Electrosynthesis of Molecularly Imprinted Polymer Films on Quartz Crystal Microbalances for the Detection of Some Biomolecules

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by

HONG Shuangjin

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Declaration

I hereby declare that this thesis summarizes my own work carried out since my registration for the degree of Doctor of Philosophy in November, 1998, and that it has not been previously included in a thesis, dissertation or report presented to this or any other institution for a degree, diploma or other qualification.

HONG Shuangjin

September, 2004

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Abstract of thesis entitled "Electrosynthesis of Molecularly Imprinted Polymer Films on Quartz Crystal Microbalances for the Detection of Some Biomolecules"

submitted by HONG Shuangjin

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Abstract

Molecular imprinting, in which specific binding sites are created by polymerization of functional monomers in the presence of template molecules followed by their subsequent removal, has been recognized as a powerful tool for the preparation of artificial receptors for specific binding with target molecules. Though molecular imprinting can be employed for the preparation of the recognition sites in chemical sensors, the development of sensors based on molecularly imprinted polymer (MIPs) is relatively slow mainly due to the difficulties in integrating the imprinted polymers to the sensor transducer. The objective of this thesis is to develop sensors for biomolecules based on electrosynthesized imprinted polymer films.

In the first part of this study, molecularly imprinted polymer films of polypyrrole and poly(o-phenylenedamine) have been prepared by electropolymerization on EQCM electrodes in the presence of adenosine-5'-triphoshate disodium salt (ATP). These ATP sensors exhibit high selectivity against ADP and AMP. A pyrrole:ATP mole ratio of 2:1 was found to be the best for the preparation of the MIP polypyrrole films by electropolymerization. On the other hand, the poly(o-phenylenediamine) film has low conductivity and therefore only very thin polymer film could be obtained. Nevertheless, this allows the poly(o-phenylenediamine) MIP films to have short response time towards the analytes. The poly(o-phenylenediamine) MIP film was found to exhibit better selectivity but lower sensitivity towards ATP.

In the second part of this project, a functionalized monomer, glucose-boronate-substituted aniline, was synthesized by cyclic esterification of glucose with

aminophenylboronic acid. Glucose imprinted co-polymer films of boronate-substituted monomer and aniline were prepared by electropolymerization in non-aqueous media and gradually transferred into aqueous media. At physiological pH, the EQCM sensor based on this glucose imprinted polymer film showed linear frequency response toward glucose solution in the concentration range of 2.0×10^{-5} to 7.5×10^{-4} mol dm^{-3}. Substantial uptake of fructose by the glucose imprinted polymer film was also observed. This was due to the high association constant (K_a) between fructose and bronic acid. The effect of various parameters such as electrode potential, solution pH and functionalized monomer to aniline ratio on the analytical performance of glucose imprinted polymer film were examined.

In the third part of this study, molecularly imprinted polymer films of acrylamidophenylboronic acid were prepared by electropolymerization on EQCM Au electrodes and Au wire electrodes in the presence of dopamine. After removal of the template molecules, a MIP film with both high affinity towards dopamine and permiselectivity against ascorbic acid (AA) was prepared. The electrochemical sensor based on the MIP film was used to detect dopamine at different concentrations at physiological pH by means of preconcentration-stripping. Using this dopamine sensor, a linear relationship was observed between the electrochemical redox peak current intensity and dopamine concentration from 5×10^{-6} to 1×10^{-4} mol dm^{-3}. This sensor shows negligible interference from ascorbic acid, even when ascorbic acid's concentration is as high as 1×10^{-3} mol dm^{-3}. The mechanism of the sensor's selectivity was discussed. The effect of various parameters such as electrode potential and solution pH on the performance of the sensor were also investigated.

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Chapter 1

Introduction

1.1 Molecularly imprinted polymers

1.1.1. Naturally occurring molecular recognition

In nature, molecular recognition plays a decisive role in biological activity[1]. For example, the receptors on the surface of cell membranes bind hormones and are responsible for cell to cell communication. When the receptor binds a hormone, its conformation is changed and the message of the hormone binding is passed to the cell in terms of this conformational change. In response to this change, the corresponding biological reaction is triggered in the cell. The most important property in these systems is that one receptor accepts only one specific hormone and it does not interact significantly with other hormones. Furthermore, this receptor/hormone interaction is very strong. Thus, even very small amount of hormone can correctly deliver its information to the target cell[2].

It is well known that enzymes show high specificity towards their substrates. Each enzyme exclusively binds a certain class of substrates and transforms the substrate into a predetermined product. All other compounds in the system, even if they resemble the specific substrate, remain intact. Only the specific substrate is efficiently transformed into the desired product.

This substrate specificity primarily comes from the selective binding by the active sites of enzymes. Results of X-ray crystallography and NMR spectroscopy [3] indicate that the substrate-binding sites of enzymes are apolar pockets or clefts, which are formed from a number of amino acid residues. In the pockets or clefts,

several functional groups such as OH, NH_2, COOH and imidazole are precisely placed to interact with the functional groups of a specific substrate. For the specified substrate, it interacts with all the functional groups in the binding site satisfactorily, which results in a stable enzyme-substrate complex.

1.1.2. Mimics of natural receptor

The elegance of molecular recognition in nature has spurred many scientists to design and develop artificial receptors to mimic the natural receptor. One of the greatest advantages of artificial receptors over naturally occurring ones is the freedom of molecular design. The framework of artificial receptors can be extended from protein to a variety of skeletons such as carbon chains and fused aromatic rings. Thus, the stability, flexibility and other properties of artificial receptors can be freely modulated according to the purpose of application. Furthermore, various functional groups that are not found in natural receptor can be employed in these man-made receptors.

Based on their pioneering works[4], Cram, Lehn, and Pedersen (Nobel Prize winners in 1987) had pointed out that the following factors are crucial for successful molecular recognition: -

1. Functional residues of guest and receptor must be complementary to each other.

2. Conformation freedom of both components should be minimized.

3. Chemical circumstance should be appropriately regulated.

In the early research on artificial receptors, low molecular weight ring or cage systems such as crown ethers[5], cryptates[6], cyclodextrins[7], cyclophanes[8] and concave molecules[9] are usually used. Figure 1.1 shows some typical cyclic host molecules used as receptors for specific guests. In many cases, various functional residues are present on the host molecules. Although each of the interactions (e.g., hydrogen-bonding, electrostatic, and Van de Waals force) is rather weak, remarkably high selectivity and binding strengths are accomplished when all of them work in a cooperative manner.

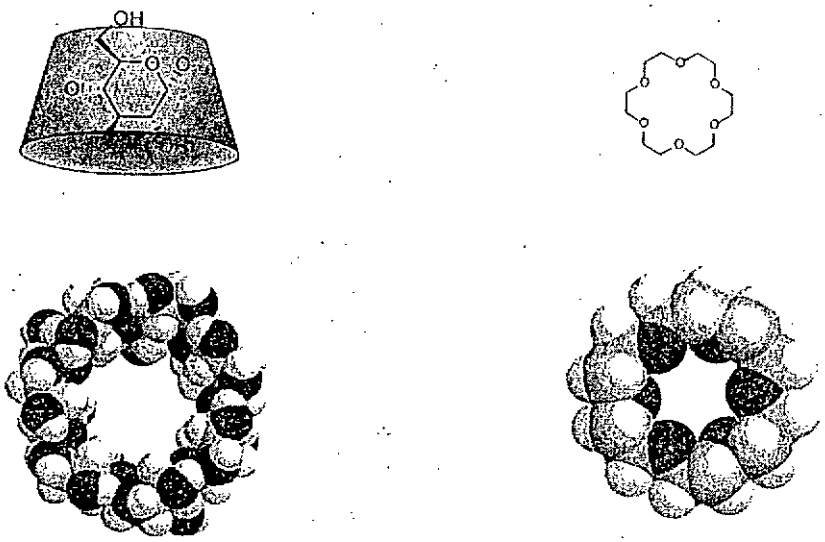


Figure 1.1 Typical cyclic host molecules used for recognition of specific target guests[2].

1.1.3. General principle of molecular imprinting

As mentioned in the foregoing section, some elegant receptors, mainly with cyclic structures, have already been synthesized by aligning functional groups on appropriate carbon backbone. These man-made receptors have self-defined molecular structures and show both high selectivity and high binding activity toward the target guest compounds. However, these synthesized single molecule receptors also have several drawbacks. Firstly, the synthesis of the receptor often requires many reaction steps which results in high preparation cost. Secondly, it is difficult to prepare receptors for large and complicated guest molecules due to the lack of large molecular framework which can accommodate two or more functional groups.

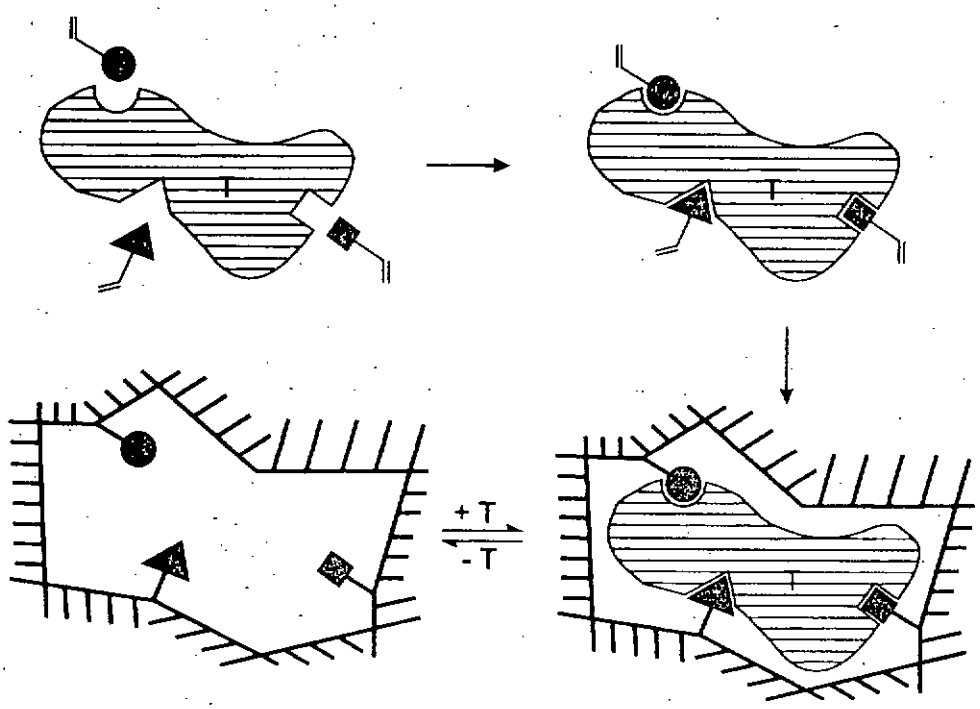


Figure 1.2 Schematic representation of the molecular imprinting and recognition of a template (T) with monomers containing three binding groups[10].

The molecular imprinting method, which is shown by the schematic presentation in Figure 1.2, is the most promising solution to these problems. Generally, this method involves a process where functional and cross-linking monomers are copolymerized in the presence of a target molecule (the imprint molecule), which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Subsequent removal of the imprint molecule results in binding sites that are complementary in functionality, size and shape to the template molecule. By this way, a molecular memory is produced in the polymer, which is capable of selectively rebinding the template.

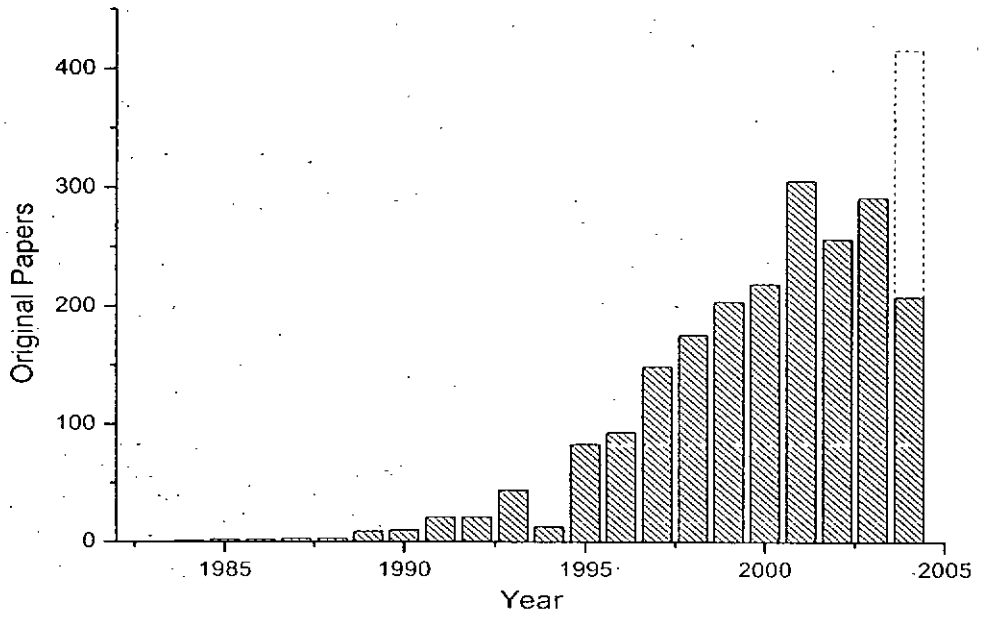


Figure 1.3 Graphical representation illustrating the number of original papers published within the field of molecular imprinting between 1984 and 2004.

The concept of molecular imprinting was claimed to date back to the early 1930s. However, it is widely accepted that 1972 is the start of molecular imprinting technology, when Wulff[11] and Klotz[12] independently reported the preparation of organic polymers with predetermined ligand selectivities. The exponential expansion in research on molecular imprinting during the last two decades is clearly shown by the increasing number of original papers published between 1984 and 2004 (Figure 1.3). In addition, the progress of this filed is reviewed by a large number of review articles[10, 13-39] and monographs[1, 2, 40-42].

1.1.4. Interactions between monomer and imprint molecules

A complex between monomers and imprint molecule can be formed via reversible covalent bonds or non-covalent interactions such as hydrogen bonds, electrostatic attraction, hydrophobic interactions, van der Waals forces, etc. A combination of two or more types of interaction can also occur. Accordingly, molecular imprinting can be classified as either covalent imprinting or non-covalent imprinting, depending on the nature of interaction between functional monomer and imprint molecule (template).

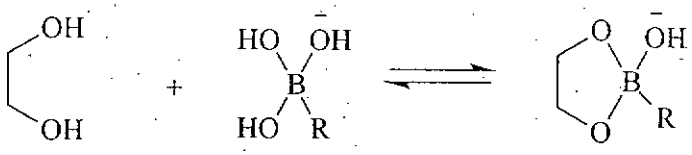
The covalent molecular imprinting was developed primarily by Wullf and co-workers[11]. In this approach, a polymerizable derivative of the imprint molecule has to be synthesized. In the polymerization step, the covalent conjugate is polymerized under the conditions where the covalent linkage is well protected. Then, the covalent linkage is cleaved and the template is removed from the polymer matrix. Owing to the greater stability of covalent bonds, covalent imprinting should yield a more

homogeneous population of binding sites. Moreover, the yield of binding sites relative to the amount of imprint molecule used (imprinting efficiency) should be higher than that with non-covalent imprinting. On the other hand, this approach also suffers from several drawbacks. First, the synthesis of the polymerizable derivative of template is often troublesome and less economical. Secondly, the number of reversible covalent linkages available is limited. Finally, due to the nature of covalent bonding, binding and release of analyte are relatively slow.

One of the keys to successful covalent imprinting is a good choice of the functional monomer and the template moiety. The covalent bonds formed between the functional monomer and the template molecules must be both stable and reversible, which are contradictory to certain extent. Therefore, the number of examples which fulfill both of these requirements is relatively small. In the published works, samples of covalent imprinting include acetals/ketals[43-46], Schiff bases[47-51], disfulfide bonds[52, 53], coordination bonds[54-61] and boronic acid esters[62-68].

The boronic acid group is very suitable for covalent binding. Ester formation between boronic acid and diol occurs with complete stoichiometric conversion. The boronic ester moieties can be readily cleaved in water/alcohol by a complete and fast reaction, so that the template molecules can be released from an imprinted polymer matrix in 85-95% yield[1]. The re-uptake of template in the imprinted polymer is relatively slow, but more than 90% of the free cavities can be reoccupied. Fortunately, in aqueous alkaline solution or in the presence of certain nitrogen bases, tetragonal boronic esters are formed[69], which equilibrate extremely rapidly with

tetragonal boronic acid and diol (Equation 1.1). Under such conditions, the rate of equilibration is comparable to typical values for non-covalent interactions.



(1.1)

Figure 1.4 shows the preparation of an imprinted polymer employing boronic acid-diol interaction and its successful application as stationary phase for chromatographic separation of racemates of the template molecules[70]. By using such a stationary phase in HPLC with gradient elution technique, complete racemate resolution of phenyl-α-mannopyranoside was performed with R_s equal to 4.3.

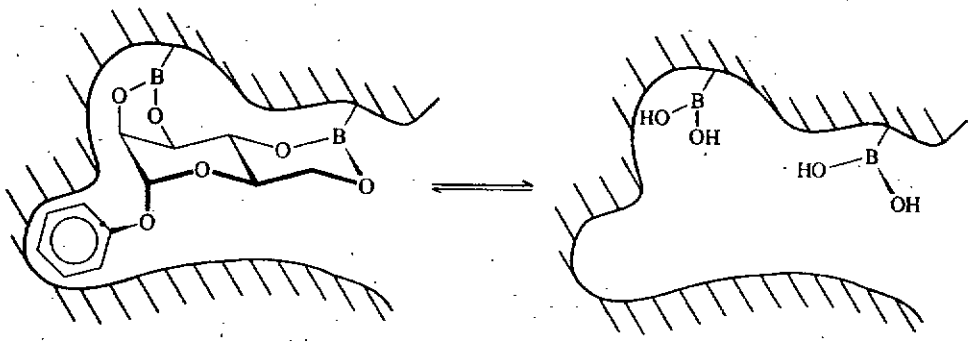


Figure 1.4 Schematic representation of a specific cavity prepared by molecular imprinting via covalent linkage between boronic acid and diol[70].

Boronic acids are capable of forming reversible and strong covalent bonds with the diol functionalities in the form of cyclic esters. It is more desirable that the rate of

this reversible reaction is much faster than other covalent boding reactions. Due to its advantageous characters, covalent formation of boronic acid esters is widely used in molecular imprinting of various template molecules such as saccharides[67, 71], nucleotides[72-74] and sialic acid[62, 64, 75, 76].

In 1984, Mosbach and co-workers reported the first application of non-covalent interaction in molecular imprinting[77]. Thereafter, the scope of molecular imprinting was remarkably extended and the research of imprinted materials was accelerated[40]. In this pioneering work, electrostatic interaction between the carboxylic acid on monomer (p-vinylbenzoic acid) and the amine group on template molecule (phenylalanine ethyl ester) was employed in molecular imprinting. By measurement of radioactivity, the polymer prepared by non-covalent imprinting was found to favor the uptake of the enantiomer used as template.

Due to its simplicity and versatility, non-covalent molecular imprinting has been widely attempted. In principles, any kind of non-covalent interactions such as hydrogen bonding, electrostatic interaction, and hydrophobic interactions can be employed for the imprinting process. Among these, hydrogen bonding is most suitable for precise molecular imprinting, since it is highly dependent on both distance and direction between monomers and templates. Various monomers which bear the required functional groups (e.g., carboxyl, amino, pyridine, hydroxyl and amide groups) complementary to the template can be used. As an example, the pesticide atrazine has been imprinted in methacrylic acid polymer[78, 79]. The hydrogen boding between the nitrogen atoms (amino groups and triazine) of atrazine and the carboxylic group of methacrylic acid serves as non-covalent interaction in

the formation of monomer-template complex. The resultant molecular imprinting polymer has been used as HPLC station phase for separation of atrazine from its analogues.

In addition to hydrogen bonding, electrostatic attraction is another interaction applicable in molecular imprinting. The degree of electrostatic interaction is simply determined by the distance between the opposite charges and is independent of direction, thus this interaction is unfavorable for precise molecular recognition compared to hydrogen bonding. On the other hand, unlike hydrogen bonding, electrostatic interaction can be used for imprinting in polar solvent. The selectivity can be improved by combining it with other interactions. An amino acid imprinted polymer film of overoxidized polypyrrole has been prepared by Nagaoka and co-workers[80-82]. The imprinting of polymer and enantioselective uptake of template amino acid is mainly dependent on electrostatic interaction. The ability of the imprinted polymer film to discriminate enantiomers of the template amino acid was evaluated by a quartz crystal microbalance.

In general, compared to covalent imprinting, the non-covalent imprinting approach is more flexible in the choice of functional monomers, possible target molecules and the use of imprinted materials. After polymerization, the imprinted molecules can be removed from the polymer by simple solvent extraction. However, the prepolymerization complex is an equilibrium system, the stability of which depends on the affinity constant between the imprint molecules and the functional monomers. This may yield certain heterogeneity in the binding sites. The advantages

and disadvantages associated with covalent and non-covalent imprinting are summarized in Table 1.1.

Table 1.1 Comparisons of Covalent and Non-covalent Imprinting Methods

	Covalent	Non-covalent
Synthesis of polymerizable template derivative	Required	Not necessary
Polymerization conditions	More choices	Restricted choices
Removal of template	Difficult	Easy
Guest-binding and release	Slow	Fast
Structure of binding site	Well defined	Less well defined

1.2 Analytical applications of molecularly imprinted polymer

1.2.1 Separation

One of the most desirable features of molecularly imprinted polymers lies in their specific binding towards the template molecules. A direct application of this specific binding is in separation or preconcentration of the template molecules from a mixture. Molecularly imprinted polymers have been used in various separation techniques such as chromatography, capillary electrochromatography (CEC), and solid phase extraction, which will be briefly reviewed in the following sections.

Chromatography

The first application of molecularly imprinted polymers was as stationary phases in affinity chromatography, in particular for the resolution of racemic mixtures of chiral compounds. Much of the early work on molecularly imprinted polymers was devoted to this field. Pure enantiomers were used to create cavities with special affinity to the imprinted enantiomer. The particularity of molecularly imprinted polymers compared with conventional chiral stationary phases is that they are tailor-made for a specific target molecule, hence their selectivity is predetermined. For example, if a polymer is imprinted with the L-enantiomer of an amino acid, an HPLC column packed with the MIP will retain the L-enantiomer more than the D-enantiomer and vice versa, whereas a column containing an identical but non-imprinted polymer will not be able to separate the enantiomers.

The selectivity of the separation process is fairly high, typical values for the separation factor are between 1.5 and 5. In some cases much higher separation factor and baseline resolution have been obtained with carefully optimized conditions. A successful application of imprinted polymer in enantio-separation of cinchonidine and cinchonine has been demonstrated by Takeuchi's group, with chiral separation factor as high as 31[83]. Despite the fairly high resolution factors usually obtained, in most cases enantiomers are not completely resolved due to the often large peak broadening and tailing, especially of the more retained enantiomer. Obviously, this drawback becomes more severe when a separation of more than two compounds is required. The observed peak broadening and tailing have been attributed to the heterogeneity of binding sites, in terms of both affinity and accessibility, and different association and dissociation kinetics. Accordingly, research on this field is

mainly focused on the synthesis of uniformly shaped and sized particles and the development of molecularly imprinted polymers with better quality binding sites[37].

Capillary electrochromatography

Capillary electrochromatography (CEC) is one of the most successful applications of imprinted polymer in chromatography[26, 33, 37, 84, 85], especially for chiral separations[86, 87]. By taking advantage of the inherent separation power of imprinted polymer, high resolution ($> 1\times 10^5$ plates m^{-1}) and separation factors of capillary electrochromatography can be obtained. In one study[88], enantioseparation of the β-adrenergic antagonists propranolol and metoprolol was achieved with imprinted-polymer-based CEC. The polymer was cast *in situ* in the capillary in the form of a macroporous monolith attached to the inner wall, and the capillary could be prepared and conditioned within a few hours. The racemate of propranolol was resolved within only 120 s. When non-racemic samples containing mainly the R-enantiomer were injected, S-enantiomer of very small amounts (1%) can be distinguished. Other applications of imprinted polymer in CEC include continuous polymer rods[89], particles included in a gel matrix[90] and small particles suspended in the carrier electrolyte[91].

Solid phase extraction

The need for efficient methods for sample preconcentration and clean-up in medical, food and environmental analyses is constantly increasing. Compared with traditional liquid-liquid extraction, solid phase extraction is superior in many aspects,

including speed, reproducibility, reduced solvent consumption and smaller amount of sample required. Moreover, solid phase extraction can be easily incorporated into automated analytical procedures. In this context it is not surprising that much of the current research in molecular imprinting is focused on solid phase extraction. In this separation application the advantages of imprinted polymer, especially their low price and their stability in different environments, are highly desired, whereas some of the limitations are less important than other separation techniques. Therefore, solid phase extraction is regarded as one of the most possible uses for imprinted polymer and has been intensively studied[92-102]. Molecularly imprinted polymers are more selective than common sample treatment methods using traditional materials, and at the same time more stable than biological matrices used in immunoextraction. Another advantage is that imprinted polymers are compatible with organic solvents, so that solid phase extraction can be applied directly after a solvent pre-extraction step. On the other hand, the low resolution factors (compares to biological matrices) are not an issue as solid phase extraction works in the adsorption-desorption mode. Therefore, solid phase extraction is regarded as one of the most promising application ideal for imprinted polymer application and at the same time the application that is closest to commercialization. This is also reflected in the relatively large number of reports dealing with real samples. Imprinted polymers have been used in solid phase extraction of the target analytes from blood plasma and serum[103, 104], urine[105], bile[103], liver extract[97], chewing gum[93], environmental water and sediment[106], plant tissue[107], etc. A successful application of imprinted polymer in solid phase extraction is reported by Stanker and co-workers[97], in which the imprinted polymer was used to further concentrate herbicide atrazine from a chloroform extraction obtained from liquid extraction of

the herbicide from beef liver. The binding capacity of the imprinted polymer for atrazine in chloroform was found to be 19 μmol g^{-1}. The analyte was eluted from the polymer with a suitable solvent and quantified by reverse phase HPLC or ELISA. The solid phase extraction step with the imprinted polymer considerably improved the accuracy and precision of the HPLC method and lowered the detection limit from 20 to 5 ppb. This was achieved by the removal of interfering components in the sample, resulting in baseline resolution of the atrazine peak. Furthermore, the analyte recovery was increased with this technique.

One of the serious obstructions for the application of imprinted polymer in solid phase extraction is template leakage. In general, once an MIP has been synthesized, it is subjected to exhaustive solvent extraction to remove the template from the polymer matrix. However, it is difficult to remove 100% of the template molecule from an imprinted polymer. Slow leakage of a portion of the remaining template from the template-removed polymer matrix over a period of time was found in some cases. As demonstrated by Arvidsson and co-workers[102], a possible resolution for template leakage problem is to use an analogue molecule instead of the analyte in molecular imprinting process.

1.2.2 Binding assays

Mimic of antigen-antibody interaction is one of the driving forces for the research in molecular imprinting. Since molecularly imprinted polymers shared with antibodies one of their most important features, the ability to specifically bind a target molecule, they could conceivably be employed in immunoassay-type binding

assays in place of antibodies. This was first demonstrated by Monsbach's group[19, 108]. Competitive radioassays for theophylline, diazepam and human growth hormone were prepared with molecular imprinting technique. The assays not only showed a very good correlation with an antibody-based enzyme immunoassay currently used in analytical laboratories in hospitals but also showed a cross-reactivity profile very similar to that of the natural antibodies. From a selection of closely related substances, only 3-methylxanthine, which has one methyl group less than theophylline, was bound to the polymer to some extent, whereas caffeine, which has one more methyl group, showed no noticeable binding. The assay for the tranquilizer diazepam was also highly specific, with cross-reactivities comparable to those of antibodies. This molecularly imprinted sorbent assay format has been used to develop assay systems for several other target compounds such as drugs[109, 110], herbicides[111, 112] and corticosteroids[113]. It has even been shown that imprinted polymer assays can be performed directly with diluted blood plasma[114].

More recently, alternative assay formats that avoid the use of radiolabels have been reported. A competitive fluorescence immunoassay has been proposed that uses a fluorescent probe for detection of herbicide 2,4-dichlorophenoxyacetic acid[115]. Piletsky developed a method in which the polymer is synthesized *in situ* in the wells of a microtiter plate[116]. Aminophenylboronic acid was polymerized in the presence of epinephrine (the target analyte) using oxidation of the monomer by ammonium peroxodisulfate. This process resulted in the grafting of a thin polymer layer on the polystyrene surface. The polymer was then used in a competitive enzyme-linked assay with a conjugate of horseradish peroxidase and norepinephrine, and improved affinity towards the target was found.

1.2.3 Sensors with imprinted polymer as recognition elements

Generally, in a sensor system, a chemical or physical signal is generated in response to the binding of the analyte to the recognition element. This signal is then translated into a measurable output signal. Since molecularly imprinted polymer is capable of specific binding towards according molecule, it is widely used as recognition elements for various sensors. Depending on the nature of the signal translation, imprinted-polymer-based sensors can be classified into electrochemical sensors, microgravimetric sensors, etc.

Electrochemical sensors

Electrochemical sensing could offer sensitive detection of a large variety of analytes with or without electroactivity at low cost[16, 117, 118]. This type of transduction is particularly attractive considering the possibility of easy miniaturization and automation.

The integration of imprinted polymer on the transducer surface is an important aspect of the design of imprinted-polymer-based electrochemical sensors. However, despite significant improvement in molecular imprinting, the processability of these materials remains limited. Most imprinted polymers are prepared as a monolith, they have to be ground and sieved as polymeric particles. Some studies have been focused on immobilization of polymeric particles on the transducer surfaces with the aid of agar gel[119, 120], polyvinyl chloride (PVC) membrane[121] and carbon paste[122]. An integrated sensor was developed by coating the imprinted polymer particles with

agar gel directly onto the working electrode and used in detection of 2,4-dichlorophenoxyacetic acid[120]. In the applications of imprinted polymer in the form of particle, the slow mass diffusion of analyte usually leads to long response time.

The integration strategy most frequently used for imprinted-polymer-based electrochemical sensor is *in-situ* preparation of molecularly imprinted polymer film on the transducer surface by electropolymerization. The big advantage of electropolymerization in comparison with other integration techniques lies in the ability to deposit an imprinted polymer uniform film on detector surface. The film thickness and deposition density can be regulated by polymerization conditions.

