in water samples for environmental analysis are co-extracted and complex extraction procedures may be required prior to analysis to isolate specific contaminants. MIP technologies offer a simultaneous preconcentration and extraction method because the binding sites, or imprinted sites, on the MIP's are designed to remove a specific contaminant from the water system (Masque et al, 2001).

MIP technologies are founded on imitation of biological antibody systems. Antibodies function based on specific binding sites where the antigen binds strongly and non-covalently to the antibody (Bauman, 2004).

MIP's are synthetic antibodies created from cross-linked polymers containing a cavity specific to an analyte. These cavities are created by copolymerization of cross-linking monomers

and functional monomers along with an imprinting molecule or template. Following polymerization, the template is removed, leaving a cavity specific to the analyte. The MIP's then selectively re-bind to this analyte compound (Masque et al, 2001; Sellergren, 1999; Alexander, et al, 2006)

Figure 2-1, shows how MIP's are formed and used. The monomer forms around the template in either non-covalent, covalent, or ligand binding, and is polymerized with the cross-linker. The template can then be removed, leaving a template-specific cavity which can then be used for removal of template molecules from pure or contaminated samples.

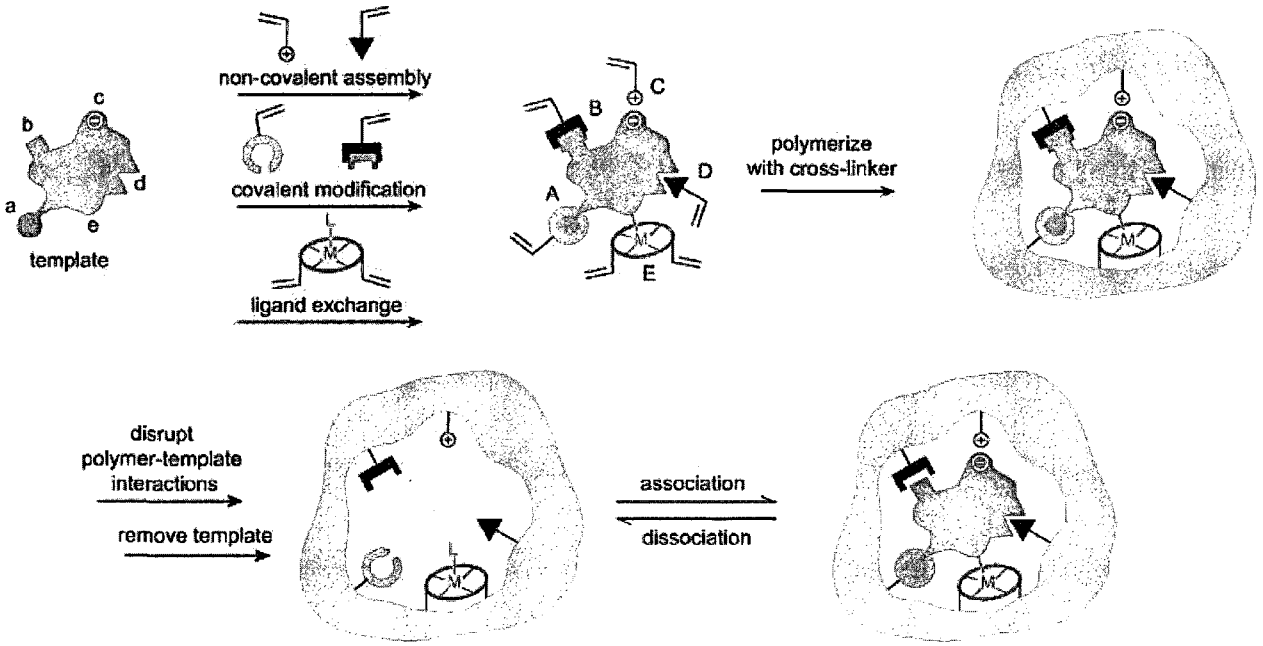


Figure 2-1: Schematic Showing the Formation, Design, and Use of MIP Particles (Alexander, et al, 2006)

2.6.1 Design of Molecularly Imprinted and Non-Imprinted Particles

There are several approaches for the design of MIP. The optimum approach depends on the template to be used and the intended purpose of the MIP. These approaches include: the

covalent approach, the non-covalent approach, the stochiometric non-covalent approach, the semi-covalent approach, and the ionic species approach. The non-covalent approach was used to create the MIP particles used for this study. Each of these approaches are discussed in detail below.

2.6.1.1 The Covalent Approach for Design of Molecularly Imprinted and Non-Imprinted Particles

The covalent approach uses templates which are covalently bound to one or more polymer groups. A covalent bond is a chemical bond where there is a sharing of electrons. Following polymerization, the template is removed and the binding site is left with the capability of re-forming covalent bonds with template molecules. An advantage of the covalent approach is that functional groups are only associated with the template sites, meaning that binding is highly specific. However, the approach can only be used for a limited number of template molecules such as alcohols, aldehydes, ketones, amines, and carboxylic acids (Alexander et al, 2006).

2.6.1.2 The Non-Covalent Approach for Design of Molecularly Imprinted and Non-Imprinted Polymers

The non-covalent approach functions by non-covalent forces such as hydrogen-bonding, ion-pairing, and dipole-dipole interactions. The interactions between the functional monomer and template during polymerization are the same as those between the polymer and the template during rebinding. This is the most widely used method for MIP production (Alexander et al, 2006). Methacrylic acid (MAA) is the most commonly used functional monomer for the non-covalent approach and ethylene glycol dimethacrylate (EGDMA) is the most commonly used crosslinker monomer (Masque et al, 2001; Sellergren, 1999). Low molecular weight templates

with functional groups capable of hydrogen bonding or acid-base reactions with the monomer can be used as templates for the non-covalent approach.

2.6.1.3 The Stochiometric Non-Covalent Approach for Design of Molecularly Imprinted and Non-Imprinted Polymers

The stochiometric non-covalent approach is a modification of the non-covalent approach for which the association constant for the reaction between the template and monomer is high and the equilibrium favours polymerization and inclusion of the template into the polymer structure (Alexander et al, 2006).

2.6.1.4 The Semi-Covalent Approach for Design of Molecularly Imprinted and Non-Imprinted Polymers

The non-covalent approach uses a combination of the covalent and non-covalent approaches. Initial polymerization takes place with covalent binding between the template and monomer. Following removal of the template from the polymer structure, rebinding takes place non-covalently. An advantage is higher specificity than the non-covalent approach and a more uniform distribution of binding sites. The template and monomer may be connected directly, or using an additional spacer group to avoid crowding of the template site (Alexander et al, 2006).

2.6.1.5 Imprinting of Ionic Species for Creation of Molecularly Imprinted Polymers

A fifth approach is imprinting with metal ions. This approach uses cross linking of linear polymers containing functional groups capable of binding to metal ions. Interactions are ionic but can vary in strength depending on the metal ion (Alexander et al, 2006).

2.6.1.6 Design Considerations for Design of Molecularly Imprinted and Non-Imprinted Polymers

The ratio of monomer to template used for construction of MIP is also very important for selectivity. Increasing the amount of monomer increases the amount of analyte bound, but beyond an optimum ratio, decreases the selectivity. This occurs because excess monomer can accumulate on the surface and provide additional non-specific adsorption sites (Liao et al, 2005).

The yield of imprinted binding sites, and hence the binding capacity of the MIP is directly related to the strength of the interaction between the functional monomers and the template both before and during polymerization. The thermodynamics of the system, the number and type of monomer interaction sites, the shape of the template, and the rigidity of the cavity are all important factors in determining the strength of interaction (Sellergren, 1999).

The number of monomer interaction sites is important because the number of interactions will increase both the binding strength and specificity. Therefore, the functional monomers should be chosen which allow for a maximization of the number of interactions and the strength of these interactions are also important. Care should also be taken to ensure that the monomer-monomer interactions do not compete with template-monomer interactions because, especially if there is no preference for the interactions with the template over the monomer, competition can substantially decrease the number of cavities formed (Sellergren, 1999).

Andersson et al (1996) studied the formation of MIP using pyridine, which is only capable of forming one hydrogen bond site with carboxylic acid groups in the monomer. It was expected that there would be no evidence of specific binding for this polymer because previous research had indicated that two hydrogen bonds or at least other hydrophobic or Van der Waals

interactions must occur. However, there was limited specificity but increased adsorption for pyridine in comparison with similar compounds. The conclusion was that there were unknown interactions occurring, but the strength of the specificity was dependent on the number and type of interactions (Andersson et al, 1996).

Another consideration is template shape. Templates other than the analyte of interest may be used (for reasons discussed in Section 2.6.2 below) however; the shape must be close to that of the analyte for interaction between the monomer and analyte to occur. Rebinding will occur with a much lower entropy loss if the template and the analyte are similar in shape. This is because entropy is lost due to shape changes in the site and template upon bonding (Sellergren, 1999).

Structural integrity or rigidity of the template sites is also important. Functional groups must be positioned in a way that allows for access by the template. This is achieved by cross linking, and cross linking must be above 50% for recognition between the cavity and the template to occur (Sellergren, 1999). Cross linking between 70-90% is ideal and will help the polymer cavities retain their shapes (Masque et al, 2001). The porosity of the polymer-cavity system must also be maintained to ensure that binding is not diffusion-limited (Sellergren, 1999).

2.6.2 Types of Binding and Adsorption that may occur on Molecularly Imprinted and Non-Imprinted Polymers

Zhang and Hu (2008) proposed a model containing three separate types of binding to explain interactions between estrogenic contaminants (E1, E2, EE2, and BPA) and MIP. The binding types included: (1) specific adsorption, (2) semi-specific adsorption, and (3) non-

specific adsorption (Zhang and Hu, 2008). Figure 2-2, illustrates the types of interactions that can occur for each of the aforementioned types of binding sites.

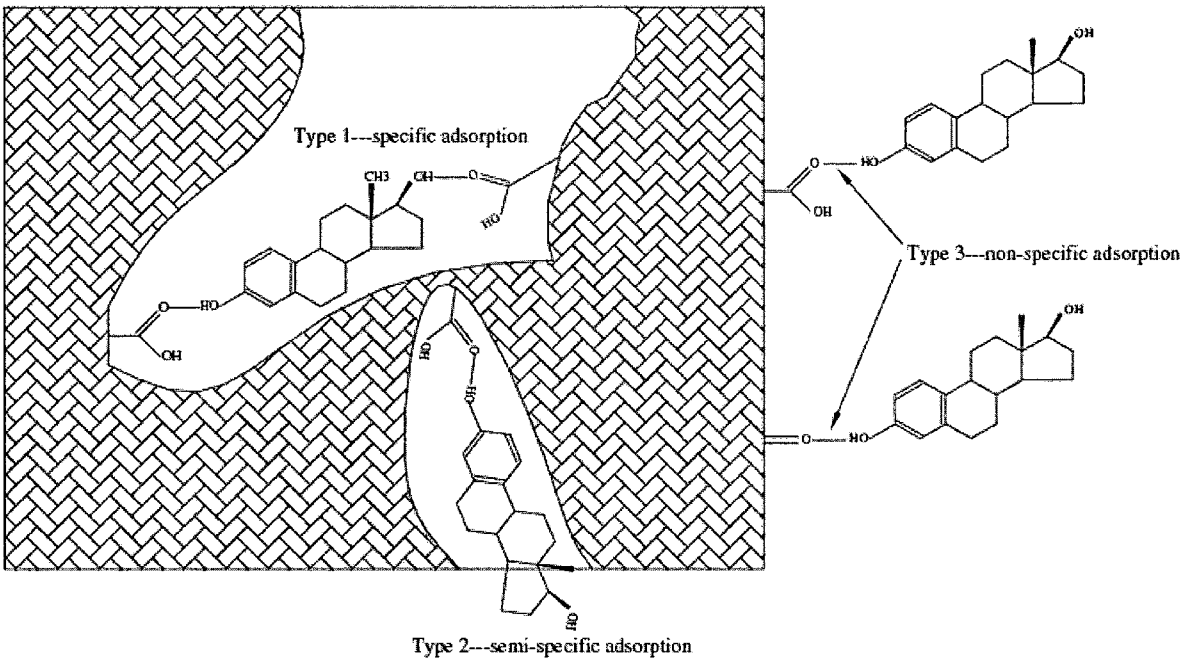


Figure 2-2: Specific, semi-specific and non-specific adsorption of EDCs to MIP prepared with an E2 template (Zhang and Hu, 2008)

Specific adsorption, as shown in the figure, can only be used for binding of the template molecule, which for the work done by Zhang and Hu was E2. For site-specific binding to be effective the shape of the binding site must closely match that of the template. There are also several thermodynamic considerations which determine whether or not binding will take place, as well as how specific the binding will be. The type and strength of the bond formed determines the selectivity of the cavity. For example, if the template binds to the receptor using hydrogen bonding or electrostatic interactions, these are much stronger than Van der Waals or hydrophobic interactions and there will be a preference for that template over other competing compounds (Koinig, 1995).

Thermodynamic considerations are important to determine whether or not any binding of the template will take place. Therefore there are three factors which must be considered in determining whether or not specific binding will occur: (1) The template and cavity must have complimentary molecular shapes, (2) there must be available non-covalent binding sites in the template (this will lead to a favourable entropy of reaction) (3) molecular rotation following binding should be minimized (this will lead to a favourable enthalpy) (Koinig, 1995).

Semi-specific adsorption sites can host either specific or non-specific adsorption due to the shape of their binding cavities. Both estrogenic and other compounds could bind to these sites; however, those compounds with a benzene ring would have a competitive advantage for the occupation of these sites. Zhang and Hu (2008) attributed semi-specific binding to the use of excess functional monomers during polymer synthesis. Functional monomers were added at a ratio of 4.8:1 compared to the E2 template. This was done to encourage the creation of more specific binding sites. The stochiometric requirement for the functional monomer to template ratio is 2:1 for the polymer formulation used. Excess functional monomers could remain at the surface of the polymer, thus providing sites for either semi-specific or non-specific adsorption (Zhang and Hu, 2008).

Non-specific adsorption refers to surface adsorption and can occur due to the hydrophobicity of the EDC and Vander Waals forces in aqueous solutions since most EDCs are hydrophobic. However, non-specific interactions were also attributed to the presence of the cross-linker which contained carbonyl groups which may have created conditions for hydrogen bond formation on the surface of the polymer where any compound capable of forming hydrogen bonds with a carboxyl or carbonyl group may have adsorbed (Zhang and Hu, 2008).

2.6.2.1 Thermodynamics of Specific and Non-Specific Binding for Molecularly Imprinted and Non-Imprinted Polymers

Thermodynamically, there are two aspects of binding which must be considered: enthalpy and entropy. Enthalpy results from energy changes which occur with interactions between the adsorbent and substrate. For imprinted polymers, this energy is very high because it is the result of the imprinted memory of the polymer. The adsorption entropy is the result of a decreased disorder following the interaction between the substrate and MIP, therefore, the enthalpy is the guiding thermodynamic factor and the energy of the binding site must be high enough for spontaneous binding to occur. Increased temperature can further increase the energy available and results in a higher level of binding because lower energy binding sites will become available to the substrate (Fang and Li, 2007).

Fang and Li (2007) studied NIP and MIP imprinted with bisphenol-A. They found that there was a higher desorption energy for BPA from MIP versus NIP, indicating specific binding sites with a higher energy than non-specific adsorption sites. They took SEM (scanning electron microscope) images indicating a smooth surface for NIP and a pitted surface for MIP, indicating clearly visible specific binding sites. Furthermore, infrared spectrum from each of the MIP with the template removed and NIP showed that MIP with the template removed were similar to NIP, indicating complete removal of the template. Also, for binding on NIP, BPA and structurally similar compounds exhibited similar binding efficiencies but BPA exhibited higher binding efficiencies for MIP, indicating specific binding sites.

Li et al (2009) also studied the interactions and thermodynamic differences for specific and non-specific binding. They found two different energy levels indicated by two kinetic adsorption steps for adsorption of 2.4-dichlorophenol to MIP. The adsorption exhibited a quick

adsorption step followed by a relatively slower step. The quick step was attributed to ionic interactions and the slow to less-specific hydrogen bonding. They gave two reasons for specificity. The first was that 2,4-dichlorophenol was larger in size than the other compounds tested and thus could fit more specifically into the holes left by the removal of the template, and the second was that the interaction energies were highest for 2,4-dichlorophenol.

Adsorption also depends on the cohesive energy holding the EDCs in the mobile phase. Successful adsorption requires a force field surrounding the surface of the adsorbent which will reduce the potential energy of the adsorbate below that experienced in the fluid phase (Karge and Weitkamp, 2008). The cohesive forces typically experienced by molecules in the liquid phases are Van der Waals or hydrogen bonding forces (Chiou, 2002). The forces typically exerted by the surface of imprinted or non-imprinted polymers are similar in nature, but the specific binding sites on MIP typically form at least two hydrogen bonds with the template molecules, which are stronger in nature, and provide a greater attraction than that of the non-specific sites.

2.6.3 Molecularly Imprinted Particles for Solid Phase Preconcentration for Analytical Analysis

The precursor to molecularly imprinted polymers (MIP) was first created in the 1930's using silica matrices. Silica particles were prepared in the presence of benzene, toluene, or xylene. The additive was removed, and subsequent adsorption was shown to be greater than for non-imprinted particles. At the time of this work, little was understood about how antibodies worked, and the mechanisms behind these synthetic antibodies were not well understood. However, since then a great deal of work has been done in the field and the research has reached a point where it has many and diverse applications including analytical solid phase preconcentration (Alexander et al, 2006).

Solid phase extraction (SPE) is the most advanced application of molecularly imprinted polymers (Alexander et al, 2006). SPE involves selective adsorption of analyte compounds into the cavities of MIP particles followed by desorption with a desorption agent and subsequent analytical analysis. The use of MIP for SPE has the advantage that it can simultaneously be used for sample cleanup and preconcentration. The sample is cleaned because the analyte is selectively adsorbed from the solution onto the MIP. Furthermore since a lower volume of the desorption agent can be used than the initial sample volume preconcentration is achieved (Alexander et al, 2006).

Rachkov et al (1998) studied separation of several EDCs using HPLC as the stationary phase for HPLC. They found that the degree of separation and resolution was highly dependent on both the mobile phase used and the structures of the EDCs to be separated. For example, they found that switching from a mobile phase with a 9:1 mixture of acetonitrile and methylene chloride instead of methanol (although the polarity of the mobile phase was not significantly changed) led to a greater degree of dipole-dipole interactions and a decrease in binding efficiency when compared to pure acetonitrile.

Additionally, they found that use of MIP for a mobile phase for HPLC separation generally lead to a broadening of the peaks. This is because of the different energy levels of the binding sites, resulting in desorption of the analyte over a period of time. So, even though the selectivity of their MIP was very good, achieving complete separation was not easy. However, separation of β-estradiol, testostereone, diethylstilbestrol, and propionate was possible. Estrone and β-estradiol were not separated as easily. This was attributed to their OH groups at the third carbon (Rachkov et al, 1998).

Watabe et al (2005) used surface treated MIP successfully to separate and pre-concentrate bisphenol-A from river water samples. They used a pretreatment column with surface modified MIP for HPLC analysis and found a high recovery of bisphenol-A from river water samples with reduced interference. Surface modification of MIP was completed to eliminate non-specific adsorption and thus interference (Watabe et al, 2005).

San Vicente et al (2004) used MIP for separation of bisphenol-A from river water samples for subsequent analysis with HPLC. An in-line column was used for separation and recoveries between 92 and 101% were obtained from river water. The recovery value greater than 100% was due to experimental error. In this case, rather than employing surface modifications to eliminate interferences, solvents which favor selective binding and remove hydrophobic surface binding were used. Acetonitrile, dichloromethane, and chloroform were all tested. Acetonitrile worked well to increase the specificity of binding and to disrupt non-specific adsorption. For chloroform and dichloromethane, bisphenol-A was retained on both NIP and MIP, which indicated non-specific binding and these solvents were not used (San Vicente et al, 2004).

Matsui et al (1995) developed a molecularly imprinted polymer selective for atrazine which exhibited at least two different types of binding sites. The MIP in this study had a binding 60 times higher than that for NIP. Several other herbicides, which were similar in structure to atrazine, were tested; a retention of less than 1% was revealed, indicating a highly specific form of binding.

Bravo et al (2007) demonstrated 72% and 83% recovery of diethylstilbestrol from river and tap water samples for an on-column solid-phase extraction for HPLC analysis. The column

was washed with acetonitrile and water to remove non-specific interactions and acetonitrile and methanol for removal of the analyte.

Sanbe et al (2002) also used an MIP column for separation of bisphenol-A from hexestrol, diethylstilbestrol, dienestrol, and beta estradiol in line with HPLC. They found no separation with NIP and complete separation of bisphenol-A from the other compounds for MIP.

Amalric et al (2008) used offline cartridges to isolate atrozine for analysis of stream water. The method applied is similar to the online method. First interfering compounds were removed using dichloromethane, then the target analyte was removed by washing with methanol. An advantage of this is that a greater binding time can be given, there is no concern about peak broadening, and actually several different washings can be completed and mixed to increase the washing efficiency (Amalric et al, 2008).

2.6.3.1 Common Problems Encountered During use of Molecularly Imprinted Polymers for Solid Phase Preconcentration and Analytical Analysis

2.6.3.1.1 Bleeding of the Template Molecule during Analytical Preconcentration and Analysis

Complete removal of the template from MIP can be very difficult and involves numerous washing steps. In fact, often even following extensive washing steps, leaching of template from the polymer will still occur (Pichon, 1997). Generally more than 1% of the template remains bound following extensive washing. Bleeding of the template following solid-phase preconcentration and subsequent desorption of the analyte, will lead to falsely high results (Sellergren, 1999).

One solution to this phenomenon is the use of a structural analogue similar to the analyte for use as a template during the preparation of the polymer. A template which does not interfere with the analyte during analysis should be chosen, and therefore it would not matter if contamination or leaching of the template occurred, the analyte could still be effectively measured (Masque et al, 2001). Thermal treatment or more effective washing can also be used to reduce bleeding (Sellergren, 1999).

Peng-Ju et al (2007) developed a procedure of accelerated solvent extraction for more efficient template removal to remove a nicotine template from MIP. They found an increase in extraction efficiency with elevated temperature up to a temperature of 80 C and were able to remove 94.2% of the template in 70 minutes using an optimized mixture of methanol and acetic acid.

2.6.3.1.2 Interferences from Non-Specific Binding During Analytical Preconcentration Using Molecularly Imprinted Polymers

Binding of MIP is generally somewhat non-specific. The MIP will bind to the template molecule, but may also bind easily to similar compounds in solution. This can be either an advantage or a disadvantage for analytical analysis. For example, in HPLC if the peaks do not overlap, then this could be an advantage because it means that several related analytes could be simultaneously extracted from an environmental sample. However, if the analytes do overlap during analysis, this could be a disadvantage because it would interfere with the analysis of the target analyte and make the isolation of this compound very difficult (Masque et al, 2001).

2.6.3.2 The Effect of the Medium on Binding and Types of Binding for Molecularly Imprinted Polymers

Binding of contaminant molecules to MIP for preconcentration is dependent on the medium in which the molecules are contained. Extraction from an aqueous solution, for example, is generally non-specific. This can be a problem for preconcentration purposes. One solution to this problem is to use a column capable of switching between water and solvent. The aqueous solution containing the analyte is first run through the column. The analyte becomes trapped by selective and non-selective binding onto the MIP. The column is then switched to an organic solvent containing an acid or a base. In this solvent, selective binding occurs, and the MIP strongly adsorbs the analyte, but non-specifically bound contaminants are washed off in the solvent (Alexander et al, 2006).

Pap et al (2002) studied the effect of the solvent on the separation of contaminants bound to MIP between the template and interfering species. The basis of the experiments was that the MIP could be added to a sample with a mixture of contaminants, each of these would bind to the MIP, and then a solvent could be used to wash interfering compounds bound to the surface of the polymer by hydrophobic interactions from the polymer prior to using a different solvent for removal of the template contaminant of interest.

Problems to consider for the application of this principle are that it is important to ensure that analyte molecule is bound only to the specific template sites and that none of the template molecules are bound to the surface of the polymer. This is dependent on the energy distribution and the concentration ratio of the analyte and MIP. Specific binding sites have a higher energy and affinity for the template than do the non-specific surface binding sites. However, if these sites become "full" then the template will bind to the surface with non-specific hydrophobic

interactions and would be removed along with the interfering compounds leading to artificially low concentration measurements. The specificity of the MIP used for these purposes is also important. If structurally similar species have the ability to bind to the specific binding sites, then they will not be removed with the interfering species and will be retained and measured with the analyte, leading to artificially high concentration measurements.

Pap et al (2002) also concluded that the choice of the solvent was very important for the selectivity of the MIP. This is because the type of solvent controls the energy distribution between the solution and sorbed phases and the type of binding favoured. They found that binding of terbutylazine was highest for pure acetonitrile and high aqueous concentrations of acetonitrile. This was because at high acetonitrile concentrations, binding was highly specific, and at high water concentrations, hydrophobic interactions became very strong, leading to strong but non-specific binding. Optimization of the solvent, and use of the solvent to control the type and extent of binding is therefore important in isolating the analyte.

2.7 Molecularly Imprinted and Non-Imprinted Particles for Water Treatment

Meng et al (2005) prepared an MIP for estrogenic pollutants using noncovalent imprinting. They studied the removal of α-estradiol from spiked lake water representing a contaminated water sample. Regeneration of the MIP was also investigated. They first tested the use of diethylstilbestrol as a template due to the high toxicity of estrogenic compounds. However, this template did not have a strong affinity for the MIP and less than 5% of the total template added was adsorbed. When α-estradiol was tested, more than 80% of α-estradiol was adsorbed from a deionized water sample in a 50 minute reaction time. The NIP exhibited a α-estradiol binding of less than 10% in the same time. They studied non-specific binding and found that MIP was specific to α-estradiol, diethylstilbestrol, and estriol, while NIP was also capable of

a high level of binding for β-estradiol and estrone, suggesting strong non-specific binding. Non-specific binding was attributed to hydrophobic interactions between the compounds of interest and the polymers. Non-specific binding is considered disadvantageous for preconcentration and sample cleanup applications. The feasibility of using MIP for water cleanup is also highly dependent on its effectiveness on complicated water matrices. α-estradiol removal was tested with lake water samples to evaluate this feasibility. Adsorption capacities were found to be 5 to 9 times lower than that for deionized water. This was attributed to a high pH and high concentration of organics in the lake water samples. However, the binding efficiencies were still considered high enough to effectively remove many EDCs from complex lake-water matrices. They also found that the particles could be effectively re-generated by washing with a methanol/acetic acid solution followed by methanol alone for up to five cycles.

MIP has been shown to be highly effective at removing EDCs at trace concentrations from water systems. However, clogging is frequently a problem for treatment of wastewater effluents. Another problem with treatment lies in treating large volumes of wastewater necessary for municipal treatment. Le Noir et al (2007) used MIP embedded in macroporous gels to process large volumes of water while avoiding clogging problems for treatment of wastewater. Monoliths are rigid but have an ideal combination of large and small pores. The large pores allow liquid to pass through freely while the small pores provide surface are for adsorption or binding onto the MIP. The pores are highly interconnected and allows for high flowrates to process high amounts of wastewater without clogging problems. Introduction of MIP into the monolith structure led to a reduction of flow paths within the system, but not significantly. They tested several different types of MIP macroporous systems, and found E2 recoveries from a 2 μg/L solution of between 75.4 – 100%. Flow rates through the macroporous gel structure were as

high as 50 mL/minute while the flowrate through a MIP column was 1 mL/minute. The column also became clogged after only 55 mL of a particulate containing fluid had been passed through it.

The use of macroporous gels for housing of MIP provides one potential application method for treatment of water or wastewater with MIP. For water systems, in which there are generally low concentrations of particulate matter, a macroporous gel structure is probably unnecessary. However, for treatment of wastewater, a high-flow, non-clogging system to house MIP during treatment is necessary, and a macroporous gel structure may provide this (Le Noir et al, 2007).

Le Noir et al (2007) compared the use of MIP and NIP with a commercial C18 agent and granular activated carbon. Columns were constructed from 6mL glass columns filled with 100 mg of MIP or NIP or 200 mg of C18 or GAC. Two liters of a 2 μg E2 solution were then percolated through the columns at a flow rate of 5 mL per minute to determine the removal efficiencies for each of the solid-phase extraction agents. Removal efficiencies were 100%, 77%, 87%, and 19% were achieved for MIP, NIP, C18, and GAC, respectively, indicating a superior removal efficiency using MIP. Testing was then conducted with a 100 mL wastewater sample percolated through MIP and NIP columns. A yeast assay was used to measure the resulting estrogenicity from elutants from the columns. The estrogenicity from the MIP column was similar to the highest E2 concentrations measured in wastewater characterizations in both a German and a Japanese study, so the conclusion was that the MIP was effective for removal of E2. No significant E2 concentrations were measured in the NIP elutant but the authors suggested that this was due to interference from inhibitory substances associated with the NIP which interfered with the assay. The high level of estrogenicity measured from the MIP elutant is

positive, however, a more comprehensive study with measures of the column influent estrogenicity for the samples being tested and more effective controls on the assay would be necessary to make any concrete conclusions as to the feasibility of the application of MIP to real wastewater samples.

Lin et al (2007) studied the removal of several estrogens from water using MIP particles imprinted with bisphenol-A. The effects of pH and humic acid concentrations were studied. The highest removal efficiency was found to occur at a pH of around 5, which is unlikely to occur in a water-treatment environment, and the lowest treatment efficiency occurred at a neutral pH. The MIP were shown to successfully remove phenolic estrogen pollutants bisphenol-A bisphenol-C bisphenol-Z and diethylstilbestrol (DES). Binding for each of these pollutants was higher on MIP than on NIP (because these are all similar in structure to the bisphenol-A template); however, binding did also occur on the NIP, indicating that non-specific binding or surface adsorption was also a factor. Ions were found to favorably affect removal efficiencies, which is promising for treatment of real water samples, because water or wastewater samples are likely to contain some concentrations of ions. This effect was attributed to the promotion of hydrophobic interaction by increased ionic strength. The effect of humic acid on removal was also investigated because this is another common component of natural water systems. Humic acid was found not to inhibit the binding. However they suggested that this was due to the lack of interactions with the functional monomer used in the study, so this may not be true for all types of MIP. When removal was tested on distilled water, tap water, lake water, and river water, the removal efficiency for distilled water was the lowest, which is very promising for use as a treatment technology. This suggests that rather than being inhibitory, other naturally occurring components of these water

systems actually improve the treatment. Regeneration was also tested and the MIP could be used more than 30 times without losing any adsorption efficiency (Lin et al, 2008).

Fernandez-Alvarez et al (2009) studied the removal of estrone, estradiol, and ethinylestradiol using MIP designed with an E2 template. They then tested the MIP for removal of the EDCs and regenerated the MIP incuding a phototreatment step to destroy the EDCs. This is important because removing EDCs from water is one step for environmental control of EDCs, but the MIP with concentrated EDCs would then become a hazardous waste stream which would require treatment. The researchers found good (73%) removal of E2 from aqueous solution using the MIP they developed and complete regeneration and re-use using UV-vis irradiation and an acetone elutants.

MIP have also been suggested as a possible method of treatment for heavy metals. Randhawa et al (2007) studied removal of cadmium ions during water treatment using cadmium imprinted resins. The resins were shown to remove 90-95 % of cadmium from a wastewater sample. They had a high capacity for cadmium, but due to large non-specific binding, this capacity was somewhat reduced in practice.

3 OBJECTIVES

The overall goal of this research was to evaluate the use of MIP and NIP particles for the removal of EDCs and other trace organic compounds during water and wastewater treatment. More specific objectives for this study are listed below:

- To investigate the use of molecularly imprinted polymers (MIP) and non-imprinted polymers (NIP) for analytical preconcentration of 17-β estradiol (E2)
- To evaluate the use of MIP and NIP for water and wastewater treatment applications. This includes:
    - An evaluation of the use of MIP and NIP for removal of E2.
    - An evaluation of the use of MIP and NIP for the removal of several other EDCs such as 17α- ethinylestradiol, bisphenol-A, atrazine, and diethylstilbestrol.
    - An evaluation of the use of MIP and NIP for treatment of surface water and wastewater.
    - An evaluation of the use of MIP and NIP for the removal of disinfection by-products.
    - Development of physical and chemical methods for the removal of MIP and NIP particles from water after the treatment is complete.

4 HYPOTHESES

- MIP's can be used effectively for solid phase extraction and sample preconcentration of E2 and other endocrine disrupting compounds as they will selectively bind to the compound of interest. Selective binding occurs because when the MIP are synthesized, polymerization occurs around a template molecule, which is later removed, leaving a template specific to the compound of interest.

- MIP and NIP are effective for removal of endocrine disrupting compounds, their disinfection by-products, and various other hydrophobic contaminants from both surface water and wastewater.

- MIP and NIP can be removed from water by incorporating physical and chemical separation methods at the end of the treatment.

- MIP and NIP provide a tool as a polishing step in water and wastewater treatment.

5 MATERIALS AND METHODS

5.1 Preparation of MIP and NIP Particles

The MIP and NIP were prepared as suggested by Wei et al (2006). 17β-estradiol (E2) methacrylic acid (MAA) which is the functional monomer, and the cross-linker ethylene glycoldimethacrylate (EGDMA) were dissolved in the porogen (or solute) with the molar ratio of 1mmol:8mmol:6.7mmol. For NIP, no 17β-estradiol was added. The porogen was composed of 40 mL of 1:3 (v:v) acetone and acetonitrile. 2% (w:w) of the monomer was then added as 2-isobutryonitrile (AIBN). The mixture was sonicated (2510 Branson sonificator) deoxygenated with nitrogen for five minutes, and then placed in a 60°C hot water bath for 24h (Hoake GH Fisons hot water bath).

The resulting particles had an average diameter of 580 μm for MIP and 354 μm for NIP (or an average overall diameter of 467 μm). This diameter was measured using dynamic light scattering nano size analysis.(Zackery De Maleki, Unpublished work). Figure 5-1 and Figure 5-2, show scanning electron microscope images of the particles. The particles were spherical in shape and the majority were in the same size range although they were not uniform in size.

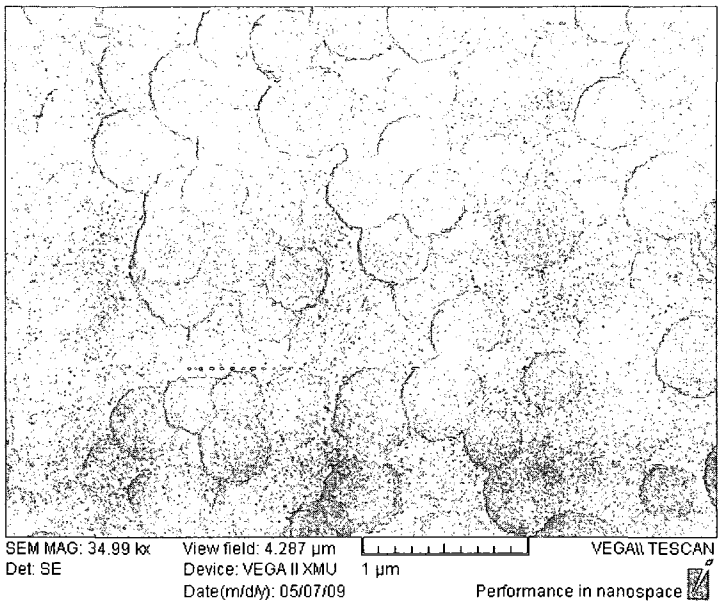


Figure 5-1: Scanning electron microscope image of molecularly imprinted polymers (average size 580 nm) (Zackery De Maleki, Unpublished Work)

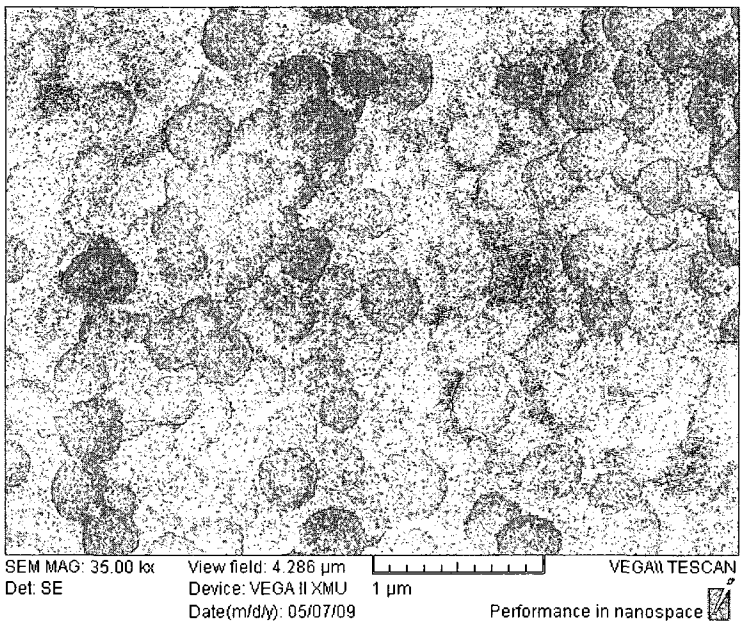


Figure 5-2: Scanning electron microscope image of non-imprinted polymers (Average size 354 nm) (Zackery De Maleki, Unpublished work)

The resulting polymers were dewatered using a centrifuge (Revolutionary Science) at 10,000 RPM, dried at 104°C overnight, and ground manually. Manual grinding was required because the particles had a tendency to clump together following drying. The particles were then rinsed four times with acetonitrile and once with water before use. MIP were then cleaned three times with 100% TEA to remove the template, rinsed once with acetonitrile, and then once with distilled-deionized water (DDW). NIP particles were prepared in the same way as MIP except that the template E2 was added during polymerization and hence template removal with TEA was not necessary.

5.2 Analytical Preconcentration of Various EDCs onto MIP and NIP Particles

5.2.1 Analytical preconcentration of E2 onto MIP particles

Solid phase preconcentration is important because it has the potential to lower the analytical detection limits for EDCs. Molecularly imprinted polymers have some advantages for analytical preconcentration because they can form strong, specific bonds to the template molecule. Thus, they can be used both for sample preconcentration by using a smaller volume for desorption than the initial sample volume and for sample cleaning.

Sample preconcentration involves two major steps: adsorption (or binding) and desorption. Both of these processes must have good but also consistent efficiencies for analytical preconcentration applications. If the efficiency is good but not consistent, it would be difficult to relate the results to a calibration curve, whereas if the efficiency is consistent but not good, the ratio of desorption agent to the initial sample would have to be very high to provide effective preconcentration.

5.2.1.1 Binding Efficiency for Binding of E2 onto MIP Particles

Binding experiments were conducted by preparing standard solutions of the EDCs in deionized water. Standard solutions were prepared using serial dilutions from a 1000 ppm standard solution prepared in methanol. Standard solutions were prepared in a 1:1 mixture of methanol and water for higher concentrations (20-100 ppm) corresponding to E2/particle ratios of 1-5 mg EDC/ g particles.

MIP samples were prepared by weighing 20 mg of the MIP (synthesized as outlined in Section 5.1) into 2 mL centrifuge tubes. 1 mL of each of the standards prepared as outlined above was added to the particles in the centrifuge tubes. The tubes were shaken manually to mix followed by 5 minutes of sonication and an additional 5 minute waiting period. Following the waiting period, the tubes were centrifuged (Revolutionary Science) at 10,000 RPM. The centrate was removed and analyzed for remaining EDC concentrations using high performance liquid chromatography (HPLC) as outlined in Section 5.3.1.

Centrate was analyzed to determine the amount of E2 remaining. In other words the amount not bound to the MIP particles. The initial amount of 17β-estradiol (E2) in each of the samples was also determined using HPLC analysis of the initial standard solutions.

The binding efficiency was then calculated based on the initial concentration of the standard, as shown below.

$$Pecent\ binding = 100\% - \left(100 * \frac{HPLC\ area\ for\ centrate}{HPLC\ area for\ standard}\right),$$

Where the expression in the bracket refers to the percent of E2 remaining in solution. Therefore the percent binding can be calculated by calculating 100% - *percent remaining.*

5.2.1.2 Desorption Efficiency for Desorption of Bound E2 from MIP Particles

Desorption experiments were conducted with MIP particles following binding experiments and were dependent on the binding efficiencies. Once the centrate had been removed from the particles with the bound EDCs, 1 mL of 1% triethylamine (TEA) in a 1:40 mixture of methanol in distilled deionized water (DDW) added to each of the centrifuge tubes. Reasons for using this desorption mixture are explained in Section 6.1.1.1.1. The tubes were manually shaken, and sonificated for 5 minutes, followed by a 5 minute waiting period. The tubes were then centrifuged at 10,000 RPM. The centrate was removed and the E2 concentration was measured using high-performance liquid chromatography as outlined in Section 5.3.1 to determine the total amount desorbed. A calibration curve was also created with E2 in a 1% TEA solution with a 1:40 mixture of methanol in DDW. The desorption efficiency and overall efficiencies were then calculated as shown below.

$$Desorption\ efficiency = \frac{desorption\ centrate\ HPLC\ area}{\frac{pecent\ binding}{100} * TEA\ calibration\ curve\ HPLC\ area} * 100\%$$

The desorption efficiency was calculated separately from the overall efficiency to better separate out parts of the process requiring optimization. Since the desorbed EDCs were contained in the desorption mixture instead of water, a calibration curve was created using the TEA solution. The values from this calibration curve were then adjusted such that only the efficiency of the desorption step was accounted for by multiplying the total area from the calibration curve by the percent that actually bound to the particles. The overall efficiency, as shown below, did not include this adjustment.

$$Overall\ efficiency = \frac{desorption\ centrate\ HPLC\ area}{TEA\ calibration\ curve\ HPLC\ area} * 100\%$$

5.2.1.3 Overall Analysis of the Binding and Desorption Efficiencies to Determine the Feasibility of Analytical Measurement of EDCs Using MIP Particles

All HPLC analysis was conducted in duplicate. Three different columns were used with a variety of mobile phases and flowrates to obtain good resolution for each of the EDCs. A Waters Scanning Fluorescence detector with a 16μL cell was used for E2 and EE2 while a 115 UV detector was used for atrazine, bisphenol-A, and diethylstilbestrol. Peak Simple software from SRI Instruments and N2000 Chromatostation Version 3.50 from Zhejiang University were used for analysis.

HPLC analysis was used for all analytical measurements. All analysis was conducted in duplicate. A Shimadzu LC-6A Liquid Chromatography system was used (Kyoto, Japan). A scanning fluorescence detector (Millipore, Waters 470) was used for detection. An injection valve (Valco Cheminert Vigi C2XL, Huston, TX, USA) was used with a sample loop volume of 20 μL. Inadequately filled sample loops lead to falsely low measurements. The HPLC column was a Spherosorb ®3 μm 50x2mn extraction column.

100 μL of sample were injected with each injection, allowing 80 μL of sample to be wasted. This was done to ensure that the sample loop was completely filled. Deionized water was injected between each sample at least once or until the peak from the sample was no longer visible. DDW was also run at the beginning of each day, analysis period, or upon the substitution of a newly prepared mobile phase for at least half an hour. A mobile phase of

acetonitrile(ACN):Methanol(MeOH):distilled-deionized water (DDW) (1:1:2) and a flowrate of 0.6 x0.1 mL/minute were used for all analytical preconcentration analysis.

Data processing was completed using Peak 238 software. Peak areas were determined using Peak 238 and directly recorded.

5.2.1.4 Evaluation of the Feasibiliity of Analytical Preconcentration of E2 onto NIP Particles

Analytical preconcentration onto NIP was also tested. NIP are similar to MIP except that they are not prepared in the presence of a template and do not contain a cavity specific to the template. Preconcentration onto NIP has a disadvantage over preconcentration onto MIP because NIP works via non-specific adsorption. Therefore, preconcentration onto NIP can be used for preconcentration purposes but not for sample extraction. MIP can specifically remove the template of interest from a sample simultaneously preconcentrating and extracting the sample. However, because the energy of adsorption for the non-specific adsorption is lower than the energy associated with the specific binding of E2 into the MIP template cavity, it is expected that the desorption efficiency, and therefore the overall efficiency, would be higher.

The procedure for analytical preconcentration of E2 onto NIP was the same as that for MIP outlined in Section 5.2.1 except that NIP was used in the place of MIP.

5.3 Comparison of the Binding Efficiencies of MIP and NIP

For water or wastewater treatment applications, the goal when applying the particles is different than that for analytical preconcentration. The selectivity of the particles is less important for water treatment because there are many different EDCs present in drinking water or wastewater which should be removed during treatment. In fact, non-selective or semi-selective

binding is preferred for water treatment applications because it would allow for the removal of a variety of EDCs. However, there are also other interfering compounds in surface water and wastewater. Hence, it is important to ensure that the particles have some preference for EDCs and that the capacity of the particles is not taken by non-trace contaminants.

A comparison of the efficiency of MIP and NIP can also provide information about the selectivity and degree of specific binding exhibited by the MIP and is therefore important to gain a full understanding of the system.

The data required for the comparison of the binding efficiencies of MIP and NIP for E2 was actually already collected as part of the analytical preconcentration study, as outlined in Section 5.2.1. The binding study, as outlined in Section 5.2.1.1 was then repeated for EE2, except that only the upper concentration range 1-5 mg E2/g particles was tested. Since NIP was not specific, E2 should not have had a competitive advantage for binding over EE2. However, the binding of EE2 was also tested with MIP because EE2 is very similar in structure to E2 and there was a potential for some binding of EE2 into the E2 template.

5.3.1 Analytical Procedure

High-performance liquid chromatography was completed as outlined in Section 5.3.1 with the exception that the column used was a Scientific Inc. 3 μm 50 x2 mm column.

5.4 Application of NIP Particles for the Removal of Atrazine, Bisphenol-A, and Diethylstilbestrol

The procedure followed again matched that outlined in Section 5.2.1.1, except that atrazine, bisphenol-A and diethylstilbestrol were tested instead of E2 and NIP particles were tested instead of MIP particles. As in Section 5.2.1.1, all experiments were completed in a 50:50

mixture of methanol and deionized water for the higher concentration range (1- 5 mg EDC/ g particles).

There are a wide variety of EDCs typically present in drinking water or wastewater. Not all of these have structures similar to that of E2. Therefore, it was also important to test the removal of several other and more varied EDCs using NIP particles if these particles are to be applied for generalized treatment of EDCs.

5.4.1 Analytical Procedure

High-performance liquid chromatography was completed as outlined in Section 5.3.1except that the column used was a Scientific Inc. 3 μm 50 x2 mm column. Also, a UV detector (Gilson 115 UV Detector) was used instead of the fluorescence detector for atrazine and diethylstilbestrol. The fluorescence detector was used for bisphenol-A.

5.5 Treatment of River Water for Removal of Unidentified Contaminants Using NIP Particles

It is important to test the removal of contaminants from a natural water sample to better evaluate the effectiveness of the particles. A river water sample was drawn from the Rideau River (Ottawa, ON) and tested for treatability with NIP particles.

The river water sample was first filtered through a 200-nm micro syringe filter. This was done to ensure a homogenous sample with no suspended particles and to avoid causing damage to the HPLC column. Binding experiments were conducted on the river water samples without spiking. A naturally occurring HPLC peak was visible with the fluorescence detector (FD) and the particles were tested for their ability to remove this peak. Note that the peak was not identified and remained an unknown water contaminant. Identification of the contaminant

requires liquid chromatography with mass spectrometry (LC-MS) which were not available during the course of this study.

NIP samples were prepared by weighing varying amounts of NIP into 2 mL centrifuge tubes (5mg, 10mg, 15mg, and 20 mg). A zero sample was also prepared with no NIP. This sample was treated by sonication and centrifugation along with the other samples as a control. 1 mL of the river water sample was added to the particles in the tubes centrifuge tubes. The tubes were shaken manually to mix followed by 5 minutes of sonication and an additional 5 minute waiting period. Following the waiting period, the tubes were centrifuged at 10,000 RPM. The centrate was removed and analyzed for remaining EDC concentrations using HPLC as outlined in the following section.

Centrate was analyzed to determine the amount of the unidentified contaminant remaining. This is the amount not bound to the MIP particles. The initial amount of E2 in each of the samples was also determined using HPLC analysis of the initial standard solutions.

The binding efficiency was then calculated based on the initial concentration of the standard, as shown below.

$$Pecent\ binding = 100\% - \left(100 * \frac{HPLC\ area\ for\ centrate}{HPLC\ area\ for\ standard}\right),$$

Where the expression in the bracket refers to the percent remaining and the percent binding is 100% - percent remaining.

5.5.1 Analytical procedure

High-performance liquid chromatography was completed as outlined in Section 5.3.1 except that the column used was a Scientific Inc. 3 μm 50 x2 mm column.

5.6 Treatment of Secondary Wastewater Effluent Using NIP Particles

5.6.1 Treatment of Unidentified Existing Contaminants Within the Secondary Wastewater Effluent

NIP particles were also tested for their ability to remove contaminants from secondary wastewater samples. Treatment of secondary wastewater is important for water reuse applications or for treatment of heavily contaminated wastewater prior to release into the environment. Secondary wastewater effluent was collected just prior to chlorination following the secondary clarifiers from the Robert O. Pickard Environmental Centre in Ottawa, Ontario.

The procedure followed mirrored that outlined in Section 5.5 except that the wastewater was used instead of the Rideau River sample.

5.6.2 Treatment of Spiked Secondary Wastewater Effluent with Varying Concentrations of NIP Particles

NIP particles were tested for the removal of unidentifiable contaminants and spiked E2 from secondary wastewater effluent. Spiked samples were tested because it is possible that the unidentifiable contaminants were not EDCs and the purpose of this study was to test the removal of EDCs from wastewater.

The wastewater sample was first filtered through a 200 nm micro syringe filter. It was then spiked with 10 ppm E2 prepared from a 1000 ppm stock solution. The procedure was then followed as outlined in Section 5.5.

5.6.3 Removal of Varying Concentrations of E2 from Secondary Wastewater Effluent Using NIP Particles

Section 5.6.2 tested the removal of spiked concentrations of E2 from wastewater. However, the procedure used varied slightly from that used to test the removal of spiked concentrations of E2 from deionized water in the first phase of experiments. Section 5.6.2 was designed to mirror sections 5.5 to 5.6.2 where unidentified contaminants were removed from surface and secondary effluent wastewater. Because the contaminants were unidentified and naturally occurring within the samples, the concentrations of these contaminants could not be changed and the NIP concentration applied was varied instead. However it is also important to compare the results obtained from the spiked wastewater with those obtained from spiked deionized water in the first phase of experiments. Therefore, NIP was also tested for its ability to remove E2 from spiked wastewater following the same procedure applied for deionized water where the concentration of E2 was varied for a constant particle concentration. This was done for comparison purposes to investigate the effects of other wastewater contaminants on removal of E2 using the particles.

The procedure was followed as outlined in Section 5.2.1.1 except that NIP particles were used instead of MIP particles. Also, secondary wastewater was used in the place of DDW. For the higher concentration solutions of E2 (20-100 ppm) above the solubility limit of E2, a 50:50 solution of secondary wastewater and methanol was used to extend the concentration range of the tests.

5.7 Evaluation of the Improvement in Overall Wastewater Quality after NIP Treatment

NIP particles bind non-selectively to a variety of contaminants primarily by surface adsorption. Therefore, the particles should be capable of removing a variety of hydrophobic

water contaminants from wastewater. This could be an advantage for wastewater treatment because the particles could improve the quality of the wastewater beyond simply the removal of EDCs which was the reason for their application. Therefore, the overall quality of the wastewater following the application of NIP particles was evaluated by measuring several wastewater quality indicators prior to and following the application of the particles. Absorbance, turbidity, ammonia, phosphate, chemical oxygen demand (COD) and total organic carbon (TOC) were all measured.

5.7.1 Sample preparation

Four different types of samples were prepared:

- Sample 1: Secondary wastewater effluent alone;
- Sample 2: Secondary effluent treated with 5 mg/mL of NIP particles and then centrifuged (for particle removal);
- Sample 3: Secondary effluent filtered through a 200 nm syringe filter; and
- Sample 4: Secondary effluent treated with 5 mg/mL of particles and then passed through a 200 nm syringe filter (for particle removal).

5.7.1.1 Preparation of Sample 2 and Sample 4

The wastewater sample was well-mixed and an 80 mL sub-sample taken for Samples 2 and 4. 400 mg of NIP particles were measured and added directly to the wastewater sample for a resulting particle concentration of 5 mg particles/ mL. The sample was sonicated for 5 minutes, and then allowed an additional 5 minute reaction time. Following the reaction time, the samples were split in half and labeled Sample 2 and Sample 4. Sample 2 was centrifuged using a Hamilton Bell Van Guard V 6500 Centrifuge at a speed of 3400 RPM for half an hour. Sample 4

was passed through a 200 nm syringe filter. The filter was changed at any point where the pressure became too great to easily pass additional sample through it.

5.7.1.2 Preparation of Sample 1 and Sample 4

Sample 1 was analyzed directly and Sample 4 was passed through the 200 nm syringe filter prior to analysis.

5.7.2 Absorbance Measurements

Absorbance measurements were taken for each of the four samples using a DR 2800 Spectrophotometer. Measurements were taken in triplicate and the sample was retained for future analysis.

5.7.3 Turbidity Measurements

Turbidity measurements were taken in triplicate using well-mixed samples. A Micro TPI Turbidimeter from Scientific Inc (Ft. Meyers Fl.) was used. The samples were retained following turbidity measurements for further analysis.

5.7.4 Ammonia Measurements

The ammonia test was completed in duplicate. The ammonia test used Hach High Range Test 'N Tube AmVer™ Nitrogen Ammonia Reagent and the Hach DR 2800 Spectrophotometer. The Ammonia program was selected for the spectrophotometer. For each of the four samples, 0.1 mL was added to a separate ammonia reagent test tube. One blank was also prepared by adding 0.1 mL of DDW to an additional ammonia reagent test tube. One Ammonia Salicylate Reagent Pillow was added to each of the test tubes. The test tubes were shaken well for at least 20 seconds or until the reagent was dissolved. A 20 minute reaction time was allowed. Following

the 20 minute reaction time, the zero was placed in the spectrophotometer and used to zero the instrument before the other samples were read in the spectrophotometer.

5.7.5 Phosphate Measurements

The phosphate test was completed in duplicate. First the phosphate program was selected for the spectrophotometer. Then the phosphate test used Hach High Range Phosporous Reactive Test N'Tube Vials. 5.0 mL of each of the samples were placed in separate test vials. One blank vial was also prepared with 5.0 mL of DDW. The tubes were mixed by inverting and a 7 minute reaction time was allowed. The blank was used to zero the spectrophotometer and then the samples were read.

5.7.6 Total Organic Carbon (TOC) Measurements

The TOC test was conducted in triplicate. The test used a Shimadzu TOC-V CPN Total Organic Carbon Analyzer in accordance to the directions provided in the user's manual.

5.7.7 Chemical Oxygen Demand (COD) Measurements

The COD test was conducted in duplicate. The test used the Hach DR 2800 Spectrophotometer. First the COD program was selected from the list of programs available in the Spectrophotometer. Nine COD Digestion Reagent Vials and the DR 2800 reactor were also used. The DR 2800 reactor was set to the COD program as well and was pre-heated prior to use. 2.0 mL of each of the samples were placed in duplicate in 8 of the vials, and the 9^{th} vial was used as a blank and filled with 2.0 mL of DDW. The vials were capped and inverted to mix before being placed in the DR 2800 reactor for two hours. Following the 2 hour reaction time, the samples were allowed to sit for 20 minutes before they were again mixed and allowed to sit for an

additional hour before analysis using the pre-set program in the Hach DR 2800 Spectrophotometer.

5.8 Chlorine Disinfection and Subsequent Removal of Disinfection By-Products with NIP Particles

NIP particles can potentially be applied either prior to, or immediately following disinfection during water or wastewater treatment. One advantage of application following disinfection is that other disinfection by-products could also be removed using NIP. Tests were completed using chlorine because this is the most common disinfection agent applied during water and wastewater treatment, and previous research has shown that chlorine is effective for the oxidation of EDCs (American Waterworks Association Research Foundation, 2007).

All experiments were completed using spiked concentrations of E2 (10 ppm) in deionized water. This was done because following oxidation, the E2 will be converted into oxidized byproducts. These byproducts were not identified because this was not possible using HPLC alone. However, since DDW was used with spiked concentrations of E2, it was assumed that any of the peaks visible using the HPLC following treatment with chlorine were oxidized byproducts of E2 treatment with chlorine and were therefore important to remove from the water.

Two chlorine concentrations were tested for the removal of E2 from DDW (5mg/L and 10 mg/L). A 1000 ppm sodium hypochlorite stock solution was prepared from Javex ®. 0.15 and 0.3 mL of this solution were then added to 30 mL of the 10 ppm E2 solution, for the 5 and 10 mg/L samples, respectively. The chlorine stock solution and all samples to which chlorine was to be added were covered with aluminum foil to avoid light exposure. Once the chlorine was added, the samples were sonicated (2510 sonicator) for 15 minutes to allow a 15 minute reaction

time during which the samples were well-mixed. Following the 15 minute contact time, a 20 mL sample was drawn and tested for free and total chlorine residuals (Refer to Section 5.8.2). Then, 40 and 80 μL of sodium thiosulfate were added to the 5 and 10 mg/L chlorine samples, respectively. Sodium thiosulfate as well as the chlorine used were both tested with the HPLC-FD prior to analysis to ensure that they did not generate any peaks which may have interfered with the analysis. The purpose of adding the sodium thiosulfate to the samples was to react with any remaining chlorine. This would stop the remaining chlorine from continuing to react with the EDCs in solution, thus extending the reaction time beyond 15 minutes. The chlorinated sample was then analyzed using HPLC analysis as outlined in Section 5.8.1.

Following chlorination, 1 mL samples were extracted and added to 2mL centrifuge tubes containing 5 mg or 10 mg of NIP particles. The tubes were shaken manually, sonicated (2510 Bronson sonication device) for 5 minutes, and then allowed to sit for an additional 5 minutes. Following this binding time, the samples were centrifuged using a Revolutionary Science Microcentrifuge at 10000 RPM for 3 minutes or until the centrate was no longer cloudy. The centrate was then removed and analyzed using HPLC analysis as outlined in Section 5.8.1.

The procedure outlined above was completed using both 10 ppm E2 in DDW and 10 ppm E2 in DDW with 5 mg/L humic acid added. Humic acid was added to simulate natural surface water and to investigate changes in efficiency which might occur in water samples with interfering species while still maintaining control over the composition of the samples. Humic acid was also tested alone with HPLC-FD to ensure that it did not create any peaks. Refer to Table 5-1, for an overview of all of the samples prepared and tested. All of the samples were tested with 10 ppm E2. In addition, chlorine concentrations, humic acid, and chlorinated humic acid were also analyzed as controls. The table describes whether or not the sample was prepared

in deionized water or humic acid and each cell defines the treatment method. The intersections represent treatment with both chlorine and particles.

Table 5-1: Tests conducted for treatment of E2 with chlorine and NIP particles

	5mg/mL NIP Particles	10 mg/mL NIP Particles	No particles
5 mg/L chlorine	Deionized water	Deionized water	Deionized water
10 mg/L chlorine	Deionized water	Deionized water	Deionized water
5 mg/L chlorine	5 mg/L humic acid	5 mg/L humic acid	5 mg/L humic acid
10 mg/L chlorine	5 mg/L humic acid	5 mg/L humic acid	5 mg/L humic acid
No chlorine	Deionized water	Deionized water	Deionized water
No chlorine	5 mg/L humic acid	5 mg/L humic acid	5 mg/L humic acid

5.8.1 Analytical Procedure

High-performance liquid chromatography was completed as outlined in Section 3.3.1.3 except that the setup used a Keystone Scientific Nucleosil C8 3µm 150x2mm column; a Perkin Elmer Fluorescence Detector; and a Rheodyne injection valve. Also, a Baseline Chromatography Data System detector and software were used.

5.8.2 Measurement of Chlorine Residuals Following Treatment

All chlorine measurements were conducted using a Hach DR 2800 Spectrophotometer. 10 mL samples were drawn from the sample beakers following the chlorine reaction time but prior to the addition of sodium thiosulfate.

