



SYNTHESIS AND DETERMINATION OF DIAZONIUM COMPOUNDS FOR
MODIFICATION SILVER NANOPARTICLES IN ESTROGEN DETECTION



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A Thesis Submitted in Partial Fulfillment of the Requirements
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THE THESIS TITLED

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In humans, synthetic estrogens are used for medical purposes, such as hormone therapy for treatment of menopausal symptoms and the use of the combined oral contraceptive pill (COCP). However, long-term estrogen use may lead to serious health risks, such as ovarian cancer and breast cancer. Furthermore, contamination of estrogens are dangerous for fish because estrogens affect male fish. This study focused on the development of diazonium ligand as a colorimetric sensor in estradiol detections. In addition, computational chemistry was used to calculate the structures and energy of molecules and complexes. AgNPs were synthesized by reducing silver(I) nitrate solution with sodium borohydride. Diazonium salts of sulfanilamide (Azo1) and sulfanilic acid (Azo2) were synthesized by dissolved sulfanilamide or sulfanilic acid in nitric acid before adding sodium nitrite to the solutions. After that, Diazonium salt and AgNPs solutions were applied for estradiol detection. The structural optimization and binding energies were determined using the density functional (DFT) approaches. The B3LYP functionals with a mixed basis set were made up of 6-311G(d,p) for nonmetal atoms and 3-21G for silver atoms. The calculations consisted of structural optimizations of the Azo compounds linked to metal atoms.

Keyword : DFT, 17beta-Estradiol, Silver nanoparticles, Diazonium salt

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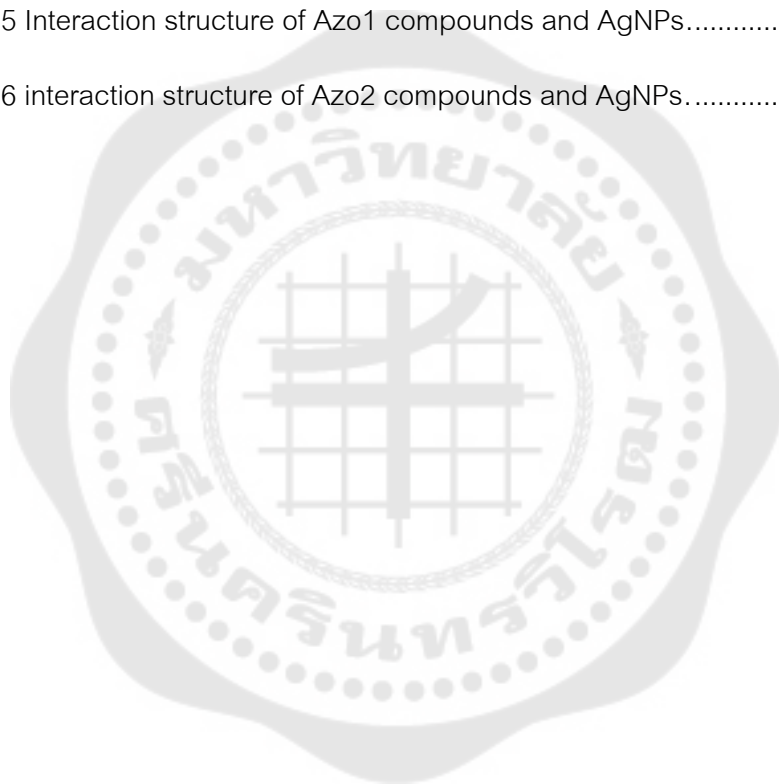
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CHAPTER 1

INTRODUCTION

1.1. Background

Hormone is biochemical agent, produced by endocrine system for signal to specific function of organism in human body. They have many types and targets for controlling metabolism system and human body growth. Estrogens are hormone produced by ovary and adrenal gland with the main function to regulation of female characteristic expression and stimulate body growth. In addition, they help balance of calcium, storage of lipid and having the function in metabolism system. There are three groups of estrogens including Estrone (E1), Estradiol (E2) and Estriol (E3) (Adeel, Song, Wang, Francis, & Yang, 2017). Estrogens have been used in medical and livestock because they protect and treat reproductive system disorder in female livestock. In human, all most estrogens used in medical applications such as hormone therapy for treat menopausal symptoms and combined oral contraceptive pill (COCP). However, estrogens used for long term can cause ovarian cancer and breast cancer. (Laurenson, Bloom, Page, & Sadrieh, 2014). The excess estrogens are eliminated from the body via urine and fecal that are possibly released into the waste water treatment system. However, there is no study to report about elimination of estrogens from wastewater. The contaminated estrogens in natural water are dangerous to fish population because estrogens effect to male fish regulation to express the female characterization. The result shown the decreasing in breeding of fish in ecology system. (Wang et al., 2018). In addition, effect on human consume polluted water and get too much concentrate of estrogens risk to diseased as mention above.

Nowadays, the analytical methods for estrogen detection are necessary for the high performance instrument and specialist to analyze data such as the high performance liquid chromatography (HPLC), gas chromatography (GC) and enzyme-linked immunosorbent assay (ELISA). (Fang et al., 2016). In recent years, sensors have been developed for analytical methods with high selectivity because the specific binding sites have the ability to recognize the template from a mixture of closely related

compounds. For example, in the detection of estrogen, molecularly imprinted polymer (MIP) grafted paper-based and modified gold nanoparticles were used to detect estrogens. The specific molecular recognition sites on the MIP identified the target material. (Xiao, Zhang, Wu, Han, & Zhang, 2017). In addition, AuNPs were modified by Aptamer, which were used for specific recognition of targets. (Pu, Huang, Sun, Xie, & Zhou, 2019)

Nanotechnology is the development of small material in nanometer scale. Nanomaterial property is very different from bulk material such as sensitivity of conductivity, fast chemical reaction, antibacterial activity and responsibility to light. Nanomaterials have two methods for development or synthesis such as top-down approach and bottom-up approach. Top-down approach is a process transforming bulk material to nanometer size by optical lithography process. Bottom-up approach involves to a synthesis of nanomaterial from molecule or metal ion by chemical reaction. (Gubala et al., 2018)

Silver nanoparticles (AgNPs) are the synthesized nanomaterial by reducing silver ion with a reducing agent or photochemical reaction. They have many unique physical and chemical properties. These include optical, electrical, and thermal, conductive, and biological properties. (Kim & Lee, 2018) They have been used for several applications, including as antibacterial agents, household products, healthcare-related products, medical device coatings, and cosmetics. One of property of AgNPs is changing optical responsibility following the change of size and chemical composites that are the advantage of AgNPs to develop optical sensor because they are easy to synthesis and detect by naked eye and photometry instrument. These detection techniques are based on the interaction of light and nanostructured materials. It was called "surface plasmon resonance (SPR)" phenomenon. In general, there are three distinct mechanisms that can trigger the SPR sensing of AgNPs including aggregation, oxidation and changing in the dimension/morphological features of nanostructures. (Amirjani & Haghshenas, 2018)

In addition, computational chemistry is used to calculate or simulate the property of complexes and the interaction between molecules helping to understand chemical mechanisms. It uses theoretical chemistry incorporated into efficient computer programs to assist in solving complex chemical problems. Today, the computational chemistry is useful in many fields of chemistry such as drug design for increasing selectivity and efficiency in medical, predicting path way or product in chemical reaction and calculating to characterization in physical and chemical properties. In silver nanoparticles, computer chemistry has been used for explaining the behavior of synthetic AgNP sensors relating to geometrical structures binding energies, and the thermodynamic property changes of complexation between sensors with analyte. (Keawwangchai, Morakot, & Wannoo, 2013)

This study focuses on the sensor development based on modified AgNPs as colorimetric sensor in 17β -estradiol detections. In addition, computational chemistry use to calculate structure and energy of complex to explain interaction between modified AgNPs and 17β -estradiol. It is expected that work will provide the new development for estrogen detections in environment.

1.2. Objective

1. To develop the ligand, metal and nanoparticle complexes for detection of 17β -estradiol based on silver nanoparticles
2. To apply the synthesized for ligand, metal and nanoparticle complexes detection of 17β -estradiol
3. To explain energy, structure and interaction of ligand, metal and nanoparticle complexes with 17β -estradiol by computation calculation

1.3. Importance of research

1. To synthesize ligand, metal and nanoparticle complexes with easy rapid experiment for development of stable silver nanoparticles.

2. To apply the synthesized ligand, metal and nanoparticle complexes to detection 17β -estradiol

3. To study energy, structure and interaction of ligand, metal and nanoparticle complexes by computation calculation.

1.4. Scope of research

This research focuses on development of ligand, metal and complexes for detection of 17β -estradiol. The modified AgNPs are characterized by spectroscopy techniques such as UV-Visible spectrophotometry (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR). The ligand, metal and complexes are tested with 17β -estradiol for detections. The accuracy of method is confirmed using UV-Visible spectrophotometry (UV-Vis). Then, this method will be applied for detection of 17β -estradiol. Computational chemistry was used to explain the behavior of synthesized ligand, metal and complexes relating to geometrical structures binding energies, and the thermodynamic property changes of complexation between sensors with analysts.

CHAPTER 2

LITERATURE REVIEWS

1. Estrogens and effect on environment
2. Nanoparticles
3. Diazonium salts
4. Computational chemistry
5. Literature reviews

2.1 Estrogens

Estrogen hormones are synthesized by ovary and adrenal gland in a type of lipid molecule called steroid form cholesterol in animal's body. Estrogens are the high molecular weight molecule and poorly dissolve in water. Role of estrogens are to regulate female characteristic, promote growth of body and control balance of metabolic system in body. Figure 1 shows two types of estrogen hormones including (Adeel et al., 2017). Natural estrogens hormone is synthesized from animal's body while synthetic estrogens are produced for medical to cure disorder of hormone systems.

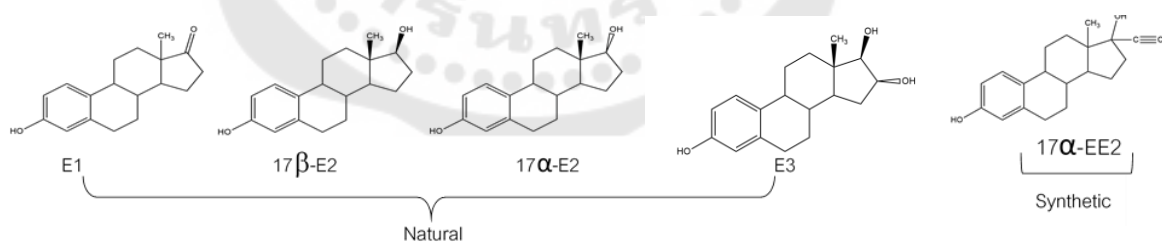


Figure 1 Chemical structure of natural and synthetic estrogenic compounds. Key: E1, estrone; 17β-E2, 17β-estradiol; 17α-E2, 17α-estradiol; E3, estriol; 17α-EE2, ethinyl estradiol.

Reference: Adeel M., Song X., Wang Y., Francis D., & Yang Y. (2017). Environmental impact of estrogens on human, animal and plant life: A critical review. *Environment International*. 99: 107–119.

2.1.1. Effect of Estrogens in Environment

Today, estrogen hormone is used in many fields such as agriculture, livestock and fishery for growth promoting, sex reversal inducing and animal disease prevention. Human use hormone in medical for treatment of diseases or birth control, but a large amount of receiving estrogen or long-term using of oral contraceptives increase the risk of blood clotting causing the breast, ovarian and endometrial cancer. (Filby et al., 2007)

In order to prevent the problems of excessive estrogen accumulation in humans and animals, the body releases excess hormones by urine and fecal that are possibly released into the waste water treatment system. However, there is no study to report about elimination of estrogens from wastewater. The contamination in natural water due to releasing of water from the water treatment system that cannot completely eliminate estrogens. Therefore, estrogens are absorbed into soil and seeped into the groundwater as shown in Figure 2. Groundwater is water resource to use in the house hold production and drinking water industry. In addition, researches were found that the use of natural fertilizers or manure in agricultural areas can bring estrogens to surface water and ground water as well. (Laurenson et al., 2014).



Figure 2 Schematic illustration of main sources of estrogen release to the environment.

Reference: Adeel M., Song X., Wang Y., Francis D., & Yang Y. (2017). Environmental impact of estrogens on human, animal and plant life: A critical review. *Environment International*. 99: 107–119.

Estrogen hormones that contaminated into the water source can cause the behavior of sexual organisms in decreasing and feminized male fish. All of the above results cause the extinction of fish in natural water sources as shown in Figure 3. Moreover, the decreasing fish population affect the ecosystem within the water source. Impacts of contaminated estrogen on endocrine system are becoming more concerned problems, although there is no clear study of the effects of estrogen on humans. However, the endocrine systems of fish, birds and humans are similar. It is absolutely necessary to study the long-term effects. (Wang et al., 2018)

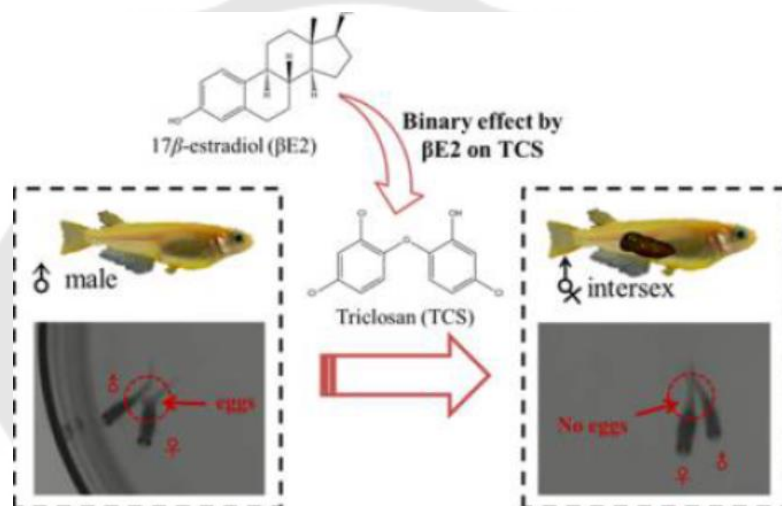


Figure 3 Effects of Triclosan and Binary Mixtures with 17β-Estradiol on Testicular Development and Reproduction in Japanese Medaka (*Oryzias latipes*)

Reference: Wang C., Li Y., Zheng G., Zhang S., Wan Y., & Hu J. (2018). Adverse Effects of Triclosan and Binary Mixtures with 17β-Estradiol on Testicular Development and Reproduction in Japanese Medaka (*Oryzias latipes*) at Environmentally Relevant Concentrations. *Environ. Sci. Technol. Lett.* 5: 136-141.

2.1.2. Analytical Techniques for Estrogens Determination

Environment concerns are interested over ultra-trace levels of steroid estrogens concentrations in water samples. They are increased because of their adverse effects

on human and animal life. Special attention to the analytical techniques uses to quantify steroid estrogens in water samples with increasingly important in the present. The analytical techniques can be either instrumental or non-instrumental techniques. Instrumental analytical techniques including Liquid Chromatography Mass Spectrometry (LC-MS) and Gas Chromatography Mass Spectrometry (GC-MS), which were developed for both separation and difference of steroid estrogens. The LC-MS separation was contained out by chemically bonded mobile phase working together with stationary phases. The GC-MS separation was used by controlling temperatures varying between 45 and 300 °C, using helium as carrier gas, and a variety of capillary columns. Non-instrumental analytical techniques are categorized into immunoassays and bioassays. Bioassays affected both in vivo and in vitro assay that based on the fact binding of free estrogens to estrogen receptors and result in response but immunoassays are techniques based on the reaction with biologically derived reagent antibodies and are thus physical assays based on the laws of mass action. (Fang et al., 2016)

2.2. Nanoparticles for Sensor

In these days, nanotechnology is a great popular researches topic in many fields and other inter disciplinary field. They studied about small materials in nanometer size because of the properties relating to optical, electrical and chemical properties are very different from bulk materials. Commercial materials include homopolymers, self-assembled organic molecules, copolymers or biopolymers, nanocarbon materials, inorganic oxides of silica or titanium dioxide, quantum dots, metals, metalloids, and constructs consisting of several of these materials. The various materials are designed to expose desire properties, such as specific band gap energies, super-paramagnetism, size-tunable emission, textile protection, biocompatibility, antibacterial activity, and biodegradability. Typically, the composition, size, pore size, and surface functionalization of the engineered nanomaterials are the main factors that govern their suitability for a particular application. (Gubala et al., 2018)

This study focuses on silver nanoparticles (AgNPs) as the noble metal nanoparticle and particles between 1 to 100 nanometers in size. Their extremely small

size, large surface area, variety structure and chemical surface modifying lead to many novel applications. Important applications of AgNPs include catalysis, chemical sensor, antibacterial. One of interesting property of AgNPs is optical property call “Surface plasmon resonances (SPR)”. It is an optical phenomenon that will occur when light from outside shined through the very small object, causing the wave interaction. Electromagnetism and electrons in the surface of the particle cause the particle to vibrate as a whole. Resulting in two interactions include scattering and absorption will cause color nanoparticles in range of visible wavelengths. The SPR character depends on the particle size, shape and chemical composition. (Kim & Lee, 2018)

2.2.1. Synthesis of Silver Nanoparticles

There are mainly 3 methods for the synthesis of silver nanoparticles including (Beyene, Werkneh, Bezabh, & Ambaye, 2017).