The possibility of inducing selectivity by the presence of the template during polymerization bas been tested with several non-crosslinked electrogenerated polymers, such as polypyrrole, polyphenol and poly(o-phenylenediamine). These polymer films can be prepared easily by electropolymerization from aqueous solutions of their monomers. Molecularly imprinted polypyrrole has recently been studied in enantioselectivity towards amino acids[80, 81]. The imprinted polymer film was prepared in the presence of optically pure amino acid by electropolymerization. After removal of the template amino acid by overoxidation, the resulting sensor showed good enanotioselectivity towards amino acid under suitable pH condition. The possibility of imprinting polymerization of poly(o-phenylenediamine) and the subsequent development of sensor based on this material was confirmed in experiments by Wang's group[123]. They performed electropolymerization of o-phenylenediamine in the presence of glucose in aqueous

solution. The imprinted polymer film obtained exhibited selective binding towards glucose in terms of capacitive response. A similar result was demonstrated for electropolymerized phenol, formed in the presence of phenylalanine, which was able to retain a "memory" for the template amino acid[124].

Despite the success in preparing electrogenerated imprinted polymer film demonstrated on the above works, development of imprinted polymer film with electropolymerization is slow. The most critical problem encountered in this field is limitation of polymerizable monomers available, especially in aqueous media in which most sensors are used.

The transduction techniques that have been used for the preparation of imprinted polymer based electrochemical sensors include conductometry, capacitive/impedance spectroscopy, potentiometry, chemical/ion-selective field effect, amperometry and voltammetry.

Conductometric detection is based on measuring the conductivity change of imprinted polymer film with ion binding. Piletsky et al. [125, 126] have prepared imprinted polymer film containing molecular recognition sites for atrazine. The electroconductivity set-up comprised a cell with platinum electrodes separated by the imprinted membrane immersed in a buffer solution. Upon the binding of analyte to the cavities on the polymer film, the permeability of the film is affected and thus results in change of the film electroresistance. By measuring the conductivity of the system, atrazine in solution in the range 4.6–231.8 mmol/dm^3 can be detected.

Interfacial phenomena can be followed by changes in capacitance or impedance of the system. The requirement is to have a totally pore-free thin, dielectric film. As mentioned in foregoing section, a capacitive sensor for glucose was developed by Wang's group[123]. Pinholes on the imprinted poly(o-phenylenediamine) was blocked by 1-dodecanethiol. Glucose in solution was measured by monitoring capacitance decrease upon injection of glucose. Capacitive sensors for creatinine and the herbicide desmetryn have been developed by photografting of acrylate derivatives[127, 128].

Voltammetry involves monitoring the current generated upon application of a potential sweep. This is the most selective and widely used electrochemical technique, since the oxidation or reduction potential of a particular substrate is its intrinsic property. There are several types of voltammetric techniques, depending on the shape of the applied potential function. For linear sweep voltammetry (LSV) and cyclic voltammetry (CV), the potential applied changes linearly with time. Pulse techniques, in which potential sweep is not a linear function but comprises constant increments on a linear ramp (differential pulse voltammetry, DPV) or a square wave function (square wave voltammetry, SWV), can offer better sensitivity, because they give better signal-to-noise ratios. However, SWV has not yet been used with imprinted polymer based sensors.

Differential pulse voltammetry was used by Pizzariello and co-worker[129] to measure clenbuterol solid-phase-extracted by imprinted polymer from bovine liver samples. In the first step, clenbuterol binds selectively to the imprinted polymer particles immobilized on electrode surface with graphite. In the second step, an

electroinactive competitor (isoxsuprine) is added in excess to enhance release of the bound clenbuterol. The released clenbuterol is then analysed using DPV. Clenbuterol in the range 0.004–25 mM, with a detection limit of 20 nM, was determined using this procedure. However, due to the slow exchange kinetics of clenbuterol to the polymer particles, release of the analyte from the polymer takes more than 35 minutes.

If the analyte itself does not exhibit any electrochemical property that can be used for detection, a competitive or displacement sensor formats may be used. This strategy was successfully employed in development of theophylline sensor based on imprinted polymer in which electroactive ferrocyanide was used as a indicator of the electroinactive analyte[130]. The anodic current of ferrocyanide in cyclic voltammogram recorded on electrode modified with imprinted polymer was found to be remarkably enhanced by the presence of the template, which was explained by the change on diffusive permeability of the thin layer of the molecularly-imprinted polymer caused by the presence of its template molecule.

Potentiometric or ion-selective electrodes (ISEs) are another approaches to electrochemical transduction of imprinted polymer based devices[131]. Generally, this approach involves the incorporation of the imprinted polymer as the active ingredient in the membrane of an ISE.

A nitrate-selective electrode based on imprinted polymer film has been reported by Hutchins et al[132]. By polymerizing pyrrole in the presence of $NaNO_3$, a film was produced with pores that were complementary to the size of the targeted

analyte ion. Both the size of the pore and the charge distribution within the polymerized film formed a host cavity for nitrate, which provided a selectivity profile different from those of conventional nitrate-selective electrodes.

Optical sensors

Optical transducer can measure changes in absorbance, reflectance, refractive index, luminescence or scattering. Many optical sensors have been developed based on indicators or fluorophores that change their absorbance or fluorescence characteristics selectively in the presence of different analytes.

Perhaps the first demonstration of the use of imprinted polymer in optical sensor was the work by Andersson et al[133], in which ellipsometry was used to quantify the amount of vitamin K_1 bound to imprinted polymer film. Ellipsometry is a technique which measure the change in the polarization of light reflected from a surface to indicate the thickness of bound organic layers. With selectivity against vitamin K_3 and other interferents, vitamin K_1 could be determined in the concentration range 0.75 – 9.0 μM.

Of the many examples in the use of imprinted polymers in optical sensors, by far the majority relies on fluorescence measurement to achieve optimal sensitivity. Wang and co-workers[134] have succeeded in developing a fluorescent sensor for L-tryptophan. Upon injection of the template molecule L-tryptophan, the bound quencher, p-nitrobenzaldehyde is replaced, which results in enhanced fluorescence. This sensor is capable of discrimination not only between the side chains of the

amino acid but also the chirality of the template. However, the incubation time of this sensor is as long as 4 hours, which is too long to be used in sensing system.

1.2.4 Determination of biomolecules with MIP based sensors

Nucleotides

The concentration of ATP can be determined by various methods such as spectrophotometry, bioluminescence, chemiluminescence, chromatography, fluorescence, and electrochemical methods [135]. Due to the intrinsic selectivity and sensitivity of firefly luciferase, bioluminescence is one of the most sensitive methods for ATP determination. However, the bioluminescent reagents are expensive and unstable. ATP can also be determined by sensors based on molecularly imprinted polymers. A QCM sensor based on ATP imprinted polyions was developed by Shinkai and co-workers [73]. The MIP was prepared by radical copolymerization of boronic acid monomer and cationic monomer. The MIP showed swelling and deswelling behaviors upon addition of analyte. The hydrogel MIP was assembled onto the surface of QCM electrodes and was used to detect AMP. Since the QCM sensor was simply prepared by putting a layer of hydrogel on the surface of electrode, the film form was relatively thick. This resulted in an excessively long sensor response time of 40 minutes. The sensitivity of such a sensor was reported to be 10^{-7} mol dm^{-3}, but no data on the selectivity was provided [73].

Glucose

As one of the most important biomolecules in human body, detection of glucose was extensively studied. Recently, a glucose sensor prepared by electropolymerization of poly(o-phenylenediamine) was reported [123]. The electropolymerization of o-phenylenediamine was performed in the presence of glucose in aqueous solution. The resulting imprinted polymer film exhibited selective binding towards glucose which can be measured by the change in capacitance. The response time of this sensor toward glucose is a few minutes whereas the detection limit is about 10^{-6} mol dm^{-3}. This result shows that MIP films prepared by electropolymerization can be applied to the construction of sensors with high selectivity and sensitivity.

Dopamine

Dopamine (DA) is one of the most important catecholamines in the family of excitatory neurotransmitters [136]. It plays an important role in the functioning of the central nervous, cardiovascular, renal and hormonal systems. Detection of dopamine by electrochemical anodic oxidation has been widely studied [137]. However, the main and foremost problem in the electrochemical determination of dopamine is the interference from other electroactive species, such as ascorbic acid (AA) and 3, 4-hydroxyphenyl acetic acid (DOPAC), which are present in biological matrices in relatively high concentration and have similar redox potential as dopamine. To the best knowledge of the author, there is no published work on the fabrication of dopamine sensor based on molecular imprinting.

In the study of the electrochemical detection of dopamine, interaction between boronic acid moiety and dopamine has been used to discriminate dopamine from ascorbic acid [138,139]. By adding phenylboronic acid into the dopamine solution, the oxidation potential of dopamine increases and becomes separated from that of ascorbic acid. With the aid of boronic acid, a selective dopamine sensor with operating oxidation potential shifted from that of ascorbic acid can be prepared. However, since addition of boronic acid to dopamine solution is a necessity, this method is not suitable for *in vivo* dopamine detection.

1.3 Aims and objectives of this project

Studies on the preparation and sensor applications of molecularly imprinted polymer films are of fundamental and practical interest. Despite its exponential increase in the last ten years, exploration on the potential application of molecular imprinting in sensor fabrication is far from exhaustive.

In most sensor systems, the analyte recognition elements are required to be in close contact with the transducer. Therefore, the preparation of molecularly imprinted polymer films firmly attached to transducer is essential for the successful application of molecularly imprinted polymers in sensors. Among the large number of molecularly imprinted polymers reported, most of them were prepared in bulk solution and thus resulted in polymers in the state of monolith. Electropolymerization is one of the methods suitable for the preparation of molecularly imprinted polymer film in sensor application. By electropolymerization, a uniform thin layer of molecularly imprinted polymer can be prepared on the surface of electrode. In this project, three kinds of molecularly imprinted polymer films based on polypyrrole, poly(*o*-phenylenediamine), polyaniline and poly(acrylamide) were prepared by electropolymerization for the purpose of sensor applications. The factors affecting the analytical performance of these imprinted polymer films will be discussed.

Most of the imprinted polymer reported so far were prepared and use in non-aqueous media. Reports on imprinted polymers for aqueous system, in which most of biological tests are carried out, are still limited. The same difficulty is also encountered in the preparation of imprinted polymer films by electropolymerization.

Monomers that are electropolymerizable in aqueous system are limited and the addition of functional groups to these monomers usually results in poor electropolymerization efficiency in aqueous media. In an attempt to overcome this problem, a molecularly imprinted polymer film has been prepared in non-aqueous medium and gradually transferred to aqueous system for use as a sensor for glucose.

As mentioned in the previous sections, fabrication of sensors by molecular imprinting is usually difficult due to the poor processability of MIPs prepared by bulk polymerization. In this study, a new approach involving assembly of MIP on electrode surface by *in-situ* electropolymerization will be employed to tackle this problem. Using this approach, a uniform MIP film firmly adhered to the transducer surface can be obtained, resulting in more efficient transduction of analytical signal and lower diffusion barrier. However, this approach also has its limitations. Firstly, the choice of electropolymerizable monomers with the appropriate functional groups is limited. Secondly, most of these monomers can only be electropolymerized in non-aqueous medium, and the resulting polymer films are not compatible with the aqueous environment in biological studies. In order to overcome these limitations, we will prepare some new electropolymerizable functionalized monomers by organic synthesis in this study. For functionalized monomer which cannot be electropolymerized in aqueous system, a new approach involving preparation of MIP film in nonaqueous system followed by gradual transfer to an aqueous solution will be tested.

Adenosine triphosphate (ATP), glucose and dopamine are important molecules in biological system. Hence, accurate measurement of these bio-molecules under

physiological conditions is of much interest. To take advantage of the specific affinity of molecularly imprinted polymers, imprinted polymer films of these bio-molecules have been prepared and used as sensors under physiological pH. The results are reported in chapter 2, 3 and 4 respectively.

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Chapter 2

Preparation of Molecularly Imprinted Polypyrrole and Poly(*o*-phenylenediamine) Films for ATP Detection

2.1 Introduction

In the preparation of molecularly imprinted polymer (MIP), complexes of the template molecule and the functional monomer are obtained by interaction between the template molecules and monomers with the spatial arrangement of the functional groups retained after polymerization of the functional monomers. This is followed by subsequent removal of template molecules to give complementary cavities with high affinity to target (template) molecules. Interactions including covalent bonding, hydrogen bonding, and electrostatic attraction are possible in the prearrangement of template molecules and functional monomers [1]. Imprinting approaches involving noncovalent interaction are usually more flexible and more similar to the natural biological processes.

Since its advent in early 1980's, the electrochemical quartz crystal microbalance (EQCM) has been widely used in mass measurement in electrochemical studies and chemical sensors[2-5]. The EQCM resonator comprises a thin quartz crystal sandwiched between two metal electrodes, the frequency of which is sensitive to mass changes of the crystal and its electrodes. According to Sauerbrey's equation[6], the frequency change of the resonator is proportional to the mass change on the surface of the EQCM electrode. For an AT-cut 9 MHz quartz crystal resonator, the sensitivity is about 0.2 ng cm^{-2} Hz^{-1}. Because of its high sensitivity to mass change, EQCM has become increasing popular for the fabrication of sensors based on molecularly imprinted polymers in the past decade. Different kinds of polymers were assembled on the EQCM electrode surface, and the EQCM

sensor has been used to detect various analytes including volatile organic vapors, glucose, amino acid and other biomolecules[7-13].

Adenine nucleotides play a crucial role in the regulation and integration of cellular metabolism. Adenosin-5'-triphosphate (ATP) is the major carrier of chemical energy in all living species. During the course of metabolism, energy is provided by enzymatic hydrolysis of ATP to adenosine monophosphate (AMP) or adenosine diphosphate (ADP). Since ATP occurs in all living cells and disappears rapidly in dead cells, it has been widely used as an indicator of living organisms[14-19]. For example, the ATP content is frequently used as an indicator of microbial activity in soils[15-25], the freshness of a wide variety of fish[26-28], and as a quality control of blood prior to transfusion[29]. Recently, because of the increasing threat of bio-attack, detection of bacteria by ATP measurement has attracted much attention[30-32]. In transplantation, the ATP content of transplanted tissue, which has been shown to correlate with graft function and survival[33], is used to assess the viability of the tissue to be transplanted[34, 35].

ATP content can be determined by spectrophotometry[36], bioluminescence[30, 32, 34, 37, 38], chemiluminescence[39], chromatography[23, 26, 40-44], fluorescence[35, 37, 45-49], and electrochemical methods[50-52]. Due to the intrinsic selectivity and sensitivity of firefly luciferase, bioluminescence is one of the most sensitive methods for ATP determination. However, the bioluminescent reagents are expensive and unstable. ATP can also be determined by sensors based on molecularly imprinted polymers. A QCM sensor based on ATP imprinted

polyions was developed by Shinkai and co-workers, the sensitivity of such a sensor is reported to be 10^{-7} mol dm^{-3}, but no data on the selectivity was provided[53].

Electroactive polymers such as polypyrrole and poly(*o*-phenylenediamine) have been used in molecular imprinting [10, 54-61]. By electropolymerization, a uniform polymer film can be easily prepared on the electrode surface and the thickness of the film can be controlled by altering the amount of charge during electropolymerization. Pyrrole and *o*-phenylenediamine both have amine-like function groups. In neutral or slightly acidic media, these monomers are positively charged. As ATP is negatively charged at pH $\leq$7, electrostatic attraction between the monomers and ATP would lead to the formation of complex between the monomer and the template molecule, thus molecularly imprinted polymer can be prepared by electropolymerization of these mixtures. The charge on the surface of these polymers can be controlled by application of a negative voltage to the EQCM electrode, therefore the negatively charged ATP template molecules can be removed by electrostatic repulsion. Here we report our studies on the preparation of the EQCM sensors based on molecularly imprinted polypyrrole and poly(*o*-phenylenediamine) and their application as sensors for ATP. The factors affecting imprinting efficiency and the mechanism of molecular recognition are discussed.

2.2 Experimental

2.2.1. Materials

Pyrrole, *o*-phenylenediamine, sodium dihydrogen phosphate and sodium hydroxide were purchased from Aldrich. Pyrrole was distilled immediately before use and *o*-phenylenediamine was used as received. Adenosine-5'-triphosphate disodium salt (ATP), adenosine-5'-diphosphate disodium salt (ADP), adenosine-5'-monophosphate disodium salt (AMP) were purchased from Sigma.

2.2.2. Instrumental

Electrochemical measurement and electropolymerization were performed on a PAR model 273 potentiostat (EG&G, USA) interfaced to a personal computer using the EG&G M270 software. Piezoelectric measurement was conducted on a QCA917 quartz crystal analyzer (Seiko EG&G). Frequency signal was collected by a personal computer with a home-developed data acquisition program (Figure 2.1). The electrochemical cell used in this work is a home-designed Teflon cell similar to that of QCA 917 (Figure 2.2). An AT-cut 9.0 MHz EQCM Pt electrode (Seiko EG&G, area = 0.2 cm^2) with only one side exposed to the electrolyte solution was used as the working electrode whereas a Pt wire and a saturated calomel electrode (SCE) were used as counter electrode and reference electrode respectively.

UV-visible absorption spectra were recorded on a Milton Roy Spectronic 3000 diode array spectrophotometer.

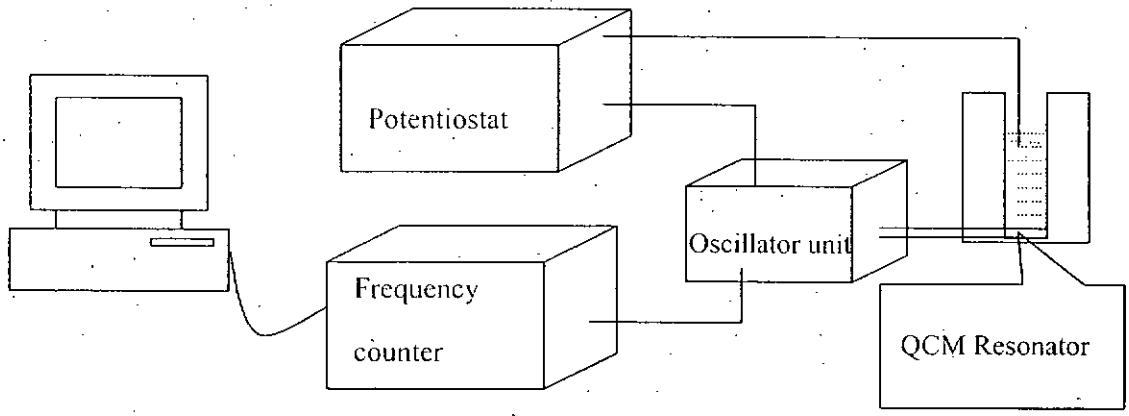

Figure 2.1. Schematic diagram showing the electrochemical quartz crystal microbalance (EQCM) setup.

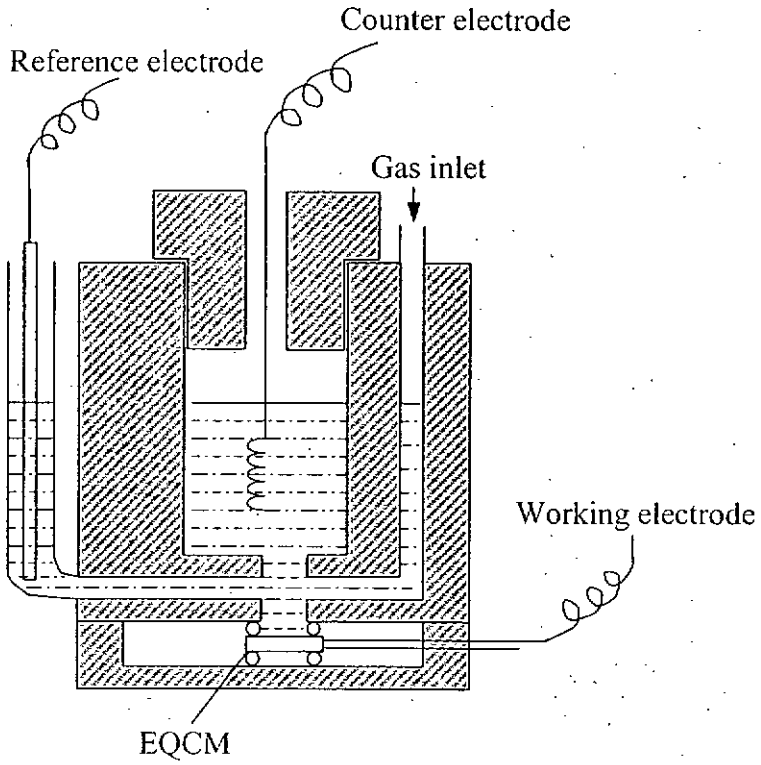

Figure 2.2. Diagram of the home-made EQCM cell.

2.2.3. Preparation of molecularly imprinted polymer films

Molecularly imprinted polypyrrole films were prepared by potentiostatic electrolysis at 0.8V of a pH 7.0 phosphate buffer solution containing 0.1 mol dm^{-3} pyrrole and 0.2 mol dm^{-3} ATP with an EQCM Pt electrode for about 1 hr. The polypyrrole films were then overoxidized by repeatedly scanning the potential between –0.2 to 1.1 V at a scan rate of 50 mV s^{-1} until stable i-E curves were obtained. Non-imprinted polymer films were prepared with the same procedures except in the absence of ATP.

Poly(*o*-phenylenediamine) films were prepared by continuously scanning the potential between 0.0 V and 0.8 V of an EQCM Pt electrode in a pH 5.0 buffer solution containing 5.0×10^{-3} mol dm^{-3} *o*-phenylenediamine and 0.01 mol dm^{-3} ATP for 16 cycles. After extensive washing with water, a negative voltage of –0.20 V was applied to the polymer films for 20 minutes, followed by extensive washing with water again to remove the template molecules. Non-imprinted polymer films were prepared in the same way but no ATP molecules were added to the electrolyte solution for electropolymerization.

Molecularly imprinted polymer films of overoxidized polypyrrole and poly(*o*-phenylenediamine) prepared by the above procedures are abbreviated as OPy/ATP and PPd/ATP, respectively.

2.2.4. Analytical measurement with EQCM

For EQCM sensor based on overoxidized polypyrrole, frequency change of the sensor was recorded during continuous potential scan of the electrode between –0.2 to 0.9 V at 50 mV s^{-1}. The supporting electrolyte was a pH 7.4 phosphate buffer containing an analyte (ATP, ADP or AMP) of known concentration. The sensor was re-generated by the overoxidation procedure mentioned above followed by extensive rinse with deionized water.

For EQCM sensor based on poly(*o*-phenylenediamine), measurement was performed by holding the sensor electrode at +0.5 V. Analytes of different concentrations were added to the cell by a micro-syringe. After each measurement the sensor was re-generated by applying a negative potential of –0.2 V to the working electrode for 20 minutes in a well-stirred electrolyte solution. This was followed by extensively washing the electrode with deionized water.

2.3 Results and discussion

2.3.1. Preparation of MIP film of overoxidized polypyrrole (OPy/ATP)

Pyrrole can be easily electropolymerized on a Pt electrode. In a pH 7.0 buffer solution, pyrrole is oxidized when the potential is higher than 0.4 V (Figure 2.3) and substantial electropolymerization can be observed at 0.5 V. Figure 2.4 shows a cyclic voltammogram of 0.01 mol dm^{-3} ATP in pH 7.0 phosphate buffer recorded on a Pt working electrode in the potential range of –0.3 to 1.0 V. The electrochemical behavior of the ATP containing solution is almost identical to that of the blank supporting electrolyte. A rise in anodic current can be observed when the potential is higher than 1.1 V, which is the background current due to electrolysis of the supporting electrolyte. Based on the above data, the potential for electropolymerization of pyrrole in the presence of ATP was chosen to be 0.8 V, a potential at which ATP is electrochemically stable and electropolymerization of pyrrole can be conducted. Molecularly imprinted polypyrrole films were prepared by potentiostatic electrolysis of a pH 7.0 phosphate buffer solution containing 0.10 mol dm^{-3} pyrrole and 0.2 mol dm^{-3} ATP at 0.8 V on an EQCM Pt electrode for about 1 hr. The frequency change of EQCM during the course of electrolysis was about 10 kHz.

It is generally believed that overoxidation of polypyrrole results in loss of cationic charge on the pyrrole nitrogen and introduction of oxygen-containing groups to the pyrrole ring [62, 63]. Therefore, release of the negatively charged ATP molecules from the polymer matrices can be expected. The ATP template molecules

on such prepared polypyrrole films were removed by over-oxidizing the polypyrrole film through repeatedly scanning the potential between –0.2 to 1.1 V at a rate of 50 $mV \cdot s^{-1}$ until the i-E curve became stable. The electrode was subsequently washed extensively with deionized water. Removal of ATP molecules from the imprinted polymer film by overoxidation was confirmed by measuring the UV-Vis absorption at 258 nm of the electrolyte after the overoxidation process. An absorption band at 258 nm attributable to ATP absorption was observed in the UV-Vis spectrum of the electrolyte after the first overoxidation process which disappeared slowly in subsequent overoxidation cycles.

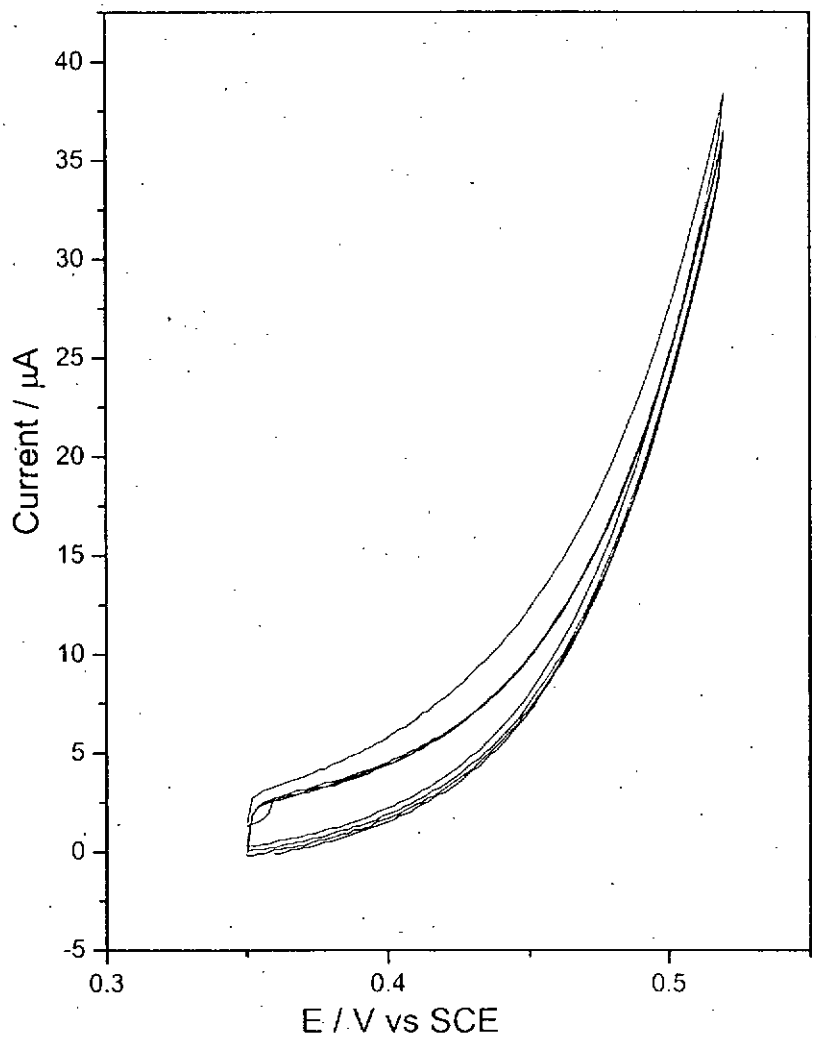

Figure 2.3. Cyclic voltammogram of a Pt working electrode in pH 7.0 phosphate buffer containing 0.2 mol dm^{-3} pyrrole, scan rate = 50 mVs^{-1}

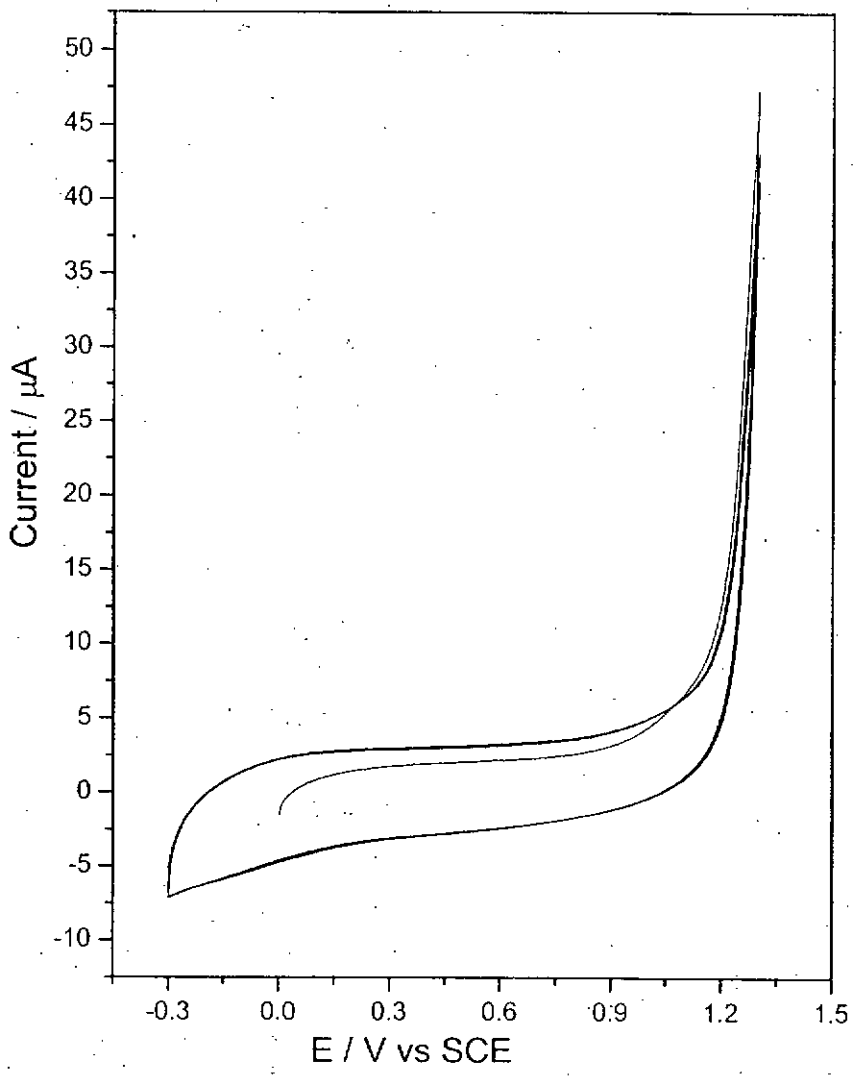

Figure 2.4. Cyclic voltammogram of a Pt working electrode in pH 7.0 phosphate buffer containing 0.01 mol dm^{-3} ATP, scan rate = 50 mV s^{-1}

2.3.2. Molecular recognition of ATP by OPy/ATP

In order to test the molecular recognition ability of the molecularly imprinted polypyrrole film towards ATP, the EQCM electrode coated with OPy/ATP film was immersed in 5 ml of pH 7.4 phosphate buffer containing an analyte (ATP, ADP or AMP) of known concentration and the frequency change of the sensor was recorded during continuous potential scan of the EQCM electrode between –0.2 to 0.9 V at 50 $mV\ s^{-1}$.

