MOLECULARLY IMPRINTED POLYMER MODIFIED OPTICAL FIBER SENSOR BASED ON SURFACE PLASMON RESONANCE SPECTROSCOPY FOR DETERMINATION OF ATRAZINE

Miss Chidchanok Tabtimhin

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	MOLECULARLY IMPRINTED POLYMER MODIFIED OPTICAL FIBER SENSOR BASED ON SURFACE PLASMON RESONANCE SPECTROSCOPY FOR DETERMINATION OF ATRAZINE
By	Miss Chidchanok Tabtimhin
Field of Study	Chemistry
Thesis Advisor	Associate Professor Dr. PAKORN VARANUSUPAKUL

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ชิดชนก ทับทิมหิน : ตัวรับรู้เส้นใยนำแสงฐานเซอร์เฟซพลาสมอนเรโซแนนซ์สเปกโทรสโกปีดัดแปรด้วยพอลิเมอร์พิมพ์แบบโมเลกุลสำหรับการตรวจวัดสารอะทราซีน. (MOLECULARLY IMPRINTED POLYMER MODIFIED OPTICAL FIBER SENSOR BASED ON SURFACE PLASMON RESONANCE SPECTROSCOPY FOR DETERMINATION OF ATRAZINE) อ.ที่ปรึกษาหลัก : รศ. ดร.ปกรณ์ วรานุศุภากุล

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งานวิจัยนี้เป็นการพัฒนาตัวรับรู้เส้นใยนำแสงฐานเซอร์เฟซพลาสมอนเรโซแนนซ์ดัดแปรด้วยพอลิเมอร์พิมพ์แบบโมเลกุลสำหรับการตรวจวัดอะทราซีน โดยขั้นแรกจะทำการตรึงอนุภาคทองระดับนาโนลงบนพื้นผิวของเส้นใยนำแสงเพื่อทำให้เกิดปรากฏการณ์เซอร์เฟซพลาสมอนเรโซแนนซ์ การกระจายตัวและขนาดของอนุภาคทองระดับนาโนถูกควบคุมโดยความเข้มข้นของสารละลายโกลด์ (III) คลอไรด์และระยะเวลาที่ใช้ในการแช่ ขั้นต่อมาเส้นใยนำแสงที่มีการตรึงอนุภาคทองระดับนาโนจะถูกดัดแปรด้วยพอลิเมอร์พิมพ์แบบโมเลกุลเพื่อนำไปใช้ในการตรวจวัดอะทราซีนในสารละลายตัวอย่าง ศึกษาการสังเคราะห์พอลิเมอร์พิมพ์แบบโมเลกุลสองวิธี วิธีแรกเป็นการเตรียมโดยให้ความร้อนเพื่อทำให้เกิดสารละลายพอลิเมอร์ที่มีโครงร่างสั้น (pre-polymer) ก่อนจะหยดลงไปบนพื้นผิวของใยแก้วนำแสงและนำไปผ่านการบ่มโดยใช้แสงอัลตร้าไวโอเลต วิธีที่สองคือการสังเคราะห์พอลิเมอร์โดยใช้เทคนิคอะตอมทรานสเฟอร์เรดิคอลพอลิเมอไรเซชั่น (atom transfer radical polymerization, ATRP) โดยใช้โบรโมไทโอฟีนอลเป็นตัวเริ่มต้นปฏิกิริยาให้ความร้อนที่ 80°C เป็นเวลา 1 ชั่วโมง การสังเคราะห์ผ่านวิธี ATRP สามารถทำได้โดยตรงด้วยการใช้ประโยชน์จากการสร้างพันธะระหว่างอนุภาคทองระดับนาโนและหมู่ไทอัลในโครงสร้างของตัวเริ่มต้นปฏิกิริยา จากการศึกษาพบว่าการสังเคราะห์ผ่านวิธี ATRP นั้นสามารถควบคุมค่าการกระจายตัวของน้ำหนักของพอลิเมอร์พิมพ์แบบโมเลกุลบนใยแก้วนำแสงแต่ละชิ้นได้ดี และพบสัญญาณเซอร์เฟซพลาสมอนเรโซแนนซ์ที่ความยาวคลื่น 569.27 นาโนเมตร ศึกษาปริมาณวิเคราะห์ด้วยการสร้างกราฟมาตรฐานช่วงความเข้มข้น 0-30 ppm พบว่าสารละลายตัวอย่างที่มีการเติมอะทราซีนที่ความเข้มข้นต่างๆให้ค่าการกลับคืนอยู่ในช่วง 88-104 เปอร์เซ็นต์ และขีดจำกัดของการตรวจวัดของวิธีนี้เท่ากับ 4.6 ppm

สาขาวิชา	เคมี	ลายมือชื่อนิสิต .....
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Chidchanok Tabtimhin : MOLECULARLY IMPRINTED POLYMER MODIFIED OPTICAL FIBER SENSOR BASED ON SURFACE PLASMON RESONANCE SPECTROSCOPY FOR DETERMINATION OF ATRAZINE. Advisor: Assoc. Prof. Dr. PAKORN VARANUSUPAKUL

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A sensitive sensor device based on surface plasmon resonance (SPR) spectroscopy was developed on an optical fiber and made to be selective to atrazine by molecularly imprinted polymer. The SPR probe was fabricated by deposition of gold nanoparticles on the uncladed surface of an optical fiber by self-assembly method. The size and distribution of gold nanoparticle was optimized by varying the soaking time of the optical fiber. The surface was further modified for selective sensing of atrazine with the molecularly imprinted polymer (MIP). The MIP was synthesized via 2 methods, the first was thermal polymerization method, the MIP was then in-situ polymerized by dropping the pre-polymer solution on the surface of the SPR probe. The second method was atom-transfer radical polymerization or so calls ATRP. The MIP with ATRP was in-situ synthesized on the SPR probe by using thiol-gold bonding initiator of 4-bromo thiophenol. The polymer further grew by heating at 80°C for 1 hr in the oven. The ATRP showed that the amount of polymer could be controlled by incubation time, and showed the dip of resonance wavelength at 569.27 nm. The calibration of SPR probe modified with MIP via ATRP was established in the range of 0-30 ppm of atrazine for quantitative analysis. The % recoveries of spiked atrazine standard in real water sample were in the range of 88-104%. The limit of detection was 4.6 ppm.

Field of Study: Chemistry Student's Signature .....

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LIST OF ABBREVIATIONS

%	percentage
ºC	degree Celsius
LOD	Limit of detection
mL	milliliter
µL	microliter
g	gram
ppm	Part per million
mM	millimolar
RSD	Relative standard deviation
UV-Vis	Ultraviolet-Visible
WHO	World Health Organization
SPR	Surface plasmon resonance
ATRP	Atom transfer radical polymerization
MIP	Molecularly imprinted polymer
NIP	Non- Molecularly imprinted polymer
AOAC	Association of official analytical chemists

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CHAPTER I

INTRODUCTION

Surface plasmon resonance (SPR) spectroscopy is a type of spectroscopy technique that offers highly sensitive, accurate and fast analytical tool. SPR takes place at the interface between nano-metal/dielectric interfaces caused by an incident light in the total internal reflection condition. The SPR spectrum is recognized by the dip of the resonance wavelength. This phenomenon is very sensitive to any change such as the adsorption of molecules to the surface that causes the shift of the resonance wavelength.

To take advantages from SPR spectrum, the SPR probe has been fabricated. The probe is consisted of three parts; light guidance (glass or plastic), metal surface and dielectric surface. In 2008, Greg and et al. developed general SPR probe based on prism or so called Kretschmann's configuration, using gold nanorod as metal surface to study bimolecular binding activity without any label [1].

Generally, SPR probe or device is based on Kretschmann's configuration, which is a prism configuration. Recently, SPR probe using optical fiber has been developed instead of prism based on the same principle [2]. The advantages of fiber optic SPR probe are small and portable. SPR probe was developed for detection of analyte by modification of the dielectric interface with antigen or antibody in biosensor works [3]. The change in dielectric surface led to the shift of resonance wavelength, which was directly corresponding to the amount of the analyte. In 2011, Jeong and et al. [4] developed the optical fiber SPR sensor for study of antigen-antibody reaction of interferon-gamma. The optical fiber SPR was developed by assembling gold nanoparticles on the end-face of an optical fiber. The density of the gold nanoparticle was optimized by varying dipping time of the optical fiber in the gold nanoparticle colloid solution. The interferon-gamma antibody was immobilized on the surface of the gold nanoparticle. The resonance wavelength shifted when the concentration of antigen increased, according to the activity of antibody/antigen.

The conventional SPR technique has been limited to biosensor works. Recently, SPR probe has been developed for environmental analysis. In 2016, Gupta and et al. [5] used molecularly imprinted polymer (MIP) instead of antigen and antibody in SPR works. The optical fiber SPR probe was developed by assembled with MIP to analyze profenofos in water sample. The probe was fabricated by coating silver nanoparticle on the unclad part of the optical fiber. The uniformity of silver nanoparticle was controlled by thermal evaporation method. Then the MIP was assembled on the silver film by using thermal polymerization path way. The shift of the resonance wavelength was observed when the concentration of profenofos in the sample changed. The probe showed high sensitivity for detection of profenofos.

Molecularly imprinted polymer or MIP is the synthesized material resembling to lock and key model providing binding sites that have specific size and shape with the template analyte. The advantages of MIP are easy to synthesize, low cost and tunable with desire types of analytes. There are many path ways to synthesize MIP. The conventional polymerization has been done by heat and UV light, using metacrylic acid as monomer. All of chemicals are prepared in a small vial then flushed with nitrogen gas for 5 min before heated at 90 degree in an oven until the short chain of polymer is formed. This path way is very easy and fast to synthesize but the main drawback is the thickness of the polymer on the SPR probe cannot be easily controlled. So, another synthesis path way has been developed. Atom transfer radical polymerization is an in-situ polymerization of the polymer on a substrate surface, so that the thickness of the polymer can be controlled [6].