5.8.2.1 Measurement of Free Chlorine

This procedure used Hach DPD Free Chlorine Reagent Powder Pillows and a 10 mL sample cell. The Hach cell adapter was first inserted into the spectrophotometer. The sample was diluted in a 1:1 (distilled-deionized water: sample) ratio. 5 mL of the diluted sample was added to the sample cell, which was wiped and used to zero the instrument. The cell was removed and the entire contents of the powder pillow was added to the sample .The cap was placed on the cell and it was shaken for approximately 20 seconds until the contents of the pillow were dissolved. The cell was placed back into the spectrophotometer and the free chlorine concentration was read. Two readings were taken with the same sample and the final concentration was multiplied by two to find the initial free chlorine concentration for the undiluted sample.

5.8.2.2 Measurement of Total Chlorine

This procedure used Hach DPD Total Chlorine Reagent Powder Pillows and a 10 mL sample cell. The Hach cell adapter was first inserted into the spectrophotometer. The sample was diluted in a 1:1 (distilled-deionized water: sample) ratio. 5 mL of the diluted sample was added to the sample cell, which was wiped and used to zero the instrument. The cell was removed and the entire contents of the powder pillow was added to the sample .The cap was placed on the cell and it was shaken for approximately 20 seconds until the contents of the pillow were dissolved. A five minute reaction time was allowed. Then the cell was placed back into the spectrophotometer and the free chlorine concentration was read . Two readings were taken with

the same sample and the final concentration was multiplied by two to find the initial total chlorine concentration for the undiluted sample.

5.9 Removal of NIP Particles from Water Following Treatment

It is important to test the removal of EDCs from water and wastewater using NIP nanoparticles; however, in order for these particles to be applied to real water treatment, removal of the particles from the water following treatment is also essential. Several removal mechanisms were tested: chemical coagulation, physical separation using centrifugation, and incorporation of the particles into a column set-up.

5.9.1 Coagulation for Removal of NIP Particles

Previous research conducted by Zackery De Maleki (unpublished work) indicated that the NIP particles were negatively charged. Therefore, positively charged coagulants were tested for removal. A cationic polymer, calcium chloride, and pH change were all tested. The turbidity and absorbance of the solutions were both measured with time following the addition of the coagulant.

5.9.1.1 Determination of the Linear Range of Absorbance for Absorbance Tests

Prior to beginning the coagulation experiments, it was important to ensure that the absorbance range was linear, and could therefore be directly related to particle concentrations or reductions in NIP concentrations. Otherwise, the efficiencies of the coagulants would be difficult to compare. Since the initial particle concentration used was flexible to some degree, the linear range of the absorbance was the basis for choosing this concentration. A high concentration in this linear range was chosen so that after coagulation and removal of some of the particles, the particle concentration would still fall in the linear range.

To determine the linear range for absorbance a high particle concentration (5mg/mL) was used to start. Triplicate absorbance measurements were taken of the sample, and it was then diluted by half with DDW. This procedure was repeated until the readings became linear, and then again became non-linear at very low concentrations. The concentration chosen was 1 mg/mL

5.9.1.2 Cationic Polymer for Removal of NIP from Water

FloPolymer CA 4600 (SNF Canada) is a high charge density, high molecular weight, dry cationic polymer, and was prepared by mixing for 1 hour with a magnetic bar mixer followed by 10 seconds with an electric hand mixer and let to sit for an additional hour before use. The polymer solution was prepared at a concentration of 0.5 %.

5.9.1.3 Calcium Chloride for Removal of NIP from Water

The calcium (II) solution was prepared from anhydrous calcium chloride salt ($CaCl_2$). The stock solution was prepared at a concentration of 3 molar by dissolving the salt in DDW and shaking the solution. The stock solution was then allowed to sit for 1 hour to allow any bubbles to leave the solution. However, since the optimum dose of calcium (II) for coagulation was high, the total volume of the sample was maintained at 20 mL. Therefore the NIP particles were prepared at higher concentrations than 1 mg/mL in DDW and diluted to 20 mL with the addition of the calcium chloride solution.

5.9.1.4 pH Changes for Optimum Settling of NIP from Water

Since the concentrations of the coagulants to be added were very high, pH change was also investigated as a possible coagulant because any pH changes could be easily reversed

following the removal of the particles. The pH was changed using additions of hydrochloric acid, and then tested using a pH meter (Oakton pH 11 Series).

5.9.1.5 Coagulation Procedure for Testing of the Cationic Polymer, Calcium Chloride, and pH Changes

The same coagulation procedure was followed for the cationic polymer, calcium chloride, and hydrochloric acid additions. Coagulation was performed by placing 10 mL samples of the 1 mg/mL solution of NIP particles into 25 mL beakers. Prior to the addition of the coagulant, the solutions were sonicated 2510 Bronson sonication device) for 5 minutes to ensure that the samples were well mixed. Then the coagulent was added and the sample was again sonicated (for 5 minutes. It was then allowed to sit and turbidity and absorbance measurements were taken after 1 hour.

Following the 1 hour waiting period, the top 5 mL of the sample was carefully drawn off and well mixed for turbidity and absorbance measurements. Turbidity measurements were taken using a turbidity meter (Scientific Inc. Micro TPI Turbidimeter) which was calibrated each day. Absorbance measurements were taken at 350 nm using a Hach DR 2800 Spectrophotometer with a program setting for absorbance.

5.9.1.6 Centrifuge Removal of NIP Particles from Water Following Treatment

Two different centrifuges were compared for different centrifuge times for removal of the particles from solution. 1 mg/mL particle solutions were prepared and dewatered using two different centrifuges with different maximum speeds and maximum centrifugal forces. The first centrifuge used was a Revolutionary Science Microcentrifuge. It was used at a speed of 10,000 RPM and applied a centrifugal force of 6238 RCF. The second centrifuge used was a Hamilton

Bell Van Guard V 6500 and was applied at a speed of 3400 RPM and with a centrifugal force of 1318 RCF. The centrifuge time required by the second centrifuge was longer than that required by the microcentrifuge, but centrifuges applied in practice might have varying centrifugal forces, so it is important to test different types of centrifuges.

5.9.2 Application of the NIP particles in a Column Set-Up

A bench scale column was created by filtering 8 mg of NIP suspended in water through a 200 nm microfilter. Both polyethersulfone and nylon 25 mm 0.2 micron filters were tested from Chromatographic Specialties Canada. As indicated previously, the diameter of the NIP nanoparticles was approximately 580 nm. The filter was then dried overnight at 104°C. The filter was then attached to a 500-9000 RPM Micropump manufactured by IDEX Corporation. 1 L of 0.2 ppm E2 solution was then prepared in water and pumped through the filter for treatment. Effluent samples were collected every five minutes for a total of 35 minutes. The ratio of E2/particles used for this experiment was chosen based on the estimated binding capacity of the particles calculated with the Scattchard Equation as outlined in Section 6.3.2.1.1.

6 RESULTS AND DISCUSSION

This study included three phases:

(1) The use of MIP and NIP for analytical preconcentration;

(2) The use of NIP for water and wastewater treatment applications; and

(3) The removal of the particles from water after treatment.

The first phase was completed because one of the main challenges faced when studying removal of EDCs from water and wastewater is the analytical measurement of EDC concentrations. Trace analysis of EDCs is difficult and development of a preconcentration strategy in the first phase of experiments would enable the study of treatment of typical concentrations of EDCs found in water or wastewater for the second phase of study. The second phase of study focused on the removal of a variety of EDCs from water and wastewater using NIP particles. The third phase then focused on the removal of the aforementioned NIP particles from water following treatment of EDCs.

6.1 Phase 1: Use of MIP and NIP Particles for Analytical Preconcentration

One of the primary hindrances to EDC research is difficulties measuring EDC (endocrine disrupting compound) concentrations at trace levels. EDCs are typically found at trace levels in water and wastewater treatment plants. Analytical equipment, such as high performance liquid chromatography (HPLC) used to measure the concentrations of E2, does not have adequate detection limits to easily measure concentrations in natural water samples. The use of MIP or NIP as preconcentration agents was tested in the first phase of experiments in an attempt to determine whether or not the particles could be used for preconcentration of E2 and/or other

EDCs, which would enable the measurement of much lower concentrations of EDCs with HPLC. Molecularly imprinted polymers are designed with binding sites specific to a given compound, which for this study was E2. They can be used for preconcentration of that compound from a sample with many different interfering substances, such as bisphenol-A, commonly found in wastewater or natural water samples. The polymer selectively binds to the compound of interest, removing it from the water sample. The sorbed analyte may then be desorbed from the particles using a volume of desorption agent one or several orders of magnitude smaller than the initial sample volume, effectively pre-concentrating the sample for analytical measurements.

This method has two distinct roles. It functions to clean the sample for analytical measurement because only the analyte of interest is extracted from the sample solution. It also simultaneously pre-concentrates the analyte (Alexander et al, 2006).

6.1.1 Preconcentration of E2 on Molecularly Imprinted or Non-Imprinted Nanoparticles for Analytical Analysis:

6.1.1.1 The Use of MIP Particles for Preconcentration of E2:

To test the use of MIP for preconcentration of E2, E2 was bound onto the MIP particles as outlined in the Section 5.2.1.1. A volume of a 3% solution of triethylamine (TEA) in water equal to the initial sample volume was then used to desorb the E2 from the particles. The efficiencies of both the adsorption and desorption were measured separately to derive an overall efficiency. In this case, the particles were used neither for sample cleanup, because samples consisted of distilled water spiked with E2, nor for preconcentration since the sample volume and desorption agent volumes were equivalent. The purpose was simply to determine the adsorption and desorption efficiencies and to test the feasibility of using MIP for sample preconcentration for analytical purposes in trace analysis.

6.1.1.1.1 Desorption Agent Tests for Optimum Desorption of E2 from MIP for Analytical Preconcentration:

Several tests were conducted to test TEA alone as a pure organic liquid; however, the TEA produced an HPLC peak which interfered with that of E2. Additionally, there was a possibility that the analysis of the TEA, which is a strong base, in the HPLC could damage the stationary phase of silica particles. Low concentrations of TEA were then tested. By decreasing the concentration of TEA, the size of the TEA peak decreased and the interference in chromatography was minimized. Since the E2 and TEA peaks were directly on top of each other, changing the mobile phase or flow rates was not tried to improve the separation because this approach is only effective if the peaks are partially resolved. Furthermore, TEA, which was available as a pure organic liquid, is not soluble in water, so it was dissolved in a 1:40 mixture of methanol and water. Although there was still an existing TEA peak, this peak was consistent for all measurements and a calibration curve was created with the same TEA concentration so the accuracy of the measurements was not affected. Use of a higher concentration of TEA also would not have added to the measurements but would have reduced the sensitivity of analysis due to problems identifying small changes in the magnitude of the E2 peak due to interferences from TEA. Finally, a desorption agent composition of 1% TEA in a 1:40 mixture of methanol and water was used.

6.1.1.1.2 Determination of the Binding Efficiency for Preconcentration of E2 onto MIP Particles

Two ranges of E2 to MIP ratios were tested to evaluate the effectiveness of MIP for preconcentration of E2 for analytical purposes. The first range was from 0.01-0.05 mgE2/g MIP

and the second from 1- 5 mg E2 /g MIP. Binding efficiencies for each of the two concentration ranges are shown in Figure 6-1 and Figure 6-2, respectively.

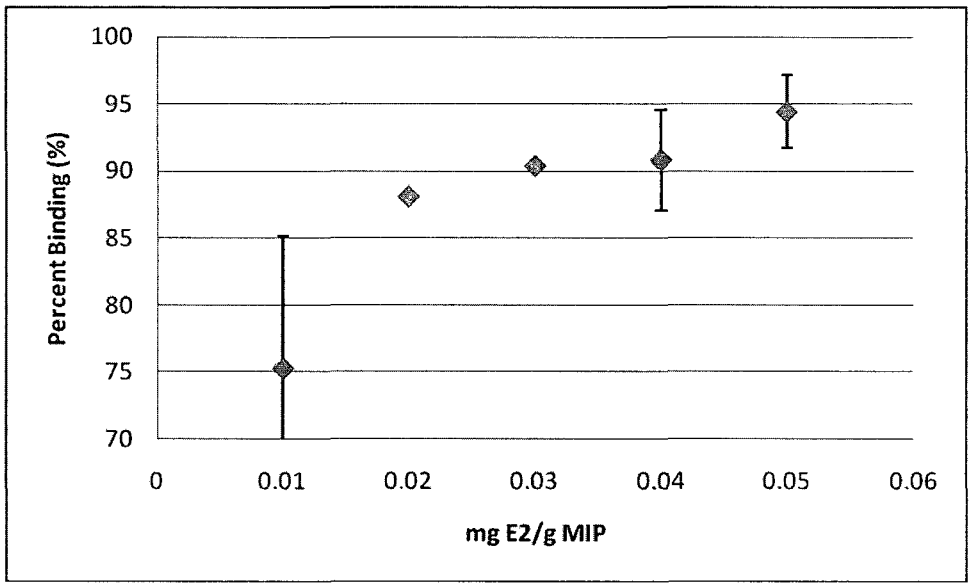


Figure 6-1: Binding Efficiencies for binding of E2 onto MIP Particles for E2 to MIP Ratios of 0.01-0.05 mg E2/ g MIP

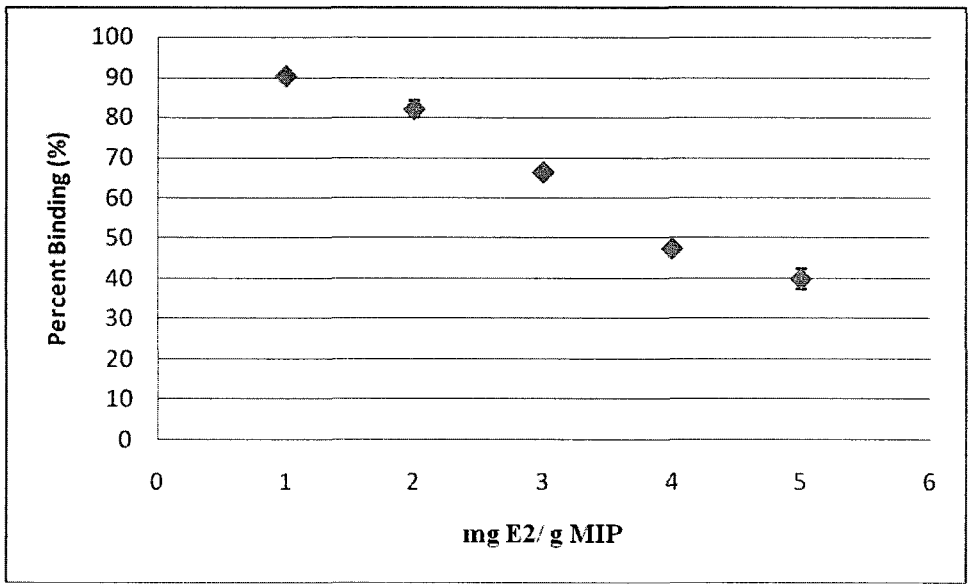


Figure 6-2: Binding Efficiencies for binding of E2 onto MIP Particles for E2 to MIP Ratios of 1-5 mg E2/ g MIP

Figure 6-1 shows the results of binding tests for the binding of E2 on MIP particles. Two replicates were used for HPLC analysis and data points represent the average of the two replicates. Error bars are shown and represent the standard deviation. The percent binding increased throughout the E2 to particle ratio shown in Figure 6-1. However, the increase was greatest between ratios of 0.01 mg E2/ g MIP and became less for larger ratios. The error bars shown in Figure 6-1 also appear to be large particularly at 0.1 mg E2 /g MIP. This is because, for the lower concentration range, E2 concentrations, both before and after binding, were approaching the detection limit for the HPLC equipment, thus leading to greater relative error in detection and hence decreased reproducibility.

Figure 6-2 shows the results of binding experiments for binding of E2 onto MIP for E2 to MIP ratios of 1 -5 mg E2/ g MIP. Two replicates were taken for all HPLC measurements, and error bars are shown as the standard deviation. The error bars are much smaller for Figure 6-2 as compared to Figure 6-1. This is because the concentrations measured were further from the detection limit for the HPLC system used. For the 1-5 mg E2/ g MIP ratio range, binding efficiencies decreased following a nearly linear trend with a slope of 13.5 %/ (mg E2/ g MIP). The percent binding was initially 90% at 1 mg E2/ g MIP and decreased to approximately 40% for 5 mg E2/ g MIP.

The binding efficiency increased with increasing E2 concentration for the lower concentration range, (0.01-0.05 mg E2 /g MIP) and decreased with increasing E2 concentration for the higher concentration range (1-5 mg E2 /g MIP). It is expected that the binding efficiency

would decrease with increasing E2 concentrations for high concentrations of E2 because the molecularly imprinted binding sites and/or non-specific surface adsorption sites would have been quickly filled. Adsorption is based on the energy difference between the solid and liquid phases. If a compound can exist at a lower energy state adsorbed onto the solid phase, then adsorption will occur. The energy balance between the solid and aqueous phases shifted for the 1-5 mg E2/ g MIP range because the energy available from the binding or adsorption decreased, making further binding less favorable (Gao et al, 1998). It is possible to shift the equilibrium energy balance by increasing the concentration of E2 in the solution, making it less energetically favorable for the E2 to remain in solution. In this case, a larger total amount of E2 may adsorb to the MIP, but the efficiency or percent binding would continue to decrease.

The amount of E2 bound onto the MIP decreased with decreasing E2 concentrations for very low concentrations of E2 as shown in Figure 6-1. This phenomenon has also been observed by other researchers for PAC adsorption experiments, although it has not been adequately explained (Chiou, 2002). One explanation provided was that it is due to binding kinetics. For binding to occur, contact is required between the E2 molecules and the MIP particles. If there is a very low concentration of E2 in the solution, it would take a much longer time for this contact to occur, leading to a slower rate of binding and hence a longer time required to reach equilibrium.

To test this theory, a kinetics study was completed where a full 24 hours was provided for binding. However, the results of this test shown in Figure 6-3 showed very little increase in binding efficiency between the 10 minute (Figure 6-2) and 24 h binding times (Figure 6-3) indicating that binding can be assumed to be complete after ten minutes and that the kinetics explanation was not applicable for this case. The reason for the decrease in binding efficiency

with decreased E2 concentrations in the lower range remains unknown. Another plausible explanation may be the contamination of samples by impurities from the plastic microtubes which may have contained bisphenol-A. However, overall, the binding was good and the steep increase followed by a steep decrease in percent binding suggests an optimum range for the E2 to MIP ratio which could be used for design purposes.

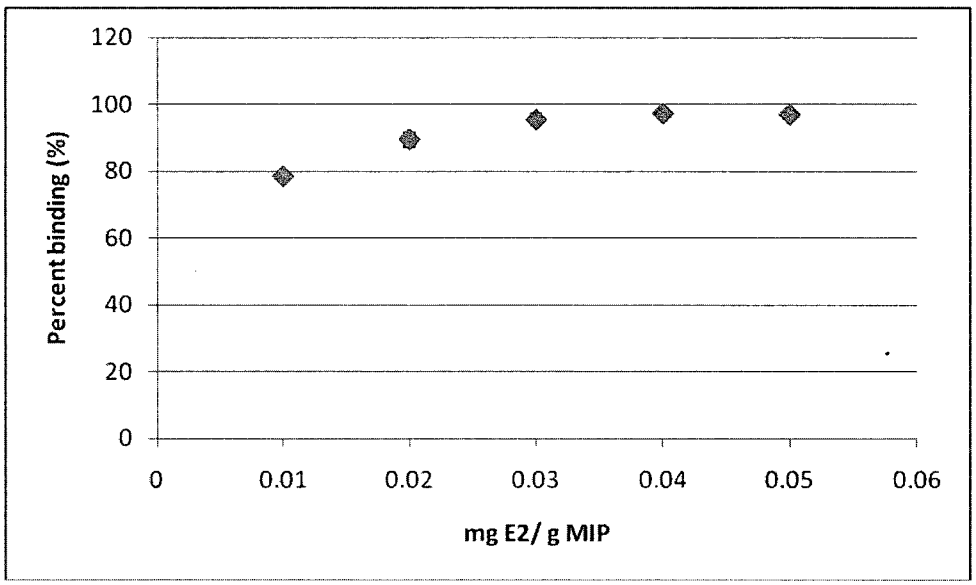


Figure 6-3: Binding Efficiencies for binding of E2 onto MIP Particles for E2 to MIP Ratios of 0.01-5 mg E2/ g MIP with a Binding Time of 24 Hours

The medium used during binding studies is also very important. The solubility of E2 in water is only 13 mg/L or approximately 13 ppm (Lintelmann et al, 2003). For this reason, E2 solutions above 10 ppm, which was used for E2 to MIP ratios greater than 0.5, were prepared in 50:50 solutions of deionized water and methanol while lower concentrations were prepared in water alone. This was necessary to study a wider range of E2 concentrations and E2 to MIP ratios. It was not possible to decrease both the E2 concentration the amount of MIP used while maintaining the same ratio to study these higher ratios because the detection limits for E2 in

HPLC would not have allowed for this. Therefore, the use of methanol was a necessary concession to gain some information about the upper binding limits for binding of E2 onto MIP. However, the use of methanol might have affected the binding.

Changing the binding medium affects both the amount of binding and also the type of binding observed. Alexander et al (2006) noted that binding from aqueous solutions was generally non-specific. This was also reported by Pap et al (2002) who noted that the solvent controlled the energy distribution between the solution and the sorbed phases due to differences in the solubility of analyte in different mediums. However, the type of binding favored was also controlled by the solvent in this study. Binding of terbutylazine was highest for pure acetonitrile and high aqueous concentrations of acetonitrile, and had intermediate binding efficiencies for intermediate concentrations. This was because, at high acetonitrile concentrations, binding was highly specific whereas at high water concentrations, hydrophobic interactions became very strong, leading to strong but non-specific binding (Pap et al, 2002).

Methanol leads to an increased solubility of E2 in the sample solution, and therefore decreased adsorption. Methanol also has an OH group capable of forming hydrogen bonds with carboxyl groups on the MIP and excluding E2 from the imprinted cavities (Yan and Ramstrom, 2005). In fact, methanol has been used by some researchers for the removal of templates from MIP's. Amalric et al (2008) used methanol to selectively remove atrazine from MIP and San Vicente et al (2004) used methanol for removal of bisphenol-A.

The presence of methanol might have impacted the binding. However, the results obtained through the study of higher range E2 concentrations with the use of methanol was significant because the trends observed are expected to be the same. Predictions can be made

about the binding efficiency trends for deionized water. Decreased efficiency would also have occurred for deionized water at a certain concentration where it was no longer energetically favourable for binding to occur. The energy balance may have shifted slightly due to the use of methanol because E2 is more soluble in methanol and thus the methanol has a stronger attraction for E2 than deionized water does. This means that for deionized water, the efficiencies should decrease to a lesser extent and at a higher initial ratio than for the methanol because the energy differential between lower energy binding sites and the dissolved phase would be greater. Therefore the methanol data can be interpreted as a worst-case scenerio and put a lower limit on the decreased efficiency.

Overall the binding efficiency was shown to increase for the E2 to MIP ratios 0.01-0.05 mg E2 /g MIP and decrease for the 1-5 mg E2 /g MIP ratios. Binding efficiencies were greater than 90 percent above 0.03 mg E2 /g MIP and below 1 mg E2 /g MIP.

6.1.1.1.3 Determination of the Desorption Efficiency for Preconcentration of E2 onto MIP Particles

Desorption efficiency also affects the use of MIP for analytical purposes. Desorption efficiencies for each of the two concentration ranges studied above are shown in Figure 6-4 and Figure 6-5. Desorption efficiencies were calculated for the desorption step alone, representing the efficiency of desorption from the amount of E2 which bound to the MIP but not the total amount initially added to the MIP. The adsorption and desorption steps were separated analytically and graphically to better understand which parts of the preconcentration procedure was least efficient and most in need of optimization.

All desorption tests were completed using a 1% TEA solution in a 1:40 (v:v) mixture of methanol and water. Ten minutes were allowed for desorption. The solution was sonicated for the first five minutes of this desorption time and then allowed to sit for an additional five minutes. The TEA mixture was then centrifuged and analyzed for desorbed E2 using HPLC-FD.

Figure 6-4 shows the desorption curve for E2 to MIP ratios ranging from 0.01 to 0.05 mg E2/ g MIP. All the points represent the average of two measurements and the error bars represent the standard deviation. Figure 6-4 shows decreasing desorption efficiency for E2 to MIP ratios between 0.01 and 0.03 mg E2/ g MIP and increasing desorption efficiency for E2 to MIP ratios between 0.03 and 0.05 mg E2/ g MIP.

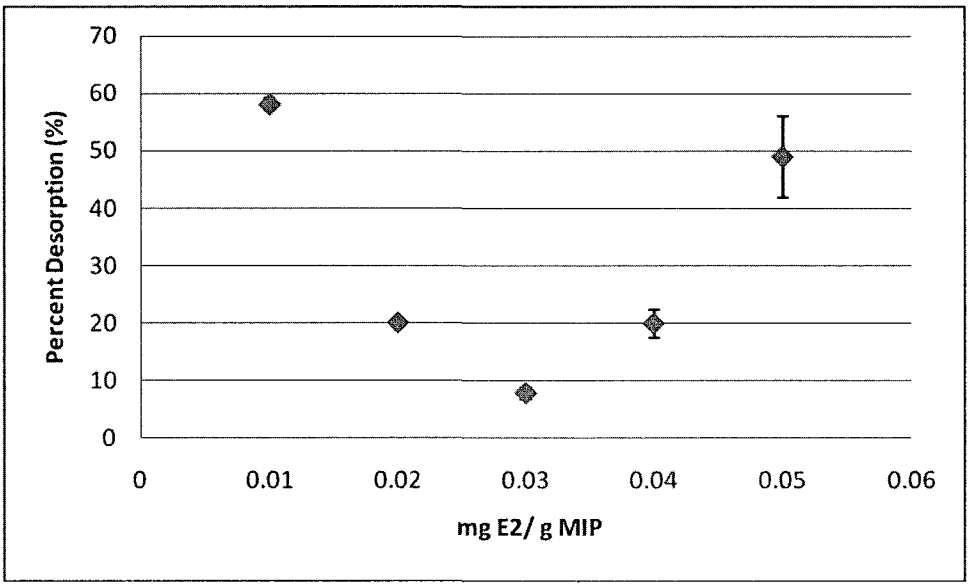


Figure 6-4: Desorption Efficiencies for Preconcentration of E2 onto MIP Particles for E2 to MIP Ratios of 0.01-0.05 mg E2/ g MIP

Figure 6-5 shows the desorption of E2 from MIP for E2 to MIP ratios of 1 – 5 mg E2/ g MIP. Points shown are averages of two replicates and the standard deviations are shown as error bars. Figure 6-5 shows a trend of increasing desorption efficiency for ratios between 1- 4 mg E2/

g MIP and decreasing desorption efficiency between 4 and 5 mg E2/ g MIP. It should be noted that the desorption efficiency for ratios of 3 – 5 mg E2/ g MIP were all higher than 100%. This is due to bleeding of the template from the MIP and can occur for MIP when the template is not completely removed following polymerization.

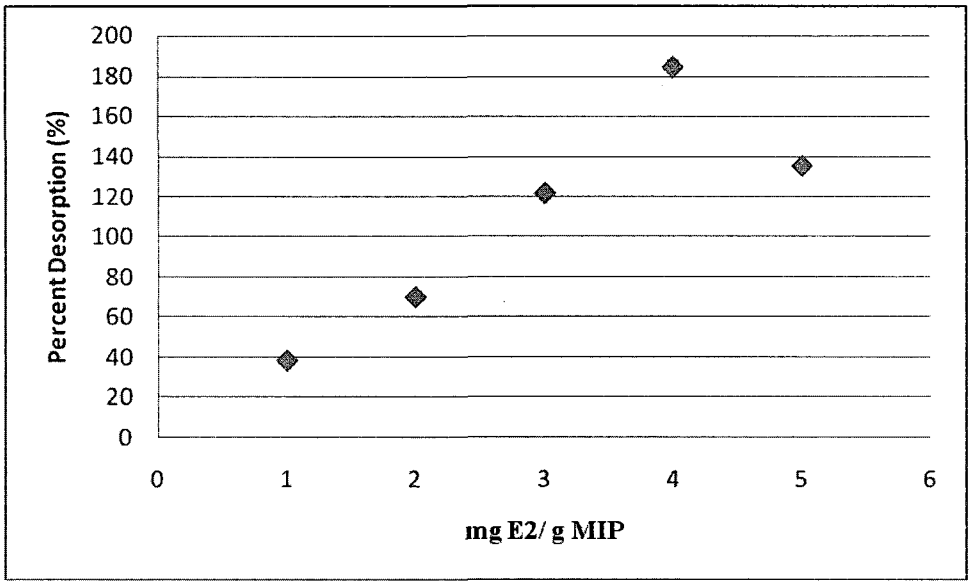


Figure 6-5: Desorption Efficiencies for Preconcentration of E2 onto MIP Particles for E2 to MIP Ratios of 0.01-0.05 mg E2/ g MIP

The desorption efficiencies (as shown in Figure 6-4 and Figure 6-5) are somewhat misleading. For the lower concentration range, desorption efficiencies were very low, and not sufficient for preconcentration purposes. For the upper range however, desorption efficiencies were in some cases low (<70%) and in other cases more than 100%. It is not possible to desorb more E2 from the MIP than was on the MIP to begin with and thus the efficiencies greater than 100% indicate an error. This suggests that there was E2 present on the MIP prior to the testing. This phenomenon is quite common and occurs because complete removal of the template from the MIP is very difficult. Bleeding of the template during the desorption step frequently leads to

falsely high results (Masque et al, 2001; Sellergren, 1999; Pichon, 1997). Use of a structural analogue (Masque et al, 2001) thermal treatment, or more effective washing have all been suggested as possible strategies to reduce non-specific binding and thus reduce bleeding (Sellergren, 1999).

6.1.1.1.4 Determination of the Overall Preconcentration Efficiency for Preconcentration of E2 onto MIP Particles

The binding and desorption efficiencies were plotted to see where the preconcentration procedure could be optimized. However, it is the overall binding efficiency that will determine the applicability of MIP to analytical measurement of EDCs. The overall efficiencies are shown for both concentrations ranges studied in Figure 6-6 and Figure 6-7.

Figure 6-6 shows the overall preconcentration efficiency of E2 onto MIP particles for E2 to MIP ratios from 0.01-0.05 mg E2/ g MIP. This figure is a combination of the data taken from Figure 6-1 and Figure 6-4 and is influenced both by the binding efficiency and the desorption efficiency. The trend observed in Figure 6-6; however, more closely follows that from Figure 6-4. Figure 6-1 demonstrated increasing binding efficiencies for increasing E2 to MIP ratios for 0.01 – 0.05 mg E2/ g MIP. Figure 6-6, like Figure 6-4, shows decreasing efficiency from 0.01 – 0.03 mg E2/ g MIP and increasing efficiency from 0.03 – 0.06 mg E2/ g MIP.

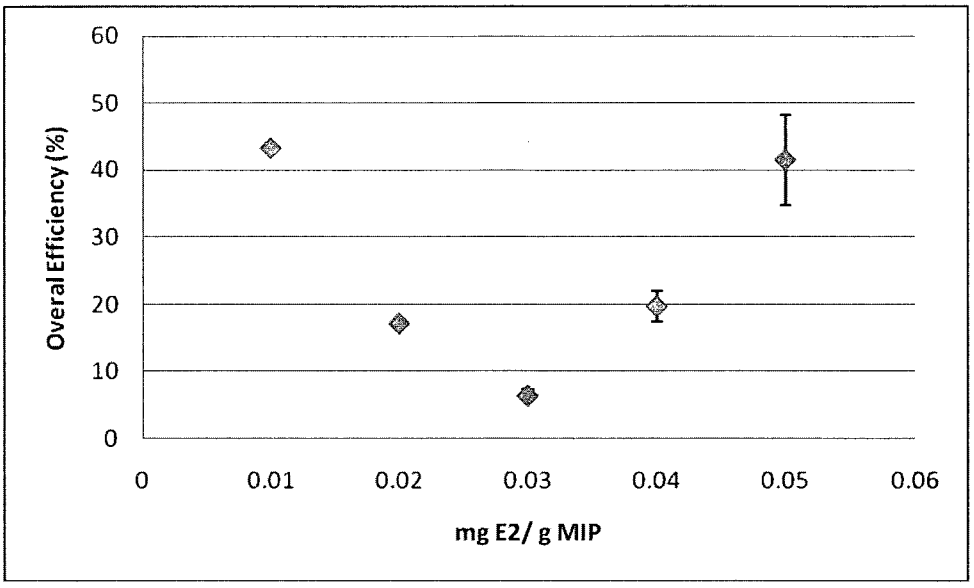


Figure 6-6: Overall Preconcentration Efficiencies for Preconcentration of E2 onto MIP Particles for E2 to MIP Ratios of 0.01-0.05 mg E2/ g MIP

Figure 6-7 shows the overall preconcentration efficiency of E2 onto MIP particles for E2 to MIP ratios of 1-5 mg E2/g MIP. This figure is a combination of the data taken from Figure 6-2 and Figure 6-5 and is influenced both by the binding efficiency and the desorption efficiency. Error bars are shown as the standard deviation. The trend, again, more closely follows that of the desorption curve than the binding curve. The binding curve, shown in Figure 6-2 presented constantly increasing efficiencies whereas the desorption curve showed increasing efficiencies for E2 to MIP ratios between 1 – 4 mg E2/ g MIP and decreasing efficiencies from 4 to 5 mg E2/ g MIP.

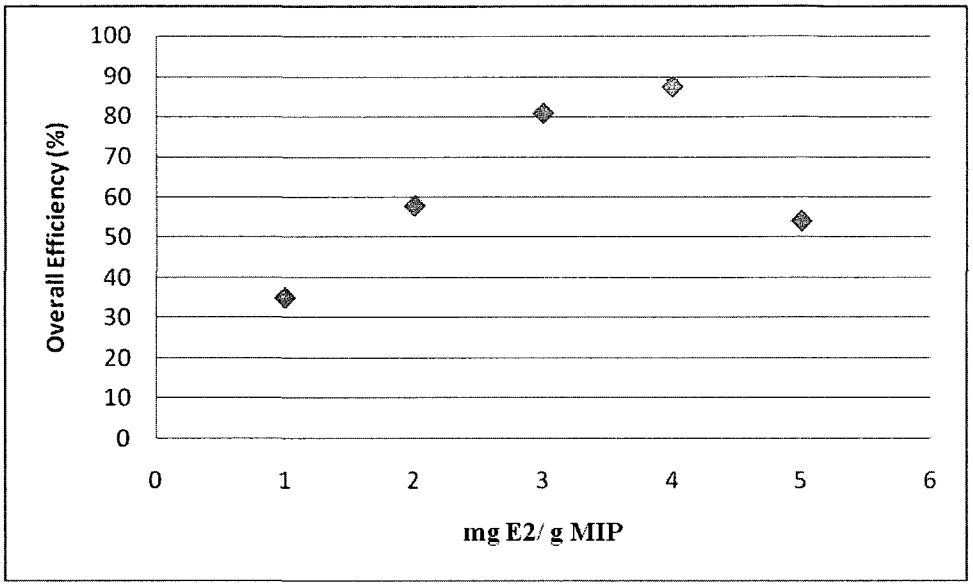


Figure 6-7: Overall Preconcentration Efficiencies for Preconcentration of E2 onto MIP Particles for E2 to MIP Ratios of 1-5 mg E2/ g MIP

The desorption efficiencies for the lower concentration range (shown in Figure 6-6) were very low and were not adequate for preconcentration purposes. Desorption efficiencies decreased to less than 10% overall efficiency for an E2/MIP ratio of 0.03 mg E2 /gMIP and then increased again. The TEA solution prepared seemed to be ineffective for removal of E2 from the MIP.

For the higher concentration range (Figure 6-7) it is possible that the desorption efficiency of the TEA was not adequate because the E2 from the template was not adequately removed. It is likely that bleeding of the template occurred during analysis. Although the overall efficiency was higher for this test, the results are suspected to be superficially high due to bleeding. This indicates that the template was ineffectively removed and the availability of specific binding sites for the re-binding tests was lower than it should have been. This would have contributed to inaccuracies in the binding efficiency tests as well. Sellergren et al (1999) found that generally more than 1% of the template always remains behind following even very

extensive washing. Bleeding was much greater in this study due to difficulties optimizing removal of the template from MIP. Yu et al (2007) found that bleeding of the template molecule from MIP was almost unavoidable and could only be reduced. This was done by creating a sample loop with MIP which was in-line with the HPLC and continuously washing the MIP until there was no longer any signal on the HPLC chromatogram. (Yu et al, 2007)

Prior to application of the particles for analytical purposes, it is possible to use IR spectroscopy to test the particles for complete template removal. Li et al (2007) and Fang and Li (2007) found that the IR spectrum of MIP with the template removed was the same as that for NIP, and used this as an indication of as complete template removal as afforded by the detection limit.

TEA was used both for removal of the template E2 from the MIP prior to use, and for removal of re-bound E2 during desorption tests for analytical preconcentration. TEA has been used successfully for template removal from MIP in the past. (Yu and Lai, 2006; Yu and Lai, 2005; Yu et al, 2007; Yu et al, 2005) As an organic base, TEA can interfere with the MIP to E2 binding equilibrium and thus remove the template (Yan and Ramstrom, 2005).

It should also be considered that the TEA solution was prepared in a mixture of water and methanol since the methanol peak interfered with the E2 peak for HPLC analysis. In previous research, either acetonitrile (Yu and Lai, 2005) or methanol (Yu and Lai, 2006; Yu et al, 2007; Yu et al, 2005) were used for the preparation of TEA solutions. The methanol would aid desorption of the template by interfering with hydrogen bonds between the MIP and E2, and E2 also has a greater solubility in methanol than in water (Yan and Ramstrom, 2005). It is possible that for the TEA solution prepared in the 1:40 mixture of methanol and water, the specific

adsorption sites are reduced and hydrogen bonds between the E2 and MIP cavities are broken; yet, since the solubility of E2 in water is low, the E2 which forms non-specific bonds due to hydrophobic interactions, is not affected by the desorption agent. TEA also becomes protonated in water, which reduces its ability to interfere with binding between E2 and the MIP. Thus it is conceivable that the template was never effectively removed from the specific binding sites.

Whether the template remained in the specific binding sites or on non-specific adsorption sites, it was not removed effectively enough from the MIP for analytical preconcentration. Desorption using 1% TEA solution in a 1:40 mixture of methanol and water was not effective enough for analytical preconcentration purposes. Further development of MIP was deemed necessary prior to use of this method for environmental analysis and a different approach was taken for the remainder of the study. 100% preconcentration efficiency is not expected, but for an analytical procedure to be effective, the preconcentration efficiency should be consistent so that the initial concentration can be determined, and the overall efficiencies found in these studies were too small of trace analysis.

6.1.1.2 The use of NIP Particles for Preconcentration of E2

Problems with desorption of E2 from MIP particles both for removal of the template, and for desorption of E2 added during rebinding tests, appeared to be limiting for use of MIP for analytical preconcentration of E2. Therefore, analytical preconcentration of E2 onto NIP was also tested. This would ensure no bound E2 prior to re-binding experiments because no template molecules are used for preparation of NIP. It was also expected to allow easier desorption because non-specific binding has a lower energy than specific binding. However, one disadvantage of using NIP is that it is non-specific and will bind to any contaminant. The types of interactions are all hydrophobic and hence non-specific and interfering species could not be

adequately differentiated. Interfering species would have to be separated during HPLC or other methods of analysis. Estrogenic compounds typically have similar HPLC retention times and are not easy to separate on the column. Nevertheless, Yu et al (2007) successfully separated E2, E3, and EE2 using a flow rate of 1.0 mL/minute and a 40:30:30 v/v acetonitrile-methanol-DDW mobile phase, demonstrating that separation during HPLC is possible, although difficult. (Yu et al, 2007) Since the MIP had shown itself to be ineffective without further development work, NIP was tested as another option with the intention of using HPLC to separate interfering compounds if necessary for new method development.

Figure 6-8 shows the results of binding experiments for binding of E2 onto NIP particles for E2 to NIP ratios of 1 -5 mg E2/ g NIP. This is the same as the upper range of concentrations studied for binding of E2 onto MIP. Two replicates were taken for all HPLC measurements, and error bars are shown as the standard deviation. For the 1-5 mg E2/ g NIP ratio range, binding efficiencies decreased following a nearly linear trend similar to that obsereved for MIP in Figure 6-8. However the slope of the decrease was less than for MIP. The MIP binding curve had a linear shape with a slope of 13.5 %/ (mg E2/ g MIP) whereas the slope for the NIP curve is 9.3 %/(mg E2/g NIP). The percent binding initially approached 95% at 1 mg E2/ g NIP and decreased to approximately 60% for 5 mg E2/ g NIP. For MIP the binding efficiency for 1 mg E2/ g MIP was 90% and decreased to 40% for 5 mg E2/ g MIP.

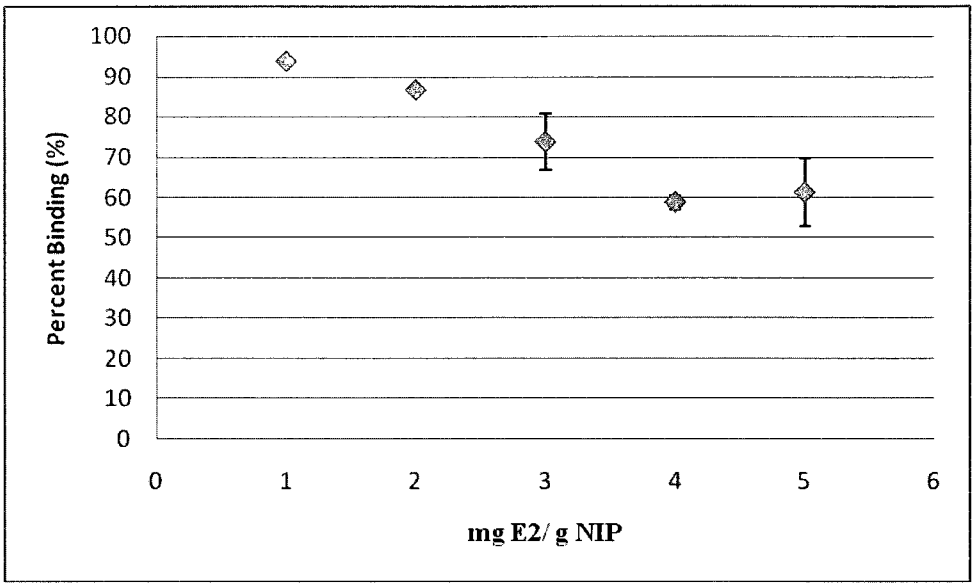


Figure 6-8: Binding Efficiencies for binding of E2 onto NIP Particles for E2 to NIP Ratios of 1-5 mg E2/ g NIP

The binding efficiency for NIP, as shown in Figure 6-8, is actually higher than that for MIP as shown in Figure 6-2 for some points. For example, at a E2/MIP ratio of 2 mg E2/ g NIP NIP exhibited 88% binding whereas MIP had a binding efficiency of 87%. Theoretically, the MIP binding should be superior to NIP binding because MIP are capable of both general and specific binding whereas NIP are only capable of non-specific binding. Yet as previously explained, there is reason to believe that the template was not completely removed from the MIP particles before each binding test. NIP should have only non-specific binding and should have the same capacity for non-specific binding as MIP. In other words, NIP and MIP binding capacities should be equivalent if the MIP's specific binding sites were all fully occupied. Wei and Mizaikoff (2007) found that most of the specific binding sites were at the surface of MIP, and attributed some of the additional binding capacity of MIP to a greater surface area created by the specific template cavities (Wei and Mizaikoff, 2007). However, in the case where the

template could not be sufficiently removed, the fact that these template cavities were full would lead to a decreased surface binding area and decreased binding efficiency for MIP. It is also possible that some of the non-specific sites for adsorption on MIP were also fully occupied prior to binding experiments leading again to decreased binding efficiency for MIP and superior binding efficiency for NIP.

In addition, there is a potential for the shape of the binding curves to be different for MIP and NIP. Adsorption is governed by the energies of the adsorption sites in comparison to the energy potential of the E2 in solution. If the energies of the non-specific adsorption sites were different between the MIP and NIP, this would lead to different curve shapes that make it appear as if NIP were more efficient than MIP.

Since the adsorption efficiency for NIP was superior to that of MIP, NIP may be a better preconcentration agent than MIP. Figure 6-9 shows the desorption efficiency for E2 on NIP. The data points shown are averages of two and error bars represent the standard deviation. The desorption of E2 from NIP is not influenced by template bleeding as the desorption of E2 from MIP was. Therefore no desorption efficiencies greater than 100% were observed. The desorption efficiency shown in Figure 6-8 increases with increasing E2 to NIP ratio in an exponential trend. Therefore there was a very steep increase in desorption efficiency between 4 and 5 mg E2/ g NIP.

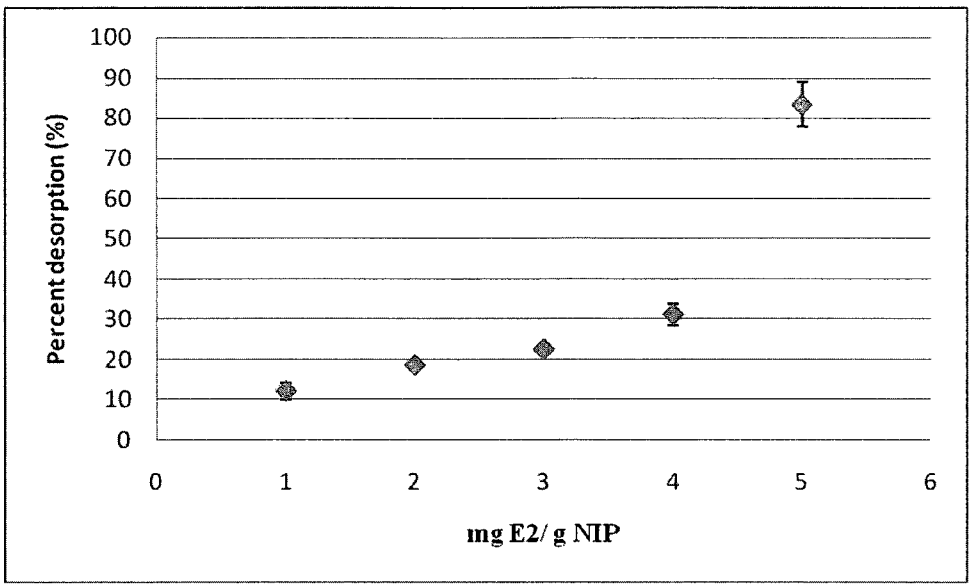


Figure 6-9: Desorption Efficiencies for Preconcentration of E2 onto NIP Particles for E2 to NIP Ratios of 1-5 mg E2/ g NIP

Figure 6-9 shows that NIP particles do have a better desorption efficiency than MIP particles (see Figure 6-5). This is promising for the preconcentration of E2 using NIP particles as a solid preconcentration surface. Increased desorption efficiency for NIP over MIP can be due to weaker, non-specific binding, indicating that some specific binding occurred for MIP. If all of the template sites were full prior to the binding study would have resulted in an artificially high desorption efficiency as template was removed during desorption, and not a lower desorption efficiency than NIP.

Figure 6-10 shows the overall preconcentration efficiency for the preconcentration of E2 onto NIP particles. Data points are averages of two and error bars represent the standard deviation. Figure 6-10 shows increasing overall efficiency between 1 and 3 mg E2/ g NIP, decreasing efficiency between 3 and 4 mg E2/ g NIP, and increasing efficiency between 4 and 5 mg E2/ g NIP. This trend is influenced both by the binding efficiency and the desorption

efficiency. The binding efficiency decreased with increasing E2 to MIP ratios whereas the desorption efficiency increased with increasing E2 to MIP ratios. There was little increase in the desorption efficiency between 3 and 4 mg E2/ g NIP so the overall efficiency decreased in this range; however, between 4 and 5 mg E2/ g NIP there was a large increase in the desorption efficiency, causing an increase in the overall efficiency.

The overall preconcentration efficiency for E2 on NIP particles is shown in Figure 6-10. The overall efficiency is low and not more than 16% for any of the concentrations studied. 16% recovery in preconcentration would only increase the efficiency of HPLC detection very slightly.

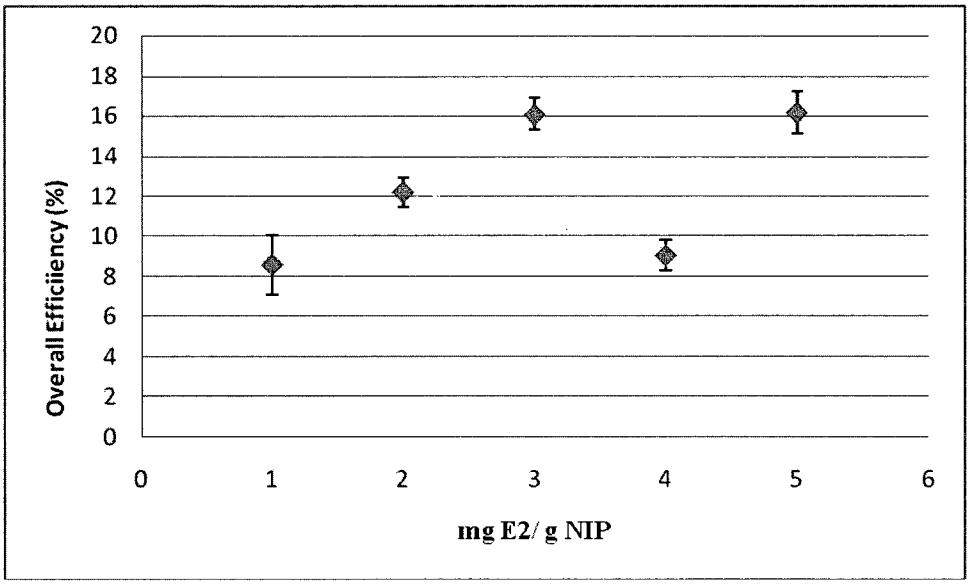


Figure 6-10: Overall Preconcentration Efficiency for Preconcentration of E2 onto NIP Particles for E2 to NIP Ratios of 1-5 mg E2/ g NIP

The preconcentration efficiencies shown in Figure 6-10 are not sufficient for HPLC analysis. This does not mean that neither MIP nor MIP could potentially be used for the preconcentration of E2 for analytical purposes. However, it does mean that further development work must be done prior to their inclusion in an analytical method. Therefore, the use of the

particles for analytical measurements for this project was considered not feasible and was dropped.

6.2 Phase 2: Use of MIP and NIP Particles for Removal of EDCs During Drinking and Wastewater Treatment

6.2.1 Comparison of MIP and NIP for Removal of EDCs for Drinking Water or Wastewater Treatment

In the second phase of experiments, the use of MIP or NIP for water or wastewater treatment applications was investigated. MIP particles were tested because it was possible that they would exhibit stronger binding and higher removal efficiency for E2 than the NIP particles. However, the attraction of the NIP polymers for water treatment lies in the fact that they are non-specific and may be capable of providing a general cleanup step by removing many different EDCs from the water. NIP are also considerably less expensive to manufacture because the most expensive component of the MIP is the E2 used to create the imprint in the polymers. The cost for the raw consumable materials for making MIP is about $1.17/100 mg while that for NIP is $0.11/100 mg.

MIP and NIP were compared using both E2 and EE2 for a concentration range of 1-5 mg E2/ g particles as well as E2 at a concentration range of 0.01-0.05 mg E2/ g particles, and the results are shown in the following sections.

6.2.1.1 Comparision of MIP and NIP for Removal of E2 from Distilled-Deionized Water

Figure 6-11 shows a comparison for the binding efficiencies of MIP and NIP for E2 to particle ratios of 0.01 to 0.05 mg E2/ g particles. The points shown are averages of two replicates and the error bars represent the standard deviation. Two replicates were considered to be sufficient since the nature of this study is that of a general feasibility study and exact values are

not required. Although only two replicates were completed, error bars were shown as opposed to showing values for both replicates for clarity. The trends are similar for MIP and NIP. Both show increasing efficiencies for this ratio range. However, the increase in efficiency was greater for the NIP than the MIP between 0.02 and 0.03 mg E2/ g particles. This led to a greater efficiency for NIP as compared to MIP for 0.03 – 0.05 mg E2/ g particles.

Similarly to MIP, NIP showed increasing efficiency for very low E2 concentrations followed by a region of high efficiency and then an area of decreasing efficiency as higher energy adsorption sites were filled. Also reported in the literature, Choi et al (2002) observed this area of increasing efficiency for very low concentrations when working with powdered activated carbon (PAC) but no explanation was given.

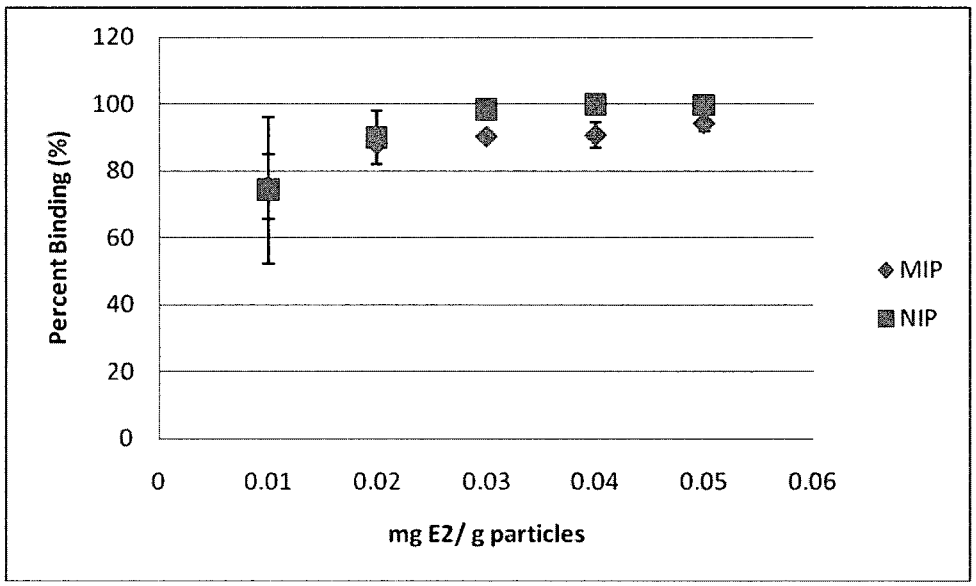


Figure 6-11: Comparison of MIP and NIP for Binding of E2 for E2 to Particle Ratios of 0.01-0.05 mg E2/g particles

Figure 6-12 shows a comparison for the binding efficiencies of MIP and NIP for E2 to particle ratios of 1 to 5 mg E2/ g particles. The points shown are averages of two replicates and

the error bars represent the standard deviation. Both MIP and NIP efficiencies possess a decreasing trend for this concentration range. MIP demonstrated a greater decrease than NIP did, leading to a wider range of higher efficiency, although the initial binding efficiency at an E2 to MIP ratio of 1 mg E2/ g particles was comparable.

For removal of E2 from water or wastewater samples, there appears to be no advantage to using MIP over NIP (Refer to Figure 6-11 and Figure 6-12). In fact, NIP showed a slightly higher binding efficiency for the lower E2 concentration range. This is counter to the theory that MIP exhibit both specific binding cavities and non-specific binding sites while NIP exhibit only non-specific binding sites. There are two possible reasons for this. The first reason is that the template may have remained in the specific cavities, blocking them to further adsorption of E2, and the second is that the particles may have been damaged by the template removal procedure. As discussed, there is evidence that the template was never adequately removed from the MIP particles completely, which would reduce their binding ability.

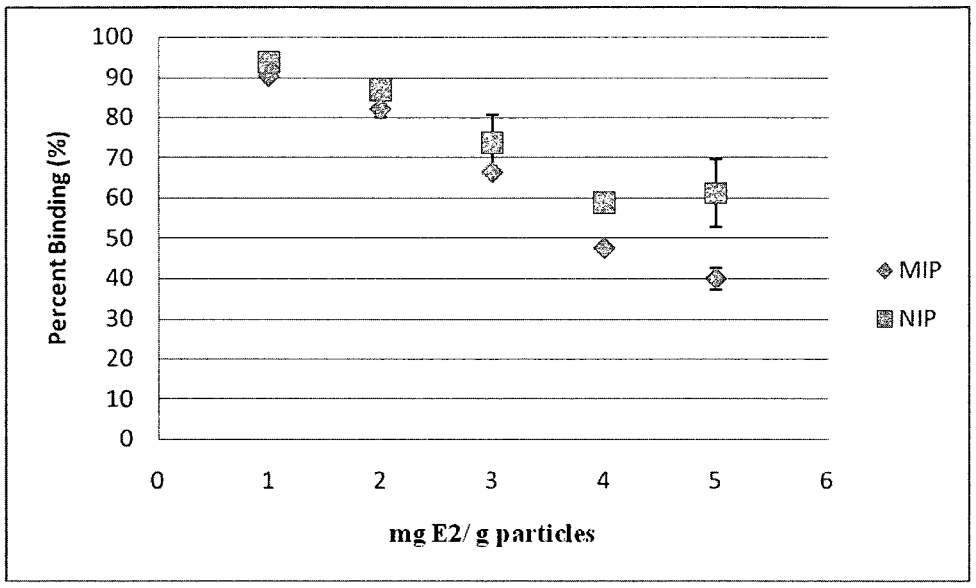


Figure 6-12: Comparison of MIP and NIP for Binding of E2 for E2 to particle ratios of 1-5 mg E2/g particles

6.2.1.2 Comparison of MIP and NIP for Binding of EE2 from Distilled-Deionized Water

Although the MIP was created specifically for E2 using E2 as a template, the high-energy specific binding sites can also have interactions with molecules similar in structure to E2. An example of such a compound is EE2. Therefore, a comparison of the binding with MIP and NIP was also completed for EE2. The results are shown in Figure 6-13.

Figure 6-13 shows the binding efficiency for binding of EE2 onto both MIP and NIP particles. The data points are shown as averages of two points and the error bars represent the standard deviation. Both MIP and NIP demonstrate increasing efficiency for this range of EE2 to particle ratios for binding efficiency. It should be noted; however, that the values for binding of EE2 onto MIP for 1 and 2 mg EE2/ g particles were negative.

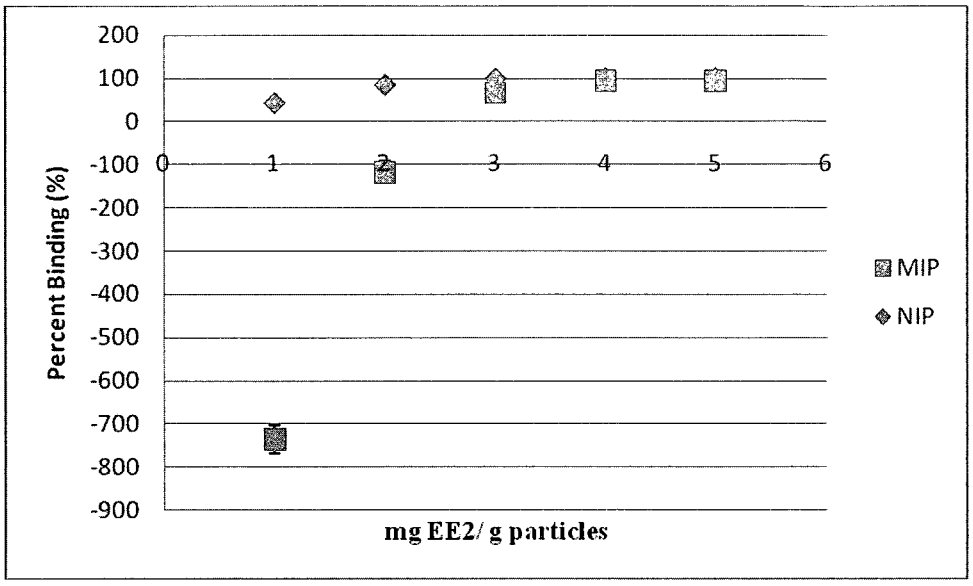


Figure 6-13: Binding Efficiency for EE2 on MIP and NIP for EE2 to Particle Ratios of 1-5 mg EE2/g particles

Figure 6-13 shows negative binding efficiencies for lower concentrations of EE2 onto MIP particles. This was caused by template bleeding out of the MIP. Yet, it was not visible on the binding curves for E2. There are several possible explanations for the negative binding observed, which is likely due to a combination of factors. The first explanation is that with the HPLC mobile phase and flow rate used for these tests, E2 and EE2 were not separated. Also, E2 exhibited a stronger HPLC signal than EE2. Therefore, any E2 which may have bled from the MIP would have contributed to a stronger signal or larger peak area than an equal amount of EE2. When compared against an EE2 calibration curve, the concentration would seem very high. Another explanation is that although MIP are capable of binding structural analogues or compounds with similar structures to the template, the energy for binding of the template is much stronger than that for structurally similar compounds (Fang and Li, 2007). For this reason, binding of EE2 to the specific binding sites of MIP in the presence of E2 leftover from

polymerization may have been very low. The energy distribution of the entire system would also be changed because there would be no E2 in the EE2 solution. However, the EE2 solution was prepared in methanol, so it is possible that much greater amounts of E2 would desorb into this methanol solution than into the aqueous E2 solution.