Figure 2.5A depicts the time dependence of frequency change (ΔF) of the OPy/ATP coated EQCM resonator during the course of potential sweep in a phosphate buffer solution containing 5.0×10^{-3} mol dm^{-3} ATP. It can be seen that the frequency continuously decreased during the course of potential sweep until it reached a steady state after about 5 minutes. The frequency change of the EQCM sensor indicated a mass gain on the electrode surface which can be ascribed to the migration of species from solution into the OPy/ATP film during the potential scan. As the first dissociation constant (pKa_1) of ATP is equal to 3.7, ATP is negatively charged in neutral media. Therefore ATP can be adsorbed onto the molecularly imprinted polymer film by electrostatic interaction. Figure 2.5A also shows the zigzag nature of the frequency response of the resonator. The frequency change of the resonator during a potential scan cycle versus the change in electrode potential is plotted in Figure 2.6. From this figure, one can see that the resonator frequency shifts negatively during the positive scan and vice versa, suggesting migration of solution species into and out of the polymer film during the positive and negative scan

respectively. This is another indirect evidence for ATP migration towards and away from the electrode surface.

In order to confirm the molecular imprinting effect, a nonimprinted overoxidized polypyrrole film was prepared with similar procedure except no ATP was present in the electrolyte for electropolymerization. The response of the EQCM resonator coated with nonimprinted polymer film is shown in Figure 2.5B. Compared with Figure 2.5A, the frequency of the resonator also shifted negatively but with a much smaller slope, indicating that uptake of ATP into the nonimprinted polypyrrole film was much slower. The large frequency drop of the imprinted polypyrrole film compared to the non-imprinted one confirms the presence of the molecular imprinting effect.

There is a chance that the frequency change during the potential sweep might arise from the EQCM Pt electrode itself. In order to eliminate this possibility, a bare EQCM Pt electrode was used in a control experiment. No substantial frequency shift from the baseline was observed. Only a horizontal baseline with a regular waveform of small amplitude due to the electrochemical oxidation and reduction of Pt metal was observed. Another control test was also carried out by monitoring the frequency change of the OPy/ATP coated EQCM electrode in the absence of ATP, in a blank buffer solution. Again, no substantial frequency shift can be observed. These control experiments indicate that the mass gain (frequency drop) is due to the interaction of ATP with the MIP film on EQCM electrode.

ADP and AMP are products of ATP decomposition with similar molecular structure with ATP, therefore clear discrimination of ATP from ADP and AMP is challenging but essential for ATP determination. Figure 2.7 depicts the correlation of the frequency change of the EQCM sensor with various concentrations of ATP, ADP and AMP. As shown in the figure, in the range of 1×10^{-4} to 2×10^{-2} mol dm^{-3}, ΔF increases proportionally with ATP concentration. This result shows the potential of this MIP based EQCM sensor as an ATP sensor. It is noteworthy that the frequency response of OPy/ATP to both ADP and AMP is much less than that to ATP. This may partly be due to the fact that ADP and AMP have lower molecular weight than ATP. If calculated with molar unit, which can normalize the attribution from molecular weight, the response of the sensor towards 1.0×10^{-3} mol dm^{-3} ATP is 730 Hz, which is much larger than that of ADP (337 Hz) and AMP (42 Hz).

The above result shows that the EQCM sensor based on OPy/ATP is both sensitive and selective towards ATP. On the other hand, as can be seen in Figure 2.4, the response time of this sensor is as long as 5 minutes, which is fairly long considering the high turnover rate of ATP *in vivo*.

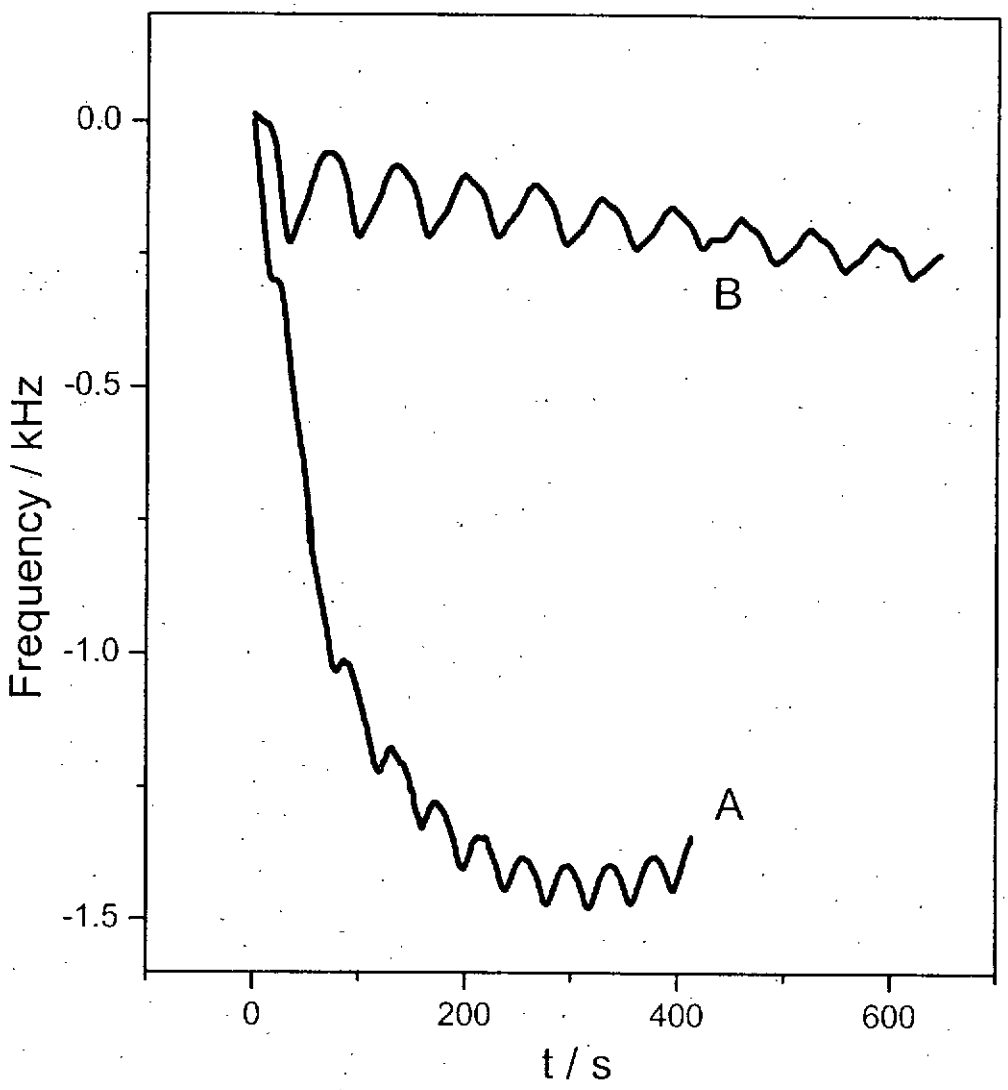

Figure 2.5. Frequency response of the EQCM resonator coated with (A) molecularly imprinted OPy/ATP and (B) non-imprinted polypyrrole upon addition of 5.0×10^{-3} mol dm^{-3} ATP.

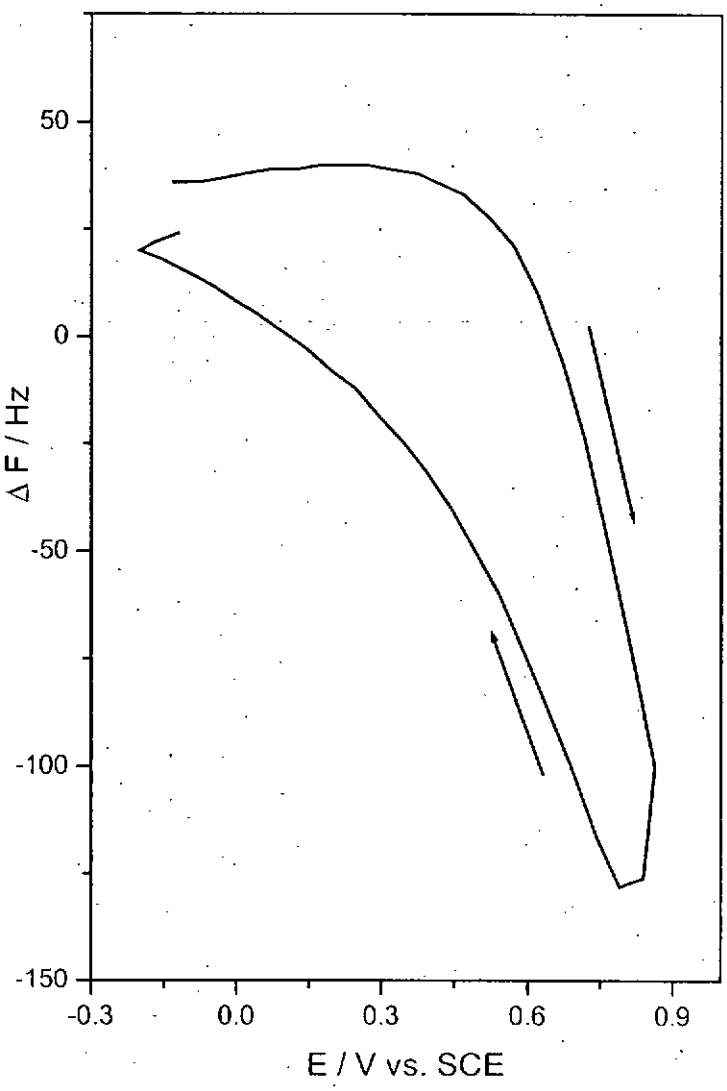

Figure 2.6. Frequency change of the OPy/ATP coated EQCM resonator in one potential sweep cycle.

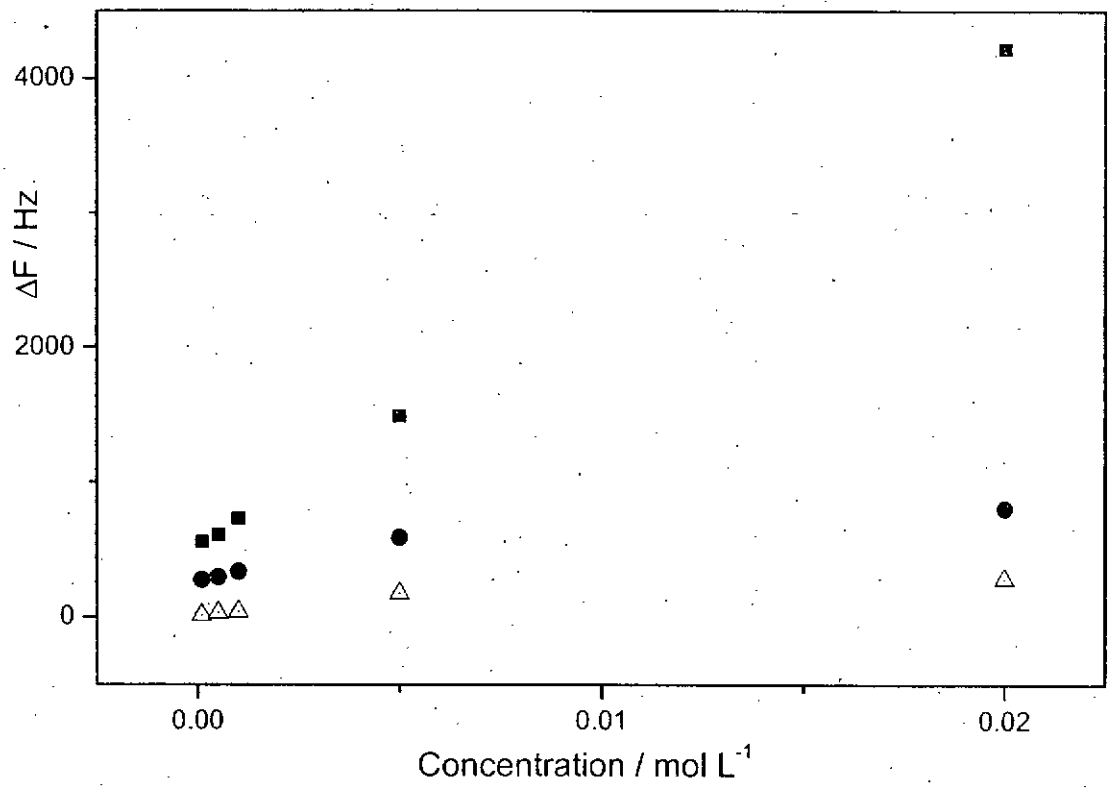

Figure 2.7. Dependence of frequency change (ΔF) of the OPy/ATP coated EQCM resonator on concentration of ATP (■), ADP (●) and AMP(Δ).

2.3.3. Optimization of parameters in the preparation of the sensor

ATP to pyrrole mole ratio. In the preparation of molecularly imprinted polymer films, non-covalent adducts of template molecules ATP and pyrrole monomers are formed through hydrogen bonding and electrostatic interaction. Thus, the ATP to pyrrole mole ratio is one of the most important parameters that may affect the performance of the MIP film. A series of MIP films were prepared from electrolyte solutions containing various ATP to pyrrole mole ratios. Electropolymerization was performed by potentiostatic electrolysis of these solutions at 0.8 V until the negative frequency shift of the EQCM resonator reached 10 kHz. These series of MIP films were used to measure the frequency shift of 5.0×10^{-3} mol dm^{-3} ATP and AMP.

Table 2.1 summarizes the analytical performance of these MIP films prepared with different ATP to pyrrole mole ratios. The sensitivity of the MIP films towards 5.0×10^{-3} mol dm^{-3} ATP (ΔF) increases with the mole ratio of ATP to pyrrole. It is likely that a higher ATP to pyrrole mole ratio can enhance the sensitivity of the MIP films towards ATP. However, the stability of the MIP film drops at very high ATP to pyrrole mole ratio. At an ATP to pyrrole mole ratio of 5:1, the polymer film prepared is only stable enough to perform one ATP incorporation-overoxidation cycle. At high ATP to pyrrole mole ratio the film prepared has lower selectivity but the response time is shorter. When the ATP to pyrrole mole ratio is increased from 1:3 to 5:1, the selectivity of the MIP film decreases more than one fold. At the same time, the response time of the MIP film decreases from 720 s to 240 s. Based on the above data, an optimized ATP to pyrrole mole ratio of 2:1 was selected.

Table 2.1 Frequency change towards 5×10^{-3} mol dm^{-3} ATP (ΔF), ATP / AMP selectivity ratio and response time of a series of MIP films prepared with various ATP : pyrrole mole ratios.

ATP : Pyrrole (mole:mole)	ΔF (kHz)	$\Delta F_{ATP} : \Delta F_{AMP}$	Response time (s)
1 : 3	0.1	10.5	720
1 : 2	0.4	9.8	480
1 : 1	0.9	9.6	420
2 : 1	1.48	8.1	300
3 : 1	1.52	7.3	300
5 : 1	1.62	4.3	240

2.3.4. Preparation of MIP film of poly(*o*-phenylenediamine) (PPy/ATP)

Molecularly imprinted polymer of poly(*o*-phenylenediamine) was prepared by continuous potential scan of an EQCM Pt electrode in a pH 5.0 acetate buffer solution containing 5.0×10^{-3} mol dm^{-3} *o*-phenylenediamine and ATP of predetermined concentration. ATP with concentration from 2.5×10^{-3} to 1.25×10^{-2} mol dm^{-3} was used, but little difference was found in sensitivity and selectivity of the sensors based on these MIP films. Thus molecularly imprinted polymer films prepared from 5.0×10^{-3} mol dm^{-3} *o*-phenylenediamine and 1.0×10^{-2} mol dm^{-3} ATP was used in further investigations. Figure 2.8 shows a typical cyclic voltammogram during the electropolymerization process. As shown in the figure, the peak current decreased drastically after the first cycle and continually decreased upon repetitive scanning. This indicates that the poly(*o*-phenylenediamine) polymer formed has low conductivity. As a result, the growth of polymer almost stopped after about 15 scan cycles and the polymer film prepared by this way was relatively thin, as evidenced by the frequency change of ca. 800 Hz in the scanning process (Figure 2.9). The cyclic voltammogram on the polymerization of o-phenylenediamine is almost indifferent to the presence or absence of ATP in the solution, which is reasonable as ATP is electrochemically inactive in the range of scanning potential. The MIP film was then extensively washed with water and a negative potential of -0.2 V was applied to the working electrode for 20 minutes to facilitate the removal of the negatively charged template molecules.

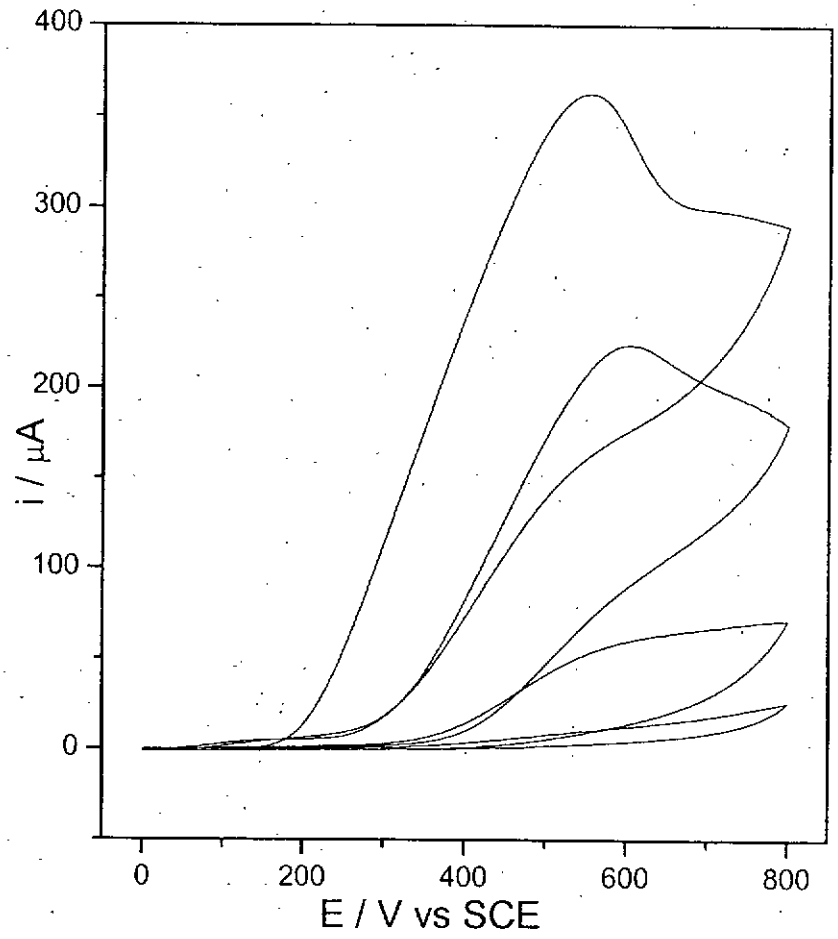

Figure 2.8. Cyclic voltammogram during electropolymerization of 5×10^{-3} mol dm^{-3} poly(*o*-phenylenediamine) in the presence of 0.01 mol dm^{-3} ATP in pH 5.2 acetate buffer.

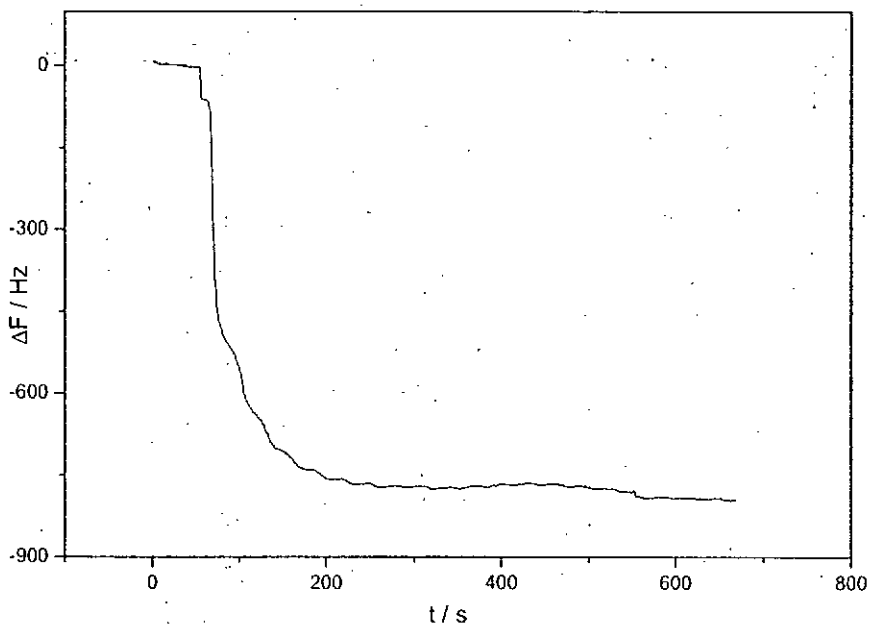

Figure 2.9. Frequency response of the EQCM resonator in the course of electropolymerization with *o*-phenylenediamine.

2.3.5. Molecular recognition of ATP by PPd/ATP

In order to test the molecular recognition ability of the PPd/ATP based sensor, EQCM measurement of the sensor was performed by holding the working electrode at +0.5 V, until a stable frequency reading was obtained. To avoid disturbance of the electrolyte, aliquots of analytes of known concentration were added carefully and quickly to the cell by a micro-syringe.

The frequency change of the PPd/ATP based EQCM sensor at 0.5 V upon addition of 1.0×10^{-5} mol dm^{-3} of ATP is shown in Figure 2.10A. The frequency of the resonator sharply decreased upon addition of ATP and the steady state was reached rapidly in as short as 10 seconds. The frequency change was measured as 130 Hz. In order to eliminate the possibility that the frequency change was a result of the addition of buffer solution, a blank buffer solution without ATP was added and the frequency change of the resonator was found to be less than 3 Hz, which is negligibly small. The response of an EQCM electrode coated with non-imprinted PPd as well as that of a bare EQCM electrode under the same experimental conditions were also shown in Figure 2.10B and 2.10C respectively. It can be seen that both resonators showed response to ATP, the frequency change was 15 Hz for the non-imprinted resonator and about 6 Hz for the bare resonator. During measurement, a positive potential was applied to the working electrode so that it could be assumed that electrostatic attraction occurred between the positively charged electrode surface and the negatively charged ATP molecules. Despite the frequency change caused by electrostatic interaction, the resonator coated with

PPd/ATP shows much higher sensitivity towards ATP, which can be attributed to the molecular imprinting effect.

The frequency response of the PPd/ATP based EQCM resonator to 1.0×10^{-3} mol dm^{-3} of ADP and AMP were recorded and shown in Figure 2.11. The frequency change of the resonator was measured to be 15 Hz for ADP and 9 Hz for AMP respectively. It is evident that the sensitivity of the molecularly imprinted resonator for ATP is higher than those for ADP and AMP. It is also noted that the time required for the frequency response to reach a steady state for ATP is about 50 s, which is much shorter than that for ADP (ca. 100 s) and AMP (130 s). The difference in response time can be attributed to the specific affinity of the MIP film to ATP as well as the difference in charges of the nucleotides (ATP carries a higher negative charge than ADP and AMP).

Figure 2.12 shows the correlation of the frequency change with various concentrations of ATP, ADP, and AMP. In general, the sensitivity of the resonator coated with MIP is much higher towards ATP than ADP and AMP. This indicates that the ATP-imprinted poly(*o*-phenylenediamine) prepared in this work has good selectivity toward ATP. As can be seen in the figure, the frequency change of the resonator is almost proportional to the concentration of ATP in the range $2 \times 10^{-5} \sim 2 \times 10^{-3}$ mol dm^{-3}. The detection limit of this sensor to ATP is about 5×10^{-6} M. Compared to the result of AMP detection published by Shinkai and coworkers using hydrogels containing boronic acid [53], the response time of this PPd/ATP based sensor (ca. 50 s) is much better than theirs (> 50 minutes), but the detection limit of Shinkai's sensor is much lower (10^{-7} mol dm^{-3}). It should be noted that the PPd/ATP

film prepared in this work is much thinner than the polyion film reported by Shinkai and co-workers. The thin film leads to fast response of the sensor at the expense of lower sensitivity.

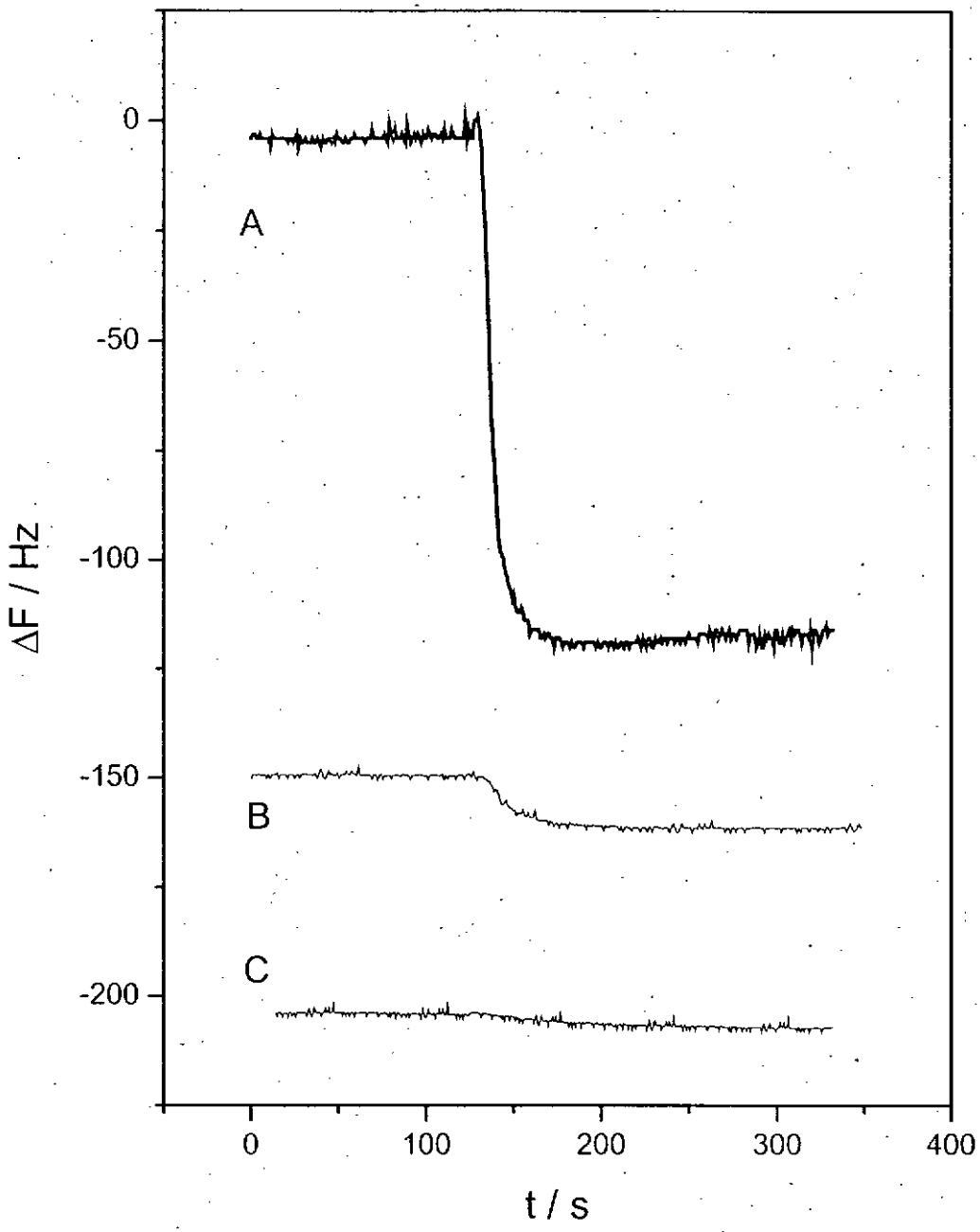

Figure 2.10. Frequency response of (A) molecularly imprinted PPd/ATP, (B) non-imprinted PPd and (C) bare EQCM resonator upon addition of 1.0×10^{-5} mol dm^{-3} ATP.