In this research, we are interested in development of an optical fiber sensor based on SPR modify by MIP for sensing of atrazine as a model of this study. Atrazine is a carcinogenic compound and also toxic with endocrine system and reproductive system. There were reports that atrazine contamination in crop water at the northern part of Thailand was around 12.9 ppb and in drinking water was around 18.8 ppb [7]. Those contamination levels were higher than the standard reported reports by World health organization (WHO) [8]. The polymerization methods for MIP were studied. Finally SPR probe assembled with MIP was tested for sensing of atrazine in water samples.

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CHAPTER II

THEORY

2.1 Surface plasmon resonance (SPR)

Surface plasmon resonance (SPR) is a spectroscopic feature of a conductive metal nanoparticle that provides an optical technique for highly sensitive analytical sensor. SPR takes place at the interface between metal nanoparticle and dielectric triggered by an incident light causing resonant oscillation of electrons (surface plasmon) at the interface resulting in a sharp absorption band being observed (Figure 1). The resonance is recognized by a sharp dip in the reflected light intensity (Figure 2). This phenomenon is very sensitive to any change to the surface causing the shift of the resonance wavelength.

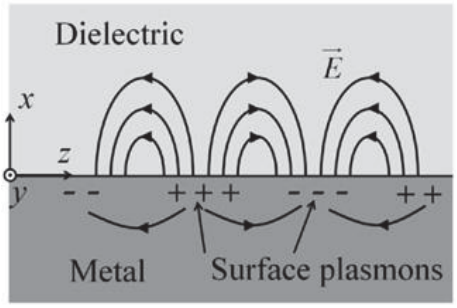

Figure 1 Surface plasmon propagation along the interface between electric and dielectric surface [9]

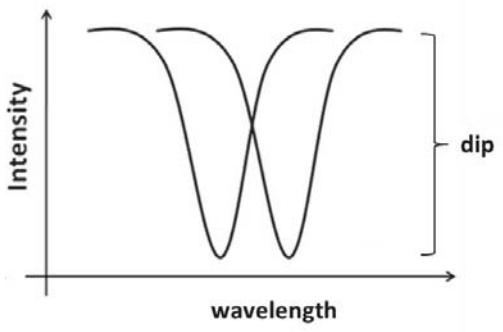

Figure 2 The dip of resonance wavelength of Surface plasmon resonance [9]

2.2 Surface plasmon resonance spectroscopy

There are two configurations being used to achieve SPR; Kretschmann's configuration and optical fiber configuration

2.2.1 Kretschmann's configuration

In general, SPR spectroscopy has used in the form of Kretschmann's configuration, which composes of prism, metal sheet and dielectric medium (air, water, vacuum or polymer). The general role of prism is a light guidance to keep the light in the total internal refraction (TIR) condition. When the incident light hits around the surface of nano-metals sheet at the right angle (or critical angle), the electron around surface will absorb the energy from the incident light. The absorbed energy is transferred to cause resonance, then the signal is observed. The drop of transmitted light so called dip of resonance wavelength can be seen. The SPR spectroscopy is developed for sensing analyte by immobilizing substrate (antigen or antibody) on the surface of the nano-metal. When the refractive index of those immobilized substrate changes, causing the shift of the resonance wavelength (shown in Figure 3). The delta shift of the resonance wavelength is directly corresponding to the amount of the analyte.

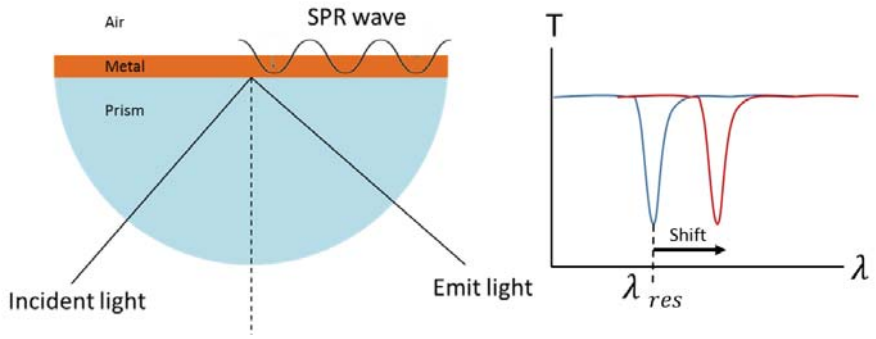

Figure 3 Kretschmann's configuration of SPR spectroscopy

SPR device based on Kretschmann's configuration has been used for biosensor works, for example study of kinetic energy of antigen and antibody

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[10] and chemical sensor for gas and volatile organic compounds (VOCs) such as toluene [11].

2.2.2 Optical fiber surface plasmon resonance spectroscopy

Recently, the optical fiber configuration of SPR spectroscopy has been developed. This configuration uses the optical fiber instead of prism with the same principles. The optical fiber itself is designed as a light guidance in the total internal refraction condition. The main parts of SPR probe consists of light guidance (optical fiber), nano-metal and immobilized substrate which is dielectric substrate. In case of optical fiber SPR, the cladding around the middle of optical fiber is removed. The bare optical fiber is coated with nanoparticles, and then further coated with dielectric substrate. The optical fiber is working the same role as prism in Kretschmann's configuration. The optical fiber surface plasmon resonance is illustrated in Figure 4.

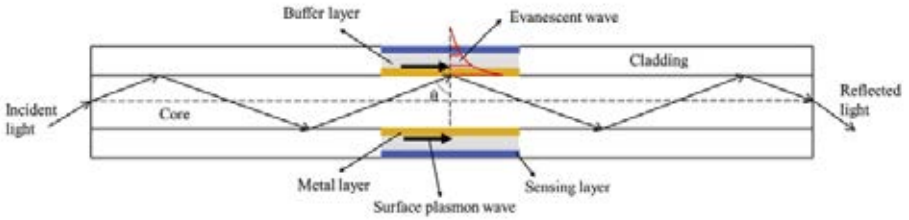

Figure 4 Optical fiber SPR spectroscopy [12]

There are many types of dielectric substrate depending on the role of SPR. For example, in biosensor work, antigen or antibody was bound on a nanometal surface for study of the activity of those antigen and antibody. For now a day, SPR has been used in environmental analysis to detect some types of herbicides and pesticides, where the molecularly imprinted polymer has been assembled on the SPR probe.

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2.3 Molecularly imprinted polymer

Molecularly imprinted polymer (MIP) is synthesized as a lock and key model like antigen and antibody in bio sensing works. The advantages of MIP are easy to be synthesized, low cost and tunable to many types of analytes. There are three easy steps in synthesis of MIP. First adding the template, which is the analyte of interest, monomer, crosslinker, and solvent (if necessary). The mixture is then polymerized by using heat or light depending on type of initiator. Lastly the template and remaining compounds are removed by washing with appropriate solvent or adjusting pH [13]. The principle step of polymerization is schematically shown in Figure 5.

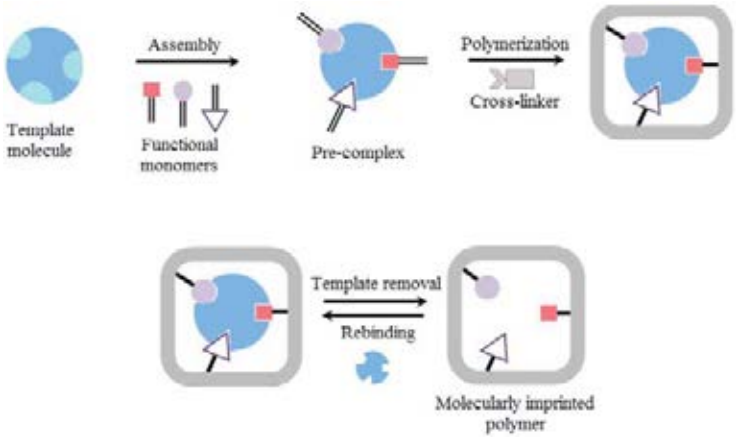

Figure 5 The principle steps of molecular imprinting polymer polymerization [14]

The main advantage of MIP is selectivity, because it's synthesized from the template having cavity size and shape as same as of the analyte. The main interactions between MIP and analyte are H-bonding and ¶-¶ stacking.

There are many path ways to synthesize MIP, such as radical polymerization which can be classified as thermal polymerization, UV-irradiated polymerization and atom transfer radical polymerization, which are different in methodology and also type of initiators.

2.3.1 Thermal polymerization

Thermal polymerization is the path way that converts monomer to polymer by thermal energy. Normally, this synthesis method is used in bulk

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condition (only monomer, cross linker and initiator). The initiator for thermal polymerization is normally contains Azo group, for example Azobisisobutyronitrile and 2,2′-Azobis(2-methylbutyronitrile).

There are 3 steps of polymerization; initiation step, when the initiator is become free radical (active species) by thermal energy; propagation step, when monomer and cross linker are attached; and termination step, when the monomer and cross linker in system are consumed or the active species are attached by itself and the reaction stops. The advantages of the thermal polymerization are easy to synthesize, high yield of polymer and also low cost. The step of polymerization is shown in figure 6.

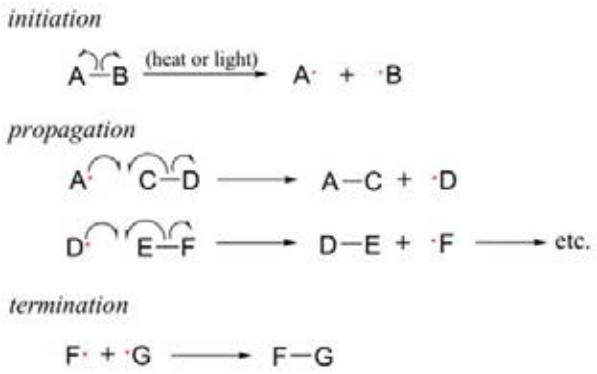

Figure 6 Three steps of polymerization [15]

2.3.2 UV-irradiated polymerization

In case of UV-irradiated polymerization, there are the same steps with thermal polymerization but it needs photo energy in the initiation step. The initiator that used for UV-irradiated polymerization is called photoinitiator, for example 2,2-dimethoxy-1,2-diphenyl-ethan-1-one and Benzophenone. However the main drawback of both thermal and UV-irradiated polymerization is difficult to control molecular weight of polymer. Recently new polymerization method called atom transfer radical polymerization (ATRP) has been used to overcome the disadvantage of the common methods.