Figure 6-14 shows only the positive binding range for Figure 6-13. There is one immediately noticeable difference when compared to Figure 6-12. For increasing analyte/particle ratios, E2 exhibited decreasing efficiencies while EE2 exhibited increasing efficiencies. This is likely due to differences in the type of binding occurring. The specific binding sites have a higher binding energy and are more accessible to E2 than EE2. Therefore E2 is capable of binding at lower concentrations than EE2 because the difference in energies between the solid and liquid phases is greater.

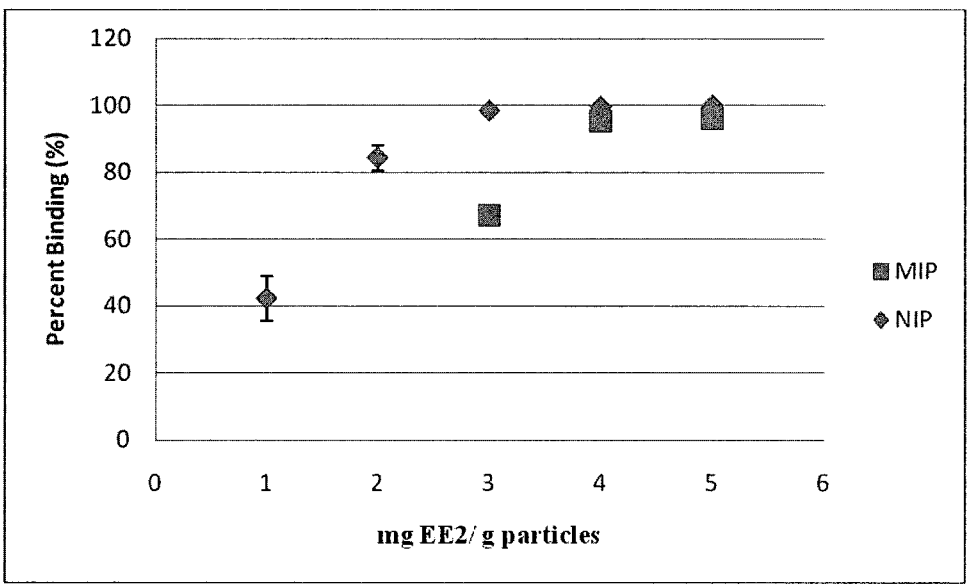


Figure 6-14: binding efficiency for EE2 on MIP and NIP for EE22 to particle ratios of 1-5 mg EE2/g particles, showing only positive binding efficiencies.

Li et al (2008) demonstrated that adsorption of a structural analogue onto MIP can occur but the energy of this adsorption was less than that of the template. Sanbe et al (2002) established selectivity factors showing the ratio of attraction of MIP for several different EDCs over that for NIP. For related EDCs, the selectivity factors were all greater than one. The retention of EDCs on NIP varied and was not highest for the template (Sanbe and Haginaka, 2002). Nevertheless, the NIP exhibited equal binding for both which supports the findings of this study. Yet, compounds with similar structures to the template can bind with MIP. It is possible that clean MIP would be more efficient than NIP. The structures of E2 and EE2 are shown in Table 6-1. The structures are almost identical except that EE2 has an additional ethyne group. Since both E2 and EE2 have an OH group, it is likely that both would be able to interact with the MIP, although the size of the molecules may also play a role (Li et al, 2007). For this study, EE2 is larger than E2 and contains an additional ethyne group, therefore it may not have been capable of fitting into the template cavity although this size difference is slight. Rachkov et al (1998) found that only EDCs with an OH-group at C17 could interact effectively with MAA and both E2 and EE2 have this OH group .

6.2.2 Use of NIP for Treatment of a Variety of EDCs

Three other EDCs (diethylstilbestrol, bisphenol-A, and atrazine) were tested with NIP to evaluate the ability of NIP to remove a wide variety of EDCs from contaminated water. These EDCs were selected based on three criteria: (1) prevalence in the natural environment, (2) variety of chemical structure, and (3) ability to conduct analysis with HPLC and either fluorescence detection (FD) or ultraviolet detection (UV). E2 was chosen because it is the natural estrogen and is common in wastewater. EE2 was chosen because it is the form of estrogen used in birth control pills and is also common in wastewater. EE2 is also very similar in structure to E2 and was used to test the selectivity of the MIP particles. Bisphenol-A, atrazine, and diethylstilbestrol

were chosen because they come from a wide range of chemical groups, have different structures, and exhibit different properties. E2 and EE2 could be measured using HPLC-FD and bisphenol-A, atrazine, and diethylstilbestrol could all be analyzed with HPLC-UV.

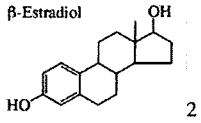
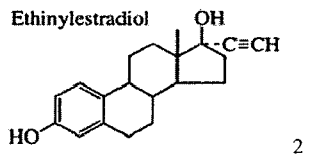
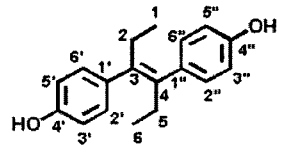
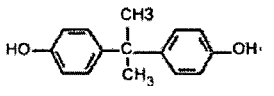
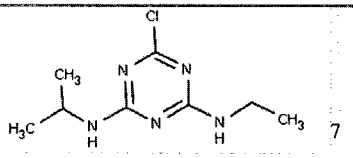
Since NIP does not contain specific binding sites and was not created with a template, interactions between the particles and EDCs will be non-specific and based solely on hydrophobic effects. Hence, binding should be influenced by the solubility and octanol/water partitioning coefficients of the contaminants. Any contaminant with a low solubility and/or high partitioning coefficients will bind with a higher efficiency. Addition of methanol to the water matrix would also cause some changes to the binding properties, primarily by making the EDCs more soluble and reducing the partitioning coefficients. The addition of methanol may not have the same effect on all of the EDCs studied, but it is difficult to quantify the effect within the scope of this thesis research.

It is this section of the research for which the use of methanol to increase the solubility of the EDCs posed the greatest disadvantage. Because different EDCs behaved differently in the methanol and water matrix as opposed to water alone and the responses to methanol additions were not the same for all of the EDCs studied, the use of methanol for this part of the study made it difficult to compare the results of different EDCs in water. The results provided below are only applicable to the specific solvent matrix studied and cannot be applied to water matrices. However, since all of the EDCs studied are hydrophobic, and methanol, being less polar than water, is more capable of dissolving hydrophobic solutes; individually the efficiencies determined represent a lower limit for the binding efficiency of that compound and thus provide valuable information for the feasibility of treating bisphenol-A, atrazine, and diethylstilbestrol with NIP particles.

Although the use of methanol as part of the solvent mixture does limit the amount of information that can be obtained from this part of the study, it was also unavoidable for this part of the study. The detection limit for the HPLC-UV analytical set-up used to measure the concentrations of bisphenol-A, atrazine, and diethylstilbestrol were approximately 1 ppm, 2 ppm, and 4 ppm, respectively. For this reason it was necessary to use higher concentrations of these compounds and thus the range of 20 to 100 ppm was chosen, but it is this range that requires the addition of methanol to increase solubility.

Table 6-1 illustrates the water solubilities and partitioning coefficients for the EDCs used in this study. The results presented in Table 6-1 and Figure 6-15 through Figure 6-17, demonstrate that the binding characteristics did not closely follow the solubility or $\log(K_{OW})$ trends. The highest $\log(K_{OW})$ value was for diethylstilbestrol, but this EDC exhibited the lowest binding efficiency. The discrepancy can probably be explained by its hydrophobic property above and beyond what solubility and partitioning coefficient predict, although the exact mechanisms responsible are difficult to determine due to the mixed nature of the solvent matrix.

Table 6-1: Physical properties of E2, EE2, atrazine, diethylstilbestrol, and bisphenol-A

EDC	Water Solubility (mg/L)	Log(K_{OW}) (Octanol/water partitioning coefficient)	Structure
17β-estradiol (E2)	13 [1]	4.01 [1]	 2
17α-ethynylestradiol (EE2)	11.3 (27C) [3]	3.67 [1]	 2
Diethylstilbestrol	Not Available	5.07 [1]	 4
Bisphenol-A	129 [3]	3.32 [3]	 5
Atrazine	Not Available	2.61[6]	 7

[1] Lintelmann, J., Katayama, A., Kurihara, N., Shore, L., and Wenzel, A. (2003) Endocrine disruptors in the environment. *Pure and Applied Chemistry*, 75 (5) 631-681.

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6.2.2.1 Removal of Atrazine from Distilled-Deionized Water Using NIP Particles

Figure 6-15 provides the binding efficiencies for binding of atrazine onto NIP particles. Data points are given as the average of two replicates and the error bars represent the standard deviation. Figure 6-15 shows decreasing binding efficiency with increasing atrazine to NIP ratios for the range of ratios 1 – 5 mg atrazine/ g NIP. The decreasing trend was approximately linear with a slope of 5.4 %/(mg atrazine/ g NIP). This decreasing slope is less than that observed for binding of E2 onto either MIP or NIP. The binding efficiency for atrazine over this range was lower than that for E2 or EE2 for the same concentration range. Nevertheles atrazine exhibited binding efficiencies greater than 50% for the entire range of atrazine to NIP ratios tested.

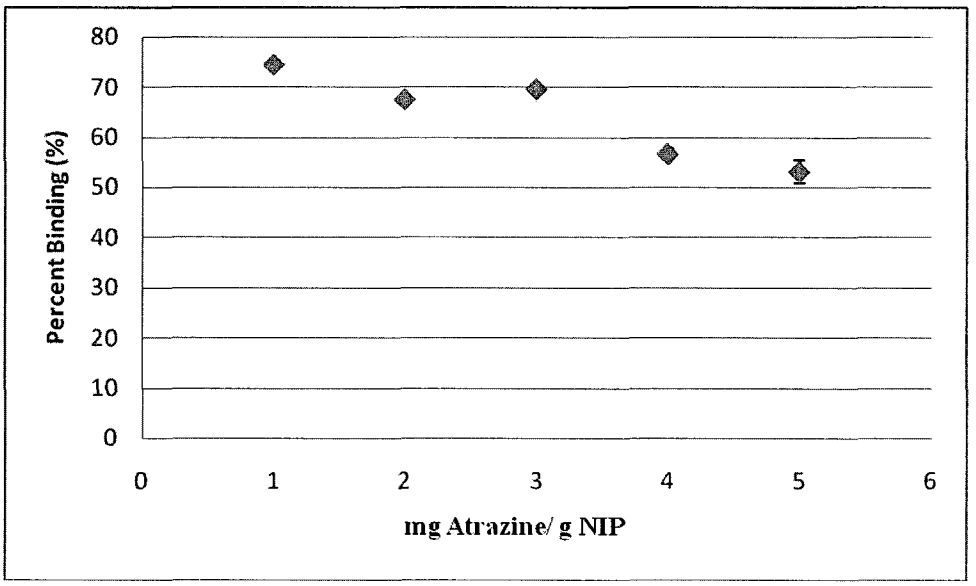

Figure 6-15: Binding efficiency for binding of atrazine on NIP for atrazine to NIP ratios of 1-5 mg atrazine/g NIP

6.2.2.2 Removal of bisphenol-A from distilled-deionized water using NIP particles

Figure 6-16 shows the binding efficiency for binding of bisphenol-A onto NIP particles. Data points are shown as the average of two replicates and the error bars represent the standard

deviation. Figure 6-16 shows an area of relatively consistent binding between 1 and 3 mg bisphenol-A/ g particles, followed by a linear decreasing trend between 3 and 5 mg bisphenol-A/ g particles. It should be noted that the binding efficiency for bisphenol-A onto NIP was very high for all of the bisphenol-A to NIP ratios studied and that the y-axis shown in Figure 6-16 shows binding efficiencies ranging only from 91 to 97%.

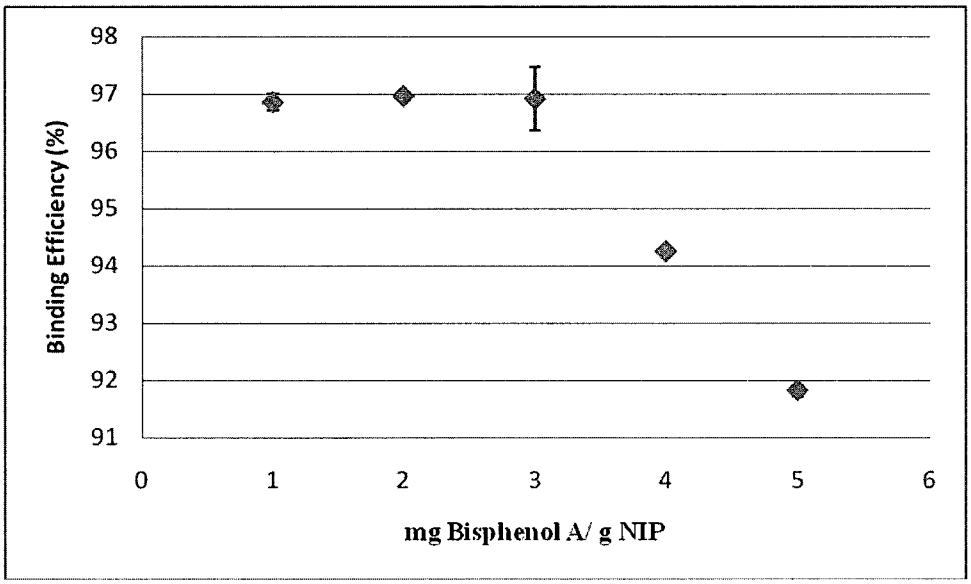

Figure 6-16: Binding efficiency for binding of bisphenol-A on NIP for atrazine to NIP ratios of 1-5 mg bisphenol-A/g NIP

6.2.2.3 Removal of Diethylstilbestrol from Distilled-Deionized Water using NIP Particles

Figure 6-17 shows the binding efficiency for binding of diethylstilbestrol onto NIP particles. Data points are shown as the average of two replicates and the error bars represent the standard deviation. Figure 6-17 displays an area of increasing binding efficiency between 1 and 3 mg diethylstilbestrol/g NIP followed by decreasing efficiency between 3 and 5 mg diethylstilbestrol/ g NIP. The maximum binding efficiency was 46% at 3 mg diethylstilbestrol/ g NIP.

The binding efficiencies for diethylstilbestrol are much lower than for the other EDCs studied. However, the NIP particles were still capable of removing some diethylstilbestrol from solution. Also, the optimum ratio of diethylstilbestrol to particles occurred at a higher EDC to particle ratio of 3mg/g particles than for the other EDCs.

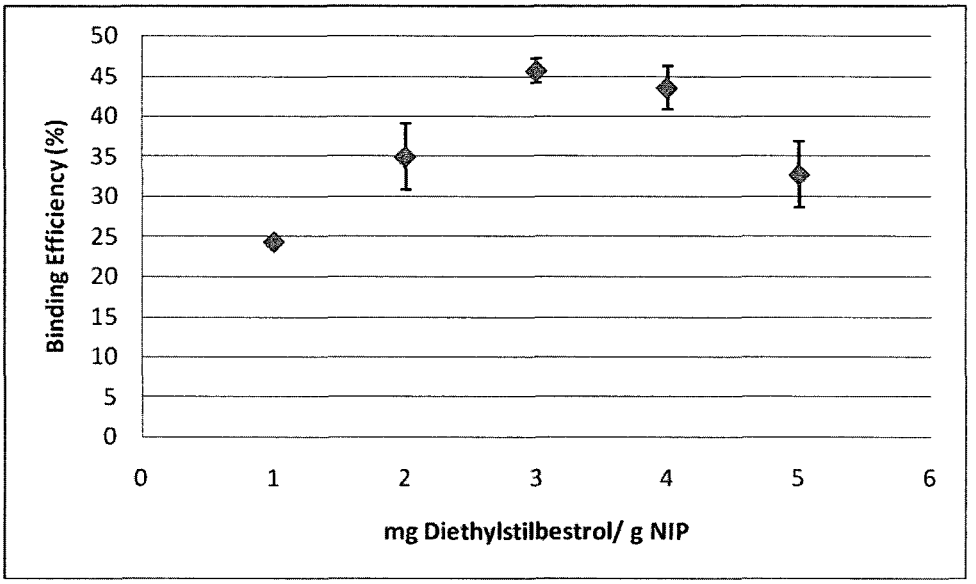

Figure 6-17: Binding efficiency for binding of diethylstilbestrol on NIP for atrazine to NIP ratios of 1-5 mg diethylstilbestrol/g NIP

6.2.2.4 Competitive Interactions between EDCs

Competitive interactions between many different EDCs within a wastewater or drinking water sample would change the binding efficiencies for each of the EDCs studied. Competitive binding would likely result in EDCs with greater affinity for NIP exhibiting a smaller reduction in binding efficiency while those with an already weak attraction from the NIP would likely exhibit a greater reduction in binding efficiency when treated in the presence of competitors.

Substances other than EDCs would also be capable of binding to the NIP particles as well. Hydrophobic organic matter within the wastewater or surface water would also have an attraction to the NIP and could be removed, to some degree, by the particles. There is a potential problem for treatment because the NIP particles are meant to clean micro-pollutants and if their capacity is wasted cleaning contaminants which are present in water in higher concentrations, they may not be effective for removal of trace EDCs. Note that this general problem is common to most water treatment technologies or methods, however.

6.2.3 Use of NIP as an Overall Water Treatment Method for EDCs:

6.2.3.1 Treatment of Unidentified Surface Water Compounds with NIP

After the binding efficiency and performance tests with laboratory water were completed, NIP was tested to determine if it was capable of treating real environmental samples. A river water sample was taken from the Rideau River in Ottawa (Ontario, Canada) and passed through a 200-nm syringe filter prior to analysis. The filtration was completed for protection of the HPLC column and injection valve from suspended particulate materials in the river water sample. The HPLC analysis chromatogram obtained from the filtered river water is shown in Figure 6-18.

Figure 6-18 imparts the chromatograms for treatment of river water with NIP particles. The data represents the average of two HPLC analyses but error bars were not shown for clarity. The peak visible at a retention time of 2.5 +/- 0.1 minutes was shown to decrease with increasing particle applications from 5 mg of NIP applied per mL of river water to 20 mg of NIP applied per mL of river water.

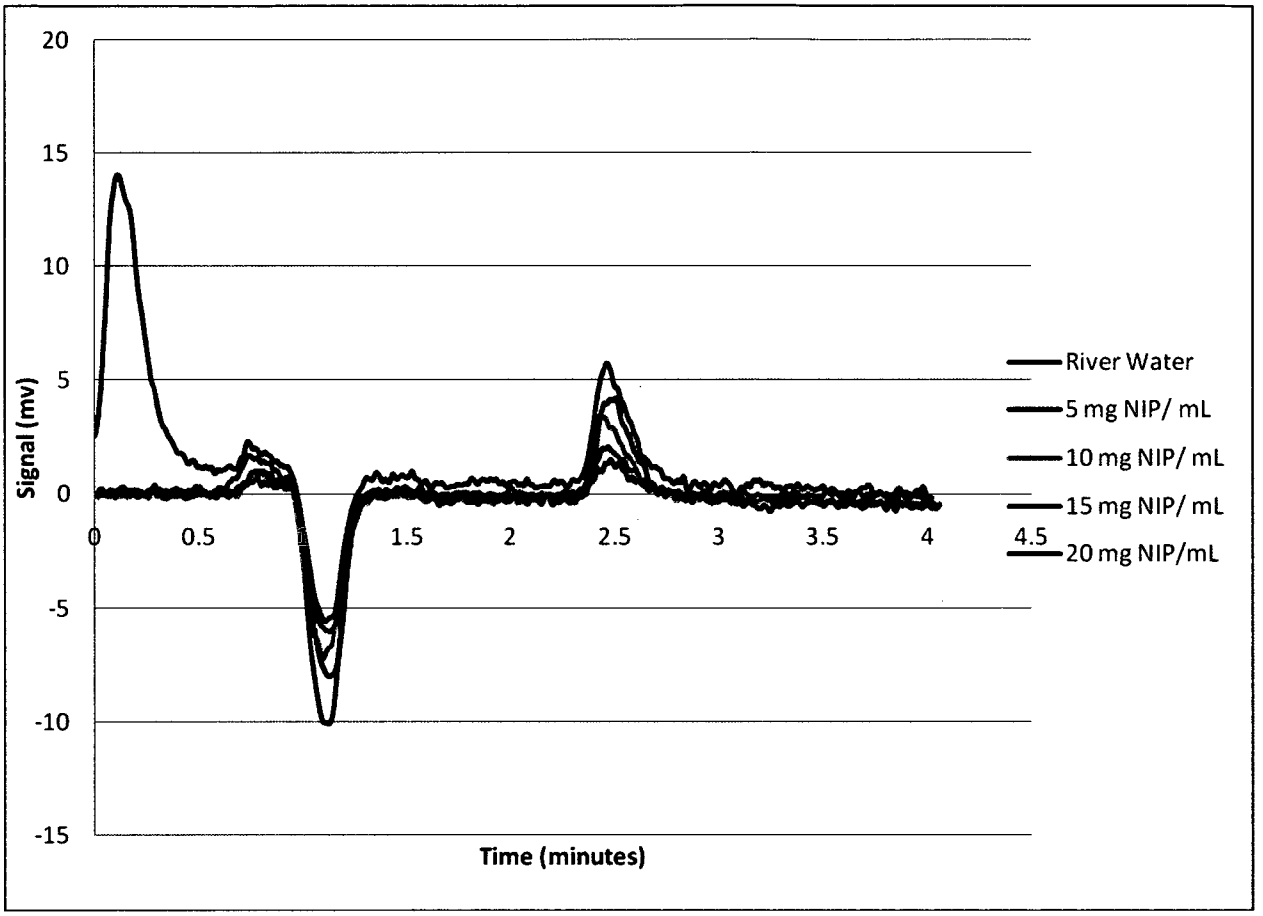

Figure 6-18: Fluorescence detector chromatogram for treatment of unidentified compounds in surface water treated with varying NIP particle concentrations

Following filtration, the river water sample was relatively clear of trace organic compound peaks visible to fluorescence detection. It is conjecturable that many of the contaminants in the river water were sorbed onto the membrane inside the syringe filter and thus removed during the filtration step. From the chromatogram, several peaks are visible prior to the negative water/methanol peak with a retention time of 1.1 +/- 0.1 minutes. The negative water/methanol peak was visible because the composition of the solute differed from that of the mobile phase used for HPLC analysis and there were interactions between these two phases. The compounds contributing to the aforementioned peaks are not measurable. That they were eluted

prior to the water peak means that they were not retained or separated in the column and can neither be identified nor analyzed. The magnitude of the negative water peak also appeared to decrease with increasing NIP particle concentrations. This was plausibly due to interactions with the particles, which were not measurable because they were not retained on the column. Only the peaks eluted following the water peak can be retained by the column and analyzed with HPLC.

Consequently, there was only one peak detected for the river water sample and it was eluted at a retention time of approximately 2.5 minutes. From Figure 6-18, this peak decreased with increasing application of the NIP particles. Although the specific compound is unidentifiable using HPLC analysis with fluorescence detection alone, this shows that NIP particles are capable of removing even unknowns from river water. These unknowns can be considered contaminants for the purposes of this study because they represent something other than pure water. Also, the contaminant, or possibly contaminants, shown by this peak was visible following filtration of suspended materials. This indicates that the contaminant was in the aqueous phase for river water and was not sorbed to suspended materials, thus requiring removal. The removal of dissolved organic compounds is of particular importance and a main challenge for water treatment plants.

6.2.3.2 Treatment of Wastewater with NIP

6.2.3.2.1 Treatment of Unidentified Wastewater Compounds with NIP Particles

Following treatment of river water with the NIP, performance of NIP for the removal of contaminants was tested on secondary wastewater. Wastewater samples were taken following the secondary clarifiers, but prior to disinfection from the Robert O. Pickard Environmental Centre (ROPEC) in Ottawa (Ontario, Canada). All samples were filtered with a 200-nm syringe filter

prior to analysis for protection of the HPLC instrument. Chromatograms acquired with both fluorescence and UV detectors are shown in Figure 6-19 and Figure 6-20.

Figure 6-19 provides the fluorescence detector output for treatment of secondary wastewater with NIP particles. The chromatograms are averages of two replicates, but error bars were not shown for clarity. In Figure 6-19, several peaks are visible prior to and above the water peak, but no peaks are visible following the water peak from the fluorescence detector. The initial wastewater sample was allowed to run for longer in HPLC analysis than the other samples which were stopped sooner at 3.0 minutes +/- 0.5 minutes since there were no visible peaks. It is possible that the wastewater did not contain any compounds which were visible with the fluorescence detector, but more likely that they were sorbed to the suspended particles and removed during filtration or else were not retained by the column and appeared as a broad peak prior to the water peak.

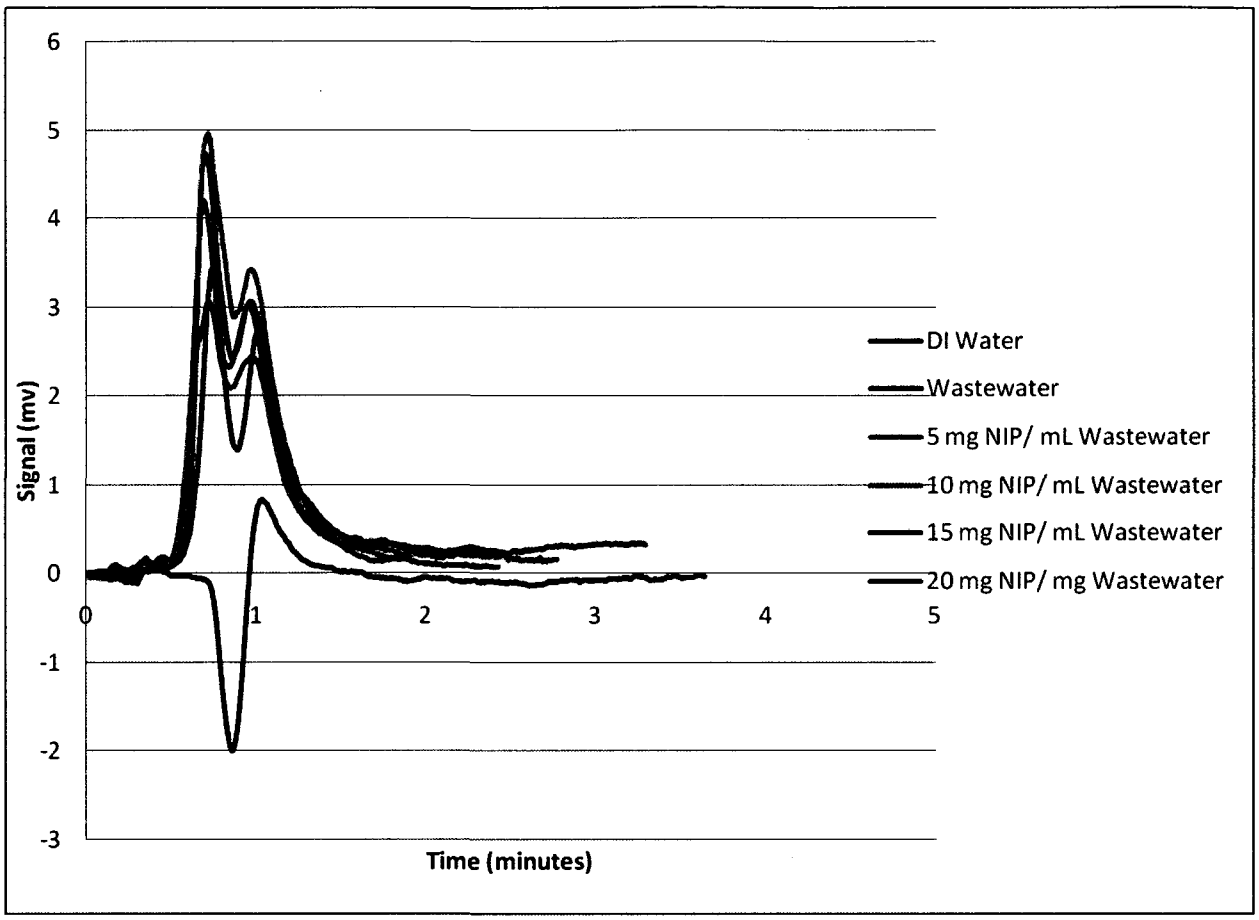

Figure 6-19: Fluorescence chromatogram for treatment of unidentified compounds in secondary clarifier wastewater effluent with NIP particles

Since there are many compounds which are not visible with a fluorescence detector, HPLC analysis with a UV detector was also completed for the secondary wastewater treated with NIP particles. Fluorescence detectors are very effective for analysis of fluorescent compounds but many compounds are not fluorescent but can be detected with a UV detector. Figure 6-20 shows the results of the secondary wastewater treated with varying concentrations of NIP particles. The chromatograms shown are averages of two replicates, but error bars are not shown for clarity. There is a peak clearly visible at an elution time of approximately 1.3 minutes which appears to decrease with application of particles, although the peak and also the decrease was

more subtle than for the river water shown in Figure 6-18. The particles were capable of removing an unknown contaminant from a complex wastewater sample with many interfering species.

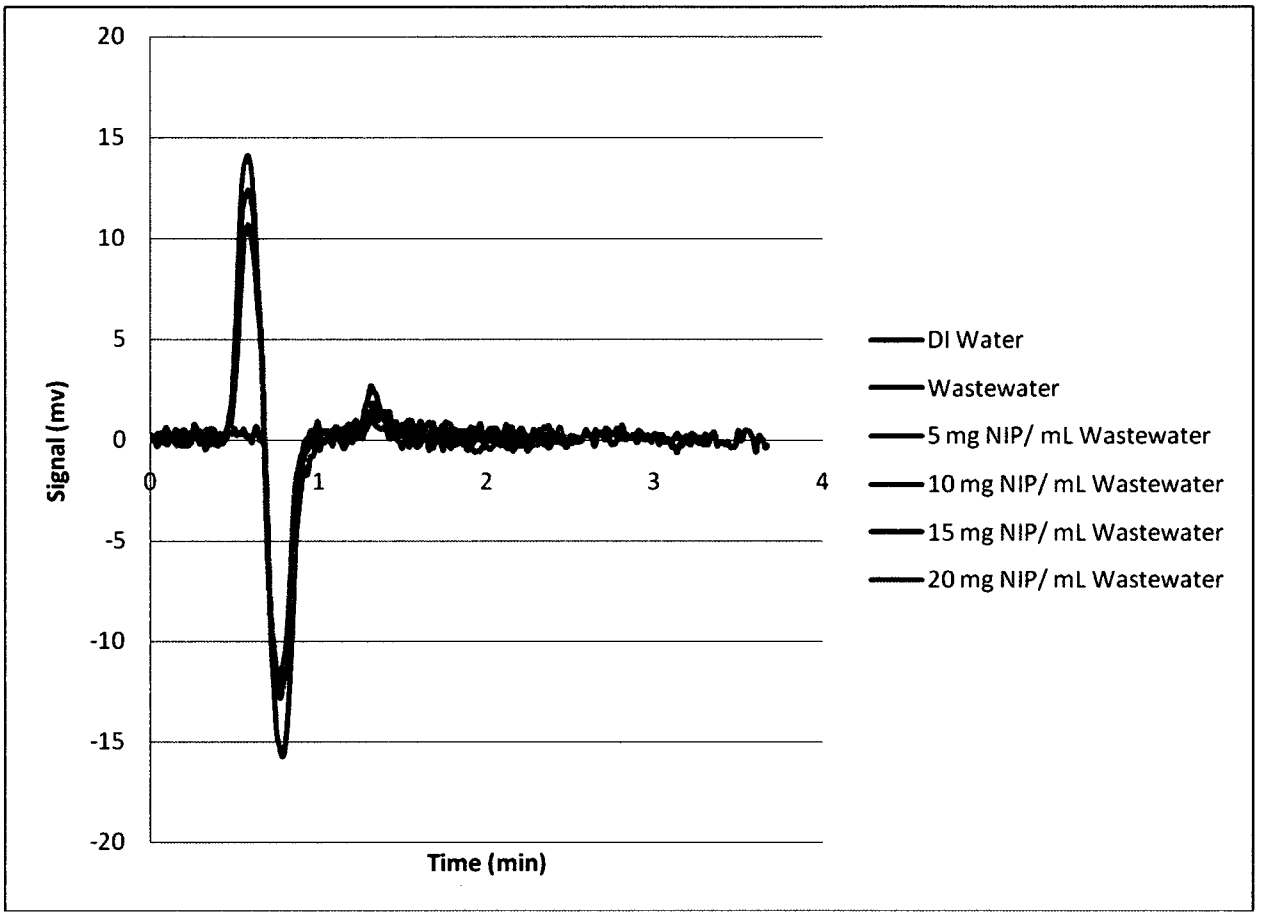

Figure 6-20: UV detector chromatogram for treatment of unidentified compounds in secondary clarifier effluent with NIP particles

6.2.3.2.2 Treatment of Spiked E2 in Secondary Wastewater Effluent with NIP Particles

Treatment of unknown contaminants in secondary wastewater and surface water has shown that commonly found trace organic contaminants can be successfully removed. Most contaminants of a hydrophobic nature would be removed using the NIP particles. However, we cannot identify these contaminants with HPLC and fluorescence or UV detection alone. It is

important to test the removal of EDCs from complex wastewater samples in the presence of interfering species as well. Figure 6-22 shows the results of HPLC-FD analysis completed with secondary clarifier effluent taken from ROPEC and spiked with 10 ppm E2. As shown in Figure 6-19, the peaks from the wastewater sample alone were very small and they occurred before and above of the water peak. Therefore the peak at a retention time of 1.4 +/- 0.1 minutes came only from the spiked E2.

Figure 6-21 shows the fluorescence detector chromatograms for the spiked wastewater treated with varying amounts of NIP particles. The chromatograms shown are averages of two replicates although error bars are not shown for clarity. The NIP particles were highly efficient at removing E2 from the wastewater medium and a steep decrease in peak size was observed following application of 5 mg of particles/ mL of water. The peak size then continued to decrease for increasing particle additions. Increased removal for increasing particle additions, indicate that treatment could be adjusted to the desired final concentration by increasing the NIP quantity.

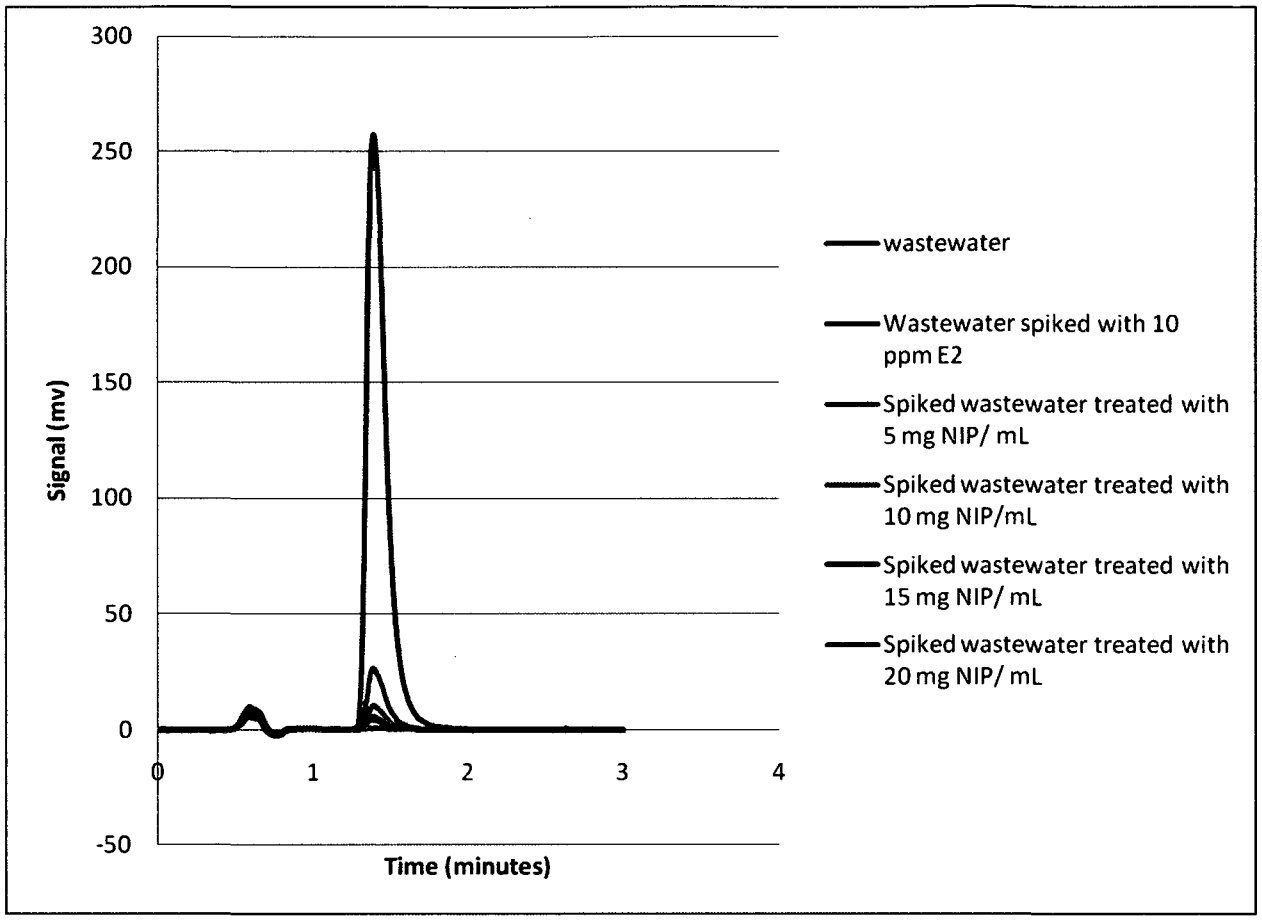

Figure 6-21: fluorescence detector chromatograms for treatment of spiked E2 in secondary wastewater effluent with varying concentrations of NIP particles

Figure 6-21 shows that differing particle concentrations are capable of removing spiked E2 from wastewater with different efficiencies, however, comparison to the results obtained from deionized water is also important. Therefore tests were then completed in which wastewater was spiked with varying E2 concentrations and treated with 20 mg/mL of NIP particles.

Figure 6-22 provides the results of binding studies conducted with secondary clarifier effluent spiked with E2 for E2 to NIP ratios between 0.1 and 5 mg E2/ g NIP. The results are averages of two replicates and the error bars represent the standard deviation. For these ratio

ranges, the efficiency was greater than 95% for ratios between 0.1- 0.5 mg E2/ g NIP before decreasing between 0.5 and 5 mg E2/ g NIP. It should be noted that the point for 0.5 mg E2/ g NIP was repeated both with and without methanol as part of the sample matrix. The sample containing methanol led to a much lower binding efficiency.

Gao et al (1998) studied the effect of dissolved organic carbon on adsorption in natural water bodies and found that the concentration of dissolved organic carbon both decreased the initial amount of the EDC adsorbed and increased the amount of the EDC desorbed with time. Lin et al (2008) studied the effect of humic acid concentration on removal of bisphenol-A from contaminated water and found that there was no effect. Though they attributed this to the lack of adsorption sites on their MIP capable of interacting with humic acid and said that results might vary for different constructions of MIP particles. Hence, it is important to repeat some of the tests which were performed in deionized water in natural water and wastewater samples.

The low concentration range (from 0.01 – 0.05 mg E2/ g NIP) showed very similar results to those obtained using deionized water (Figure 6-3). However, in the higher concentration range (1 – 5 mg E2/ g particles) where methanol was added to increase the solubility of E2, the removal efficiencies were different. Theoretically, the solubility of E2 in a deionized water and methanol mixture should be very similar to that in a wastewater and methanol mixture so it appears that the interfering species in the wastewater decreased the efficiency of the particles for E2 removal only when methanol was added. The point at 0.5 mg E2/ gNIP was repeated twice because E2 was still soluble in water at this concentration. There was a sudden drop in efficiency at this concentration. The chemistry of the sample mixture should be investigated further to further understand the interferences from other compounds.

However, what is important is that within the optimum treatment range, the particles were capable of removing typical concentrations of E2 present in water and wastewater (97±2%).

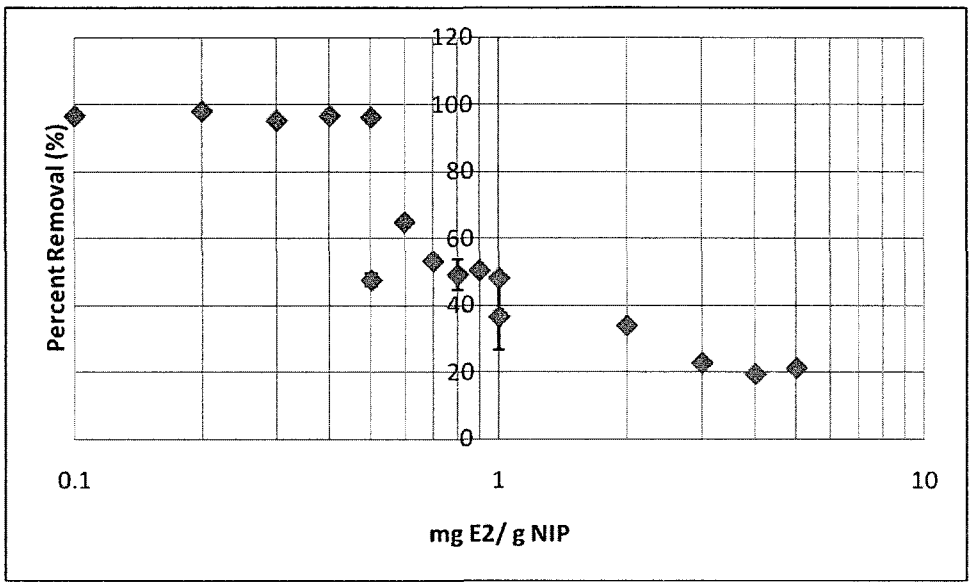

Figure 6-22: Removal efficiency for removal of Spiked E2 from secondary wastewater effluent for varying E2 concentrations

6.2.3.2.3 Removal of Typical Wastewater Contaminants from Secondary Wastewater Effluent Samples

Adsorption of spiked EDCs from wastewater was expected to be less efficient than the adsorption of the same concentrations of those EDCs from distilled-deionized water samples because there were interfering substances within the wastewater. These substances interfere with the adsorption of E2, because they themselves are adsorbed onto the surface of NIP particles. There are many substances which are partially removed during wastewater treatment and further treatment or removal with NIP would be beneficial as it would lead to a greater removal of these contaminants. Wastewater characterization tests were completed both before and after application of the particles to evaluate the types of contaminants removed by the particles. Tests

were also completed with filtered wastewater samples because it was these samples which were spiked with E2 for previous tests. Ammonia, phosphate, total organic carbon (TOC) and chemical oxygen demand were all measured. Absorbance and turbidity were also measured to evaluate the degree of removal of NIP particles following centrifugation (for unfiltered samples) or filtration (without centrifugation). Ammonia and phosphate are both constituents of concern in wastewater because high levels of these chemicals can contribute to eutrophication of water bodies receiving wastewater effluent. TOC and COD were chosen because they are both measures of the overall organic content of the wastewater. TOC and COD can include organic matter such as proteins, carbohydrates, and fats but also complex synthetic or natural organic molecules (Metcalf and Eddy Inc., 2003). Centrifugation was used to remove the particles for unfiltered samples. Figure 6-23 through Figure 6-28 show the results from absorbance, turbidity, ammonia, phosphate, TOC and COD, respectively.

Figure 6-23 shows the effects of NIP treatment on wastewater adsorbance. All of the data shown are averages of three replicates and the error bars represent the standard deviations. The figure shows that the initial absorbance for the wastewater was greatly increased following addition of NIP particles which were removed using the Hamilton Bell Van Guard V 6500 centrifuge. It should be noted that this centrifuge was different than that used for the binding studies which employed a Revolutionary Science Microcentrifuge. A larger centrifuge was required for this part of the study due to the larger sample size required and thus the removal efficiency was much lower. The absorbance for the filtered wastewater was decreased following filtration. The sample which was treated with NIP particles which were then removed with filtration exhibited higher absorbance than both the initial wastewater sample and the filtered wastewater sample, but the absorbance was much lower than that for the sample where the NIP

particles were removed using the centrifuge. The higher absorbance for the sample for which the particles were removed with filtration is a reflection of the heterogeneity of particle sizes for NIP. Although the filter size was 200 nm, it was not capable of capturing 100% of the particles. These results indicate that for further wastewater characterization experiments, the results for the filtered samples may be more accurate than those for the centrifuged samples because the wastewater characterization measurements, excluding total organic carbon (TOC) were all taken using spectrophotometric tests and there was less interference due to particles for the filtered sample.

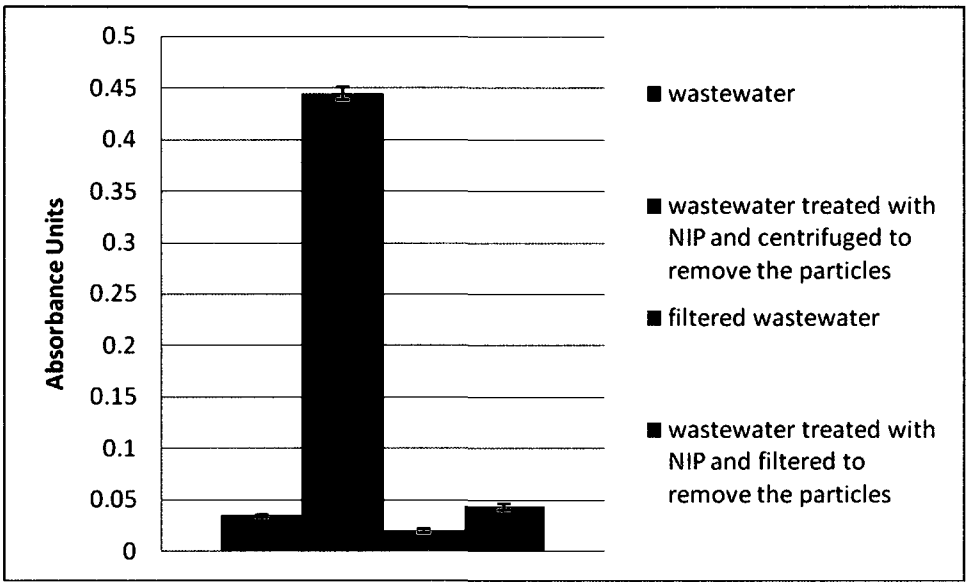

Figure 6-23: Effect of NIP treatment on wastewater absorbance

Figure 6-24 demonstrates the effects of treatment with NIP particles on the turbidity of the wastewater samples. Note that the y-axis scale of Figure 27 is given in logarithmic units. The data was plotted in logarithmic units because the increase in turbidity for the sample in which the particles were removed with centrifugation was very large and the other turbidity values were not clear on a linear scale. Turbidity, like absorbance is a measure of the level of removal of the

particles. The values given are averages of three measurements and error bars represent the standard deviation. Figure 6-24 shows that there was a substantial increase in turbidity following the addition of the NIP particles and that these particles were not adequately removed with the centrifuge. As explained for Figure 6-23, one explanation for this was that for the other phases of the study, a Revolutionary Science Microcentrifuge with a speed of 10,000 rpm was used to separate the samples, but since a larger sample size was required for wastewater characterization, a Hamilton Bell Van Guard V 6500 was used for this study and particle removal was less effective. The figure also shows a decrease in turbidity following filtration of the wastewater sample and a slight increase in turbidity between the wastewater sample and the sample which was treated with NIP which were removed via filtration. Although filtration was not 100% efficient for particle removal, it did remove a significant amount of the particles to use spectrophotometric tests with some degree of confidence whereas the results for the centrifuged sample cannot be interpreted with confidence.

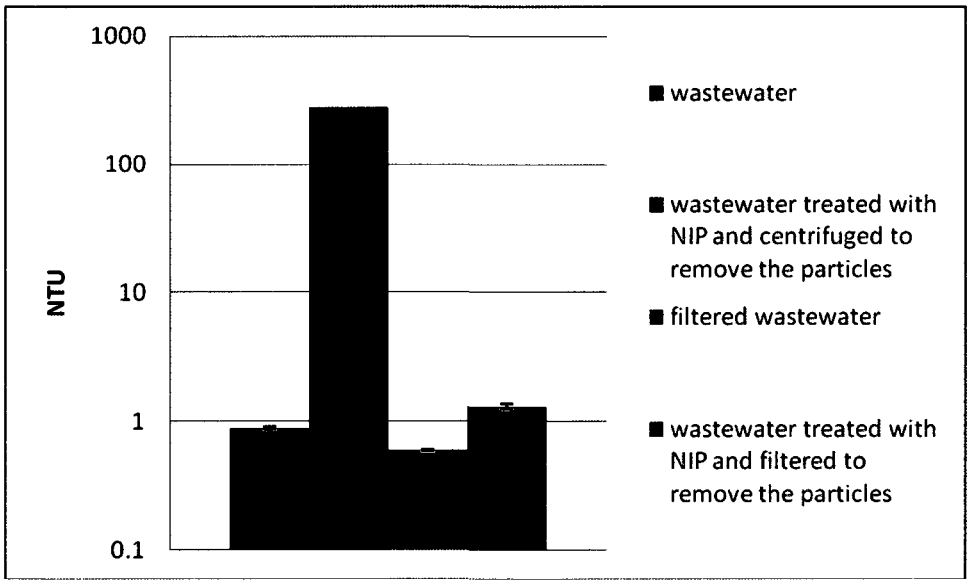

Figure 6-24: Effect of NIP treatment on wastewater turbidity

Figure 6-25 illustrates the effect of NIP treatment on ammonia concentrations of the wastewater. The data shown are averages of two replicates and the error bars represent the standard deviations. In Figure 6-25 decrease is visible in ammonia concentrations between the wastewater sample and the NIP treated and centrifuged wastewater sample. A decrease was also observed between the filtered wastewater sample and the sample which was treated with NIP and then filtered. Yet this decrease may not have been significant due to the large standard deviation for the NIP treated and filtered sample. Figure 6-25 was not an in-depth study into the potential for ammonia removal with NIP particles but as a feasibility study to determine whether or not there was any possibility for reductions in ammonia concentrations and the results were positive, indicating an area for future study.

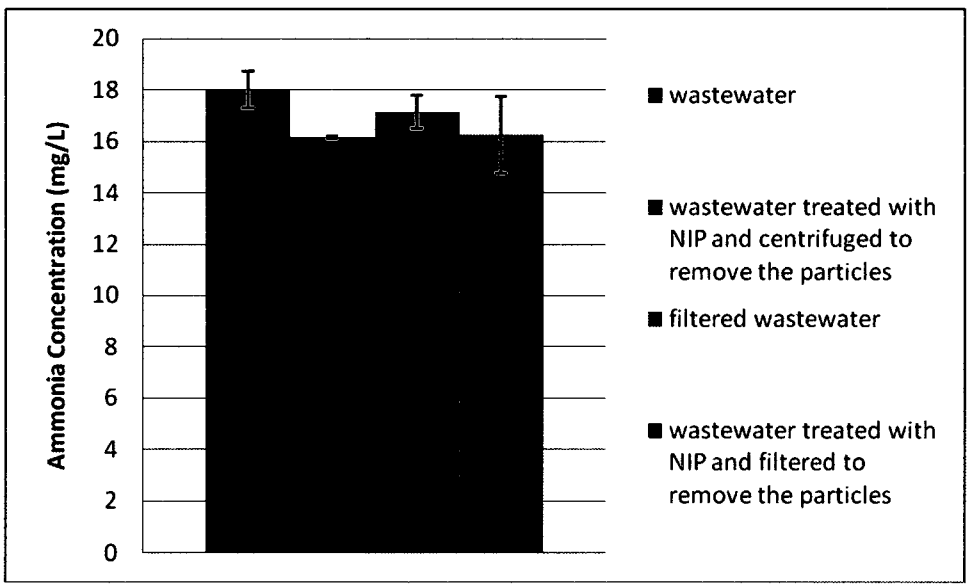

Figure 6-25: Effect of NIP treatment on wastewater ammonia concentrations

Figure 6-26 shows the effects of NIP treatment on the phosphate concentrations of the wastewater. The data shown are averages of two replicates and the error bars represent the standard deviation. From Figure 6-26, the measured phosphate concentrations appeared to

increase following treatment for both filter separation and centrifuge separation. However, it is likely that the increases were caused by increased sample absorbance rather than actual increases in phosphate concentrations. The trends observed in phosphate concentrations too closely resemble the absorbance trends to be considered accurate measurements of phosphate. More accurate measurements of phosphate concentrations by ion chromatography would be necessary to confirm whether or not NIP particles are capable of removing phosphate from wastewater.

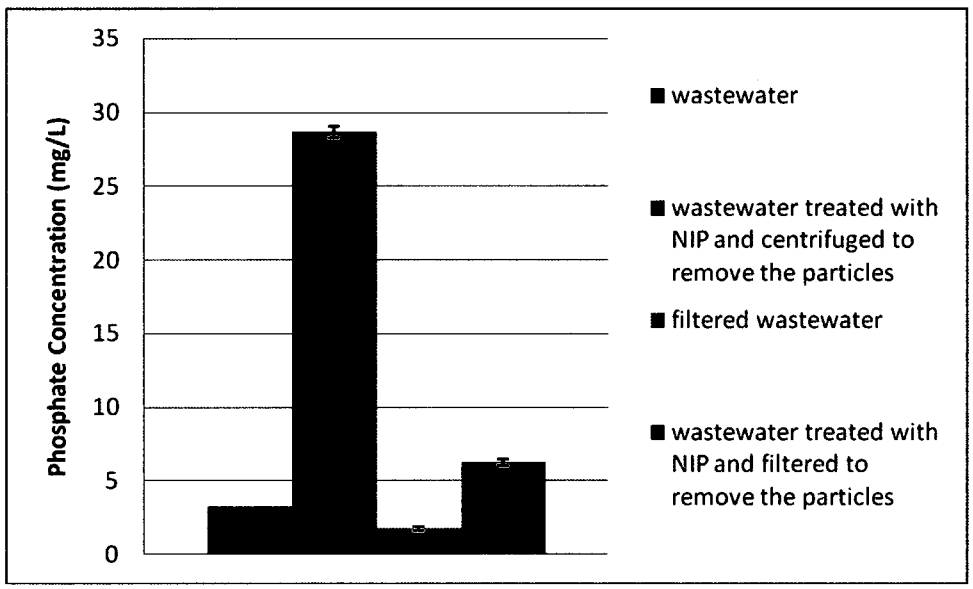

Figure 6-26: Effect of NIP treatment on wastewater phosphate concentrations

Figure 6-27 shows changes in total organic carbon (TOC) following treatment with NIP particles. Measurements were taken using a Shimadzu Total Organic Carbon Analyzer and were not the result of spectrophotmetric tests. Therefore the results were not influenced by the absorbance of the samples. The data shown are averages of three replicates and the error bars represent the standard deviation. The sample which was treated with NIP and then centrifuged showed a decreased TOC concentration in comparison with the sample which was not treated. Filtration of the sample appeared to have no effect on the TOC of the wastewater sample, but the

sample which was treated with NIP and then filtered had a lower TOC concentration that the sample for which the particles were removed by centrifugation. It is possible that the particles themselves contributed to the TOC concentration of the NIP treated and centrifuged sample.

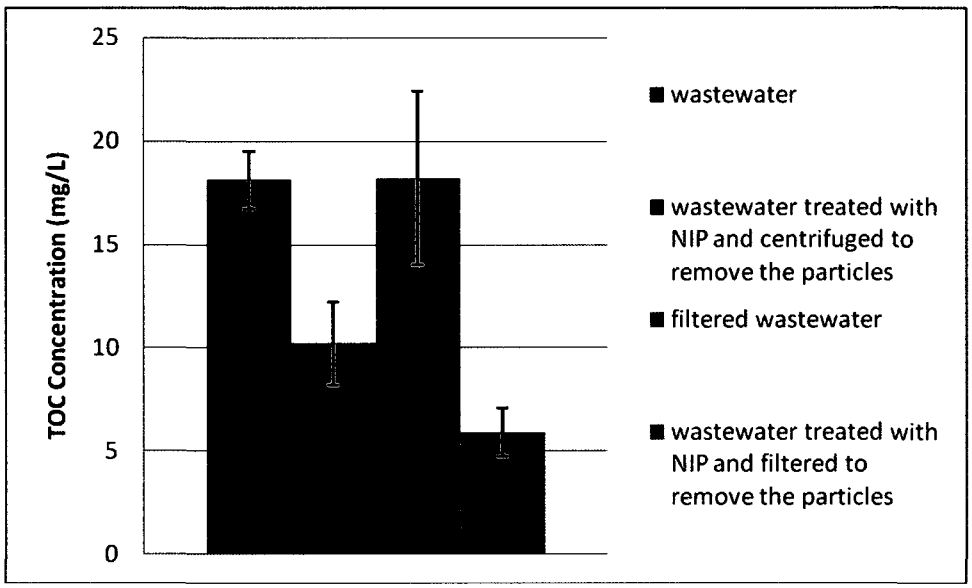

Figure 6-27: Effect of NIP treatment on wastewater TOC concentrations

Figure 6-28 shows the chemical oxygen demand (COD) for the wastewater alone, the wastewater treated with NIP particles and then centrifuged, the filtered wastewater, and the wastewater treated with NIP particles and then filtered. The results shown are averages of two replicates and the standard deviation is shown by the error bars. From Figure 6-28, there appears to be a decrease in COD between the wastewater sample and the wastewater sample which was treated with NIP and then centrifuged. However, due to the size of the standard deviation for both of these samples, it is difficult to tell if the decrease is significant or not. A decrease was also observed between the unfiltered and filtered wastewater samples, but the sample which was treated with NIP and then filtered had the same COD concentration as the filtered wastewater

sample. These results are unclear as to whether or not NIP treatment reduces the COD of a wastewater sample.

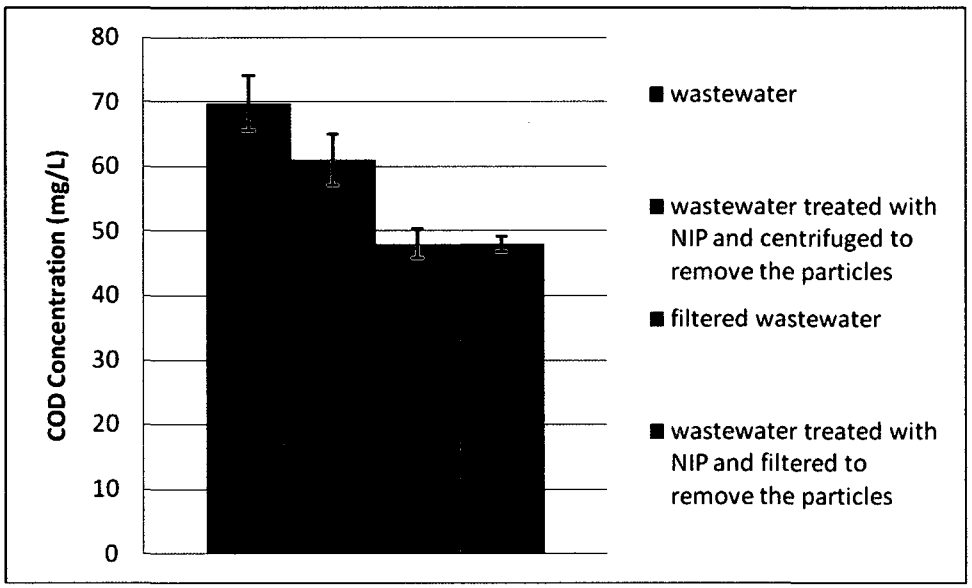

Figure 6-28: Effect of NIP treatment on wastewater COD concentrations

Overall, treatment with NIP particles was shown to increase both the absorbance and turbidity of the samples due to incomplete removal of the particles from the samples following treatment. Although removal of the particles by filtration was more effective than removal with the centrifuge, the efficiency was still not 100%. Decreases in ammonia and TOC concentrations were observed following treatment with NIP, which is beneficial for wastewater treatment applications. However, results investigating the removal of phosphate and COD were inconclusive and require further study with more accurate concentration measurement.