1. **Physical methods** are the synthesis that uses external energy to change silver ions in the solution to become silver nanoparticles. Advantages of these method are appearance of silver nanoparticles by controlling the energy of the laser. They are also methods that does not require chemicals. But disadvantages of these methods are complex methods, using expensive tools and high energy in the synthesis.

2. **Chemical methods** are synthesis by using the organic or inorganic compounds in reduction reaction to change silver ion (Ag^+) to silver atom (Ag^0). These methods can increase the stability of AgNPs by adding the stabilizing agents into reaction. Advantages of these method includes the rapidly synthesized, easily control condition. The synthesized AgNPs have small particles size and usually dispersion. From above, chemical methods are the most popular method for the synthesis of AgNPs method.

3. **Green synthesis** or bio-base methods are synthesized by using the reducing agent from biological mass such as plants, algae, bacteria and fungi. They can be transformed the inorganic metal ions into metal nanoparticles via the reductive capacities of the proteins and metabolites present in these organisms.

2.2.2. Silver Nanoparticles Sensor

In the recent decades, new techniques are based on the unique properties of nanostructured materials that have determined and improved for the facile, sensitive, selective and low cost detection of different analytes. A survey of previous literature presents that AgNPs based sensors have been developed for the determination of various biochemical and chemical analytes. These determination techniques are the interaction of light with nanostructured materials on the surface plasmon resonance (SPR) phenomenon. In the presence of an analyte, there are three different mechanisms that can activate the SPR sensing of AgNPs such as changing the dimension/morphological properties of nanostructures, aggregation of nanoparticles and oxidation of nanoparticles. (Amirjani & Haghshenas, 2018)

The roles of ligands increase the selectivity of the AgNPs sensors towards the different heavy metal ions. For example, citrate capped AgNPs selectively detect Cr^{3+} while melamine-functionalized AgNPs showed high selectivity to Hg^{2+} (Amirjani & Haghshenas, 2018). Moreover, the biofunctionalized silver nanoparticles (triazole-carboxyl Ag-NPs) are synthesized through a click reaction having a cooperative effect on recognition of Co^{2+} (Yao, Tian, & Li, 2010). The conjugated polyelectrolyte (CPE) stabilized silver nanoparticles was used to detect the melamine. From the above research, the changing ligands increases the ability to analyze both organic and inorganic compounds.

2.3. Diazonium salt

The sensor development requires substances to interact with the targets. It generates signals for measurements such as electrical changes in light signals. In previous researches, there were found specific coupling reaction between phenolic estrogens and azo dye such as diazonium salt. Diazonium compounds or diazonium salts are a group of organic compounds sharing a common functional group $\text{R}-\text{N}^+\text{X}^-$ where R can be any organic group, such as an aryl or an alkyl, and X is an organic anion or inorganic, such as a halogen. Diazonium salts are usually prepared by the diazotization reaction of primary amines with nitrous acid as shown in Figure 4. The

aliphatic diazonium salts exist only as passing intermediates, rapidly decomposing into a nitrogen molecule and a carbonium ion, however, the certain aromatic diazonium salts are stable enough to be isolated, but they react promptly either by loss of nitrogen or by formation of azo compounds.

Diazonium salts were first found from aromatic amines in 1858, and their utilities in the preparation of azo compounds were widely used in the dye industry. By varying the chemical structures of the amines, they are diazotized (the diazo components) which they react with the coupling components. The colors throughout the visible spectrum are exposed to dyes applicable with varieties of fibers by several techniques. (Vaiano, Mari, Busardo, & Bertol, 2014)

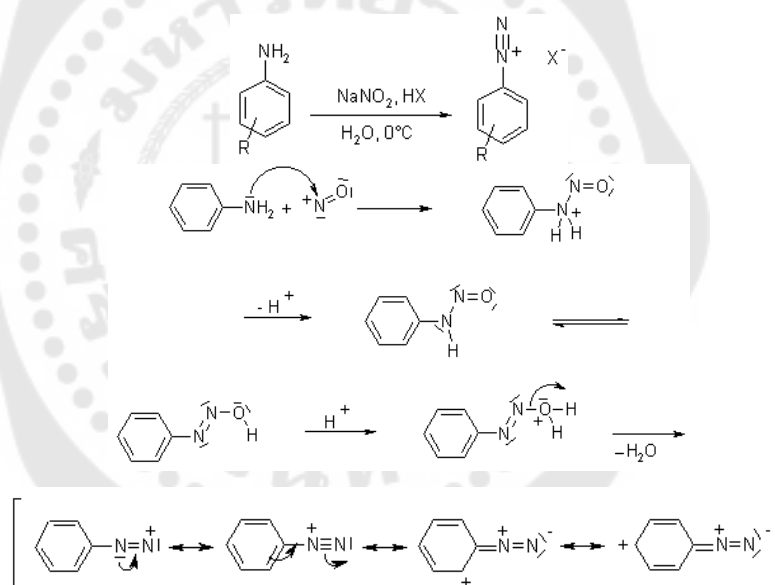


Figure 4 Mechanisms for the diazotization

Reference: Vaiano F., Mari F., Busardo P. F., & Bertol E. (2014).

Enhancing the sensitivity of the LC-MS/MS detection of propofol in urine and blood by azo-coupling derivatization. *Anal Bioanal Chem.* 406: 3579–3587.

2.4. Computational chemistry

Computational chemistry or calculation chemistry is branch of chemistry that study by using the computer calculation and stimulation to understand about energy in

mimic condition or angle, bond length of complexes structures. It is helpful to predict the opportunity in real reaction. However, the results from calculated method will be difference from real test. Therefore, it is used as basic information for planning the experiment, increasing the efficiency or reducing the parts that are not necessary for the experiment. The main types of computer chemical research are as follows (Goh, Hodas, & Vishnu, 2017)

1. Computer simulation is a study of the structure that changes with time, often used to calculate the reaction of substances or deformation of the biological molecules.

2. Energy calculation usually studies and optimizes the structure. In this form of calculation, it calculates the energy level in orbital level with the high-resolution calculation. As a result, it takes a long time for analysis. Therefore, it is suitable for small molecules with not much atom calculation.

3. Molecular design is one of the very popular studies in molecular design of medicine. It is used to simulate the structure of the drug and calculates the energy values with the target of protein structure in the drug to analyze the effectiveness of the drug.

The computer chemistry has been used in research on silver nanoparticles for explaining the behavior of synthetic AgNPs sensors about binding energies, geometrical structures and the thermodynamic property changes of complexation between sensors with analytes. Moreover, Density functional theory is calculated for interpretation of the Surface-enhanced Raman scattering (SERS) data based on model systems constituted of molecules bound to structure of nanoparticles with a few metal atoms. They are able to simulate the active sites present on the surface of the colloidal nanoparticles (Muniz-Miranda, Pedone, & Muniz-Miranda, 2018). However, there is not much type of research that the additional studies are needed in order to find an efficient method for future research.

In 2017, Tasawan Keawwangchai and group (Keawwangchai et al., 2013) focused on the synthesis and design of BODIPY-based fluorescence sensors for metal

ions. Target fluorescence sensors were modified to carry BODIPY moiety as fluorescence signaling subunit. The 4-hydroxyphenyl (L1) and 3,4-dihydroxyphenyl (L2) groups at BODIPY meso-position were developed for the metal ion binding subunit. The binding properties of the complexation between metal ions and sensors were examined by fluorescent and UV-vis spectrophotometry. Computational chemistry was used to give information for explaining about the behavior of synthetic fluorescent sensors and the complexation of metal ions. The computational calculation included binding energies, geometrical structures and the thermodynamic property changes of complexation between sensors and metal ions were calculated using the density functional theory (DFT) method as shown in figure 5.

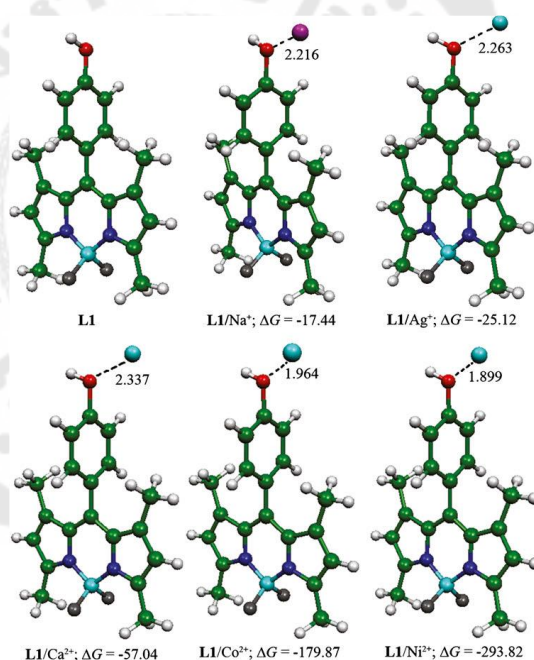


Figure 5 The B3LYP/LanL2DZ optimized structures of sensor L1 and its metal ion complexes. The presented bond distances and binding free energies are in Å and kcal/mol, respectively

Reference: Han X. X., Pienpinijtham K., Zhoa B. & Ozaki Y. (2011). Coupling Reaction-Based Ultrasensitive Detection of Phenolic Estrogens Using Surface-Enhanced Resonance Raman Scattering. *Anal. Chem.* 83: 8582–8588.

2.5. Literature reviews

In 2011, Xiao Xia Han and group (Han, Pienpinijtham, Zhao, & Ozaki, 2011) studied about detection of estrogen by using silver nanoparticles modified with diazonium derivative compound of sulfanilic acid and then measured with Surface-enhanced Raman scattering (SERS) technique. In this study, sulfanilic acid was used to make diazonium salt compound because coupling reaction between diazonium salt and phenolic group of estrogen has been proven to have strong raman activity and a propensity toward binding to AgNPs as shown in figure 5. The proposed protocol has multiplexing ability, because each SERRS signal of the azo dyes specifically represents to the related estrogen. Furthermore, it is comprehensive and great selective, not only for phenolic estrogens but also for other phenolic molecules, even in complex systems.

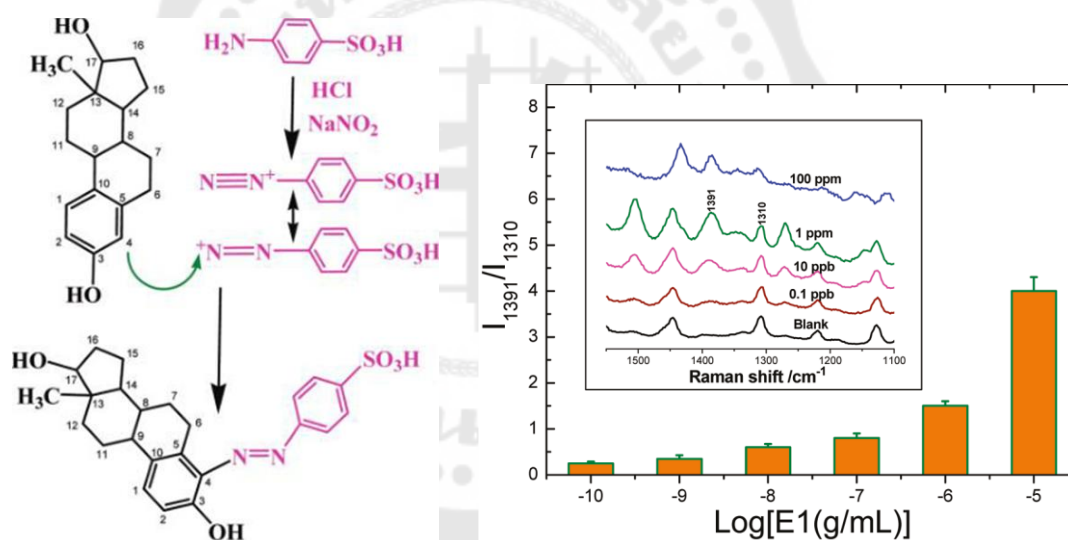


Figure 6 Coupling reaction between estradiol and diazonium ions (Left). E1 concentration-dependent SERRS spectra of the corresponding azo dyes and the intensity ratio of two SERRS bands versus the estrogen concentration (Right).

Reference: Han X. X., Pienpinijtham K., Zhao B. & Ozaki Y. (2011). Coupling Reaction-Based Ultrasensitive Detection of Phenolic Estrogens Using Surface-Enhanced Resonance Raman Scattering. *Anal. Chem.* 83: 8582–8588.

In 2017, Ying Ma and group (Ma, You, Promthaveepong, & Li, 2017) used diazonium salt compound with gold nanoparticles was used in the analysis of Bisphenol A, a substance that has the same effect on the endocrine system as estrogen. This research was used for analysis at low concentrations of Bisphenol A. In this study, the 4, 4-dithiodibenzylamine (DTDBA) is the substrate for diazotization reaction with changing to 4, 4-dithiodibenzylidiazonium (DTDBD). It was complexed with gold nanoparticles and binding to Bisphenol A which has been found to change the color of the solution from dark red to gradually fade as the Bisphenol A concentration increases as shown in figure 6. The measurement with UV-VIS spectroscopy technique observed the significantly change by decrease absorbance at 540 nm and increase absorbance at 750 nm. From this data, they generate the standard curve by use ratio of absorbance at 540 nm and 750 nm. that the gold nanoparticles modified with 4, 4-dithiodibenzylidiazonium were detection of Bisphenol A with a fast response in 4 min as shown in figure 7, a broad linear range at 0.1–4 nM and a low limit of detection at 0.02 nM.

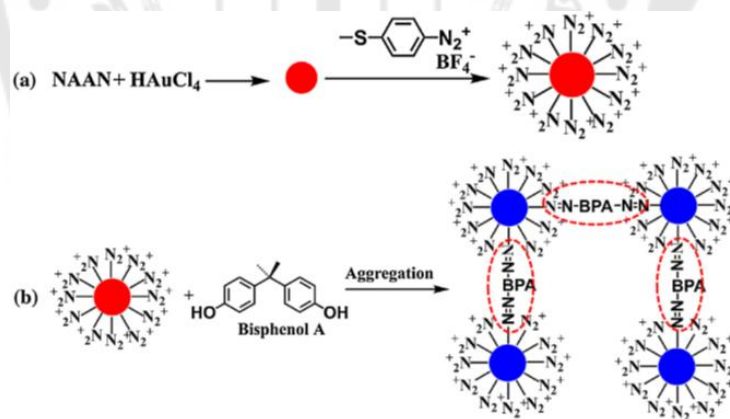


Figure 7 Schematic demonstration of (a) AuNP synthesis and their surface ligand exchange with DTDBD and (b) the BPA-induced AuNP aggregation.

Reference: Ma Y. , You F. , Promthaveepong K. , & Nan L. (2017) .
 Ultrasensitive Bisphenol A sensing based on responsive plasmonic nanoparticles.
 Sensors and Actuators B. 245: 369–374.

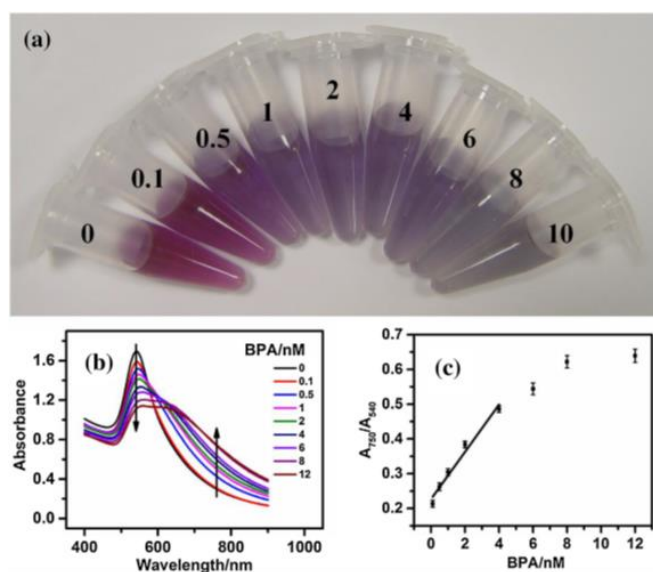


Figure 8 (a) Photographs and (b) UV absorption spectra of AuNP solutions after incubation with different concentrations of BPA. (c) AuNP absorbance ratio (A_{750}/A_{540}) vs. BPA concentrations.