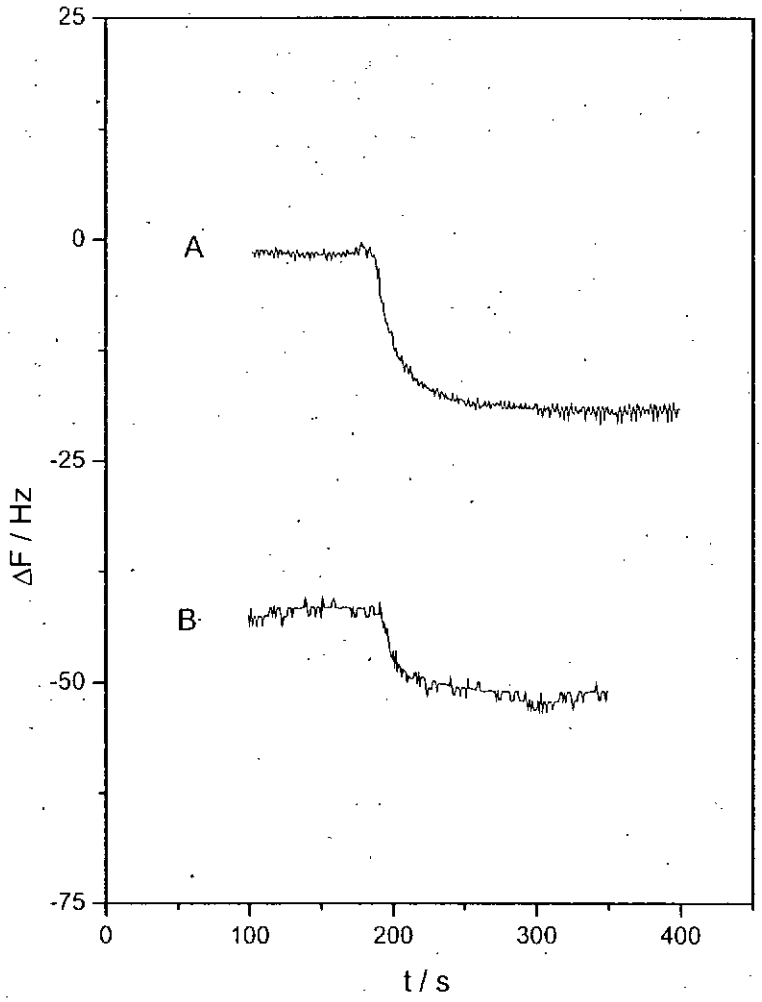

Figure 2.11. The time-resolved frequency change of the PPd/ATP coated EQCM resonator upon addition of 1.0×10^{-4} mol dm^{-3} ADP (A) and AMP (B).

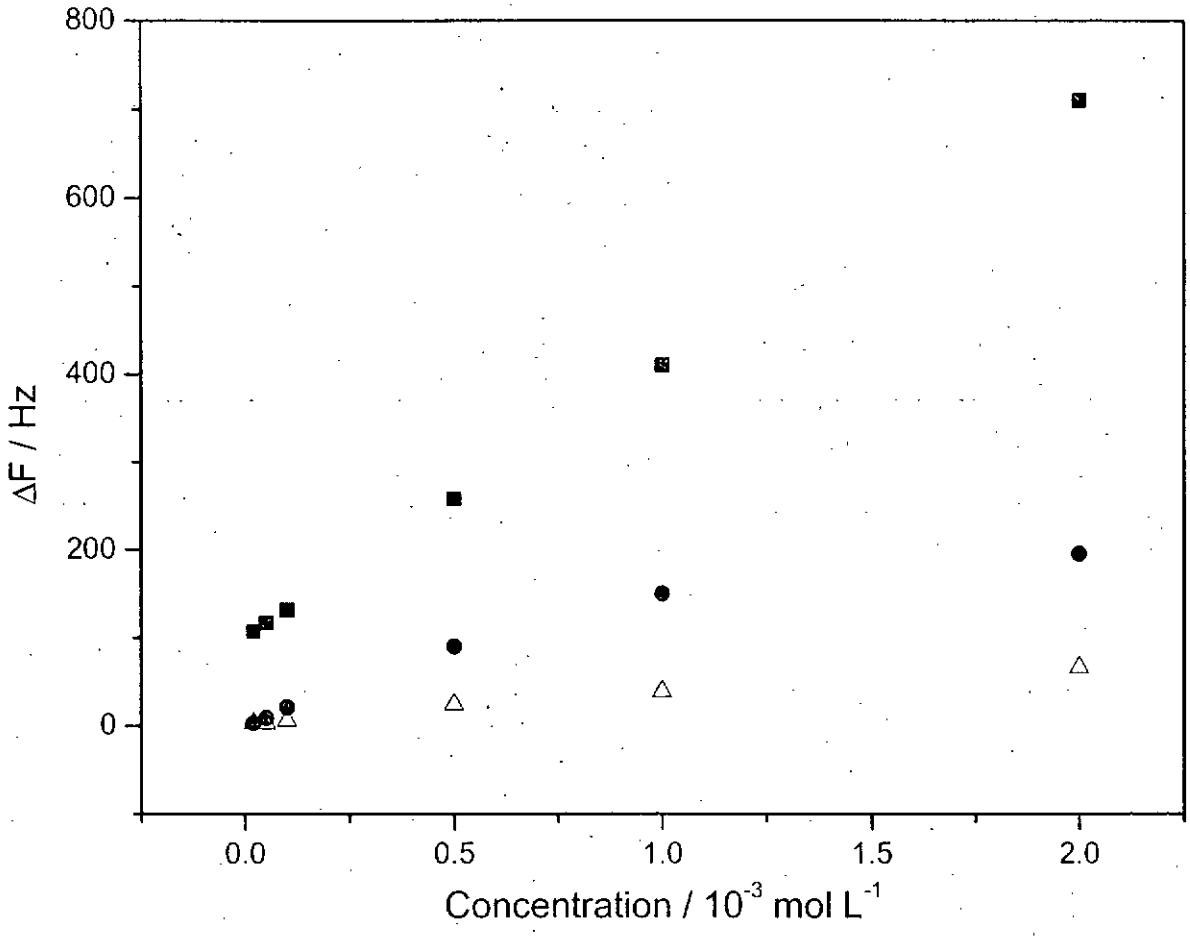

Figure 2.12. Dependence of frequency change (ΔF) of the PPd/ATP coated EQCM resonator on concentration of ATP (■), ADP (●) and AMP (Δ).

2.3.6. Comparison of molecularly imprinted OPy and PPd polymer films

On comparing the analytical performance of the OPy/ATP and PPd/ATP based sensors, it is found that the latter is superior both in response time and detection limit. The difference in response time is partly due to thinner polymer film in the PPd/ATP sensor. The difference in sensitivity can be due to the intrinsic difference in the structure of the polymer films. The *o*-phenylenediamine monomer contains two amino groups which are retained in the MIP film. These amino groups carry positive charges in pH 7.4 phosphate buffer. In contrast, the positive charge on the nitrogen atoms of pyrrole was reported to be partly removed by the overoxidation process [63]. The loss in the positive charge of the overoxidized polypyrrole can decrease the affinity of the MIP film to ATP. Moreover, the amino groups on poly(*o*-phenylenediamine) are more out-reaching than the N-H groups in polypyrrole. Thus it is reasonable to assume that amino groups in PPd/ATP films can bind the ATP molecules much more readily.

2.4 Conclusions

Molecularly imprinted polymer films of polypyrrole and poly(*o*-phenylenedamine) have been prepared by electropolymerization on EQCM electrodes in the presence of adenosine-5'-triphoshate (ATP). These ATP sensors are selective against ADP and AMP. A pyrrole to ATP mole ratio of 2:1 was found to be the best for the preparation of the MIP polypyrrole films by electropolymerization. On the other hand, the poly(*o*-phenylenediamine) film has low conductivity, thus very thin polymer film would be obtained. This allows the MIP films to have short response time towards the analytes. Compared to the polyion film reported by Shinkai and co-workers, the poly(*o*-phenylenediamine) MIP films were found to exhibit better selectivity but lower sensitivity towards ATP.

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Chapter 3

Preparation of Molecularly Imprinted Poly(boronate-substituted aniline) Films and their Application as Glucose Sensor

3.1 Introduction

As described in chapter 1, depending on the nature of interaction between functional monomer and template molecules, there are two types of molecular imprinting methods: covalent imprinting and non-covalent imprinting[1]. In the case of covalent imprinting, functional monomer and template molecule are bound to each other by covalent bonds. This aggregation of monomer and template molecule is then polymerized under the conditions where the covalent bond remains intact. The covalent bond is subsequently cleaved under appropriate conditions and the template molecules are removed from the polymer matrix. When the imprinted polymer film is used for analysis, the guest molecules form the same covalent bond with the functional groups on the polymer.

The covalent imprinting method possesses certain advantages over the non-covalent method[2]. The most obvious one is that the imprinting effect is more precise due to the strong interaction between imprinted polymer backbone and template molecules. The strong interaction of the covalent bonding also enhances the stability of the imprinted polymer thus enables its utility under a wide variety of experimental conditions. Moreover, monomer-template aggregations are stable and stoichiometric so that the imprinting processes and the structure of the binding site are relatively well-defined. On the other hand, the synthesis of the monomer-template conjugate is often troublesome and less economical. Compare to non-covalent binding, uptake and release of guest molecule by covalently imprinted polymer are usually slower.

One of the keys to success in covalent imprinting is a good choice of the functional monomer and the template moiety. The covalent bonds formed between the functional monomer and the template molecules must be both stable and reversible, which are contradictory to a certain extent. Therefore, the number of examples which fulfill both of these requirements is relatively small. In the published works, samples of covalent imprinting include acetals[3-6], ketals[7-11], Schiff bases[7-11], disfulfide bonds[12, 13], coordination bonds[14-21] and boronic acid esters[22-28].

Boronic acids are capable of forming reversible and strong covalent bonds with the diol functionalities of carbohydrates in the form of cyclic esters[29]. It is even more desirable that the rate of this reversible reaction is much faster than the human time-scale. Due to its advantageous characters, covalent formation of boronic acid esters is widely used in various sensing systems[30-36]. Nevertheless, most of these sensory works were confronted with the problem of low solubility of the boronic acid derivatives in aqueous media[34].

In this chapter, I will report the preparation of molecularly imprinted co-polymer film of boronate-substituted aniline (BSA) and its application as sensor for glucose, which is one of the most important analytes in human blood. By immobilization of the boronic acid moieties into a polymer film, the problem of low solubility of boronic acid derivatives can be overcome.

3.2 Experimental

3.2.1. Materials

3-Aminophenylboronic acid (APBA) hydrochloride salt, sodium dihydrogen phosphate, sodium hydroxide, sodium chloride and tetrabutylammonium hydrogensulfate (Bu_4NHSO_4) were purchased from Aldrich and used as received. D-glucose, D-fructose, D-sucrose and ribose were purchased from Sigma. Deionized water was prepared by a Milli-Q system (Millipore, MA, USA).

3.2.2. Instrumental

Electrochemical measurements and electropolymerization were performed on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by BAS 100B/W software running on a personal computer. Piezoelectric measurement was conducted on a QCA917 quartz crystal analyzer (Seiko EG&G). Frequency signal was collected by a PC computer with a home-developed data acquisition program. The electrochemical cell used in this work is a home-made Teflon cell similar to that of QCA 917 Teflon cell. A 9.0 MHz EQCM Pt electrode (Seiko EG&G, area = 0.2 cm^2) with only one side exposed to solution was used as the working electrode whereas a Pt wire and an Ag/AgCl electrode were used as the counter electrode and reference electrode respectively.

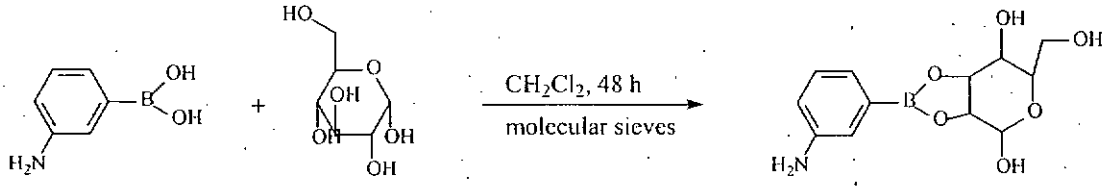
UV-visible absorption spectra were recorded on a Milton Roy Spectronic 3000 diode array spectrophotometer. FT-IR spectra were obtained as pellets in the 500-

4000 cm^{-1} region by a Nicolet Magna-IR 750 FTIR spectrometer. ^1H NMR spectra were recorded on a Bruker 400 MHz DPX 400 spectrometer.

3.2.3. Synthesis of monosaccharide-boronate-substituted aniline

Glucose-boronate-substituted aniline was synthesized according to published work[37] with slight modification (Scheme 1). 3-Aminophenylboronic acid (3.47 g, 20 mmol) and activated molecular sieves (BDH 4A, 5.0 g) were added to a 100 ml dichloromethane solution of glucose (2.74 mg, 20 mmol). After 48 hours, the sieves were removed by filtration and methylene chloride was removed under vacuum to give a light yellow solid. The raw product obtained was recrystallized from dichloromethane / hexane. Yield: 64%.

Scheme 3.1



3.2.4. Preparation of molecularly imprinted poly(boronate-subsituted aniline) films

Co-polymer films of boronate-substituted aniline (BSA) and aniline were prepared by continuous potential scan of the EQCM Pt or Pt wire electrode between -0.20 and 1.15 V in an acetonitrile solution containing BSA and aniline. The concentration of BSA is fixed at 5.0×10^{-3} mol dm^{-3} while that of aniline was varied for different purposes. Polymer films with various thicknesses can be prepared by altering the number of potential scan cycles. The molecularly imprinted polymer film thus prepared was then immersed in well-stirred mixtures of acetonitrile-acetone, acetone-water and water in sequence for 2 hours each. After that, the MIP film was immersed in well-stirred 0.1 mol dm^{-3} acetic acid solution for 4 hours, during this course, the acetic acid solution was changed every hour.

For non-imprinted polymer film, similar conditions were applied except that BSA was replaced by 3-aminophenylboronic acid. After electropolymerization, the non-imprinted polymer film was treated with the same series of mixtures and water. The step of acetic acid treatment was omitted.

Molecularly imprinted and non-imprinted polymers of BSA and aniline prepared with the above procedures are referred to as poly(BSA) and poly(aminophenylboronic acid) respectively.

3.2.5. Analytical measurement with EQCM

Generally, EQCM measurement of the sensor was performed by holding the working electrode at a positive potential of 0.5 V. Analytes of certain concentration were added to the cell by a micro-syringe. After each measurement the sensor was re-generated by dipping in a well-stirred acetic acid solution (0.1M) for 30 minutes. During this step, the acetic acid solution was changed every 10 minutes. The working electrode was subsequently washed extensively with deionized water.

3.3 Results and discussion

3.3.1. Electrochemical characterization of boronate substituted aniline (BSA) in aqueous and non-aqueous media

Glucose-boronate substituted aniline synthesized by the procedure described in the experimental section was investigated by electrochemical methods. In pH 7.4 phosphate buffer, BSA shows a substantial anodic current at about 0.6 V in the first cycle on a fresh Pt electrode (Figure 3.1). This anodic peak disappeared in subsequent cycles. Presumably, the disappearance of the peak is due to the formation of an insulated polymer film on the electrode surface. After the potential scan, no polymer on the Pt electrode could be observed. The above phenomenon indicated that BSA cannot be electropolymerized in neutral aqueous media.

It has been reported that 3-aminophenylboronic acid can be electropolymerized in acidic media[38], but electropolymerization in acidic media is not suitable for preparation of molecularly imprinted polymer of glucose boronate substituted aniline. This is due to the pH dependence of covalent bond between boronic acid moieties and glucose. At low pH value, the ester bond between boronic acid and glucose would dissociate and glucose would be released, which makes the glucose molecules unable to be imprinted onto the polymer film.

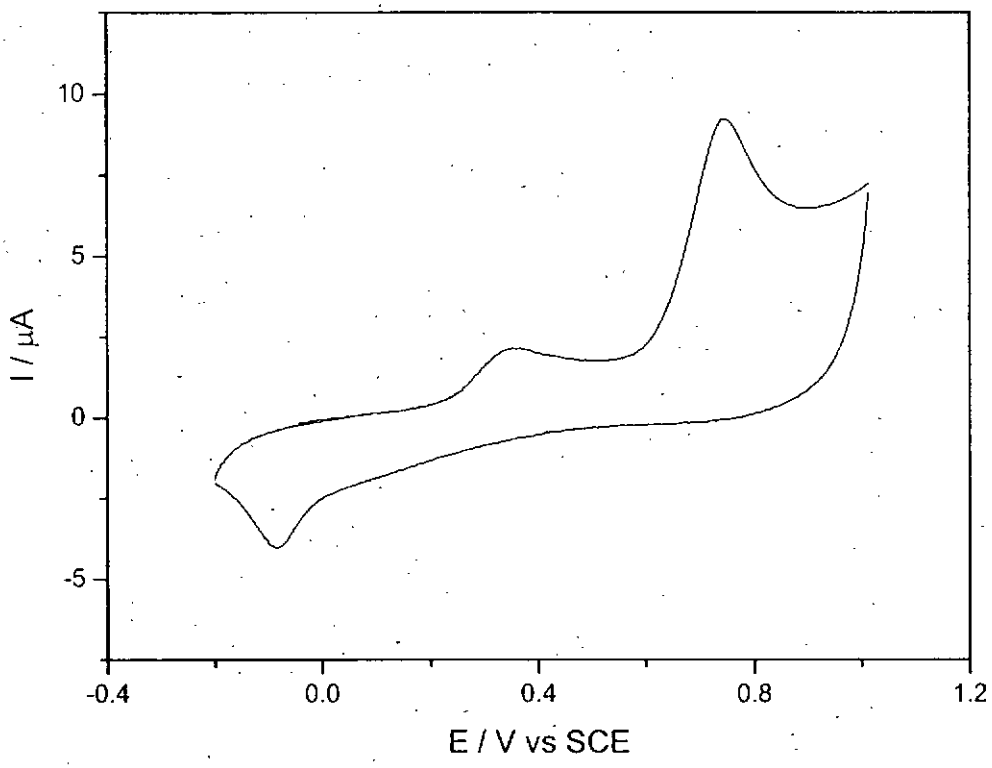


Figure 3.1. i-E curve of the first cycle of cyclic voltammogram recorded on a Pt electrode in pH 7.4 phosphate buffer solution containing 5.0×10^{-3} mol dm^{-3} BSA. Scan rate: 50 mVs^{-1}.

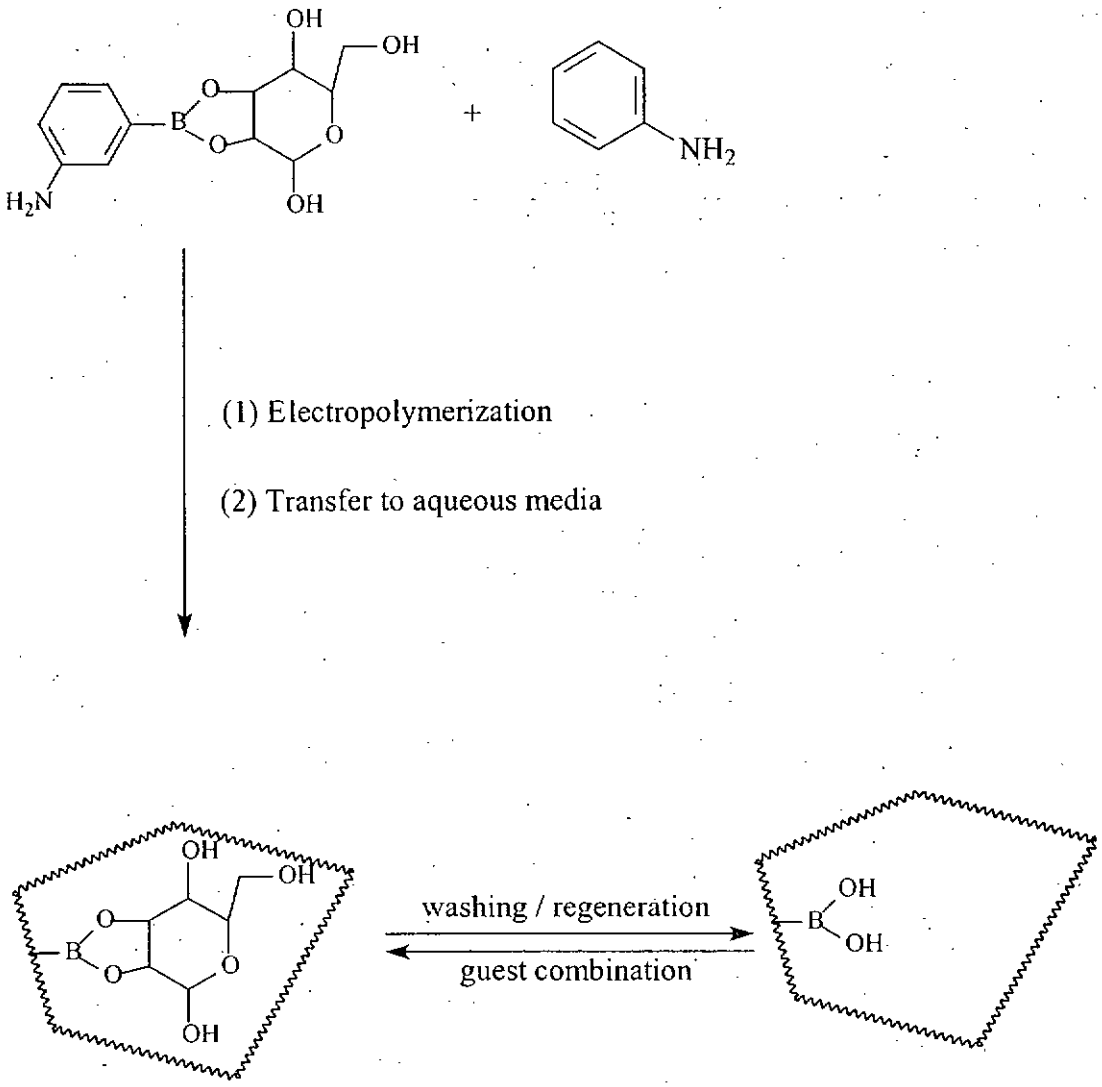
3.3.2. Preparation of molecularly imprinted polymer film of BSA

An acetonitrile solution with 0.1 mol dm^{-3} Bu_4NHSO_4 as supporting electrolyte was then employed for electrochemical investigation. Figure 3.2 presents the cyclic voltammogram of BSA recorded on a Pt electrode in such solution. A redox couple can be observed at about 0.6 V. At potential higher than 1.0 V, a significant oxidation wave due to the electroxidative polymerization of BSA can be observed. In subsequent cycles, the peak current increases with the number of cycles. The above result indicates that an electroactive polymer film has been formed.

Despite the great development achieved in the field of molecular imprinting over the past years, one of the major problems remain unsolved is the availability of suitable solvent. To date, most of the imprinted polymers reported were prepared and functioned only in organic solvents. In the case of BSA, the polymer cannot be prepared in aqueous system. One of the solutions to this problem is to prepare Poly(BSA) film in non-aqueous media before using it in aqueous system. Nevertheless, most of the literature-reported polymer films prepared in non-aqueous media lose their electrochemical activity and / or conductivity in aqueous media. Nevertheless, Miras and co-worker[39] have reported the success in transferring a poly(aniline) film from non-aqueous system to aqueous system without losing its conductivity.

The procedure to prepare a molecularly imprinted polymer film of BSA is shown in scheme 3.2. It includes the following steps: (1) continuous potential scan of EQCM Pt or Pt wire working electrode between -0.20 and 1.15 V in acetonitrile

containing 5.0×10^{-3} mol dm^{-3} of BSA and 0.013 mol dm^{-3} aniline to prepare poly(BSA), (2) immerse the polymer film thus prepared into a mixture of (i) acetonitrile-acetone (1:1 v/v), (ii) acetone-H_2O (1:1 v/v) and (iii) deionized H_2O in sequence, and (3) dissociation of the ester bond and removal of glucose from the polymer matrix by washing with 0.1 M acetic acid.



Scheme 3.2. Schematic presentation of preparation, recognition and regeneration of glucose-imprinted poly(BSA) film.

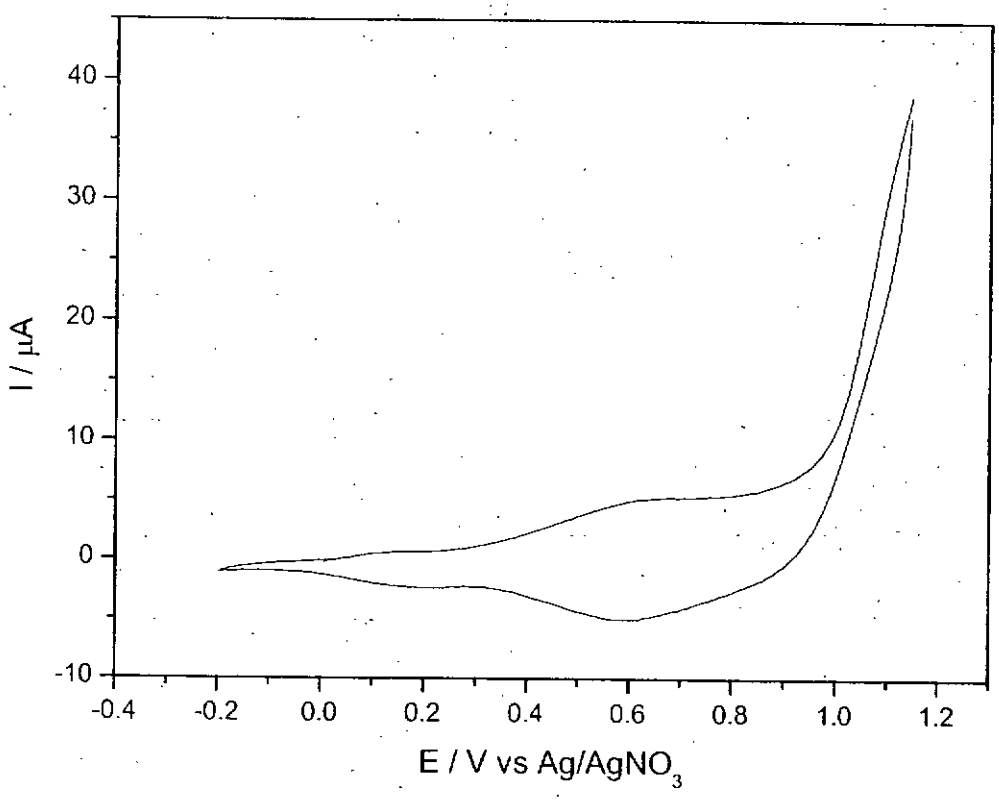


Figure 3.2. Cyclic voltammogram of 5.0×10^{-3} mol dm^{-3} BSA recorded on a Pt electrode in acetonitrile solution. Supporting electrolyte: 0.1 mol dm^{-3} Bu_4NHSO_4. Scan rate: 50 mVs^{-1}.

A typical cyclic voltammogram recorded during the electropolymerization process is shown in Figure. 3.3. A couple with increasing current indicating growth of the polymer film can be observed at about 0.5 ~ 0.7 V. During the course of potential scanning, mass change on the working electrode was monitored by EQCM. Figure 3.4 depicts a typical curve of the frequency change of the EQCM resonator. The negative shift of the frequency serves as another evidence for polymer film growth on the electrode surface. During the course of electropolymerization, the frequency displacement increases almost in a constant rate, which can be regarded as an indication of constant rate of electropolymerization. This finding indirectly suggests that the polymer film formed is fairly conductive. Under this electropolymerization conditions, the net frequency shift after 40 cycles of potential scan is about 2.8 kHz.

In order to transfer the polymer film prepared in non-aqueous solution to aqueous media, the polymer film was immersed in a mixture of (i) acetonitrile-acetone (1:1 v/v), (ii) acetone-water (1:1 v/v) and (iii) deionized water in sequence. Removal of template molecule from polymer film was performed by washing the polymer film with 0.1 mol dm^{-3} well-stirred acetic acid solution. The removal of glucose template molecules was monitored by measuring the frequency change of the resonator. The frequency increased quite rapidly during the first 40 minutes, after that it increased slowly (less than 10 Hz) for the next 2.5 hours. After transferring from nonaqueous media to aqueous media and removal of glucose, the imprinted polymer film was subject to electrochemical investigation. A typical cyclic voltammogram of Au electrode coated with glucose imprinted poly(BSA) film

recorded in pH 7.4 phosphate buffer solution is shown in Figure 3.5. As can be seen from the i-E curve, the conductivity of the imprinted polymer film is maintained after transfer from nonaqueous to aqueous system and removal of glucose template molecules.

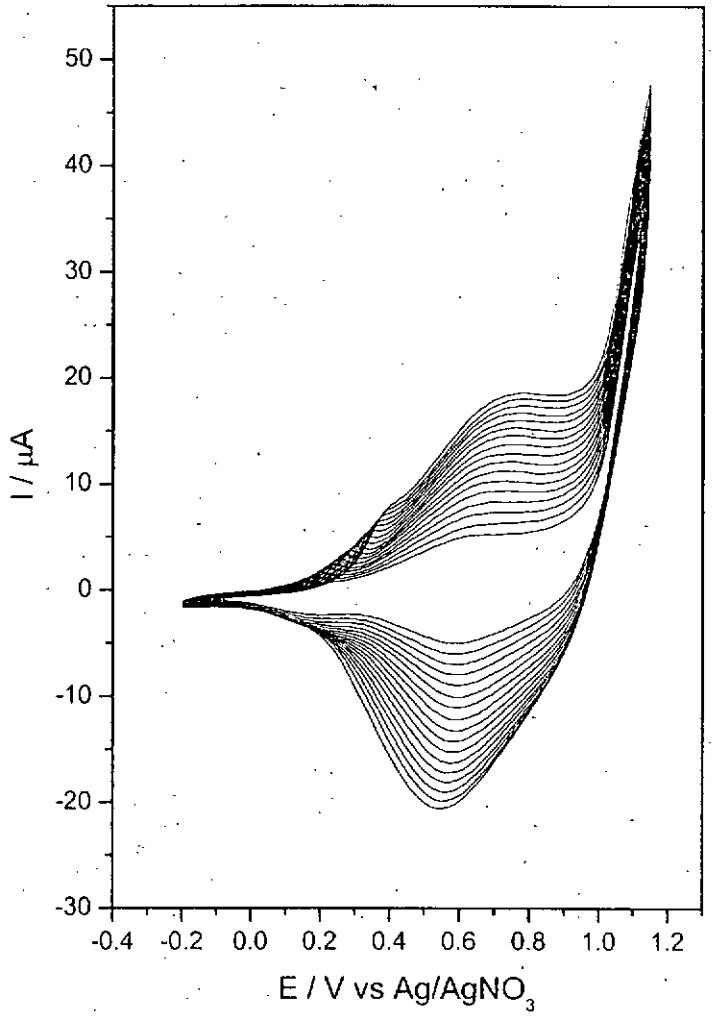


Figure 3.3. Cyclic voltammogram of 5.0×10^{-3} mol dm^{-3} BSA recorded on Pt electrode in acetonitrile solution. Supporting electrolyte: 0.1 mol dm^{-3} Bu_4NHSO_4. Scan rate: 50 mVs^{-1}.