6.2.3.3 Application of NIP Particles for Removal of EDCs Following Disinfection with Chlorine

There are two possible points for application of NIP particles during water or wastewater treatment: before or after disinfection. After disinfection NIP particles can be used to remove

products of oxidized EDCs but also other disinfection by-products. The application of the particles to spiked samples is much easier to study for development purposes because the EDCs can be added to the samples in known concentrations and then removed following disinfection. The system is well defined and the peaks visible during HPLC analysis are easily identifiable as the spiked EDC. Even when surface or wastewater samples were used, the spiked concentrations were much higher than the concentrations of other peak-generating components in the samples, so there was no ambiguity as to the origin of the peaks.

Many studies have looked at the reduction of EDCs following chlorine oxidation. (American Waterworks Association Research Foundation, 2007; Benotti, Trenholm, Vanderford et al, 2009; Deborde et al, 2004). These studies all found high degrees of removal of most EDCs, although the extent of removal was dependent on the EDC and the design of the chlorination scheme. The majority of the studies on reduction of EDCs during chlorination simply measured the concentrations of the EDC of interest before and after chlorination. Chlorination is an oxidative process and it reduces the concentrations of the EDCs through oxidation. The chemical reactions lead to the formation of disinfection (or chlorination) by-products, which in many cases, are alternate EDCs. The ability of these daughter compounds to induce a biological response in humans or wildlife is not necessarily less than that of the parent compounds. Therefore, these studies do not determine whether or not chlorination makes the water safer, in terms of estrogenicity, but merely measure the decrease in the concentration of the parent compound. Korshin et al (2006) for example, found that the risk posed by bisphenol-A initially increased following chlorination before decreasing below the initial level (Korshin et al, 2006).

Lee et al (2004) studied the decrease in estrogenicity of samples containing E2, nonylphenol, or bisphenol-A and found an overall decrease in estrogenicity although they also

observed an initial increase in estrogenicity for bisphenol-A. Overall estrogenicity studies simplify the process of evaluating the effectiveness of chlorine for treatment of EDCs because they eliminate the need to identify and measure daughter products but instead measure the overall estrogenicity of samples.

Some work has been done recently to identify the daughter products of chlorination of EDCs. This is a complicated task which must be done for each natural water composition and disinfection scheme separately because many daughter products are intermediate products and the composition of the final solution is dependent on many factors such as the chlorine concentration and contact time. Hu et al (2003) identified seven daughter products for thechlorine oxidation of E2 in deionized water. These products are shown in Figure 6-29. All of these EDCs exhibited estrogenic activities lower than that of E2, but all exhibited some degree of estrogenicity. This means that measuring just E2 would have lead to an over-estimation of the decrease in estrogenicity, although a decrease was still apparent. The percent composition of these daughter products varies with chlorine dose and contact time and will change during the disinfection period (Hu et al, 2003).

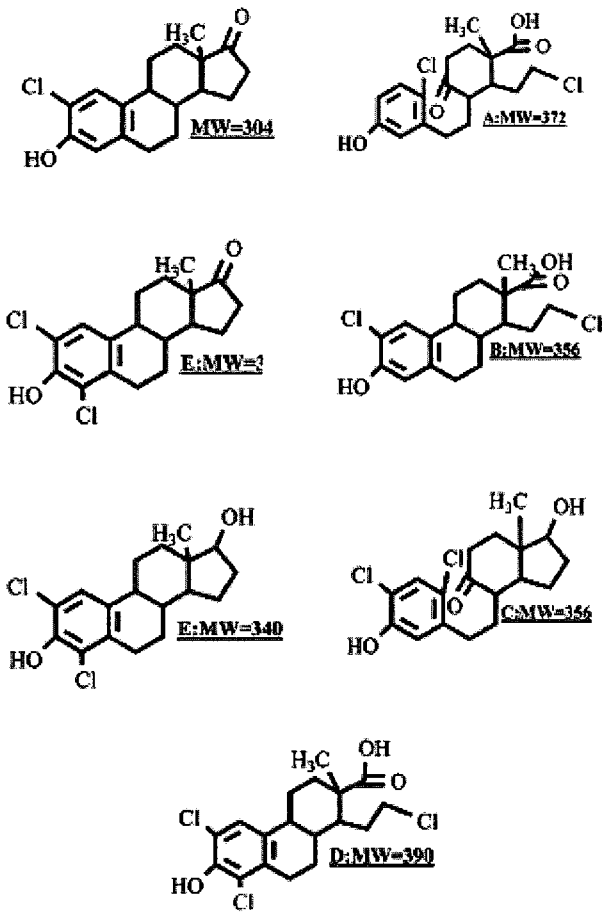

Figure 6-29: Daughter products of chlorine oxidation of E2 (Hu et al, 2003)

Chlorination experiments were carried out in deionized water samples chlorinated with 5mg/L and 10mg/L of chlorine. Experiments were also conducted both in distilled-deionized water and in distilled-deionized water with the addition of humic acid, to simulate surface water. The distilled-deionized water should have produced daughter products similar to those noted by Hu et al (2003) but the addition of humic acid would have led to the creation of different daughter products. Identification of the specific daughter compounds was not within the scope of

this work. Removal of the parent and daughter compounds after NIP application was evaluated based on the reduction in the HPLC peaks.

The first experiment was the treatment of 10 ppm E2 in distilled-deionized water first with chlorination and followed by NIP particles. The results are shown in Figure 6-30. The chromatograms shown are averages of two replicates, but error bars were not shown for clarity. There is a peak for the untreated, spiked E2 sample. This peak then decreased following chlorination. It is unclear whether this peak is E2 or whether there are daughter products contributing, but it is likely that at least part of this peak is due to E2. A second peak also became visible prior to the initial peak at a retention time of 1.2 +/- 0.1 minutes. This peak is likely to be due to a disinfection by-product, and should be removed from the water. The sample was then treated with 5mg/mL and 10 mg/mL of NIP and complete removal of both of these peaks occurred.

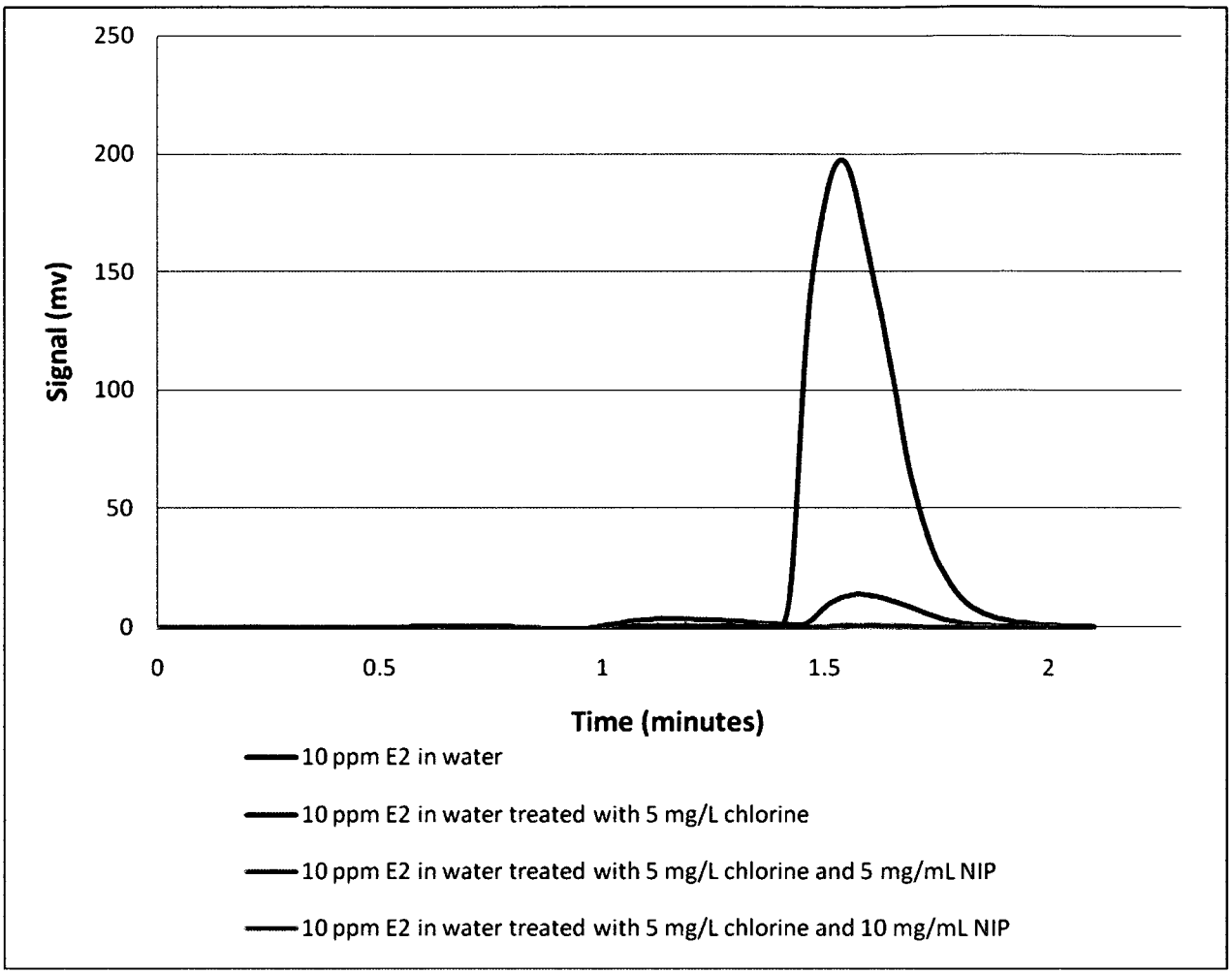

Figure 6-30: Treatment of 10 ppm E2 with 5mg/L chlorine and NIP particles

For the second set of experiments, humic acid was added to the samples together with 10 ppm E2. The samples were then again chlorinated with 5 mg/L and 10 mg/L of chlorine. The chromatograms shown in Figure 6-31 are averages of two replicates but error bars were not shown for clarity. The larger peak shown represents the spiked concentration of 10 ppm E2 into distilled-deionized water containing humic acid. The peak size then decreased with the addition of chlorine. The presence of humic acid decreased the efficiency of chlorine oxidation of E2. Small peaks were still visible following the application of 5mg/mL of NIP particles, although they completely disappeared with the application of 10 mg/mL of particles.

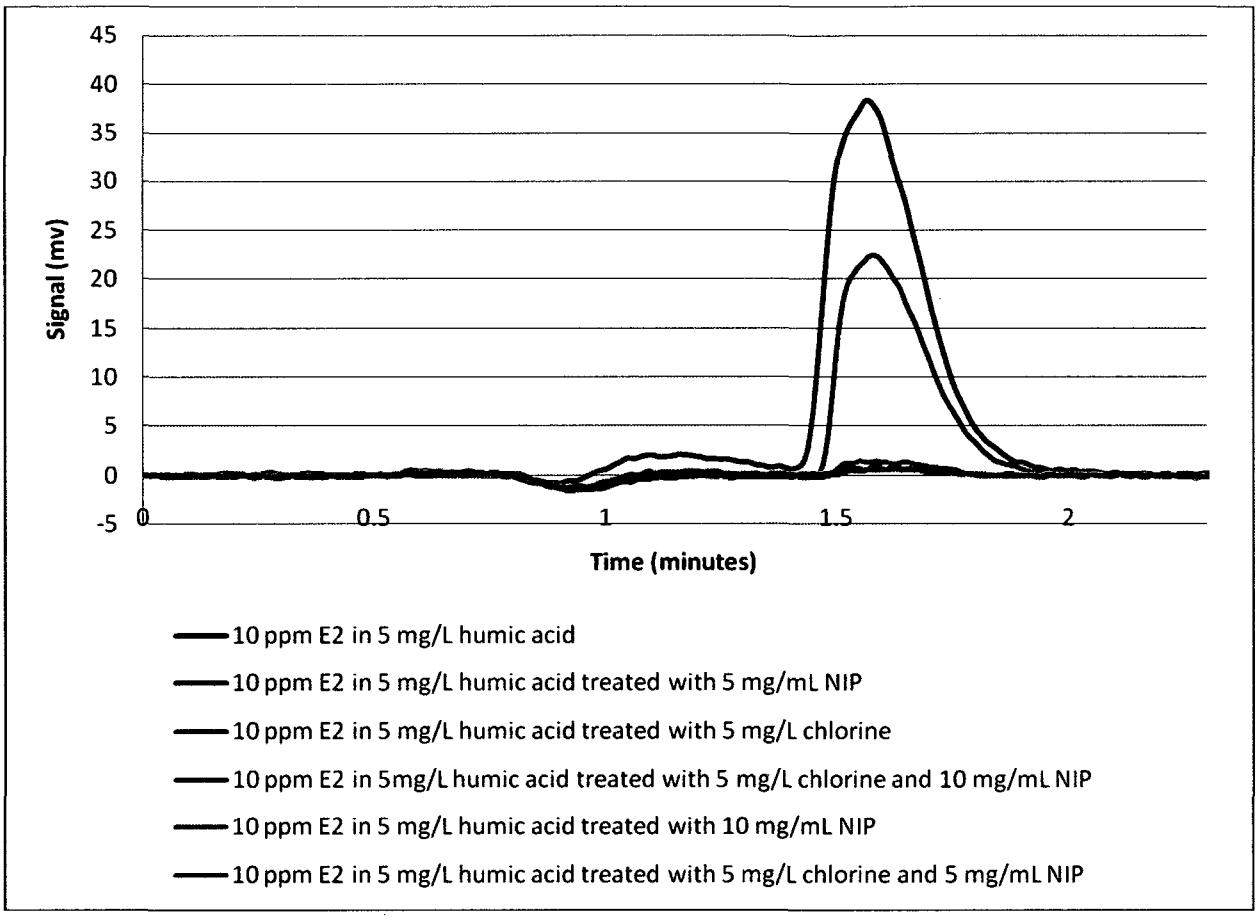

Figure 6-31: Treatment of 10 ppm E2 in distilled-deionized water with humic acid added with 5mg/L chlorine and NIP particles

Figure 6-32 shows the treatment of distilled-deionized water containing 10 ppm E2 with 10mg/L of chlorine. The chromatograms shown are averages of two replicates although error bars are not shown for clarity. The initial large peak shown shows spiked E2 in distilled-deionized water. Following chlorination with 10 mg/L of chlorine, the initial E2 peak appeared to have completely disappeared. A second peak, visible in Figure 6-30 at a retention time of 1.2 +/- 0.1 minutes, increased in size as compared to that shown in Figure 6-30 for treatment with 5 mg/L of chlorine, indicating a greater conversion of E2 to daughter products with the higher

concentration of chlorine. NIP particle concentrations of both 5 and 10 mg/mL completely eliminated the peaks.

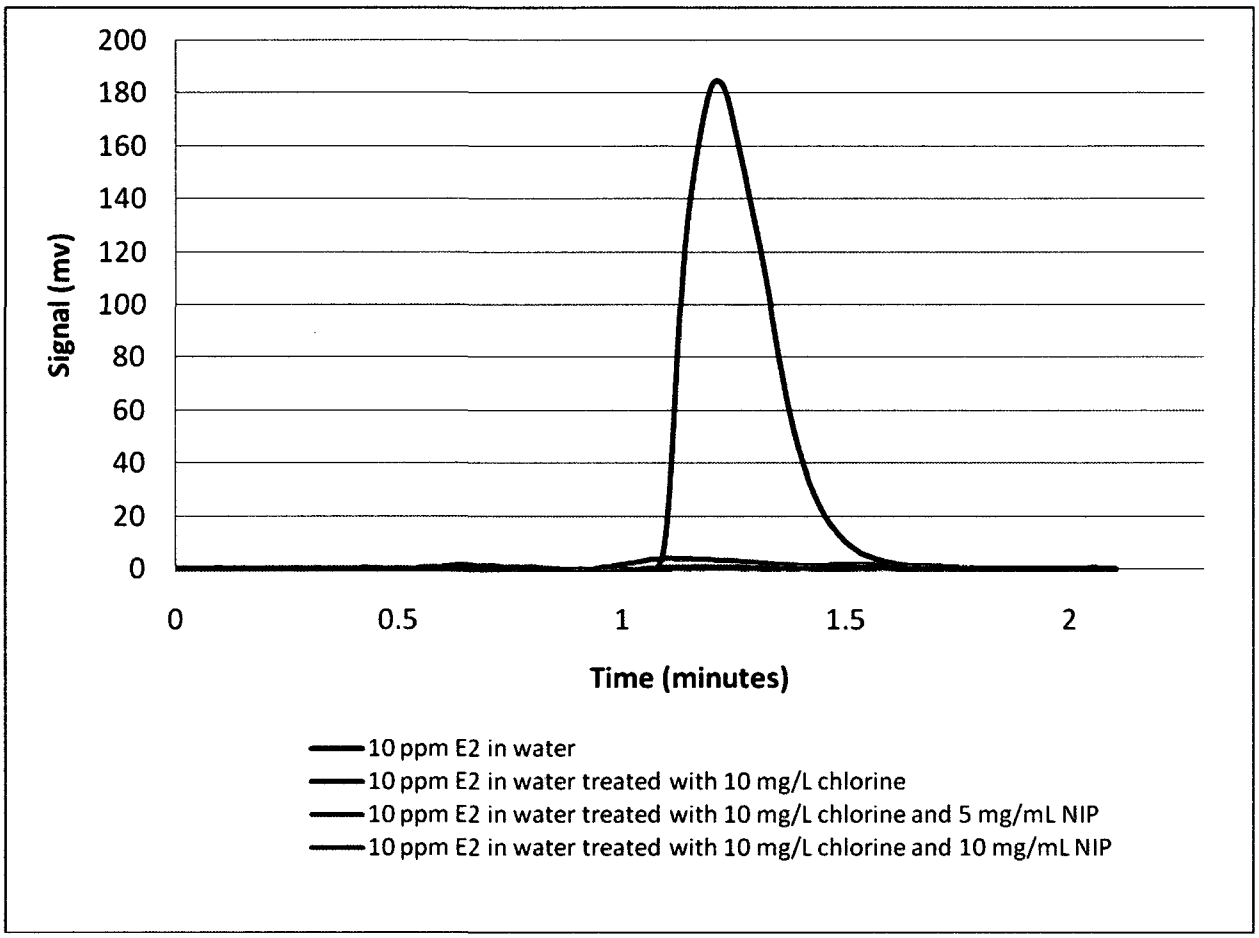

Figure 6-32: Treatment of 10 ppm E2 with 10mg/L chlorine and NIP particles

The last set of experiments involved the treatment of 10 ppm E2 in a solution of distilled-deionized water and humic acid with chlorine. The results are shown in Figure 6-33. The chromatograms shown are averages of two replicates although error bars are not shown for clarity. Humic acid did not decrease the efficiency of chlorine for removal of E2. The small peak visible was completely removed following the application of 5 or 10 mg/L of NIP particles.

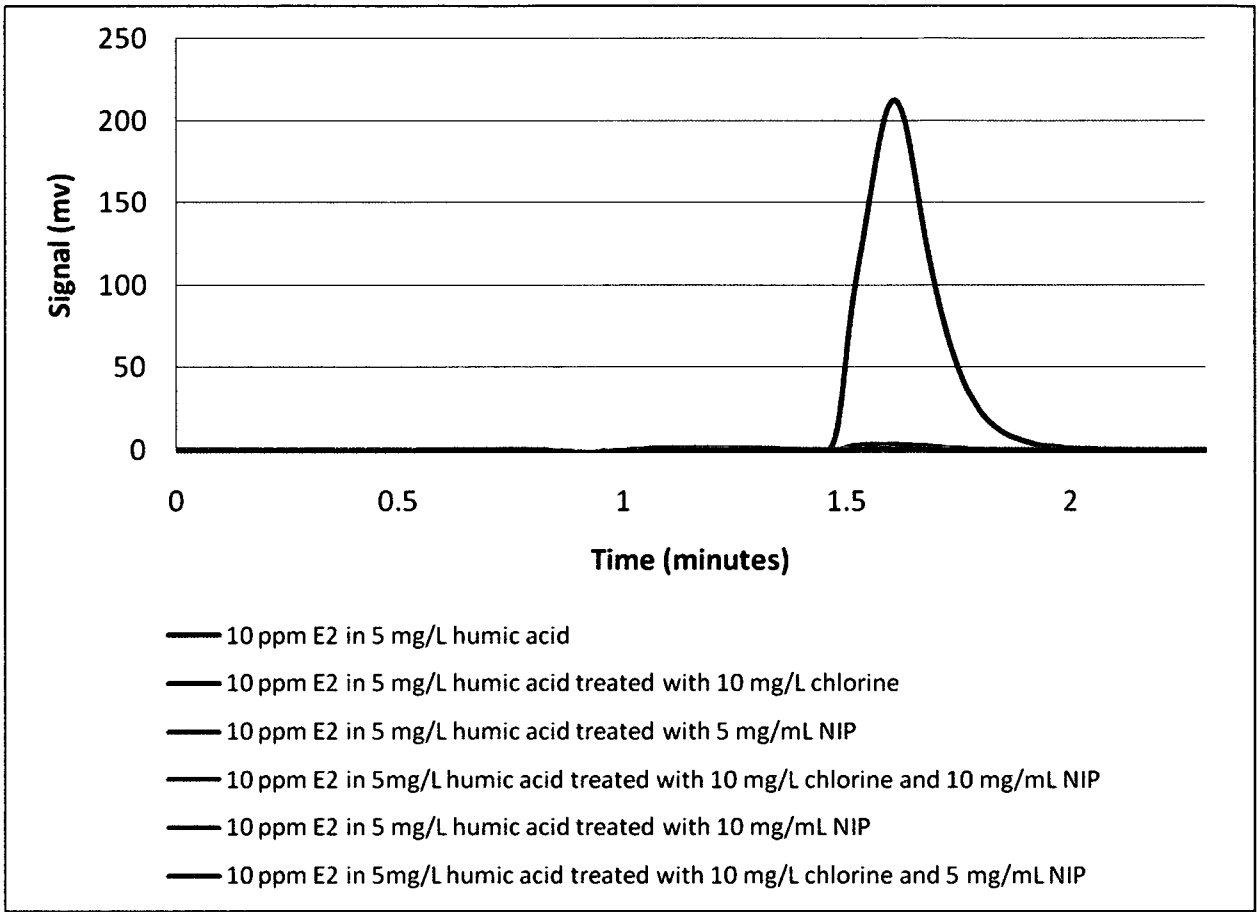

Figure 6-33: Treatment of 10 ppm E2 in distilled-deionized water with humic acid added with 10mg/L chlorine and NIP particles

6.2.3.3.1 Measurement of Chlorine Residuals Following Chlorine Oxidation of E2

Chlorine residuals were measured immediately following the chlorine reaction time and before the addition of sodium thiosulfate to stop the chlorination reactions. Chlorine residuals can be used as an indication of the amount of chlorine which was consumed during the chlorination reactions. Table 6-2 shows the results for free and total chlorine residuals measured following chlorination. Two replicates were taken for each measurement and the standard deviations are given. The table shows increasing chlorine demand for increasing concentrations of oxidizable materials. For example, the free and total chlorine residuals for the combination of

E2 and humic acid were much smaller than the residuals for E2 alone in distilled-deionized water. This is because the humic acid also exerted a chlorine demand on the solution, as can be seen by the free and total chlorine residual measurements for humic acid alone in distilled-deionized water.

Table 6-2: Free and total chlorine residuals following chlorination of E2 solutions with and without humic acid

	5 mg/L chlorine		10 mg/L chlorine	
	Average Concentration	*Standard Deviation*	*Average Concentration*	*Standard Deviation*
Chlorine and E2 in distilled-deionized water				
Free chlorine	0.97	0.007	3.78	0
Total Chlorine	1.36	0.007	>4	N/A
Chlorine and humic acid in distilled-deionized water				
Free chlorine	0.74	0.007	2.47	0.007
Total Chlorine	0.7	0	2.45	0.007
Chlorine, humic acid, and E2 in distilled-deionized water				
Free chlorine	0.03	0.007	0.2	0
Total Chlorine	0.16	0	0.28	0

The results of the chlorination study shown in Figure 6-30 to Figure 6-33 indicate good potential for application of NIP particles following disinfection. Deborde et al (2004) showed that the half lives for estrone, estradiol, β-estradiol, and 17 α ethinylestradiol were all between

6.3 and 7.3 minutes when treated with 1 mg/L of chlorine. This indicates that chlorine is efficient for the removal of EDCs from water. However, disinfection by-products and oxidized products of EDCs, although they may be present in lower concentrations than the initial EDC, can have more of an effect on the water. For example, Korshin et al (2006) found that the organic halogen concentration of a solution of bisphenol-A increased initially upon chlorination. For this reason, it is not sufficient to oxidize the EDCs, measure the concentration of the parent compound and conclude that satisfactory reduction has occurred. It is important also to monitor the formation of daughter products and to remove those daughter products from solution.

Although the daughter products in this study could not be identified, at least one daughter product was observed upon oxidation of E2 with chlorine, as indicated the formation of a new peak at a different retention time. NIP particles were then applied to completely eliminate both the peaks caused by the parent compound and the daughter product. It is not possible to conclude that all of the daughter compounds produced were eliminated because the daughter compounds were not measured and it is possible that there were daughter products produced which were not visible with HPLC-FD analysis, but this experiment does indicate that there is good potential for removal of the by-products of E2 oxidation using NIP particles. Application of NIP particles following disinfection may also have additional advantages because they may also remove other disinfection by-products following chlorination. This is a potential area for future study.

Chlorination and advanced oxidation have both been studied for their potential to remove EDCs from water. Treatment with chlorine or advanced oxidants functions by the oxidation of EDCs. This presents a disadvantage because the EDCs are chemically oxidized but are not completely removed from the water. Daughter products are formed through the oxidation of the EDCs and these daughter products have the potential to be more harmful in some cases than the

parent compounds (Korshin, et al 2006). NIP particles have an advantage over advanced oxidation because EDCs are physically removed from the water and no daughter products are created.

6.3 Phase 3: Removal of NIP Particles Following Water Treatment

One major consideration for the use of NIP particles for treatment of drinking water or wastewater is the removal of the particles from the treated water. Coagulation, centrifugation, and a column or filtration application of the particles were all investigated for this purpose. The initial particle concentration was 1 mg of particles per mL of solution for all experiments.

6.3.1 Removal of NIP Particles by Coagulation

Since the NIP particles were known to be negatively charged based on capillary electrophoresis work done by other members of the research group (Zackery De Maleki, unpublished work) several cationic coagulants were tested for their removal. A cationic polymer FloPolymer CA 4600, calcium chloride, and hydrochloric acid were all tested. These coagulants were tested because they all provided positive charges or positively charged ions to neutralize the negative surface charge of the particles. Once the negative surface charges of the particles are neutralized, the particles will no longer repulse each other and will be capable of flocculation and sedimentation and thus removal from the solution. Turbidity and absorbance measurements were taken for each applied dose and the amount of coagulant required and overall reduction in turbidity were evaluated.

Figure 6-34 shows the results of coagulation experiments conducted with FloPolymer CA 4600. Figure 6-34 provides the absorbance and turbidity for varying polymer doses. The data points shown are averages of three replicates and the error bars represent the standard deviation.

FloPolymer CA 4600 was effective for the removal of particles from solution. However, relatively large concentrations of the polymer were required for optimum removal and the consistency of the water changed. The optimum dose for the polymer, or the dose leading to the lowest turbidity and absorbance, was 0.05 mg of polymer/ g of NIP particles. Since there was 1 mg of particles per mL of water, this is equivalent to 0.05 mg of polymer/ L of solution. The water developed a gel-like consistency similar to the polymer itself and the particles were no longer capable of settling. From Figure 6-34, an 80% decrease in absorbance occurred with the application of the polymer. The turbidity did not decrease as extensively because the particles were trapped in the polymer in small flocs, creating turbidity. However the areas which did not contain these small flocs were relatively clear and thus had relatively low absorbance. Thus, the turbidity and absorbance measurements did not mirror each other.

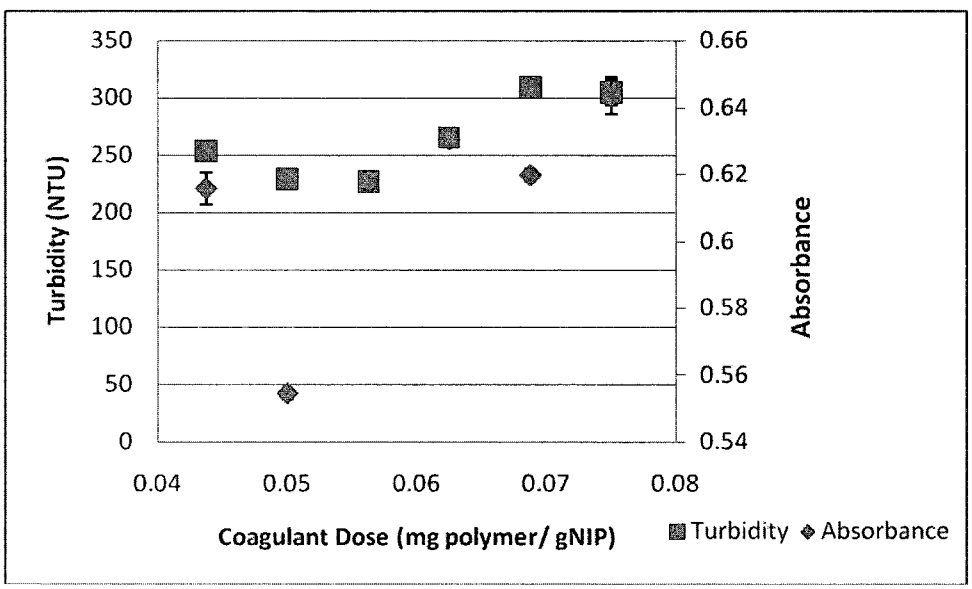

Figure 6-34: Removal efficiencies for varying coagulant doses for removal of NIP particles with cationic polymer FloPolymer CA 4600

Figure 6-35 shows the results for NIP coagulation using calcium chloride. The data points show both the turbidity and absorbance for varying calcium chloride doses. The points represent the average of three replicates and the error bars represent the standard deviation. For the calcium chloride, a decrease of just over 60% was possible (Figure 6-35) although it should be noted that the calcium chloride addition was very large and approached the solubility limit for calcium chloride. The optimum dose for calcium chloride was 0.3 mol/L of solution. This concentration represents the final concentration following dilution by addition to the solution.

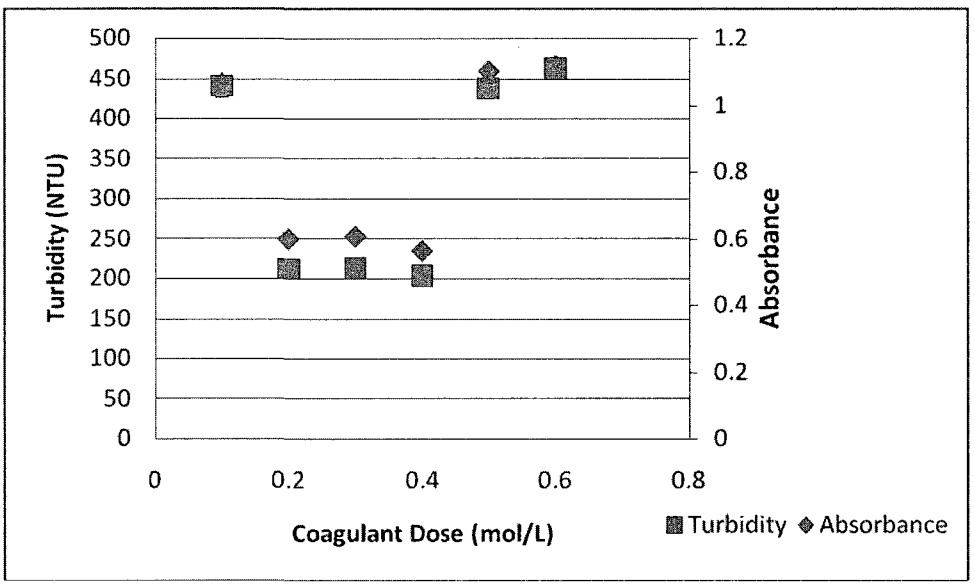

Figure 6-35: Removal efficiencies for varying coagulant doses for removal of NIP particles with calcium chloride

Figure 6-36 shows the use of acid or pH changes for coagulation of the NIP particles. Acid is not typically used as a coagulant for water treatment because divalent cations are more effective. However, because of the high doses of FloPolymer CA 4600 and calcium chloride required, acid was suggested because any changes to the composition of the water could be reversed following removal of the particles. Figure 6-36 shows the absorbance and turbidity

measurements for varying levels of acid addition. The data points represent the averages of three replicates and error bars represent the standard deviations. From the figure, increasing acidity led to increased removal of the particles and the particle concentrations continued to decrease with decreasing pH to a pH of zero, which was the lowest pH value tested.

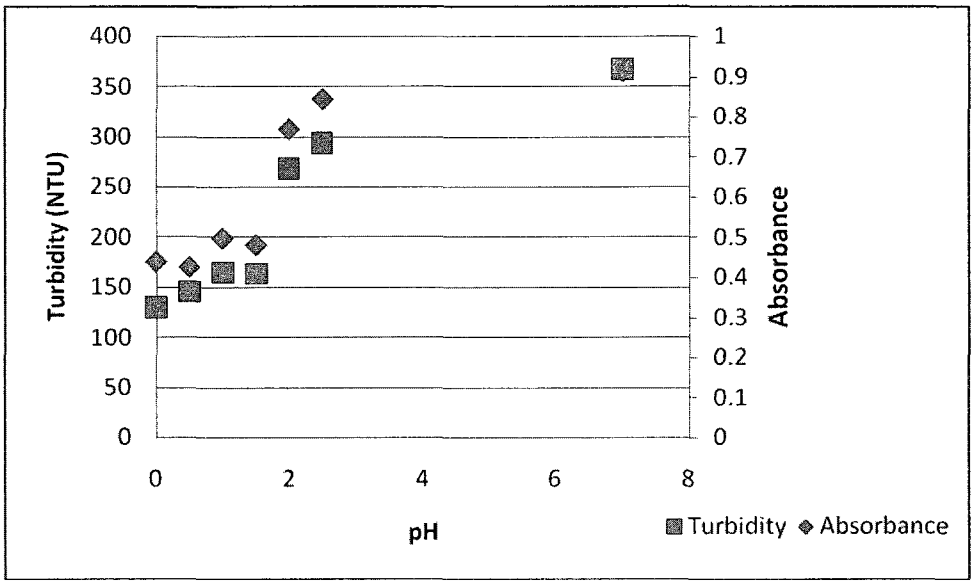

Figure 6-36: Removal efficiencies for varying pH values for removal of NIP particles with hydrochloric acid

Although coagulation was somewhat effective for the removal of NIP particles from solution and use of a cationic polymer, FloPolymer CA 4600, led to 80% removal of the particles from solution, the feasibility of coagulation with FloPolymer CA 4600 for removal of the particles from solution appears to be low. This is because large coagulant doses were required to treat the nanoparticles. These large coagulant doses are required because of the high surface area of the nanoparticles requiring neutralization. However, it is possible that a different polymer or coagulant would be more efficient for removal of the particles, and further testing is required.

6.3.2 Removal of NIP Particles Following Water Treatment with Centrifugation

Centrifugation, or other mechanical means of separation, provides an alternative to chemical separation. Two different centrifuges were compared for different centrifuge times for removal of the particles from solution. 1 mg/mL particle solutions were prepared and dewatered using two different centrifuges with different maximum speeds and maximum centrifugal forces. The first centrifuge used was a Revolutionary Science Microcentrifuge. It was used at a speed of 10,000 RPM and applied a centrifugal force of 6238 RCF. The second centrifuge used was a Hamilton Bell Van Guard V 6500 and was applied at a speed of 3400 RPM and with a centrifugal force of 1318 RCF.

Figure 6-38 shows the results of centrifugation with the Revolutionary Science Microcentrifuge. Absorbance and turbidity measurements were taken for increasing centrifuge times to provide a measure for the efficiency of the centrifuge. The data shown represents averages of three replicates and error bars are representative of the standard deviations. The removal efficiency increased for increasing centrifuge times between 1 and 5 minutes. For a centrifuge time of 5 minutes, it was possible to achieve 70% removal of the particles. However, because the increases in efficiency became less pronounced it is possible to determine an optimum centrifuge time based on the required efficiency and treatment costs for increased centrifuge time.

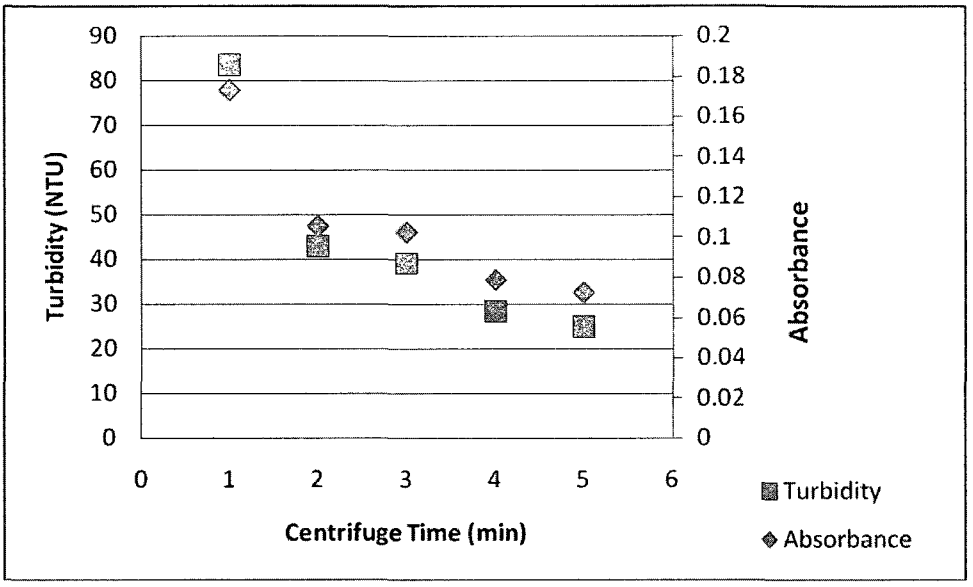

Figure 6-37: Removal of NIP particles with a Revolutionary Science Microcentrifuge for varying centrifuge times

Figure 6-38 shows the results of tests conducted with the Hamilton Bell Van Guard V 6500 centrifuge which had an applied at a speed of 3400 RPM and a centrifugal force of 1318 RCF. This centrifuge required a longer centrifuge time than the Revolutionary Science Microcentrifuge but was capable of treating larger sample volumes. Figure 6-33 shows absorbance and turbidity measurements for increasing centrifuge times. The data points shown are averages of three replicates and the error bars represent the standard deviation. Removal increased with increasing centrifuge times for all of the times tested (10-60 minutes). The centrifuge was capable of removing 90% of the NIP particles from solution with a 50 minute centrifuge time, and could achieve 69% removal after 30 minutes, which is equivalent to treatment for 5 minutes with the Revolutionary Science Centrifuge.

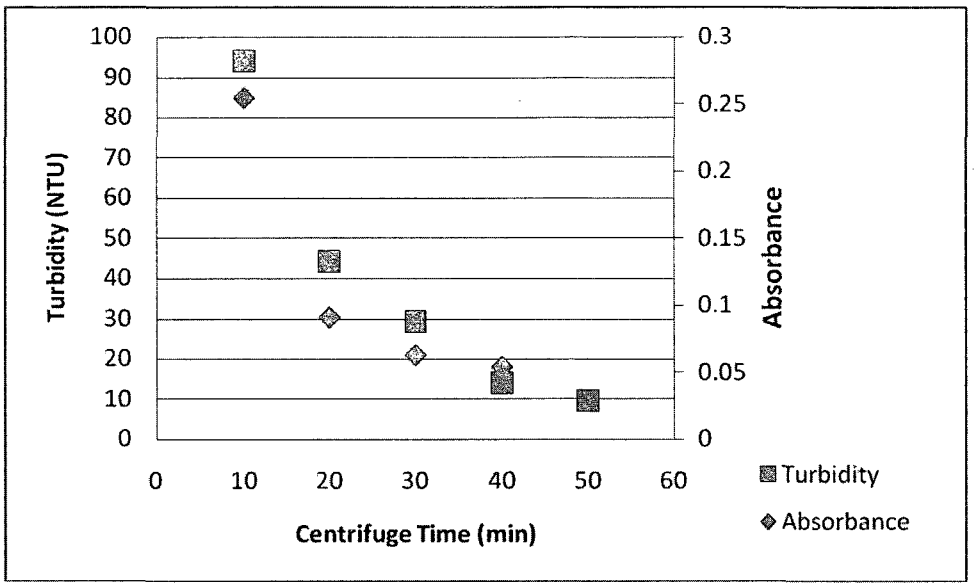

Figure 6-38: Removal of NIP particles with a Hamilton Bell Van Guard V 6500 centrifuge for varying centrifuge times

Overall centrifugation was shown to be applicable for the removal of NIP particles from water following treatment. Centrifugation for only 5 minutes led to 70% removal of NIP particles using the Revolutionary Science Microcentrifuge and centrifugation for 50 minutes led to 90% removal using the Hamilton Bell Centrifuge. Mechanical separation appears to be more applicable than coagulation for removal of the NIP particles because it does not alter the composition of the water and no chemical additions are required.

6.3.2.1 Construction of a Bench Scale Column for Removal of EDCs from Water or Wastewater

One possible application of NIP for water or wastewater treatment is in the form of a column. Columns provide both a mechanical means for separation of the particles and a treatment scheme for treatment of the water. A bench scale column was constructed to determine how the treatment efficiency changed with this type of application. The column consisted of a

200 nm syringe filter packed with NIP particles. Since the particles are nanoparticles, filters required for the column must be quite small, which is why a syringe filter was used for bench-scale testing.

6.3.2.1.1 Estimation of the Binding Capacity of NIP Particles using the Scatchard Equation

In an attempt to determine as much information as possible from the column study, the intention of the study was to filter E2 through the NIP filled filter until it reached its binding capacity. Unfortunately, although the micropump used could handle substantial backpressure, it was not capable of handling the backpressure created by the NIP filled filter for more than 35 minutes without overheating, so the capacity of the filter was not reached.

The binding capacity of the NIP was calculated based on previous results using the Scattchard equation shown below. The Scattchard equation is commonly used to model MIP because it is a good model for adsorption systems with many classes of binding sites (Garcia-Calzon and Diaz-Garcia, 2007). The equation was initially developed to model the binding of anions to albumin because the albumin contained three different types of binding sites. The Scatchard equation was developed to quantify the number of each type of site (Scatchard et al, 1957).

$$\frac{n}{[E_2]} = (NK_a - nK_a)$$

where

n = bound E2 for corresponding E2 concentrations ([E2])

Ka = the association constant

N= the number of binding sites (expressed as mg E2/ g particles)

Data from the first phase of experiments, shown in Figure 6-1 for the binding of E2 onto NIP, was used for the Scatchard Equation estimation as shown in. From this linearization, N was determined to be 6 mg E2/ g NIP. From this, use of 8 mg of NIP would require 0.048 mg of E2 to reach the binding capacity. The flow rate of the column was approximately 1L/h. Since the binding efficiency was not 100% and the column was only capable of treating operating for 35 minutes, a concentration of 0.2 mg/L of E2 was used. This is four times the capacity of the particles so that the capacity of the particles would be reached within half an hour but would not be reached too quickly to see an adsorption curve. Unfortunately, due to problems with the pump filtration system, the full liter of solution was not filtered and the column did not reach its capacity.

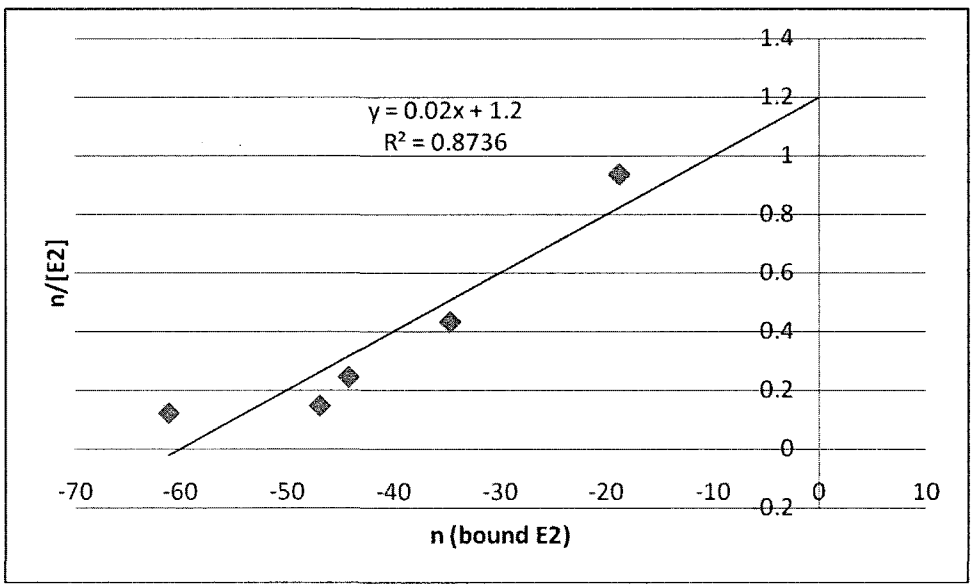

Figure 6-39: Scatchard Equation linearization of binding efficiencies for the determination of the number of binding sites

6.3.2.1.2 Performance of the Bench-Scale Column for Removal of E2 from Distilled-Deionized Water

Figure 6-40, shows the removal efficiency with time through the bench-scale column. The bench-scale column was identified as a column because the water was able to flow through the particles which were retained. However, the column consisted simply of particles which were pre-loaded onto a 200 nm syringe filter. The data points represent the averages of two replicates and the error bars show the standard deviation. The binding efficiency was lower than for the 10 minute reaction time provided in the batch scale tests. However, the efficiency was still high considering that additional removal would also occur elsewhere in a water or wastewater treatment plant. The binding efficiency continued to decrease throughout the 35 minute testing time.

Backpressure was a major problem for the bench-scale column tests. The polyethersulfone (PES) filters were first tested. However, the filters were not able to withstand the backpressure. Sonication was then added to allow the particles to remain suspended within the syringe filter and to reduce the pressure acting on the surface of the filters. However, the sonication caused quick disintegration of the syringe filter membrane. Masselin et al (2001) found that PES membranes disintegrated in the presence of sonication. The results shown in Figure 42 are from the test conducted with the PES membrane which disintegrated. For this reason, the data was not used for further analysis because its integrity could not be guaranteed.

Nylon syringe filters were also tested for use in the bench-scale column. Although no work had been done with nylon membranes and their capacity to withstand sonication, nylon was anticipated to be stronger than PES and more capable of withstanding the pressures encountered in the bench-scale set-up. The nylon filters did not show any evidence of tearing in the column.

However, the back-pressure was too great and water could not be passed through the column without causing damage to the pump. The bench-scale column was abandoned.

Since the NIP particles are nanoparticles backpressure is a major consideration for water treatment applications. This has also been considered by other researchers and Le Noir et al (2007) used macroporous gels to decrease the backpressure while still providing treatment as one solution.

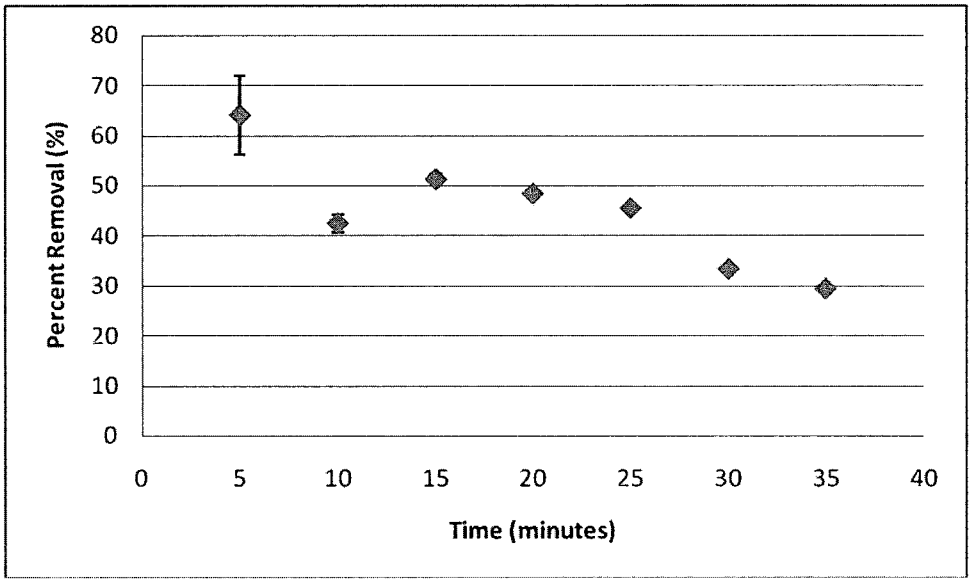

Figure 6-40: Percent removal with time for the bench-scale column

7 CONCLUSIONS

The premise of the study was to evaluate the use of molecularly imprinted (MIP) and non-imprinted polymers (NIP) both for analytical preconcentration purposes and for the removal of EDCs during water and wastewater treatment.

Both MIP and NIP polymers were evaluated for their ability to pre-concentrate E2 and EE2 from solution. Preconcentration onto MIP was found to be ineffective due to incomplete removal of the template from the MIP prior to use. Desorption of the E2 and EE2 from the particles for analytical analysis was also insufficient. The use of a triethylamine (TEA) solution created in a 1:40 mixture of methanol and distilled-deionized water (DDW) may have been the cause of this and future work should be conducted using TEA solutions prepared in methanol to allow greater solubility of the E2. Preconcentration using NIP was also found to be insufficiently efficient, and the use of a desorption agent prepared in methanol was also suggested for NIP.

NIP was shown to be more efficient for removal of both E2 and EE2 from DDW than MIP. This is advantageous for water treatment applications because NIP particles can be used to remove a wide variety of EDCs from contaminated water. NIP was effective for the removal of E2, EE2, atrazine, bisphenol-A, and diethylstilbestrol from DDW. This indicates good potential for application of NIP to remove a wide range of EDCs during water or wastewater treatment.

The NIP particles were then tested for their ability to remove unknown contaminants from both surface and wastewater (secondary effluent) samples. The NIP particles were effective for the removal of these contaminants.

To test the ability of the particles to remove E2 from wastewater which contains interferences not found in DDW, wastewater samples were spiked with E2 and the removal was

tested. Removal was found to be equally efficient for wastewater and DDW for lower concentrations, but much less efficient at higher concentrations. This may have been due to the effect of methanol which was added to increase the solubility of the E2 at higher concentrations, however, the effect of the methanol was much stronger for the wastewater sample than for the DDW sample.

The NIP particles were also capable of removing ammonia, TOC, and COD from wastewater samples. The removal of these contaminants was not complete or sufficient for treatment, but some removal was observed, which is an aid to treatment. These tests also indicate that there was some adsorption of interfering species occurring during treatment.

Chlorine disinfection followed by treatment with NIP particles was shown to be more efficient than treatment prior to disinfection. Although this was a preliminary study, treatment following disinfection appeared to be more efficient than treatment prior to disinfection. This study was not conclusive because the analytical means to identify the disinfection byproducts and quantitatively measure their removal from solution following treatment with NIP were not available.

Chemical coagulation was shown to be ineffective for the removal of the particles from water following treatment. The chemical doses required were too high, and the efficiency of removal was not high enough for water treatment applications. However, further testing of different coagulants could prove to be successful. Physical separation was shown to be more effective for the removal of the particles and two different centrifuges were shown to be effective for removal. Incorporation of the particles into a column design is also an option for treatment that would eliminate the need for removal of the particles from water following treatment.

However, because the particles are nanoparticles, the pressure required to filter water through the column is significant and the use of a column may not be feasible.

8 FUTURE WORK

- Analytical preconcentration of E2 onto MIP particles created with a specific E2 template requires further developmental work. Future work should focus on complete removal of the template from the MIP, removal of bound E2 during analysis, and separation of E2 from other interfering compounds.

- The applicability of NIP particles for the removal of a variety of EDCs from DDW, surface water, and secondary wastewater samples appears to be good. However, this needs to be studied in greater detail. Primarily to determine:

    - The effectiveness of removal at concentrations found in typical surface water or wastewater effluent. This study could be aided by advancements in analytical methods in the future, or even by the development of an analytical preconcentration method using MIP particles.

    - Competitive effects which would be encountererd treating water samples with several different EDCs. Chromatographic separation of the EDCs studied during HPLC analysis would be necessary to accomplish this goal.

- The applicability of NIP particles for the removal of disinfection by-products following chlorination of water samples containing EDCs should be studied in greater detail. Future goals of this study would include:

    - The identification and quantitative analysis of each of the disinfection by-products prior to and following treatment with the NIP particles.

- The effects of different contact times and chlorine doses should be studied in greater detail.

- The use of NIP particles for the removal of trace contaminants such as hydrocarbons or pharmaceutical agents other than EDCs in drinking water or wastewater can also be investigated.

- Enhanced removal and recovery of the particles from solution following treatment is a priority for future studies.