Reference: Ma Y. , You F. , Promthaveepong K. , & Nan L. (2017) .
 Ultrasensitive Bisphenol A sensing based on responsive plasmonic nanoparticles.
 Sensors and Actuators B. 245: 369–374.

In 2019, Hongbin Pu and group (Pu et al., 2019) studied to develop colorimetric sensor for detection 17β -estradiol (E2) based on gold nanoparticles (AuNPs). This research was used shortening DNA to modify gold nanoparticles and measured the visible color change of solution for E2 detection. Shortening DNA or Aptamer are in vitro selected single-stranded oligonucleotides, which were applied for specific recognition of different targets, such as cells and small molecules. Unlike antibodies, aptamers are highly specific, stable, low-cost, and desirably biocompatible which promotes the development of aptamer-based detection technique. The principle of this technique was the addition of NaCl to aggregation of AuNPs. While the shortening DNA aptamers prevent AuNPs from aggregation by modifying on the surface of AuNPs through electrostatic adsorption. After that, E2 was added into the solution, DNA aptamers

specifically bind to E2 by non-covalent bonds and become enveloped, resulting in the reduction of their affinity to AuNPs. Although DNA aptamers were decreased absorption from the AuNP surface. They cannot prevent the AuNPs, leading to the aggregation of AuNPs. The experiment showed excellent performance for E2 determination with the absorbance ratio at 620 and 523 nm and great linearity between E2 concentrations from 0.2 to 5 nM, with the limit of detection of 0.1 nM. This technique exhibited great potential for on-site fast determination of E2 in environmental water samples.

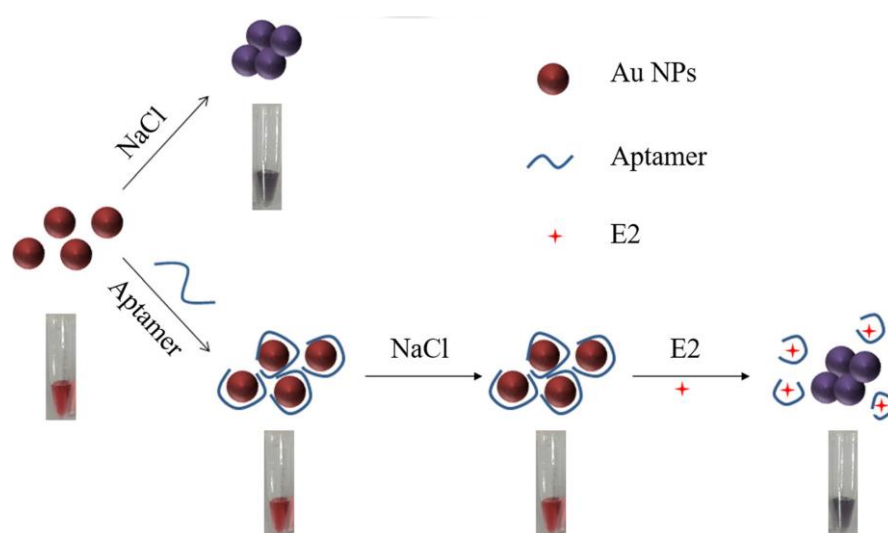


Figure 9 Schematic of the gold nanoparticle–based colorimetric approach for 17 β -estradiol detection using shortening DNA aptamers

Reference: Pu H., Huang Z., Sun D. W., Xie X., & Zhou W. (2019). Development of a Highly Sensitive Colorimetric Method for Detecting 17 β -Estradiol Based on Combination of Gold Nanoparticles and Shortening DNA Aptamers. *Water Air Soil Pollut.* 230(124). 1-9.

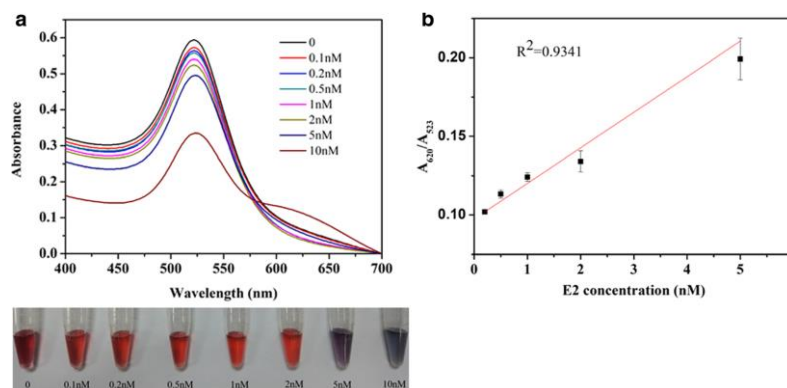


Figure 10 UV-Vis absorption spectra and images of AuNPs under the presence of various concentrations of E2. Linear calibration curve of $A_{620\text{nm}}/A_{523\text{nm}}$ against E2 concentrations from 0.2 to 5 nM.

Reference: Pu H., Huang Z., Sun D. W., Xie X., & Zhou W. (2019).

Development of a Highly Sensitive Colorimetric Method for Detecting 17β -Estradiol Based on Combination of Gold Nanoparticles and Shortening DNA Aptamers. *Water Air Soil Pollut.* 230(124). 1-9.

In 2017, Paulo A. Raymundo-Pereira and group (Raymundo-Pereira et al., 2017) studied combination of silver nanoparticles with carbon nanoballs (CNB) to improve electrochemical sensors for determining the estrogenic hormone estriol ($(16\alpha,17\beta)$ -estra-1,3,5(10)-triene-3,16,17-triol). The synergetic combination between these two nanomaterials with large surface areas developed electrocatalytic activity and led to great features for estriol sensing as shown in figure 10. The use of metallic nanostructures such as silver nanoparticles also decreases adsorption of electrooxidation estriol products on the sensor platform. The CNBAgNP/GCE sensor was then examined for estriol detection, and exhibited the excellent performance in terms of sensitivity and detection limit ($0.131 \mu\text{Mol/L}$ and $0.16 \mu\text{mol/L}$).

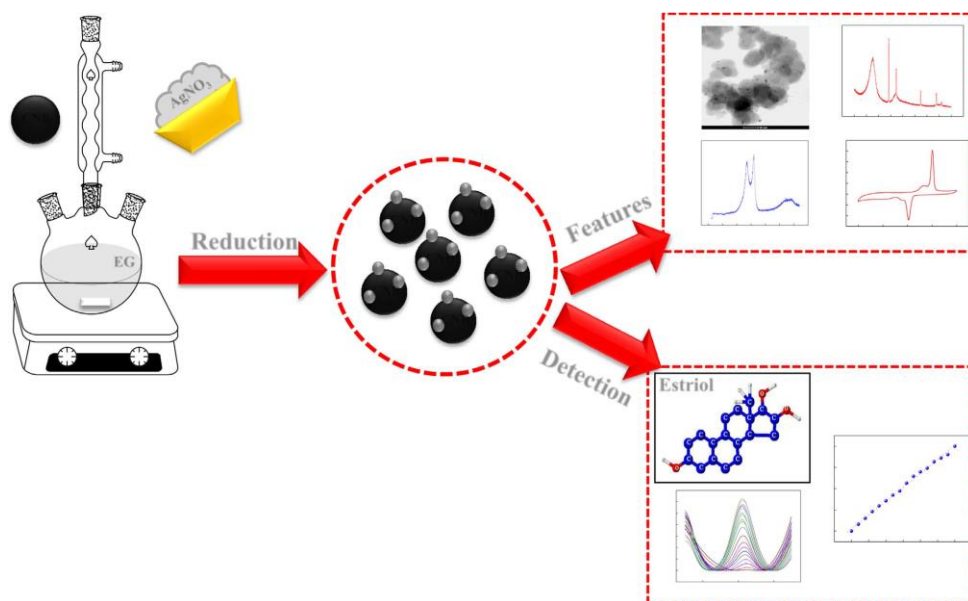


Figure 11 Schematic diagram of different steps for preparing the sensor platform to detect estriol.

Reference: Paulo A. R., Anderson M. C., Fernando P. V., Bruno C. J., Camila D. M., Leonardo N. F.; et al. (2017). Sensitive detection of estriol hormone in creek water using a sensor platform based on carbon black and silver nanoparticles. *Talanta*. 174. 652–659.

In 2017, Lei Xiao and group (Xiao et al., 2017) studied molecularly imprinted polymer grafted paper-based for detection of 17 β -estradiol. Molecularly imprinted polymers (MIPs) have been improved very quickly as simulated antibodies in recent years. MIPs recognized targets with high selectivity because its specific binding sites had the potentiality to recognize the template from a mixture of closely related compounds. 17 β -estradiol were detected qualitatively in the sample that 17 β -estradiol - horseradish peroxidase (HRP) in grafted MIP layers on the paper was applied to conjugate with 17 β -estradiol in sample solution. This method had the advantages of

easy and rapid operation, high sensitivity and selectivity, and widely used for the screening of positive samples.

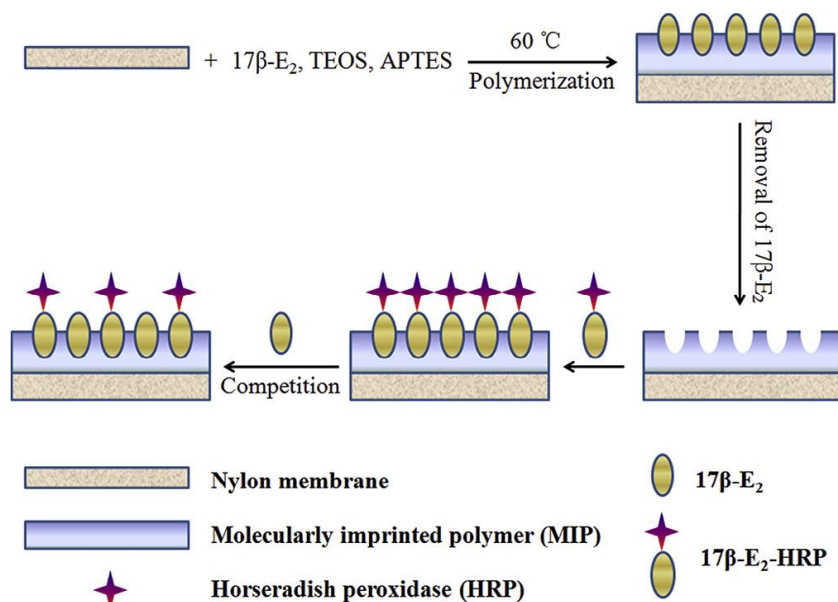


Figure 12 Schematic view of the polymerization procedure

Reference: Xiao L., Zhang Z., Wu C., Han L. & Zhang H. (2017). Molecularly imprinted polymer grafted paper-based method for the detection of 17β -estradiol. *Food Chemistry*. 221: 82–86.

In 2014, Xiaoyan Zhang and group (Zhang et al., 2014) studied molecularly imprinted electrochemical sensor for the rapid determination of estradiol. Glassy carbon electrode (GCE) was modified by molecular imprinted polymer (MIP) and gold nanoparticles (AuNPs). AuNPs were electrodeposited on the surface of GCE and used to enhance the electrode surface area for amplifying the sensor signal. Then, AuNPs was p-Aminothiophenol (ATP) combined with through gold and sulfur bonds that the high affinity and selectivity of MIP has been worked as a biological and chemical sensing element of the sensor. It is widely used for determination of estrogen because of the chemically and mechanically stable, easy to prepare and low cost. The

electrochemical sensor performed a linear detection range from 1.0×10^{-7} to 1.0×10^{-12} mg/mL, and a detection limit of 1.28×10^{-12} mg/mL.

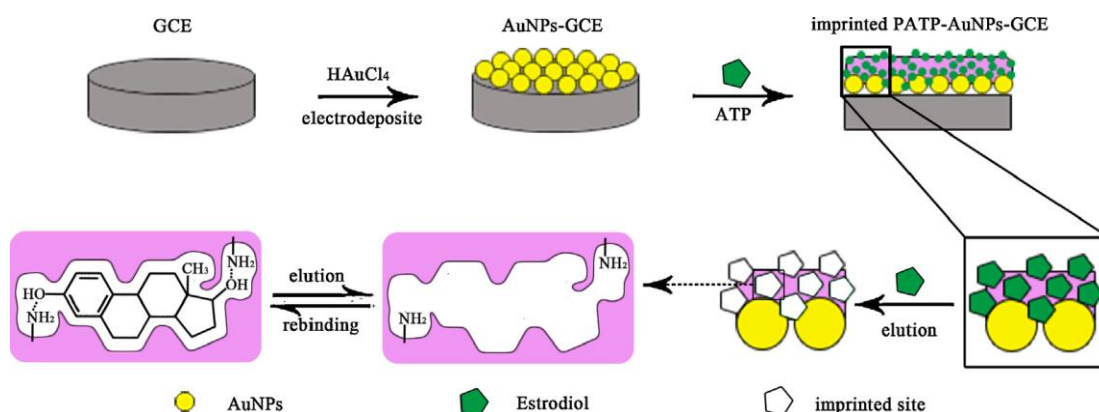


Figure 13 Schematic illustrations of the fabrication procedure for the MIPs-AuNPs-GCE.

Reference: Zhang X., Peng Y., Bai J., Ning B., Sun S., Hong X.; et al. (2014).

A novel electrochemical sensor based on electropolymerized molecularly imprinted polymer and gold nanomaterials amplification for estradiol detection. *Sensors and Actuators B*. 200: 69–75.

In 2013, Aysem Uzer and group (Uzer, Can, Akin, Ercag, & Apak, 2014) studied sensitive colorimetric method for octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Colorimetric method for the determination of HMX and RDX was proposed on the basis of differential kinetics in the hydrolysis of the two compounds. For example, the gold nanoparticles (AuNPs) were modified by 4-aminothiophenol (4-ATP) and naphthyl-ethylene diamine (NED) are as coupling agent for azo-dye formation. In this result, LOD of HMX and RDX were $0.24 \mu\text{g mL}^{-1}$ and $0.20 \mu\text{g mL}^{-1}$, respectively. This type of screening was greatly reduced analysis costs to monitor the nitro explosives-contaminated land remediation.

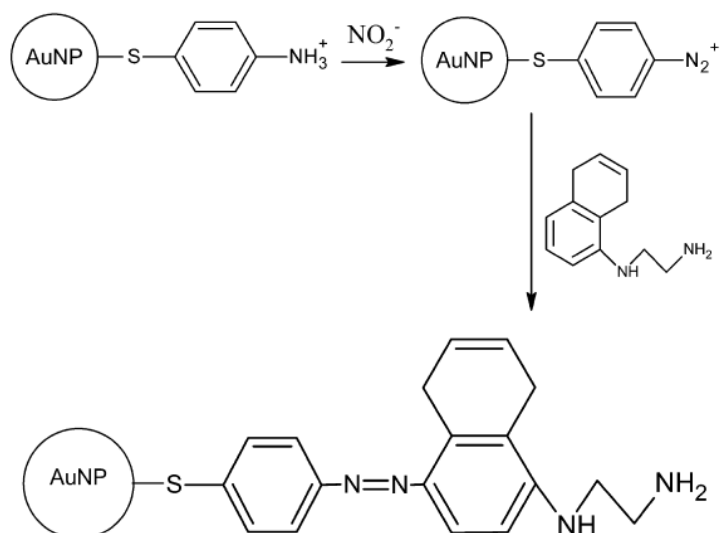


Figure 14 Schematic presentation of NO₂⁻ (produced from alkaline hydrolysis of RDX or HMX) determination with 4-ATP modified gold nanoparticles and NED coupling agent.

Reference: Aysem U., Ziya C., Ilknur A., Erol E., & Resat A. (2014). 4-Aminothiophenol Functionalized Gold Nanoparticle-Based Colorimetric Sensor for the Determination of Nitramine Energetic Materials. *Anal. Chem.* 86. 351-356.