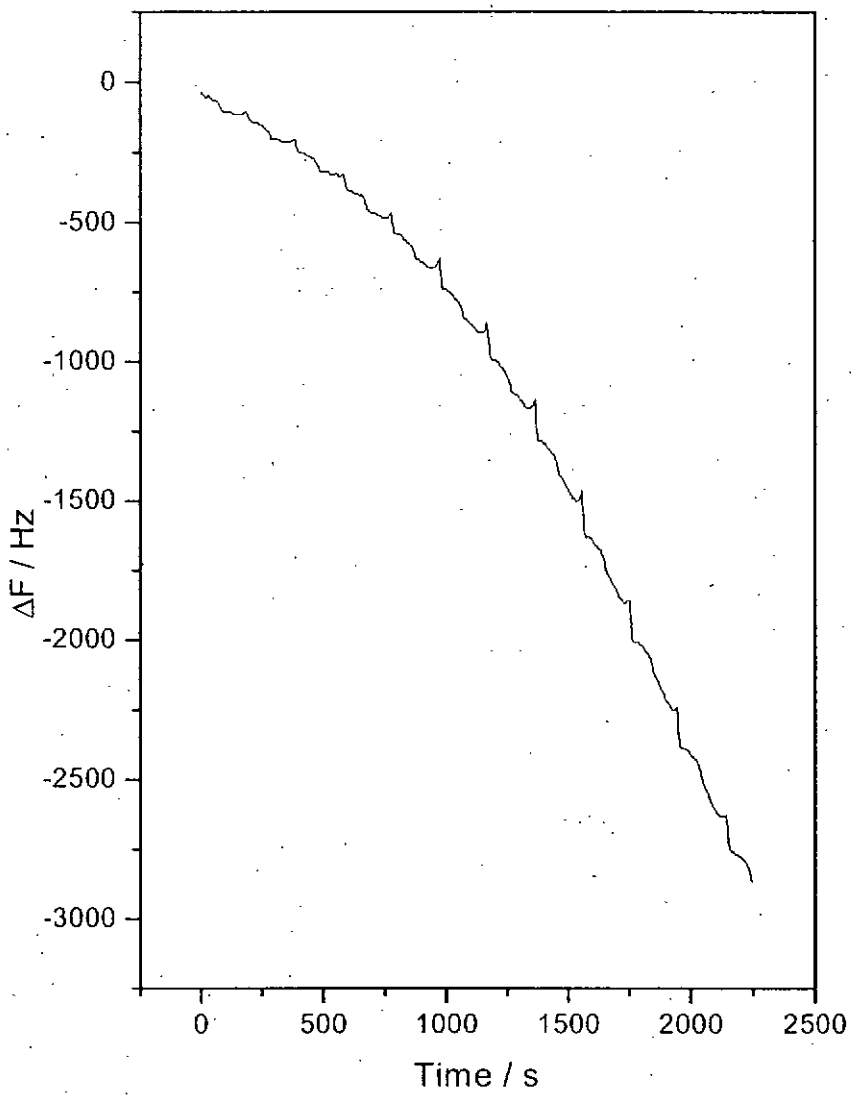


Figure 3.4. Frequency response of the EQCM resonator recorded during the course of electropolymerization of BSA.

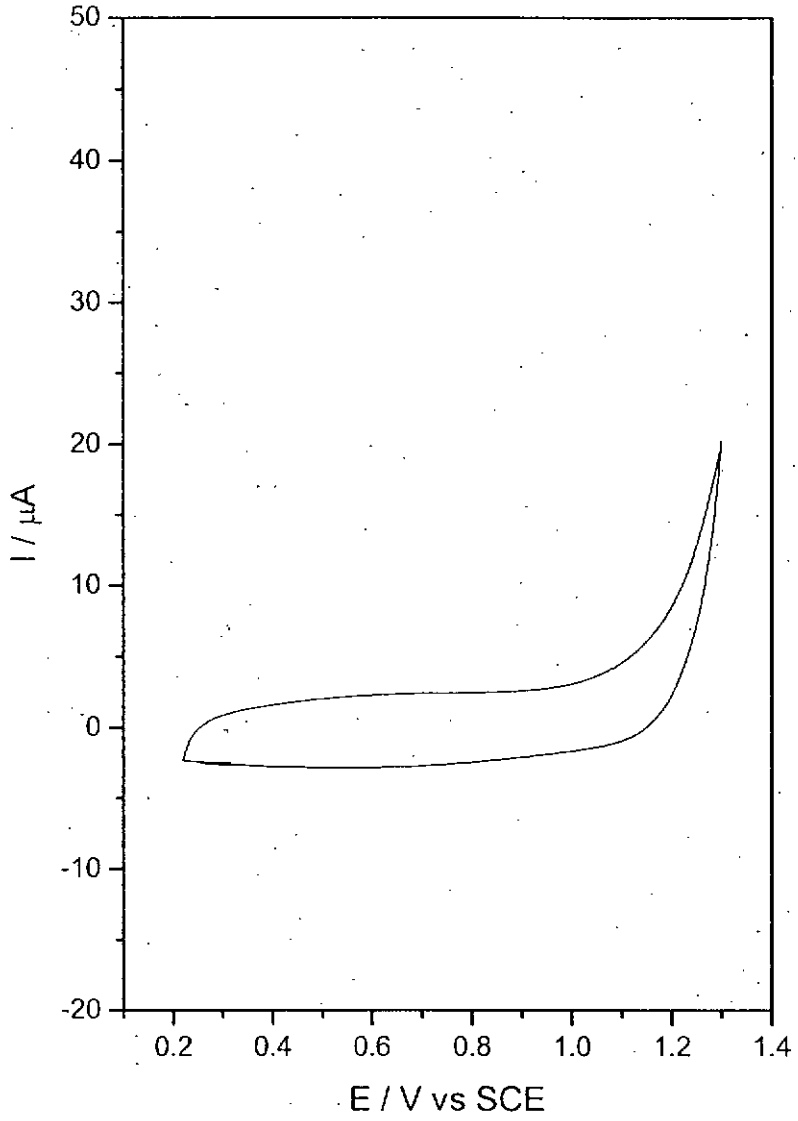


Figure 3.5. Cyclic voltammogram of Poly(BSA) film in pH 7.4 phosphate buffer. Scan rate: 50 mV s^{-1}.

3.3.3. Molecular recognition of glucose by poly(BSA)

Sensitivity towards glucose

The EQCM resonator modified with poly(BSA) showed a steady frequency for over 12 hours, indicating that the MIP polymer is stable and firmly adhered to the electrode surface. This is crucial for the long-term performance of a chemical sensor.

In general, measurement of glucose concentration with the EQCM sensor was performed by holding the sensor electrode at a positive potential of 0.5 V in a pH 7.4 buffer solution. After a steady frequency of the QCM had been reached for more than 15 min, glucose solution of known concentration was added to the cell by a micro-syringe. To facilitate diffusion of analyte, the solution was stirred for about 5 s by bubbling with nitrogen. Figure 3.6 presents the frequency change (ΔF) upon addition of 1.0×10^{-3} mol dm^{-3} glucose. As can be seen in the figure, the frequency of the resonator shifted sharply upon addition of glucose. The speed of frequency displacement slowed down after about 150s and reached a stable state after about 350 s. The final frequency change is 324 Hz. The result suggests that glucose can be taken up by the poly(BSA) MIP film.

In order to eliminate the possibility that the buffer solution causes the frequency change, control experiments were performed by adding 10 μL of buffer solution and pure deionized water instead of glucose solution. In both experiments, the frequency shift on the MIP film coated resonator is negligible. In order to evaluate the contribution of the molecular imprinting process to the frequency shift

upon addition of glucose, non-imprinted polymer films, poly(aminophenylboronic acid) and poly(aniline) were prepared with similar procedures. A series of control tests were carried out with the non-imprinted polymer films and bare EQCM Pt electrode under identical experimental conditions. As expected, no observable frequency shift was observed upon addition of glucose. For the two non-imprinted polymer films, upon addition of 1×10^{-3} mol dm^{-3} glucose, a small frequency shift of 12 Hz for resonators coated with poly(aminophenylboronic acid) and 4 Hz for poly(aniline) can be observed. The frequency response of the non-imprinted polymer films is much lower than that of imprinted one for the same glucose concentration. Moreover, the response time of the sensors based on non-imprinted polymer films (560 s and 830 s for poly(aminophenylboronic acid) and poly(aniline), respectively) are longer than poly(BSA). Combining the results of the tests mentioned above, one could be assured that the frequency change is caused by the uptake of glucose into the molecularly imprinted polymer film.

The poly(BSA) based EQCM sensor was then used to determine a series of glucose solutions with different concentrations. The frequency shift upon addition of various amount of glucose is shown in Figure 3.8. One can see a proportional increase of frequency shift with glucose concentration in the range of 2×10^{-5} to 7.5×10^{-4} mol dm^{-3}. However, the increase in frequency shift levels off for glucose concentration higher than 7.5×10^{-4} mol dm^{-3}. The linear response of the sensor toward glucose indicates that this MIP film can be used for glucose detection. The constant EQCM response to glucose solutions of concentration higher than 7.5×10^{-4} mol dm^{-3} can be rationalized by the fact that all receptor sites are saturated (see next section). The association constant (K_a) between glucose and the MIP film is

calculated to be 6250 M^{-1} based on non-linear regression analysis of the following equation:

$$\frac{\Delta F}{\Delta F_{max}} = \frac{[A]}{\frac{1}{K_a} + [A]}$$

where ΔF is the change in frequency of the MIP film upon analyte binding; ΔF_{max} is the change in frequency of the MIP film at analyte saturation; and [A] is the analyte (glucose) concentration.

Under certain conditions, the frequency change of the QCM resonator is proportional to the mass change on the surface of QCM electrode. For the 9.0 MHz QCM (surface area, A = 0.20 cm^2) resonator used in this study, the mass change can be calculated from the frequency change by a ratio R of 0.2 ng/Hz. With the assumption that the mass change during the course of glucose detection is equal to the mass of glucose bound to the MIP film, the surface density (d_b) of accessible binding sites can be calculated from the maximum frequency change (ΔF_{max} = 341 Hz) from equation 1 below:

$$d_b = R \cdot \Delta F_{max} / A \cdot M \qquad (1)$$

where A is the electrode surface area and M is the molecular weight of glucose. From the above equation, the surface density of binding sites in the electropolymerized MIP film was calculated to be 1.9×10^{-9} mol cm^{-2}.

Optimization of analytical conditions

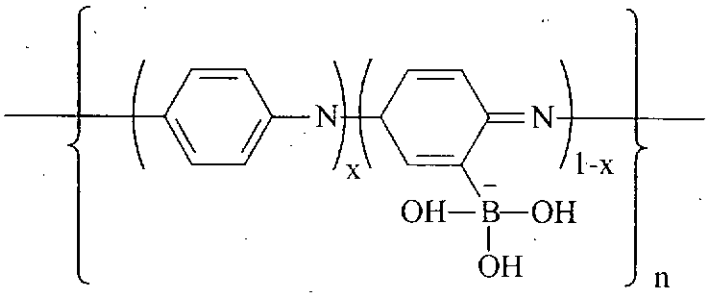
In the evaluation of the sensitivity of poly(BSA) based sensor towards glucose, uptake of glucose by poly(BSA) film was firstly carried out in an open circuit mode,

i.e., the EQCM electrode potential was not controlled. It is interesting to note that the sensitivity of the sensor can be improved by applying a positive potential to the EQCM electrode on which the poly(BSA) film was attached. To further investigate this finding, measurements of glucose with the sensor were carried out under different electrode potentials. The dependence of the frequency shift on the applied potential is shown in figure 3.8. As can be seen in the figure, the frequency change upon addition of 5×10^{-4} mol dm^{-3} glucose increases quite sharply with the applied electrode potential from 0.25 V to 0.50 V. The frequency change continues to increase but slows down in the potential range of 0.50-0.8 V. Thus a higher electrode potential results in higher sensing sensitivity. However, when the electrode potential is higher then 0.7 V, the MIP polymer film is not stable enough for 10 analytical tests. Under such conditions, a continuous shift of baseline frequency towards the positive direction followed by detachment of the polymer film from the EQCM electrode could be observed. Taking into account both sensitivity and stability of the film, 0.5 V is selected as the working potential for the glucose detection with the poly(BSA) based sensor.

It is well known that glucose occurs in both pyranose and furanose forms, with the former being the predominant species. The latter form exists only as the minor species and has not been isolated. Boronic acids are reported[40] to bind glucose preferentially in the α-furanose form and not in the more abundant pyranose form. Moreover, in aqueous media, significant formation of boronate ester only occurs between carbohydrate and the tetrahedral form of boronate[41], which necessitates the solution pH to be lower than the pK_a of boronic acid. The pK_a of aromatic boronic acid has been found to be regulated by the inductive and resonance effect of

the neighboring aromatic ring[42]. For example, 4-carboxy-3-nitrophenylboronic acid, which has electron-withdrawing substituent on the aromatic ring, was reported to have a pK_a of 7.0[43].

Based on the facts mentioned above, the dependence of frequency change on electrode potential can be rationalized by the electron-withdrawing effect from the electrode, which is induced by the potential applied. The poly(aniline) backbone of poly(BSA) film is composed of aromatic rings and with nitrogen atoms whose π orbits form a conducting pathway (Scheme 3.3). When the electrode potential is set to a higher value, the electrode surface will become electron deficient and electron-withdrawing. This electron-withdrawing effect results in the lowering of the pK_a of boronic acid.



Scheme 3.3

It is well known that in aqueous media, the formation of boronate ester from boronic acid and diols is reversible to a certain extent dependent on the pH of the solution[44]. Therefore, the uptake of glucose by the poly(BSA) based sensor can be affected by the pH value of the solution. To evaluate the effect of solution pH on the sensor, detection of 5×10^{-4} mol dm^{-3} glucose with poly(BSA) based sensor under

various pH values were carried out. The dependence of the frequency change (ΔF) on the solution pH is depicted in Figure 3.9. As can be seen in the figure, ΔF increases sharply with increase of pH from 6.5 to 8.0, indicating the enhancement of glucose uptake efficiency at higher pH. Increase of ΔF with pH can also be observed in the pH ranges of 2.5-6.5 and 8.0-9.0, though with a smaller slope. In the pH range studied, the highest sensitivity of the sensor toward glucose can be achieved in pH 9.0 solution. However, considering the advantage of physiological measurement, a pH 7.4 solution was used in most of the uptake experiment.

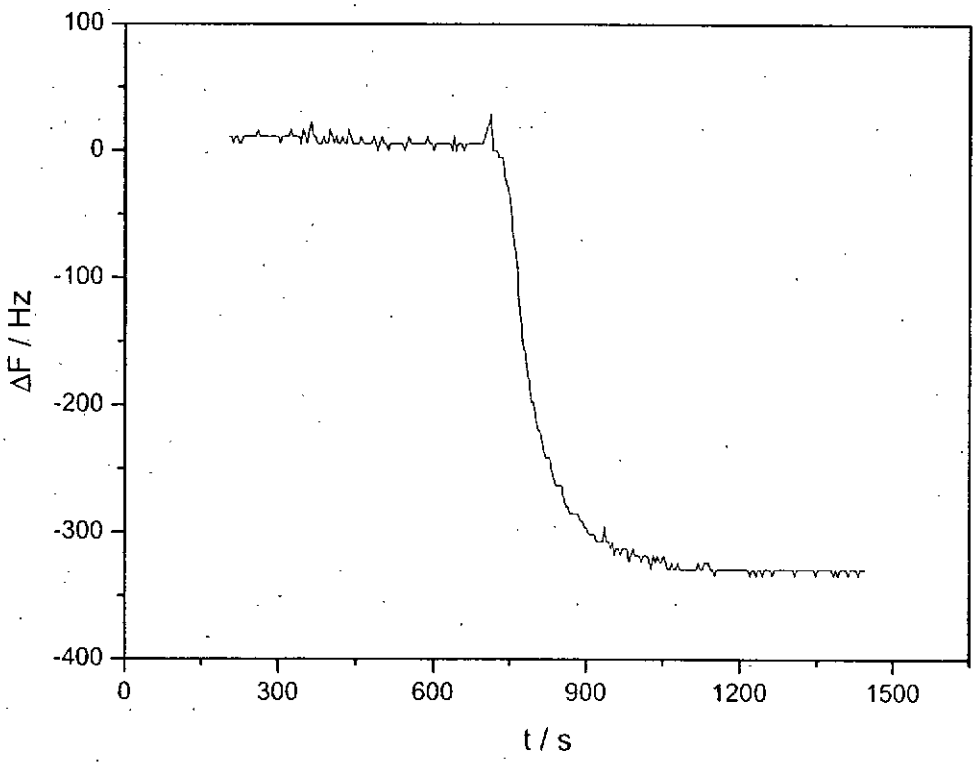


Figure 3.6. Frequency change of QCM resonators coated with poly(BSA) imprinted polymer film upon addition of 1.0×10^{-3} mol dm^{-3} glucose.

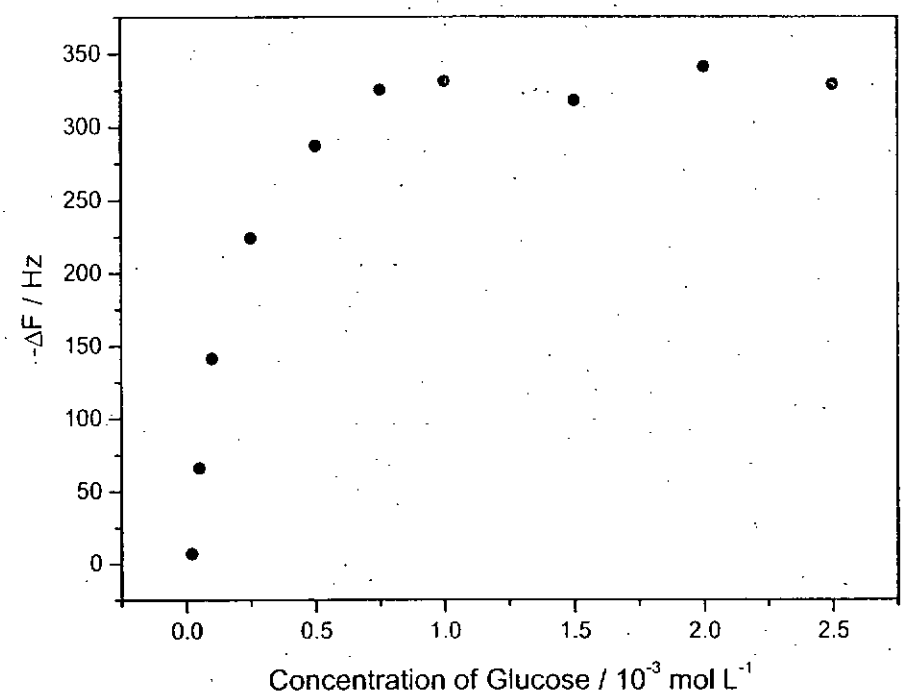


Figure 3.7. Correlation of frequency change of poly(BSA) based EQCM sensor and concentration of glucose in the concentration range of 2×10^{-5} to 2.5×10^{-3} mol dm^{-3}.

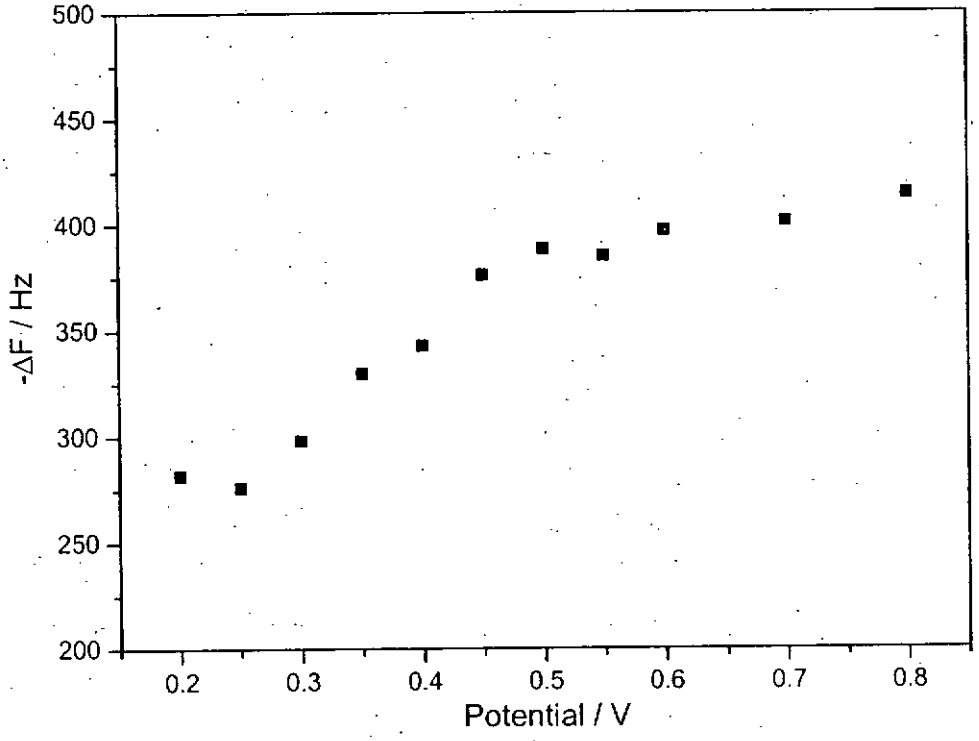


Figure 3.8. Frequency change of poly(BSA) based EQCM sensor upon addition of 5.0×10^{-4} mol dm^{-3} glucose at various electrode potential.

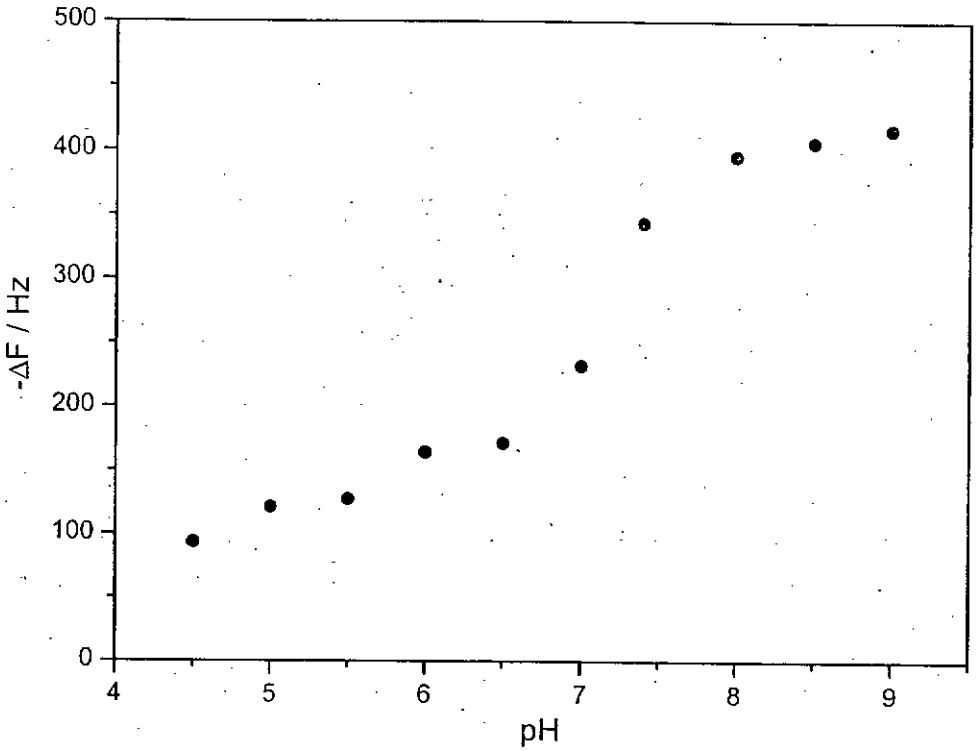
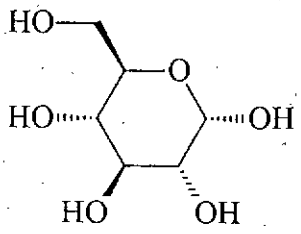


Figure 3.9. Frequency change of poly(BSA) based EQCM sensor upon addition of 5.0×10^{-4} mol dm^{-3} glucose under various pH values.

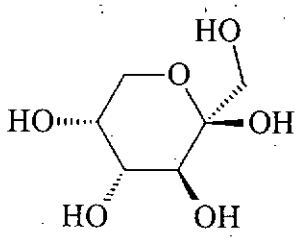
3.3.4. Selectivity of the poly(BSA) based sensor

Selectivity against fructose

Fructose is commonly found together with glucose and sucrose in honey and fruit juices. Fructose and glucose are monosaccharides while sucrose is a disaccharide. In contrast to glucose, fructose metabolism is not dependent on insulin levels and as such, it forms an important component of diabetic diets. Therefore, ability to discriminate glucose from fructose is essential for a glucose sensor.



Glucose



Fructose

To evaluate the selectivity of poly(BSA) based sensor against fructose, the frequency response of the sensor upon addition of fructose of various concentration was collected and compared with that of glucose (figure 3.10). By comparing Figure 3.10a and 3.10b, one can observe that the response of the sensor to addition of fructose is similar to that of glucose. However, if we only compare the concentration below 1.0×10^{-3} mol dm^{-3}, the difference in sensitivity is significant: the slope calculated for glucose and fructose is 485 and 292 Hz / mM, respectively. The uptake of glucose reaches a maximum at a concentration of 7.5×10^{-4} mol dm^{-3}. In the case

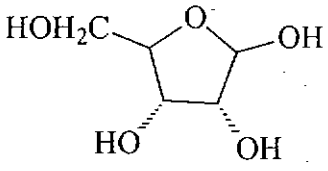
of fructose, the frequency change reaches a maximum value at a higher concentration of 1.5 mM. Nevertheless, although the poly(BSA) film based sensor does exhibit higher sensitivity towards glucose , the selectivity between glucose and fructose is not good enough for sensory application.

In general, for a molecularly imprinted polymer film based on boronate-diol interaction, the affinity of the film towards guest molecule is dependent on two factors. The first one is the size and functional groupd arrangement of the cavities of the film, which are created in the process of molecular imprinting. The second one is the ability of the boronic acid moieties to form boronate ester with the guest molecule, which is largely affected by the nature of both the boronic acid and guest molecule. In a recent report[44], the association constant (K_a) between fructose (160 M^{-1}) and boronic acid is found to be much higher than that of glucose (4.6 M^{-1}) at physiological pH. This remarkable difference in association constant might account for the relatively low selectivity. While the boronic acid moieties can bind fructose more readily, on the other hand, the cavities of the film are structurally complementary to glucose and hence favor its uptake. These two counter-acting effects result in comparable affinity of the molecularly imprinted polymer film towards glucose and fructose.

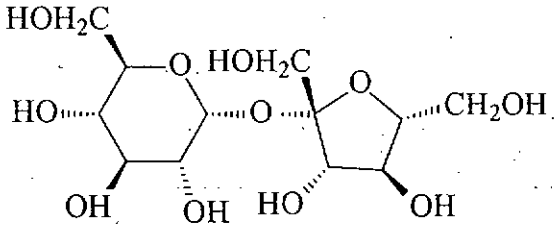
Selectivity against ribose and sucrose

Ribose is a five-carbon sugar (pentose) found primarily in ribonucleic acid. It is a natural anti-anxiety and stress relief ingredient used to control stress-related symptoms and has the added value of being non-sedating with potential anti-

depressant properties. It is not an essential nutrient, since it can be made in the body from other substances, such as glucose. However, D-ribose is very essential for life. Some of the most important biological molecules contain D-ribose, including ATP (adenosine triphosphate), all the nucleotides and nucleotide coenzymes and all forms of RNA (ribonucleic acid).



Ribose



Sucrose

Since D-ribose and its derivatives are ubiquitous in physiological systems, they are potential interferents for the detection of glucose. In order to further evaluate the selectivity of the poly(BSA) based sensor, solutions with various concentrations of ribose or sucrose were measured with the sensor and compared with that of glucose (Figure 3.11). The frequency shifts upon addition of ribose and sucrose is proportional to the concentration of ribose/sucrose in the range of 2.0×10^{-5} to 2.0×10^{-3} mol dm^{-3} for ribose and 2.0×10^{-5} to 2.5×10^{-3} mol L^{-1} for sucrose. Sensitivities of the sensor towards ribose and sucrose, represented by the slope of calibration curves, were calculated to be 120 and 38 Hz/mM, respectively. The sensitivities of the poly(BSA) based sensor towards ribose and sucrose are in accordance with the difference in structure with the template molecule (glucose). This serves as direct evidence for the molecular imprinting effect.

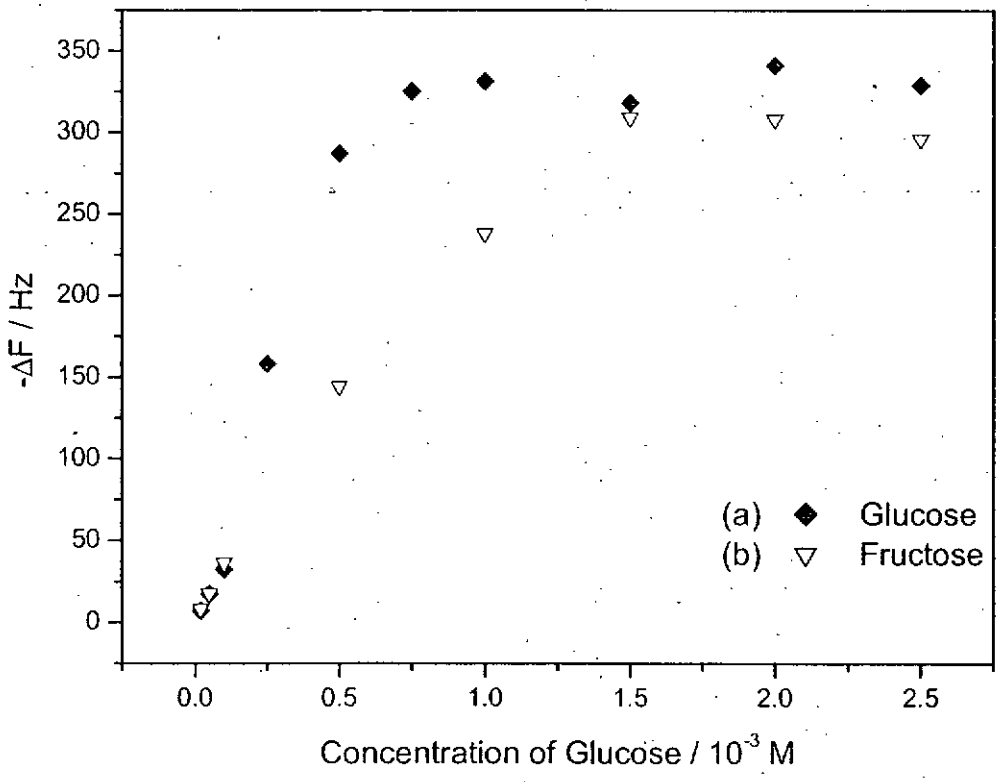


Figure 3.10. Correlation of frequency shift of imprinted poly(BSA) based EQCM sensor with concentration of glucose (a) and fructose (b).