9 REFERENCES

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10 APPENDIX A: RAW DATA CHROMATOGRAMS

11

11.1 Preconcentration of E2 onto MIP Particles

11.1.1 Calibration Curve in DDW

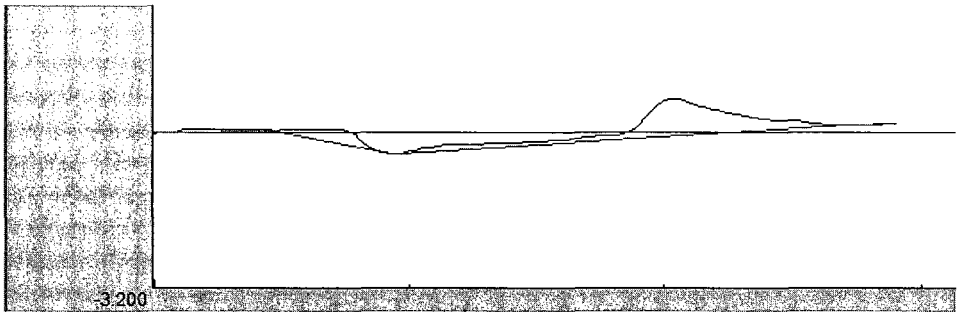

Figure 41: Calibration Curve 0.2 ppm E2 in DDW (Replicate 1)

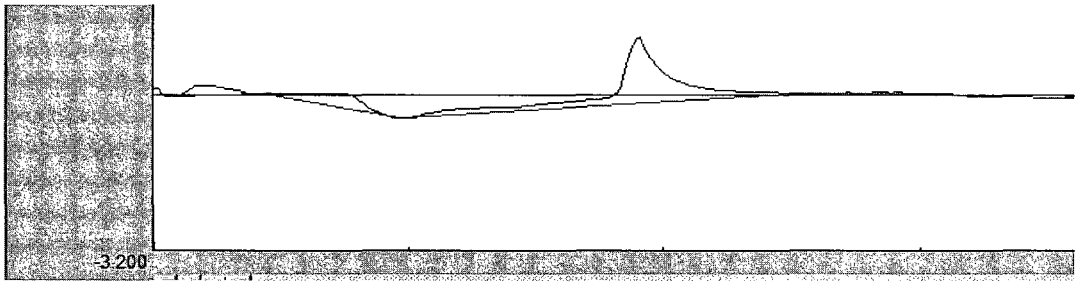

Figure 42: Calibration Curve 0.2 ppm E2 in DDW (Replicate 2)

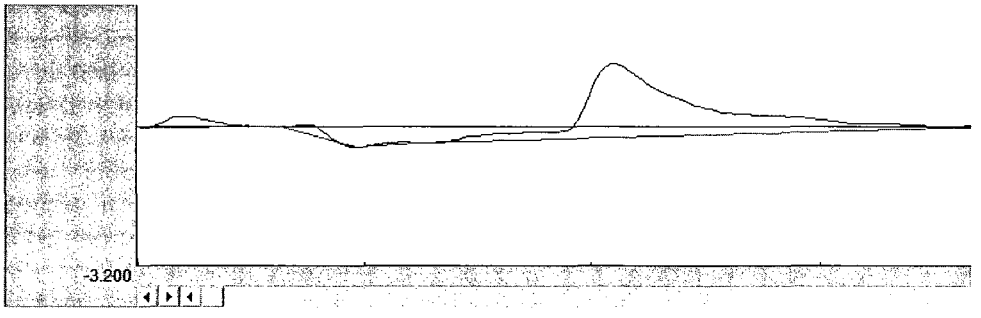

Figure 43: Calibration Curve 0.4 ppm E2 in DDW (Replicate 1)

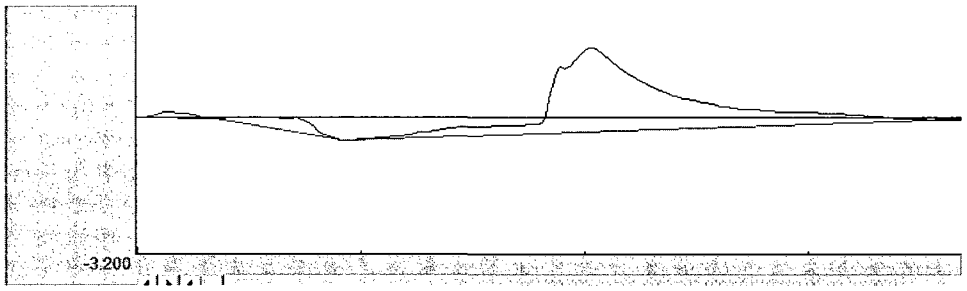

Figure 44: Calibration Curve 0.4 ppm E2 in DDW (Replicate 2)

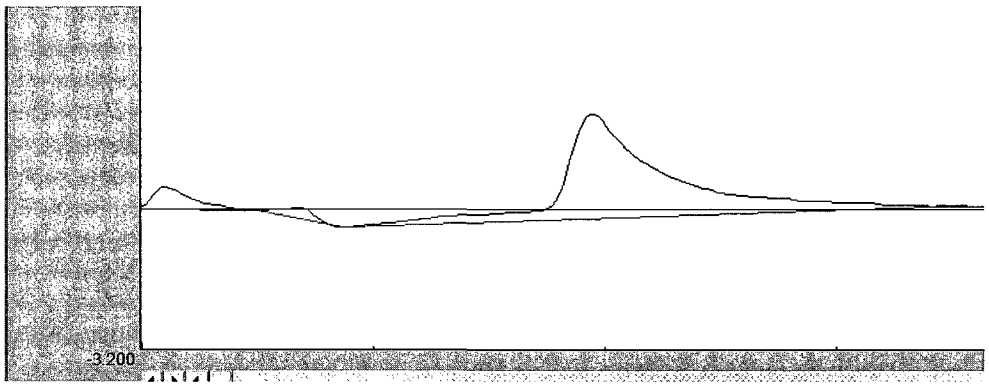

Figure 45: Calibration Curve 0.6 ppm E2 in DDW (Replicate 1)

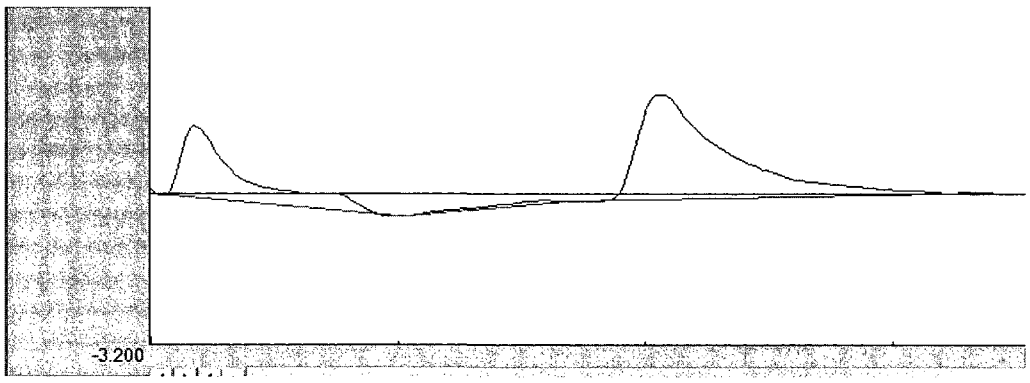

Figure 46: Calibration Curve 0.6 ppm E2 in DDW (Replicate 2)

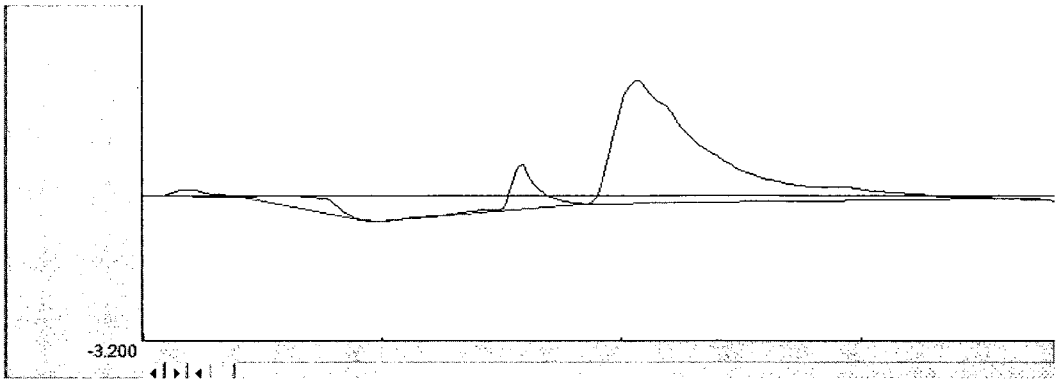

Figure 47: Calibration Curve 0.8 ppm E2 in DDW (Replicate 1)

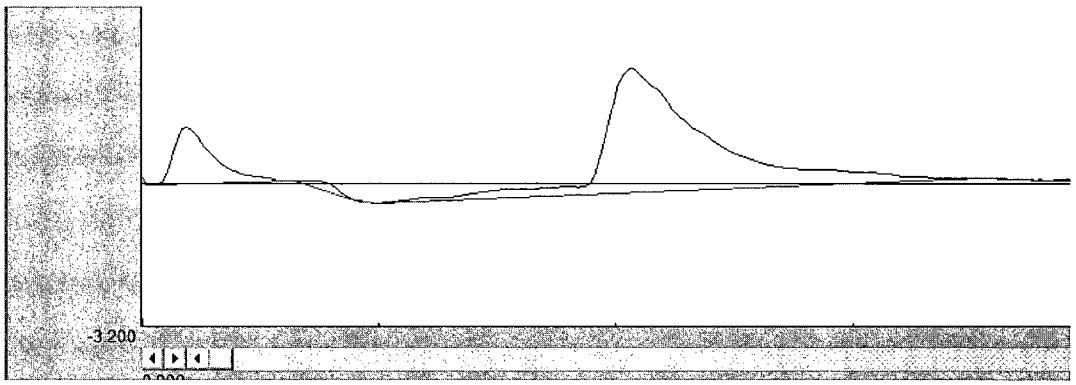

Figure 48: Calibration Curve 0.8 ppm E2 in DDW (Replicate 2)

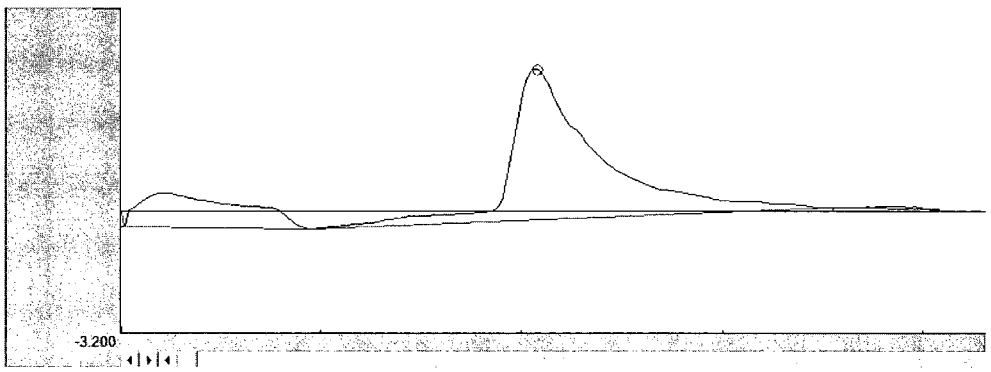

Figure 49: Calibration Curve 1 ppm E2 in DDW (Replicate 1)

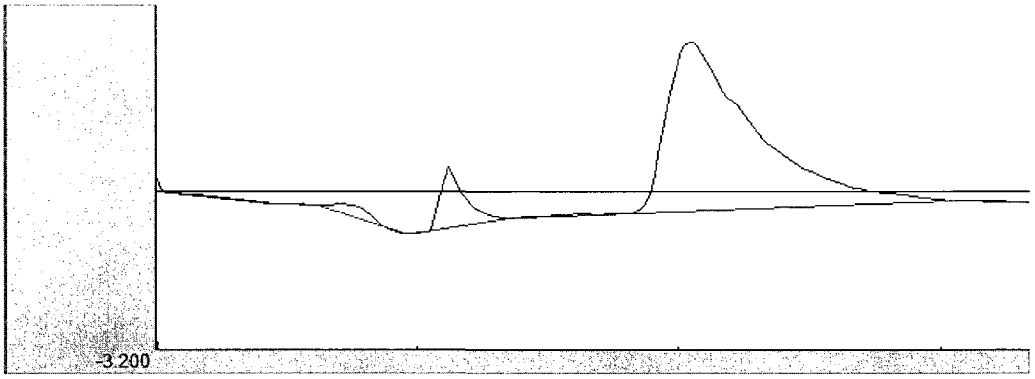

Figure 50: Calibration Curve 1 ppm E2 in DDW (Replicate 1)

11.1.2 Calibration Curve in TEA

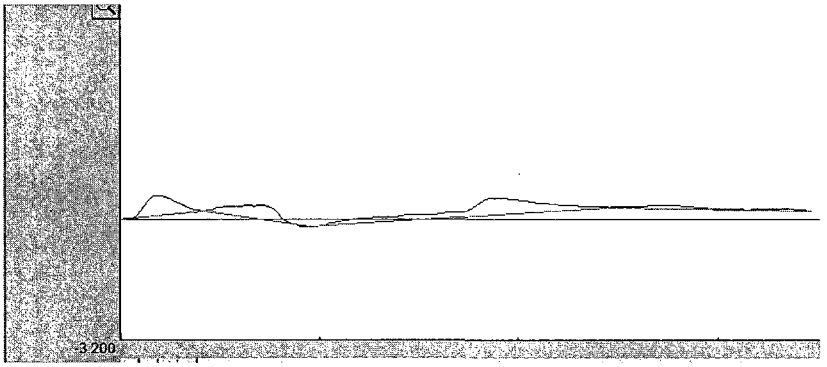

Figure 51: Calibration Curve 0.2 ppm E2 in TEA (Replicate 1)

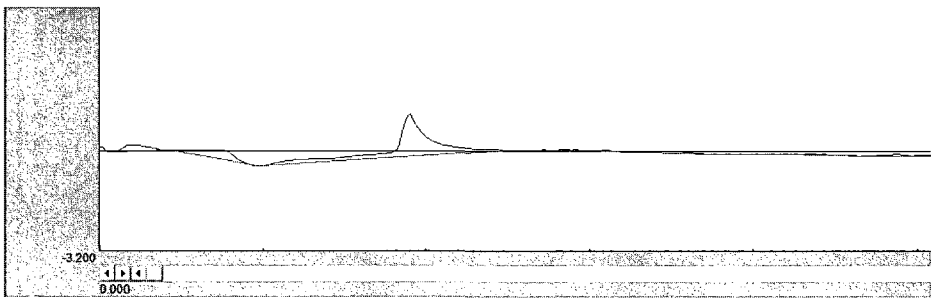

Figure 52: Calibration Curve 0.2 ppm E2 in TEA (Replicate 2)

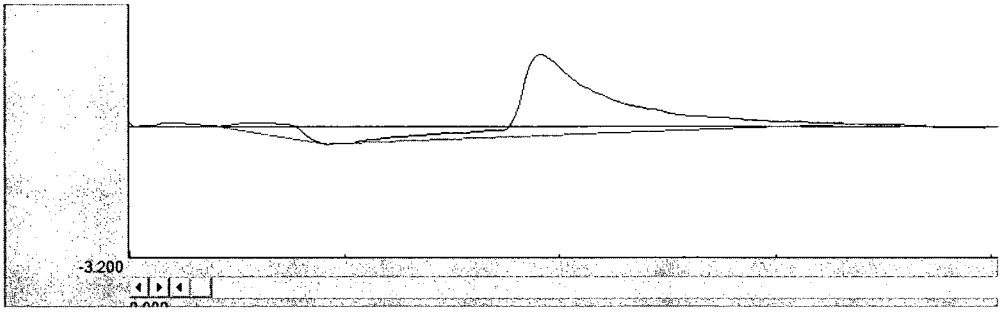

Figure 53: Calibration Curve 0.4 ppm E2 in TEA (Replicate 1)

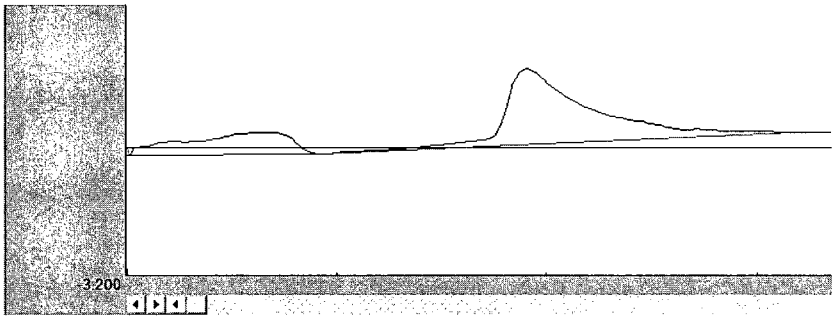

Figure 54: Calibration Curve 0.4 ppm E2 in TEA (Replicate 1)

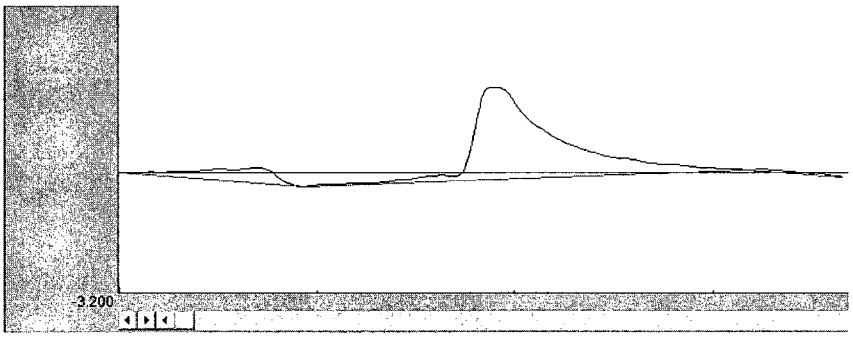

Figure 55: Calibration Curve 0.6 ppm E2 in TEA (Replicate 2)

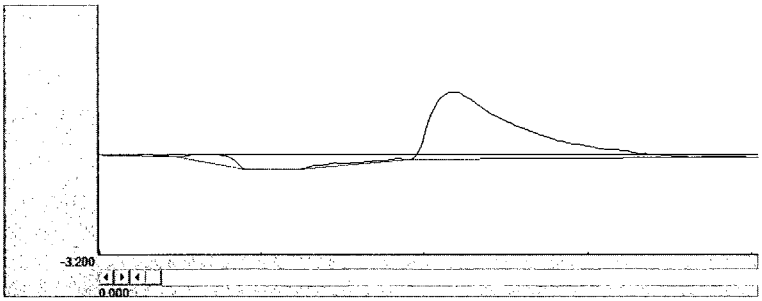

Figure 56: Calibration Curve 0.6 ppm E2 in TEA (Replicate 1)

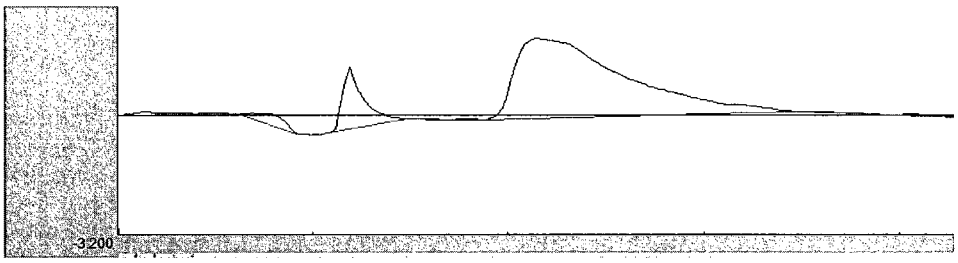

Figure 57: Calibration Curve 0.8 ppm E2 in TEA (Replicate 1)

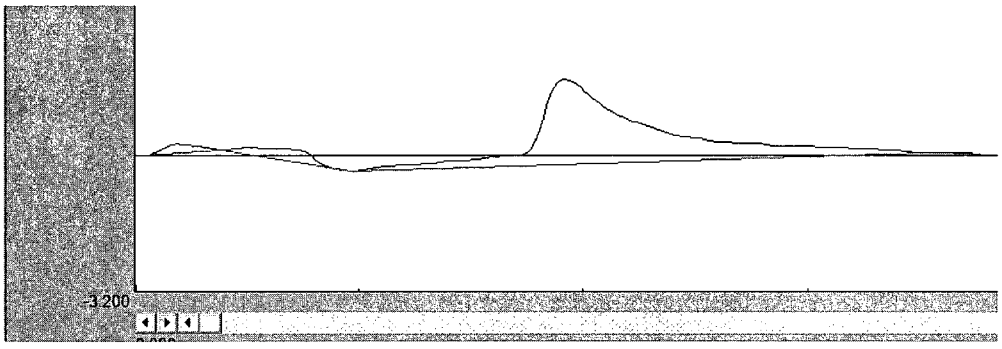

Figure 58: Calibration Curve 0.8 ppm E2 in TEA (Replicate 1)

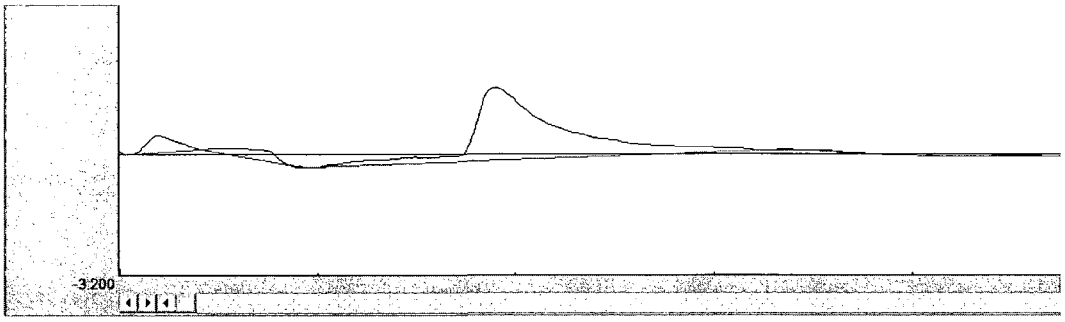

Figure 59: Calibration Curve 1 ppm E2 in TEA (Replicate 1)

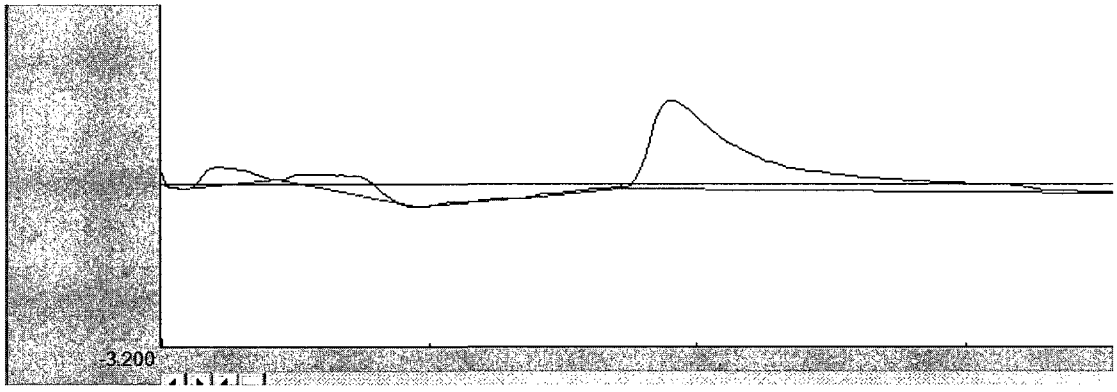

Figure 60: Calibration Curve 1 ppm E2 in TEA (Replicate 2)

11.1.3 Amount Remaining

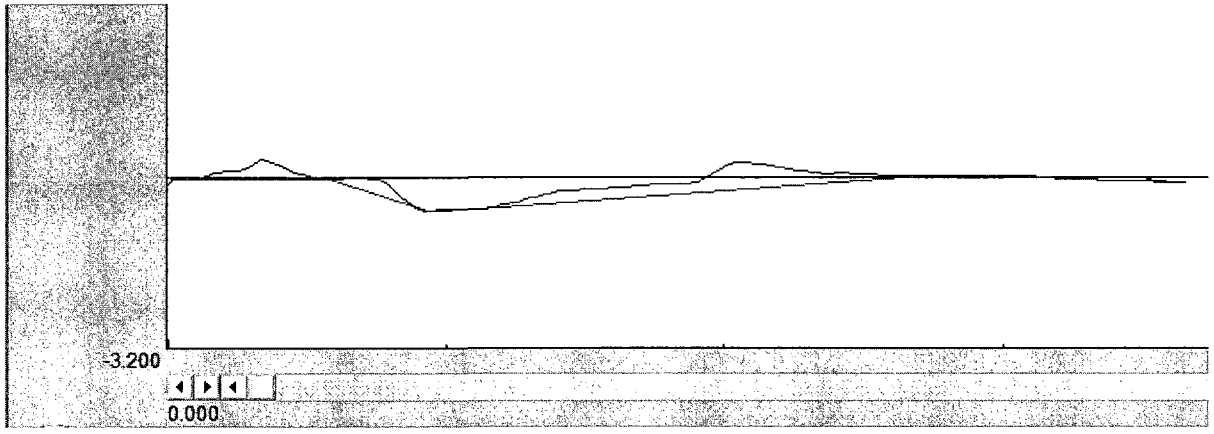

Figure 61: Remaining amount for an initial concentration of 0.1 ppm (Replicate 1)

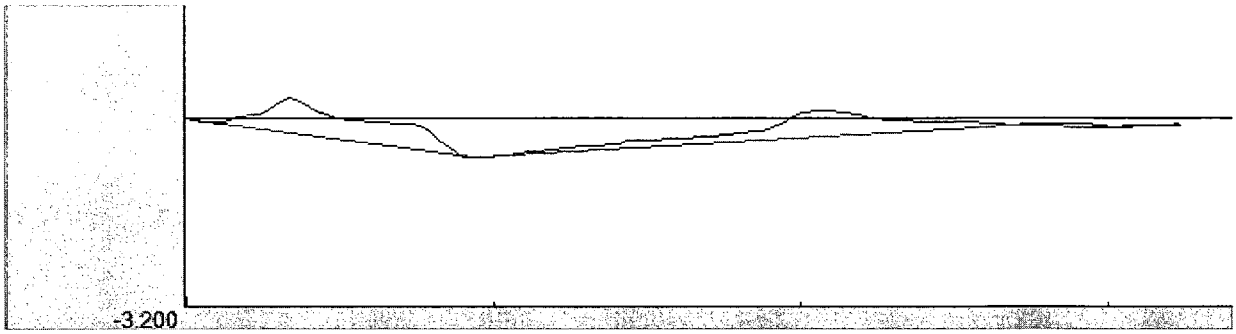

Figure 62: Remaining amount for an initial concentration of 0.1 ppm (Replicate 2)

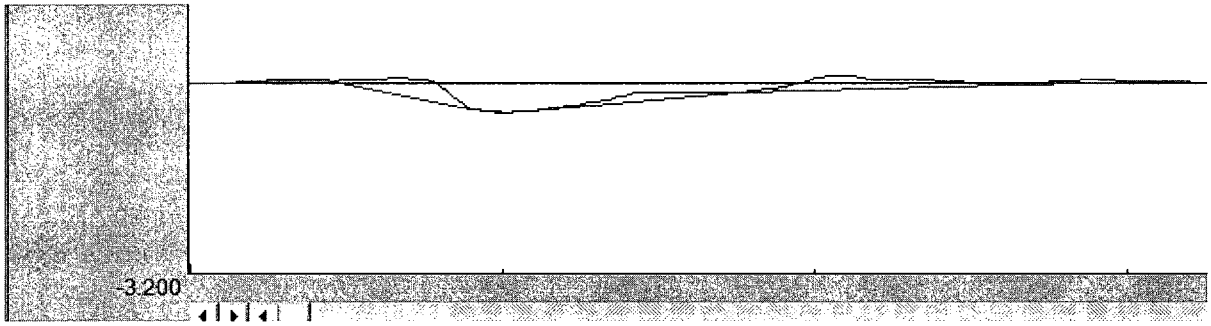

Figure 63: Remaining amount for an initial concentration of 0.2 ppm (Replicate 1)

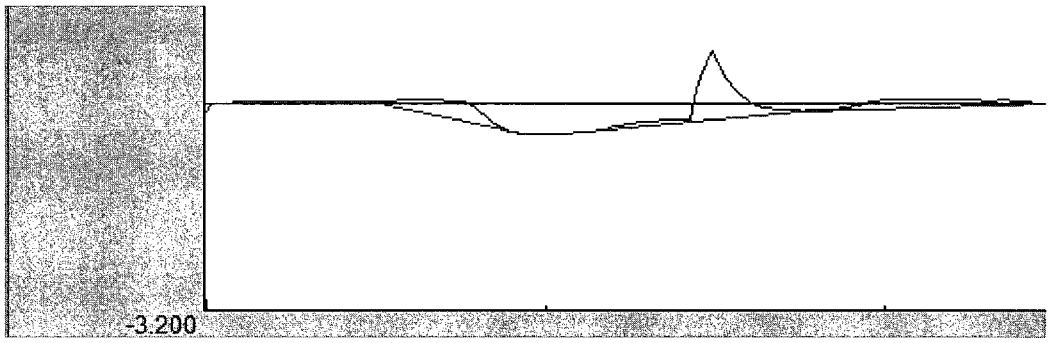

Figure 64: Remaining amount for an initial concentration of 0.2 ppm (Replicate 2)

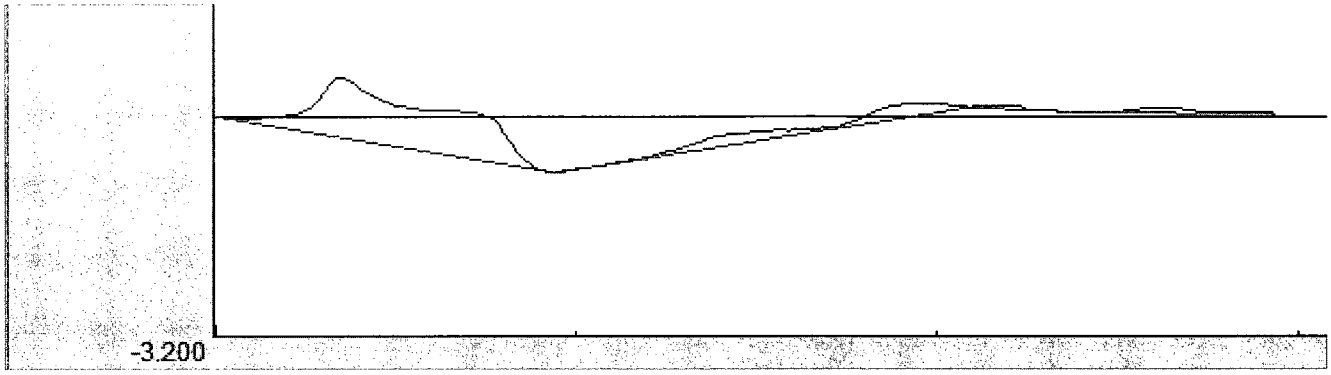

Figure 65: Remaining amount for an initial concentration of 0.4 ppm (Replicate 1)

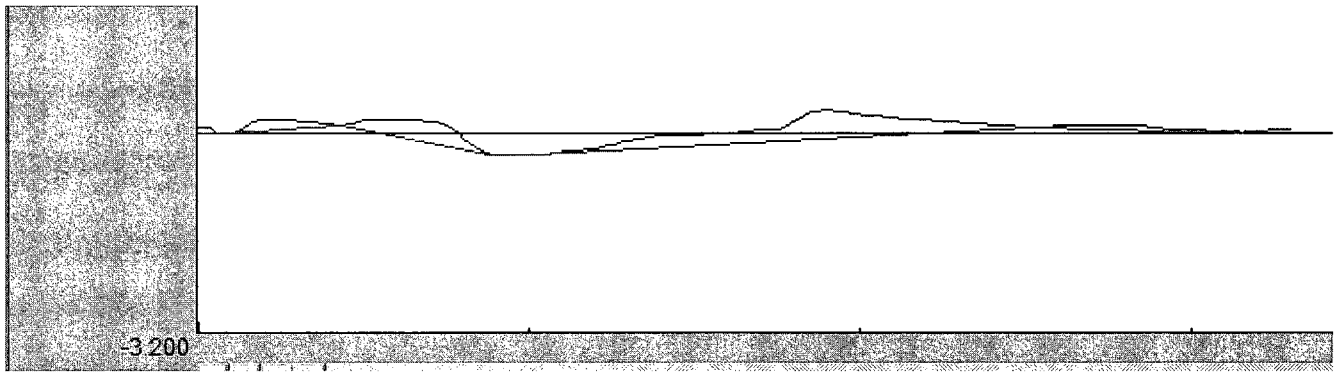

Figure 66: Remaining amount for an initial concentration of 0.4 ppm (Replicate 2)

Figure 67: Remaining amount for an initial concentration of 0.6 ppm (Replicate 1)

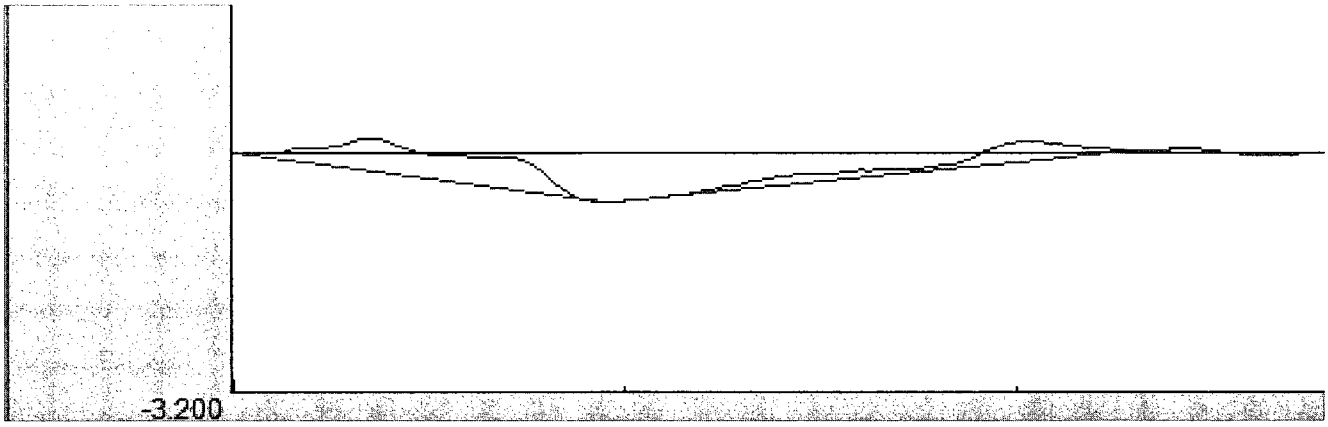

Figure 68: Remaining amount for an initial concentration of 0.6 ppm (Replicate 2)

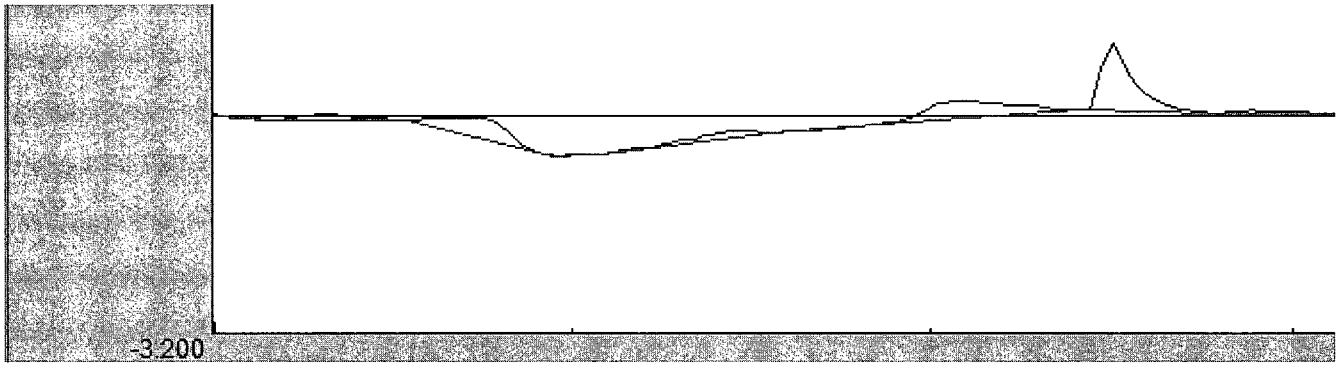

Figure 69: Remaining amount for an initial concentration of 0.8 ppm (Replicate 1)

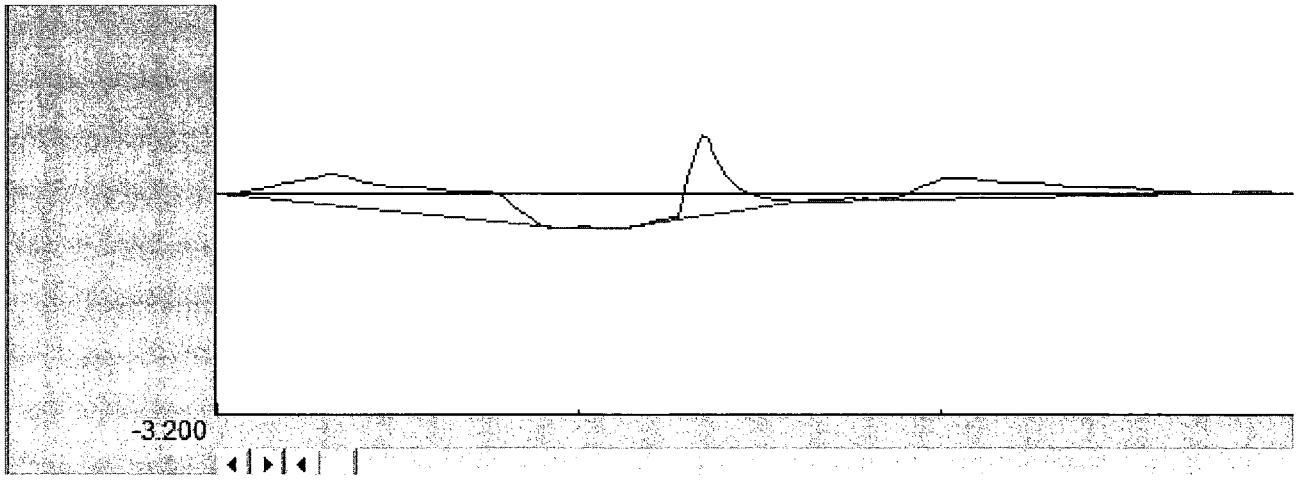

Figure 70: Remaining amount for an initial concentration of 0.8 ppm (Replicate 2)

11.2 Amount Desorbed

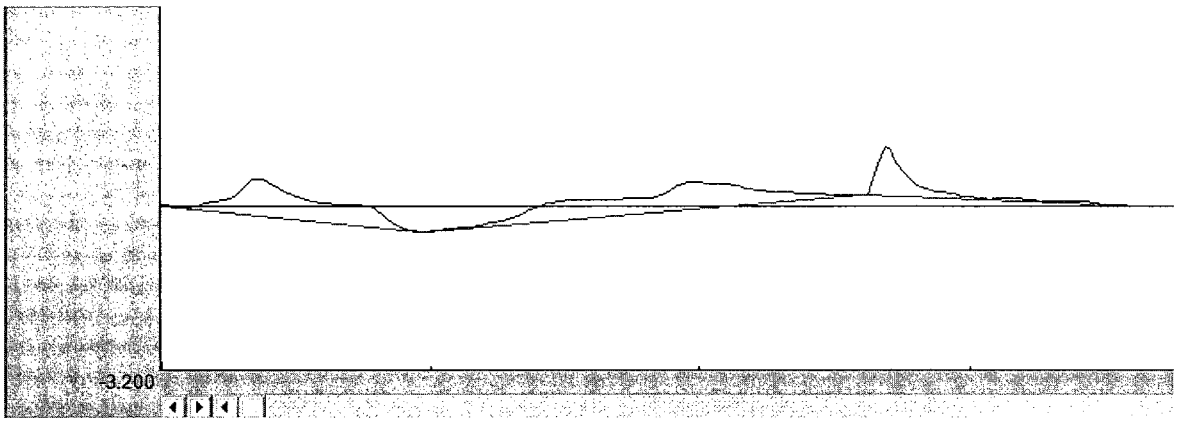

Figure 71: Desorbed amount for an initial concentration of 0.2 ppm (Replicate 1)

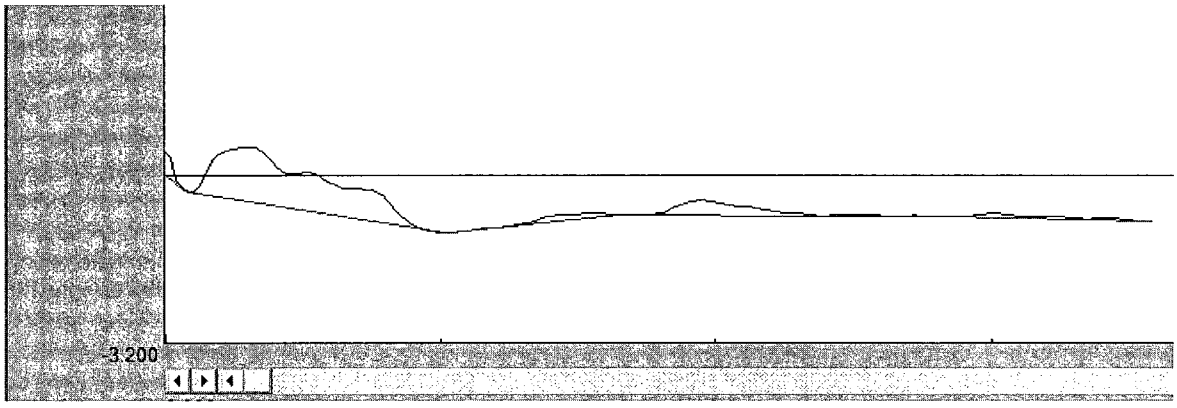

Figure 72: Desorbed amount for an initial concentration of 0.2 ppm (Replicate 2)

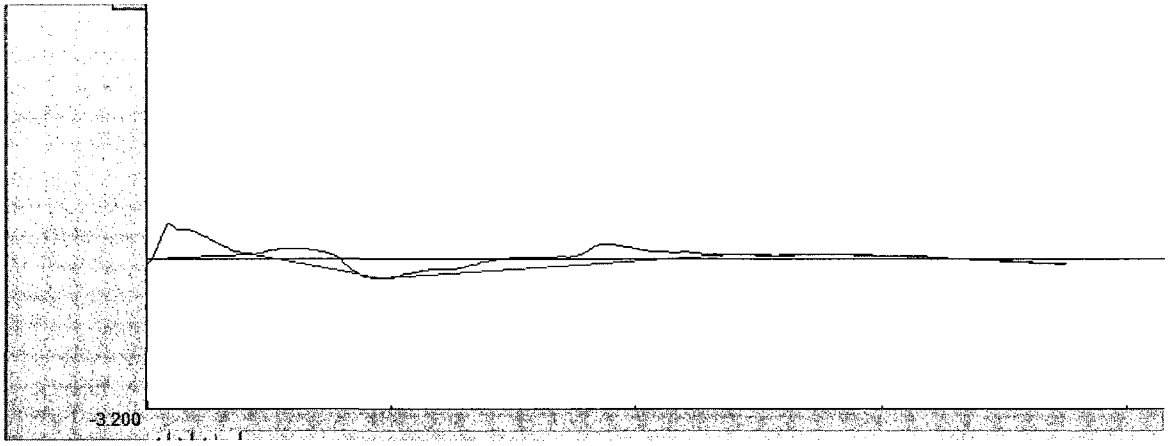

Figure 73: Desorbed amount for an initial concentration of 0.4 ppm (Replicate 1)

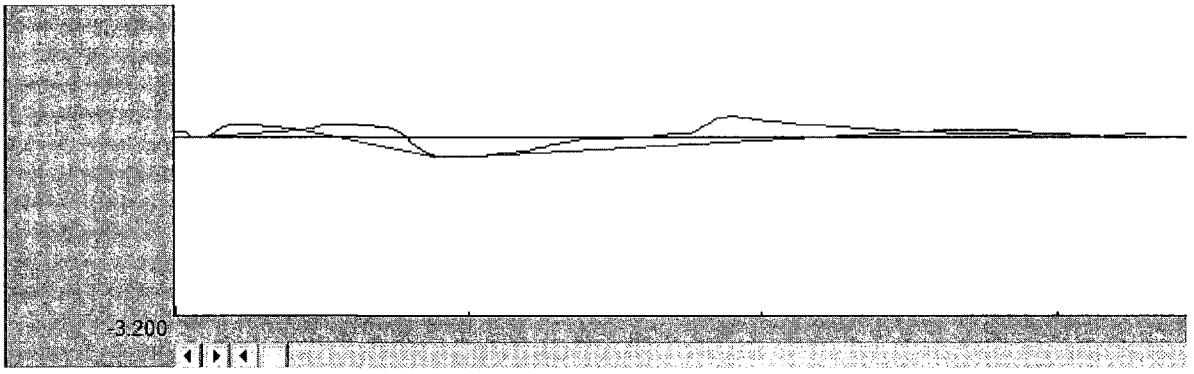

Figure 74: Desorbed amount for an initial concentration of 0.4 ppm (Replicate 2)

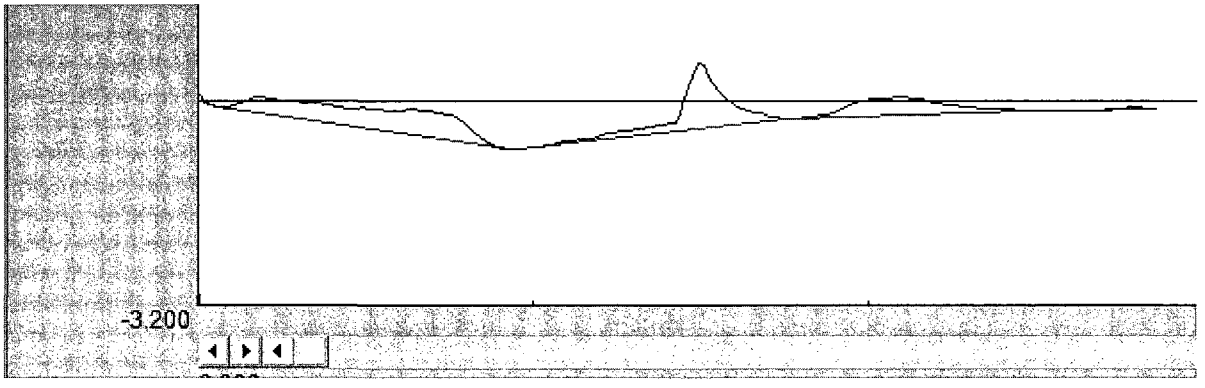

Figure 75: Desorbed amount for an initial concentration of 0.6 ppm (Replicate 1)

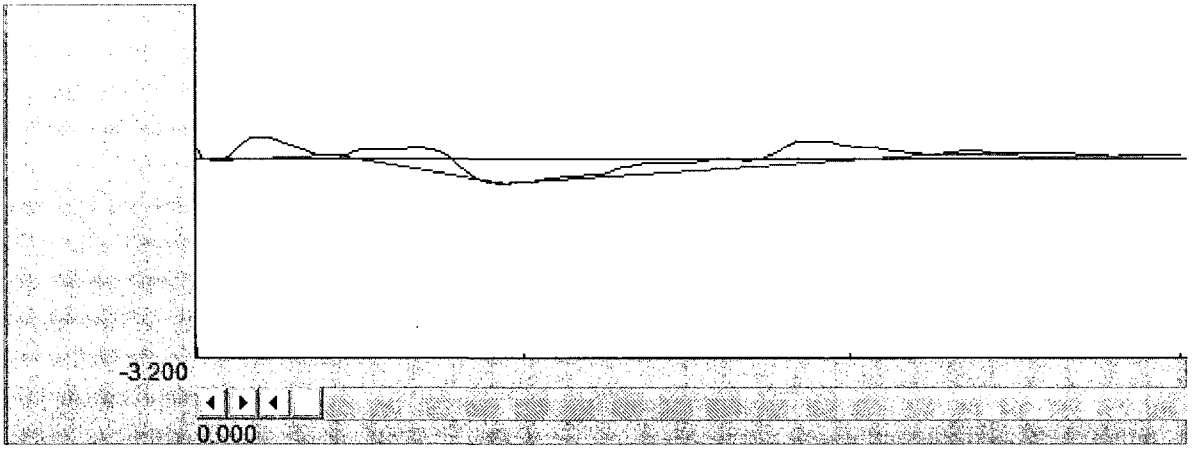

Figure 76: Desorbed amount for an initial concentration of 0.8 ppm (Replicate 1)

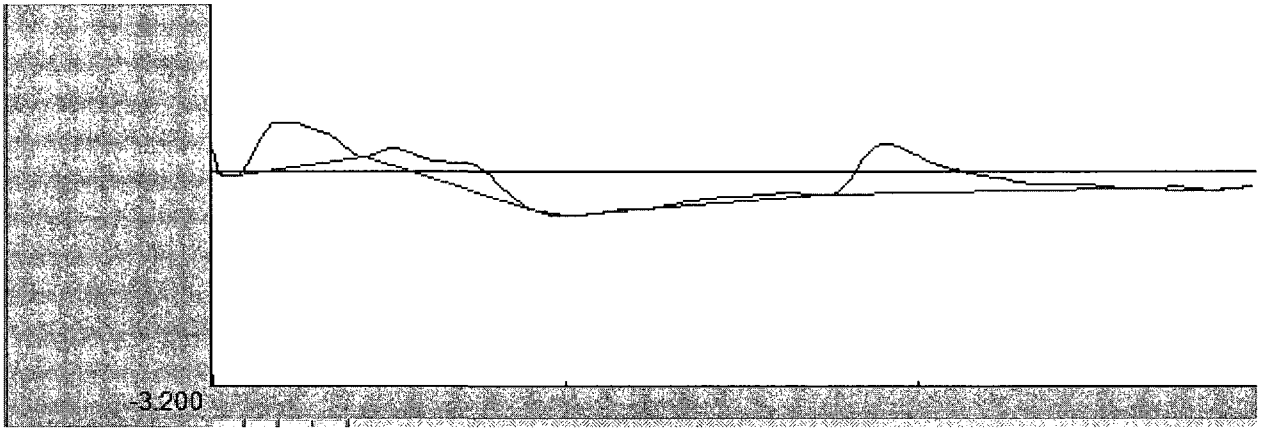

Figure 77: Desorbed amount for an initial concentration of 0.8 ppm (Replicate 2)

11.3 Preconcentration of E2 onto NIP Particles

11.3.1 Calibration Curve

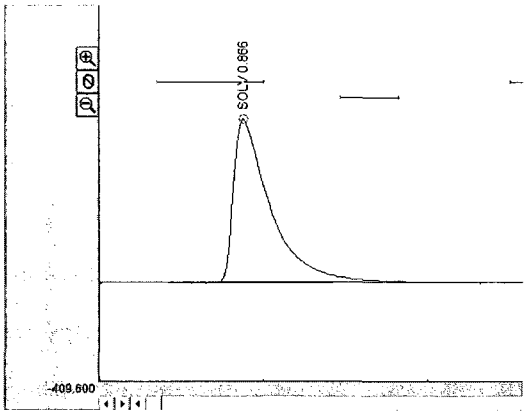

Figure 78: Calibration curve 20 ppm (Replicate 1)

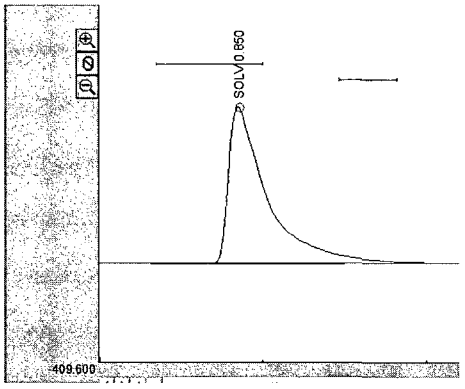

Figure 79: Calibration curve 20 ppm (Replicate 2)

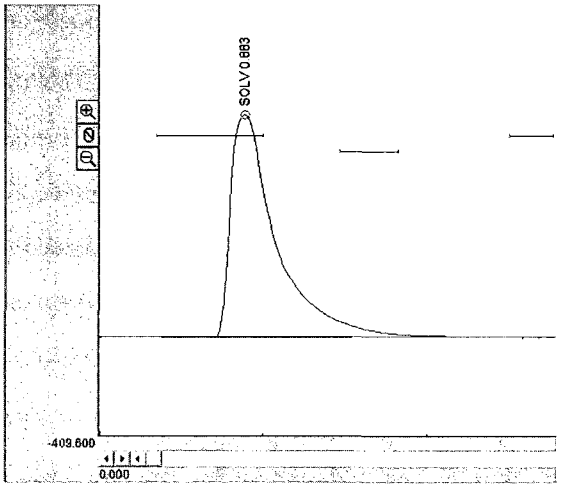

Figure 80: Calibration curve 40 ppm (Replicate 1)

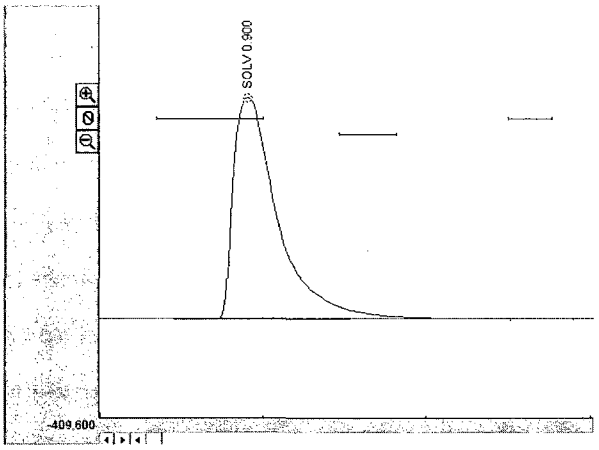

Figure 81: Calibration curve 40 ppm (Replicate 2)

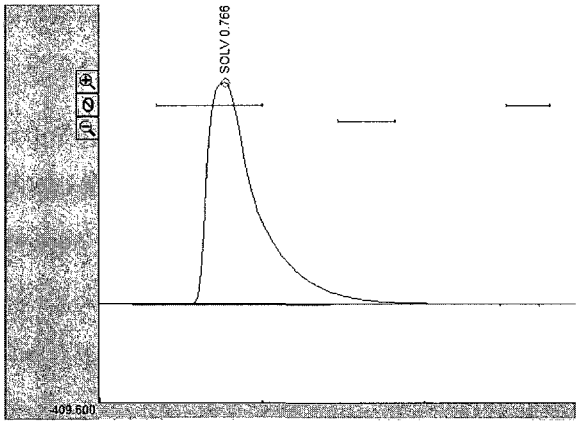

Figure 82: Calibration curve 60 ppm (Replicate 1)

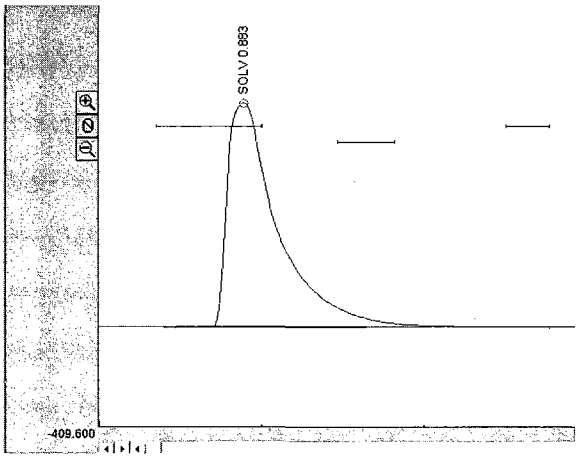

Figure 83: Calibration curve 60 ppm (Replicate 2)

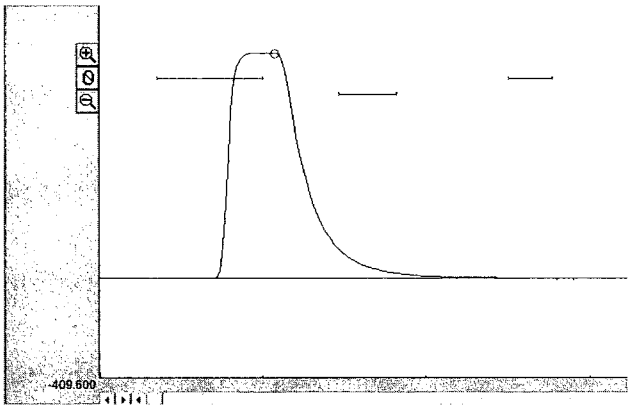

Figure 84: Calibration curve 80 ppm (Replicate 1)

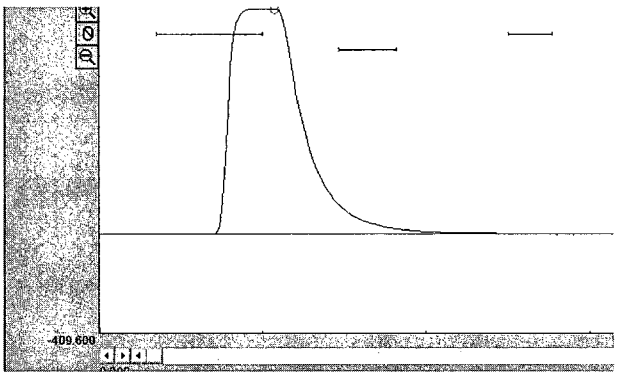

Figure 85: Calibration curve 80 ppm (Replicate 2)

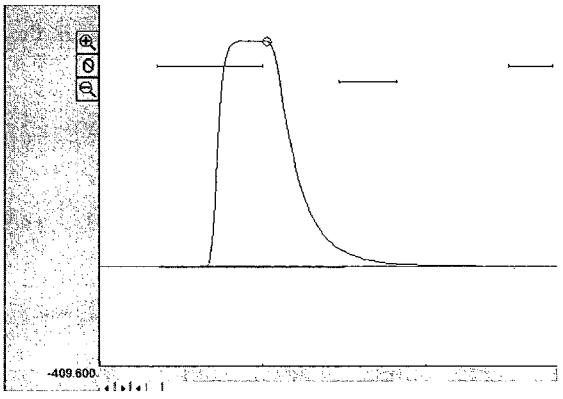

Figure 86: Calibration curve 100 ppm (Replicate 1)

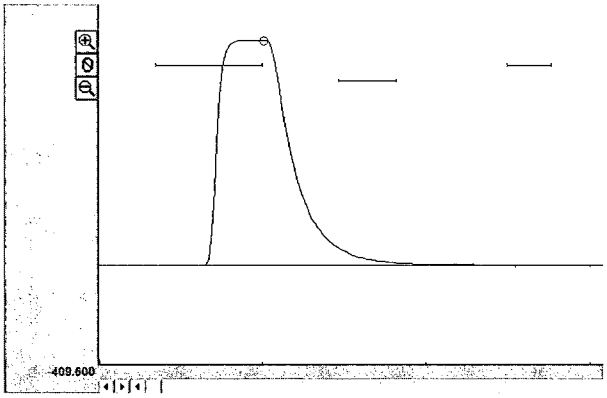

Figure 87: Calibration curve 100 ppm (Replicate 1)

11.3.2 Amount Remaining

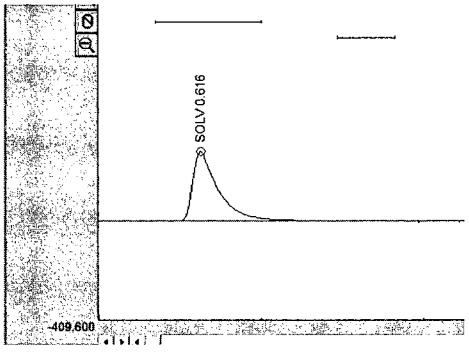

Figure 88: Remaining E2 for an initial E2 concentration of 20 ppm (Replicate 1)

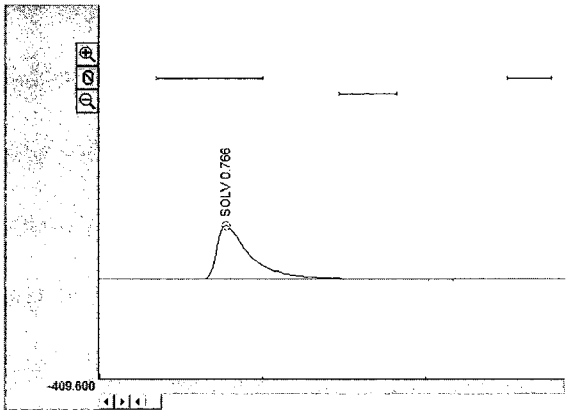

Figure 89: Remaining E2 for an initial E2 concentration of 20 ppm (Replicate 2)

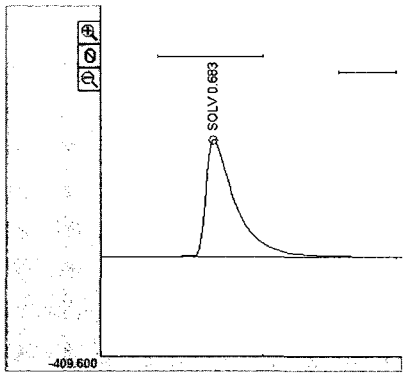

Figure 90: Remaining E2 for an initial E2 concentration of 40 ppm (Replicate 1)

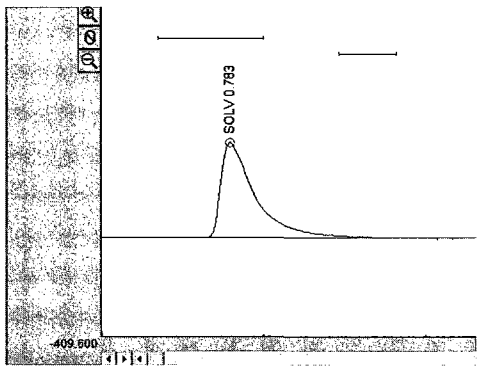

Figure 91: Remaining E2 for an initial E2 concentration of 40 ppm (Replicate 2)

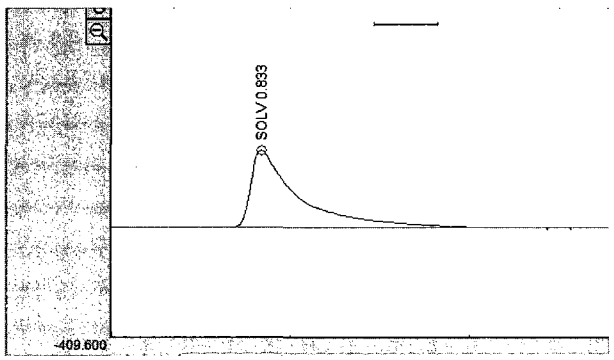

Figure 92: Remaining E2 for an initial E2 concentration of 60 ppm (Replicate 1)

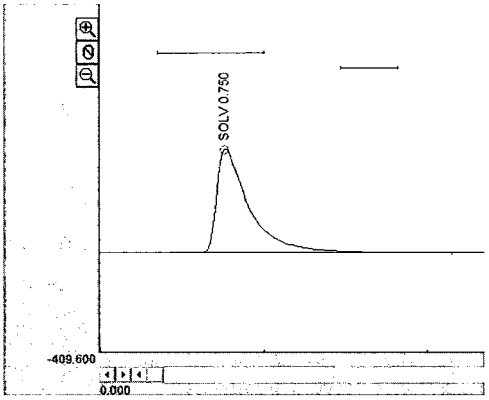

Figure 93: Remaining E2 for an initial E2 concentration of 60 ppm (Replicate 2)

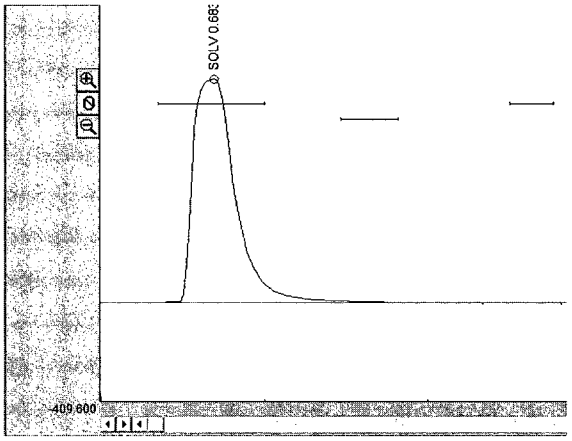

Figure 94: Remaining E2 for an initial E2 concentration of 80 ppm (Replicate 1)

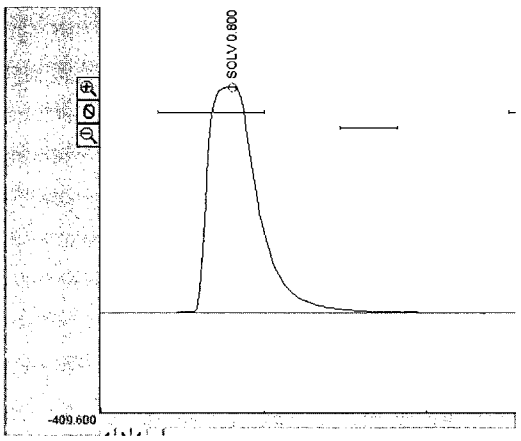

Figure 95: Remaining E2 for an initial E2 concentration of 80 ppm (Replicate 2)

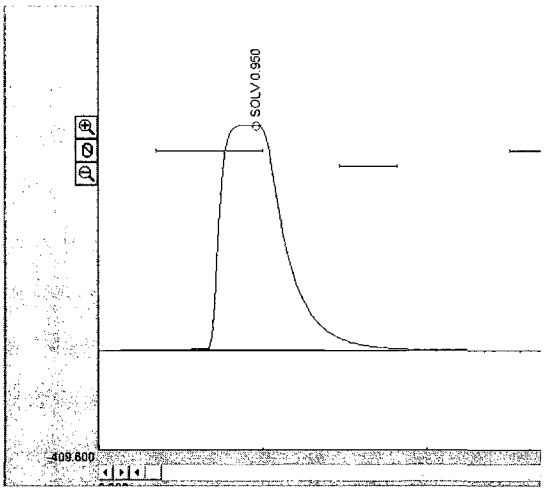

Figure 96: Remaining E2 for an initial E2 concentration of 100 ppm (Replicate 1)

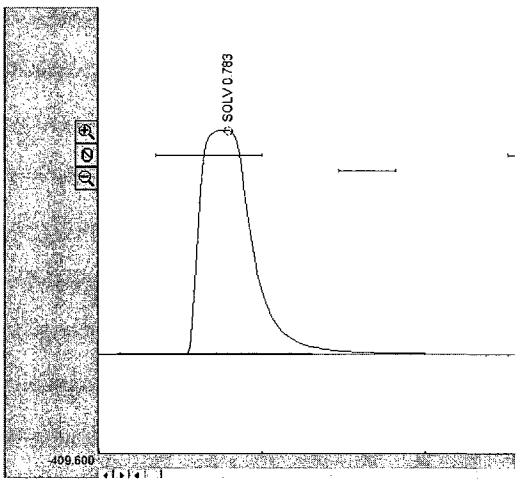

Figure 97: Remaining E2 for an initial E2 concentration of 100 ppm (Replicate 2)

11.3.3 Amount desorbed

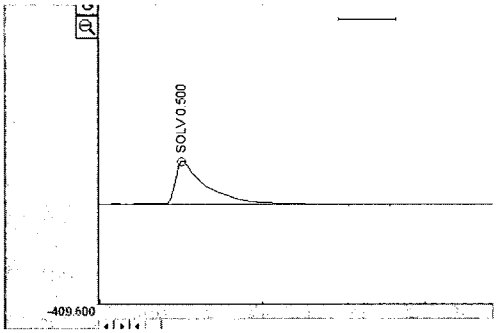

Figure 98: Desorbed E2 for an initial E2 concentration of 20 ppm (Replicate 1)