CHAPTER 3

RESEARCH METHADODOLOGY

In this research have orders of experiment as follows

1. To synthesize ligand, metal and nanoparticle complexes with easy rapid experiment for development of stable silver nanoparticles.
2. To apply the synthesized ligand, metal and nanoparticle complexes for detection of 17 β -estradiol
3. To explain energy, structure and interaction of ligand, metal and nanoparticle complexes by computation calculation

3.1. Instrumental and chemical material

3.1.1. Instrumental

- UV-vis spectrophotometer (Jasco V-750 spectrophotometer)
- Fourier transform infrared spectrometer (FT-IR)
- Analytical balance (PX224 OHAUS)
- pH meter (MP220 METTLER TOLEDO)
- Micropipette (WITOPET WITEG)
- Magnetic stirrer (MGS-1001 Harmony)

3.1.2. Chemical material

- Silver(I) nitrate (AgNO₃) (Carlo Erba)
- Sulfanilamide (C₆H₈N₂O₂S)
- sulfanilic acid (C₆H₇NO₂S)
- Sodium borohydride (NaBH₄) (ACROS)
- Concentrated Nitric acid (HNO₃) (Carlo Erba)
- Sodium hydroxide (NaOH) (Carlo Erba)
- Acetonitrile (C₂H₃N) (Carlo Erba)

- Sodium nitrite (Na_2NO_2) (Carlo Erba)
- Sodium carbonate (NaCO_3) (Carlo Erba)
- Sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) (Carlo Erba)
- citric acid ($\text{C}_6\text{H}_8\text{O}_7$) (Carlo Erba)
- 17β -estradiol ($\text{C}_{18}\text{H}_{24}\text{O}_2$) (TCI)

3.2. Research method

3.2.1. Synthesis and Characterization of diazonium compounds, AgNPs and diazo-AgNPs

3.2.1.1. Synthesis of silver nanoparticles (AgNPs)

Silver(I) nitrate 0.01 M was prepared by dissolved 0.0849 g of silver(I) nitrate in 50 mL deionized water. Silver(I) solution was diluted to 0.1 mM by pipetted 10 mL of solution to 100 mL volumetric flask and adjusted with deionized water. Then, 0.1 M of sodium borohydride was prepared by dissolved 0.1891 g of sodium borohydride in 50 ml deionized water. The 5 mL of 0.1 M sodium borohydride was added to silver(I) nitrate solution and stirred at room temperature for 4 hours at 0°C until solution changed from no color to yellow color. The UV-Vis spectrophotometer was used to obtain the absorption spectra of the prepared solution. (Adapted from Mehr, Khanjani, & Vatani, 2015)

3.2.1.2. Synthesis of 4-Aminosulfonylbenzenediazonium (Azo1)

The 0.1 M of Sulfanilamide solution was prepared by adding 0.4305 g of sulfanilamide in 100 ml of 0.5 M HNO_3 . Sodium nitrite 0.1725 g was added into sulfanilamide solution. Mixture was stirred at room temperature for 1 hours until the solution color was changed from no color to orange. UV-vis spectrometer and Fourier transform infrared spectrometer were used to characterize the diazo ligand. (Adapted from Khan et al., 2019)

3.2.1.3. Synthesis of 4-sulfobenzenediazonium (Azo2)

The 0.1 M of sulfanilic acid solution was prepared by adding 0.4330 g of sulfanilamide in 100 ml of 0.5 M HNO_3 . Sodium nitrite 0.1725 g was added into sulfanilamide solution. Mixture was stirred at room temperature for 1 hours until the solution color was changed from no color to orange. UV-vis spectrometer and Fourier transform infrared spectrometer were used to characterize the diazo ligand.

3.2.1.4. Effects of pH on diazonium compounds

Nitric acid 0.5 M was prepared by dissolved 3.30 mL of 65 % nitric acid in 100 mL of deionized water. Then, sodium hydroxide 0.5 M was prepared by dissolved 2 g of sodium hydroxide in 100 mL of deionized water. The 3 mL of both diazonium solution were added in to small vials and adjust pH 1-10 with solution 0.5 M nitric acid or 0.5 M sodium hydroxide. The UV-Vis spectrophotometer was used to obtain the absorption spectra of the prepared solution.

3.2.1.5. Interaction between silver nanoparticles and diazonium compounds

The titration of silver nanoparticles and Azo1 or Azo2 were measured that the prepared AgNP 0.1mM solution 2 mL was pipetted to small vial, then the prepared Azo1 or Azo2 solution 1mM was titrated to AgNPs by adding solution between 50-500 μL . UV-Vis spectrometer were measured absorption spectra of mixture at the wavelength of 250 – 600 nm. In addition, the IR spectra of mixture were studied to confirm the complexes.

3.2.1.6. Modification of diazo-AgNPs

Azo1 and Azo2 solutions were adjusted to pH 5 by using citrate buffer. The diazo ligand solution was added into AgNPs at different ratio and then the solution was stirred at room temperature for 30 minutes before UV-Vis spectrometer was used to measure the absorption spectra.

3.2.2. Applying the diazo-AgNPs to detected 17β -estradiol by UV-Vis spectrometry method.

3.2.2.1. Interaction of 17β -estradiol with Diazonium compounds by UV-Vis spectrophotometer

Diazonium solutions 0.5 mM of Azo1 or Azo2 2 mL was pipetted to small vial. Then, 17β -estradiol 500 ppm in 50% acetonitrile was added into Diazonium salt solution 10-70 ppm. The solution was measured the absorption spectra by UV-Vis spectrometer at the wavelength between 300-600 nm. This method was time controlled at 0, 30 and 90 minute.

3.2.2.2. Interaction of 17β -estradiol with mixture of silver ion and Diazonium compounds

Mixture of silver ion and Diazonium salt compounds were prepared by mixed 1 mM of silver nitrate with 1 mM diazo ligand at mol ratio 1:1 for Azo1 and 1:2 for Azo2. After that mixture 2 mL was pipetted to small vial. Then 17β -estradiol 500 ppm in 50% acetonitrile was added into mixture 1-70 ppm. The solution was measured the absorption spectra by UV-Vis spectrometer at the wavelength between 250-600 nm. This method was time controlled at 0, 30 and 90 minute.

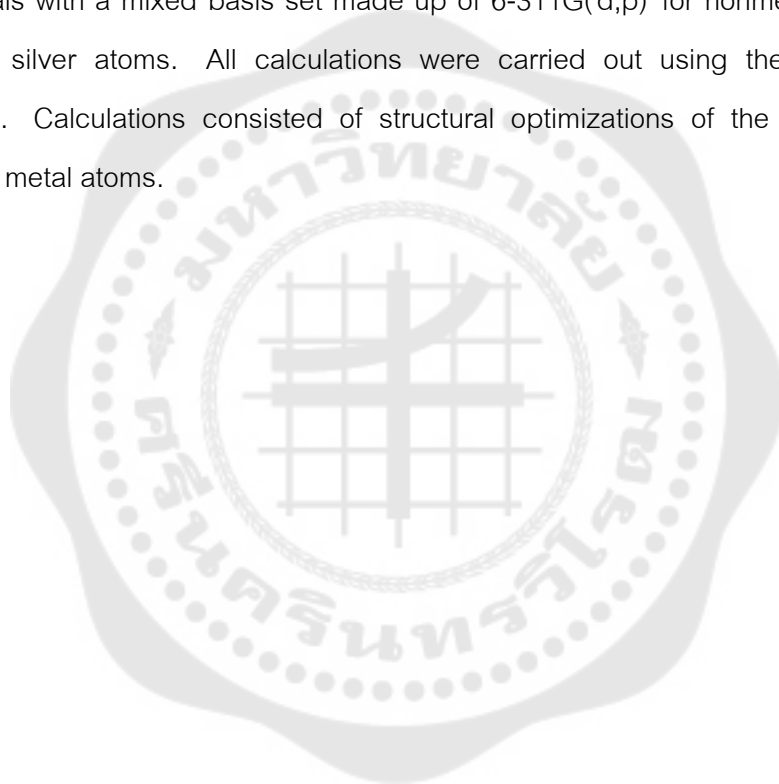
3.2.2.3. Interaction of 17β -estradiol with diazo-AgNP by UV-Vis spectrophotometer

The standard solution of 1000 ppm 17β -estradiol was prepared by dissolved 0.0125 g of 17β -estradiol 98% in 25 ml volumetric flask and adjusted with 50% acetonitrile. Then, the prepared 17β -estradiol solution was diluted to 500 ppm in 10 ml volumetric flask by 50% acetonitrile. The diazo-AgNPs were prepared by mixed 1 mM of AgNPs with 1 mM diazo ligand at mol ratio 1:1 for Azo1 and 1:2 for Azo2. After that, the prepared standard 17β -estradiol solutions were added into diazo-AgNPs and measured by UV-Vis spectrometer.

3.2.3. Calculation interaction between diazonium compounds, silver nanoparticles and 17 β -estradiol by computational chemistry

3.2.3.1. Computational method

The geometry optimization, and binding energies of diazonium compounds, silver nanoparticles and 17 β -estradiol were determined using the density functional approaches B3LYP (B3 Becke 3-parameter exchange and Lee–Yang–Parr correlation) functionals with a mixed basis set made up of 6-311G(d,p) for nonmetal atoms and 3-21G for silver atoms. All calculations were carried out using the Gaussian 2003 package. Calculations consisted of structural optimizations of the Azo compounds linked to metal atoms.



CHAPTER 4

RESULT AND DISCUSSION

4.1. To synthesize ligand, metal and nanoparticle complexes with easy rapid experiment for development of stable silver nanoparticles.

4.1.1. Synthesis of silver nanoparticles

From method synthesis AgNPs, Silver(I) nitrate solution was changed from colorless to brown color due to a phenomenon known as plasmon absorbance because of AgNPs. The spectrum of the clear yellow colloidal silver from the synthesized AgNPs shown in Figure 15 with strong absorption peak at 395 nm corresponding to the size of synthesized AgNPs 10-15 nm (Mulfinger et al., 2007).

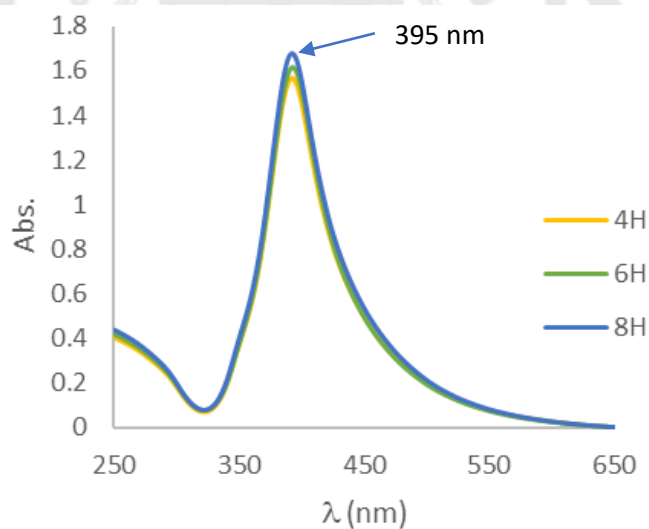


Figure 15. UV-vis absorption spectrum of silver nanoparticles.

4.1.2. Synthesis of diazonium compounds.

The diazonium salt ligands were produced from sulfanilamide (Azo1) and sulfanilic acid (Azo2) given the pale yellow solution in 1 hour and slowly change to orange solution shown in Figure 17. Absorption spectra of diazonium salt showed strong peak at 360 nm both in Azo1 and Azo2 while no spectrum of sulfanilamide and

sulfanilic acid remaining in Figure 17. This results indicated the appearance of azo functional groups ($R-N_2^+$) in synthesized ligands both Azo1 and Azo2 in Figure 16

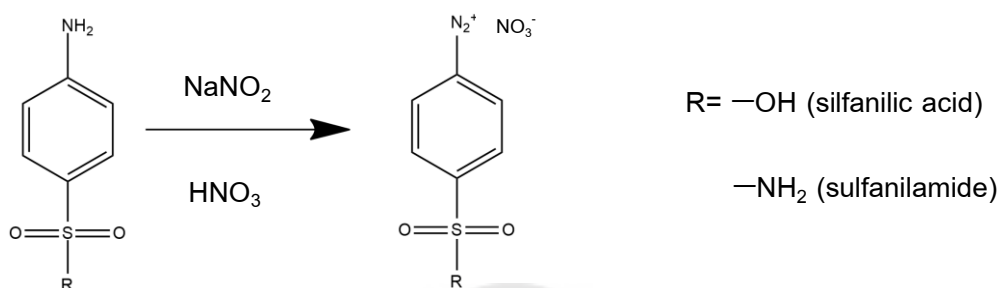


Figure 16. Schematic Synthesis diazonium salt compounds

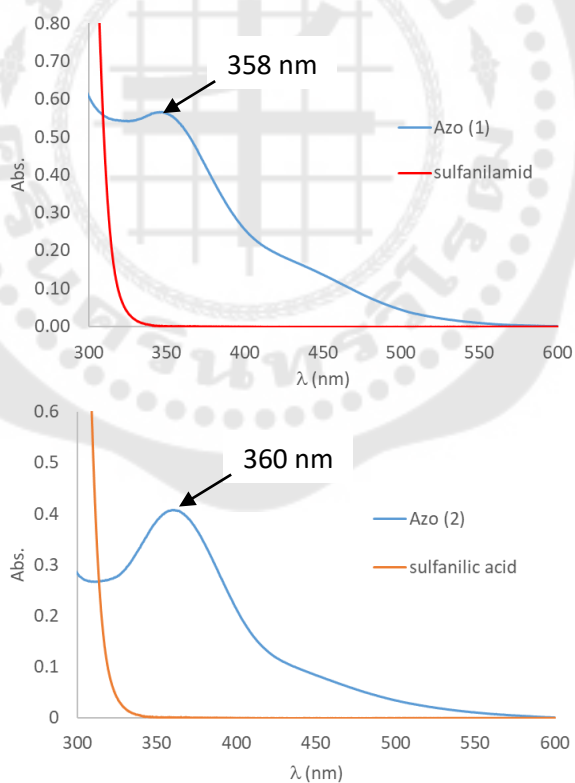


Figure 17. UV-vis absorption spectrum of Azo1 and sulfanilic acid Azo2.

4.1.3. Effects of pH on diazonium compounds.

The spectrum in figure 18 A and B showed spectrum of diazonium salt form sulfanilamide (Azo1) and sulfanilic acid (Azo2) at different pH, respectively. From spectrum of both diazonium salt compounds, there were observed the strong peak at 360 nm at value of pH equal to 7 and below. This results indicated diazonium salt compounds were stable at acid solution.

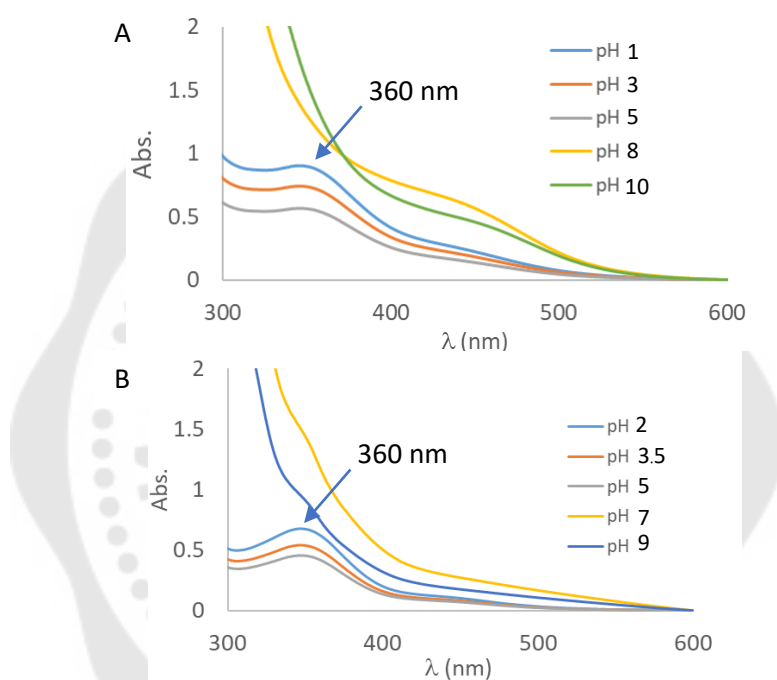


Figure 18. UV-vis absorption spectrum of diazonium salt form sulfanilamide (A) at pH 1–10 and sulfanilic acid (B) at pH 2–9.