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2.3.3 Atom transfer radical polymerization

Atom transfer radical polymerization (ATRP) is a controlled polymerization method to synthesize the polymer that has narrow molecular weight distribution. Organic initiator (R-X) containing halogen atom (Cl, Br or I) that can be interact with transition metal in ligand ($[M]^N$). The active radical (R•) interact with vinyl monomer and cross-linker in the system. This process can be controlled by the amount of ligand and initiator. The principle of ATRP is shown in Figure 7.

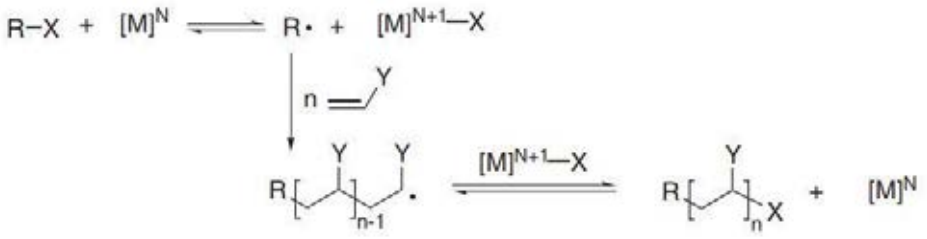

Figure 7 Atom transfer radical polymerization [15]

2.4 Atrazine

Atrazine has been used in this study as a model analyte. Atrazine is a main herbicide in Thailand, that was imported around 3,983,749 kg. per year [7]. Atrazine is a carcinogenic compound this is toxic to endocrine system and reproductive system. Contamination of atrazine in crop water at the northern part of Thailand was reported around 12.9 ppb and in drinking water was around 18.8 ppb, [7]. That contamination levels are higher than the standard recommended by world health organization (WHO) [8]. The structure of atrazine is shown below.

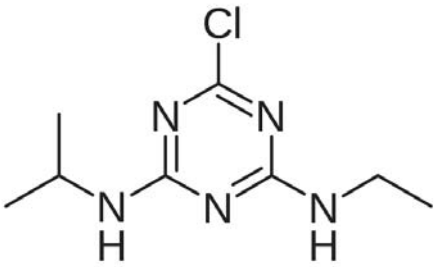

Figure 8 Atrazine structure

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CHAPTER III

EXPERIMENTAL

3.1 Instruments and Equipment

UV Solarization-Resistant optical fiber (Thorlabs, Singapore)

Step-Index Multimode Fiber (Thorlabs, Singapore)

Optical fiber cuter, Universal Bare Fiber Terminator (Thorlabs, Singapore)

Temporary connector Selection Guide (Thorlabs, Singapore)

UV-visible spectrophotometer (Ocean optic, USA)

3.2 Chemicals and Reagents

Gold (III) chloride trihydrate $\geq$99.9% trace metals basis (SIGMA-ALDRICH, Germany)

Sodium formate (Carlo-Erba, France)

Sodium hydroxide (Carlo-Erba, France)

Dimethyl sulfoxide (Fisher Chemical, China)

Acetonitrile (Fisher Chemical, China)

Methacrylic acid (MERCK, Germany)

Ethylene glycol dimethacrylate (SIGMA-ALDRICH, Germany)

Azobisisobutyronitrile (SIGMA-ALDRICH, Germany)

1-bromo thiophenol (SIGMA-ALDRICH, Germany)

Methyl α -bromo isobutyrate (SIGMA-ALDRICH, Germany)

Copper (II) chloride (SIGMA-ALDRICH, Germany)

N,N,N′,N′-Tetramethylethylenediamine (SIGMA-ALDRICH, Germany)

3.3 Preparation of chemical solution

3.3.1 Gold chloride solution

Gold chloride solutions of 5, 15 and 20 mM. were prepared by weighting gold chloride trihydrate 0.0492 gram, 0.0984 gram, and 0.1477 gram respectively, and diluting to 25 ml of MiliQ water in volumetric flask.

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3.3.2 Sodium formate solution

A 1.7002 gram of sodium formate was dissolved in Milli-Q water and diluted to 50 mL in volumetric flask to make 500 mM of sodium formate solution.

3.3.3 Stock and working standard atrazine solution

A 0.0029 gram of atrazine standard was weighted then dissolved in 1 mL of dimethyl sulfoxide to make 2900 ppm of atrazine standard.

Six working standard atrazine solution was varied into 6 concentrations (5, 10, 15, 20, 25, and 30 mM) were prepared by pipetting approximate volume of stock solution and made to the volume of 10 mL with miliQ water as depicted in Figure 9.

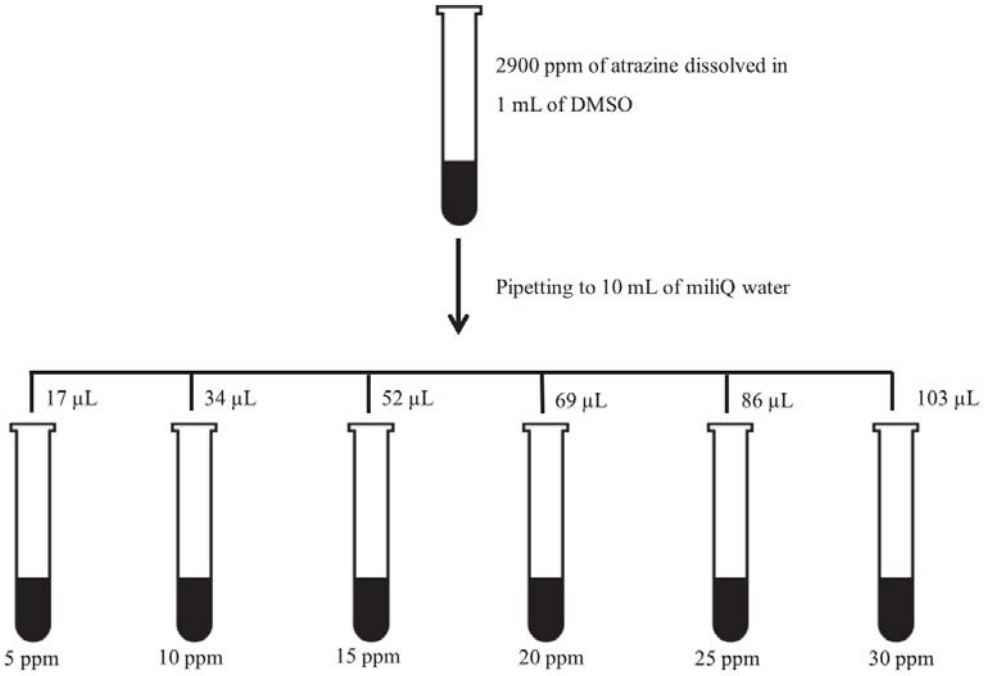

Figure 9 Preparation of atrazine standard solutions

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3.4 Probe fabrication

3.4.1 Preparation of optical fiber

The optical fiber was cut into 12 cm. About 1 cm in the middle of the optical fiber was uncladed, washed and sonicated in a surfactant for 10 min followed by DI water for 10 min prior to use.

3.4.2 Self-assembly of gold nanoparticle

The optical fiber was immersed into gold (III) chloride solution mixed with 500 mM of sodium formate at 1:5 ratios [16]. The process was summarized in Figure 10. The effect of concentration of gold (III) chloride and immersion time on SPR characteristic of the optical fiber were investigated and optimized. The concentrations of gold (III) chloride were varied from 5-20 mM and the immersion times were varied from 5-80 min.

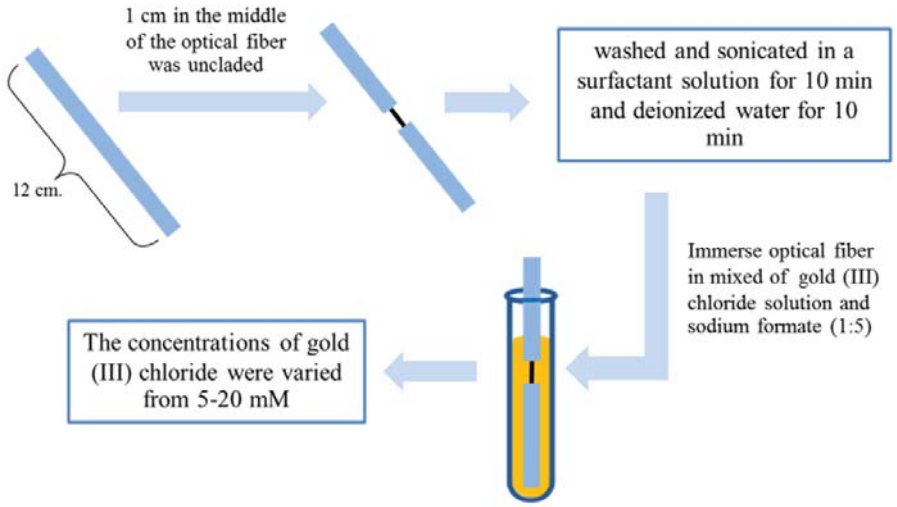

Figure 10 The process of self-assembly of gold nanoparticle on the surface of the optical fiber

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3.4.3 Synthesis of molecularly imprinted polymer

3.4.3.1 Thermal polymerization

3.4.3.1.1 Batch synthesis

The MIP was synthesized using methacrylic acid as a monomer, 2,2′-Azobis(2 methylpropionitrile) as initiator, ethylene glycol dimethylacrylate (EGDMA) as a cross linker and atrazine as a template. A 30 mg of atrazine, 2 mL of monomer, 1 mL of cross-linker were mixed under nitrogen gas for 5 min, then a 0.5 mL of initiator was added into the mixture and stired under nitrogen gas for 2 min. The mixture was heated in an oven (about 90 ℃) for 1 hr. The MIP was formed. The remaining monomer and cross linker were removed by ethanol. The atrazine template was then removed by washing with sodium hydroxide pH 8.2. The MIP was dried at 60℃ in the oven for 30 min and grounded into powder prior to uses. The non-molecularly imprinted polymer (NIP) was prepared in the same way without addition of atrazine template. The summary of these processes was shown in Figure 11. The morphology of MIP was observed by using scanning electron microscope (SEM). The absorptivity of atrazine standard was studies to evaluate the performance of synthesized MIP.