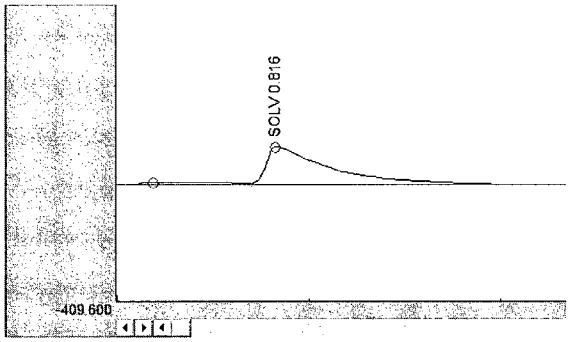

Figure 99: Desorbed E2 for an initial E2 concentration of 20 ppm (Replicate 2)

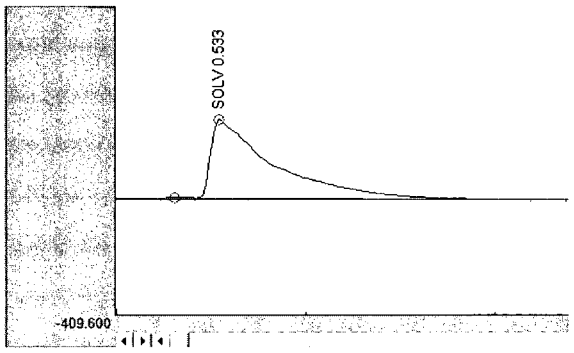

Figure 100: Desorbed E2 for an initial E2 concentration of 40 ppm (Replicate 1)

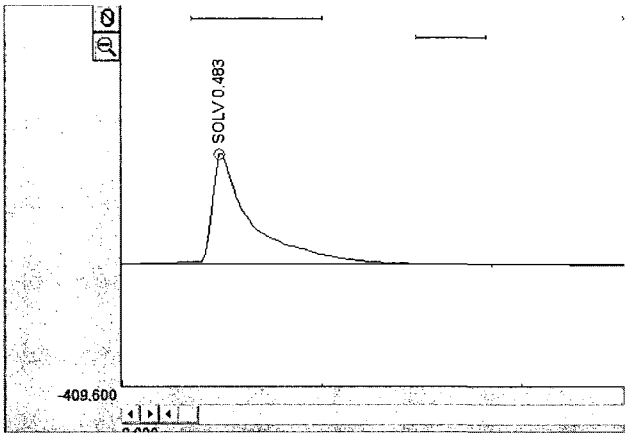

Figure 101: Desorbed E2 for an initial E2 concentration of 40 ppm (Replicate 2)

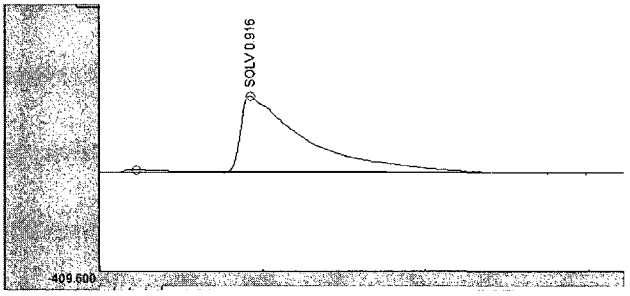

Figure 102: Desorbed E2 for an initial E2 concentration of 60 ppm (Replicate 1)

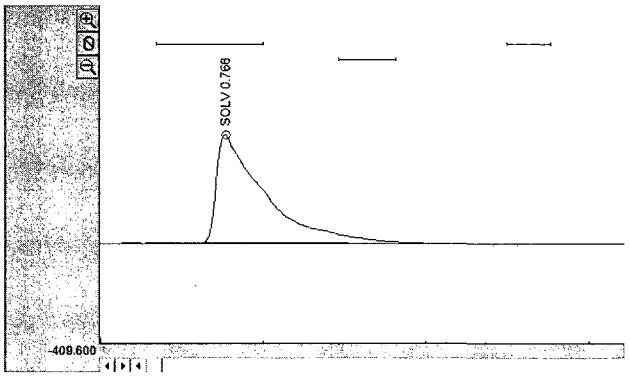

Figure 103: Desorbed E2 for an initial E2 concentration of 60 ppm (Replicate 2)

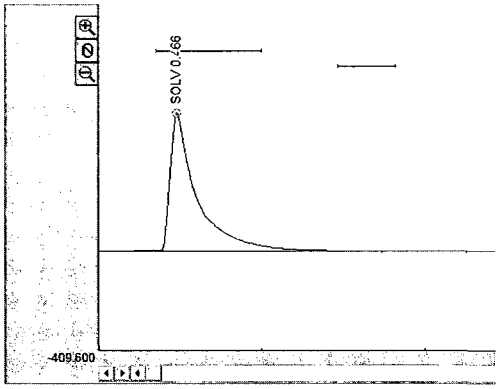

Figure 104: Desorbed E2 for an initial E2 concentration of 80 ppm (Replicate 1)

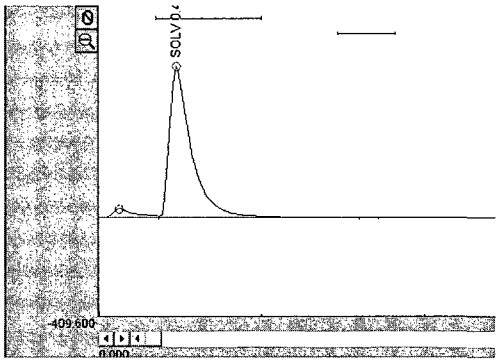

Figure 105: Desorbed E2 for an initial E2 concentration of 80 ppm (Replicate 2)

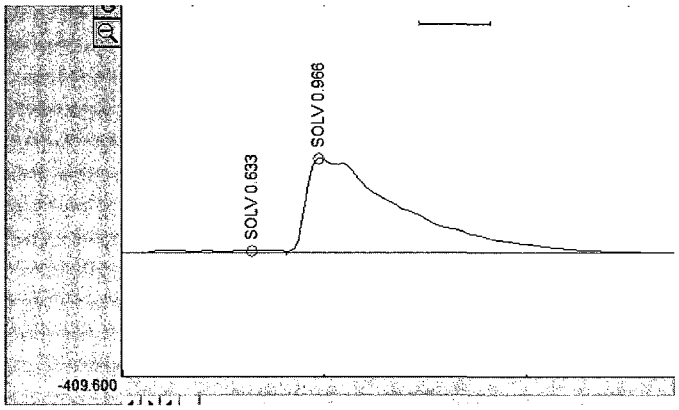

Figure 106: Desorbed E2 for an initial E2 concentration of 100 ppm (Replicate 1)

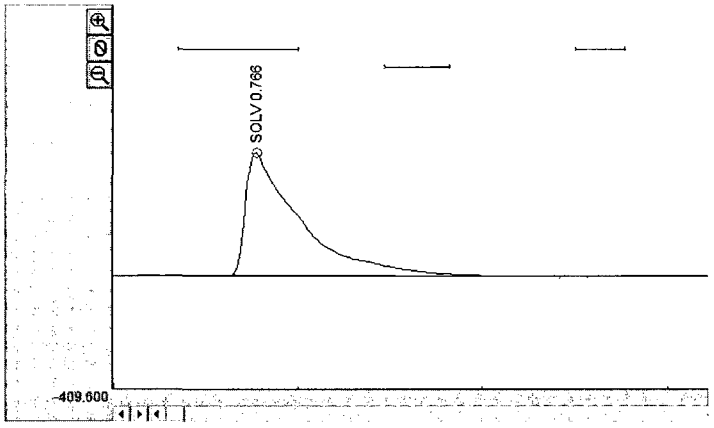

Figure 107: Desorbed E2 for an initial E2 concentration of 100 ppm (Replicate 2)

11.4 Comparison of the Binding Efficiencies of MIP and NIP for E2

11.4.1 Calibration Curve

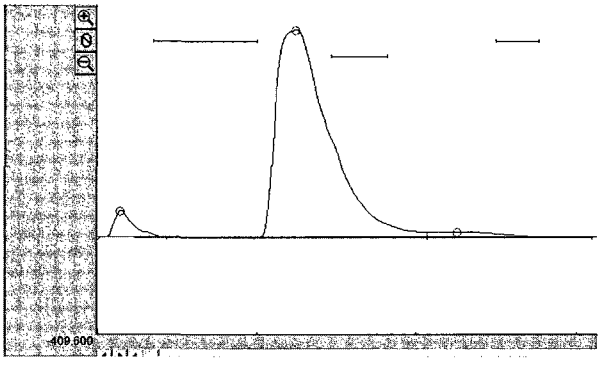

Figure 108: Calibration curve 20 ppm (Replicate 1)

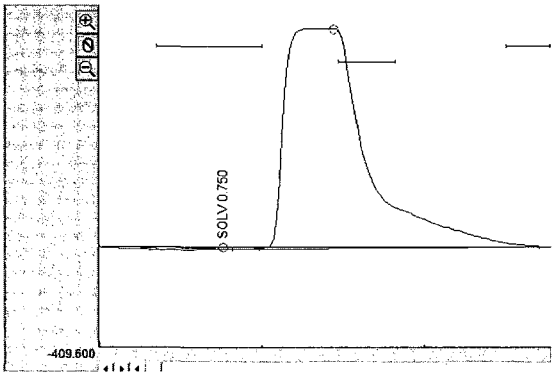

Figure 109: Calibration curve 40 ppm (Replicate 1)

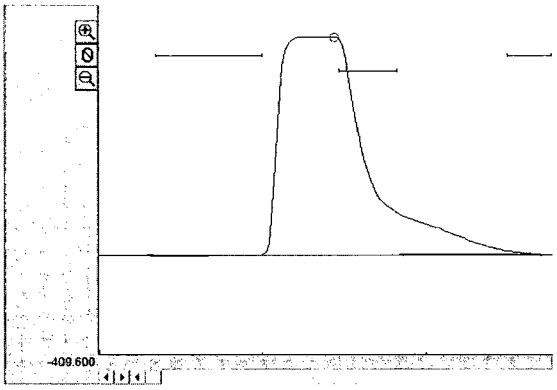

Figure 110: Calibration curve 40 ppm (Replicate 2)

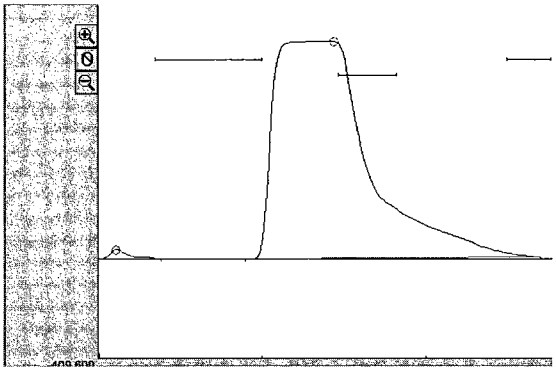

Figure 111: Calibration curve 60 ppm (Replicate 1)

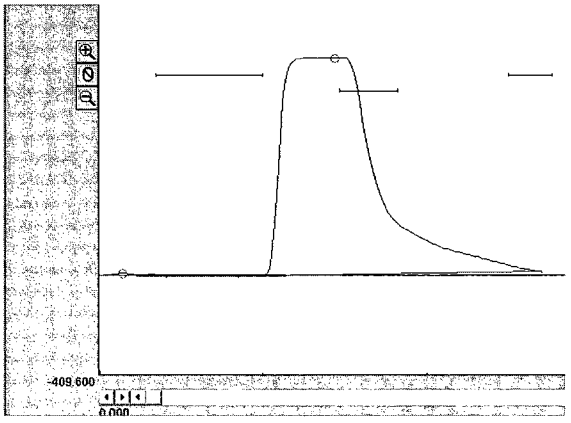

Figure 112: Calibration curve 80 ppm (Replicate 2)

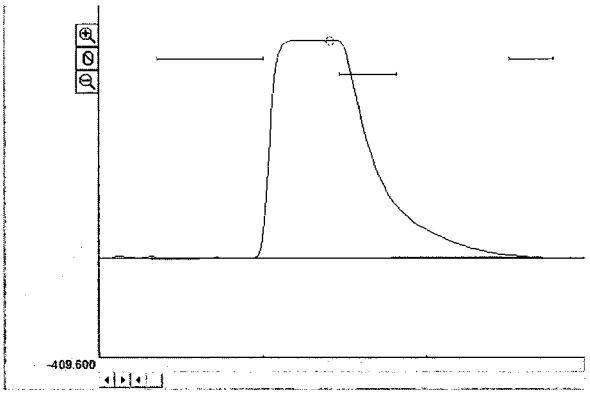

Figure 113: Calibration curve 100 ppm (Replicate 1)

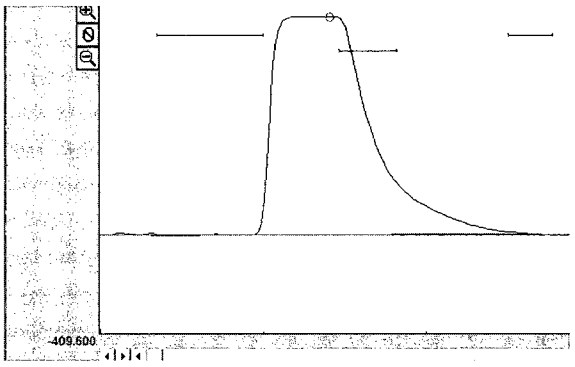

Figure 114: Calibration curve 100 ppm (Replicate 2)

11.4.2 Amount Remaining for MIP

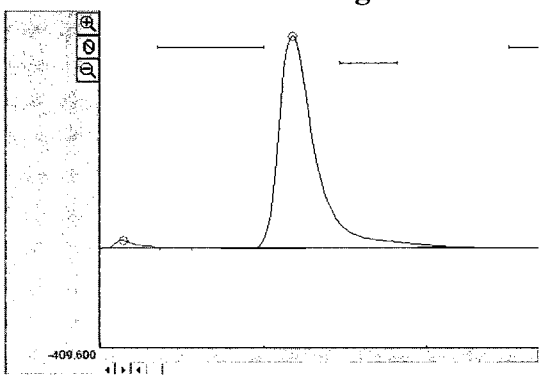

Figure 115: Remaining amount for an initial concentration of 20ppm for MIP (Replicate 1)

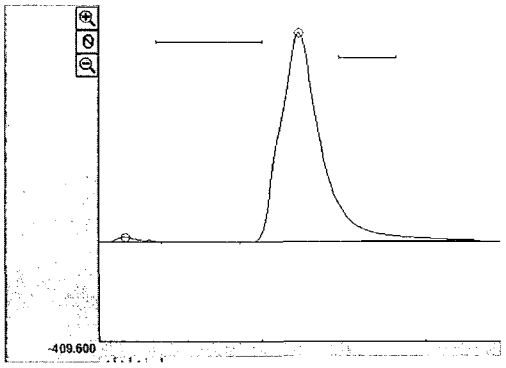

Figure 116: Remaining amount for an initial concentration of 20ppm for MIP (Replicate 2)

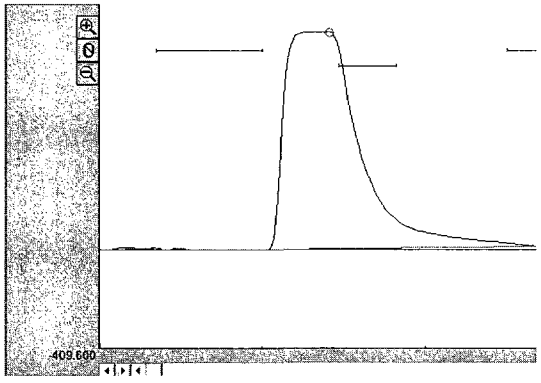

Figure 117: Remaining amount for an initial concentration of 40ppm for MIP (Replicate 1)

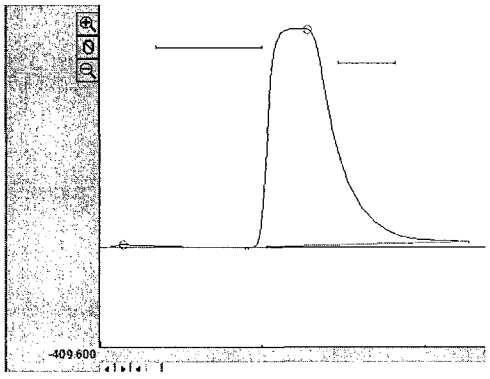

Figure 118: Remaining amount for an initial concentration of 40ppm for MIP(Replicate 2)

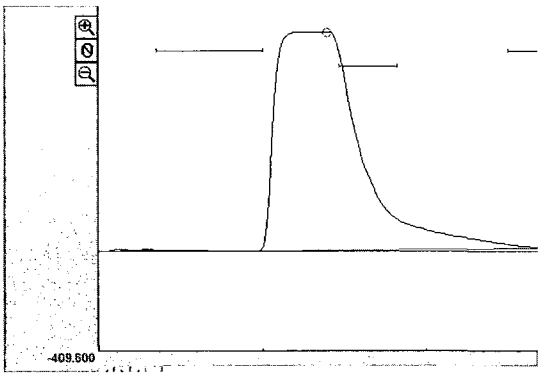

Figure 119: Remaining amount for an initial concentration of 60ppm for MIP (Replicate 1)

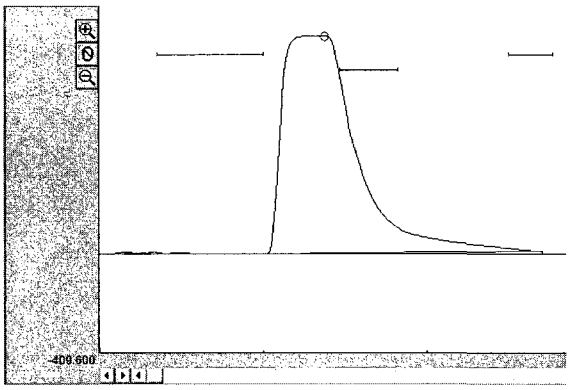

Figure 120: Remaining amount for an initial concentration of 60ppm for MIP (Replicate 2)

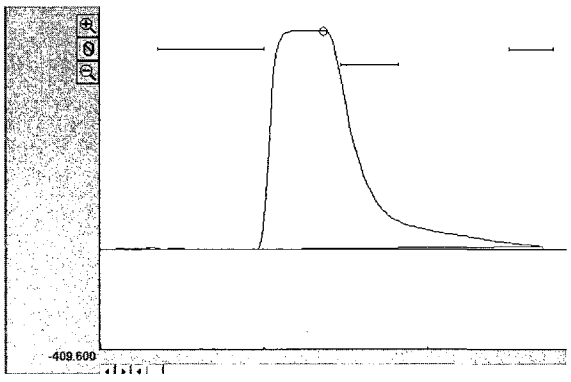

Figure 121: Remaining amount for an initial concentration of 80ppm for MIP (Replicate 1)

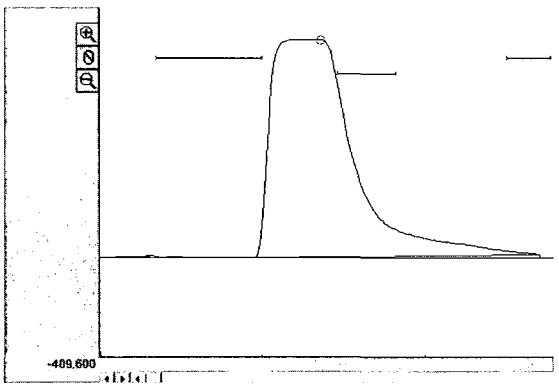

Figure 122: Remaining amount for an initial concentration of 80ppm for MIP (Replicate 2)

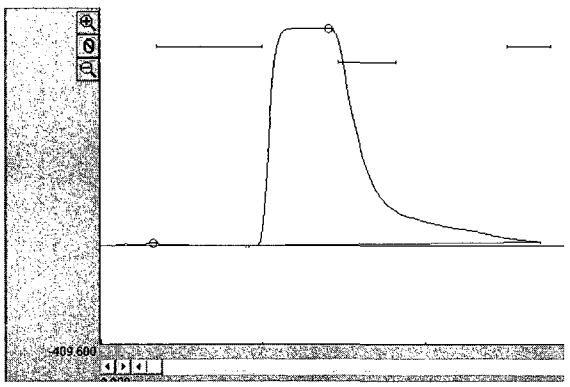

Figure 123: Remaining amount for an initial concentration of 100ppm for MIP (Replicate 1)

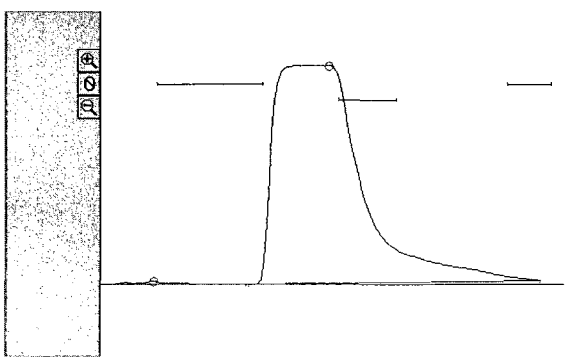

Figure 124: Remaining amount for an initial concentration of 100ppm for MIP (Replicate 2)

11.4.3 Amount Remaining for NIP

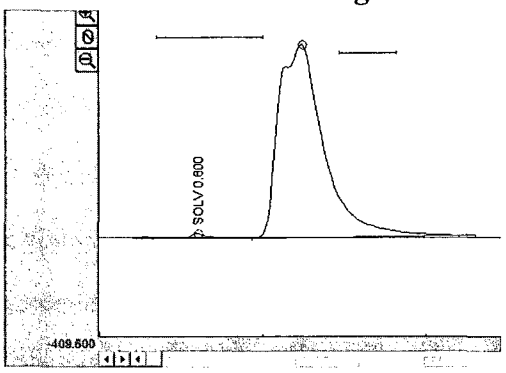

Figure 125: Remaining amount for an initial concentration of 20 for NIP (Replicate 1)

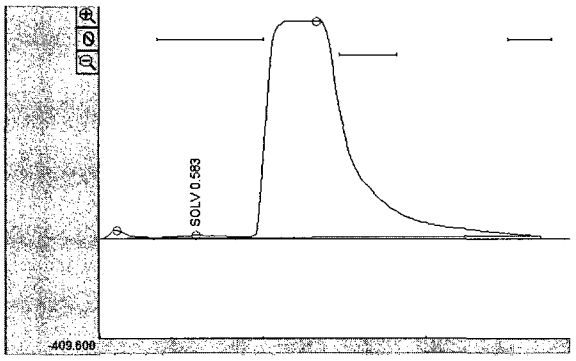

Figure 126: Remaining amount for an initial concentration of 20 for NIP (Replicate 2)

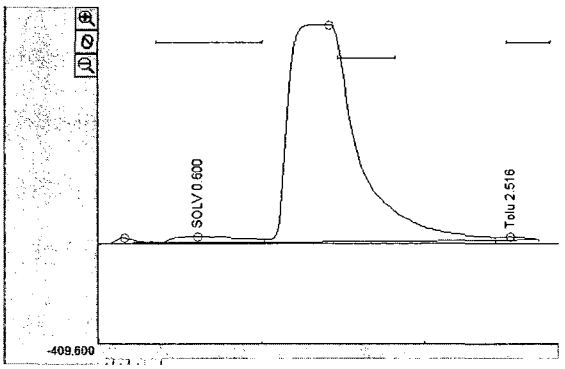

Figure 127: Remaining amount for an initial concentration of 40 for NIP (Replicate 1)

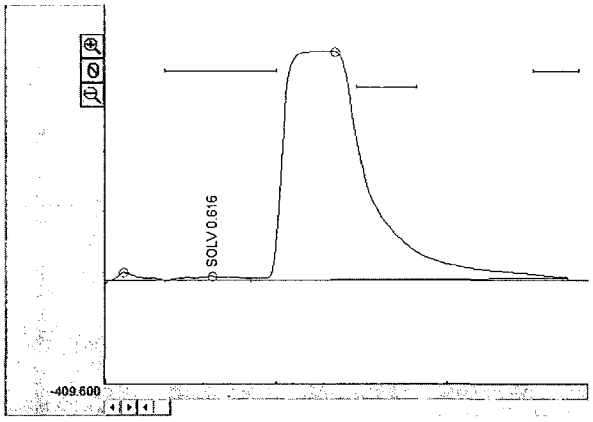

Figure 128: Remaining amount for an initial concentration of 40 for NIP (Replicate 2)

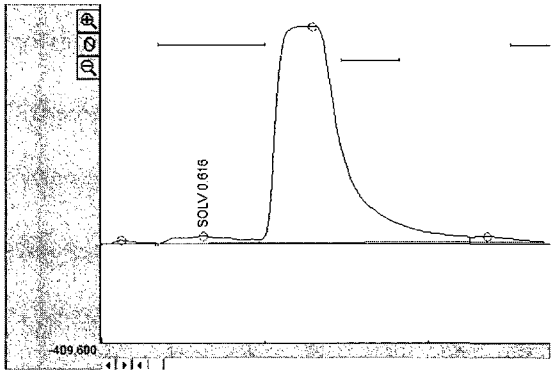

Figure 129: Remaining amount for an initial concentration of 60 for NIP (Replicate 1)

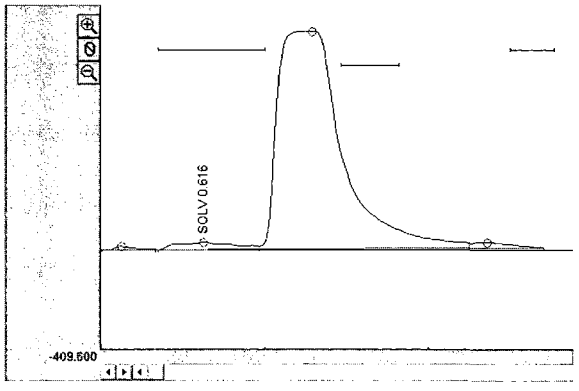

Figure 130: Remaining amount for an initial concentration of 60 for NIP (Replicate 2)

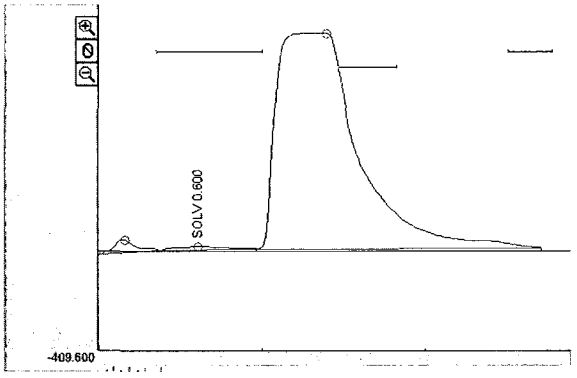

Figure 131: Remaining amount for an initial concentration of 80 for NIP (Replicate 1)

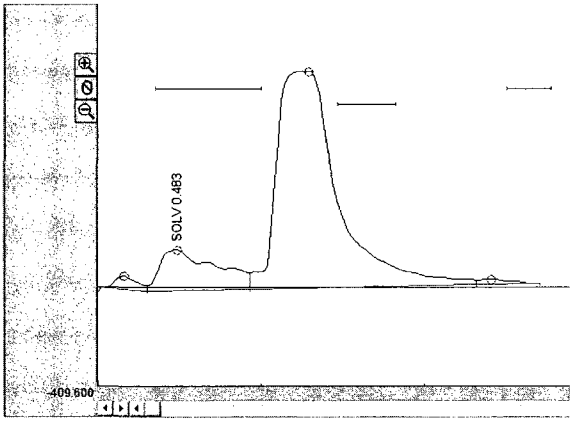

Figure 132: Remaining amount for an initial concentration of 80 for NIP (Replicate 2)

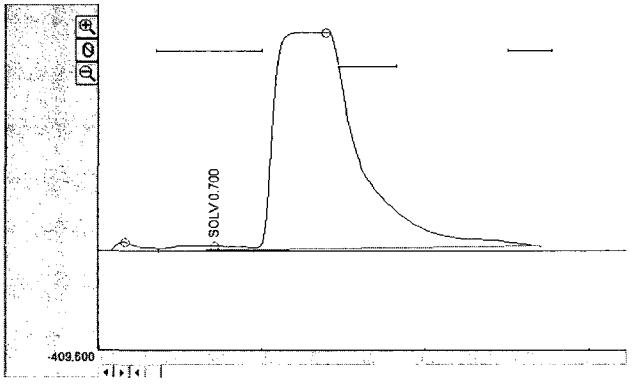

Figure 133: Remaining amount for an initial concentration of 100 for NIP (Replicate 1)

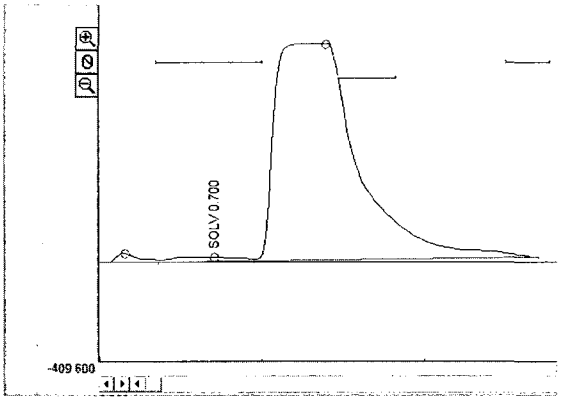

Figure 134: Remaining amount for an initial concentration of 100 for NIP (Replicate 2)

11.5 Comparison of the Binding Efficiencies of MIP and NIP for EE2

11.5.1 Calibration Curve

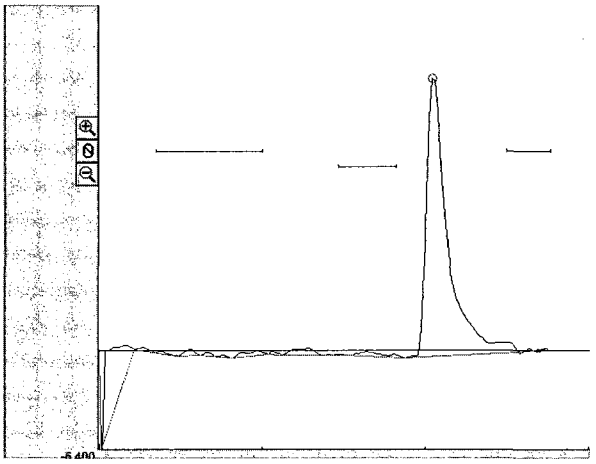

Figure 135: Calibration Curve 20 ppm EE2 in DDW (Replicate 1)

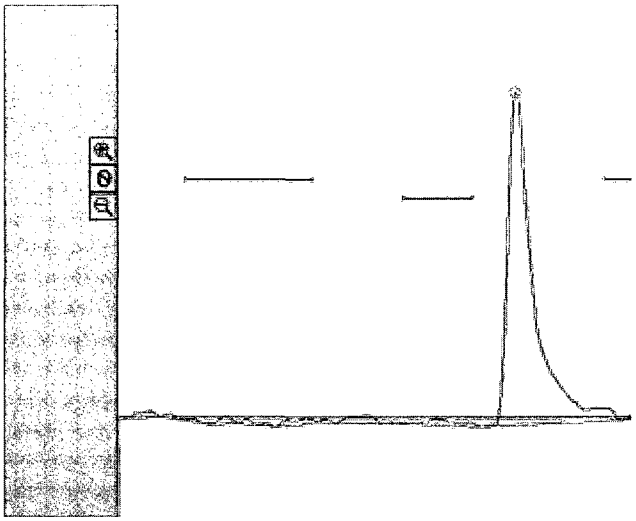

Figure 136: Calibration Curve 20 ppm EE2 in DDW (Replicate 2)

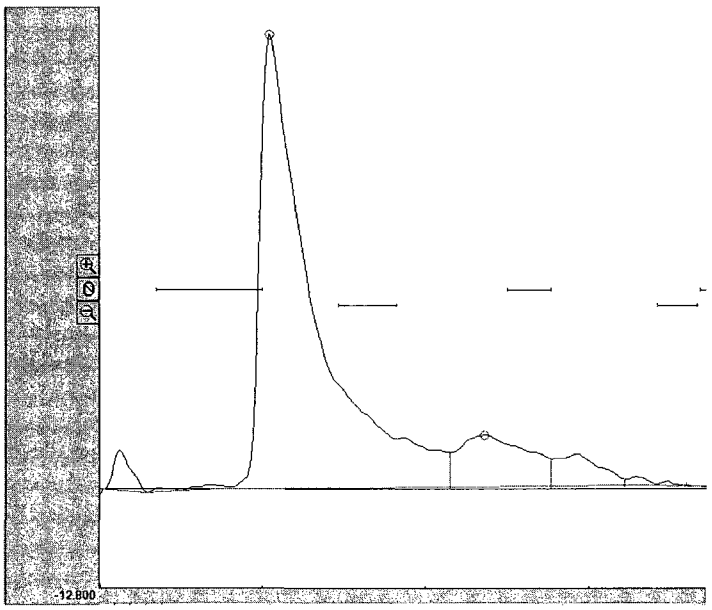

Figure 137: Calibration Curve 40 ppm EE2 in DDW (Replicate 1)

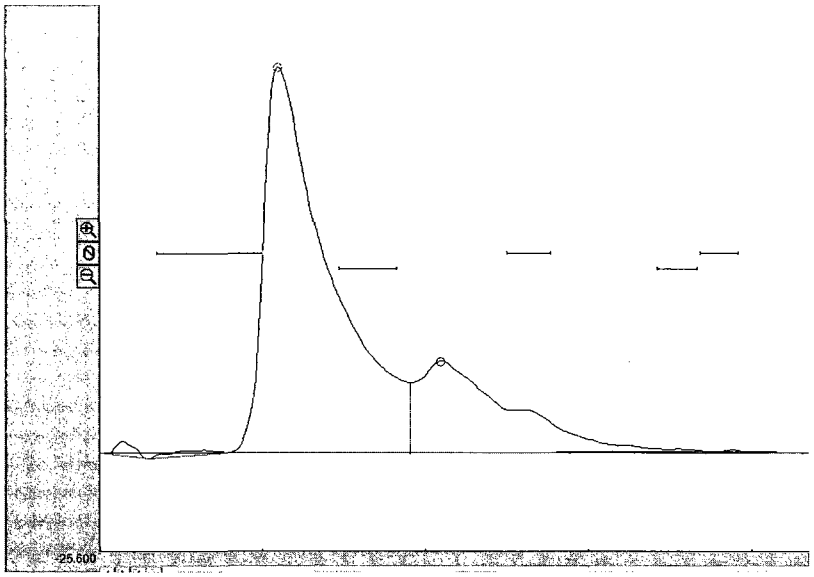

Figure 138: Calibration Curve 40 ppm EE2 in DDW (Replicate 2)

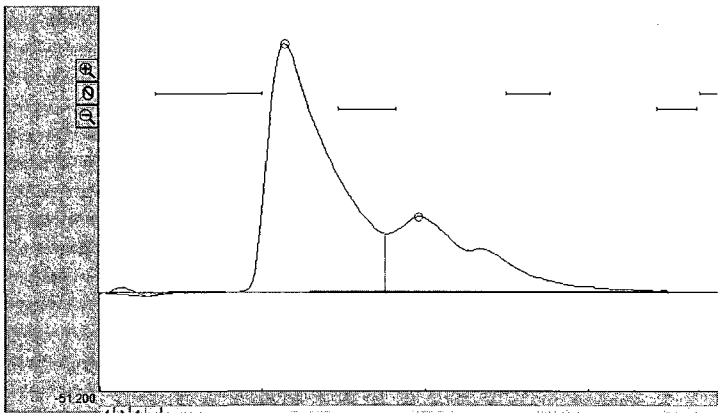

Figure 139: Calibration Curve 60 ppm EE2 in DDW (Replicate 1)

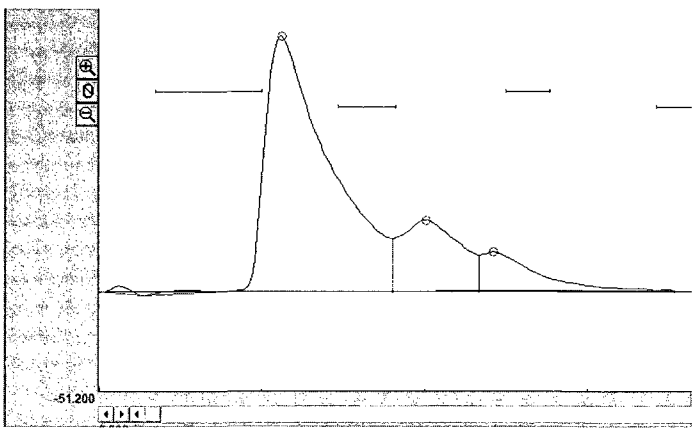

Figure 140: Calibration Curve 60 ppm EE2 in DDW (Replicate 2)

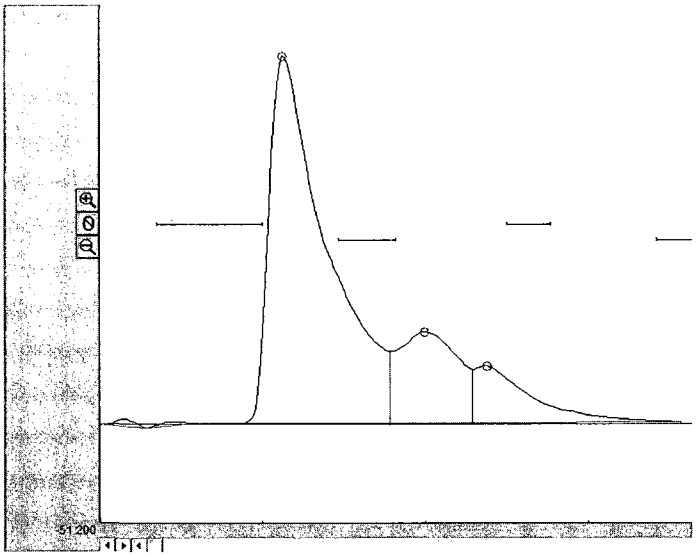

Figure 141: Calibration Curve 80 ppm EE2 in DDW (Replicate 1)

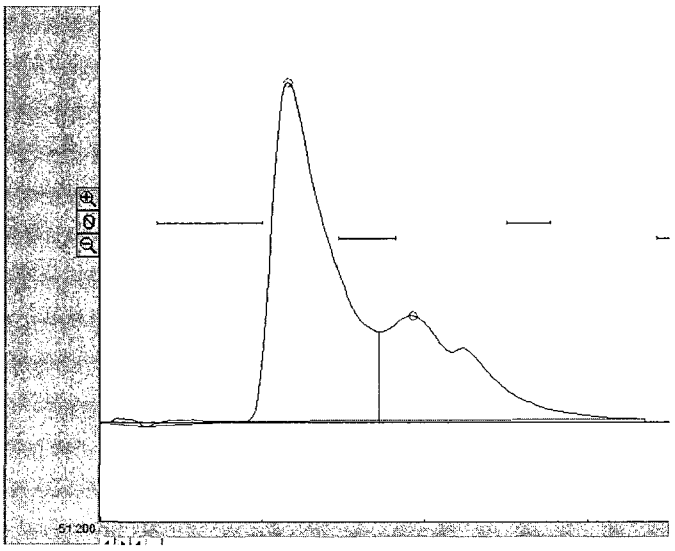

Figure 142: Calibration Curve 80 ppm EE2 in DDW (Replicate 2)

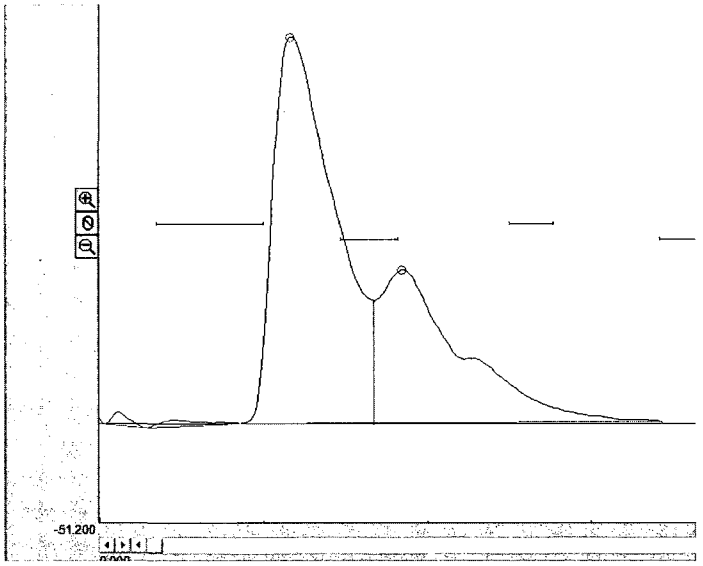

Figure 143: Calibration Curve 100 ppm EE2 in DDW (Replicate 1)

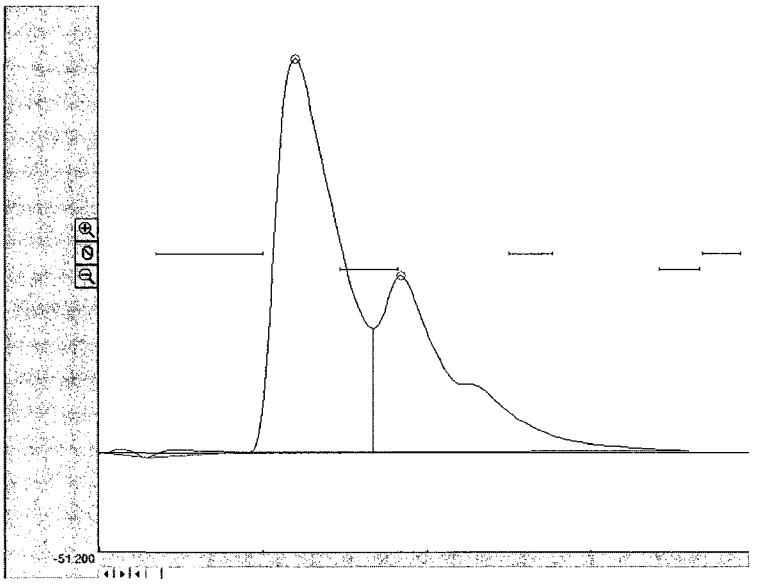

Figure 144: Calibration Curve 100 ppm EE2 in DDW (Replicate 2)

11.5.2 Remaining Concentration for MIP

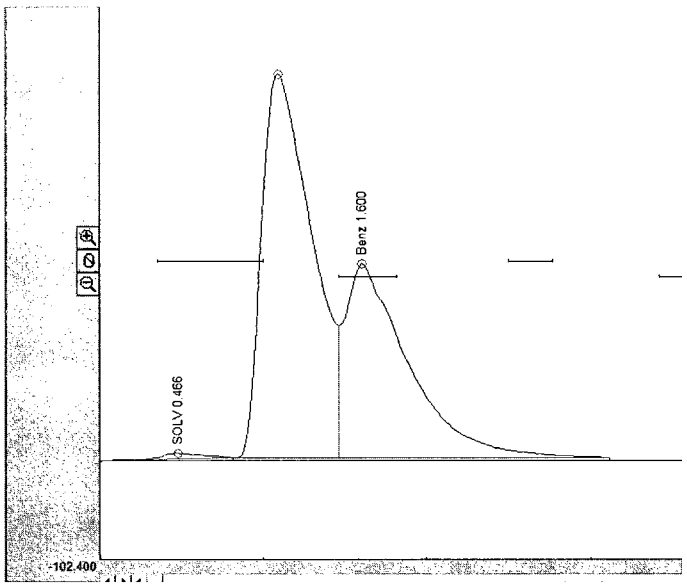

Figure 145: Amount remaining for an initial concentration of 20 ppm for MIP (Replicate 1)

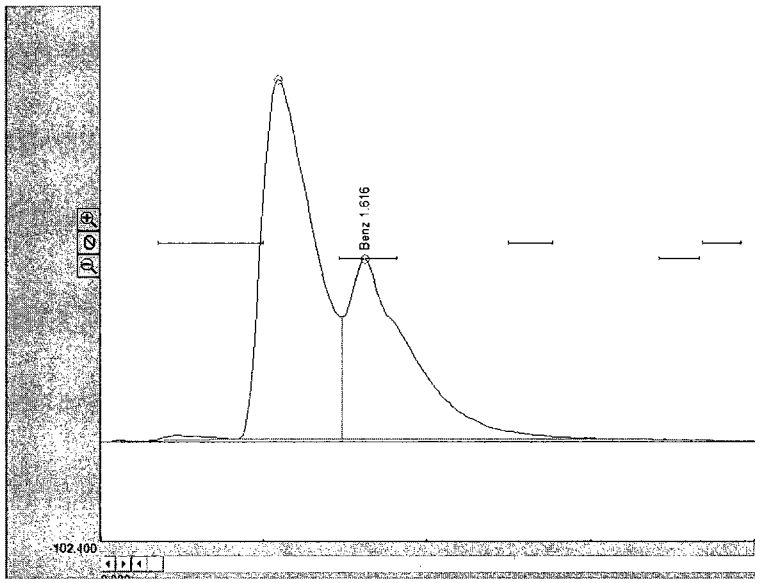

Figure 146: Amount remaining for an initial concentration of 20 ppm for MIP (Replicate 2)

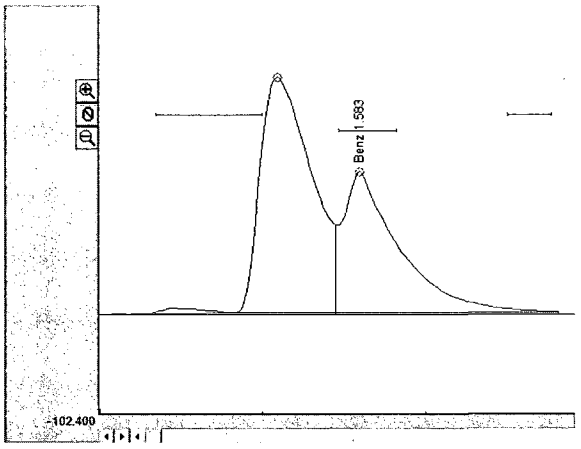

Figure 147: Amount remaining for an initial concentration of 40 ppm for MIP (Replicate 1)

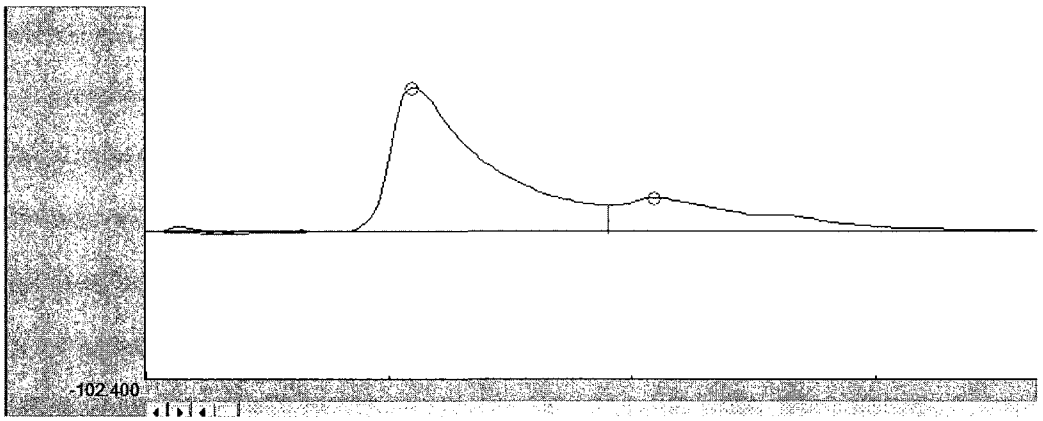

Figure 148: Amount remaining for an initial concentration of 40 ppm for MIP (Replicate 2)

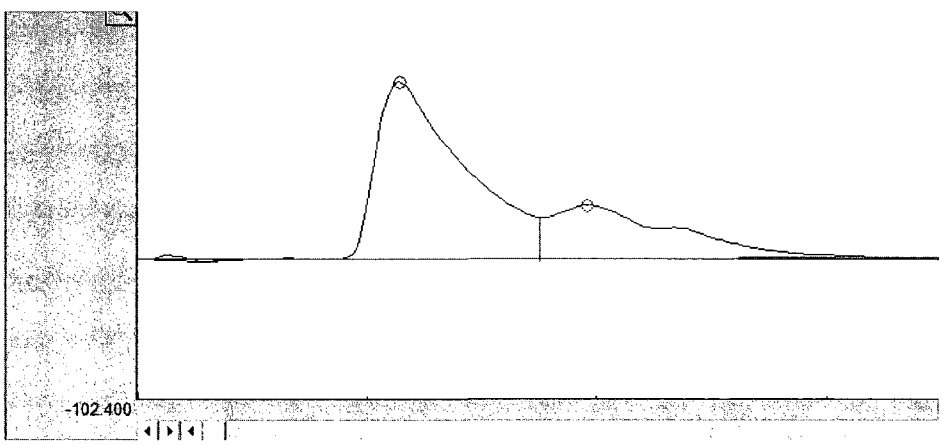

Figure 149: Amount remaining for an initial concentration of 60 ppm for MIP (Replicate 1)

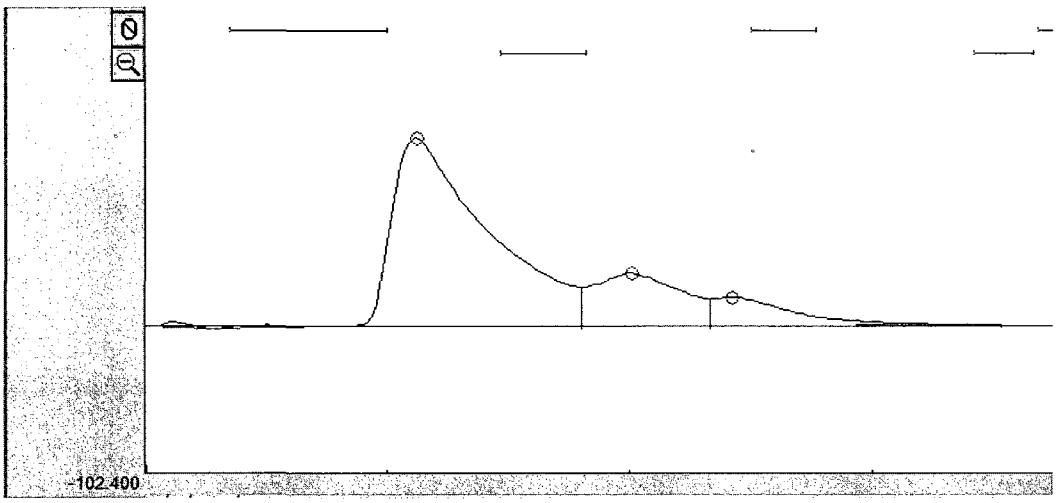

Figure 150: Amount remaining for an initial concentration of 60 ppm for MIP (Replicate 2)

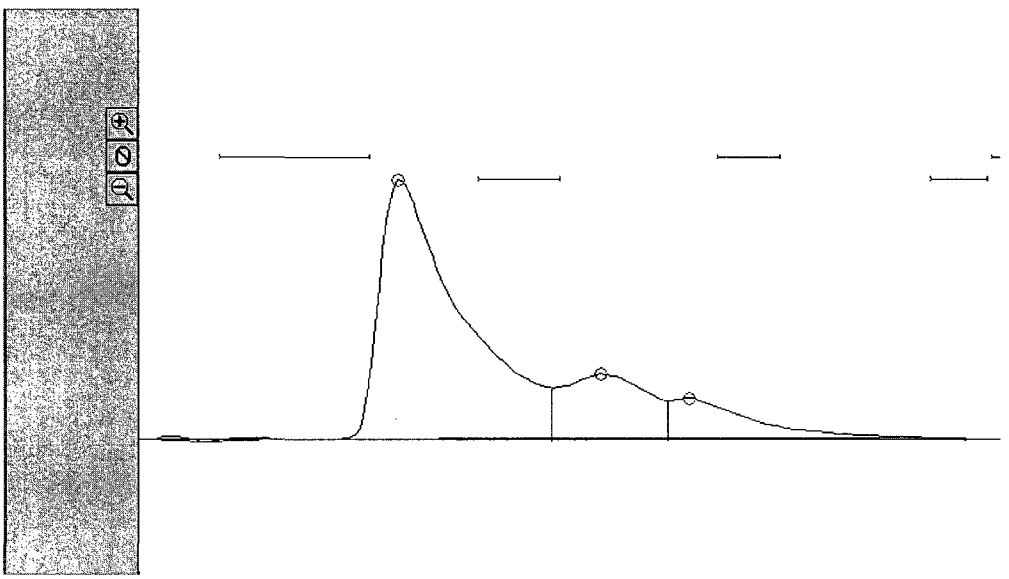

Figure 151: Amount remaining for an initial concentration of 80 ppm for MIP (Replicate 1)

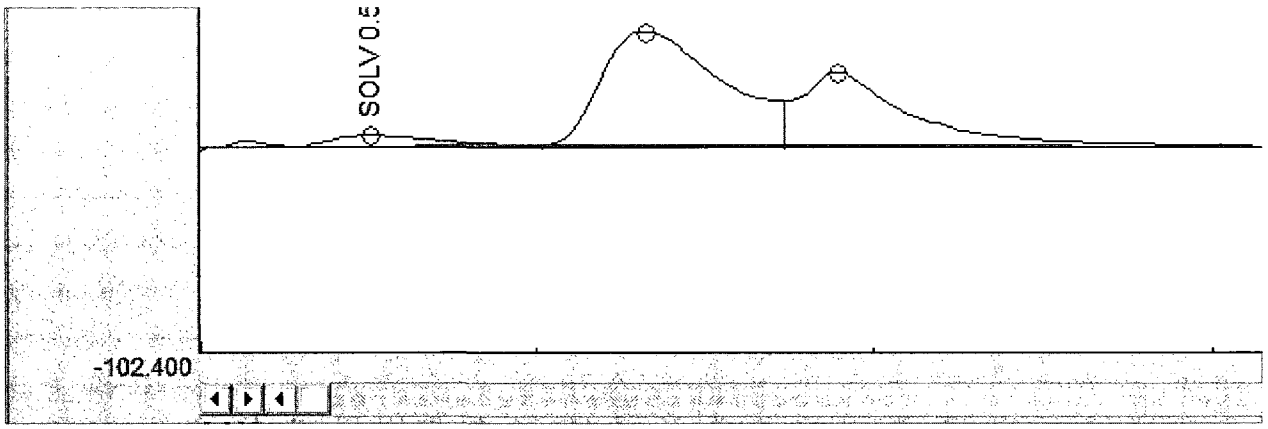

Figure 152: Amount remaining for an initial concentration of 80 ppm for MIP (Replicate 2)

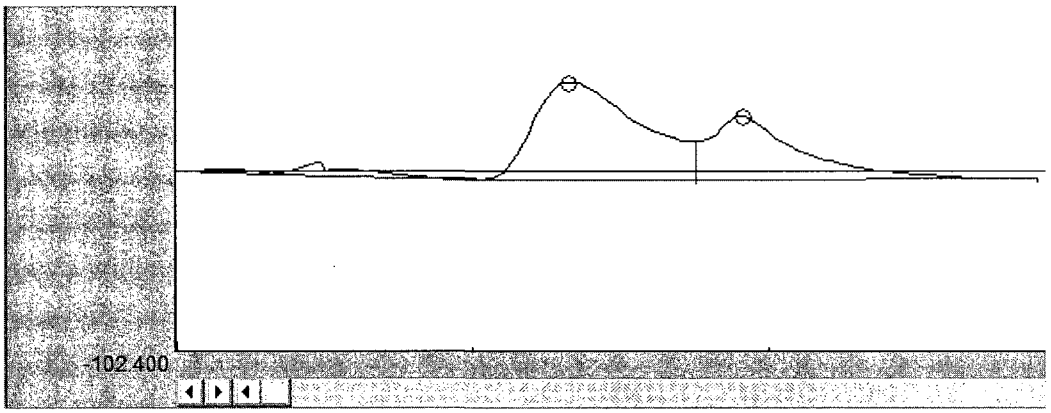

Figure 153: Amount remaining for an initial concentration of 100 ppm for MIP (Replicate 1)

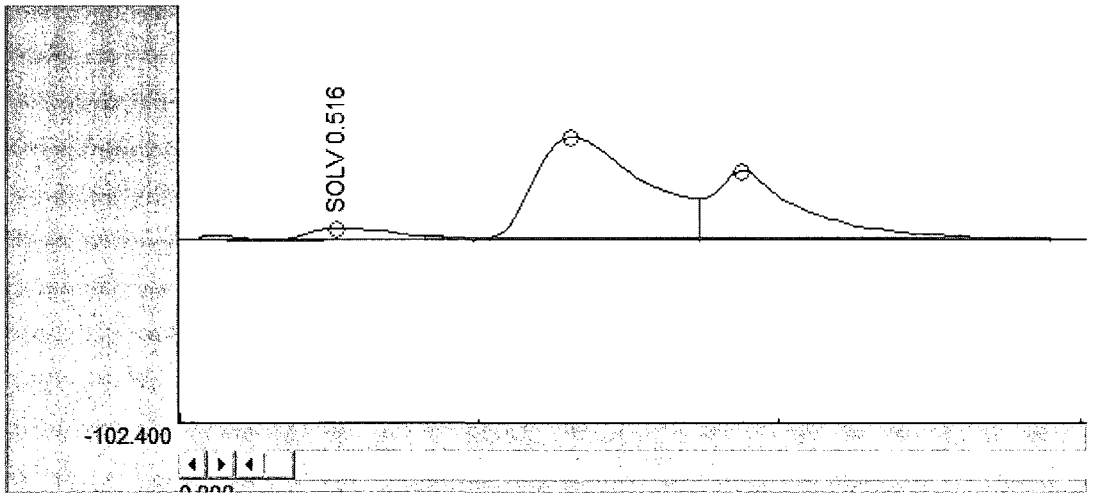

Figure 154: Amount remaining for an initial concentration of 100 ppm for MIP (Replicate 2)

11.5.3 Amount Remaining for NIP

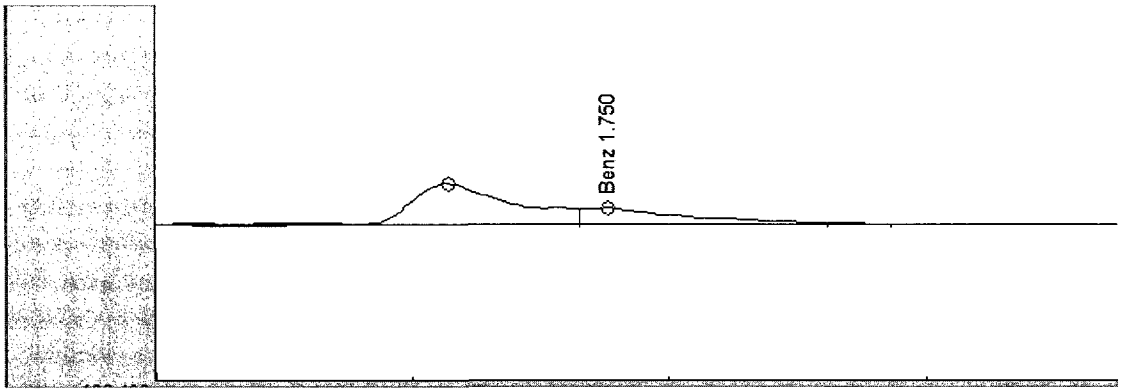

Figure 155: Amount remaining for an initial concentration of 20 ppm for NIP (Replicate 1)