4.1.4. Interaction between silver nanoparticles and diazonium compounds

In this study, 0.1 mM AgNPs were titrated with 1.0 mM solution of Azo1 and Azo2. In Figure 19(A), the spectra of mixture AgNPs and diazonium Azo1 were shown the red shift of wavelength from 395 nm to 405 nm after adding Azo1 between 50 – 200 μ L and then the absorbance at 405 nm were constant since ratio of metal: ligand equal 1:1 as shown in table 1. In addition, Figure 19(B) was showed the spectra of mixture AgNPs and Azo2 with the blue shift from 397 nm. to 394 nm. after adding between 50 –

400 μL of Azo2 before the constant absorbance at 394 nm were occurred. This result was presented interaction between AgNPs and Azo2 with the ratio of metal: ligand is 1:2 as shown in table 2.

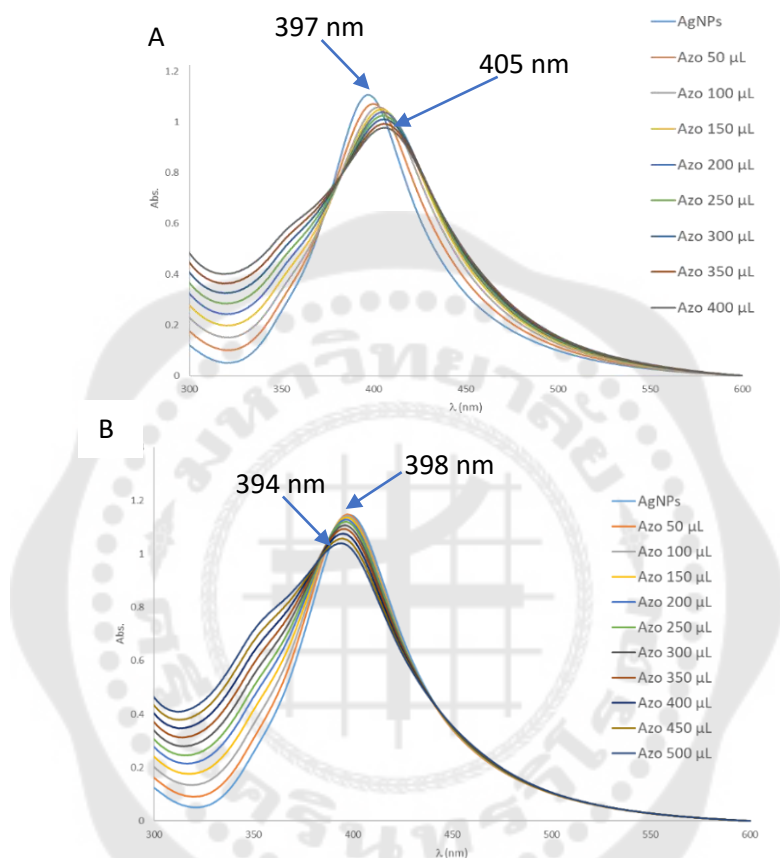


Figure 19. UV-vis absorption spectrum of titrations AgNPs with diazonium compound form sulfanilamide (A) and sulfanilic acid (B)

Table 1. Results of interaction of AgNP and Azo1 at different volume of Azo1.

Volume of Azo1	λ_{\max} (nm)	Mole ratio (AgNPs:Diazonium)
AgNPs	397.0	-
Azo1 50 μ L	399.6	1:0.25
Azo1 100 μ L	402.2	1:0.50
Azo1 150 μ L	403.8	1:0.75
Azo1 200 μ L	405.4	1:1
Azo1 250 μ L	405.4	1:1.25
Azo1 300 μ L	405.6	1:1.50
Azo1 350 μ L	405.4	1:1.75
Azo1 400 μ L	405.4	1:2

Table 2. Results of interaction of AgNP and Azo2 at different volume of Azo2.

Volume of Azo2	λ_{\max} (nm)	Mole ratio (AgNPs:Diazonium)
AgNPs	398.0	-
Azo2 50 μ L	397.4	1:0.25
Azo2 100 μ L	397.2	1:0.50
Azo2 150 μ L	397.0	1:0.75
Azo2 200 μ L	396.4	1:1
Azo2 250 μ L	396.6	1:1.25
Azo2 300 μ L	396.0	1:1.50
Azo2 350 μ L	395.0	1:1.75
Azo2 400 μ L	394.4	1:2
Azo2 450 μ L	394.8	1:2.25
Azo2 500 μ L	394.0	1:2.50

4.1.5 FT-IR spectrum

From FT-IR spectrum of Azo1, Azo2 in figure 20 and 21, they showed the band at 2295 and 2289 cm^{-1} from Azo1 and Azo2, respectively. The appearance of these bands were clearly demonstrated $\text{N}\equiv\text{N}$ stretching vibration and verified the formation of diazonium salt ($\text{R}-\text{N}_2^+$) (Bamoniri, Mirjalili, & Moshtael-Arani, 2014).

In addition, FT-IR were used to determine the brown residues from the interaction of AgNPs-Azo1 with 17β -Estradiol in 3.2.2.3. The residues were separated and measured by FT-IR spectrum. Spectra was showed in figure 22 and observed aromatic peak ($\text{C}=\text{C}$) appears in the spectrum at 1620 cm^{-1} and 1495 cm^{-1} . Moreover, the 1528 cm^{-1} and 1584 cm^{-1} frequencies are due to chromophore of azo. The 1273 cm^{-1} is due to stretching of $\text{C}-\text{N}$ (azo bond $\text{C}-\text{N}$) and the $\text{C}-\text{N}$ absorption occurs at a higher frequency in aromatic compounds due to resonance increases the double bond character between the ring and the attached nitrogen atom (Ahmed, Dewani, Pervez, Mahboob, & Soomro, 2016).

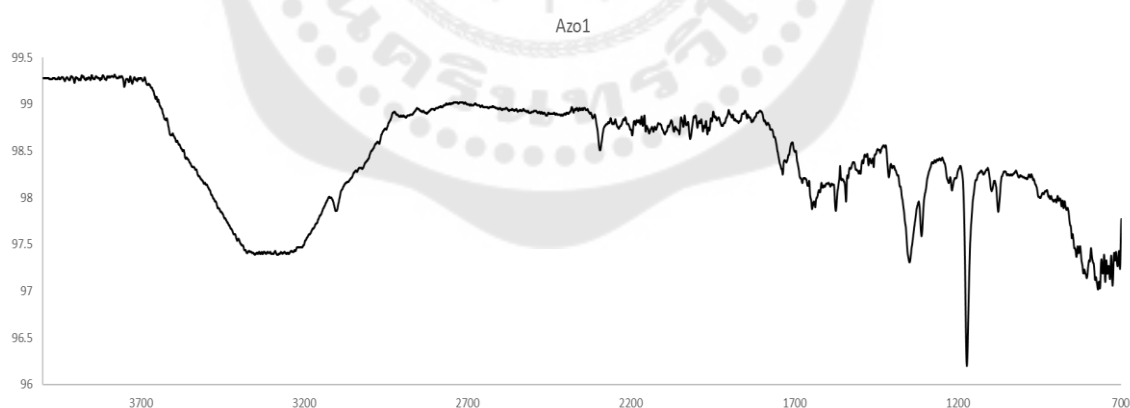


Figure 20. IR Spectrum for Azo1

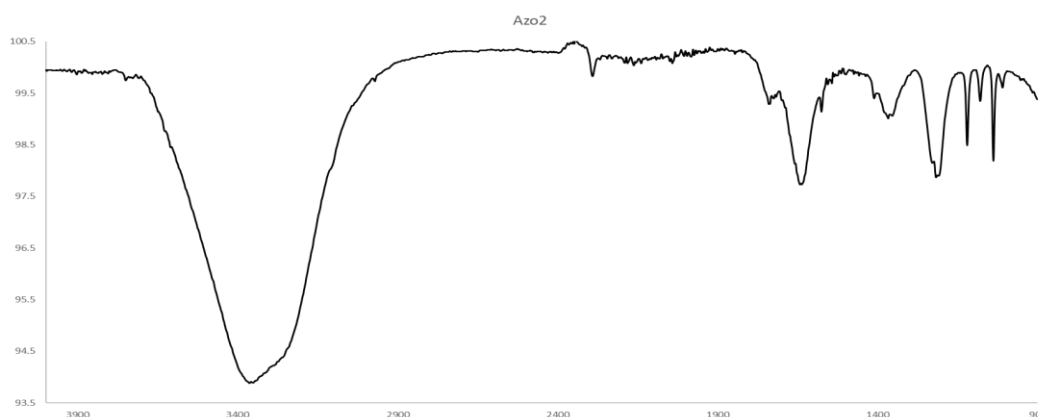
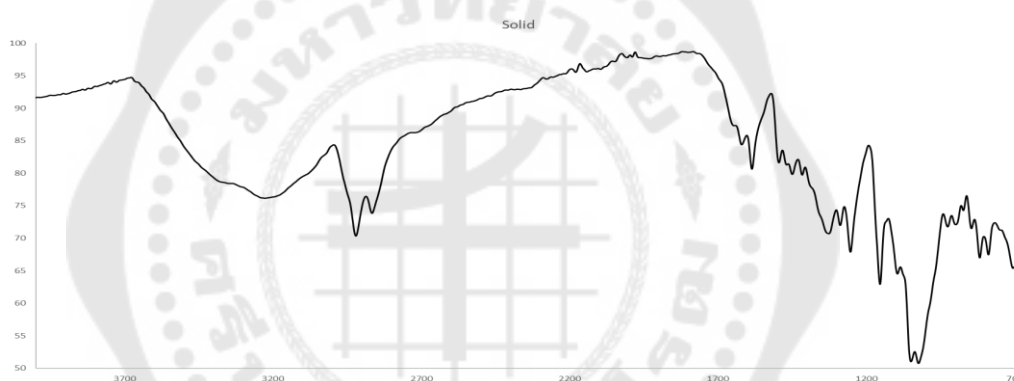


Figure 21. IR Spectrum for Azo2

Figure 22. IR Spectrum for residues from mixture of AgNPs, Azo1 and 17 β -estradiol.

4.2. To apply the synthesized ligand, metal and nanoparticle complexes for detection of 17 β -estradiol

4.2.1. Interaction of 17 β -estradiol with Diazonium compounds by UV-Vis spectrophotometer

In this study, 0.5 mM of Azo1 and Azo2 were titrated with standard 500 ppm 17 β -estradiol solution and measured the spectrum by UV – Visible at 0-90 minute. In figure 23, 24 and 25, they were showed absorption spectrum of mixture between Azo1 and 17 β -Estradiol at 0, 30 and 90 minute, respectively with in increasing of absorbance

at 360 nm and a new absorption peak at 450 nm after 0 minute. Color of solution were change form pale yellow to dark yellow followed increasing concentration of 17β -estradiol and time .

Figure 26, 27 and 28 were showed absorbance spectrum of mixture Azo2 and 17β -estradiol at 0, 30 and 90 minute, respectively, observed peak showed at 360 and 450 nm and increasing the absorbance after 30 minutes. Color of solution were change form pale yellow to dark yellow followed increasing concentration of 17β -estradiol and time after adding 17β -estradiol 10 ppm.

This result was showed Diazotization reaction between synthesized Diazonium salt compounds (Azo1 and Azo2) with 17β -estradiol by coupling between diazonium salt and phenolic group of estrogen . In addition, azo compounds are water-soluble compounds possessing the characteristic single $-N=N-$ (azo) bond. The azo compounds have vivid colors, especially yellows {Ziarani, 2018}.

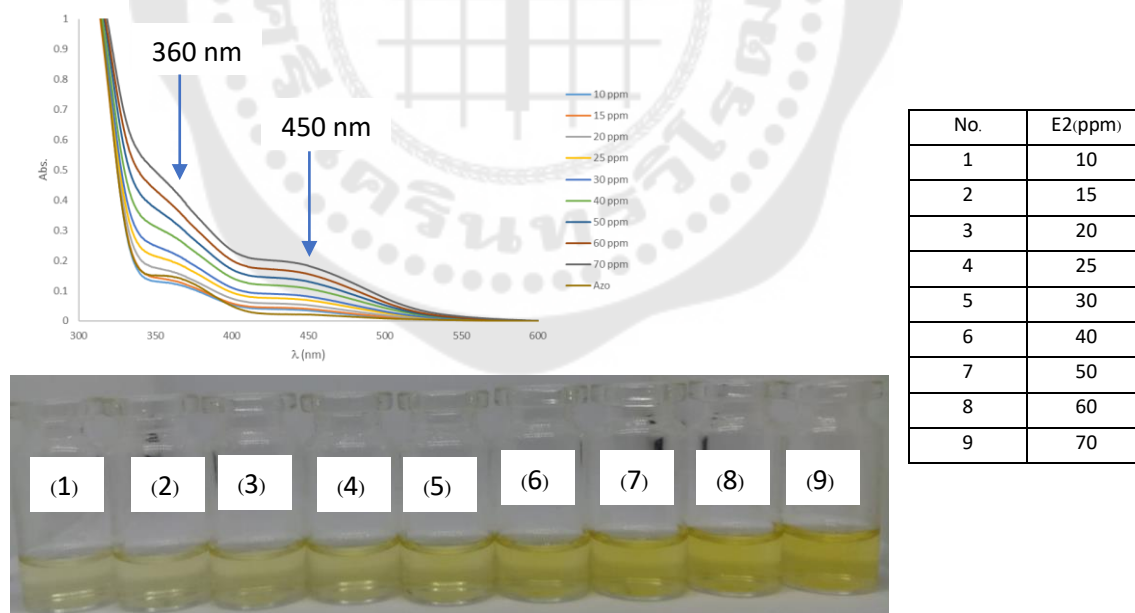


Figure 23. UV-vis absorption spectrum Azo1 and 17β -estradiol for 0 minute (A). Picture of mixture Azo1 solution were added 17β -estradiol 10-70 ppm (b)

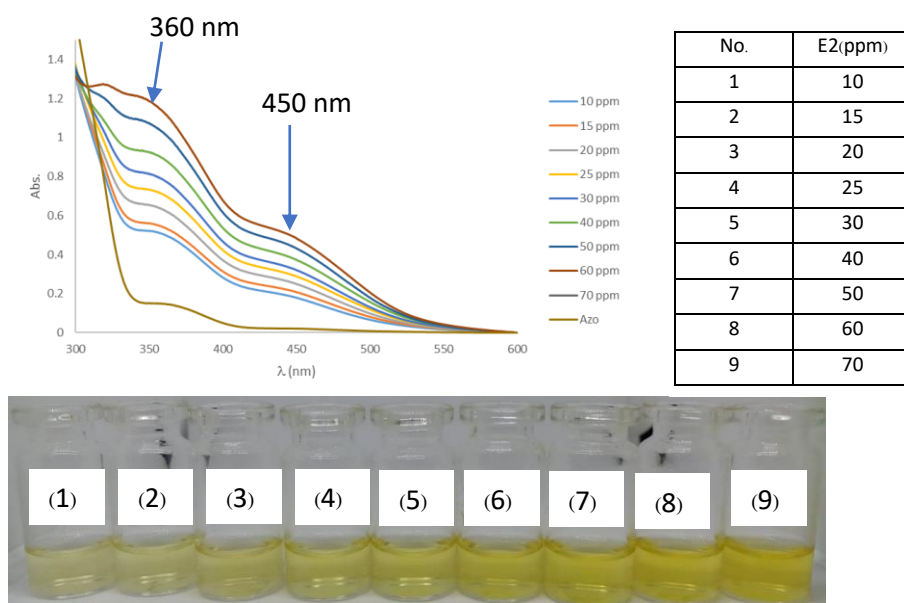


Figure 24. UV-vis absorption spectrum Azo1 and 17β -estradiol for 30 minutes (A).

Picture of mixture Azo1 solution were added 17β -estradiol 10-70 ppm (b)

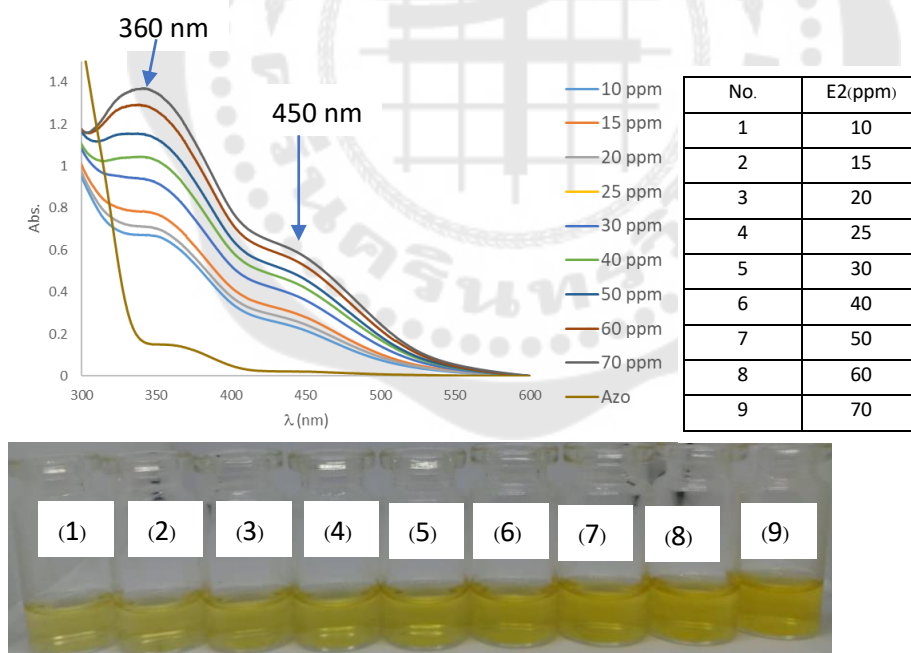


Figure 25. UV-vis absorption spectrum Azo1 and 17β -estradiol for 90 minutes (A).