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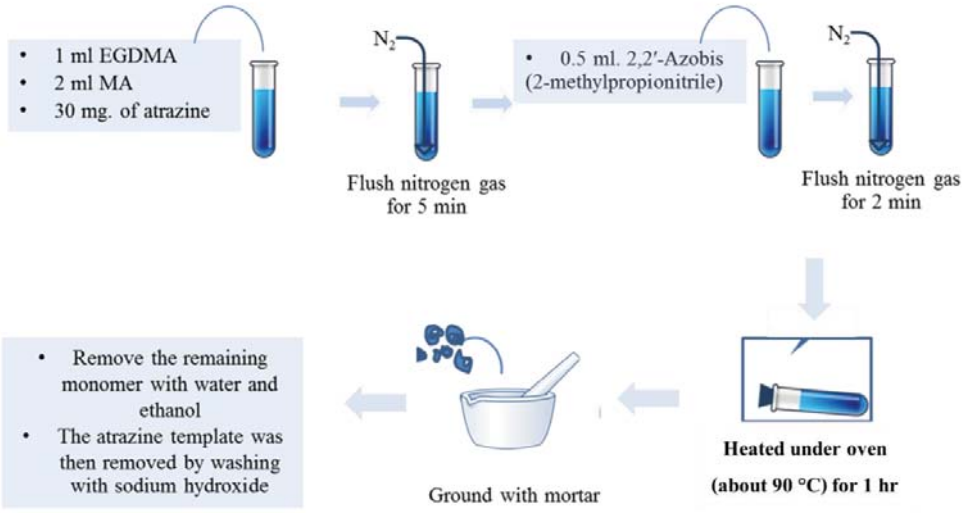

Figure 11 Synthesis of molecularly imprinted polymer via thermal polymerization method

3.4.3.1.2 Assembly of MIP on the optical fiber using thermal/UV-polymerization

The MIP was in situ synthesized on the optical fiber. The MIP was synthesized as the same with 3.4.3.1.1. The polymerization time was set to 5 min and cured under water bath. The viscous liquid of pre-polymer was formed. The pre-polymer was dropped on the layer of gold nanoparticles on the optical fiber and radiated under UV lamp for 1 hr to form the MIP. The optical fiber was washed and the template was removed and dried as described in section 2.3. The NIP was assembled on the fiber optical sensor in the same way without addition of atrazine template. The summary of these processes was shown in Figure 12. The SPR probe assembled with MIP via thermal/UV-polymerization method was used for sensing of atrazine in water sample.

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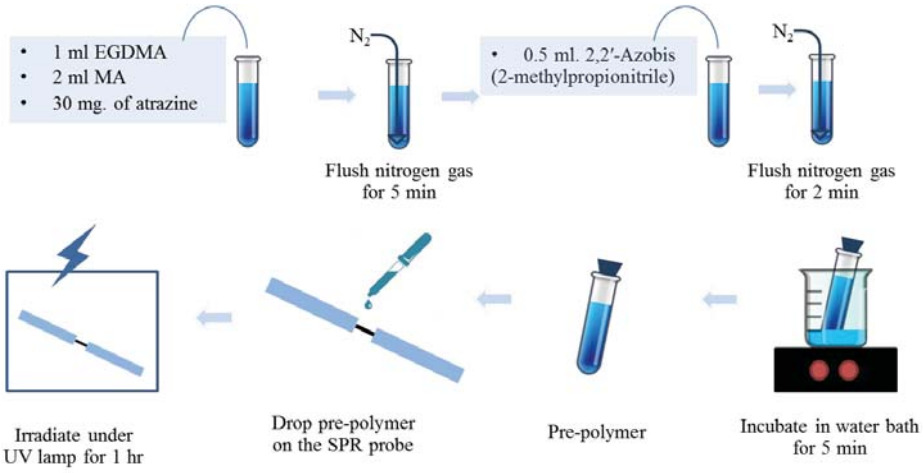

Figure 12 Assembly of MIP on the optical fiber using thermal/UV-polymerization

3.4.3.2 Atom transfer radical polymerization (ATRP)

3.4.3.2.1 Batch synthesis

The MIP was synthesized using methacrylic acid as a monomer, ethylene glycol dimethylacrylate (EGDMA) as a cross linker, Copper (II) chloride as a catalyst, N,N,N′,N′-Tetramethylethylenediamine as a ligand, methyl 2-bromopropionate (MBrP) as initiator, and atrazine as template. A 30 mg of atrazine, 315 µL of monomer, 2 mL of cross-linker were mixed under nitrogen gas for 5 min, then a 5 mL of acetonitrile was added. The mixture was stirred for 5 min then 0.7 g Copper (II) chloride was added and continued stirred until the solid was dissolved. A green solution was obtained. Then a 20 µL of ligand was added. Solution was flushed with nitrogen gas for 5 min before added 20 µL of initiator then the solution was heated at 80 °C in an oven for 1 hr. The MIP was formed. The remaining monomer and cross linker were removed by ethanol. The atrazine template was then removed by washing with sodium hydroxide pH 8.2. The MIP was kept in room temperature until dry. The non-molecularly imprinted polymer (NIP) was prepared in the same way without

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addition of atrazine template. The summary of these processes was shown in Figure 13.

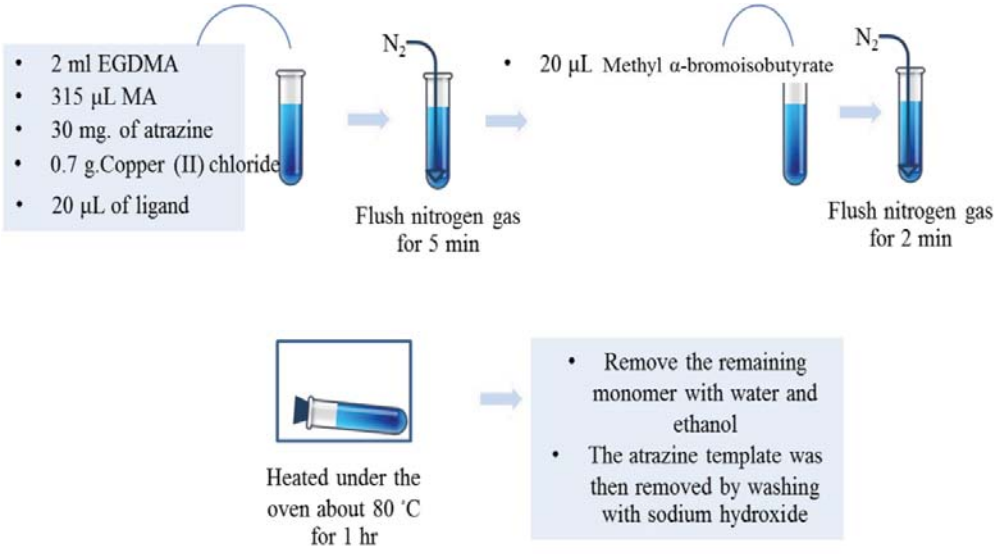

Figure 13 Synthesis of molecularly imprinted polymer via ATRP method

3.4.3.2.2 Assembly of MIP on the optical fiber using modified ATRP method

The MIP was insitu synthesized on the surface of gold coated optical fiber. To assemble the MIP on the optical fiber with gold nanoparticles, the probe was treated with 4-bromo thiophenol by dipping in the solution to create thiol-gold interaction. The pre-polymer mixture (same mixture with **3.4.3.2.1** but without initiator) with atrazine template was prepared on the small vial. The treated optical fiber was immersed in pre-polymer solution and heated in the oven for 1 hr to form the MIP. The optical fiber was washed and the template was removed by washed with the basic solution (pH 8.4). The NIP was assembled on the fiber optical sensor in the same way without addition of atrazine template. The summary of these processes was shown in figure 14.

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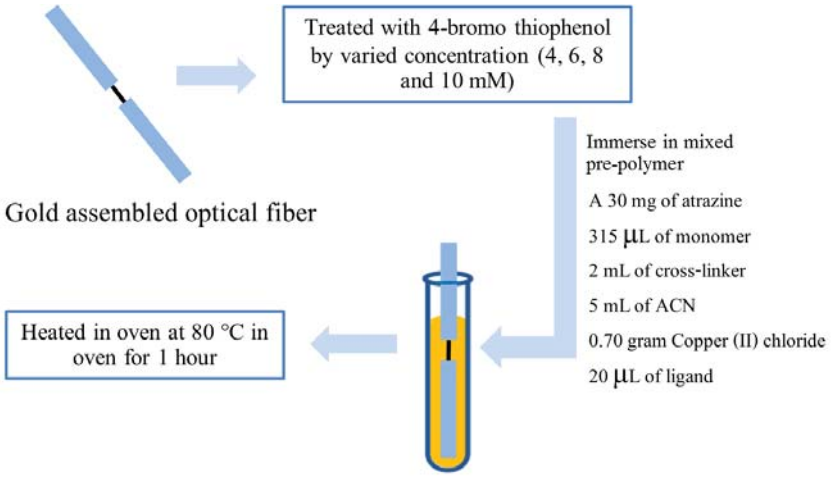

Figure 14 Assembly of MIP on the SPR probe using modified ATRP method

3.5 Set up of SPR probe

The SPR probe assembly with MIP was immersed in sample solution for 1 hr before set up with spectrometer. The SPR probe was connected with the light source and detector (UV-visible spectrophotometer) by using temporary connector Selection Guide as the connector. The SPR spectrometer configuration was shown in Figure 15.

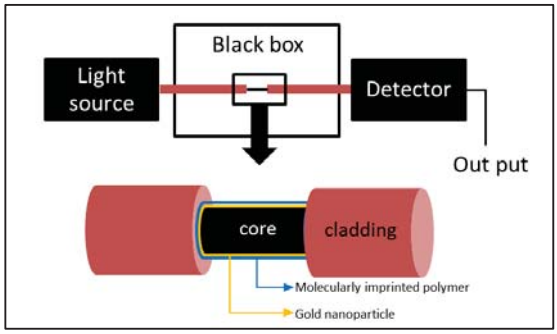

Figure 15 SPR spectrometer configuration

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CHAPTER IV
RESULTS ANS DISCUSSION

4.1 The effect of concentration of gold solution and immersion time with SPR signal

Particle size is very important to achieve the good SPR signal. In case of gold nanoparticle SPR can be observed for gold nanoparticle size ranging between 10-100 nm [17], where the resonance wavelength varied depending on size. Paramiters affecting gold nanoparticles on the surface of the optical fiber were studied such as concentration of gold (III) chloride and immersion time.