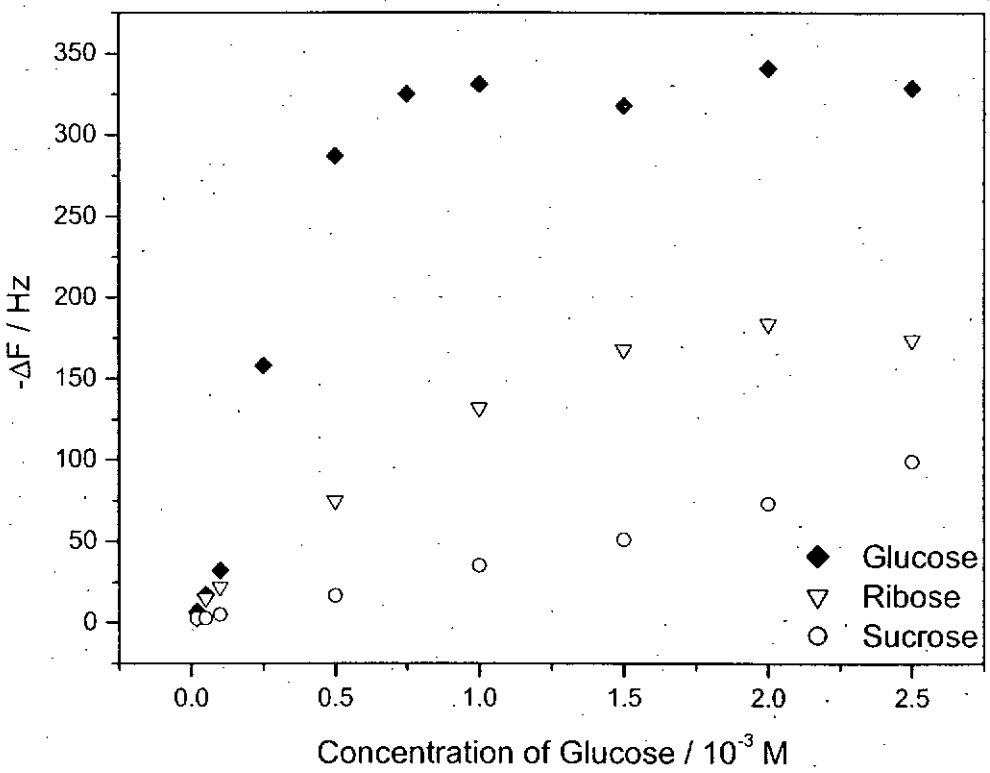


Figure 3.11. Correlation of frequency shift of poly(BSA) based EQCM sensor with concentration of glucose, ribose and sucrose.

3.3.5. Effects of monomer ratios on the efficiency and selectivity of the poly(BSA) polymer film

In the preparation of molecularly imprinted polymer film, the structurally complementary cavities are formed by co-polymerization of functionalized monomer and other complementary monomers. In the case of poly(BSA), the ratio of functionalized monomer BSA and aniline may affect the efficiency of molecular imprinting. To investigate the effect of BSA to aniline ratio on the analytical performance of imprinted polymer film, a series of molecularly imprinted poly(BSA) films were prepared with various BSA to aniline mole ratios (with BSA concentration fixed at 5.0×10^{-3} mol L^{-1}). The poly(BSA) films prepared were used in the detection of glucose and fructose. The selectivity of these films is represented by relative frequency shift ($\Delta F_{Glu}/\Delta F_{Fru}$) upon addition of 5.0×10^{-4} mol dm^{-3} glucose and fructose respectively. Figure 3.12 depicts the correlation between the relative frequency shift ($\Delta F_{Glu}/\Delta F_{Fru}$) and BSA to aniline mole ratio. It can be found from the figure that the relative frequency shift is greatly affected by the BSA to aniline ratio, implying the importance of aniline in the formation of complementary cavities. From the asymmetric peak shape of the plot, one can see that the poly(BSA) film prepared with a BSA to aniline mole ratio of 0.4 gives the maximum $\Delta F_{Glu}/\Delta F_{Fru}$. It is likely that a cavity can be more specifically defined by neighboring aniline moieties. The dependence of frequency shift on BSA to aniline mole ratio is shown in Figure 3.13. The frequency shift was found to increase with the increase in BSA to aniline mole ratios.

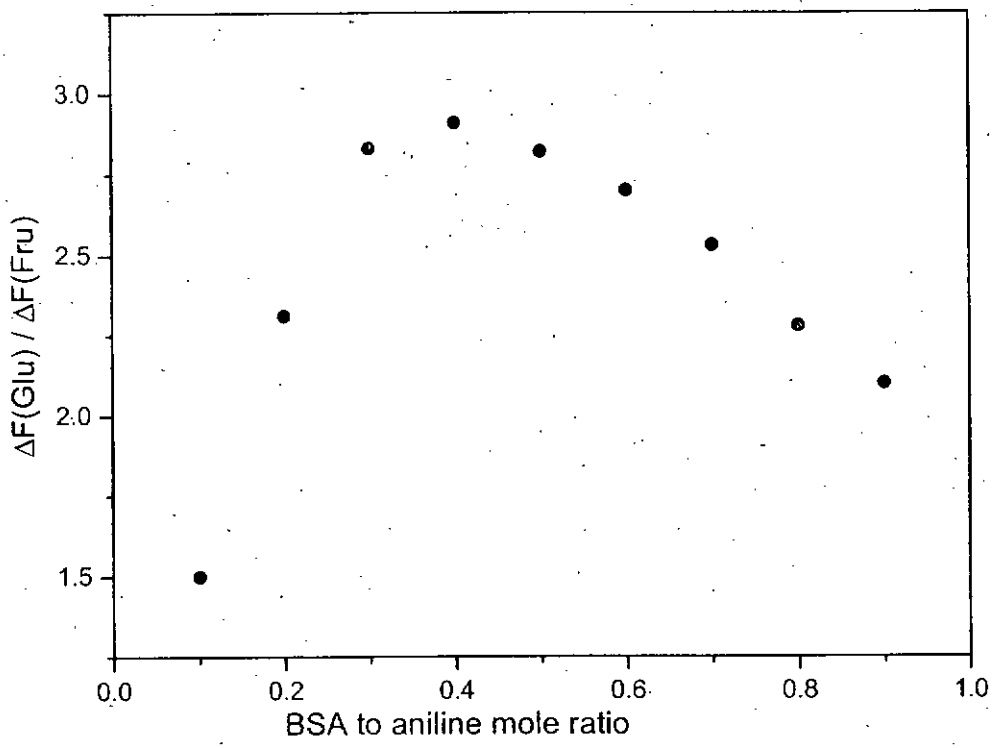


Figure 3.12. Relative frequency response ($\Delta F_{Glu}/\Delta F_{Fru}$, concentration of glucose and fructose: 5.0×10^{-4} mol dm^{-3}) of imprinted polymer films prepared with different BSA to aniline mole ratio.

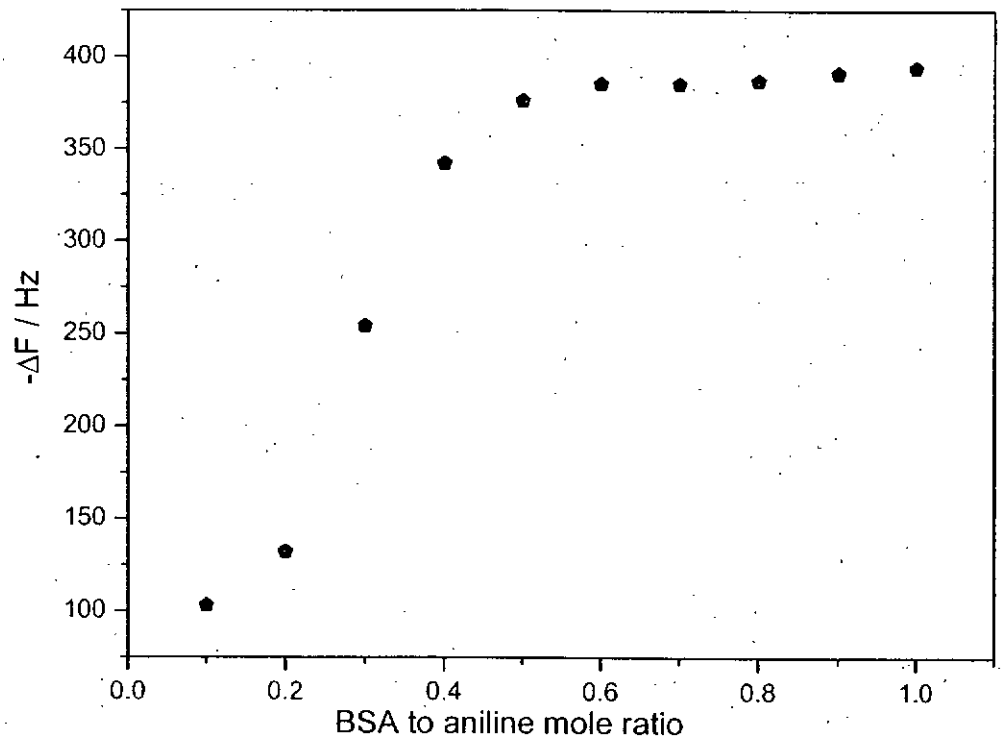


Figure 3.13. Frequency response of imprinted polymer films prepared with different BSA to aniline mole ratios towards a glucose concentration of 5.0×10^{-4} mol dm^{-3}.

3.4 Conclusions

A functionalized monomer, glucose-boronate-substituted aniline was synthesized by cyclic esterification of glucose with aminophenylboronic acid. Glucose imprinted co-polymer films of boronate-substituted monomer and aniline were prepared by electropolymerization in non-aqueous media and gradually transferred into aqueous media. At physiological pH, the EQCM sensor based on this glucose imprinted polymer film shows proportional frequency response toward glucose solution in the concentration range of 2.0×10^{-5} to 7.5×10^{-4} mol dm^{-3}. Substantial uptake of fructose by the glucose imprinted polymer film was also observed. The high association constant (K_a) between fructose and bronic acid moieties can be used to account for the significantly uptake of fructose by the MIP film. In addition, parameters such as electrode potential, solution pH and functionalized monomer to aniline mole ratio would all affect the analytical performance of glucose imprinted polymer film.

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Chapter 4

Preparation of Molecularly Imprinted Poly(acrylamidophenylboronic Acid) Films and Their Application as Dopamine Sensor

4.1 Introduction

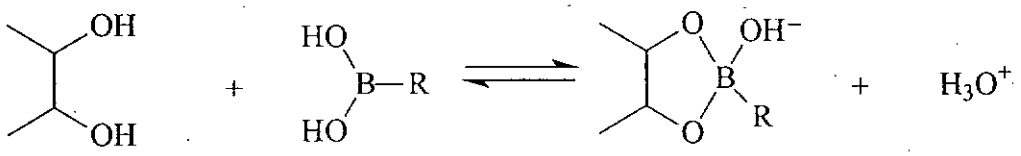
Dopamine (DA) is one of the most important catecholamines in the family of excitatory chemical neurotransmitters[1]. It plays an important role in the functioning of the central nervous, cardiovascular, renal and hormonal systems, as well as in drug addiction, schizophrenia and Parkinson's disease[2-4]. Furthermore, studies in neurobiology reveals that DA system dysfunction plays a critical role in some clinical manifestations of HIV infection[5]. Therefore, there is a need to develop sensitive, selective and reliable methods for the direct measurement of dopamine at nanomolar concentration both *in vitro* and *in vivo*.

The fact that dopamine and other catecholamines are easily electro-oxidizable makes their detection possible by electrochemical methods based on anodic oxidation[6, 7]. However, the main and foremost problem in the electrochemical determination of dopamine is the interference from other electroactive species, such as ascorbic acid (AA) and 3,4-hydroxyphenyl acetic acid (DOPAC), which are present in biological matrices in relatively high concentration and have similar redox potentials[8, 9].

One of the most common strategies to overcome these problems is to cover the electrode with a negatively charged film. This strategy has been extensively exploited and various films such as Nafion[10, 11], clay[12], overoxidized polypyrrole[13, 14], polythiophene[15] and others[16] have been used as the permselective films. These modified electrodes were reported to attract or even preconcentrate the cationic dopamine while effectively repulse the negatively

charged ascorbic acid and other anionic interfering species at the physiological pH of 7.4. However, the precise preparation of these films by dip- and cast-coating is difficult to control which results in low reproducibility and non-uniformity of such films.

Boronic acids are capable of reversible formation of strong covalent bonds with diol functionalities in the form of cyclic esters[17]. It is more desirable that the rate of this reversible reaction is a relatively fast reaction. Due to its advantageous characteristics, covalent bonding with boronic acid finds its wide application in various sensory systems based on molecularly imprinted polymer films. MIP films of various target molecules, such as nucleotides[18, 19] (AMP, ATP) and carbohydrates[20-22], have been prepared with various monomers.



The dopamine molecule contains two neighboring hydroxyl groups, which can be taken as a diol group on the aromatic ring. It is known that dopamine combines with boronic acid moiety under suitable pH value[23]. Two electrochemical sensors for dopamine employing the interaction of dopamine and boronic acid were reported recently[23, 24]. Dopamine was found to combine with both free phenylboronic acid in solution and boronic acid functionality attached to polymer backbone. It is interesting that the oxidation potential of the dopamine-boronic acid shifts substantially to higher potential compared to the free dopamine, thus avoids the

overlapping of redox potential with ascorbic acid. The selective bonding of boronic acid to dopamine opens another access to electrochemical discrimination of dopamine against ascorbic acid under physiological pH value.

In this chapter, we will report the preparation of dopamine imprinted co-polymer film of 3-acrylamidophenylboronic acid (AABA) and its application as dopamine sensor. The MIP film was used as analytical element in electrochemical dopamine sensor. The sensitivity of this sensor system and its selectivity against various interferents such as ascorbic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), tyramine and glucose was evaluated. Other factors affecting the performance of the MIP based sensor was also investigated and optimized.

4.2 Experimental

4.2.1. Materials

3-Aminophenylboronic acid (APBA) hydrochloride salt, acrylamide, N,N'-methylenebisacrylamide, sodium hydrogen, carbonate, acryloyl chloride, sodium dihydrogen phosphate, sodium hydroxide and zinc chloride were purchased from Aldrich and used as received. D-glucose, dopamine (DA), ascorbic acid (AA), tyramine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homoranillic acid (HVA) were purchased from Sigma. Deionized water purified by Milli-Q system (Millipore, MA, USA) was used to prepare all solutions..

4.2.2. Instrumental

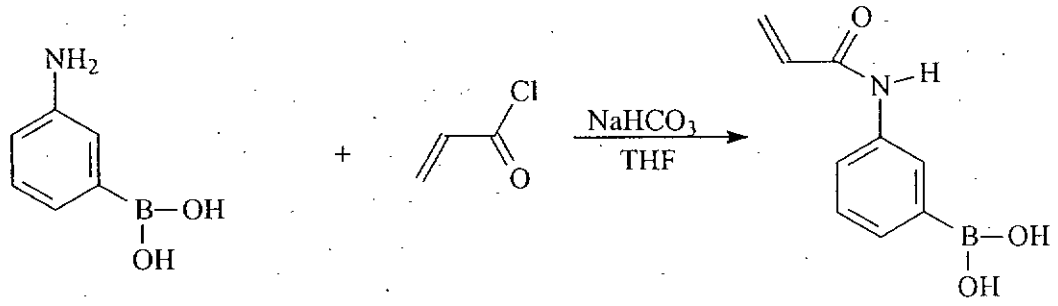
Electrochemical measurements and electropolymerization were performed on a PAR model 273A potentiostat interfaced to a personal computer using the EG&G model 270 software package. Piezoelectric measurement was conducted on a QCA917 quartz crystal analyzer (Seiko EG&G). Frequency signal was collected by a PC computer with a home-developed data acquisition program. The electrochemical cell used in this work is a home-designed Teflon cell similar to that of QCA 917 (figure 2.1). A 9.0 MHz EQCM Au electrode (Seiko EG&G, area = 0.20 cm^2) with only one side exposed to solution was used as the working electrode whereas a Pt wire and an Ag/AgCl electrode were used as counter electrode and reference electrode respectively.

UV-visible absorption spectra were recorded on a Milton Roy Spectronic 3000 diode array spectrophotometer. FT-IR spectra were obtained as pellets in 500–4000 cm^{-1} region by a Nicolet Magna-IR 750 FTIR spectrometer. ^1H NMR spectra were recorded on a Bruker 400 MHz DPX 400 spectrometer.

4.2.3. Synthesis of 3-acrylamidophenylboronic acid (AABA)

3-Acrylamidophenylboronic acid (AABA) was synthesized with a modified procedure according to published work[18] (Scheme 4.1). 3-Aminophenylboronic acid (4.65 g, 30 mmol) and sodium hydrogen carbonate (5.04 g, 60 mmol) were dissolved in a 200 ml of 2:1 vol/vol mixture of water and THF. To this solution was added acryloyl chloride (10 ml, 60 mmol) at 0 °C, and the mixture was stirred for 1 h. After this treatment, the product was extracted with ethyl acetate. After evaporation to dryness, the residue was recrystallized from water. Yield: 3.3 g, 58%.

Scheme 4.1



4.2.4. Preparation of molecularly imprinted polymers

Dopamine imprinted co-polymer films of 3-acrylamidophenylboronic acid with acrylamide and N,N'-methylenebisacrylamide were prepared by continuously

scanning the potential of the Au wire and EQCM Au electrodes between -1.4 to 0.1 V in an aqueous solution containing dopamine, acrylamidophenylboronic acid, acrylamide, $ZnCl_2$ and N,N'-methylenebisacrylamide. In each scan, the potential was held at -1.4 V for 20 s to enhance the electropolymerization reaction. The concentration of AABA is usually fixed at 0.1 mol dm^{-3} while that of dopamine, acrylamide and N,N'-methylenebisacrylamide were varied for different purposes. Polymer films with various thickness can be prepared by altering the number of potential scan cycle. The molecularly imprinted polymer film thus prepared was then extensively washed with 0.1 mol dm^{-3} HCl solution, ethanol and deionized water.

4.2.5. Analytical measurement with electrochemical oxidation

In general, determination of dopamine with potentiodynamic scan and differential pulse voltammetry involves the following steps: (1) incorporation of the target analyte molecule by the MIP film fabricated on an Au wire electrode or Au EQCM electrode in a pH 7.4 phosphate buffer solution; (2) extensive rinsing of the analyte containing film with deionized water and then soaking in a 0.1 mol dm^{-3} HCl solution (pH=2.0) for 3 minutes to allow the release of target analyte molecules; (3) stripping of analyte molecules by cyclic voltammetry or differential pulse voltammetry (DPV). After each measurement the sensor was re-generated by dipping in a well-stirred 0.1 mol dm^{-3} HCl solution for 4 × 3 minutes, followed by extensively washing with de-ionized water.

4.3 Results and discussion

4.3.1. Electrochemical properties of AABA and acrylamides in aqueous media

Acrylamide and bisacrylamide have been successfully employed to produce polymer films on glassy carbon and platinum electrodes by electrooxidative polymerization[25, 26]. However, as pointed out by Otero and co-workers[26], this electropolymerization method is not suitable to be used with active biological materials because of the low local pH and vigorous gas evolution at extreme positive potential. Gas (oxygen) evolution also results in poorly adherent polymer film.

Acrylamidophenylboronic acid (AABA) was subject to potential scan in alkaline phosphate buffer. No redox peak assignable to AABA can be observed in the potential region of -0.2 – 1.3 V. Similar electrochemical behaviors of AABA can be observed in the presence of acrylamide and bisacrylamide. This is consistent with the published results that electro-oxidative polymerization of acrylamide based monomer requires more extreme potentials. The extremely high potential required for the electro-oxidation polymerization of acrylamide prevents its application in the preparation of imprinted polymer films of oxidizable molecules such as dopamine.

4.3.2. Preparation of molecularly imprinted polymer film of AABA by electroreductive polymerization

To prepare electrogenerated polymer film from acrylamide, another approach involving electro-reductive polymerization of the monomer in the presence of zinc

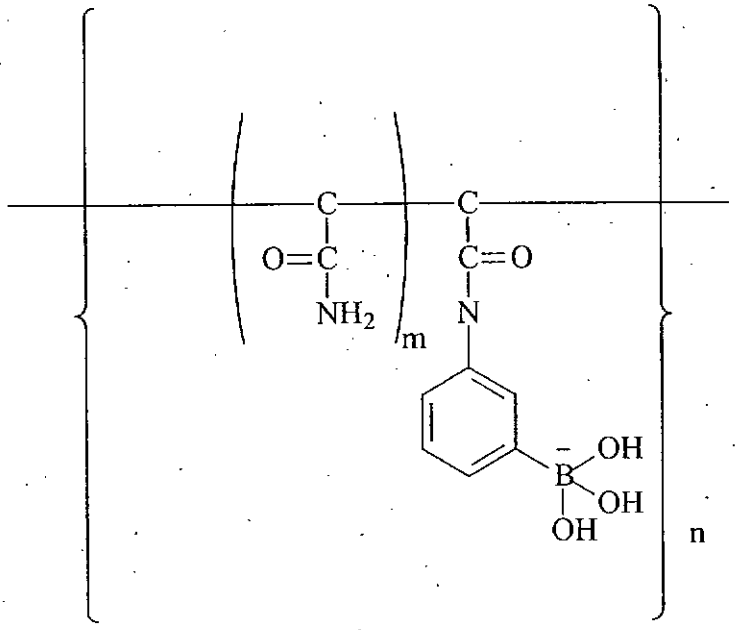
chloride was successfully adopted in the 1970's[27]. Electro-generated acrylamide polymer film has been used as analytical elements in various sensor systems[21, 28-30].

Fabrication of molecularly imprinted polymer film was performed by electropolymerization of AABA, acrylamide and N,N'-methylenebisacrylamide in the presence of dopamine. A schematic presentation of the overall approach of molecule imprinting is shown in scheme 4.2. The optimized procedure involves potentiodynamic electrolysis of an aqueous solution containing AABA (0.2 mol dm^{-3}), dopamine (0.2 mol dm^{-3}), acrylamide (1.0 mol dm^{-3}), N,N'-methylenebisacrylamide (0.2 mol dm^{-3}) and $ZnCl_2$ (0.2 mol dm^{-3}) in the potential range of 0.1 to -1.4 V at a scan rate of 50 mV s^{-1}. In each cycle of potential scans, the potential was held at -1.4 V for 20 s to improve the efficiency of polymerization. In the preparation of MIP film, one can expect the complexation of dopamine with the monomers via hydrogen bonding and formation of cyclic ester. Moreover, the electro-reductive polymerization avoids cleavage of the cyclic ester bonds caused by electrochemical oxidation. The expected structure of poly(AABA) film is shown in scheme 4.3.

A typical cyclic voltammogram recorded on Au wire electrode in the solution for electropolymerization is shown in Figure 4.1. In the negative sweep of potential, substantial cathodic current, which can be ascribed to the electrochemical reduction of acrylamide monomer and possibly $ZnCl_2$, can be observed. After 30 cycles of potential scans, a semi-transparent film can be observed on the surface of electrode. In the case of electropolymerization on QCM electrode, mass change on the

electrode was also monitored and a typical plot of frequency versus electrolysis time is shown in figure 4.2. During the course of electropolymerization, the frequency of the EQCM resonator shifted sharply to the negative direction at the first stage but eventually slowed down. The frequency change after 30 potential scans is about 12 kHz. During the electropolymerization process, the frequency of the EQCM resonator declines in a zig-zag manner, which can be attributed to the migration of ionic species in the solution and the deposition / dissolution of Zn metal.

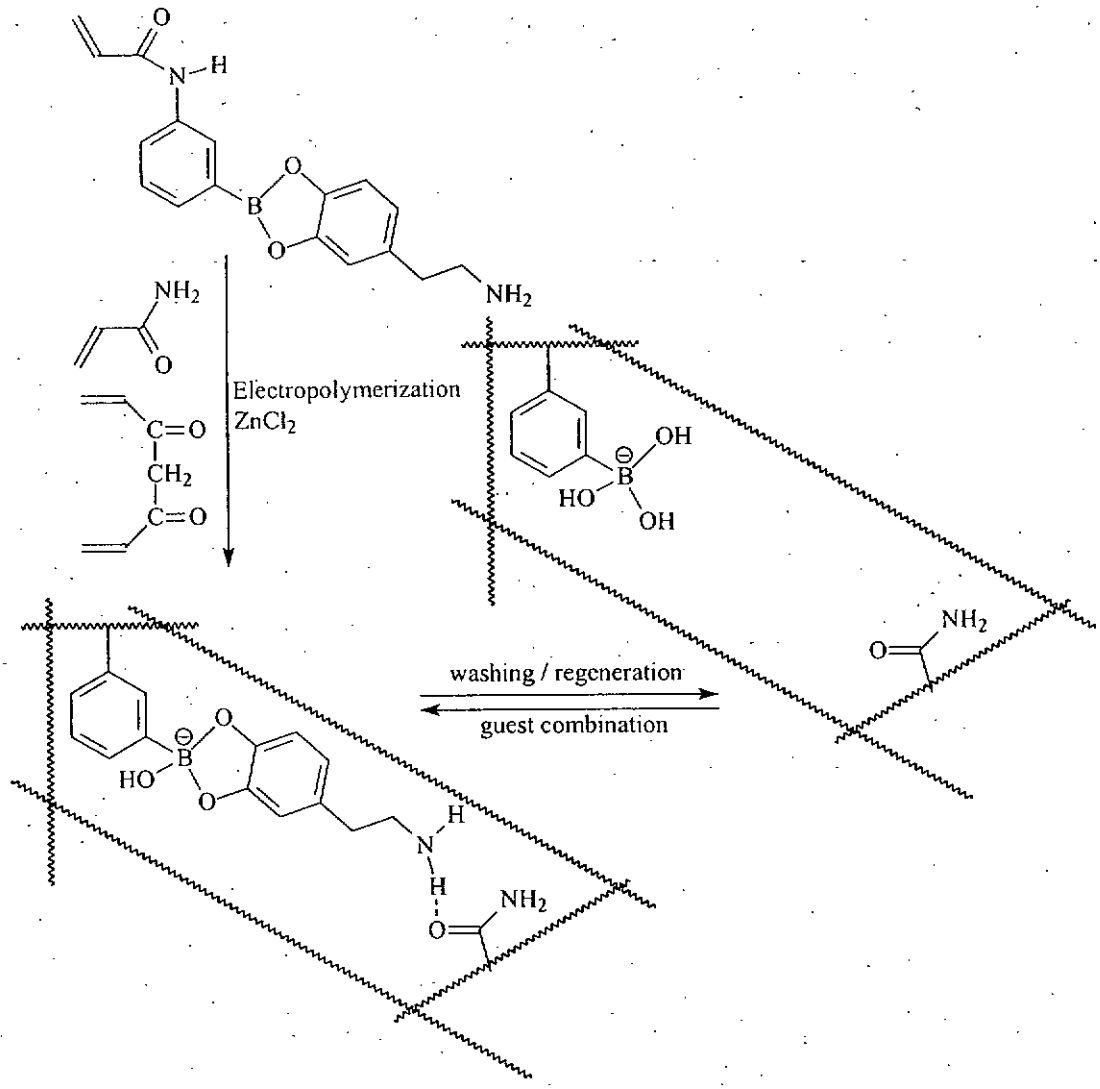
Scheme 4.3



To remove the imprinted dopamine and any Zn metal which may have deposited on the electrode surface during the course of polymerization, the polymer film prepared was extensively washed with 0.1 mol dm^{-3} HCl solution, ethanol and deionized water. The frequency change of the EQCM resonator after washing with 0.1 mol dm^{-3} HCl was measured as +0.8 kHz, indicating a mass loss of about 7%

w/w in the treatment with hydrogen chloride. Although the frequency change cannot be simply taken as contribution from the mass change caused by the dissolution of Zn metal, this result does give clear evidence that the film formed in the electrolysis is mainly composed of polymer. Similar result was obtained in a mechanistic study of acrylamide electropolymerization published by Thomas and co-workers[27].

Scheme 4.2. Schematic presentation of preparation, recognition and regeneration of dopamine-imprinted poly(AABA) film.