Picture of mixture Azo1 solution were added 17β -estradiol 10-70 ppm (B)

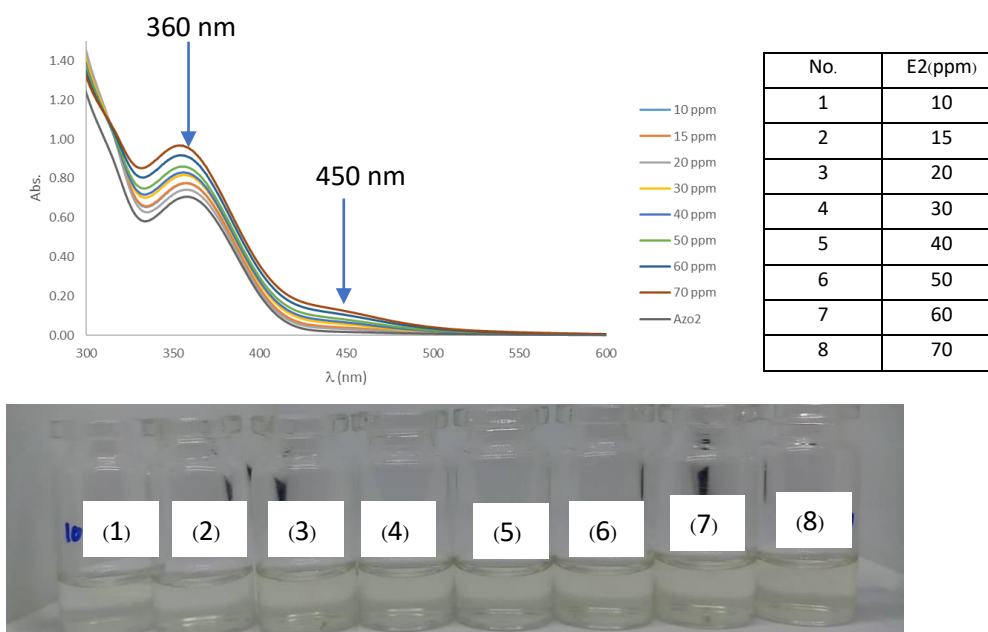


Figure 26. UV-vis absorption spectrum Azo2 and 17β -estradiol for 0 minute (A). Picture of mixture Azo2 solution were added 17β -estradiol 10-70 ppm (B)

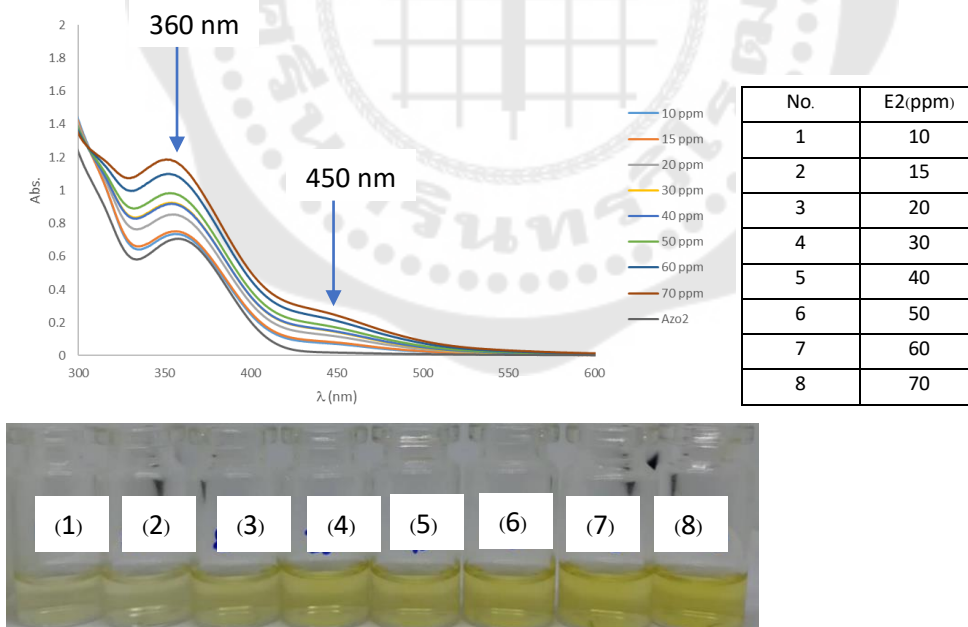


Figure 27. UV-vis absorption spectrum Azo2 and 17β -estradiol for 30 minutes (A). Picture of mixture Azo2 solution were added 17β -estradiol 10-70 ppm (B)

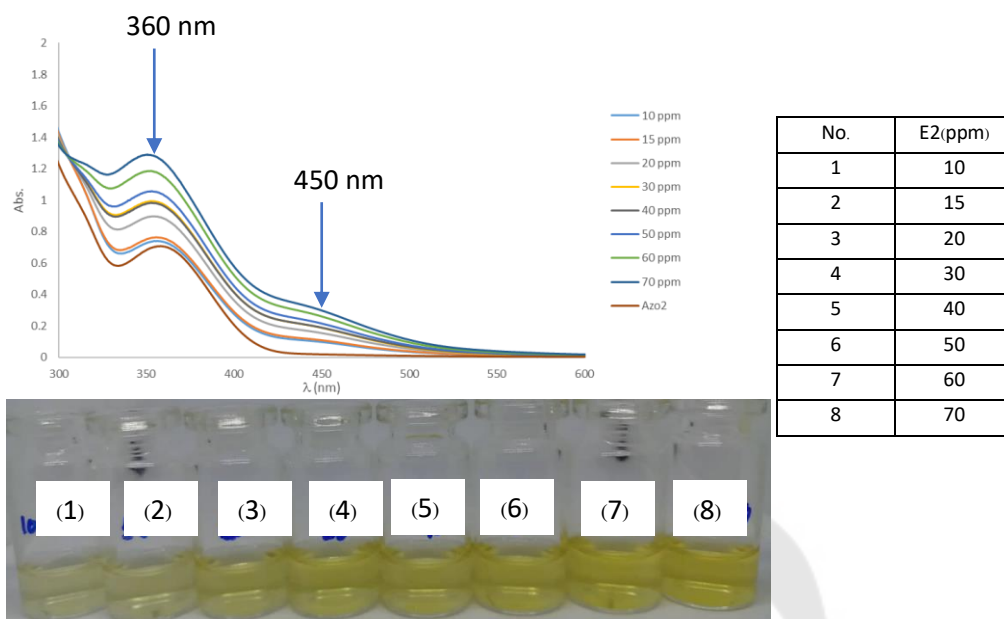


Figure 28. UV-vis absorption spectrum Azo2 and 17β -estradiol for 90 minutes (A).

Picture of mixture Azo2 solution were added 17β -estradiol 10-70 ppm (B)

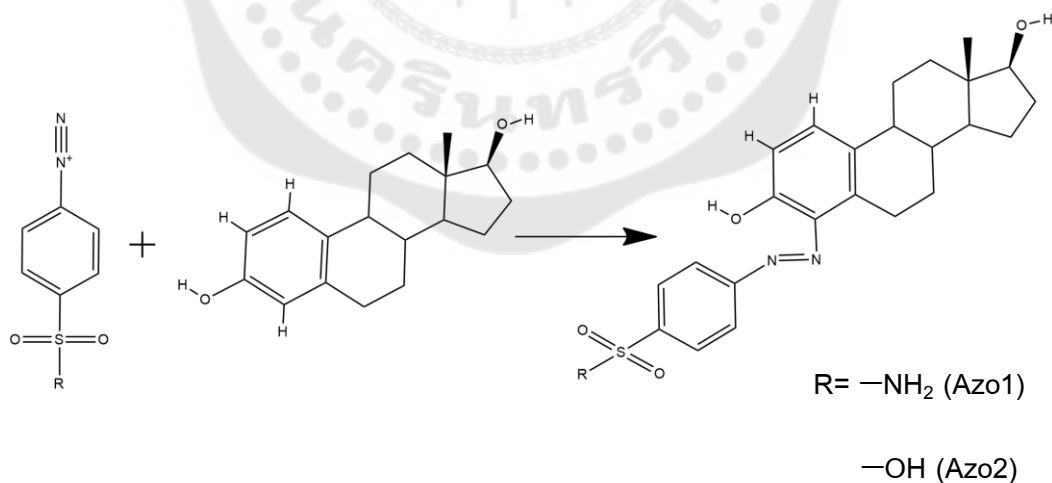


Figure 29. Schematic of Diazotization between synthesized Diazonium salt ligands and 17β -estradiol

4.2.2. Interaction of 17β -estradiol with mixture of silver ion and Diazonium compounds

In this study mixture Azo1 and Azo2 with silver ion were titrated with the standard 500 ppm 17β -estradiol before measured UV-Visible spectrum at 0-90 minute. In figure 30, 31 and 32, the spectrum of mixture Azo1 was at 0, 30 and 90 minute respectively. They were observed in increasing of absorbance at 360 nm and new absorption peak at 450 nm after 0 minute. Color of solution were change form pale yellow to dark yellow with the increasing concentration of 17β -estradiol and time after adding 17β -estradiol 10 ppm.

Figure 33, 34 and 35 were showed absorbance spectrum of mixture Azo2 at 0, 30 and 90 minute, respectively. They were observed in increasing of absorbance at 360 nm and new absorption peak at 450 nm after 0 minute. Color of solution were change form pale yellow to dark yellow followed increasing concentration of 17β -estradiol and time after adding 17β -estradiol 10 ppm.

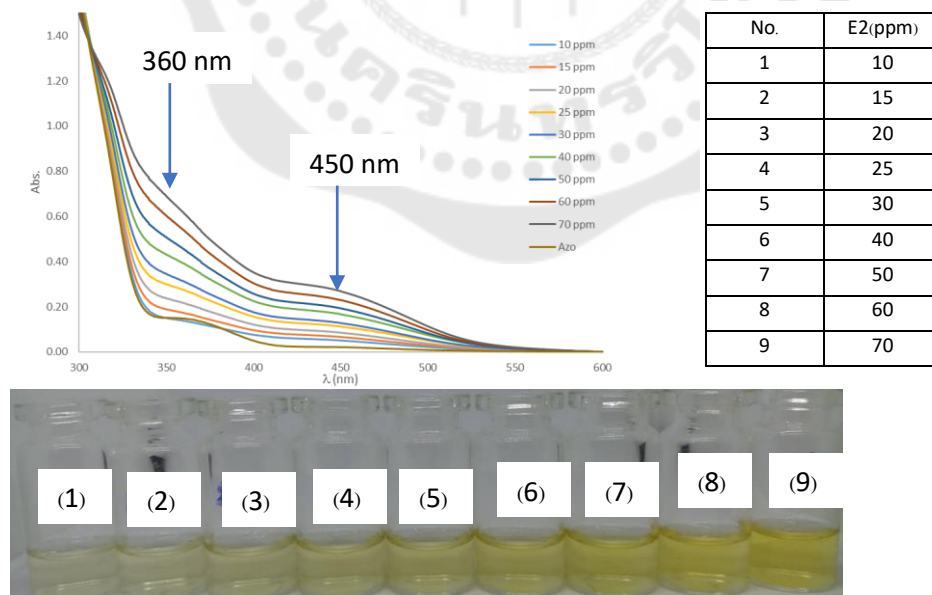


Figure 30. UV-vis absorption spectrum mixture of silver ion with Azo1 were added E2 for 0 minute (A). Picture of mixture Azo1 solution were added E2 10-70 ppm (B)

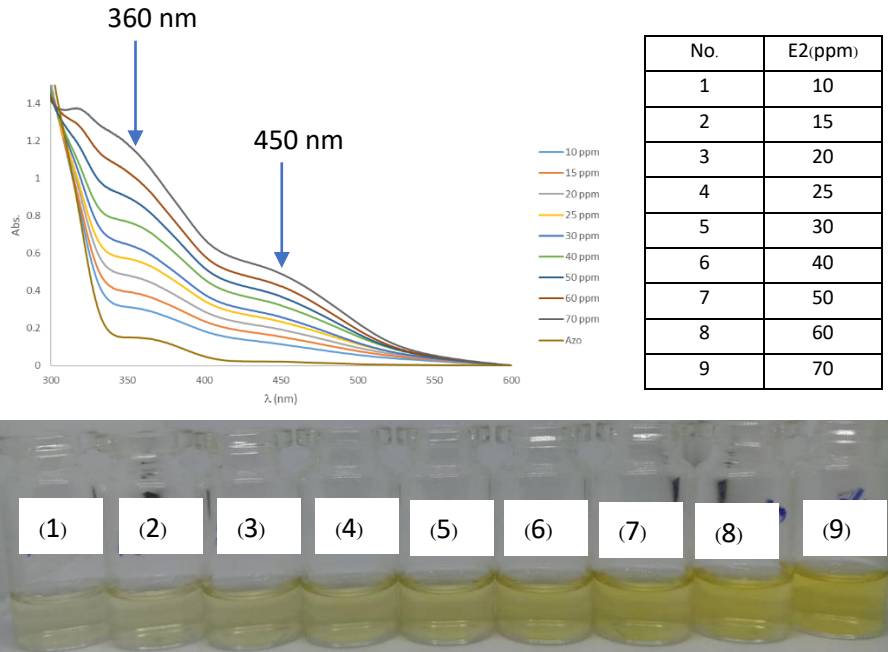


Figure 31. UV-vis absorption spectrum mixture of silver ion with Azo1 were added E2 for 30 minutes (A). Picture of mixture Azo1 solution were added E2 10-70 ppm (B)

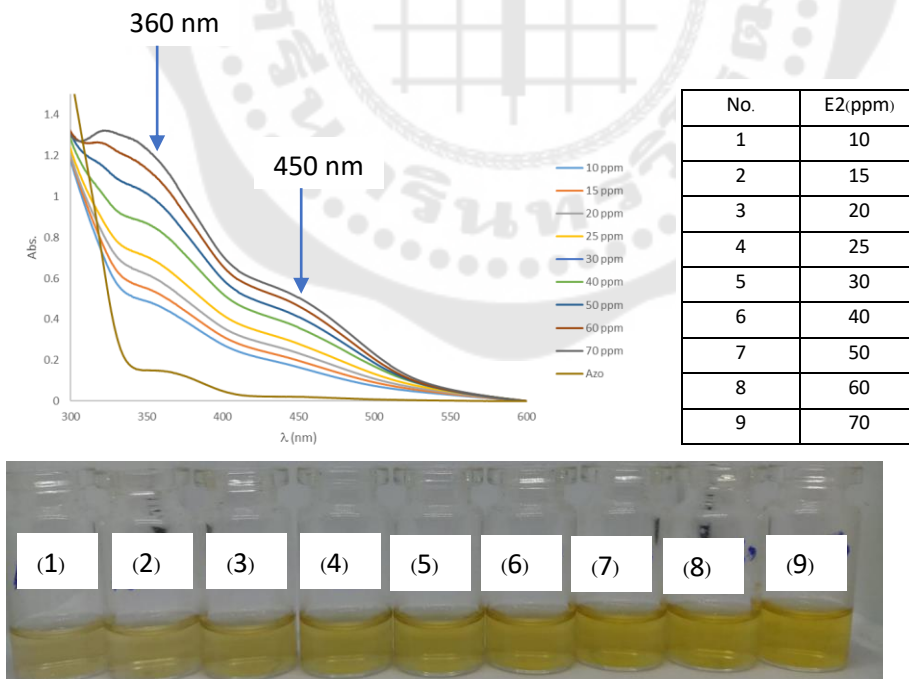


Figure 32. UV-vis absorption spectrum mixture of silver ion with Azo1 were added E2 for 90 minutes (A). Picture of mixture Azo1 solution were added E2 10-70 ppm (B)

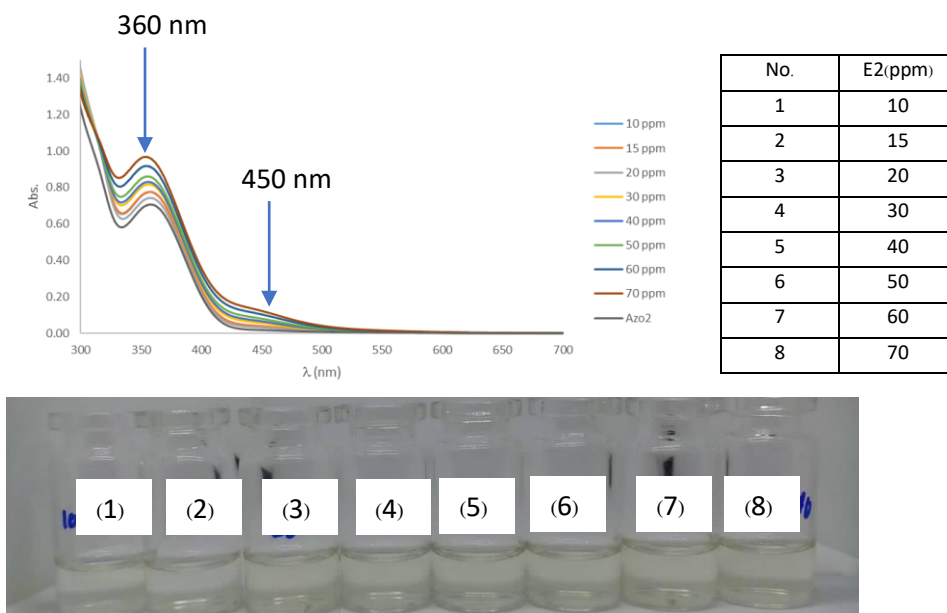


Figure 33. UV-vis absorption spectrum mixture of silver ion with Azo2 were added E2 for 0 minute (A). Picture of mixture Azo1 solution were added E2 10-70 ppm (B).