The concentrations of gold chloride solution were varied at 5, 10, and 20 mM for 15, 20, 25, 30, 40, 45, 50, 55, 60 and 80 min immersion time. The SPR signals obtained from optical fibers coated with gold nanoparticles at various concentrations of gold chloride and various immersion time were summarized in Figure 16.

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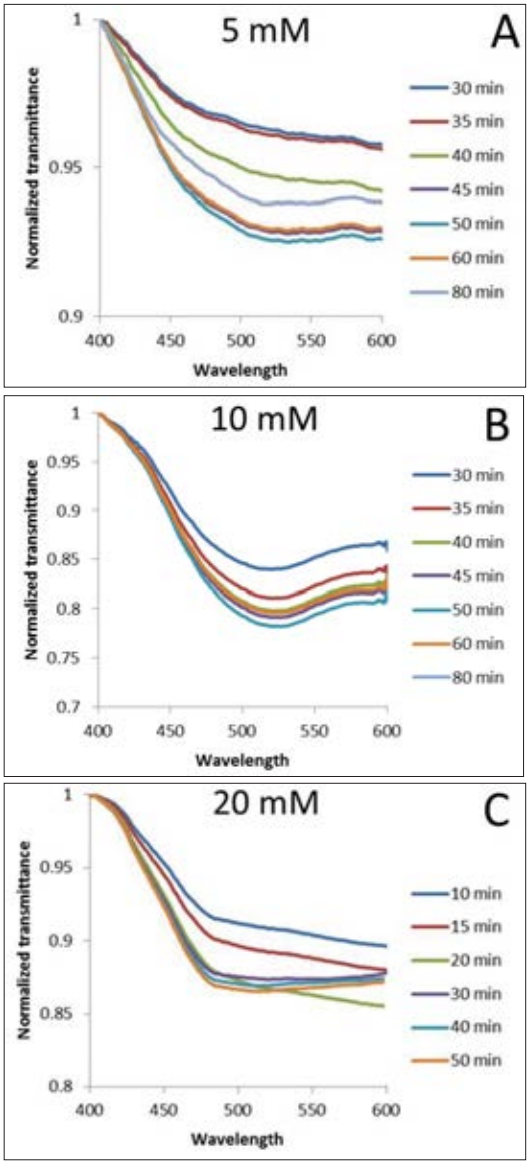

Figure 16 The SPR signals obtained from optical fibers deposited with gold nanoparticles at various concentrations of gold chloride at various immersion times (A) 5 mM of at 30-80 min (B) 10 mM at 30-80 min and (C) 20 mM at 10-50 min.

The concentration of gold chloride directly affects the shape and the dip of SPR signals. Using gold (III) chloride concentration of 5 mM (Figure 16A), the spectra were slowly decreased and the dips of SPR signals were not clearly observed. Probably, the particle size of the gold nanoparticle was not suitable for SPR. Using gold (III) chloride concentration of 10 mM (Figure 16B), the spectra were nicely

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dipped and SPR characteristic were clearly observed at 521 nm. Using gold (III) chloride concentration of 20 mM (Figure 16C), the spectra were not clearly seen than those of 10 mM. At high concentration of gold chloride, the gold nanoparticle layer could be developed relatively fast, forming large particle sizes and become to the thick film, consequently. Therefore, no SPR characteristic was observed.

Immersion time also strongly affect SPR signal. When immersion time was increased, the intensity of resonance wavelength increased, the SPR signals were clearly observed, but at immersion time above 60 min, the intensity of resonance wavelength decreased (Figure 16).

The SEM images of the optical fibers using gold chloride concentration of 10 mM at different immersion time were shown in Figure 17. The particle sizes of the gold nanoparticles were bigger and denser with increased immersion time. At immersion time of 80 min (Figure 17D), the gold particle layer started to get dense because of the compilation of particles resulting in decrease in SPR signal. Therefore, the gold chloride concentration of 10 mM at 60 min was chosen to prepare the optical fiber for SPR sensing probe.

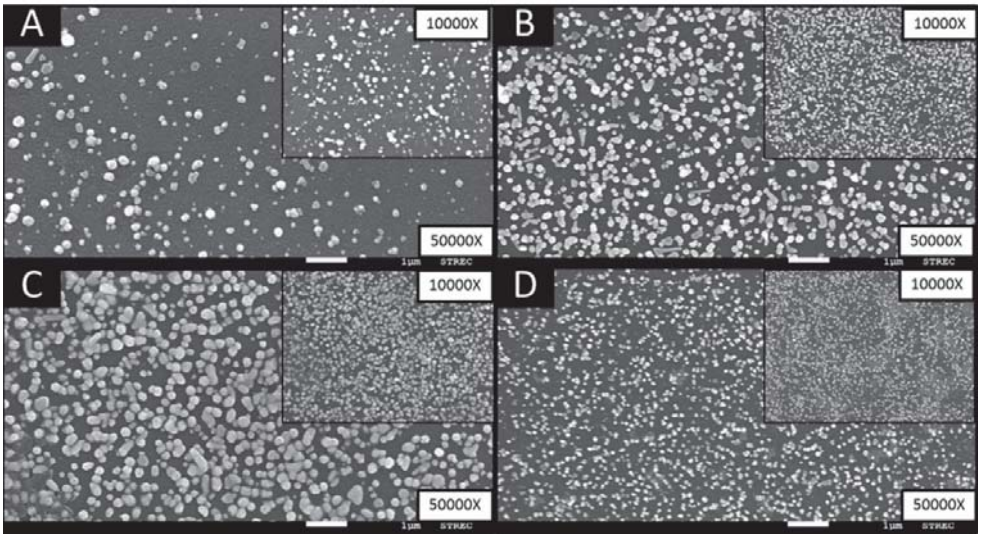

Figure 17 SEM images of the optical fiber surfaces using gold chloride concentration of 10 mM at different immersion time; (A) 35 min, (B) 50 min (C) 60 min and (D) 80 min

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4.2 Synthesis of molecularly imprinted polymer for atrazine

4.2.1 Thermal polymerization

The MIP and NIP were synthesized using thermal polymerization method. A 30 mg of atrazine, 2 mL of monomer, 1 mL of cross-linker were mixed under nitrogen gas for 5 min. Then a 0.5 mL of initiator was dropped under nitrogen gas for 2 min. The mixture was heated on water bath (about 70 ℃) for 10 min. The polymer was formed. The SEM images (Figure 18) of both MIP and NIP show different morphology of the surface and size. The MIP shows rougher surface and bigger size compared to NIP and both of them show the good morphology of 3- D structure polymer.

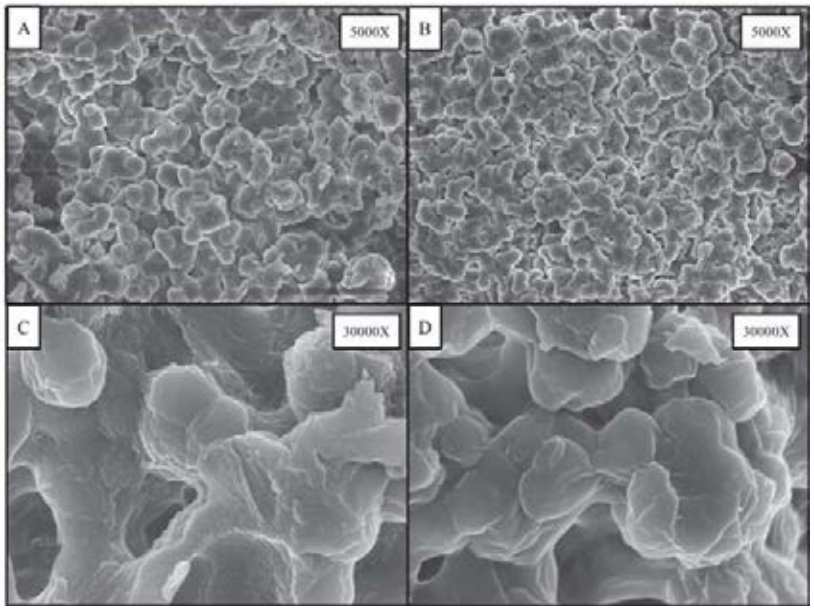

Figure 18 SEM images of MIP (A and C) and NIP (B and D) synthesis via thermal polymerization technique

The MIP was tested for adsorption of atrazine. Figure 19 shows the amounts (represented as absorbance) of remaining atrazine in the solution after treated with MIP and NIP. Apparently, atrazine could be significantly adsorbed to the MIP compared to the NIP. It suggested that the MIP was successfully synthesized providing binding sites, shape and size that fit to the target

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atrazine. In addition, the absorption of atrazine increased with increased adsorption time.

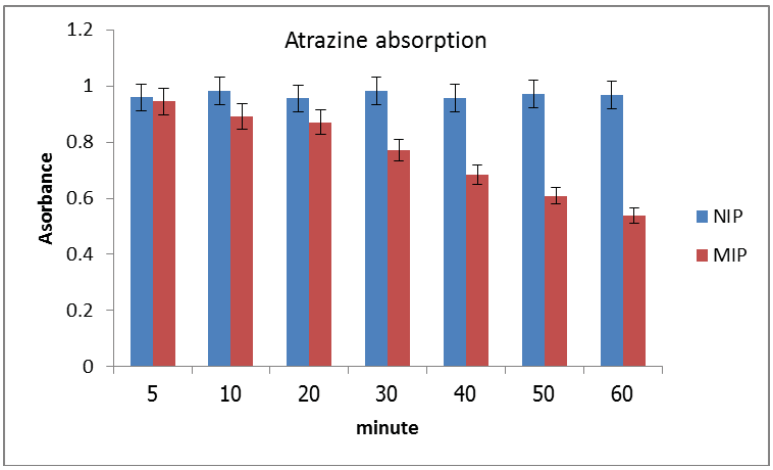

Figure 19 Amounts of atrazine standard in the solution after treated with MIP and NIP (via thermal polymerization method) at various time, using 0.1 g of polymer and 30 ppm as initial concentration of atrazine

4.2.2 Atom transfer radical polymerization

4.2.2.1 Using Methyl α-bromo isobutyrate as initiator

The MIP and NIP were synthesized using atom transfer radical polymerization method by using Methyl α-bromo isobutyrate. The synthesis process was described in section 3.4.2.1. The SEM images (Figure 20) of both MIP and NIP show different morphology of the surface and size. The MIP shows rougher surface and bigger size compared to NIP. The results are similar to those using thermal polymerization.