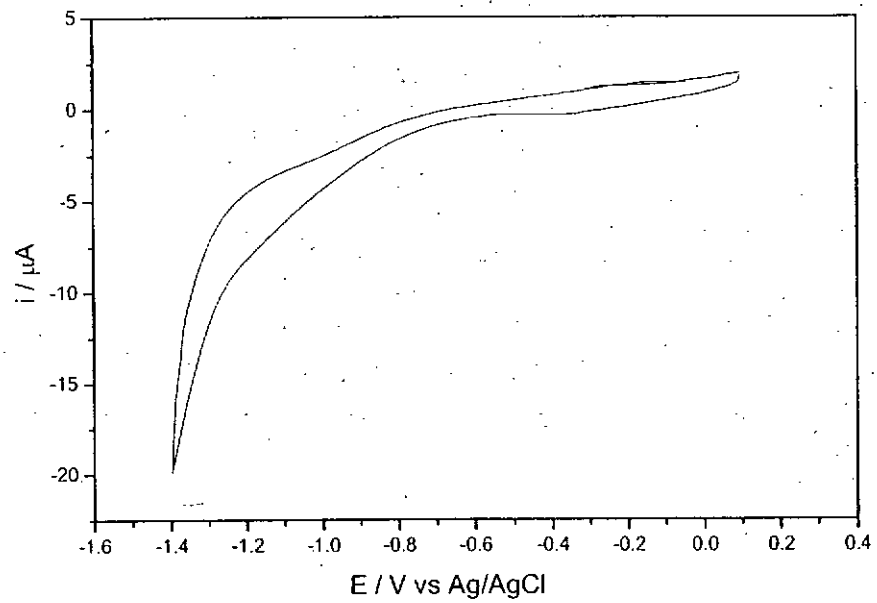


Figure 4.1. Cyclic voltammogram recorded on a Au electrode in aqueous solution containing AABA (0.2 mol dm^{-3}), dopamine (0.2 mol dm^{-3}), acrylamide (1.0 mol dm^{-3}), N,N'-methylenebisacrylamide (0.2 mol dm^{-3}) and $ZnCl_2$ (0.2 mol dm^{-3}). Scan rate: 50 mV s^{-1}.

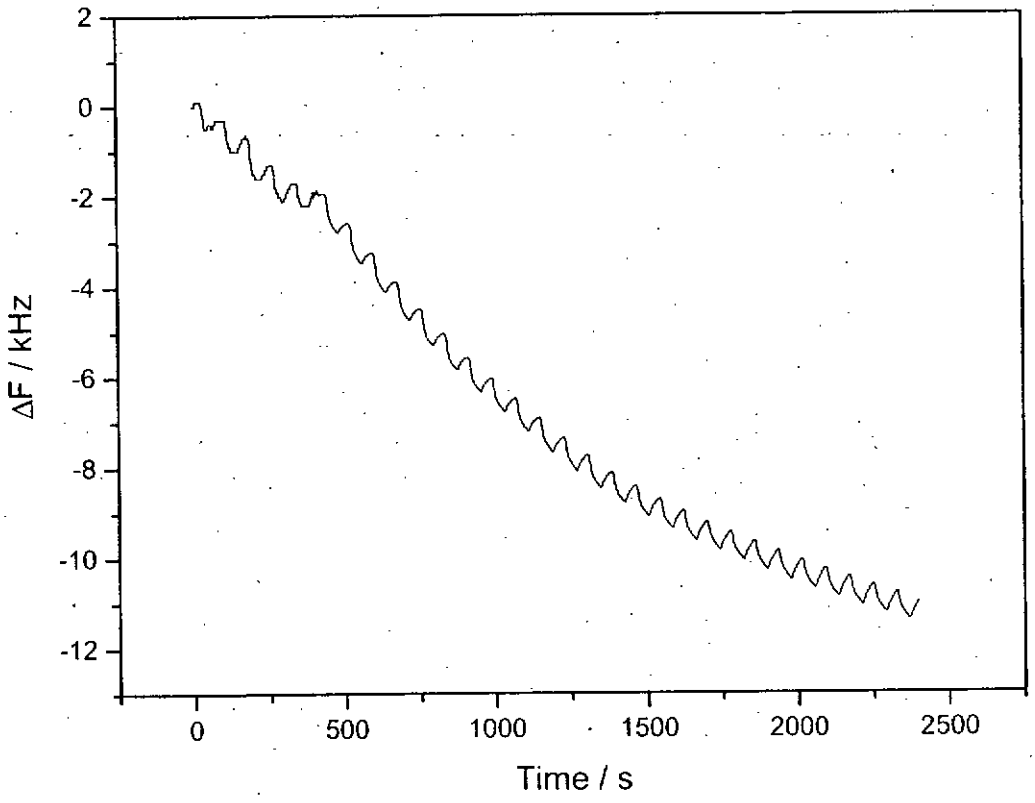


Figure 4.2. Frequency change of an EQCM resonator during fabrication of dopamine imprinted polymer film.

4.3.3. Molecular recognition of dopamine by poly(AABA)

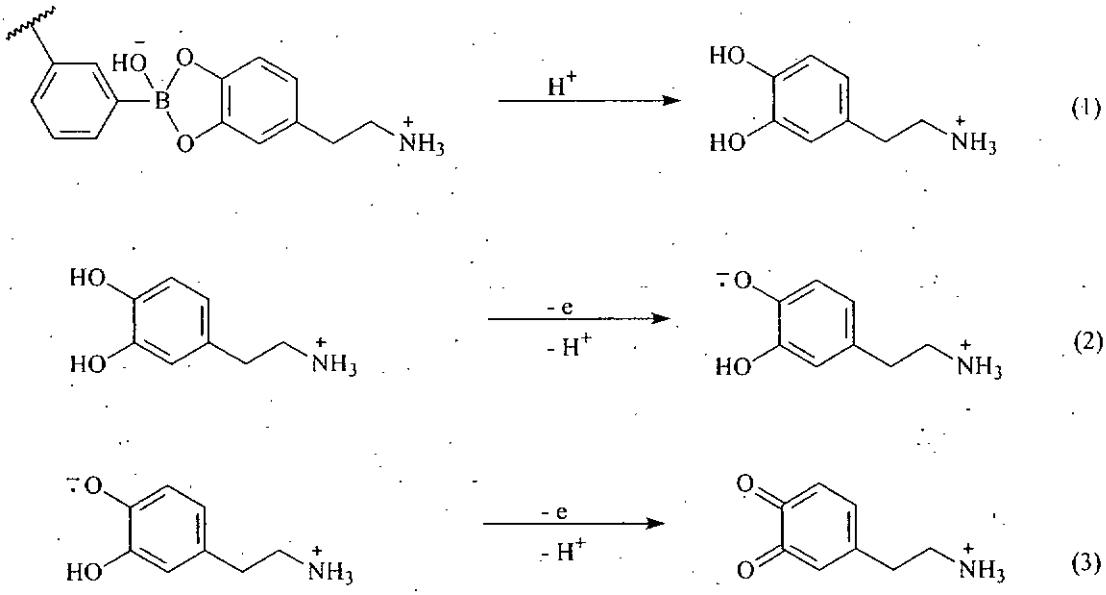
Cyclic voltammetry measurement

In general, determination of dopamine was performed by a preconcentration-stripping approach. The poly(AABA) molecularly imprinted polymer film fabricated on a Au electrode was dipped into a pH = 7.4 buffer solution of 1×10^{-4} mol dm^{-3} dopamine for 10 minutes to allow interaction of dopamine with the boronic acid moiety in the polymer matrix. Following extensive rinsing with pH 7.4 phosphate buffer solution, the electrode was transferred into an electrochemical cell containing 0.05 mol dm^{-3} HCl + 0.05 mol dm^{-3} KCl (pH=2.0). The electrode was allowed to stand in the acidic electrolyte for 3 minutes for dissociation of dopamine from the boronic acid moieties. Cyclic voltammogram or differential pulse voltammogram (DPV) was then recorded in the potential range of 0.1 to 0.85 V.

A typical cyclic voltammogram of the dopamine embedded in poly(AABA) MIP film on Au electrode is shown in Figure 4.3a. A redox couple located at 0.44 V (ΔE_p = 170 mV) was observed which can be ascribed to the oxidation and reduction of dopamine species. The peak current of the dopamine couple decreases upon repetitive scanning due to the loss of the dopamine molecules from the polymer film. A cyclic voltammogram of the same MIP film without preconcentration of dopamine was also recorded and depicted in Fig. 4.3b, in which no substantial redox peak could be observed. These results suggest that preconcentration of dopamine can be performed with imprinted poly(AABA) film under physiological pH. The

dissociation of dopamine from boronic acid moieties and its electrochemical oxidation can be represented by the equations in Scheme 4.4.

Scheme 4.4



In order to confirm the imprinting effect, a control experiment was done with a non-imprinted polymer film prepared with identical procedures except that no dopamine was placed in the electrolysis solution. With the same preconcentration-stripping procedures as the imprinted poly(AABA) film, this non-imprinted polymer film was used to detect dopamine and the cyclic voltammogram recorded was shown in Figure 4.4. Similar to Figure 4.3a, one can easily observe a redox couple at the same potential. However, the peak current of this redox couple is much less than that in Figure 4.3a, indicating that less dopamine was incorporated and transferred to the electrochemical cell. The preconcentration of dopamine in a non-imprinted polymer

film can be explained by the existence of boronic acid moieties, which can readily combine with dopamine to form cyclic ester. The difference on the preconcentration ability of imprinted and non-imprinted poly(AABA) polymer film can be ascribed to the higher affinity of the cavities formed in the imprinted film during the imprinting process. Therefore, this difference can serve as an evidence for the imprinting effect.

The dopamine sensor based on MIP film was re-generated by dipping in a well-stirred 0.1 mol dm^{-3} HCl solution for 4×3 minutes followed by extensive washing with water. The efficiency of the regeneration process is confirmed by cyclic voltammetry measurement, in which no redox peak assignable to dopamine can be observed. The cyclic voltammograms of MIP film modified electrode subjected to additional dipping treatment show no further noticeable change.

QCM measurement

As mentioned in the previous chapters, quartz crystal microbalance (QCM) is capable of measuring trace mass change occurred on the surface of QCM electrode. To investigate the mass change during the course of dopamine preconcentration on a Au EQCM electrode, dopamine imprinted polymer film was prepared with the same procedure as the Au wire electrode. The EQCM Au electrode was mounted on the bottom of the electrochemical cell containing pH 7.4 phosphate buffer solution (5.0 ml). Dopamine solutions were added to the EQCM cell with a micro-syringe and the resonator frequency was recorded and shown in Fig.4.5. Upon the addition of 1.0 x 10^{-3} mol dm^{-3} dopamine, the frequency of the resonator shifts negatively for 40 Hz and levels off after about 10 minutes. After another addition of 5.0 x 10^{-4} mol dm^{-3}

dopamine, similar but smaller frequency shift (18 Hz) can be observed. As a control test, a blank buffer solution was also added to the cell. Only a very small frequency shift could be detected. The frequency response of the imprinted polymer film modified EQCM resonator serves as another evidence for the preconcentration ability of the MIP film towards the template molecule.

It should be pointed out that the frequency change induced by the addition of dopamine is not as remarkable as the EQCM sensors mentioned in the former chapters. The low sensitivity may be ascribed to the small molecular mass of dopamine and the displacement of incorporated species during the course of dopamine uptake. It is possible that some incorporated species, such as adsorbed water and supporting electrolyte molecules, were replaced by dopamine. As a result of the displacement, the net mass change of the resonator is smaller than that of the incorporated dopamine molecules.

Differential pulse voltammetry measurement

In the above section, we have demonstrated successful detection of dopamine by cyclic voltammogram. However, as can be seen in Fig. 4.3, the redox couple of dopamine is fairly broad, which might cause problem in accurate measurement of the peak current. For accurate determination of dopamine, differential pulse voltammetry (DPV) was employed in the oxidative stripping of dopamine. With DPV, well-resolved voltammetric peak can be obtained and the analytical sensitivity can be improved.

Differential pulse voltammograms were recorded on a MIP film modified electrode after dopamine uptake in a series of solutions with various concentration of dopamine. Figure 4.6 shows the voltammograms obtained. As shown in the figure, a well-resolved positive peak assignable to dopamine oxidation can be observed at 0.47 V. In the control experiment in which a blank buffer solution was used, no observable redox peak was present in the i-E curve.

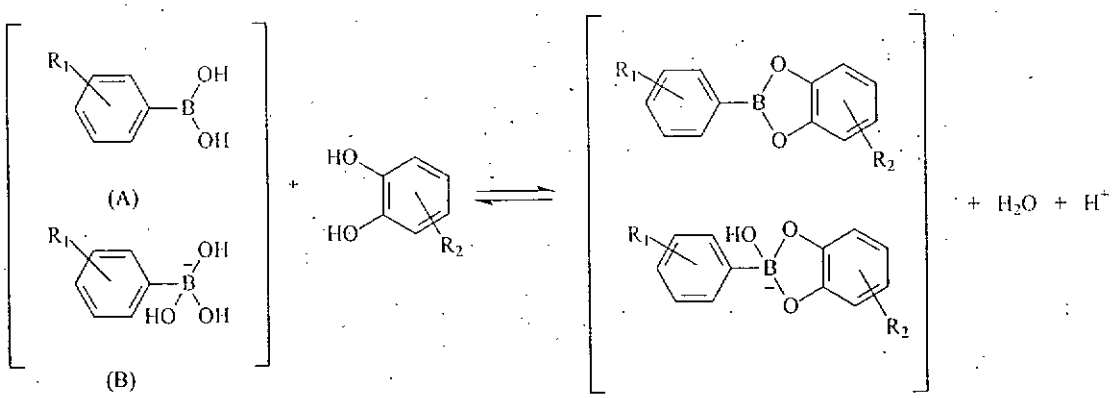
The peak current increases with dopamine concentration. Figure 4.7 shows the dependence of peak current on dopamine concentration, from which proportional increase of the peak current with dopamine concentration in the range of 5×10^{-6} to 1×10^{-4} mol dm^{-3} can be observed. This result proves the ability of quantitative measurement of the MIP film based electrode and its potential application as a dopamine sensor.

Optimization of analytical conditions

As mentioned in the previous chapter, the formation of cyclic ester between boronic acid and diols is regulated by the inductive and resonance effect of the neighboring aromatic ring[31]. In chapter 3, electrode potential is shown to affect the esterification reaction. In order to evaluate the effect of electrode potential on the preconcentration of dopamine by the MIP film, different electrode potentials were applied to the MIP modified electrode during the course of preconcentration. However, in contrast to the poly(boronate substituted aniline) system (Chapter 3), electrode potential was found to exert little effect on the uptake of dopamine: an increase in the electrode potential from -0.2 to 0.3 V shows no substantial change in

the oxidative stripping current. These different behaviors can be explained by the difference in the structure of poly(AABA) and poly(boronate substituted aniline). The backbone of poly(boronate substituted aniline) consists of aromatic rings which form a conjugated system. When a higher potential is applied to the electrode, the electrode surface becomes electron deficient and electron-withdrawing. Because of the conjugated system of poly(boronate substituted aniline), the electron-withdrawing effect of the electrode can be passed all the way down to the boronic acid moieties. On the other hand, the backbone of poly(AABA) is composed of aliphatic carbon chains. Without conjugation on the backbone, the electron-withdrawing effect of the electrode to boronic acid moieties is much weaker.

It is well known that, in aqueous media, the formation of boronate ester from boronic acid and diols is reversible depending on the pH of the solution (Scheme 4.5). Therefore, it is likely that the preconcentration of dopamine into the dopamine imprinted polymer film can be controlled by altering the pH of the buffer solution. A series of 5×10^{-5} mol dm^{-3} dopamine solutions with various pH values from 4.0 to 9.0 were used in the preconcentration step with MIP film based electrode. The dependence of the stripping current of dopamine to pH value of the buffer solution is depicted in Figure 4.8. The stripping current (i), which can be regarded as a representation of the amount of dopamine combined to imprinted polymer film, increases with pH value of the solution in the range of 5.5 – 9.0. At pH < 5.5, no substantial preconcentration of dopamine can be observed, indicating that the boronate ester tends to dissociate at low pH. The preconcentration amount increases sharply in the pH range of 5.5 to 7.0 and slows down in the range of 7.0 to 9.0.



Scheme 4.5

Scheme 4.5 shows the esterification process. As shown in the scheme, proton is produced as a result of the esterification reaction. Therefore, high pH favors estericatión (the forward reaction). In our experiments, the physiological pH (7.4) was chosen because of its potential *in vivo* application of the dopamine sensor.

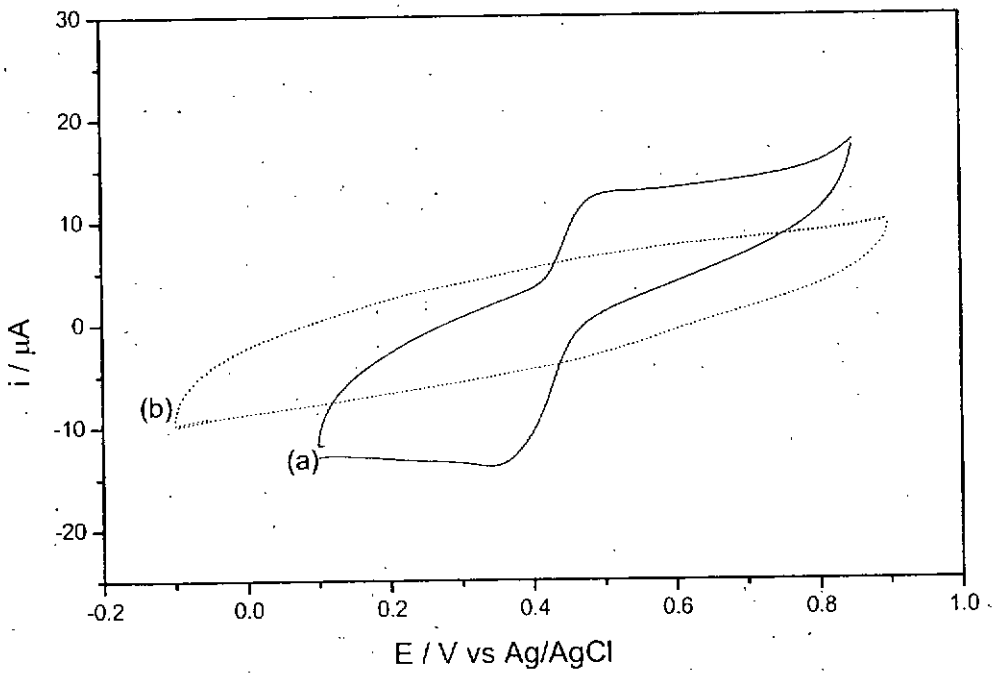


Figure 4.3. Cyclic voltammogram recorded on MIP film modified electrode with (a) and without (b) dopamine preconcentration, electrolyte: 0.05 mol dm^{-3} HCl + 0.05 mol dm^{-3} KCl (pH = 2.0). Scan rate: 50 mVs^{-1}

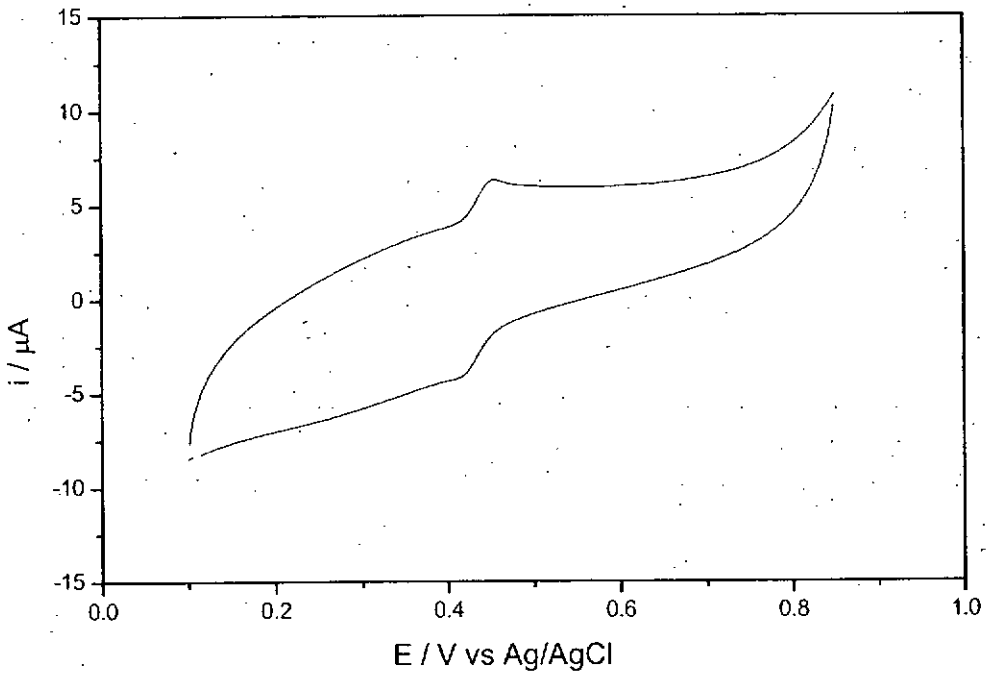


Figure 4.4. Cyclic voltammogram recorded on a Au electrode coated with nonimprinted polymer after dopamine preconcentration, electrolyte: 0.05 HCl + 0.05 mol dm^{-3} KCl (pH = 2.0). Scan rate: 50 mVs^{-1}.

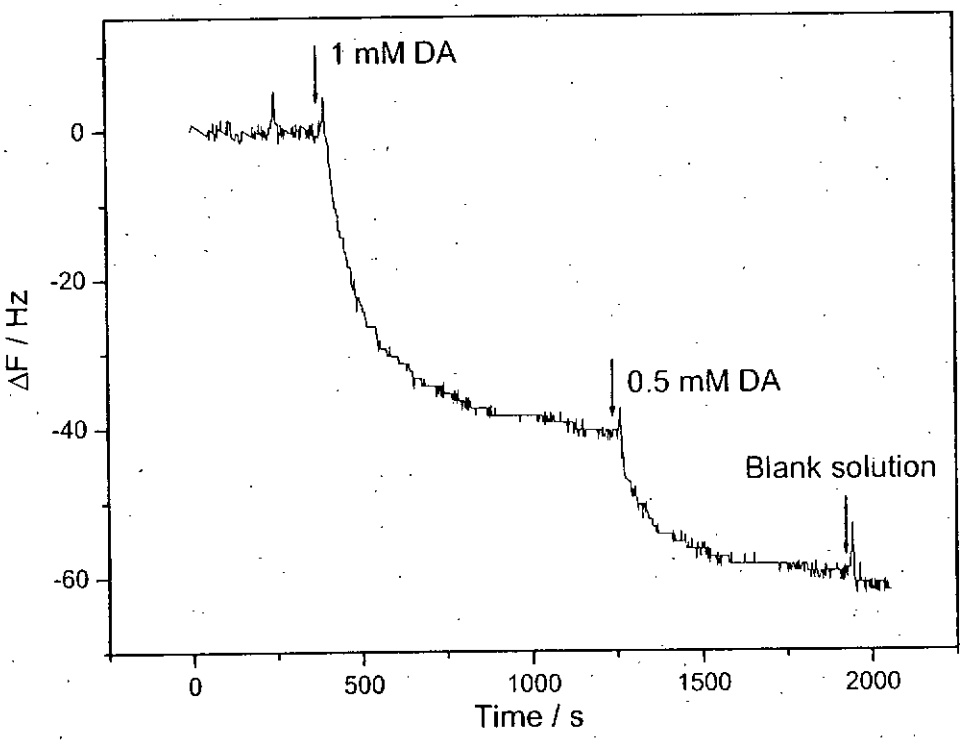


Figure 4.5. Frequency shift of an EQCM resonator modified with poly(AABA) MIP film upon addition of various amount of dopamine (DA) and buffer solution in pH 7.4 phosphate buffer solution.

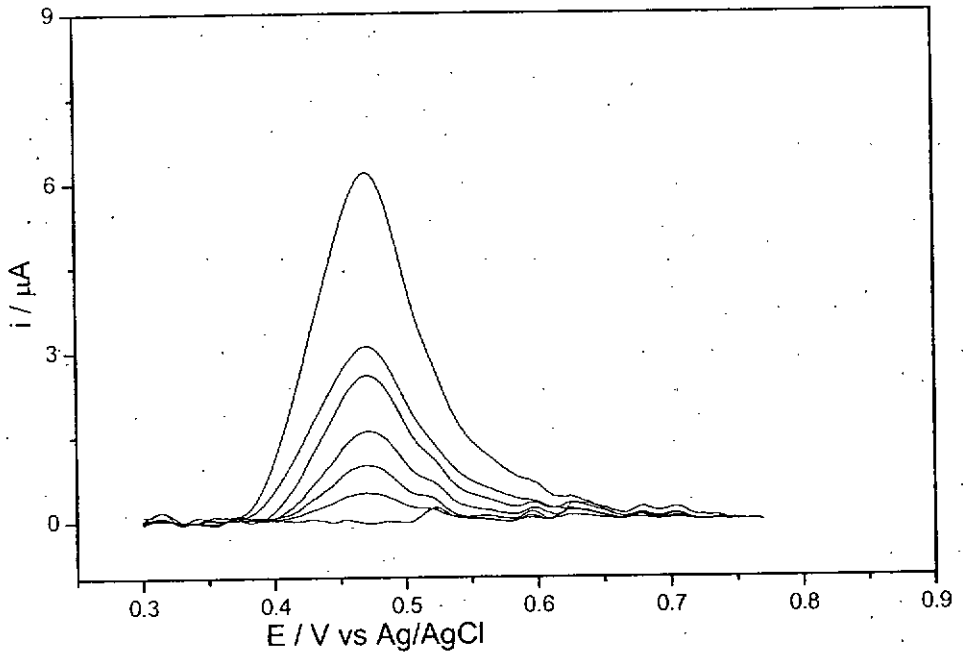


Figure 4.6. Differential pulse voltammogram recorded on MIP film modified electrode after preconcentration in solution containing various amount of dopamine (5×10^{-6} – 10^{-4} mol dm^{-3}). Electrolyte: 0.05 mol dm^{-3} HCl + 0.05 mol dm^{-3} KCl (pH = 2.0) solution. Scan rate: 20 mVs^{-1}

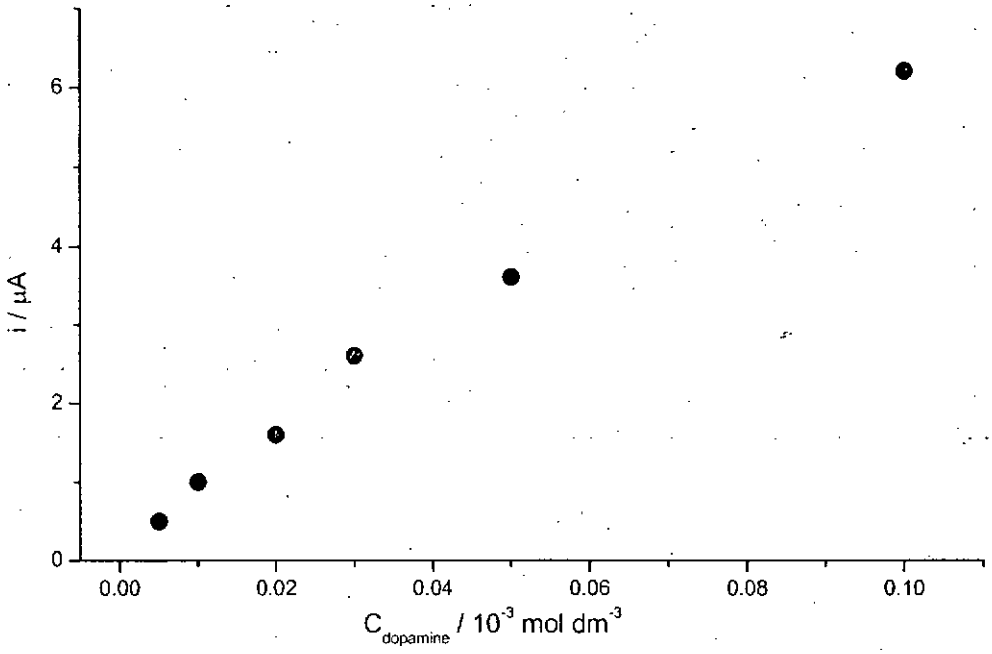


Figure 4.7. A plot of peak current in differential pulse voltammogram versus concentration of dopamine.

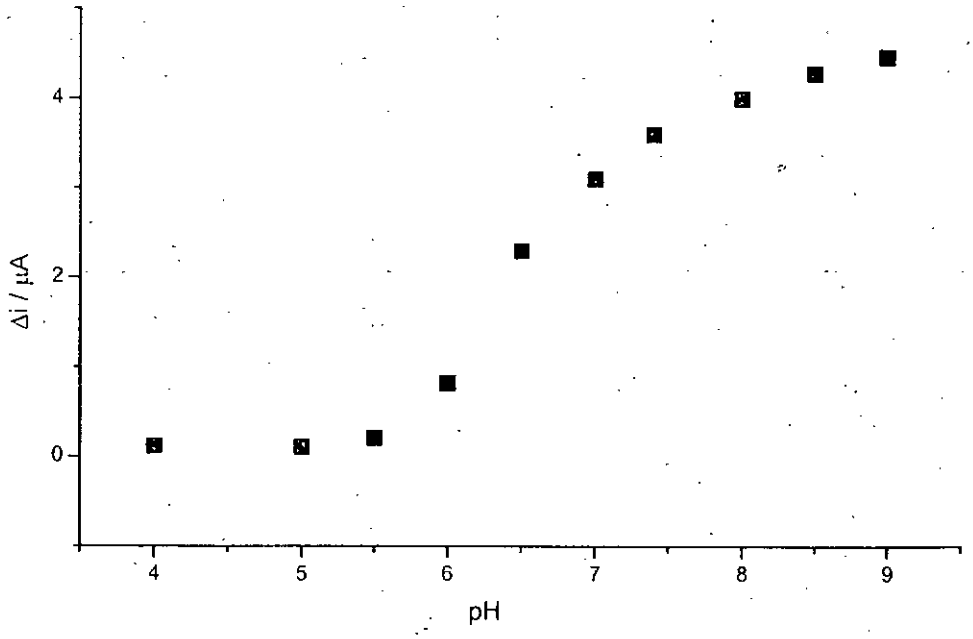
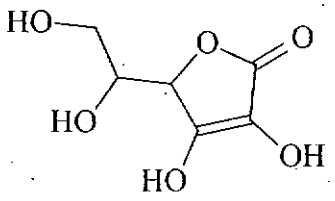


Figure 4.8. A plot of stripping peak current in differential pulse voltammogram of MIP film modified electrode versus pH of dopamine solution (5×10^{-5} mol dm^{-3}) used in preconcentration.

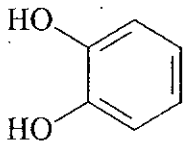
4.3.4. Selectivity of poly(AABA) MIP film based sensor

Selectivity against ascorbic acid

As mentioned in the introduction section, the main problem in the electrochemical determination of dopamine is the interference from ascorbic acid, which exists in real biological systems in relatively high concentration and has a similar redox potential as dopamine.