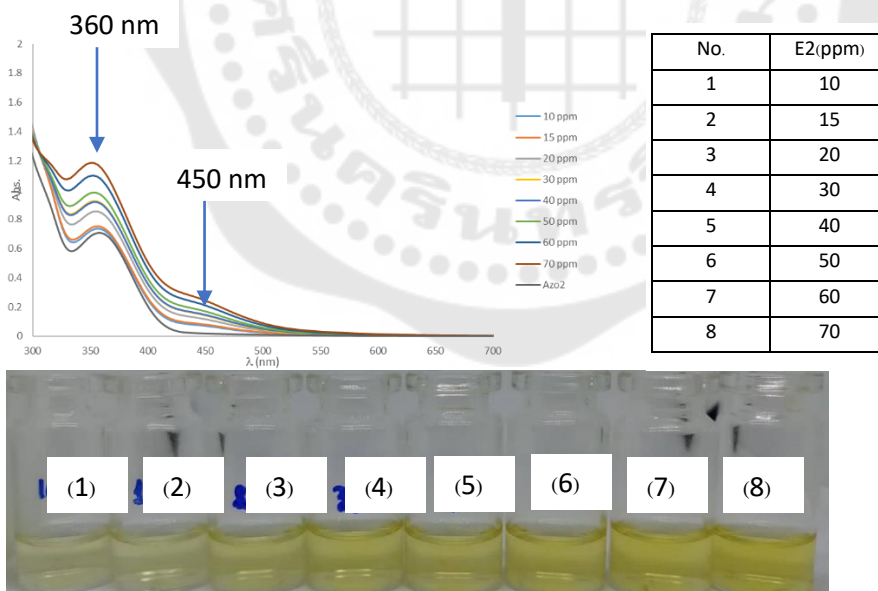


Figure 34. UV-vis absorption spectrum mixture of silver ion with Azo2 were added E2 for 30 minutes (A). Picture of mixture Azo1 solution were added E2 10-70 ppm (B).

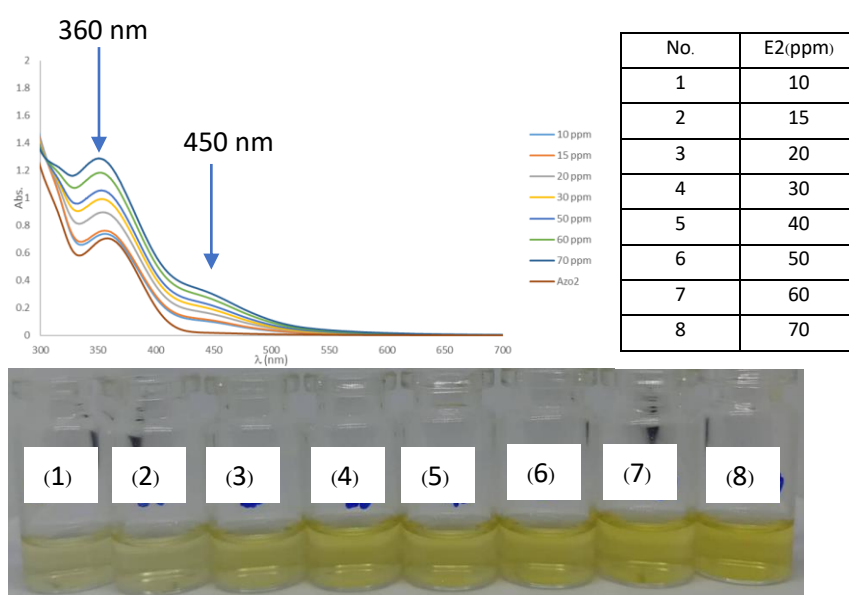


Figure 35. UV-vis absorption spectrum mixture of silver ion with Azo2 were added E2 for 90 minutes (A). Picture of mixture Azo2 solution were added E2 10-70 ppm (B).

4.2.3 Interaction of 17β -estradiol with diazo-AgNP by UV-Vis spectrophotometer

Figure 36 was showed spectrum and pictures of mixtures solution including AgNPs, Azo1 and 17β -estradiol. It was observed blue shifted of modified AgNPs with diazonium salt (AgAzo1) absorption spectra for 405 nm to 395 nm. It was decreased absorption when concentration of 17β -estradiol was increased. After 17β -estradiol solution were added to AgAzo1 for 30 minute, it was observed precipitate in mixture form figure 37. Precipitation was observed after adding standard 17β -estradiol solution since 25 ppm to the AgAzo1. From this results, the diazonium ligand (Azo1) on the surface of the silver nanoparticles were released and reacted with phenol group of 17β -estradiol. The absorbance changed from 405 nm, the value representing the interaction between AgNPs and diazonium salt compound to 395 nm which were only absorbance of AgNPs.

Figure 38, 39 was showed spectrum and pictures of mixtures solution including AgNPs, Azo2 (AgAzo2) and 17β -estradiol. It was not changed like AgAzo1. From spectrum were observed new absorption peak at estimate 350 nm. From results, this absorbance is assumed to be a result of the interaction between the substance and estrogen assuming that the reaction was different from mixture of AgAzo1 solution. Azo2 have interacts with the surface of AgNPs and makes the reaction with estradiol more difficult than Azo1 because the change that occurs is only noticeable when need more time to reaction.

In addition, from the FT-IR spectrum of residue of AgAzo1 with estrogen, they were indicated the reaction between Azo1 and 17β -Estradiol and formed azo compound. Then, azo compound was interacted with AgNPs and precipitated. After adding 17β -Estradiol into AgAzo1 solution at 1-5 ppm absorption peak at 395 there was not observed changing but when more 17β -Estradiol, the absorbance was decreased and formed of precipitation as seen in the naked eye. However, the effects on Azo2 differed from Azo1 that the further studies in important.

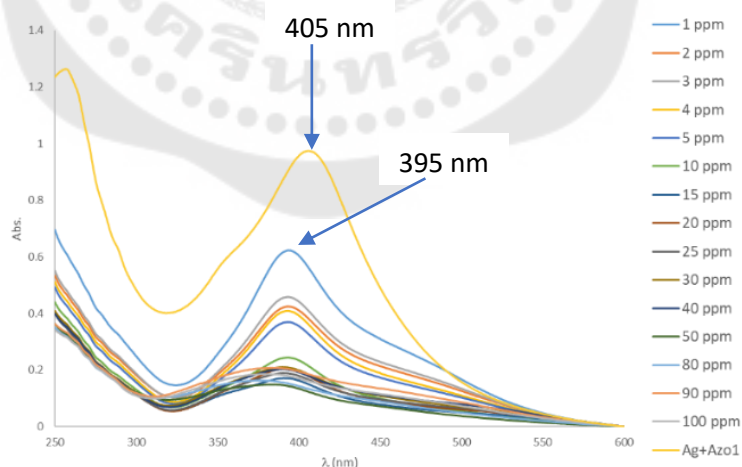


Figure 36. UV-vis absorption spectrum mixture of AgAzo1 were added E2 1-100 ppm.

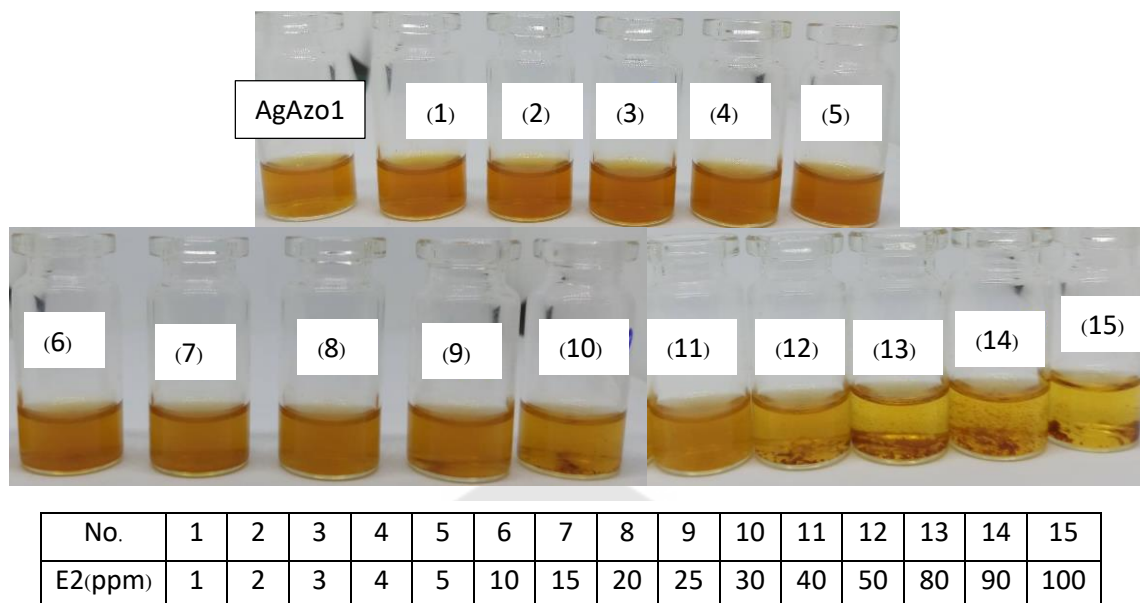


Figure 37. Picture of mixture AgAzo1 solution were added E2 1-70 ppm.

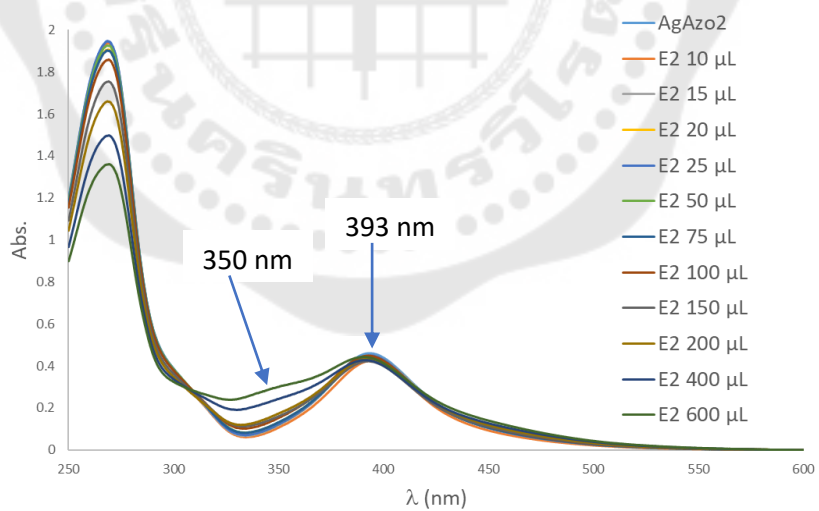
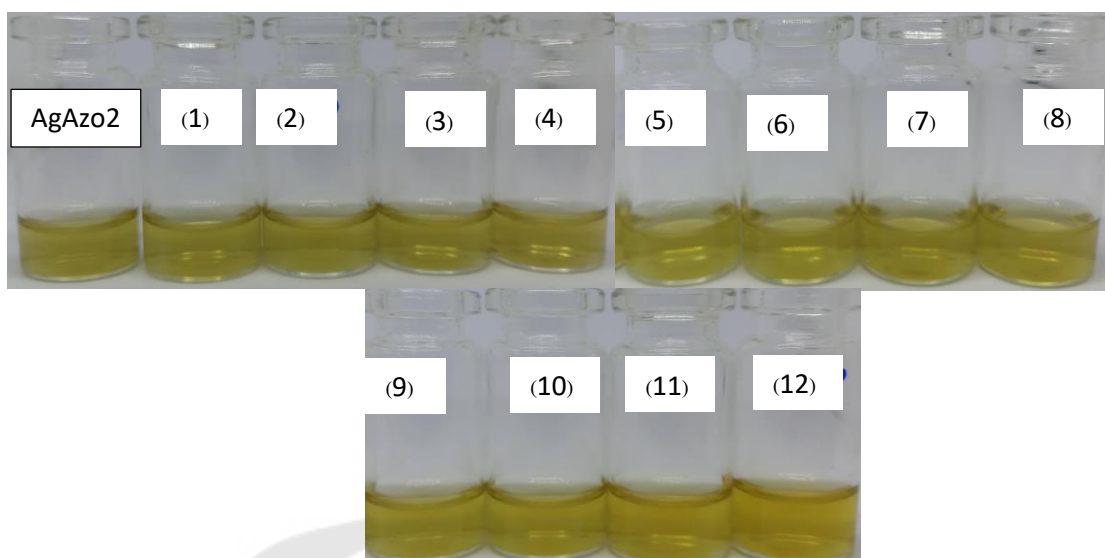


Figure 38. UV-vis absorption spectrum mixture of AgAzo2 were added E2 1-100 ppm.



No.	1	2	3	4	5	6	7	8	9	10	11	12
E2(ppm)	1	2	3	4	5	10	20	30	40	50	80	100

Figure 39. Picture of mixture AgAzo2 solution were added E2 1-70 ppm.

4.3 To explain energy, structure and interaction of silver nanoparticles sensor by computation calculation

4.3.1. Optimized structure of diazonium compounds and azo compounds

Optimized structures of Azo1, Azo2 and 17β -estradiol with density functional approaches B3LYP method and the B3LYP/6-311g (d,p) basis set. The energy values displayed that the most stable structure of Azo1, Azo2 and 17β -estradiol as shown in Figure 40. In addition, the interaction between Azo1, Azo2 with 17β -estradiol were investigated all three position (position 1, 2 and 4) as shown in Figure 41 and 42. The results of the calculation showed that the substitution reaction on the second site of the estrogen phenyl gave the lowest energy of both Azo1 and Azo2. Because the second position is influenced by the group replacement which make the substance easily react. Moreover, steric effect from 17β -estradiol is expected to be lower than other position.