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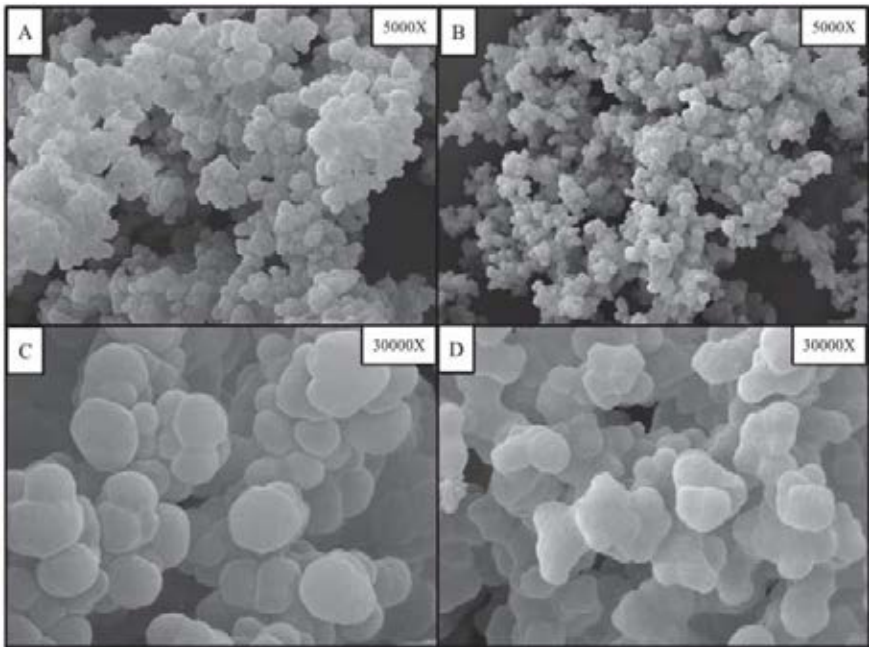

Figure 20 SEM images of MIP (A and C) and NIP (B and D) synthesis via atom transfer racial polymerization technique using 2-bromopropionate as initiator

The MIP was also tested for adsorption of atrazine with atrazine standard 30 ppm. Figure 21 shows the amounts (represented as absorbance) of remaining atrazine in the solution after treated with MIP and NIP. Apparently, atrazine could be significantly adsorbed to the MIP compared to the NIP. Noted that the absorptivity performance was less effective compared to the MIP synthesized by the thermal polymerization method.

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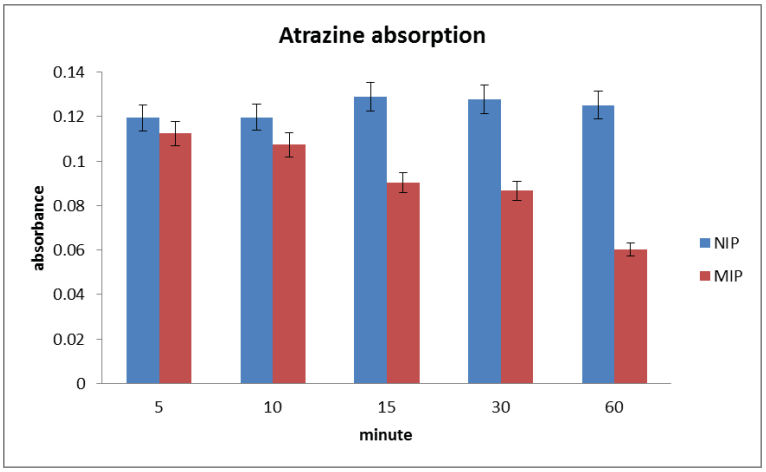

Figure 21 Amounts of atrazine standard in the solution after treated with MIP and NIP (via ATRP method) at various time, using 0.1 g of polymer and 30 ppm as initial concentration of atrazine

4.2.2.2 Using 4-bromo thiophenol as initiator

To assemble the MIP to the gold nanoparticles, the initiator with thiol group (-SH) was required [18]. Then the initiator that contains both of bromine and thiol group was used. The 4-bromo thiophenol was chosen as the initiator for this method. A chemical structure of 4-bromo thiophenol was shown in figure 22. For this reason, the initiator was changed from methyl 2-bromopropionate to 4-bromo thiophenol.

Figure 22 Chemical structure of 4-bromo thiophenol

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The synthesis processes was described in section 3.4.2.1. The SEM images (Figure 23) of both MIP and NIP show different morphology of the surface and size. The MIP shows rougher surface and bigger size compared to NIP. The results are still similar to those using methyl 2-bromopropionate as initiator, but clearly seem that this initiator gave the polymer with lower surface area than using methyl 2-bromopropionate as initiator. The polymer from ATRP usign 4-bromo thiophenol as initiator was slowly formed when compared to that using methyl α-bromo isobutyrate, because the structure of 4-bromo thiophenol was less active. So, it took long time to form the polymer and needed higher energy to generate the free radical. When the optical fiber was immersed in to the pre-polymer mixture for long time at high temperature the cladding of the optical fiber was damaged. Therefore, the synthesis condition was limited at 80 ºC for 1 hr to form the polymer on the SPR probe.

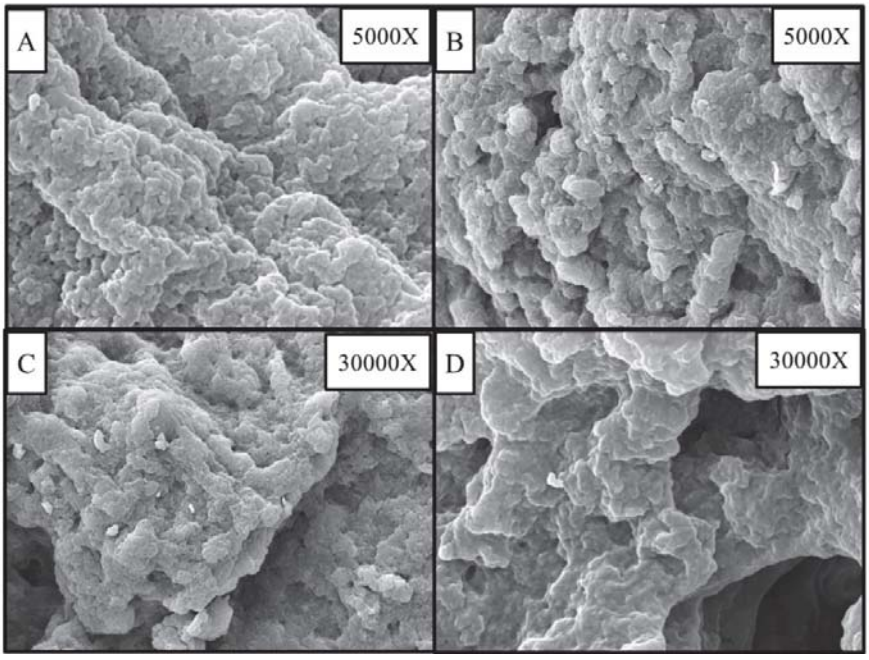

Figure 23 SEM images of MIP (A and C) and NIP (B and D) synthesis via atom transfer racial polymerization technique using 4-bromo thiophenol as initiator

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4.3 Investigation method for probe fabrication

4.3.1 Thermal/UV-polymerization

In this method MIP was attached to the gold nanoparticle layer on the optical fiber via thermal and UV-polymerization method. Thermal polymerization was done by batch synthesis before (in section 3.2.1) but the big important problem was to assemble the polymer on the SPR probe. So, UV-polymerization was used to polymerization the MIP on the SPR probe.

A mixture of 30 mg of atrazine, 2 mL of monomer and 1 mL of cross-linker were mixed under nitrogen gas for 5 min, then a 0.5 mL of initiator was added under nitrogen gas for 2 min. The mixture was heated on water bath (about 70 °C) for 10 min. The viscous liquid was formed, called pre-polymer.

The pre-polymer was dropped on the gold film surface on the SPR probe then irradiated under UV-light with wavelength of 350-400 nm for 1 hr. Then the white solid of polymer was formed on the SPR probe.

Figure 24 shows the SPR signals obtained from optical fibers attached with MIP for sensing of atrazine compared to those with NIP. In Figure 24, the sensing of the SPR probe with MIP for the atrazine is shown by the shift of the SPR wavelength from 517 (a red line) to 532 (a green line) nm in a 30 ppm of atrazine solution for 10 min by real time detection.

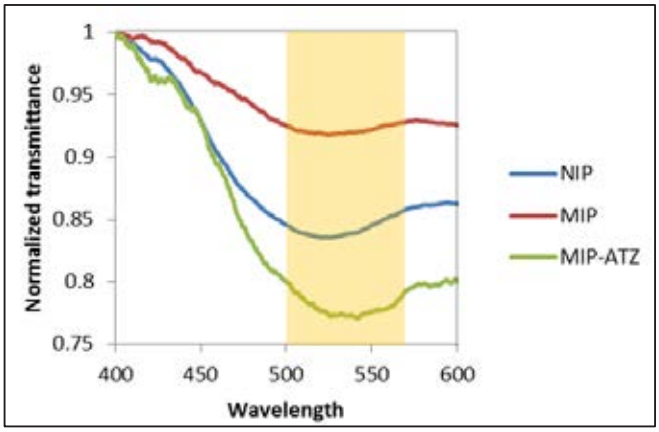

Figure 24 Optical fiber SPR with MIP for sensing of atrazine (10 min soaking time, 5 mL of 30 ppm atrazine standard)

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This method is not easy to control the mass of polymer on SPR probe resulting in poor reproducibility, so the Atom transfer racial polymerization was studies as alternative polymerization method.