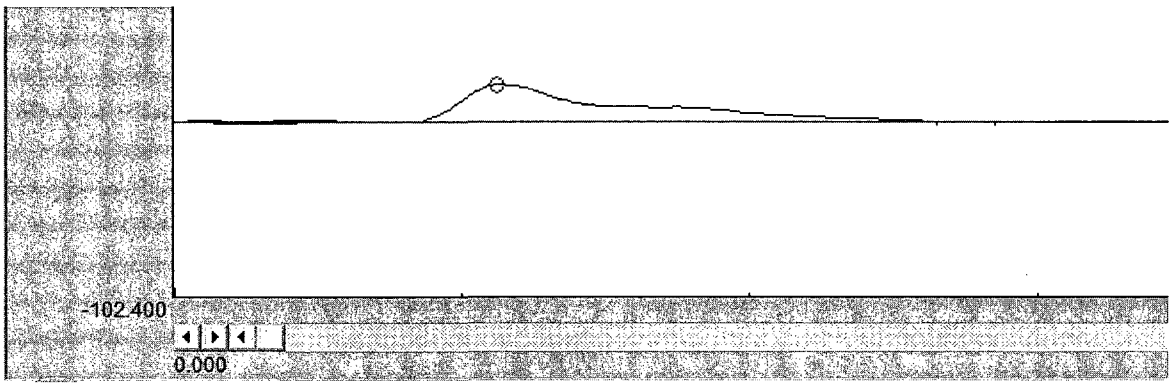

Figure 156: Amount remaining for an initial concentration of 20 ppm for NIP (Replicate 2)

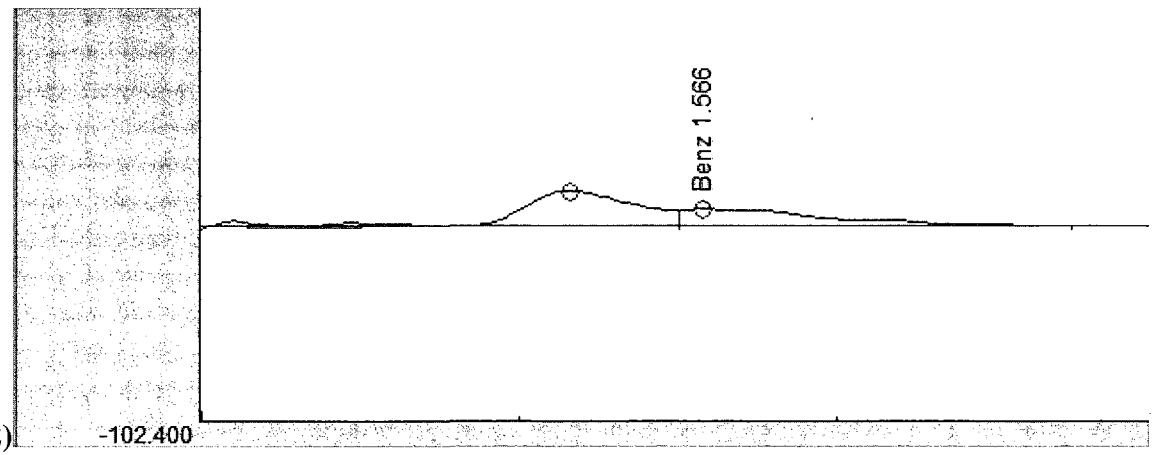

Figure 157: Amount remaining for an initial concentration of 40 ppm for NIP (Replicate 1)

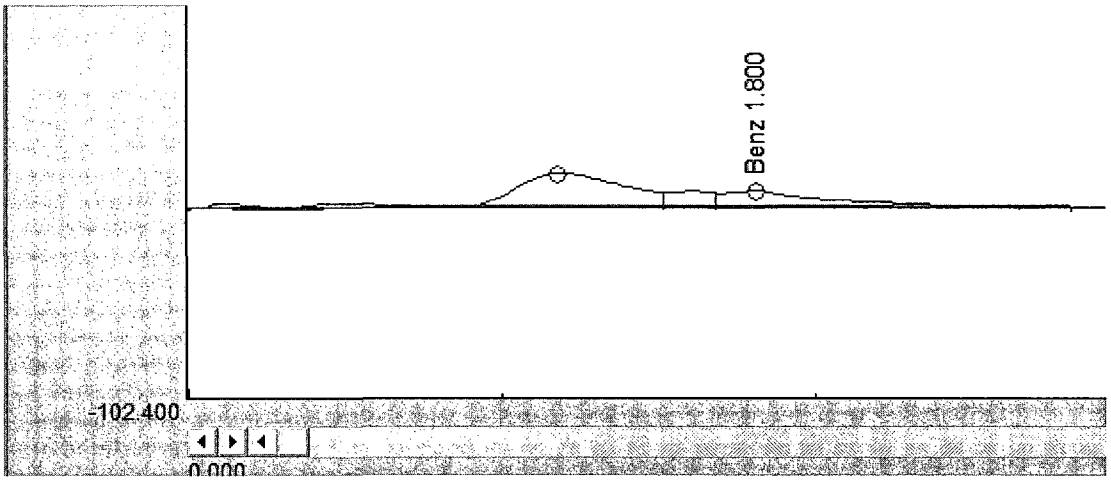

Figure 158: Amount remaining for an initial concentration of 40 ppm for NIP (Replicate 2)

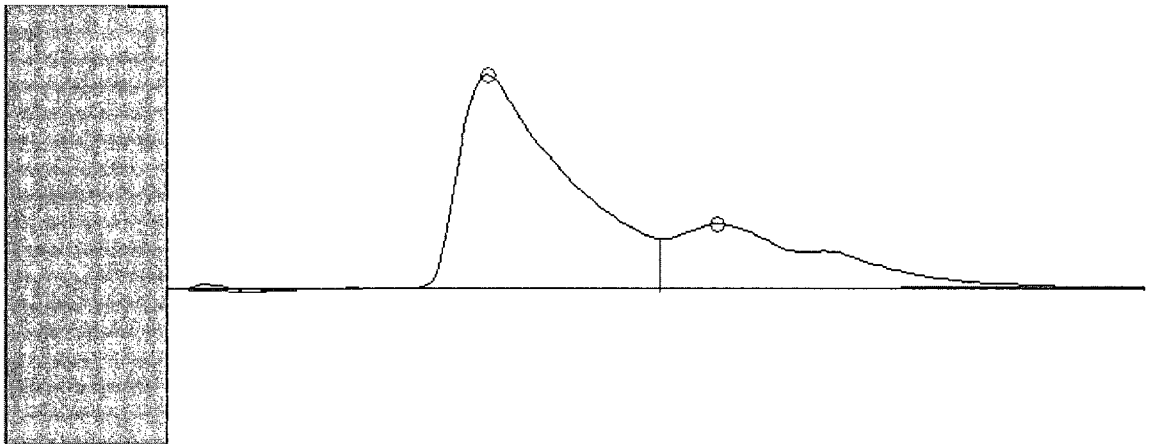

Figure 159: Amount remaining for an initial concentration of 60 ppm for NIP (Replicate 1)

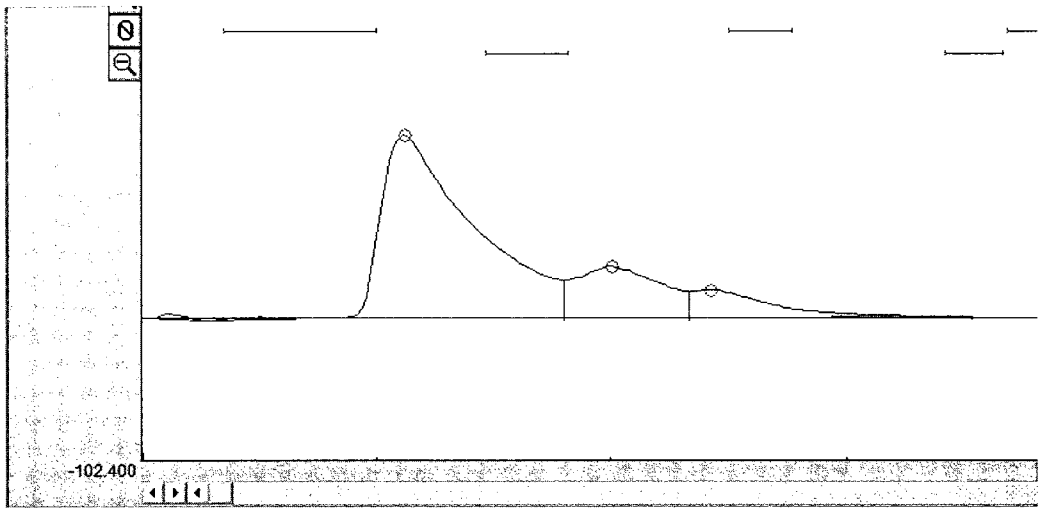

Figure 160: Amount remaining for an initial concentration of 60 ppm for NIP (Replicate 2)

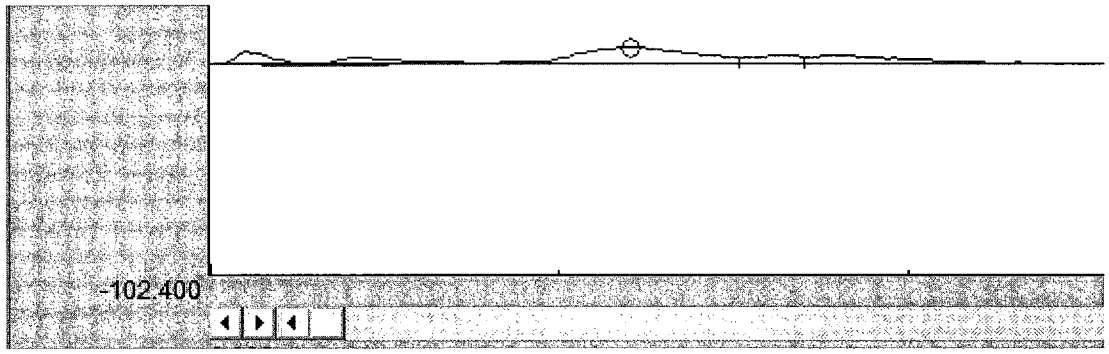

Figure 161: Amount remaining for an initial concentration of 80 ppm for NIP (Replicate 1)

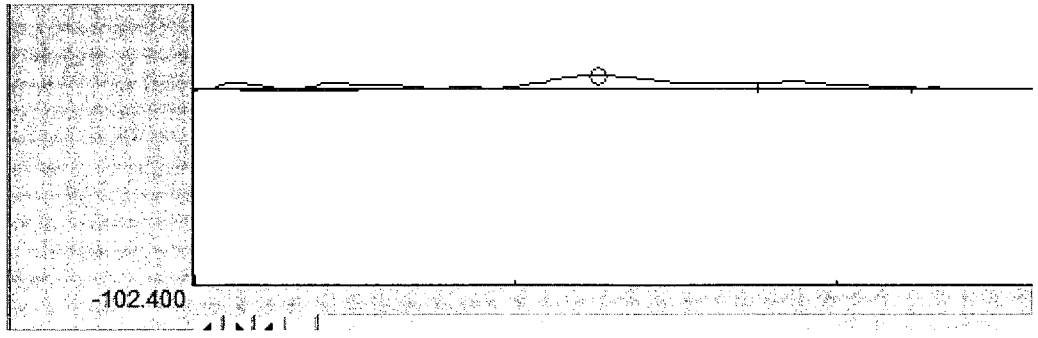

Figure 162: Amount remaining for an initial concentration of 80 ppm for NIP (Replicate 2)

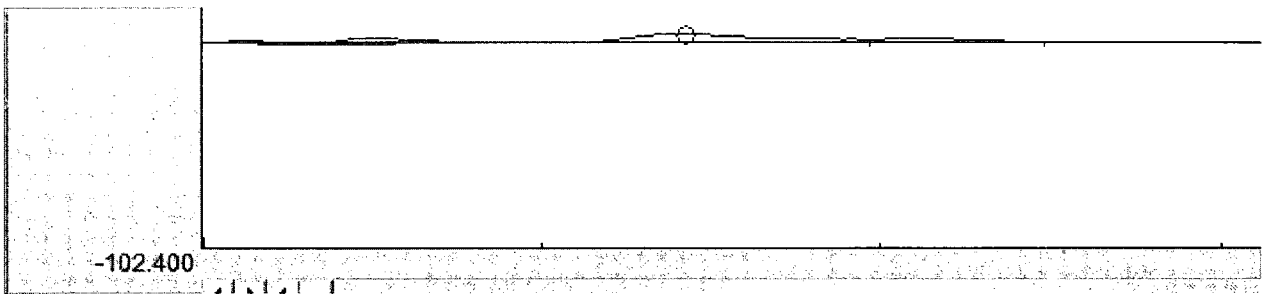

Figure 163: Amount remaining for an initial concentration of 100 ppm for NIP (Replicate 1)

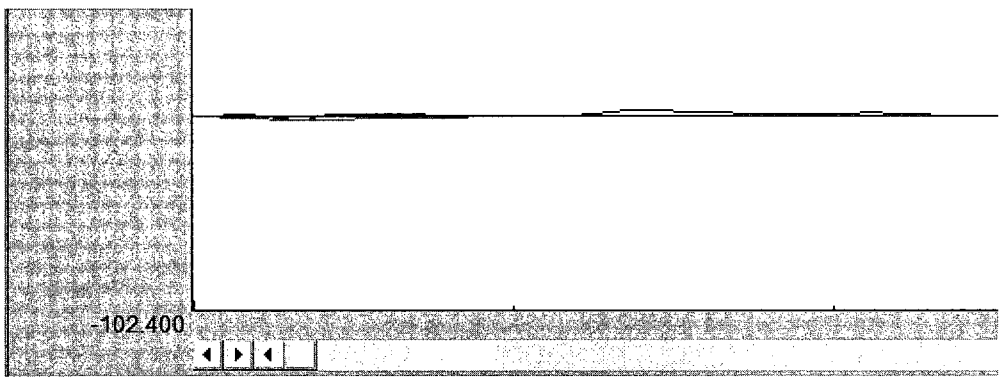

Figure 164: Amount remaining for an initial concentration of 100 ppm for NIP (Replicate 1)

11.6 Treatment of Bisphenol A with NIP

11.6.1 Calibration Curve

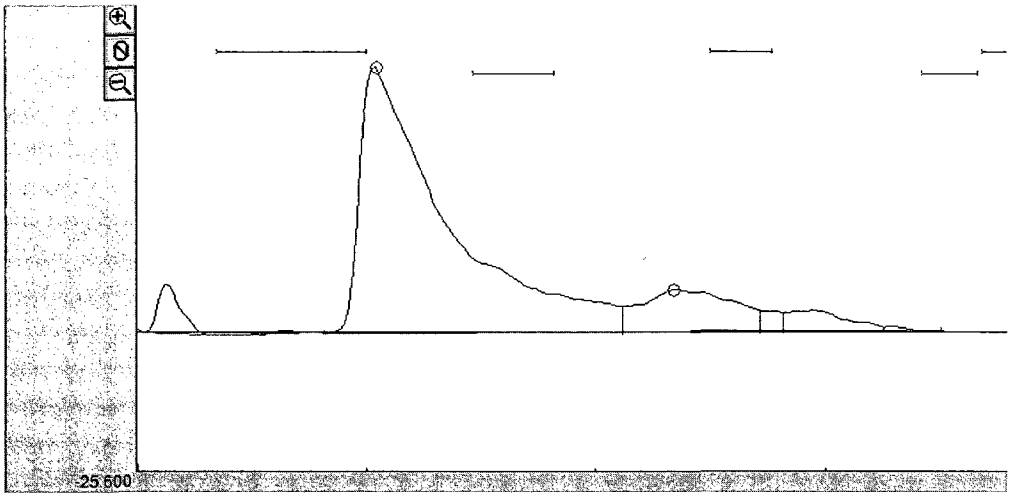

Figure 165: Calibration Curve for 20 ppm Bisphenol A in DDW (Replicate 1)

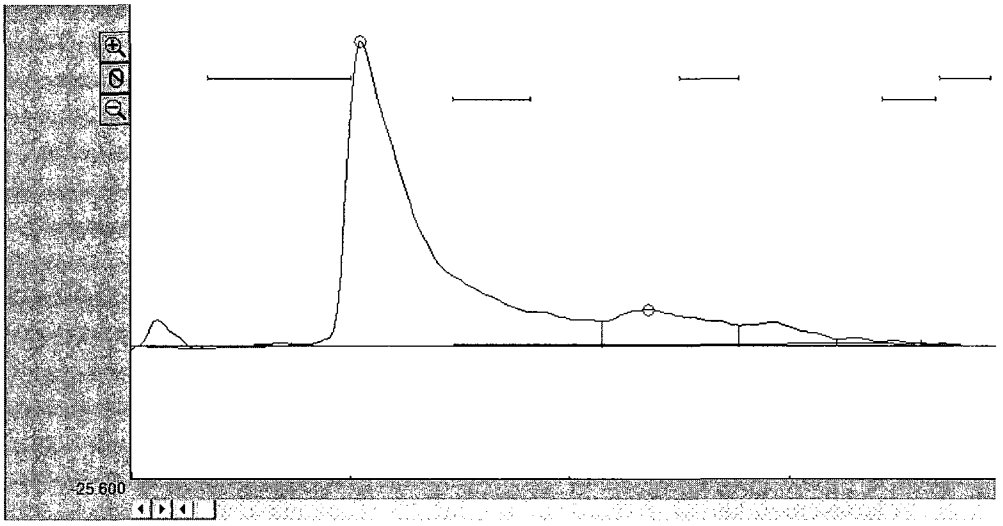

Figure 166: Calibration Curve for 20 ppm Bisphenol A in DDW (Replicate 2)

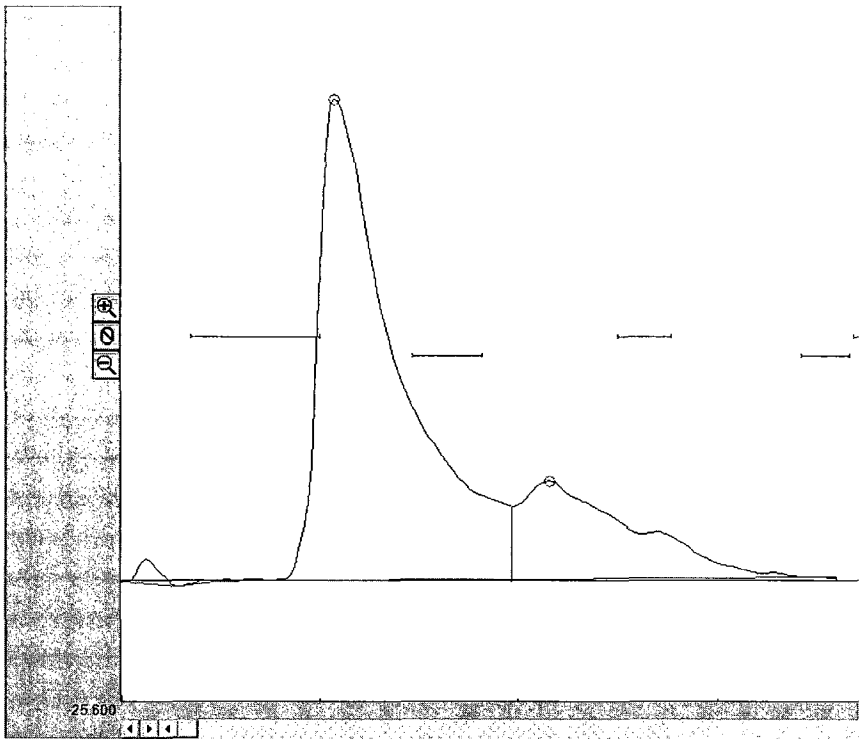

Figure 167: Calibration Curve for 40 ppm Bisphenol A in DDW (Replicate 1)

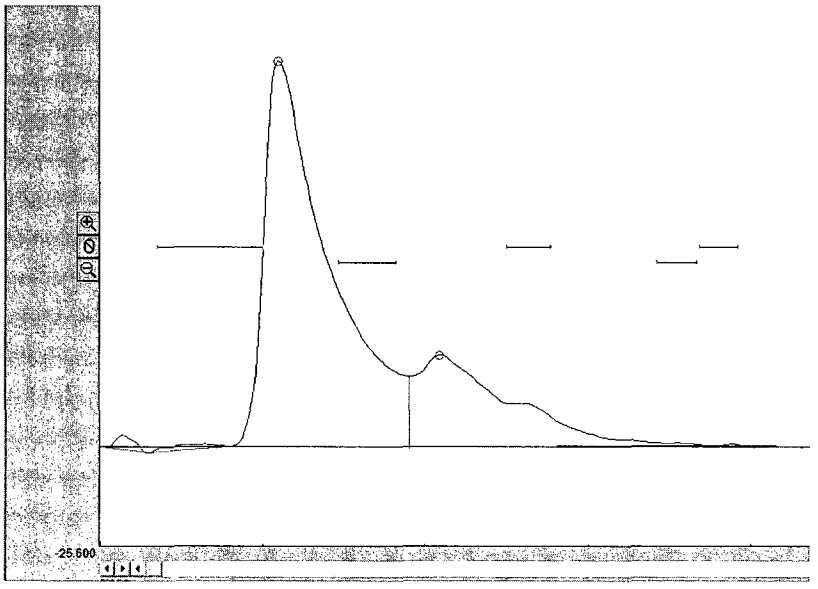

Figure 168: Calibration Curve for 40 ppm Bisphenol A in DDW (Replicate 2)

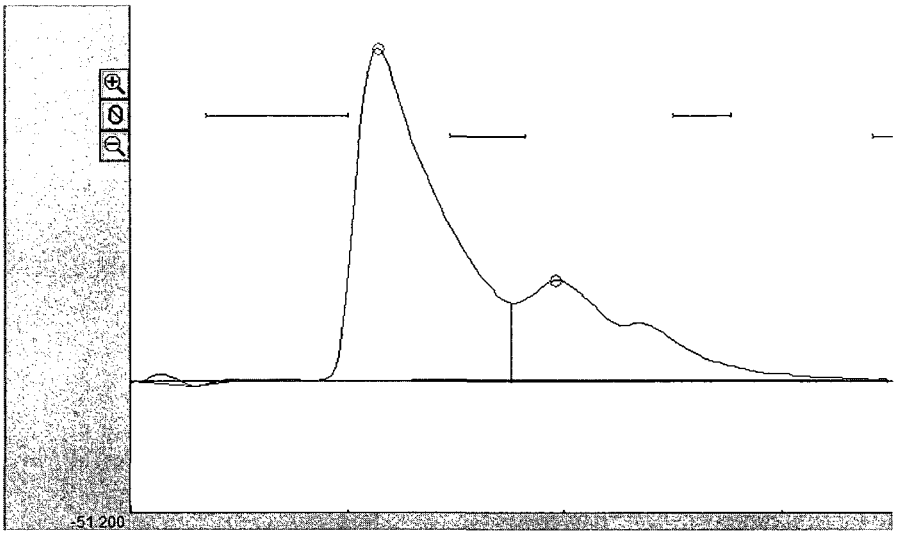

Figure 169: Calibration Curve for 60 ppm Bisphenol A in DDW (Replicate 1)

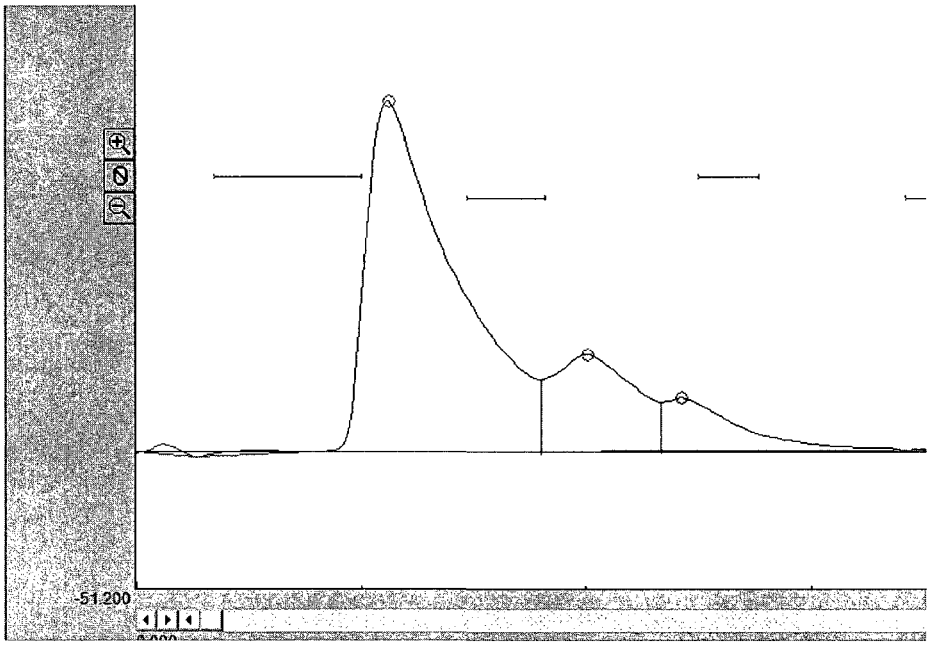

Figure 170: Calibration Curve for 60 ppm Bisphenol A in DDW (Replicate 2)

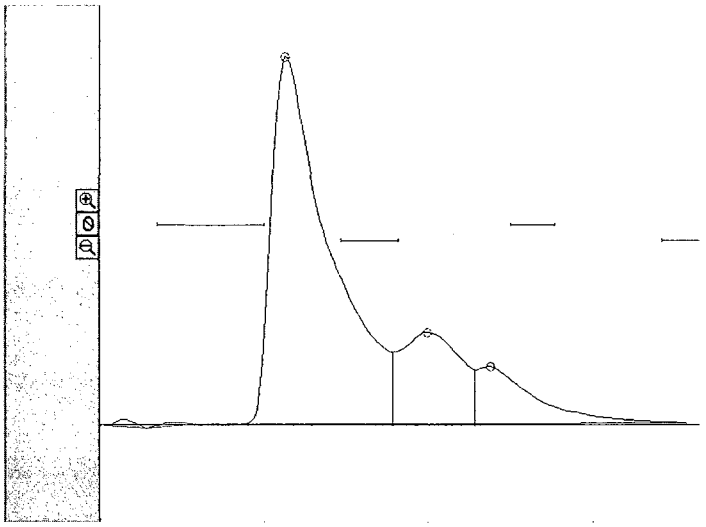

Figure 171: Calibration Curve for 80 ppm Bisphenol A in DDW (Replicate 1)

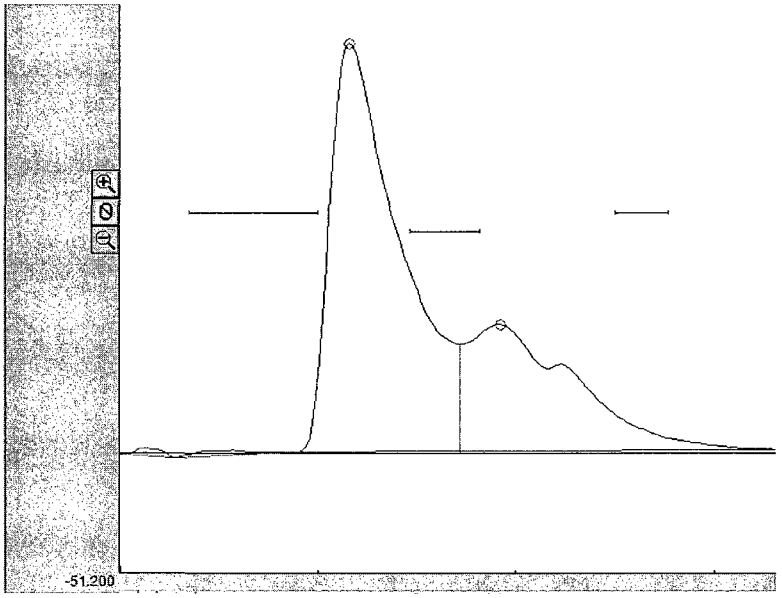

Figure 172: Calibration Curve for 80 ppm Bisphenol A in DDW (Replicate 2)

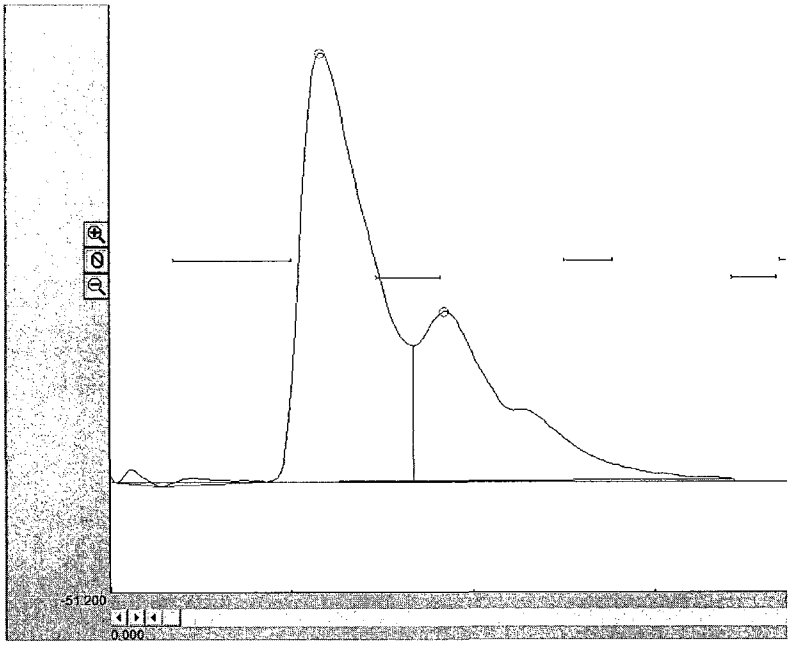

Figure 173: Calibration Curve for 100 ppm Bisphenol A in DDW (Replicate 1)

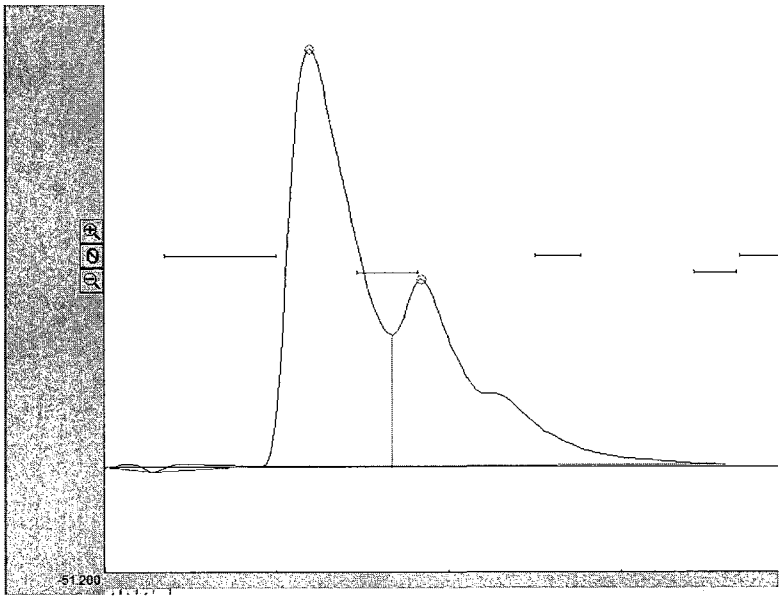

Figure 174: Calibration Curve for 100 ppm Bisphenol A in DDW (Replicate 2)

11.6.2 Remaining Concentration

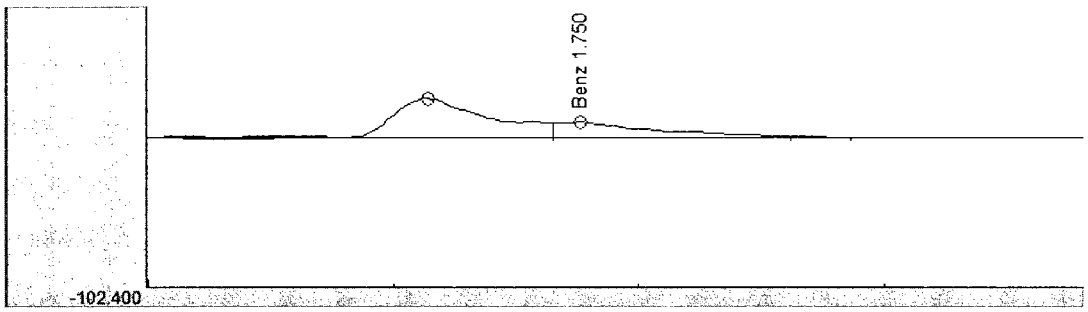

Figure 175: Remaining Bisphenol A in Solution for an Initial Concentration of 20 ppm (Replicate 1)

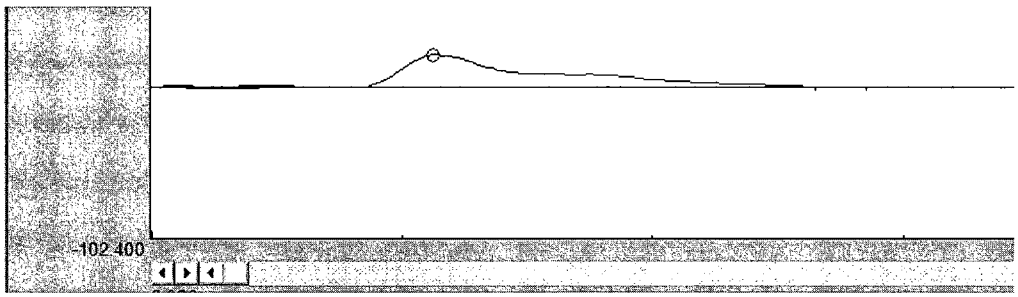

Figure 176: Remaining Bisphenol A in Solution for an Initial Concentration of 20 ppm (Replicate 2)

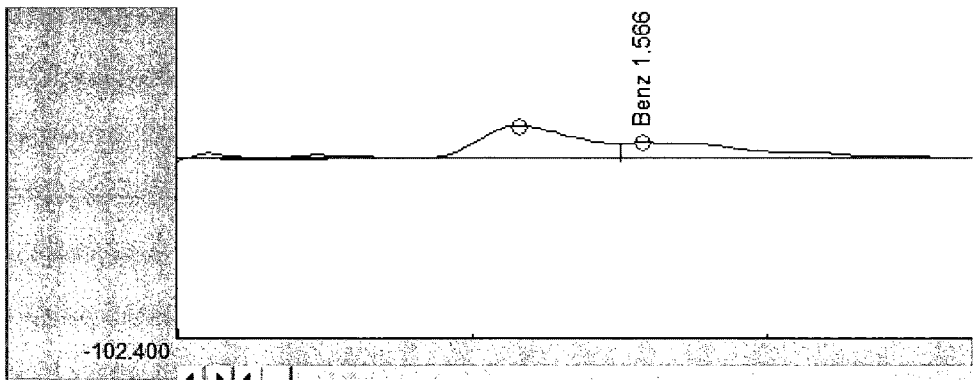

Figure 177: Remaining Bisphenol A in Solution for an Initial Concentration of 40 ppm (Replicate 1)

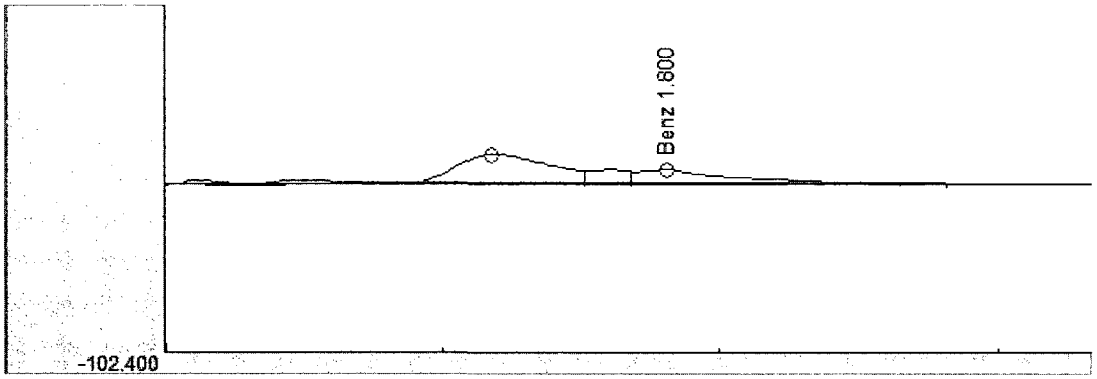

Figure 178: Remaining Bisphenol A in Solution for an Initial Concentration of 40 ppm (Replicate 2)

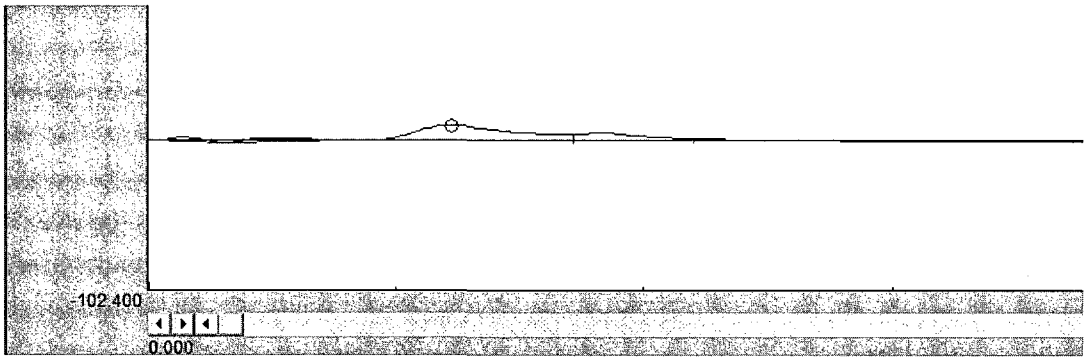

Figure 179: Remaining Bisphenol A in Solution for an Initial Concentration of 60 ppm (Replicate 1)

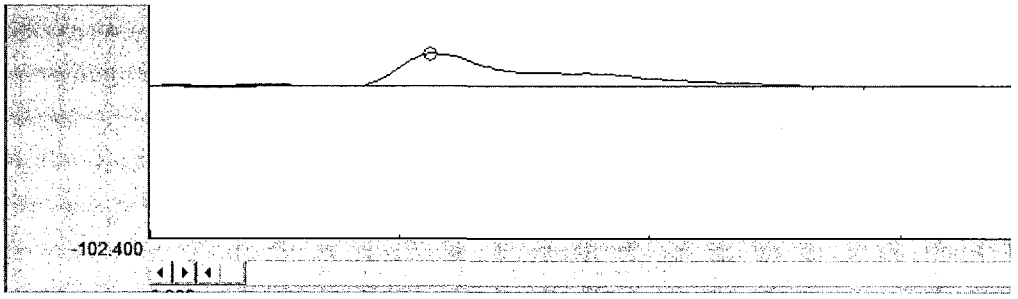

Figure 180: Remaining Bisphenol A in Solution for an Initial Concentration of 60 ppm (Replicate 2)

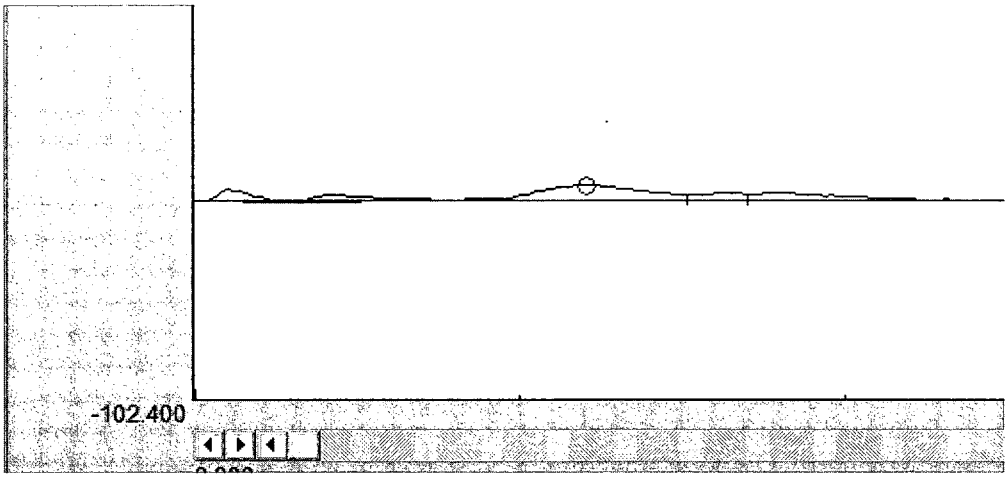

Figure 181: Remaining Bisphenol A in Solution for an Initial Concentration of 80 ppm (Replicate 1)

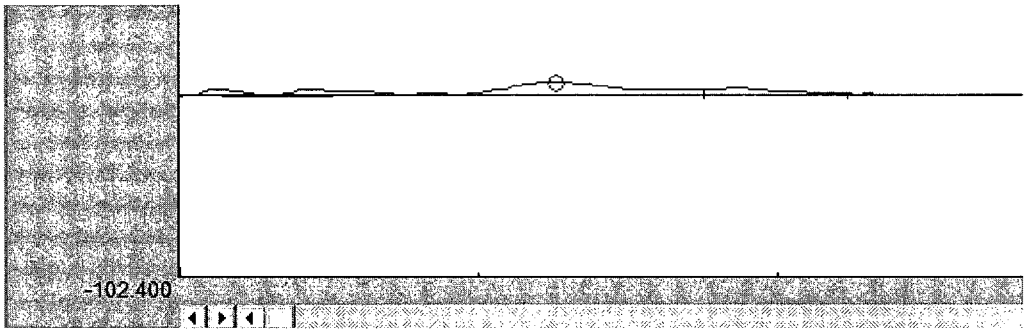

Figure 182: Remaining Bisphenol A in Solution for an Initial Concentration of 80 ppm (Replicate 2)

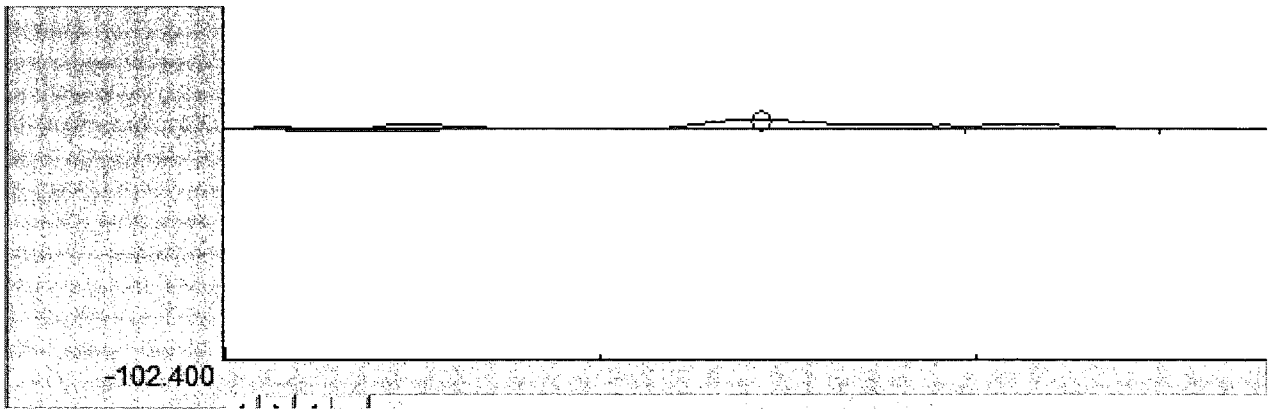

Figure 183: Remaining Bisphenol A in Solution for an Initial Concentration of 100 ppm (Replicate 1)

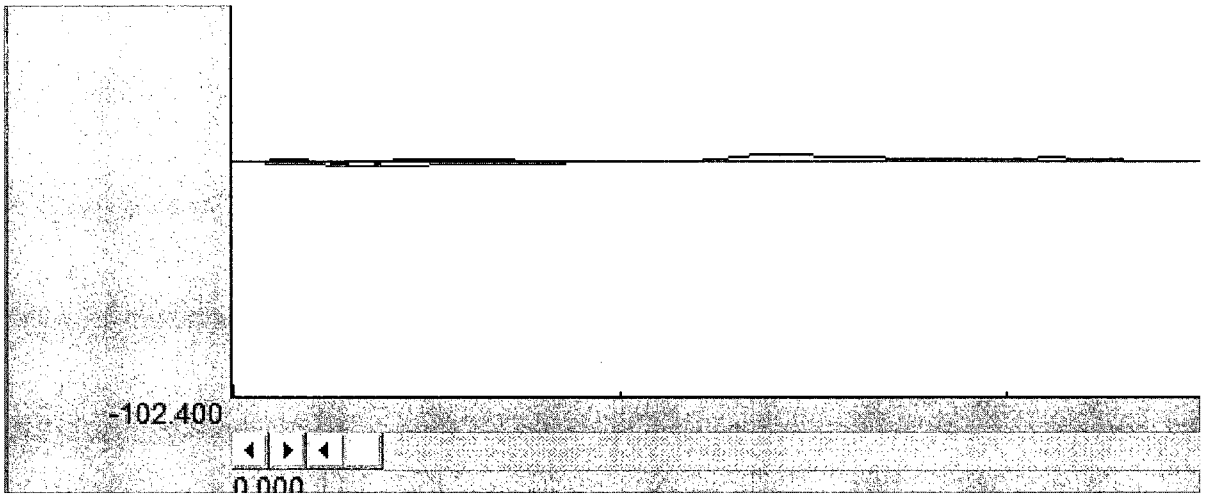

Figure 184: Remaining Bisphenol A in Solution for an Initial Concentration of 100 ppm (Replicate 2)

11.7 Treatment of Atrazine with NIP

11.7.1 Calibration Curve

21/08/08 17:22:25

FILE 1. METHOD 0. RUN 5 INDEX 5

PEAK#	AREA%	RT	AREA	BC
1	6.247	0.26	69854	02
2	6.726	0.43	75219	02
3	52.932	1.	591921	02
4	34.095	1.33	381272	03
TOTAL	100.		1118266	

Figure 185: Calibration curve for 20 ppm atrazine in DDW (Replicate 1)

21/08/08 17:57:35

FILE 1. METHOD 0. RUN 10 INDEX 10

PEAK#	AREA%	RT	AREA	BC
1	2.299	0.44	45004	02
2	0.397	0.78	7762	02
3	58.879	1.04	1152409	02
4	38.425	1.4	752082	03
TOTAL	100.		1957257	

Figure 186: Calibration curve for 20 ppm atrazine in DDW (Replicate 2)

21/08/08 17:38:56

FILE 1. METHOD 0. RUN 7 INDEX 7

PEAK#	AREA%	RT	AREA	BC
1	1.47	0.22	15096	02
2	5.401	0.43	55472	03
3	55.587	1.02	570972	02
4	37.543	1.36	385623	03
TOTAL	100.		1027163	

Figure 187: Calibration curve for 40 ppm atrazine in DDW (Replicate 1)

21/08/08 17:29:28

FILE 1. METHOD 0. RUN 6 INDEX 6

PEAK#	AREA%	RT	AREA	BC
1	3.942	0.15	81427	02
2	61.215	0.93	1264602	02
3	34.844	1.31	719821	03
TOTAL	100.		2065850	

Figure 188: Calibration curve for 40 ppm atrazine in DDW (Replicate 2)

21/08/08 10:03:03

FILE 1. METHOD 0. RUN 11 INDEX 11

PEAK#	AREA%	RT	AREA	BC
1	1.497	0.43	42620	01
2	57.715	1.02	1643399	02
3	40.789	1.35	1161437	03
TOTAL	100.		2847456	

Figure 189: Calibration curve for 60 ppm atrazine in DDW (Replicate 1)

21/08/08 10:09:53

FILE 1. METHOD 0. RUN 12 INDEX 12

PEAK#	AREA%	RT	AREA	BC
1	3.494	0.38	52257	02
2	75.883	0.92	1135047	02
3	20.623	1.24	308472	03
TOTAL	100.		1495776	

Figure 190: Calibration curve for 60 ppm atrazine in DDW (Replicate 2)

21/08/08 10:21:25

FILE 1. METHOD 0. RUN 14 INDEX 14

PEAK#	AREA%	RT	AREA	BC
1	1.005	0.53	38248	01
2	46.472	1.15	1768102	02
3	8.685	1.36	330422	02
4	39.145	1.55	1489335	02
5	3.011	2.03	114569	02
6	1.02	2.32	38818	02
7	0.439	2.44	16716	02
8	0.223	2.58	8479	03
TOTAL	100.		3804689	

Figure 191: Calibration curve for 80 ppm atrazine in DDW (Replicate 1)

21/08/08 19:40:26

FILE 1. METHOD 0. RUN 23 INDEX 23

PEAK#	AREA%	RT	AREA BC
1	0.477	0.10	18117 02
2	52.054	0.69	1979127 02
3	47.47	0.96	1804841 03
TOTAL	100.		3802085

Figure 192: Calibration curve for 80 ppm atrazine in DDW (Replicate 2)

21/08/08 19:33:04

FILE 1. METHOD 0. RUN 22 INDEX 22

PEAK#	AREA%	RT	AREA BC
1	1.298	0.15	65523 02
2	53.674	0.89	2710300 02
3	45.029	1.16	2273754 03
TOTAL	100.		5049577

Figure 193: Calibration curve for 100 ppm atrazine in DDW (Replicate 1)

21/08/08 19:47:56

FILE 1. METHOD 0. RUN 24 INDEX 24

PEAK#	AREA%	RT	AREA BC
1	0.659	0.46	26403 02
2	51.01	0.98	2042722 02
3	48.331	1.26	1935439 03
TOTAL	100.		4004564

Figure 194: Calibration curve for 100 ppm atrazine in DDW (Replicate 2)

11.7.2 Amount Remaining

21/08/08 19:55:16

FILE 1. METHOD 0. RUN 25 INDEX 25

PEAK#	AREA%	RT	AREA BC
1	51.967	0.96	2445908 02
2	48.033	1.24	2260748 03
TOTAL	100.		4706656

Figure 195: Amount remaining for 20 ppm atrazine treated with NIP particles (Replicate 1)

21/08/08 20:00:25

FILE 1. METHOD 0. RUN 26 INDEX 26

PEAK#	AREA%	RT	AREA BC
1	21.462	0.5	68407 01
2	78.538	0.94	250334 01
TOTAL	100.		318741

Figure 196: Amount remaining for 20 ppm atrazine treated with NIP particles (Replicate 2)

21/08/08 20:03:19

FILE 1. METHOD 0. RUN 27 INDEX 27

PEAK#	AREA%	RT	AREA BC
1	7.261	0.37	25125 02
2	23.281	0.47	80564 03
3	39.369	0.9	136237 02
4	30.089	1.18	104124 03
TOTAL	100.		346050

Figure 197: Amount remaining for 40 ppm atrazine treated with NIP particles (Replicate 1)

21/08/08 20:17:30

FILE 1. METHOD 0. RUN 28 INDEX 28

PEAK#	AREA%	RT	AREA BC
1	6.062	0.12	40179 01
2	93.938	0.6	622663 01
TOTAL	100.		662842

Figure 198: Amount remaining for 40 ppm atrazine treated with NIP particles (Replicate 2)

21/08/08 20:22:11

FILE 1. METHOD 0. RUN 29 INDEX 29

PEAK#	AREA%	RT	AREA BC
1	8.072	0.44	56126 02
2	52.261	0.91	363389 02
3	39.35	1.14	273619 03
4	0.317	2.01	2207 01
TOTAL	100.		695341

Figure 199: Amount remaining for 60 ppm atrazine treated with NIP particles (Replicate 1)

21/08/08 20:51:09

FILE 1. METHOD 0. RUN 30 INDEX 30

PEAK#	AREA%	RT	AREA BC
1	7.648	0.43	74944 02
2	49.466	0.88	484726 02
3	42.886	1.11	420246 03
TOTAL	100.		979916

Figure 200: Amount remaining for 60 ppm atrazine treated with NIP particles (Replicate 2)

21/08/08 20:56:37

FILE 1. METHOD 0. RUN 31 INDEX 31

PEAK#	AREA%	RT	AREA BC
1	7.022	0.44	129094 02
2	48.799	0.9	897196 02
3	44.179	1.14	812256 03
TOTAL	100.		1838546

Figure 201: Amount remaining for 80 ppm atrazine treated with NIP particles (Replicate 1)

21/08/08 21:02:53

FILE 1. METHOD 0. RUN 32 INDEX 32

PEAK#	AREA%	RT	AREA BC
1	6.455	0.42	113936 02
2	51.268	0.84	904981 02
3	42.278	1.06	746287 03
TOTAL	100.		1765204

Figure 202: Amount remaining for 80 ppm atrazine treated with NIP particles (Replicate 2)

21/08/08 21:09:21

FILE 1. METHOD 0. RUN 33 INDEX 33

PEAK#	AREA%	RT	AREA BC
1	55.11	0.15	853839 02
2	44.89	0.37	695497 03
TOTAL	100.		1549336

Figure 203: Amount remaining for 100 ppm atrazine treated with NIP particles (Replicate 1)

21/AA/AA 21:14:15

FILE 1. METHOD 0. RUN 34 INDEX 34

PEAK#	AREA%	RT	AREA BC
1	8.293	0.41	197956 02
2	50.859	0.79	1214000 02
3	40.848	1.	975028 03
TOTAL	100.		2386984

Figure 204: Amount remaining for 100 ppm atrazine treated with NIP particles (Replicate 2)

11.8 Treatment of Diethylstilbestrol with NIP

11.8.1 Calibration Curve

22/08/08 01:33:36

FILE 1. METHOD 0. RUN 1 INDEX 1

PEAK#	AREA%	RT	AREA BC
1	75.105	0.19	2691 02
2	24.895	0.27	892 03
TOTAL	100.		3583

Figure 205: Calibration curve for 20 ppm diethylstilbestrol in DDW (Replicate 1)

22/08/08 01:54:27

FILE 1. METHOD 0. RUN 3 INDEX 3

PEAK#	AREA%	RT	AREA BC
1	0.478	0.65	31691 01
2	0.743	1.06	49199 02
3	60.453	2.28	4005418 02
4	35.065	3.18	2323255 02
5	3.261	3.95	216069 03
TOTAL	100.		6625632

Figure 206: Calibration curve for 20 ppm diethylstilbestrol in DDW (Replicate 1)

22/08/08 01:41:42

FILE 1. METHOD 0. RUN 2 INDEX 2

PEAK#	AREA%	RT	AREA	BC
1	1.779	0.42	31591	01
2	62.373	1.6	1107412	02
3	35.847	2.32	636451	03
TOTAL	100.		1775454	

Figure 207: Calibration curve for 40 ppm diethylstilbestrol in DDW (Replicate 1)

22/08/08 02:08:51

FILE 1. METHOD 0. RUN 4 INDEX 4

PEAK#	AREA%	RT	AREA	BC
1	2.553	0.57	25988	01
2	94.283	2.1	959742	02
3	3.164	3.05	32206	03
TOTAL	100.		1017936	

Figure 208: Calibration curve for 40 ppm diethylstilbestrol in DDW (Replicate 2)

22/08/08 02:41:23

FILE 1. METHOD 0. RUN 5 INDEX 5

PEAK#	AREA%	RT	AREA	BC
1	0.244	0.52	16333	01
2	1.217	1.06	81499	02
3	63.73	2.14	4267854	02
4	32.514	3.06	2177384	02
5	2.296	3.8	153752	03
TOTAL	100.		6696822	

Figure 209: Calibration curve for 60 ppm diethylstilbestrol in DDW (Replicate 1)

00/00/00 02:52:49

FILE 1. METHOD 0. RUN 6 INDEX 6

PEAK#	AREA%	RT	AREA	BC
1	0.293	0.46	12760	01
2	64.572	1.88	2811889	02
3	35.134	2.7	1529975	03
TOTAL	100.		4354624	

Figure 210: Calibration curve for 60 ppm diethylstilbestrol in DDW (Replicate 2)

22/00/00 03:25:31

FILE 1. METHOD 0. RUN 9 INDEX 9

PEAK#	AREA%	RT	AREA	BC
1	1.644	0.49	174747	02
2	63.873	2.02	6787628	02
3	34.483	2.9	3664397	03
TOTAL	100.		10626772	

Figure 211: Calibration curve for 80 ppm diethylstilbestrol in DDW (Replicate 1)

22/00/00 03:36:31

FILE 1. METHOD 0. RUN 10 INDEX 10

PEAK#	AREA%	RT	AREA	BC
1	1.597	0.51	153023	02
2	63.2	1.99	6057352	02
3	35.203	2.85	3374034	03
TOTAL	100.		9584409	

Figure 212: Calibration curve for 80 ppm diethylstilbestrol in DDW (Replicate 2)

22/08/08 03:04:02

FILE 1. METHOD 0. RUN 7 INDEX 7

PEAK#	AREA%	RT	AREA	BC
1	1.773	0.48	69795	01
2	61.402	1.82	2416541	02
3	36.824	2.59	1449253	03
TOTAL	100.		3935589	

Figure 213: Calibration curve for 100 ppm diethylstilbestrol in DDW (Replicate 1)

22/08/08 03:17:51

FILE 1. METHOD 0. RUN 8 INDEX 8

PEAK#	AREA%	RT	AREA	BC
1	1.706	0.5	179284	02
2	61.518	1.99	6465688	02
3	36.777	2.86	3865324	03
TOTAL	100.		10510296	

Figure 214: Calibration curve for 100 ppm diethylstilbestrol in DDW (Replicate 2)

11.8.2 Amount Remaining

22/08/08 03:46:14

FILE 1. METHOD 0. RUN 11 INDEX 11

PEAK#	AREA%	RT	AREA	BC
1	0.462	0.48	42594	01
2	64.175	1.76	5922999	02
3	35.364	2.55	3263863	03
TOTAL	100.		9229456	

Figure 215: Amount remaining for 20 ppm diethylstilbestrol treated with NIP particles (Replicate 1)

22/08/08 03:56:08

FILE 1. METHOD 0. RUN 12 INDEX 12

PEAK#	AREA%	RT	AREA	BC
1	13.247	0.49	177268	01
2	58.454	1.84	782221	02
3	28.299	2.63	378686	03
TOTAL	100.		1338175	

Figure 216: Amount remaining for 20 ppm diethylstilbestrol treated with NIP particles (Replicate 2)

22/08/08 04:17:02

FILE 1. METHOD 0. RUN 13 INDEX 13

PEAK#	AREA%	RT	AREA	BC
1	0.666	0.39	8182	02
2	5.2	0.57	63865	03
3	59.097	1.81	725788	02
4	35.037	2.54	430297	03
TOTAL	100.		1228132	

Figure 217: Amount remaining for 40 ppm diethylstilbestrol treated with NIP particles (Replicate 1)

22/08/08 04:34:43

FILE 1. METHOD 0. RUN 14 INDEX 14

PEAK#	AREA%	RT	AREA	BC
1	0.418	0.34	15718	02
2	2.864	0.46	107745	03
3	60.919	1.89	2292146	02
4	35.8	2.6	1346997	03
TOTAL	100.		3762606	

Figure 218: Amount remaining for 40 ppm diethylstilbestrol treated with NIP particles (Replicate 2)

22/08/08 04:44:44

FILE 1. METHOD 0. RUN 15 INDEX 15

PEAK#	AREA%	RT	AREA BC
1	4.268	0.5	161910 01
2	59.935	1.89	2273840 02
3	35.798	2.69	1358116 03
TOTAL	100.		3793866

Figure 219: Amount remaining for 60 ppm diethylstilbestrol treated with NIP particles (Replicate 1)

22/08/08 04:55:18

FILE 1. METHOD 0. RUN 16 INDEX 16

PEAK#	AREA%	RT	AREA BC
1	4.664	0.44	112183 01
2	73.823	1.73	1775600 02
3	21.512	2.81	517418 03
TOTAL	100.		2405201

Figure 220: Amount remaining for 60 ppm diethylstilbestrol treated with NIP particles (Replicate 2)

22/08/08 05:04:25

FILE 1. METHOD 0. RUN 17 INDEX 17

PEAK#	AREA%	RT	AREA	BC
1	4.011	0.45	95253	01
2	63.275	1.8	1502767	02
3	32.714	2.76	776960	03
TOTAL	100.		2374980	

Figure 221: Amount remaining for 80 ppm diethylstilbestrol treated with NIP particles (Replicate 1)

22/08/08 05:14:29

FILE 1. METHOD 0. RUN 18 INDEX 18

PEAK#	AREA%	RT	AREA	BC
1	6.259	0.48	385730	02
2	56.768	1.89	3498551	02
3	36.973	2.76	2278643	03
TOTAL	100.		6162924	

Figure 222: Amount remaining for 80 ppm diethylstilbestrol treated with NIP particles (Replicate 2)

22/08/08 05:24:01

FILE 1. METHOD 0. RUN 19 INDEX 19

PEAK#	AREA%	RT	AREA	BC
1	0.362	0.29	20565	02
2	5.911	0.43	336047	02
3	56.88	1.83	3233809	02
4	36.848	2.66	2094917	03
TOTAL	100.		5685338	

Figure 223: Amount remaining for 100 ppm diethylstilbestrol treated with NIP particles (Replicate 1)