Ascorbic acid



Catechol

In order to test the ability of poly(AABA) MIP film to discriminate dopamine from ascorbic acid, a series of 5.0×10^{-5} mol dm^{-3} dopamine solutions with ascorbic acid of various concentration (5.0×10^{-5} to 1.0×10^{-3} mol dm^{-3}) was used in the preconcentration step with MIP film modified electrode. It is obvious that the change on the peak current induced by the addition of ascorbic acid is negligible; the plot shown in Figure 4.9a shows no substantial correlation between peak current and ascorbic acid concentration. For the measurement under ascorbic acid concentration of 1.0×10^{-3} mol dm^{-3}, which is two folds of the highest concentration of ascorbic acid commonly found in physiological system, the peak current is increased by 5% only compared to the one without ascorbic acid. Also shown in Figure 4.9b are the

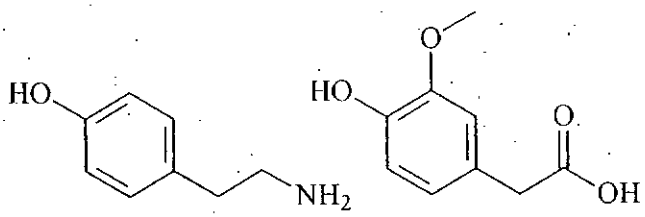
peak currents obtained with various concentrations of dopamine in the presence of 1.0×10^{-3} mol dm^{-3} ascorbic acid. A comparison of Figure 4.9b with Figure 4.7 (absence of ascorbic acid) shows similar correlation between the peak current and dopamine concentration. The small interference from excess ascorbic acid suggests the ability of the poly(AABA) MIP film in the discrimination of dopamine from ascorbic acid.

The ability of poly(AABA) MIP film to discriminate aseorbic acid can be explained by the different affinities and electronic properties of dopamine and ascorbic acid. At physiological pH, the boronic acid containing poly(AABA) polymer film is negatively charged due to the deprotonization of boronic acid. Ascorbic acid, which exists as anion at this pH ($pK_a = 4.1$), should be repulsed by the poly(AABA) film. On the other hand, the cationic dopamine ($pK_b = 8.87$) can immigrate into the polymer film and combine with boronic acid. The combination of boronic acid with dopamine and its structure analogue catechol has been proved by NMR investigation[23]. Moreover, The association constant of catechol with phenylboronic acid is found to be 40 fold higher than that of 1,2-cyclopentane diol[32].

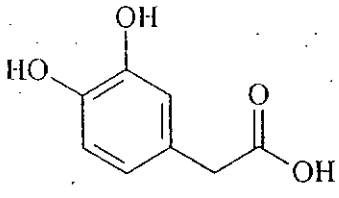
Selectivity against structurally related compounds

One of the most desirable properties of molecularly imprinted polymer is its ability in specific binding towards the template molecules. In order to further investigate molecular recognition of the dopamine imprinted polymer film, tyramine,

homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), which are structurally similar to dopamine, were used as interferents for dopamine detection.



Tyramine



Homovanillic acid

3,4-Dihydroxyphenylacetic acid

Similar to the detection of dopamine with MIP modified electrode, a solution containing 1.0×10^{-4} mol dm^{-3} tyramine was determined with the preconcentration-stripping procedure. It was reported that tyramine can be electro-oxidized on Au electrode[33]. However, no observable oxidation wave exists in the differential pulse voltammogram recorded after the preconcentration step. This indicates that uptake of tyramine into the imprinted polymer film is negligible. The interference by tyramine on the detection of dopamine was examined by measuring dopamine with concentration varied from $5.0 \times 10^{-6} - 1.0 \times 10^{-4}$ mol dm^{-3} in the presence of 1.0×10^{-4} mol dm^{-3} tyramine. The peak currents obtained are shown together with that obtained without presence of tyramine in Figure 4.10A. As can be seen, both set of data points show very similar characters with only minor difference which can be ascribed to random error. Interference from HVA to dopamine detection was also investigated and similar results were obtained (Figure 4.10B). These results prove the ability of the imprinted polymer film to discriminate dopamine from structurally similar analogues. The discrimination is mainly due to the structural difference

between dopamine and the two analogues: both tyramine and HVA have only one hydroxyl group, which is unable to combine with the boronic moieties to form cyclic ester. For the anionic HVA species, the negatively charged polymer film also functions as a permselective film. Tyramine is positively charged at physiological pH and can be entrapped in the polymer film. However, unlike covalently bound dopamine, most of the physically entrapped tyramine can be easily removed by the extensive rinse following preconcentration.

3,4-Dihydroxyphenylacetic acid (DOPAC) is one of metabolites of dopamine and has an electrochemical oxidation potential close to that of dopamine[34]. Therefore, selectivity against DOPAC is of much importance for a dopamine sensor. The differential pulse voltammogram obtained in 1.0×10^{-4} mol dm^{-3} DOPAC solution with a MIP-film-coated electrode is shown in Figure 4.11a, in which a small oxidation peak (E_p = 0.52 V) can be observed. Compared with the oxidation peak of dopamine of same concentration (Figure 4.11b), the oxidation potential of DOPAC is 0.05 V higher than that of dopamine, which is in accordance with reported result that DOPAC can be oxidized at potential slightly higher than dopamine[35]. The obvious difference in the magnitude of peak current indicates that the amount of DOPAC entrapped by the MIP film is much less than that of dopamine. It also implies that the negatively charged MIP film can serve as a permselective barrier against the anionic DOPAC, thus only a small amount of DOPAC was bound to the imprinted polymer and transferred into the electrochemical system. This may be due to the interaction of DOPAC with boronic acid moieties located on the surface of the polymer film.

To study the interference by DOPAC on dopamine detection, a series of 3.0 x 10^{-5} mol dm^{-3} dopamine solutions with DOPAC of various concentrations (1.0×10^{-5} to 2.0×10^{-4} mol dm^{-3}) was used in the preconcentration step with a MIP film modified electrode. The peak current in the differential pulse voltammogram obtained in the presence DOPAC shows a slight increment with the concentration of DOPAC (Figure 4.12a). In the presence of a DOPAC concentration which is 7 times of that of dopamine, the peak current of dopamine is increased by 6% compared to the one without presence of DOPAC. Unlike ascorbic acid, which coexists with dopamine in excess amount, DOPAC generally exists in concentration which is comparable to that of dopamine. The interference by DOPAC of the same concentration as dopamine is shown in Figure 4.12b, in which the peak currents were obtained with various concentrations of dopamine in the presence of same amount of DOPAC. A comparison of Figure 4.12b with Figure 4.7 (absence of DOPAC) shows similar correlation between the peak current and dopamine concentration.

The ability of this imprinted polymer film to discriminate structurally similar compounds demonstrates the feasibility of molecular imprinting technique in creating specific binding sites for template molecules.

Selectivity against glucose

As a source of energy in animals and plants, glucose is one of the most important carbohydrates and widely exists in extracellular fluids. As shown in chapter 3, glucose can readily combine with boronic acid moieties in polymer films. Due to the coexistence of glucose with dopamine, discrimination between glucose

and dopamine is important for the application of the imprinted polymer film in dopamine detection. Interference from glucose to detection of dopamine was examined by determining dopamine with the preconcentration-stripping approach in the concentration range of 5.0×10^{-6} to 1.0×10^{-4} mol dm^{-3} in the presence of 5.0×10^{-3} mol dm^{-3} glucose, which is typical of glucose concentration in human body. From the results shown in Figure 4.13, one can see that in the presence of glucose, the peak current obtained from dopamine oxidation is generally smaller than that in the absence of glucose. This becomes very obvious at low concentration of dopamine - for a dopamine concentration of 5.0×10^{-6} mol dm^{-3}, the decrease in peak current is as large as 52%, indicating severe interference from glucose which is 1000 times higher in concentration. With the increase in dopamine concentration, the interference from glucose becomes smaller - for a dopamine concentration of 1.0×10^{-4} mol dm^{-3}, the peak current is only slightly lowered by 1.5%, thus demonstrating the molecular recognition ability of the imprinted polymer. The decrease in peak current of dopamine oxidation is due to competition for binding sites between glucose with dopamine in the preconcentration step. Since glucose is a small neutral molecule, it can penetrate through the negatively charged imprinted polymer film and competes with dopamine on covalent bonding with the boronic acid moieties. As parts of the binding sites are now occupied by the electroinactive glucose, a lower peak current was observed in the differential pulse voltammogram.

The above results demonstrate that the imprinted polymer film possesses considerable discrimination ability against glucose. However, due to the high concentration of glucose in extracellular fluids, the interference from glucose to the detection of low concentration of dopamine cannot be ignored. A possible solution to

this problem is to create more specific binding sites for dopamine by introduction of additional interacting functional groups into the binding sites. One possible candidate is the anionic carboxylate, which can interact with the cationic amine of dopamine. In addition, the permselectivity of the imprinted polymer film can also be improved by the negatively charged carboxylate groups.

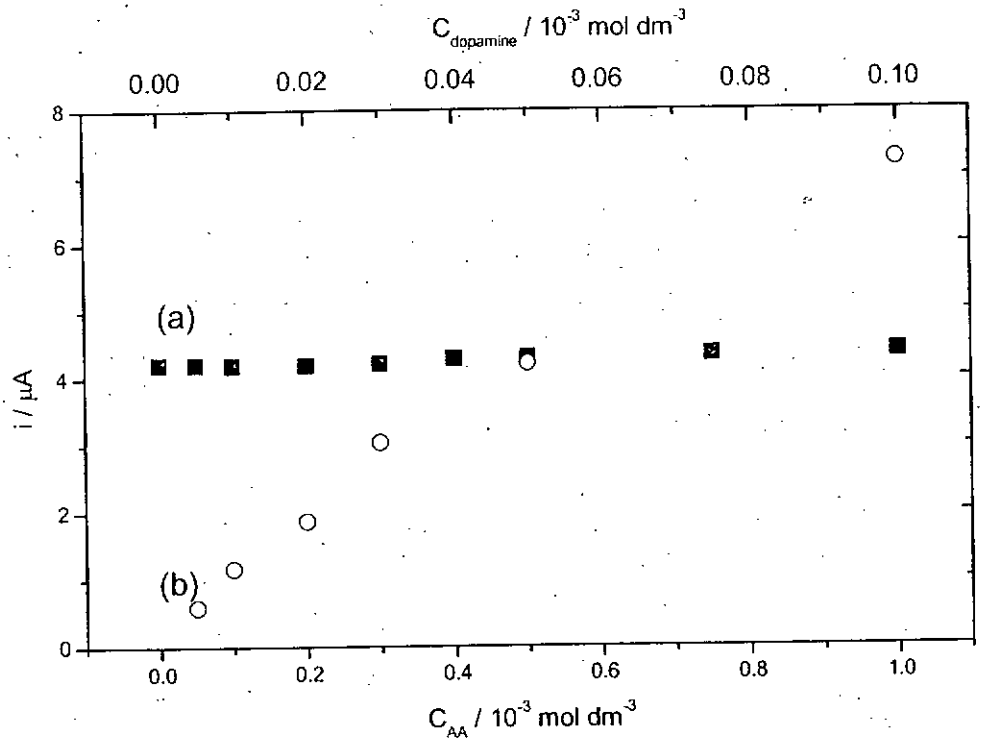


Figure 4.9. Peak current obtained in the differential pulse voltammogram under different concentration of dopamine and ascorbic acid concentration. (a) Dopamine concentration was fixed at 5.0×10^{-5} mol dm^{-3} while ascorbic acid concentration (lower X-axis) was varied from 5.0×10^{-5} to 1.0×10^{-3} mol dm^{-3}; (b) Ascorbic acid concentration was fixed at 1.0×10^{-3} mol dm^{-3} while dopamine concentration (upper X-axis) was varied from 5.0×10^{-6} to 1.0×10^{-4} mol dm^{-3}.

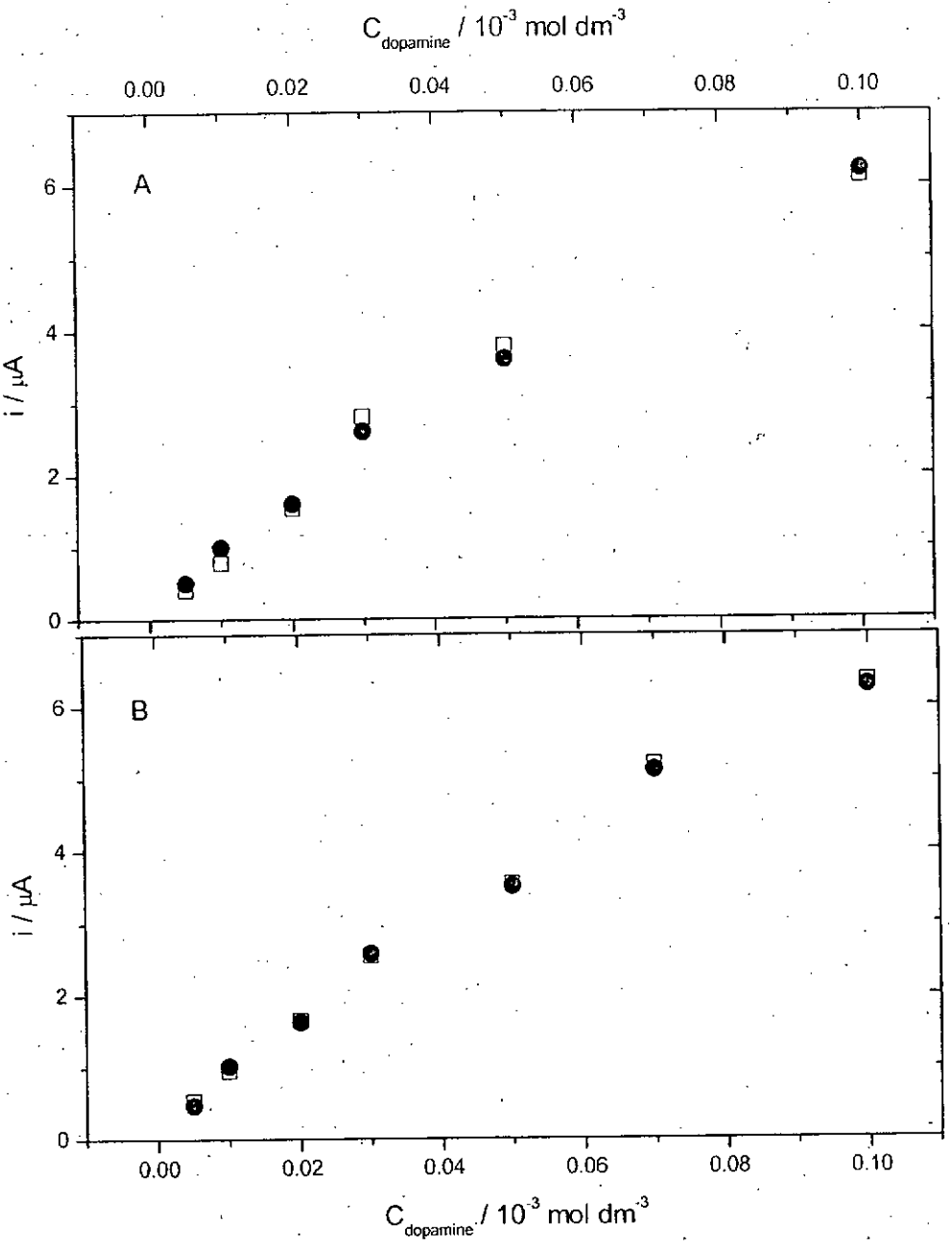


Figure 4.10. Peak current in the differential pulse voltammogram with dopamine concentration varied from 5.0×10^{-6} to 1.0×10^{-4} mol dm^{-3} in the presence of 1.0×10^{-4} mol dm^{-3} tyramine (A) or homovanillic acid (B).

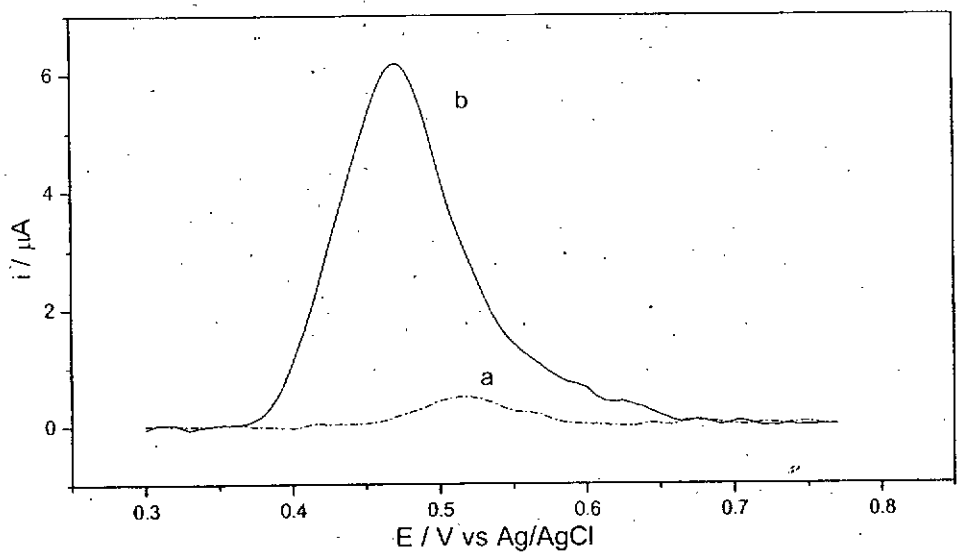


Figure 4.11. Differential pulse voltammograms recorded on a MIP film modified electrode after preconcentration in 1.0×10^{-4} mol dm^{-3} of (a) DOPAC and (b) dopamine solution. Electrolyte: 0.05 mol dm^{-3} HCl + 0.05 mol dm^{-3} KCl (pH = 2.0) solution. Scan rate: 20 mVs^{-1}.

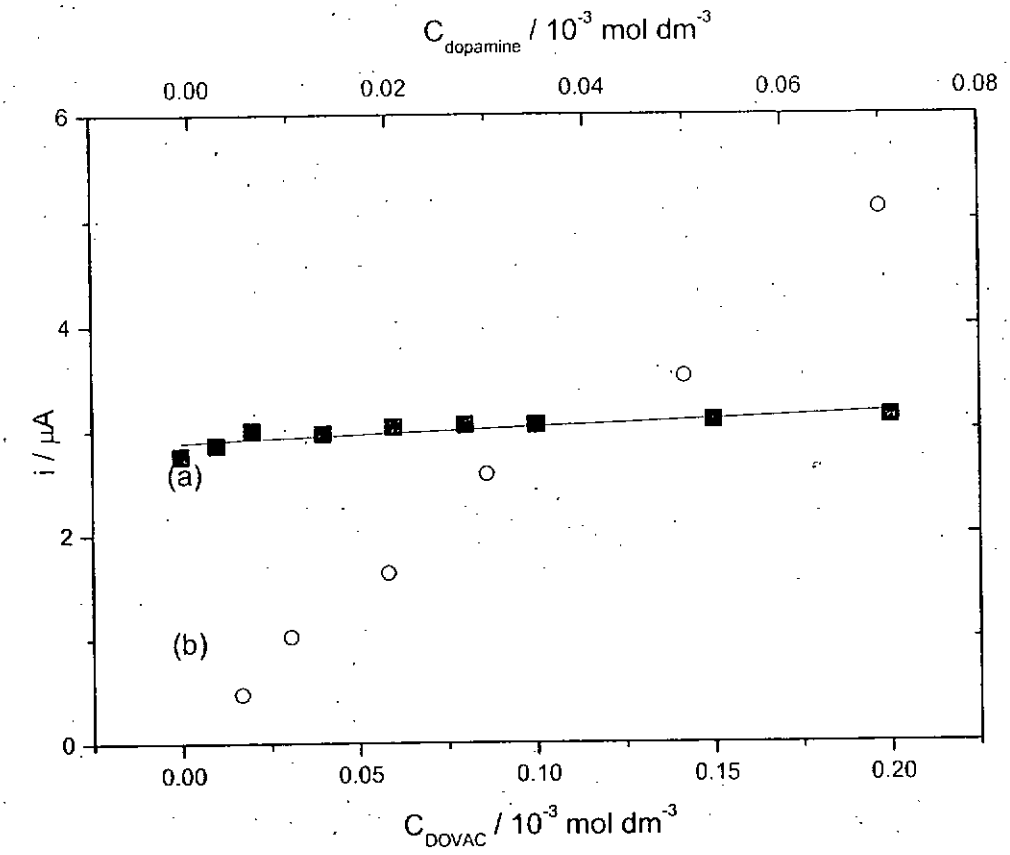


Figure 4.12. Peak current in the differential pulse voltammogram obtained under different concentrations of dopamine and DOPAC. (a) Dopamine concentration was fixed at 3.0×10^{-5} mol dm^{-3} while DOVPAC concentration (lower X-axis) was varied from 1.0×10^{-5} to 2.0×10^{-4} mol dm^{-3}; (b) A mixture containing the same concentration of dopamine and DOPAC, from 5.0×10^{-6} to 1.0×10^{-4} mol dm^{-3} (upper X-axis).

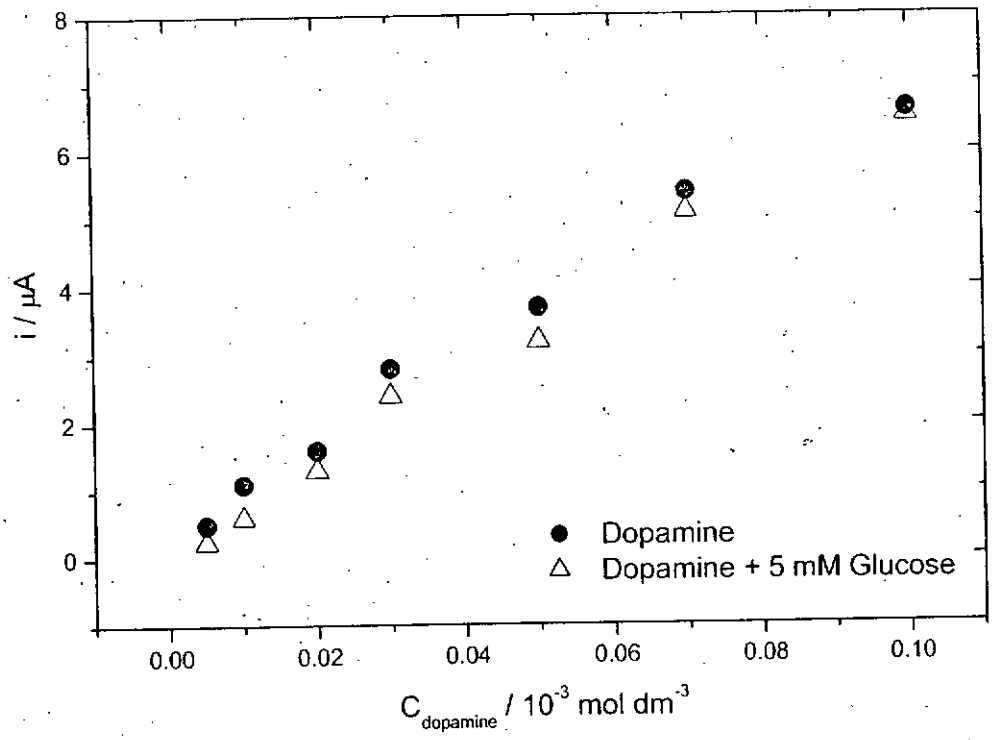


Figure 4.13. Plots of oxidative peak current in the differential pulse voltammogram of dopamine bound to MIP film by preconcentration in dopamine solution (5.0×10^{-6} to 1.0×10^{-4} mol dm^{-3}, pH 7.4) in the absence (●) and presence (Δ) of 5.0×10^{-3} mol dm^{-3} glucose.

4.4 Conclusions

Molecularly imprinted polymer (MIP) films of acrylamidophenylboronic acid were prepared by electropolymerization on EQCM Au and Au wire electrodes in the presence of dopamine. After removal of the template molecules, an imprinted polymer film with both high affinity to dopamine and permselectivity against the interferent ascorbic acid (AA) was prepared. An electrochemical sensor based on the imprinted polymer film was used to detect dopamine at physiological pH by a preconcentration-stripping approach. This dopamine sensor exhibits a direct response in terms of electrochemical redox peak current to dopamine in the range of 5.0×10^{-6} to 1.0×10^{-4} mol dm^{-3}. Interference from ascorbic acid with concentration as high as 1.0×10^{-3} mol dm^{-3} is successfully eliminated with this sensor. Ideal selectivity of this dopamine sensor against structurally similar compounds of dopamine was also demonstrated. Interference from excess amount of glucose was also examined. The mechanism of selective sensitivity of the sensor was discussed. The effect of electrode potential and solution pH on the performance of sensor was also investigated.

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Chapter 5

Conclusions

Molecularly imprinting has been recognized as one of the most promising tools for the development of synthetic receptors. Because of their capability of specific binding towards corresponding substrates, molecularly imprinted polymers are ideal as recognition elements for sensors. However, due to the difficulties in integrating the imprinted polymers to the sensor transducers, development of sensors based on molecularly imprinted polymer films are relatively slow. In this study, we have developed a series of electrochemical sensors based on molecularly imprinted polymers, including some new monomers. All these sensors show good selectivities and response towards their substrates.

An ATP imprinted polymer film was prepared by electropolymerization of pyrrole in the presence of ATP. Using the electrochemical quartz crystal microbalance (EQCM), potentiodynamic uptake of ATP, ADP and AMP into overoxidized polypyrrole film was observed. It was found that the amounts of uptake decrease in the order ATP > ADP > AMP, which indicated that the sensor is selective toward ATP against ADP and AMP. This EQCM sensor based on imprinted polypyrrole film showed linear response in frequency shift of the QCM resonator towards ATP concentration in the range of 1.0×10^{-4} to 0.02 mol dm^{-3}. An optimal pyrrole: ATP mole ratio of 2:1 was found to generate electropolymerized imprinted polypyrrole film with highest selectivity.

Another ATP imprinted polymer film was prepared by electropolymerization of o-phenylenediamine in the presence of ATP. Similar to the polypyrrole-based film, the amount of uptake into the imprinted poly(o-phenylenediamine) film was found to decrease in the order ATP > ADP > AMP. The frequency shift of the EQCM sensor

based on this imprinted film was found to vary linearly with ATP concentration in the range of 2.0×10^{-5} to 2.0×10^{-3} mol dm^{-3}. The response time of this sensor could be as short as 10 seconds, which was ascribed to the low diffusion barrier of the thin polymer film.

A newly developed functional monomer, glucose boronate-substituted aniline, was used for the preparation of glucose imprinted polymer film. A novel approach, which involved electropolymerization of the functional monomer in nonaqueous system and gradually transferring into the aqueous system, was found to be suitable for preparation of this glucose imprinted polymer film. At physiological pH, the EQCM sensor based on this glucose imprinted polymer film showed linear frequency response toward glucose concentration in the range of 2.0×10^{-5} to 7.5×10^{-4} mol dm^{-3}. The mole ratio between the functional monomer and aniline was found to remarkably affect the sensitivity and selectivity of the sensor and a functional monomer to aniline mole ratio of 0.4 was found to result in imprinted polymer film with best selectivity. It was found that the potential applied during the course of analysis and pH value of the solution could greatly affect the uptake of glucose into the imprinted polymer film. This is because the electrode potential and solution pH can both affect the association constant of the boronate ester.

A dopamine imprinted polymer film was prepared by electropolymerization of acrylamidophenylboronic acid in the presence of dopamine. This molecularly imprinted polymer film showed specific affinity towards dopamine and permselectivity against ascorbic acid. In order to minimize the interference from ascorbic acid, an alternative approach in which dopamine was first selectively

entrapped into the imprinted polymer and then analyzed by differential pulse voltammetry was used. Using this approach, the sensor based on dopamine imprinted polymer film was free from ascorbic acid interference, and showed linear current response in the concentration range of 5×10^{-6} to 1×10^{-4} mol dm^{-3}. These advantages make the sensor ideal for dopamine determination, and hence may find its application in medicine or industry.

This research work successfully demonstrates that assembly of molecularly imprinted polymers on the surface of electrochemical and microgravimetric transducers can be achieved by *in-situ* electropolymerization, which provides a convenient means to fabricate MIP films directly on the transducer surface. Properties of the MIP films such as thickness and porosity can be easily controlled by tuning the current and voltage as well as by the choice of supporting electrolyte in electrolysis. These properties of the MIP film are important for sensors with quick response time and high sensitivity. By properly choosing appropriate functional groups on the monomers, sensors with high selectivity and sensitivity can be developed. In this study, we have demonstrated that MIP films prepared in nonaqueous medium can be transferred gradually to and used subsequently in aqueous environment. However, this involves a number of tedious steps and the more convenient direct preparation of MIPs by electropolymerization in aqueous system is still much preferred. The latter approach, however, is limited by the small number of choices of monomers that can be electropolymerized in aqueous medium. Although we have prepared some new electropolymerizable monomers such as acrylamidophenylboronic acid in this study, the choice of electropolymerizable monomers with appropriate functional groups is still limited compared to the large

number of monomers available for MIP preparation by conventional chemical means. To make the electrochemical approach really practical, more effort to expand the library of electropolymerizable monomers with various functional groups through molecular design and chemical synthesis is needed. As pyrrole, aniline and thiophene are the best known monomers that can be electropolymerized in aqueous medium, a logical extension is to modify these monomers with appropriate functional groups. Monomers with various functional groups such as carboxylate, hydroxyl and amine located on different positions of the monomers can be designed and prepared by organic synthesis. To prepare more sophisticated binding sites, different functionalized monomers and cross-linkers can be used. This can be achieved by electropolymerization of a mixture monomers with similar structure but bearing different functional groups in the presence of the template molecules. For example, a dopamine-selective MIP film can be prepared by co-polymerization of acrylamidophenylboronic acid with acrylamidobenzoic acid. While the boronate functional group interacts with the hydroxyl groups on dopamine in the molecular imprinting process, the negatively charged carboxylate group is expected to interact with the ammine group on dopamine. MIP films with properly orientated boronate and carboxylate groups for higher selectivity of dopamine can then be prepared. With the expansion of choices of electropolymerizable functionalized monomers available to the chemical community, it is expected that the convenient preparation of molecularly imprinted films by direct *in-situ* electropolymerization on transducer surface will become the method of choice for the fabrication MIP sensors in the future.