4.3.2 Atom transfer racial polymerization

The polymer was insitu synthesized on SPR probe ATRP using 4-bromo thiophenol as initiator. The concentration of 4-bromo thiophenol was studied. A 4-bromo thiophenol initiator was dissolved on dimethyl sulfoxide with concentration to 4, 6, 8 and 10 mM. Then the initiator solution was dropped on the gold surface. The high concentration of initiator can dissolve the gold nanoparticle on SPR probe, so the concentration of initiator could affects SPR signal. The SPR characteristics obtained from the various concentration of initiator were shown in Figure 25. The results show that no SPR characteristic when using 8 and 10 mM of initiator. At the concentration of 4 mM, the SPR signal appeared at 576 mm that was the same wavelength obtained from the SPR probe with gold nanoparticle alone. At the concentration of 6 mM, the SPR signal was shifted to 581 nm indicating that the refractive index of the gold surface had changed. The concentration of initiator at 6 mM was chosen.

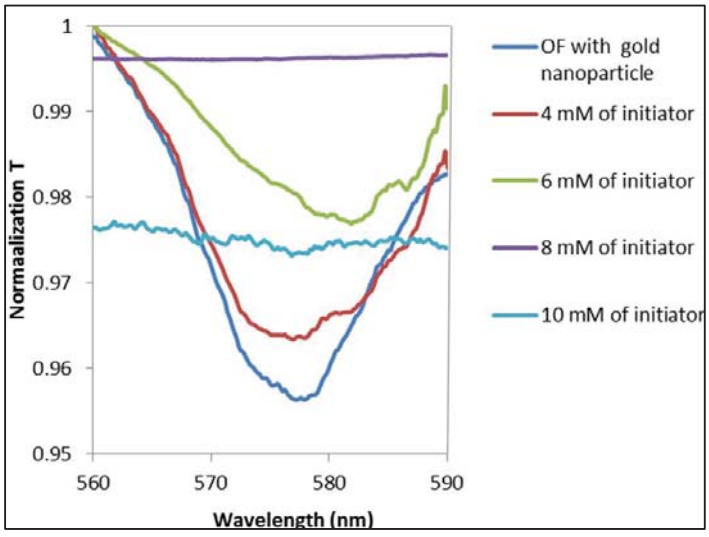

Figure 25 The SPR signal of the SPR probe using various concentration of initiator

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The MIP was insitu synthesized on the probe after treated with 6 mM of the initiator. The mixture contained methacrylic acid as a monomer, ethylene glycol dimethylacrylate (EGDMA) as a cross linker, Copper (II) chloride as a catalyst, N,N,N′,N′-Tetramethylethylenediamine as a ligand and atrazine as template. A 30 mg of atrazine, 315 μL of monomer, 2 mL of cross-linker were mixed under nitrogen gas for 5 min, then a 5 mL of acetonitrile was added and a solvent. The mixture was stirred for 5 min then 0.7 g Copper (II) chloride was added and stirred until the solid was dissolved. A green solution was obtained. Then a 20 μL of ligand was added. The probe was soaked on pre-polymer solution before incubated at 80 °C in an oven for 1 hr. The MIP was formed on SPR probe as shown in Figure 26. The atrazine template was removed before use.

Figure 26 MIP modified SPR probe via ATRP method

The reproducibility of probe fabrication can be controlled by incubation time. The probes after fabrication were weighed for determination of weight of polymer deposited on the probes. The amounts of polymer that deposited on the probe were shown in Table 1.

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Table 1 Mass of the polymer on MIP modified SPR probe via ATRP method

OF NO.	Mass before assemble with MIP (gram)	Mass after assemble with MIP (gram)	polymer mass (gram)
1	0.1023	0.1128	0.0105
2	0.1031	0.1132	0.0101
3	0.1043	0.1155	0.0112
4	0.1028	0.1123	0.0104
5	0.1035	0.1129	0.0094
6	0.1026	0.1121	0.0095
7	0.1044	0.1147	0.0103

The average of the amount of polymer that deposited on the probes was 0.0102 g, and %RSD was 0.060. This synthesis method showed that the mass of polymer deposited on SPR probe was controlled.

4.4 Quantitation analysis of atrazine

SPR probe with MIP prepared by ATRP technique was set up for the spectrometric determination as shown as in Figure 15. The probe was immersed in atrazine standard solution in various concentration 5, 10, 15, 20, 25 and 30 ppm for 1 hr. The probe was dried in room temperature prior to SPR measurement. The SPR signal of each concentration was shown in Figure 27.

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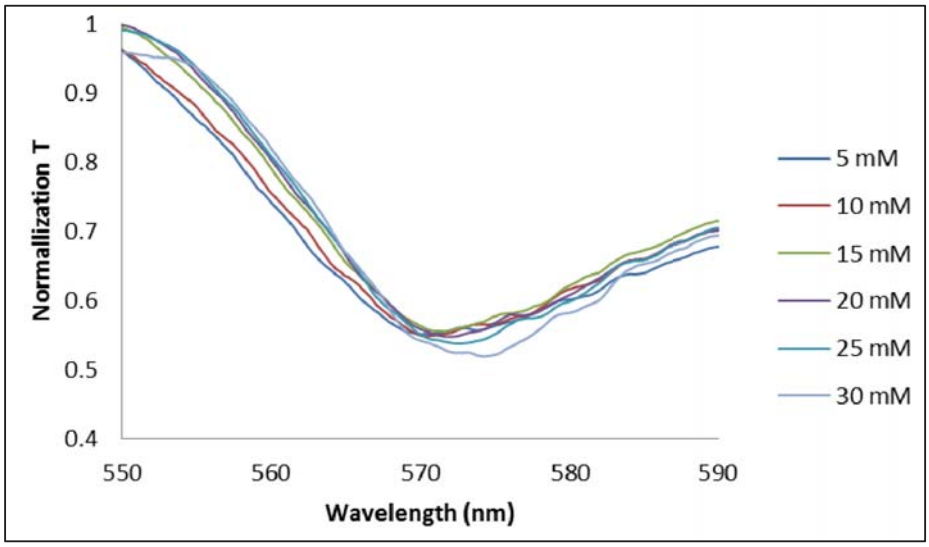

Figure 27 SPR signal at each concentration of atrazine standard using SPR probe modified with MIP via ATRP method

The results show that the red shift of SPR signal is occurred with increased concentration of atrazine. At zero concentration of atrazine the resonance wavelength is 569.27nm, the delta resonance wavelength of each concentration is shown in Table 2. The calibration curve of SPR signal plot between Δresonance wavelengths and concentrations of atrazine is shown in figure 28. The shift of resonance wavelength has linear relation with concentration of atrazine. The limit of detection of this calibration was calculated form Y_{DL} = 3S/slop; S is standard error of linear recreation line. The LOD of this calibration is 4.6 ppm.

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Table 2 Resonance wavelength of each concentration of atrazine obtained from SPR probe modified with MIP via ATRP method

concentration of atrazine (ppm)	Resonance wavelength (nm)	Δresonance wavelength (nm)
0	569.27	0
5	570.39	1.12
10	571.56	2.29
15	571.75	2.48
20	572.73	3.46
25	573.7	4.43
30	574.29	5.02

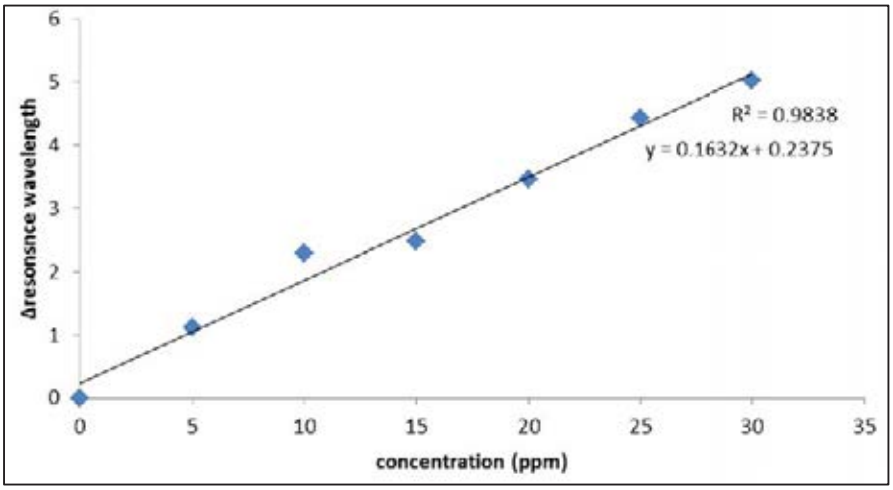

Figure 28 Calibration curve of atrazine obtained from SPR probe modified with MIP via ATRP method

The recovery of atrazine was studies. The amount 1, 2.5 and 3 mL of 30 ppm of atrazine standard were added in the real water samples from Nan province of Thailand. The sample was made up to 5 mL to obtain the concentration of atrazine of 8, 15 and 18 ppm. In Table 3 shows that the %recovery were in the range 88-104%. The probe shows high effective sensing of atrazine in real samples and with

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no the effect of matrix from the real samples. That % recovery was acceptable according to the association of official analytical chemists (AOAC) [19].

Table 3 %Recovery of atrazine in real samples obtained from SPR probe modified with MIP via ATRP method

Sample NO.	Spiked concentration (ppm)	Δresonance wavelength (nm)	Calculated concentration (ppm)	%Recovery
1	6	1.10	5.3	88
2	15	2.41	13.3	89
3	18	3.44	19.6	104

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CHAPTER V

CONCLUSSION AND SUGGESTION OF FUTUER WORK

5.1 Conclusion

The optical fiber based on surface plasmon resonance spectroscopy modified with molecularly imprinted polymer was developed for determination of atrazine in water samples. The gold nanoparticles was deposited on the uncladded optical fiber by self-assembly method. The concentration of gold (III) chloride trihydrate of 10 mM with 50-60 min immersion time was chosen.