22/AA/AA A5:33:02

FILE 1. METHOD 0. RUN 20 INDEX 20

PEAK#	AREA%	RT	AREA	BC
1	2.548	0.44	187197	01
2	63.29	1.82	4649533	02
3	34.162	2.59	2509703	03
TOTAL	100.		7346433	

Figure 224: Amount remaining for 100 ppm diethylstilbestrol treated with NIP particles (Replicate 2)

11.9 Treatment of Secondary Wastewater Effluent Spiked with E2

11.10 Calibration Curve:

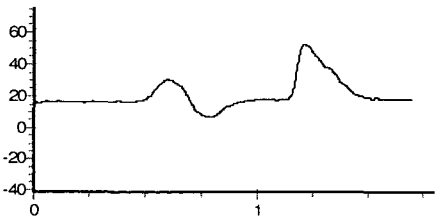

Figure 225: Wastewater spiked with 1.2 ppm E2 (Replicate 1)

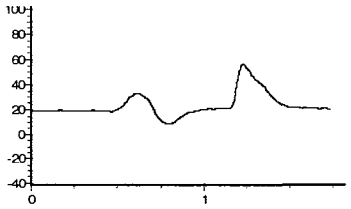

Figure 226: Wastewater spiked with 1.2 ppm E2 (Replicate 2)

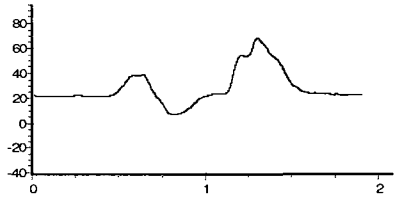

Figure 227: Wastewater spiked with 1.4 ppm E2 (Replicate 1)

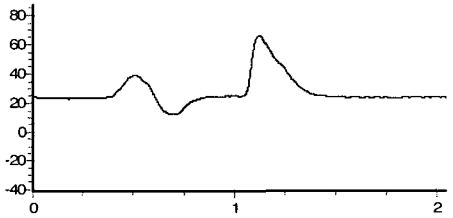

Figure 228: Wastewater spiked with 1.4 ppm E2 (Replicate 1)

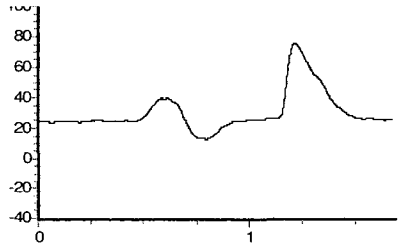

Figure 229: Wastewater spiked with 1.6 ppm E2 (Replicate 1)

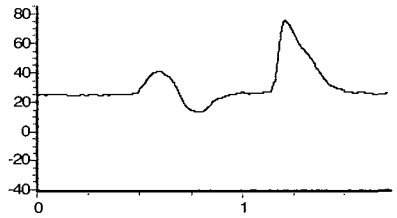

Figure 230: Wastewater spiked with 1.6 ppm E2 (Replicate 2)

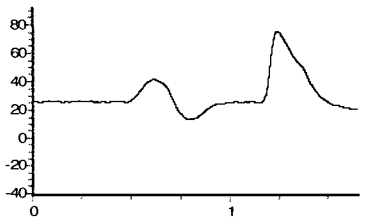

Figure 231: Wastewater spiked with 1.8 ppm E2 (Replicate 1)

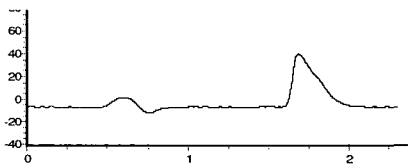

Figure 232: Wastewater spiked with 1.8 ppm E2 (Replicate 2)

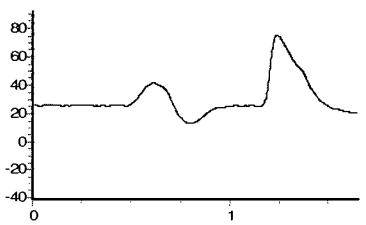

Figure 233: Wastewater spiked with 2 ppm E2 (Replicate 1)

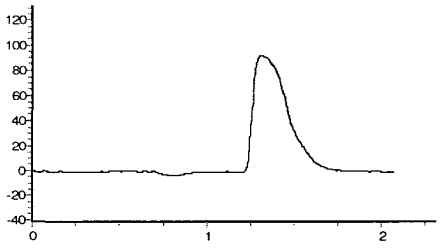

Figure 234: Wastewater spiked with 2 ppm E2 (Replicate 2)

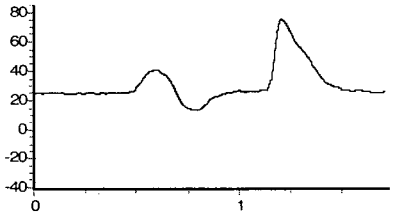

Figure 235: Wastewater spiked with 4 ppm E2 (Replicate 1)

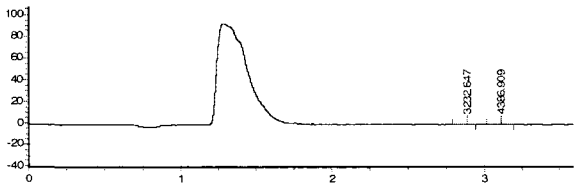

Figure 236: Wastewater spiked with 4 ppm E2 (Replicate 2)

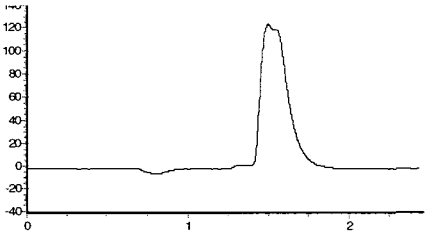

Figure 237: Wastewater spiked with 6 ppm E2 (Replicate 1)

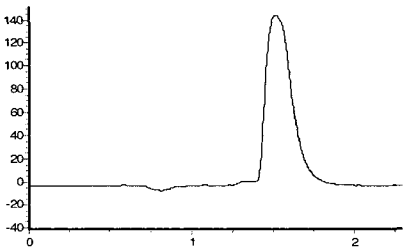

Figure 238: Wastewater spiked with 6 ppm E2 (Replicate 2)

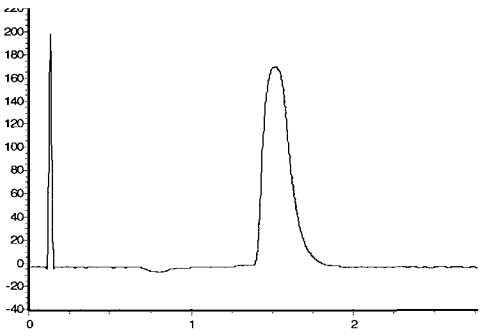

Figure 239: Wastewater spiked with 8 ppm E2 (Replicate 1)

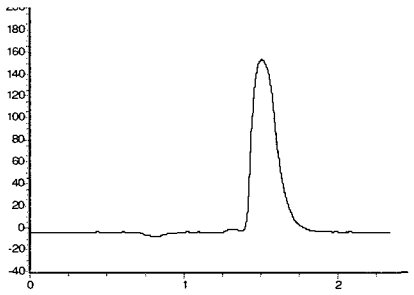

Figure 240: Wastewater spiked with 8 ppm E2 (Replicate 2)

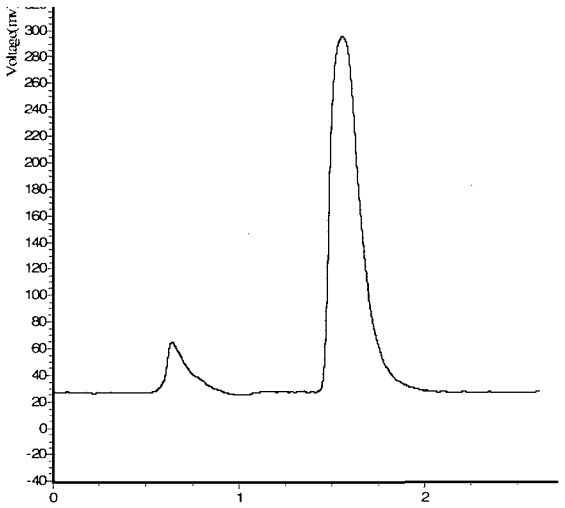

Figure 241: Wastewater spiked with 10 ppm E2 (Replicate 1)

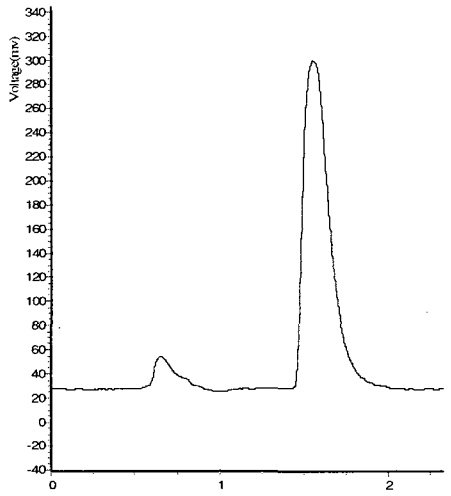

Figure 242: Wastewater spiked with 10 ppm E2 (Replicate 2)

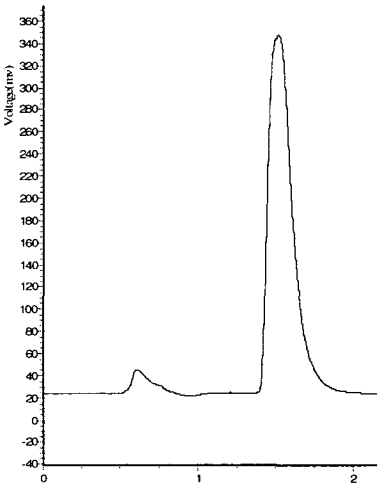

Figure 243: Wastewater spiked with 12 ppm E2 (Replicate 1)

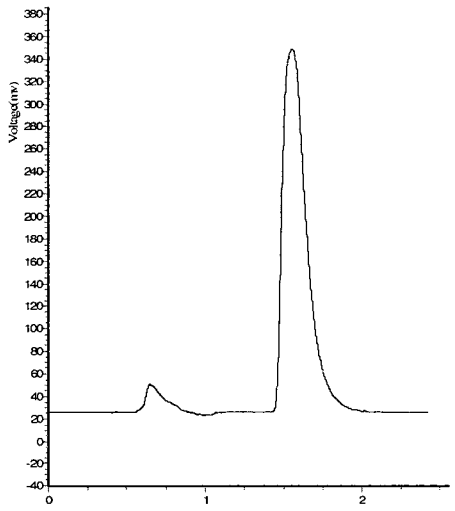

Figure 244: Wastewater spiked with 12 ppm E2 (Replicate 2)

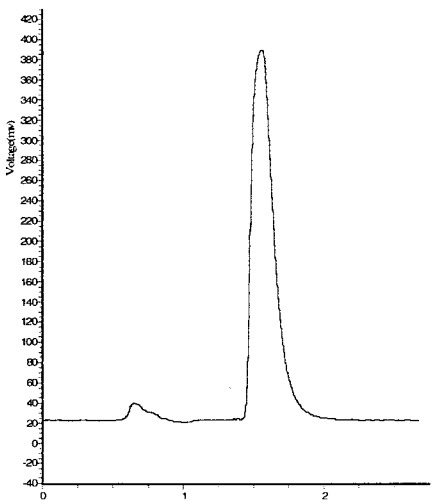

Figure 245: Wastewater spiked with 14 ppm E2 (Replicate 1)

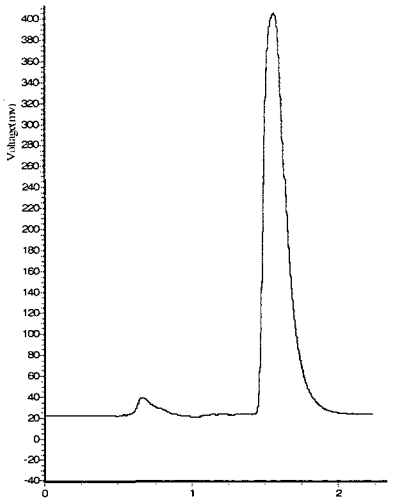

Figure 246: Wastewater spiked with 14 ppm E2 (Replicate 2)

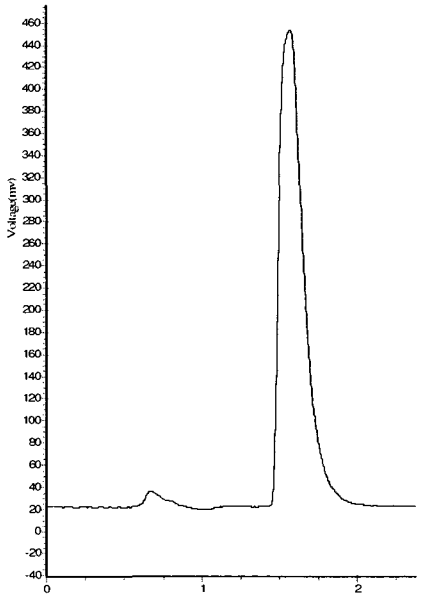

Figure 247: Wastewater spiked with 16 ppm E2 (Replicate 1)

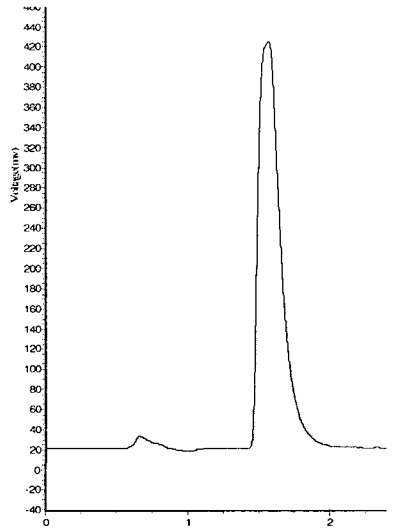

Figure 248: Wastewater spiked with 16 ppm E2 (Replicate 2)

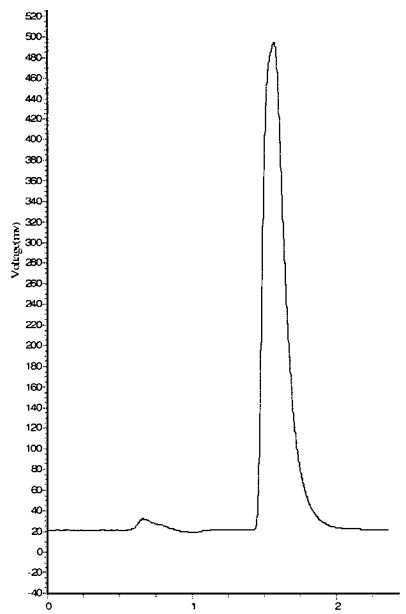

Figure 249: Wastewater spiked with 18 ppm E2 (Replicate 1)

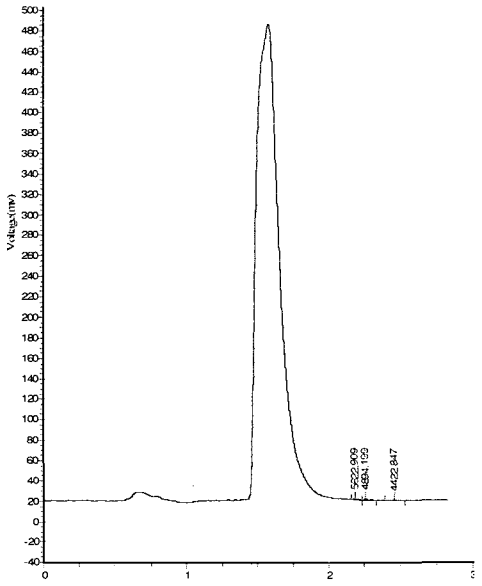

Figure 250: Wastewater spiked with 18 ppm E2 (Replicate 2)

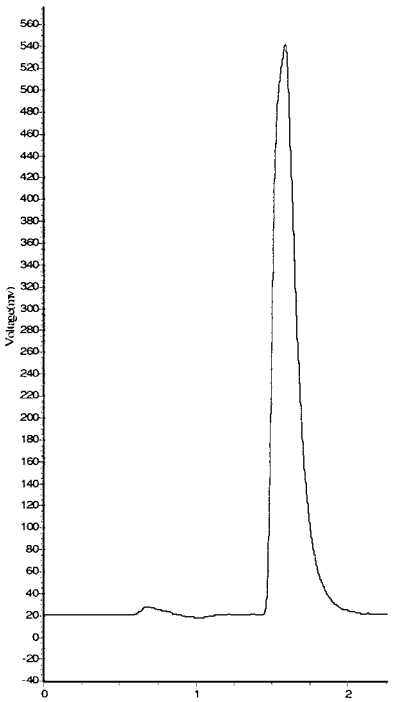

Figure 251: Wastewater spiked with 20 ppm E2 (Replicate 1)

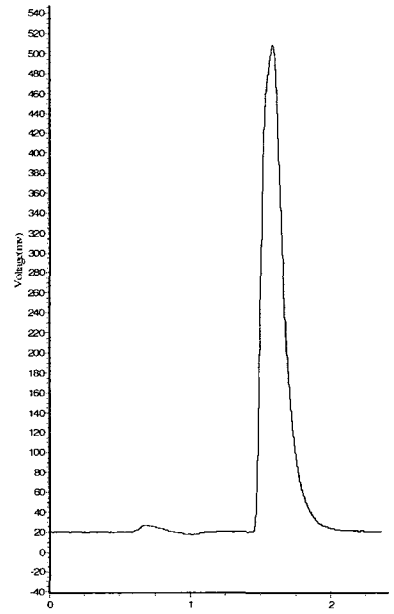

Figure 252: Wastewater spiked with 20 ppm E2 (Replicate 2)

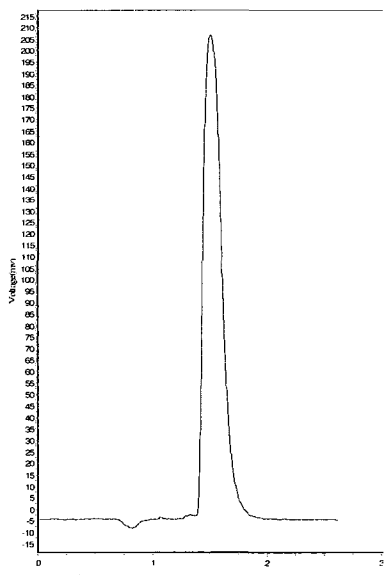

Figure 253: Wastewater spiked with 20 ppm E2 (Replicate 3)

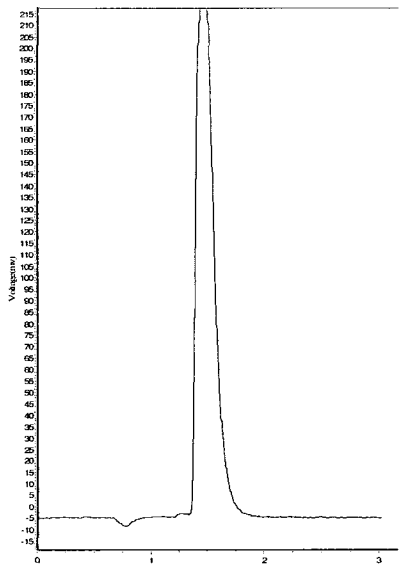

Figure 254: Wastewater spiked with 20 ppm E2 (Replicate 4)

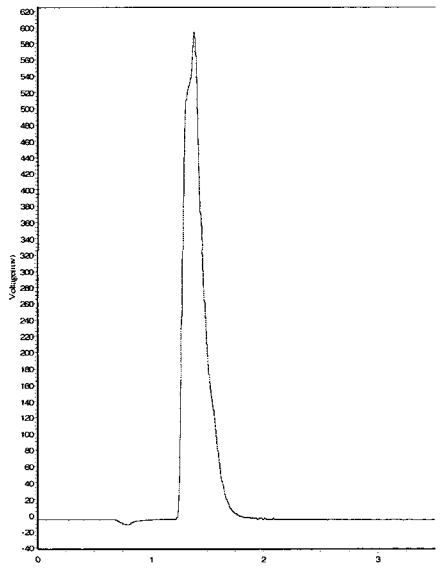

Figure 255: Wastewater spiked with 40 ppm E2 (Replicate 1)

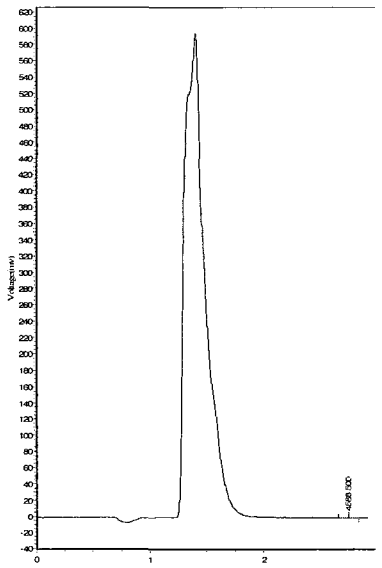

Figure 256: Wastewater spiked with 40 ppm E2 (Replicate 2)

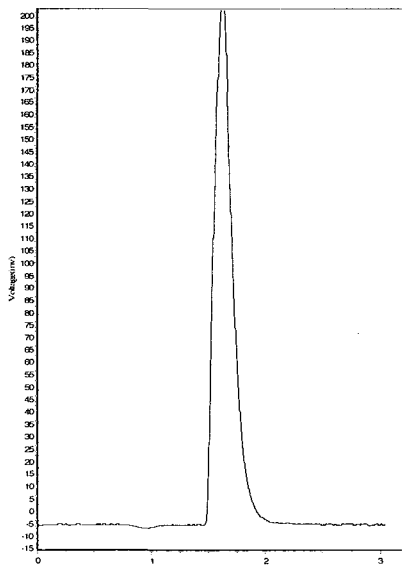

Figure 257: Wastewater spiked with 60 ppm E2 (Replicate 1)

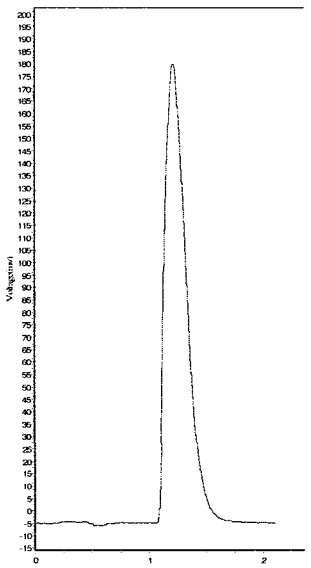

Figure 258: Wastewater spiked with 60 ppm E2 (Replicate 2)

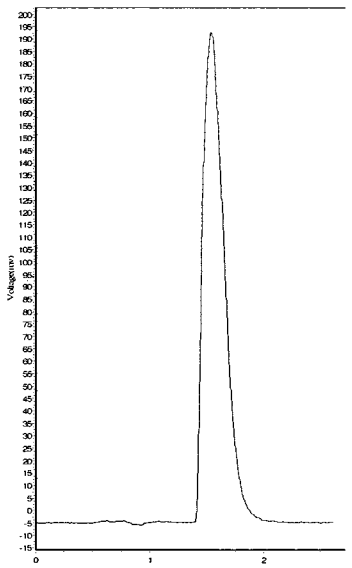

Figure 259: Wastewater spiked with 80 ppm E2 (Replicate 1)

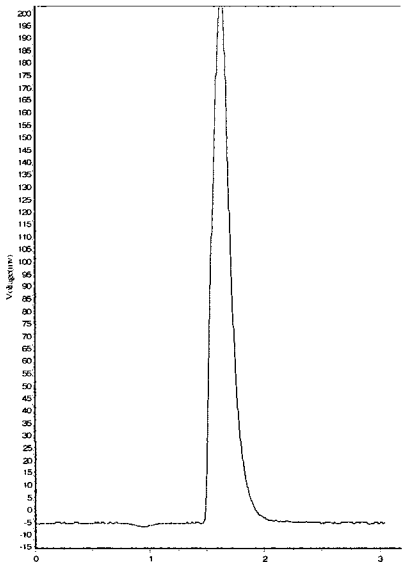

Figure 260: Wastewater spiked with 80 ppm E2 (Replicate 2)

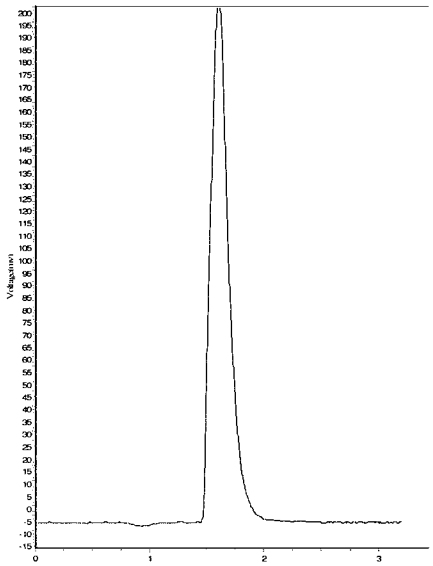

Figure 261: Wastewater spiked with 100 ppm E2 (Replicate 1)

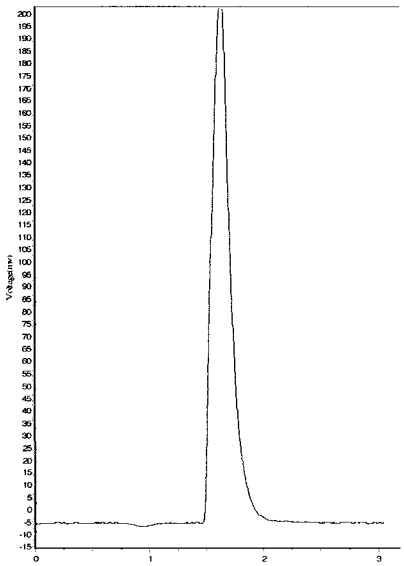

Figure 262: Wastewater spiked with 100 ppm E2 (Replicate 2)

11.11 Centrate Analysis:

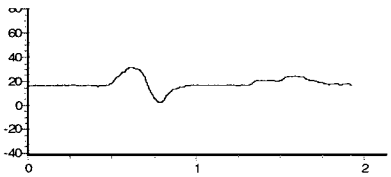

Figure 263: Remainder for wastewater spiked with 1.2 ppm E2 (Replicate 1)

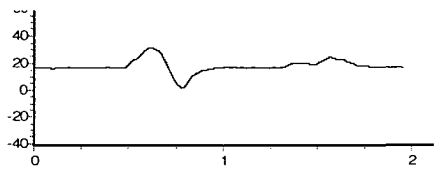

Figure 264: Remainder for wastewater spiked with 1.2 ppm E2 (Replicate 2)

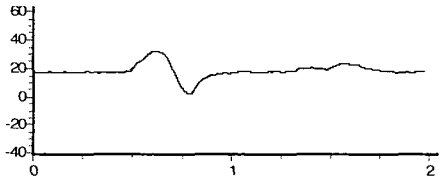

Figure 265: Remainder for wastewater spiked with 1.4 ppm E2 (Replicate 1)

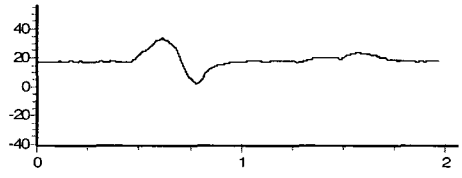

Figure 266: Remainder for wastewater spiked with 1.4 ppm E2 (Replicate 2)

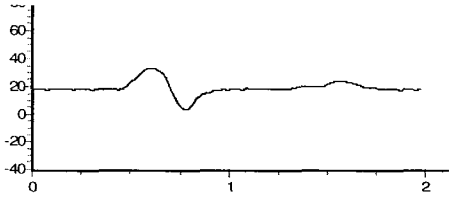

Figure 267: Remainder for wastewater spiked with 1.6 ppm E2 (Replicate 1)

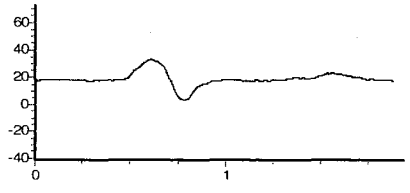

Figure 268: Remainder for wastewater spiked with 1.6 ppm E2 (Replicate 2)

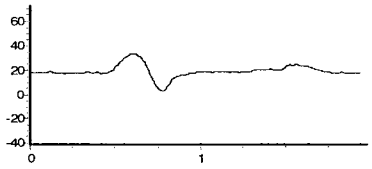

Figure 269: Remainder for wastewater spiked with 1.8 ppm E2 (Replicate 1)

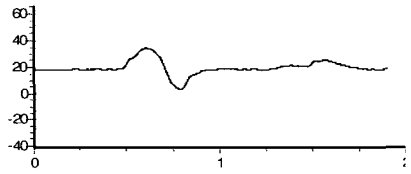

Figure 270: Remainder for wastewater spiked with 1.8 ppm E2 (Replicate 2)

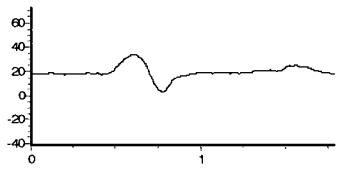

Figure 271: Remainder for wastewater spiked with 2 ppm E2 (Replicate 1)

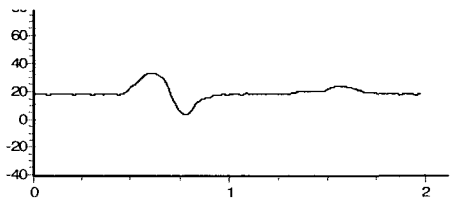

Figure 272: Remainder for wastewater spiked with 2 ppm E2 (Replicate 2)

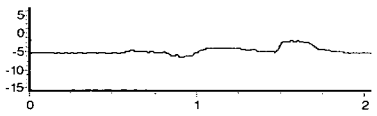

Figure 273: Remainder for wastewater spiked with 4 ppm E2 (Replicate 1)

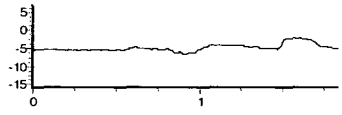

Figure 274: Remainder for wastewater spiked with 4 ppm E2 (Replicate 2)

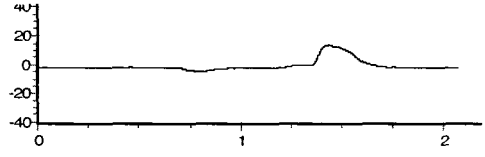

Figure 275: Remainder for wastewater spiked with 6 ppm E2 (Replicate 1)

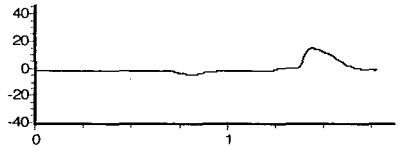

Figure 276: Remainder for wastewater spiked with 6 ppm E2 (Replicate 2)

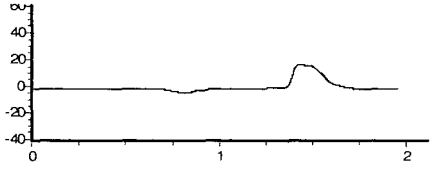

Figure 277: Remainder for wastewater spiked with 8 ppm E2 (Replicate 1)

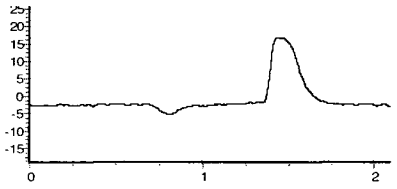

Figure 278: Remainder for wastewater spiked with 8 ppm E2 (Replicate 2)

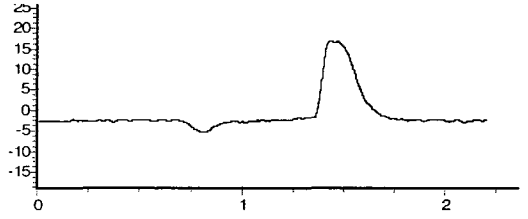

Figure 279: Remainder for wastewater spiked with 10 ppm E2 (Replicate 1)

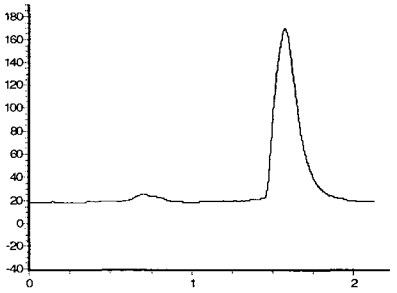

Figure 280: Remainder for wastewater spiked with 10 ppm E2 (Replicate 2)

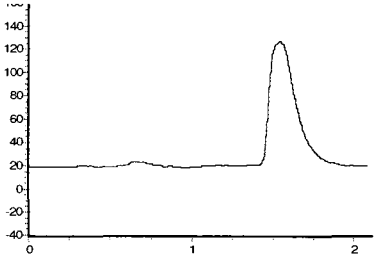

Figure 281: Remainder for wastewater spiked with 12 ppm E2 (Replicate 1)

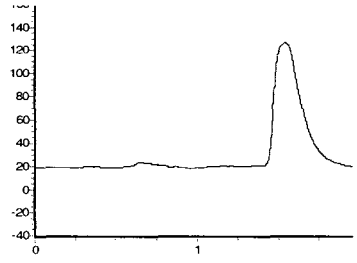

Figure 282: Remainder for wastewater spiked with 12 ppm E2 (Replicate 2)

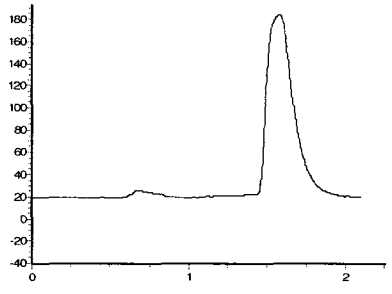

Figure 283: Remainder for wastewater spiked with 14 ppm E2 (Replicate 1)

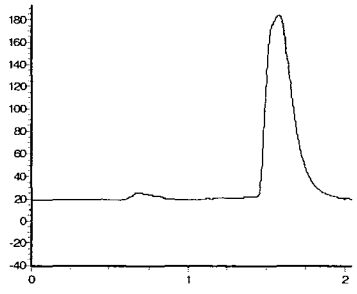

Figure 284: Remainder for wastewater spiked with 14 ppm E2 (Replicate 2)

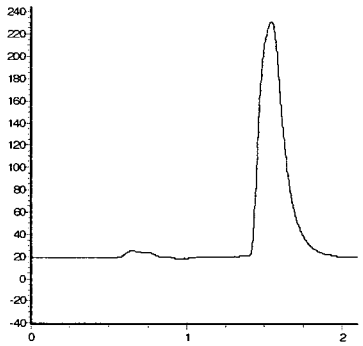

Figure 285: Remainder for wastewater spiked with 16 ppm E2 (Replicate 1)

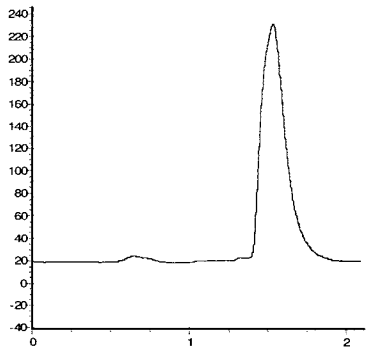

Figure 286: Remainder for wastewater spiked with 16 ppm E2 (Replicate 2)

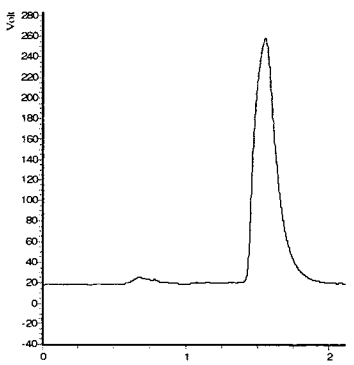

Figure 287: Remainder for wastewater spiked with 18 ppm E2 (Replicate 1)

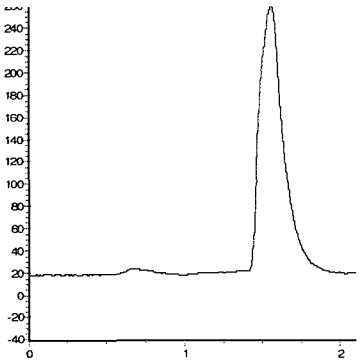

Figure 288: Remainder for wastewater spiked with 18 ppm E2 (Replicate 2)

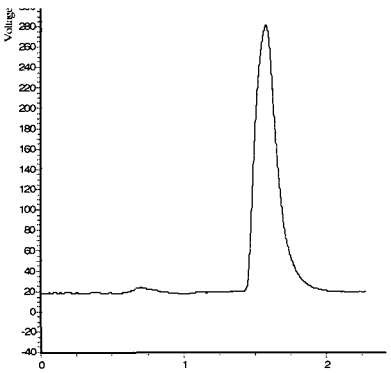

Figure 289: Remainder for wastewater spiked with 20 ppm E2 (Replicate 1)

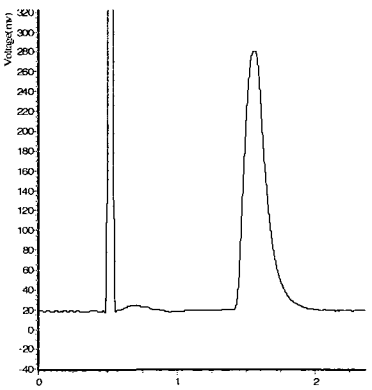

Figure 290: Remainder for wastewater spiked with 20 ppm E2 (Replicate 2)

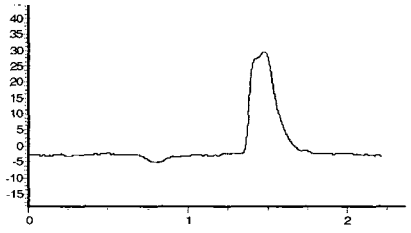

Figure 291: Remainder for wastewater spiked with 20 ppm E2 (Replicate 3)

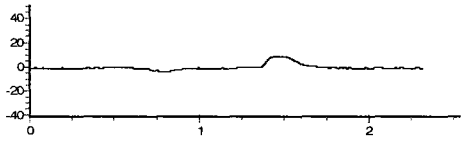

Figure 292: Remainder for wastewater spiked with 40 ppm E2 (Replicate 1)

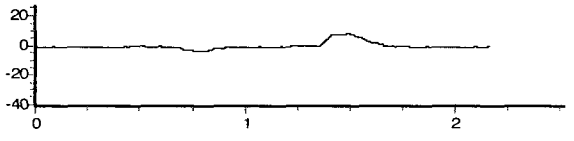

Figure 293: Remainder for wastewater spiked with 40 ppm E2 (Replicate 2)

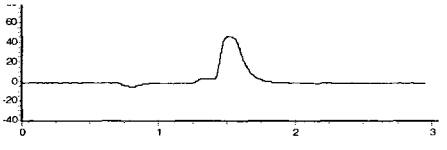

Figure 294: Remainder for wastewater spiked with 60 ppm E2 (Replicate 1)

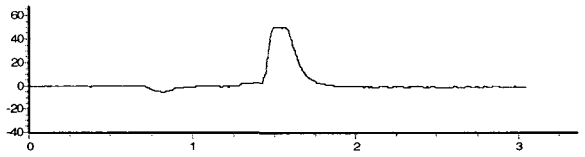

Figure 295: Remainder for wastewater spiked with 60 ppm E2 (Replicate 2)

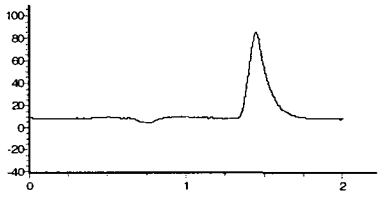

Figure 296: Remainder for wastewater spiked with 80 ppm E2 (Replicate 1)

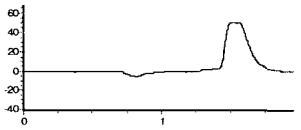

Figure 297: Remainder for wastewater spiked with 80 ppm E2 (Replicate 2)

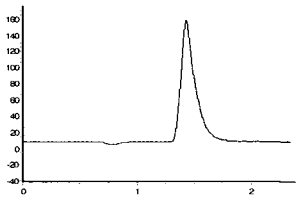

Figure 298: Remainder for wastewater spiked with 100 ppm E2 (Replicate 1)

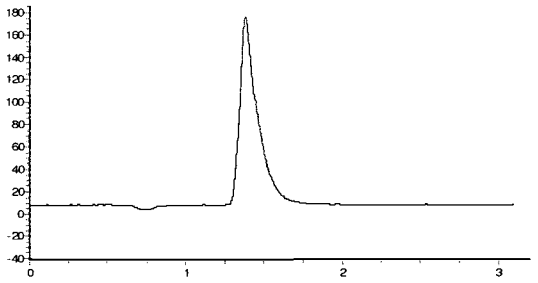

Figure 299: Remainder for wastewater spiked with 100 ppm E2 (Replicate 2)

11.12 Application of NIP Particles for Removal of Endocrine Disruptors Following Disinfection with Chlorine

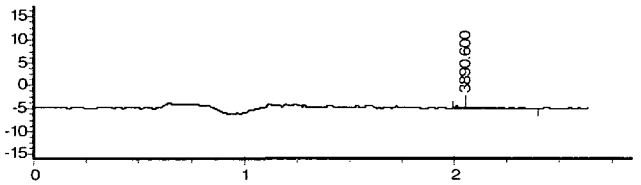

Figure 300: Treatment of 10 ppm E2 in DDW with 10 mg/L of chlorine and 10 mg/mL NIP (Replicate 1)

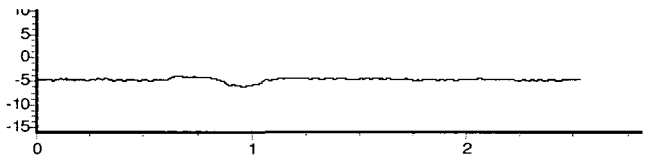

Figure 301: Treatment of 10 ppm E2 in DDW with 10 mg/L of chlorine and 10 mg/mL NIP (Replicate 2)

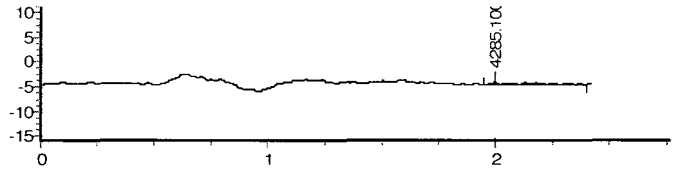

Figure 302: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine and 5 mg/mL NIP (Replicate 1)

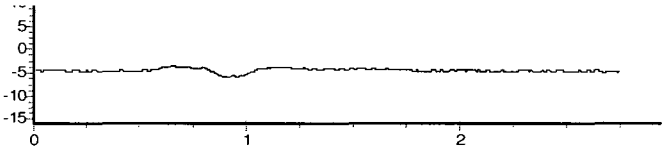

Figure 303: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine and 5 mg/mL NIP (Replicate 2)

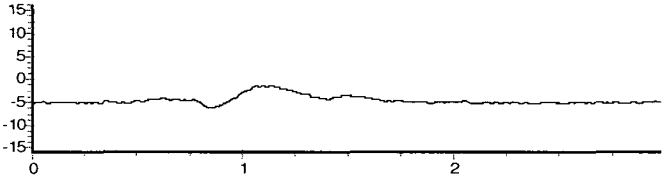

Figure 304: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine (Replicate 1)

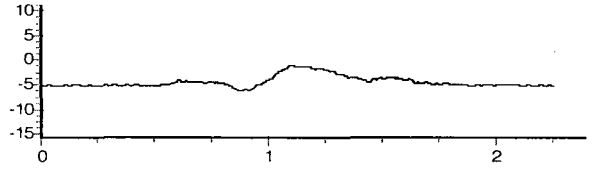

Figure 305: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine (Replicate 2)

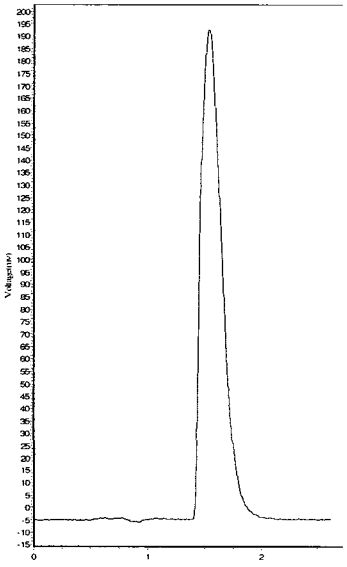

Figure 306: 10 ppm E2 in DDW (Replicate 1)

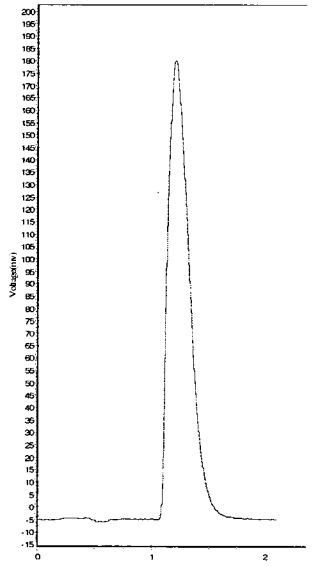

Figure 307: 10 ppm E2 in DDW (Replicate 2)

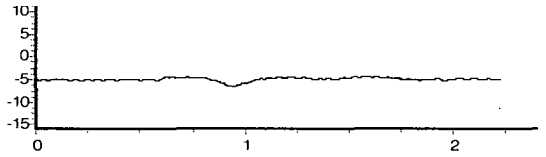

Figure 308: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 1)

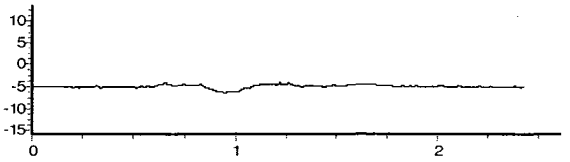

Figure 309: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 2)

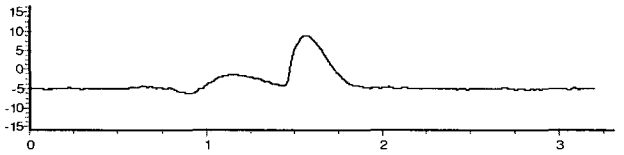

Figure 310: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine (Replicate 1)

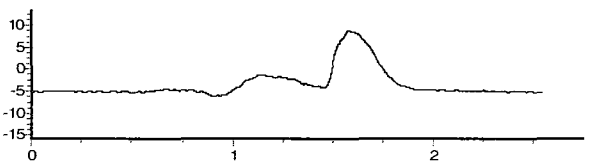

Figure 311: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine (Replicate 2)

Figure 312: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine and 5 mg/mL NIP (Replicate 1)

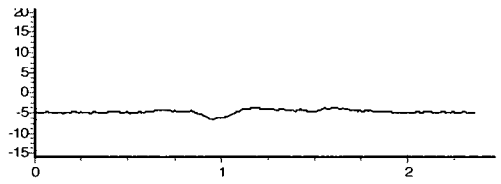

Figure 313: Treatment of 10 ppm E2 in DDW with 5 mg/L of chlorine and 5 mg/mL NIP (Replicate 2)

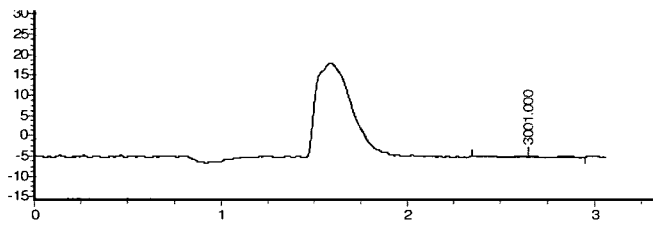

Figure 314: Humic acid solution spiked with 10 ppm E2 (Replicate 1)

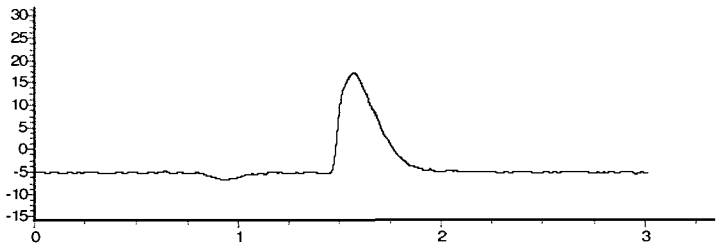

Figure 315: Humic acid solution spiked with 10 ppm E2 (Replicate 2)

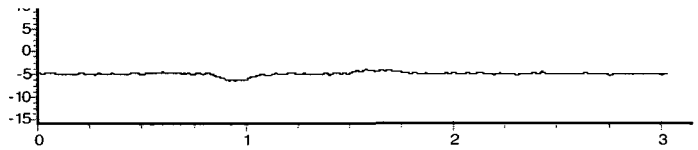

Figure 316: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/mL NIP (Replicate 1)

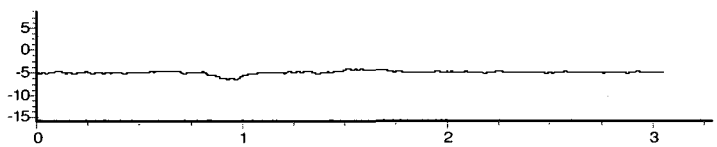

Figure 317: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/mL NIP (Replicate 2)

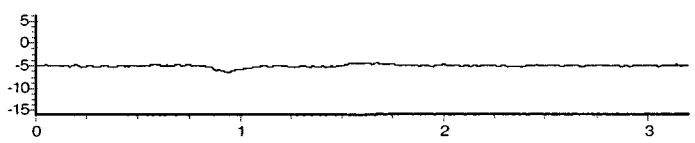

Figure 318: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/mL NIP (Replicate 1)

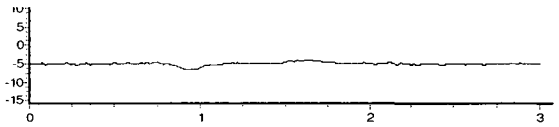

Figure 319: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/mL NIP (Replicate 2)

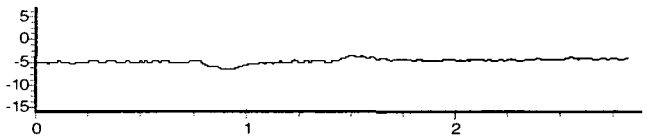

Figure 320: Humic acid in DDW (Replicate 1)

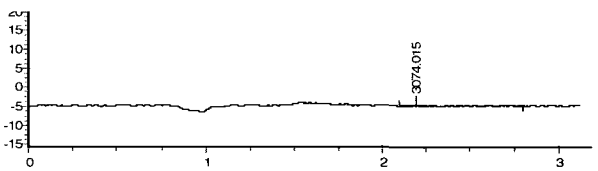

Figure 321: Humic acid in DDW (Replicate 2)

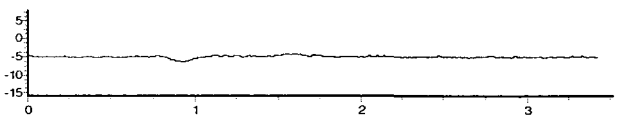

Figure 322: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/L of chlorine and 10 mg/mL NIP (Replicate 1)

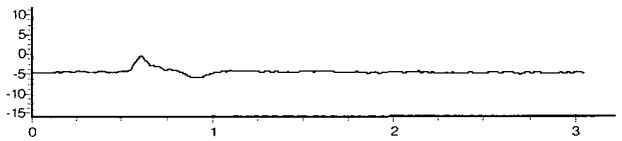

Figure 323: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/L of chlorine and 10 mg/mL NIP (Replicate 2)

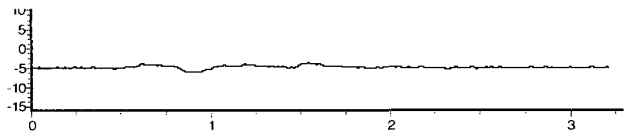

Figure 324: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 1)

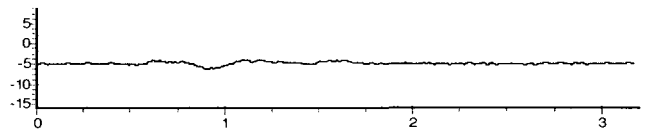

Figure 325: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 2)

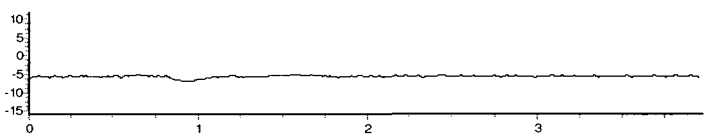

Figure 326: Humic acid solution treated with 5 mg/L of chlorine (Replicate 1)

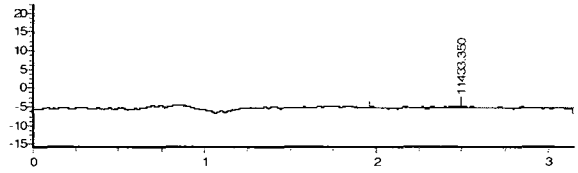

Figure 327: Humic acid solution treated with 5 mg/L of chlorine (Replicate 2)

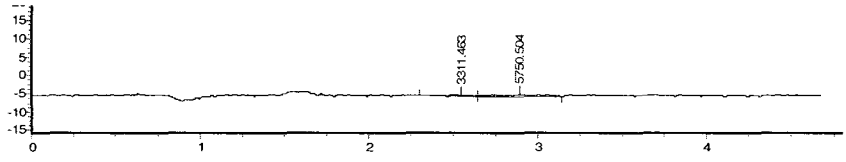

Figure 328: 10 mg/L chlorine in DDW (Replicate 1)

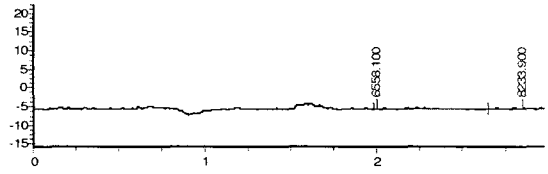

Figure 329: 10 mg/L chlorine in DDW (Replicate 2)

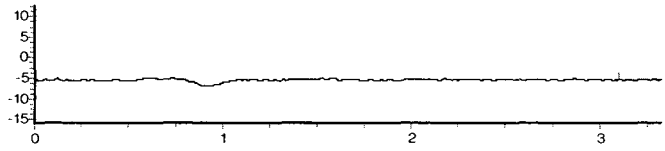

Figure 330: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 1)

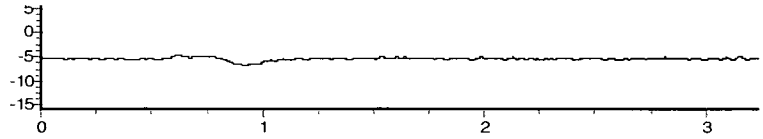

Figure 331: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 2)

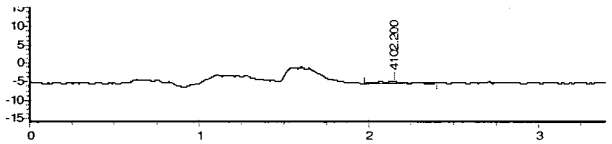

Figure 332: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/L of chlorine (Replicate 1)

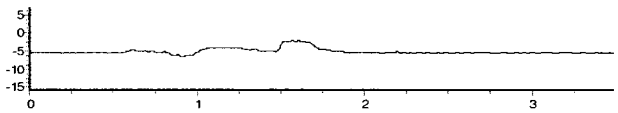

Figure 333: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/L of chlorine (Replicate 2)

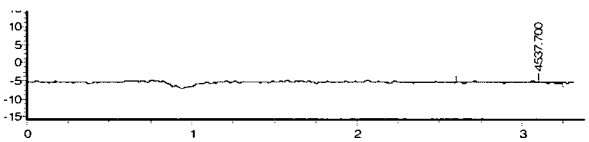

Figure 334: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/L of chlorine and 5 mg/mL NIP (Replicate 1)

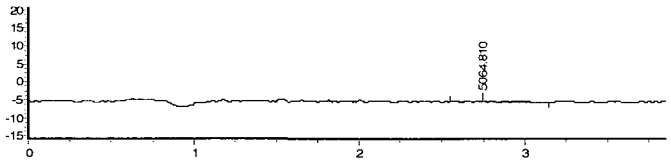

Figure 335: Humic acid solution spiked with 10 ppm E2 and treated with 10 mg/L of chlorine and 5 mg/mL NIP (Replicate 2)

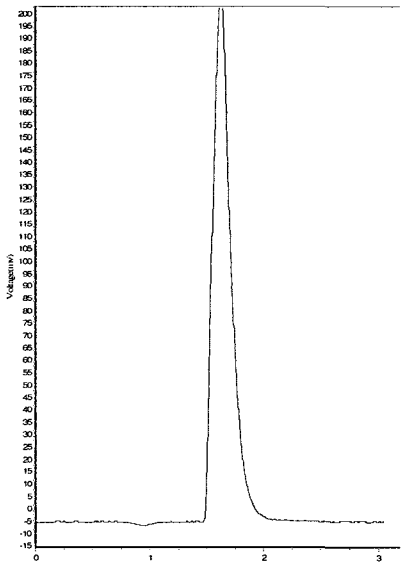

Figure 336: Humic acid solution spiked with 10 ppm (Replicate 1)

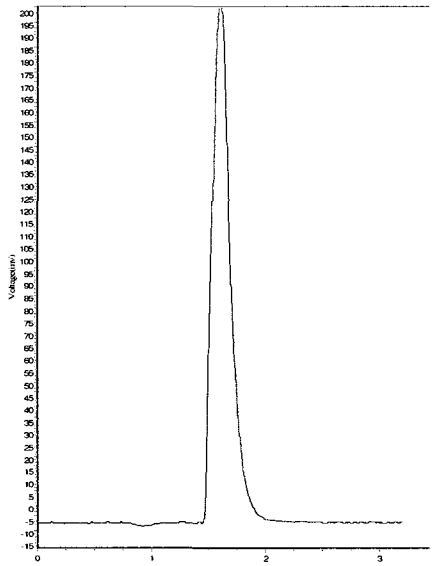

Figure 337: Humic acid solution spiked with 10 ppm (Replicate 2)

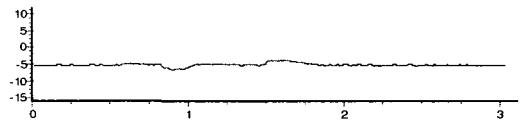

Figure 338: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 1)

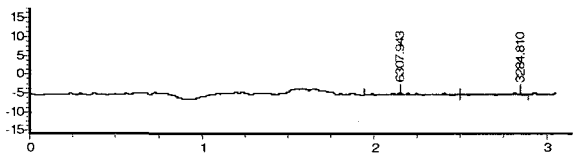

Figure 339: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 10 mg/mL NIP (Replicate 2)

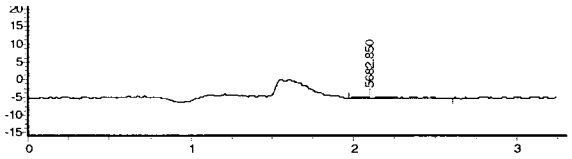

Figure 340: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 5 mg/mL NIP (Replicate 1)

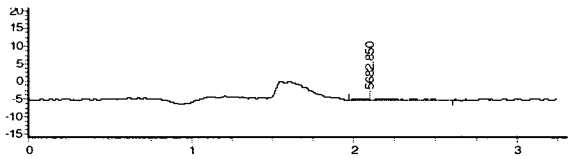

Figure 341: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine and 5 mg/mL NIP (Replicate 2)

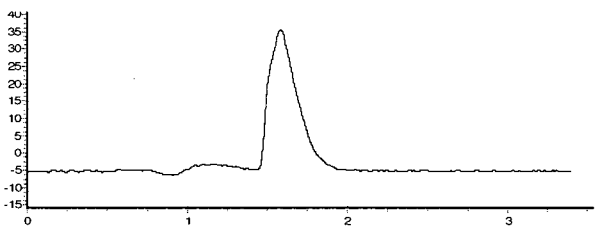

Figure 342: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine (Replicate 1)

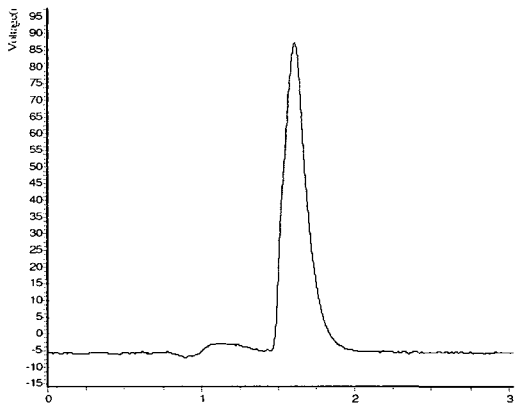

Figure 343: Humic acid solution spiked with 10 ppm E2 and treated with 5 mg/L of chlorine (Replicate 2)