Two polymer polymerization techniques were studies for synthesis of MIP that were thermal/UV-polymerization and ATRP method. The MIP was successfully synthesized and used to extract atrazine from water samples. The MIP was assembled on SPR probe. The SPR signal was obtained for sensing of atrazine by the shift of the resonance wavelength. However the polymerization processes were not easy to control in term of polymerization time and probe reproducibility.

Atom transfer radical polymerization technique was successfully used to synthesis of MIP in batch mode. SPR probe modified with MIP was successfully fabricated by using 4-bromo thiophenol as initiator, and could be used to sense atrazine in water sample. This technique gave the good reproducibility in probe fabrication. However, the sensitivity was not quite good because the initiator might not be active to generate the free radical. In addition, SPR probe modified with MIP was attempted for quantitative analysis of atrazine in water samples. The % recoveries of spiked atrazine standard in real water obtained from the SPR probe modified with MIP via ATRP method were in the range of 88-104% and the limit of detection was 4.6 ppm.

Both thermal/UV polymerization and ATRP method afforded advantage and disadvantage for modification of SPR probe with MIP. Thermal/UV-polymerization gave better sensitivity than ATRP technique, while ATRP technique was easy to synthesis than thermal/UV-polymerization.

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5.2 Suggestion in the future work

The sensitivity of SPR probe modified with MIP via ATRP method was not quite satisfactory. The initiator might not be suitable for this method, because it was less active to generate the free radical. The initiator containing to aliphatic group of bromine and thiol group may be considered. Moreover, in ATRP method, the processes required catalyst; it may have to be optimized to improve the synthesis process of MIP.

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APPENDIX

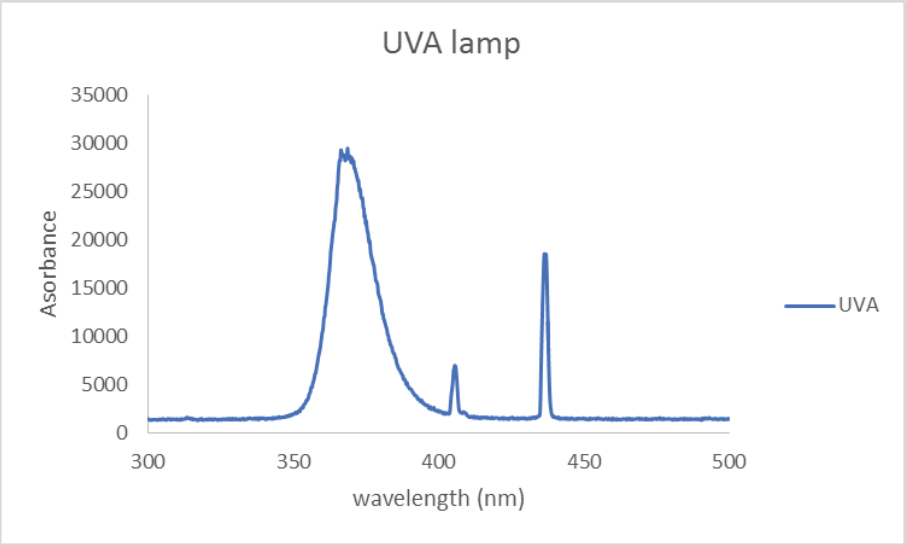

Figure 29 The wavelength of UVA lamp

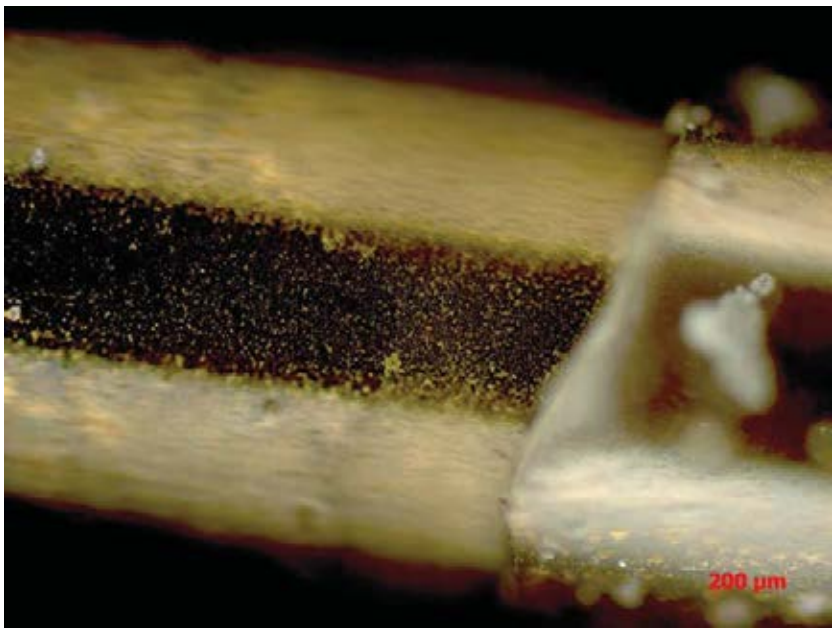

Figure 30 The image of SPR probe assembly 10 mM with gold nanoparticle for 60 min

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Table 4 The remaining absorbance of 30 mM atrazine after extracted with MIP and NIP synthesized via thermal polymerization method

time (min)	MIP 1	MIP 2	MIP 3	Average
5	0.095	0.072	0.17	0.112333
10	0.093	0.067	0.162	0.107333
15	0.09	0.065	0.116	0.090333
30	0.077	0.062	0.121	0.086667
60	0.052	0.06	0.069	0.060333
time (min)	NIP 1	NIP 2	NIP 3	Average
5	0.134	0.137	0.087	0.119333
10	0.128	0.135	0.096	0.119667
15	0.123	0.139	0.125	0.129
30	0.121	0.13	0.132	0.127667
60	0.11	0.136	0.129	0.125

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Table 5 The remaining absorbance of 30 mM atrazine after extracted with MIP and NIP synthesized via ATRP method

time (min)	MIP 1	MIP 2	MIP 3	Average
5.000	0.934	0.966	0.934	0.014
10.000	0.887	0.921	0.867	0.892
20.000	0.870	0.870	0.919	0.022
30.000	0.748	0.742	0.827	0.772
40.000	0.702	0.704	0.647	0.025
50.000	0.630	0.614	0.582	0.609
60.000	0.588	0.489	0.540	0.033
time (min)	NIP 1	NIP 2	NIP 3	Average
5.000	0.986	0.859	1.519	1.121
10.000	0.933	0.885	1.044	0.954
20.000	0.903	0.779	1.519	1.067
30.000	0.900	0.916	1.519	1.112
40.000	0.859	1.362	0.785	1.002
50.000	0.916	0.888	0.793	0.866
60.000	0.779	0.964	1.290	1.011

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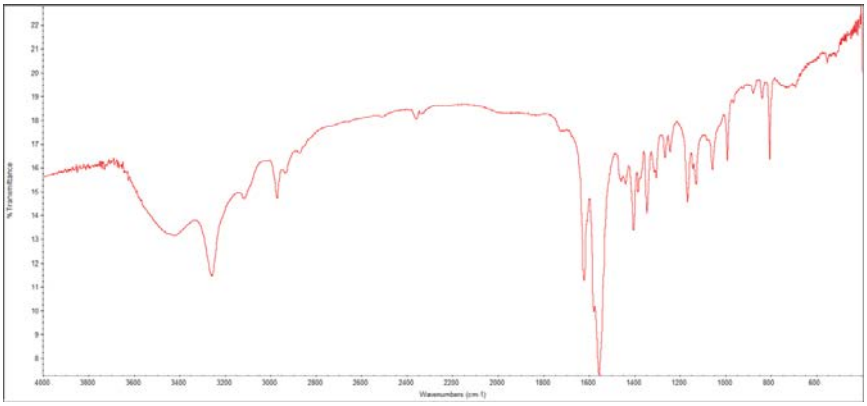

Figure 31 IR spectrum of atrazine

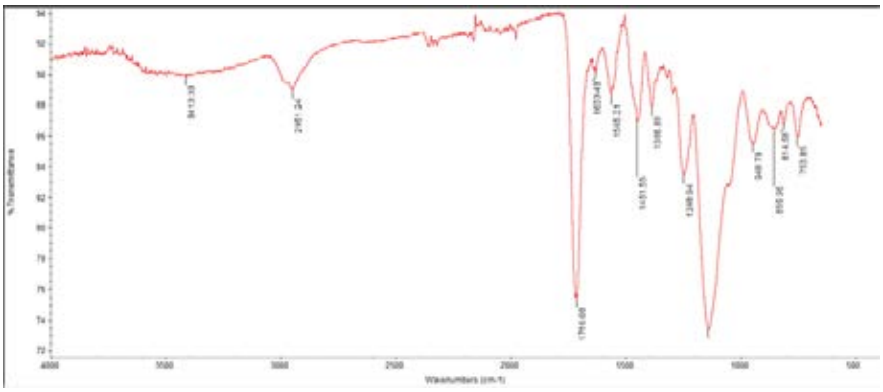

Figure 32 IR spectrum of MIP synthesized via thermal polymerization method

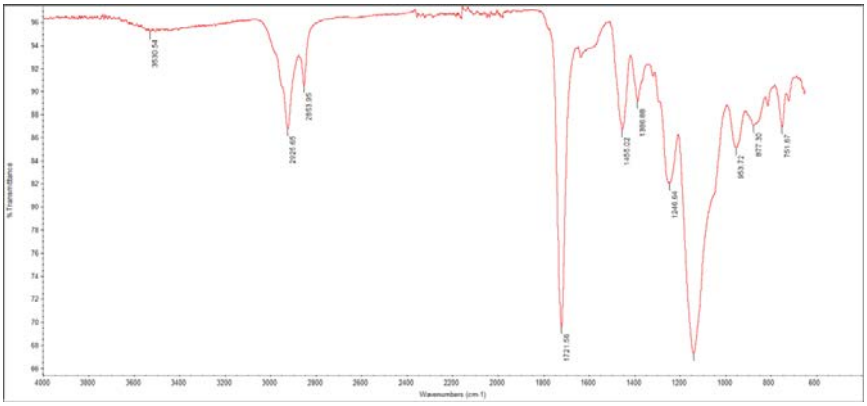

Figure 33 IR spectrum of MIP synthesized via ATRP method

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