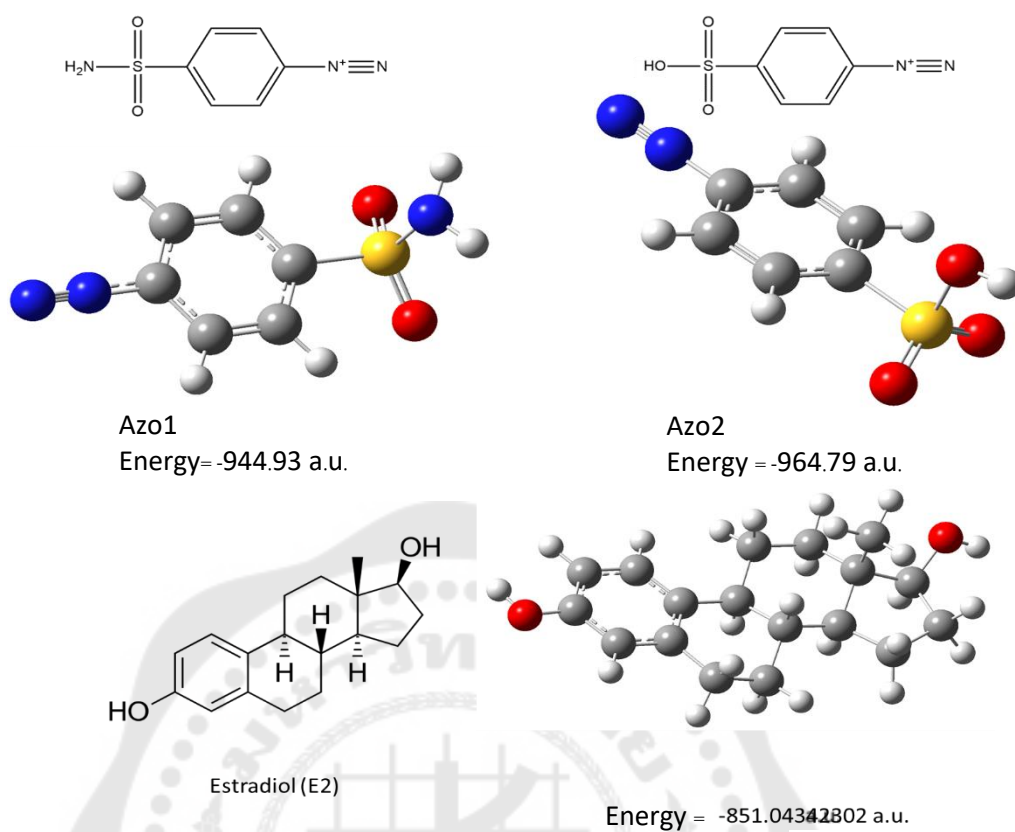


Figure 40. optimized structure of Azo1, Azo2 and 17 β -estradiol.

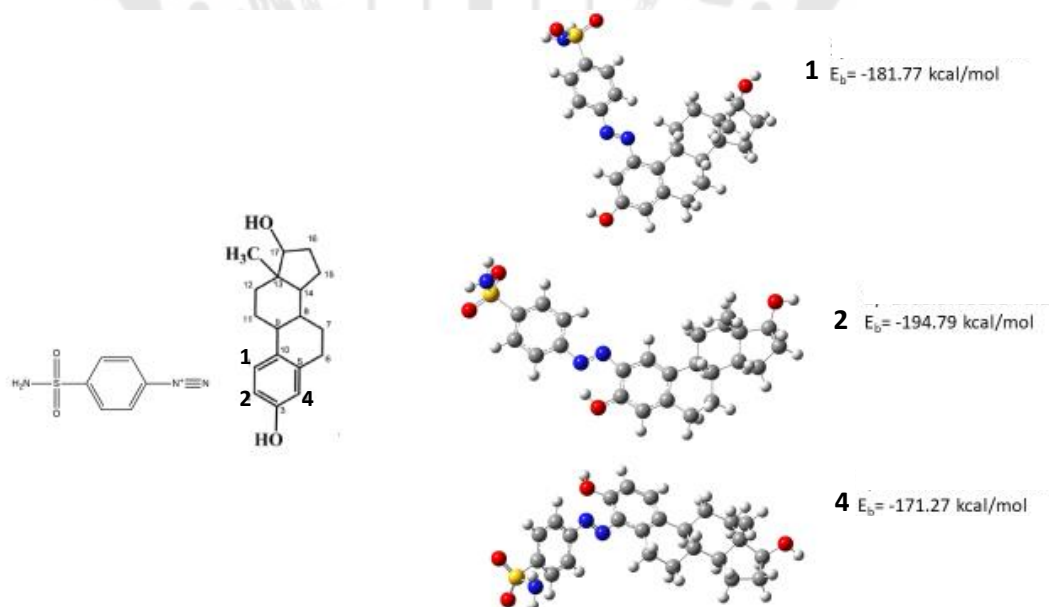


Figure 41. optimized structure of Azo compound were formed by Azo1 and 17 β -estradiol.

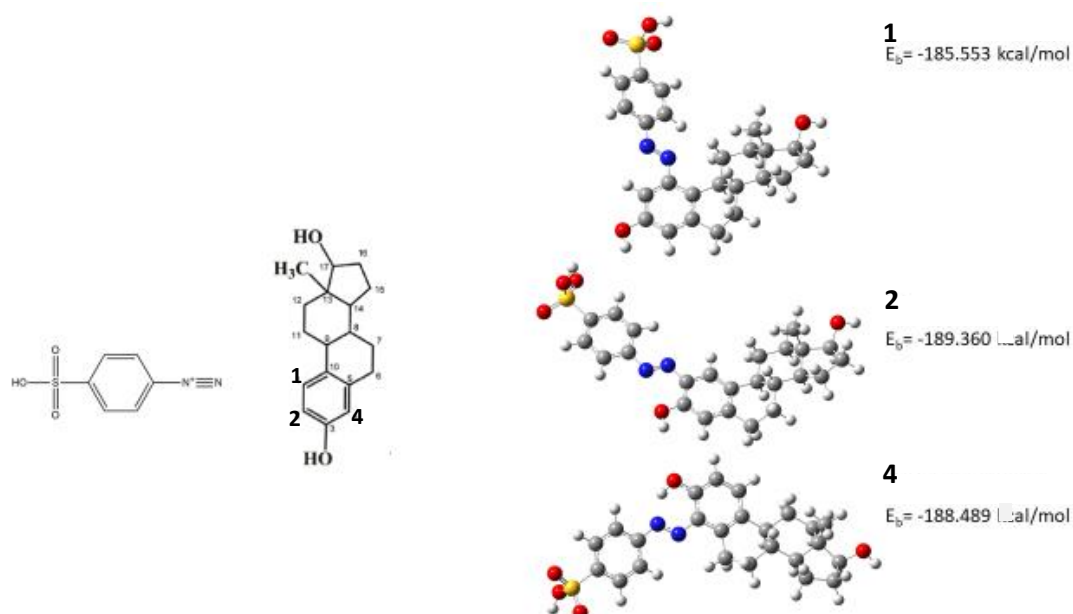


Figure 42. optimized structure of Azo compound were formed by Azo2 and 17β -estradiol.

4.3.2. Optimized structure of diazonium compounds and AgNPs

The interactions of the silver and Azo1 complexes were studied on the three different initial position of AgNPs such as between N12 and O17 atom (position 1) or between N12 and O18 atom on the side of Azo1 molecule (position 2) or between O17 and O18 atom below of Azo1 molecule (position 3). The structure and energy of complexes between AgNPs and Azo1 were shown in figure 44. It was observed changing of all complexes to position 3 and calculation energy of three structures were -45.24 kcal/mol. Table 3 showed bond length and bond angles of complexes between AgNPs with Azo1. Bond lengths of the Ag–O17 bond in three complexes of AgNPs and Azo1 ((1a), (1b) and (1c) in figure 44.) were 2.46, 2.41 and 2.46 Å, respectively and bond lengths of the Ag–O18 bond were 2.41, 2.46 and 2.41 Å, respectively.

The interactions of the AgNPs and Azo2 complexes were studied on the three different initial position of AgNPs such as between O_H and O13 atom (position 1) or between O_H and O14 atom on the side of Azo2 molecule (position 2) or between O13

and O14 atom below of Azo2 molecule (position 3) . The structure and energy of complexes between AgNPs and Azo1 were show in figure 45. Calculation energy of structure in position 1 and 2 were -202.22 kcal/mol but calculation energy of structure in position 3 was decreased to -202.24 kcal/mol. Table 4 showed bond length and bond angles of complexes between AgNPs with Azo2. Bond lengths of Ag-O_H bond in complexes of AgNPs and Azo2 ((2a), (2b) In figure 45.) were 2.47 Å . Bond lengths of Ag-O13 bond in complex (2a) was 2.34 Å and bond lengths of Ag-O14 bond in complex (2b) was 2.34 Å . Bond lengths of Ag-O13 and Ag-14 bond of complex (2c) were 2.46 and 2.45 Å .

Table 3. Interaction structure of complexes between AgNPs and Azo1 (AgAzo1) for bond length and bond angle

structures	Angle(deg) (N-Ag-O)	Bond Length (Å)	
		Ag-O18	Ag-O17
1a	60.57	2.46	2.41
1b	60.57	2.41	2.46
1c	60.56	2.46	2.41

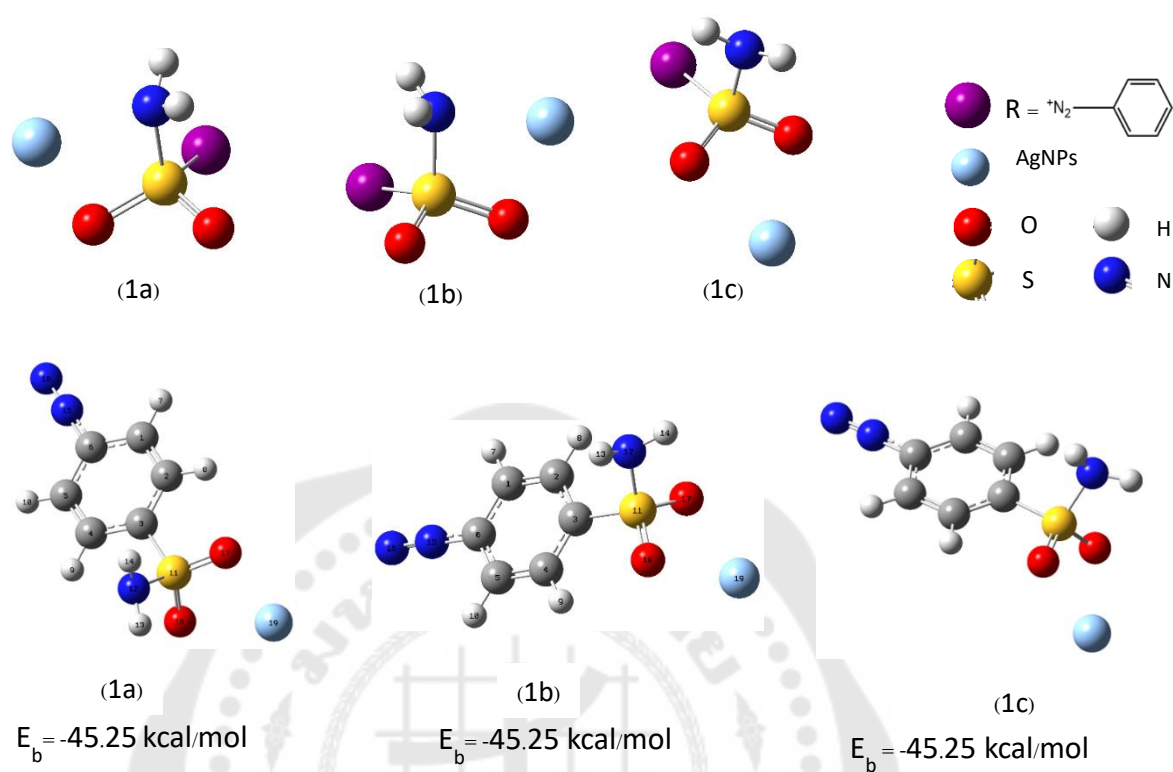


Figure 43. Interaction structure of Azo1 and AgNPs.

Table 4 Interaction structure of complexes between AgNPs and Azo2 (AgAzo2) for bond length and bond angle

structures	Angle(deg)			Bond length (\AA)		
	(O _H -Ag-O13)	(O _H -Ag-O14)	(O13-Ag-O14)	Ag-O13	Ag-O14	Ag-O _H
2a	59.39	—	—	2.34	—	2.47
2b	—	59.40	—	—	2.34	2.47
2c	—	—	60.16	2.46	2.45	—

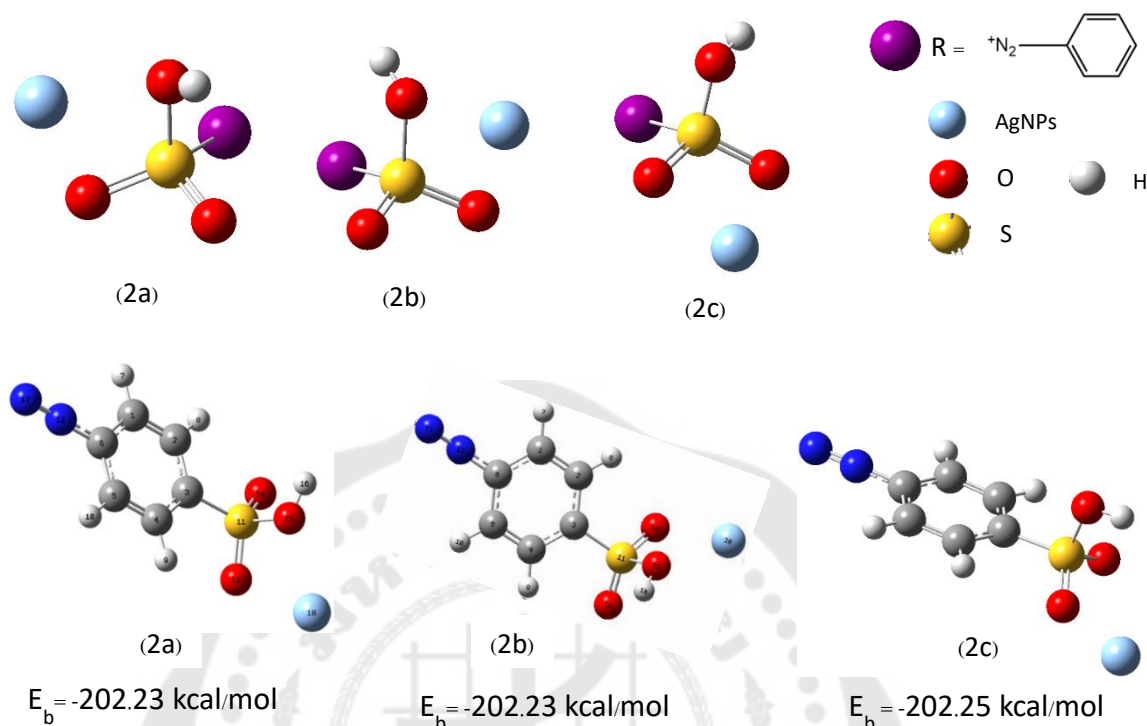


Figure 44. Interaction structure of Azo2 and AgNPs.

4.3.2. Optimized structure of Azo compounds and AgNPs

The optimized bond lengths and bond angles for the nine possible structure complexes of Azo1 compound and AgNPs were showed in Table 5. The Ag–N, Ag–O57 and Ag–O58 bond length of the complexes were observed in the range of 3.31–3.50 Å, 2.54–3.44 Å and 2.4601–3.396 Å respectively. The bond angle of N–Ag–O57, N–Ag–O58 and O57–Ag–O58 were observed in the range of 46.91°–48.70°, 47.65°–48.02° and 46.74°–47.24°, respectively. In addition, the structures of nine complexes and binding energy of complexes were showed in Figure 45 that the binding energy of complexes were observed in the range of -17.62 to -32.08 kcal/mol.

The optimized bond lengths and bond angles for the nine possible structure complexes of Azo2 compound and AgNPs were showed in Table 6. The Ag–O_H, Ag–O57 and Ag–O58 bond length of the complexes were observed in the range of 3.23–

3.32 Å, 2.59–3.84 Å and 2.51–3.10 Å respectively. The bond angle of N–Ag–O57, N–Ag–O58 and O57–Ag–O58 were observed in the range of 47.49°– 47.74°, 43.49°– 47.97° and 40.91°– 54.43°, respectively. In addition, the structures of nine complexes and binding energy of complexes were showed in Figure 46. binding energy of complexes were observed in the range of -14.39 to -30.69 kcal/mol.



Table 5. The optimized structure of complexes between AgNPs and Azo compounds (E2Azo1) for bond length and bond angle

Structures	Angle (deg)	Bond Lengths (Å°)		
		Ag-N	Ag-O ₅₇	Ag-O ₅₈
1d	46.91	3.50	2.55	—
1e	48.01	3.35	—	2.52
1f	46.74	—	3.40	2.94
1g	47.69	3.35	2.54	—
1h	47.66	3.35	—	2.55
1i	47.24	—	3.44	2.46
1j	48.70	3.33	2.54	—
1k	48.03	3.31	—	2.57
1l	46.91	—	2.91	3.39

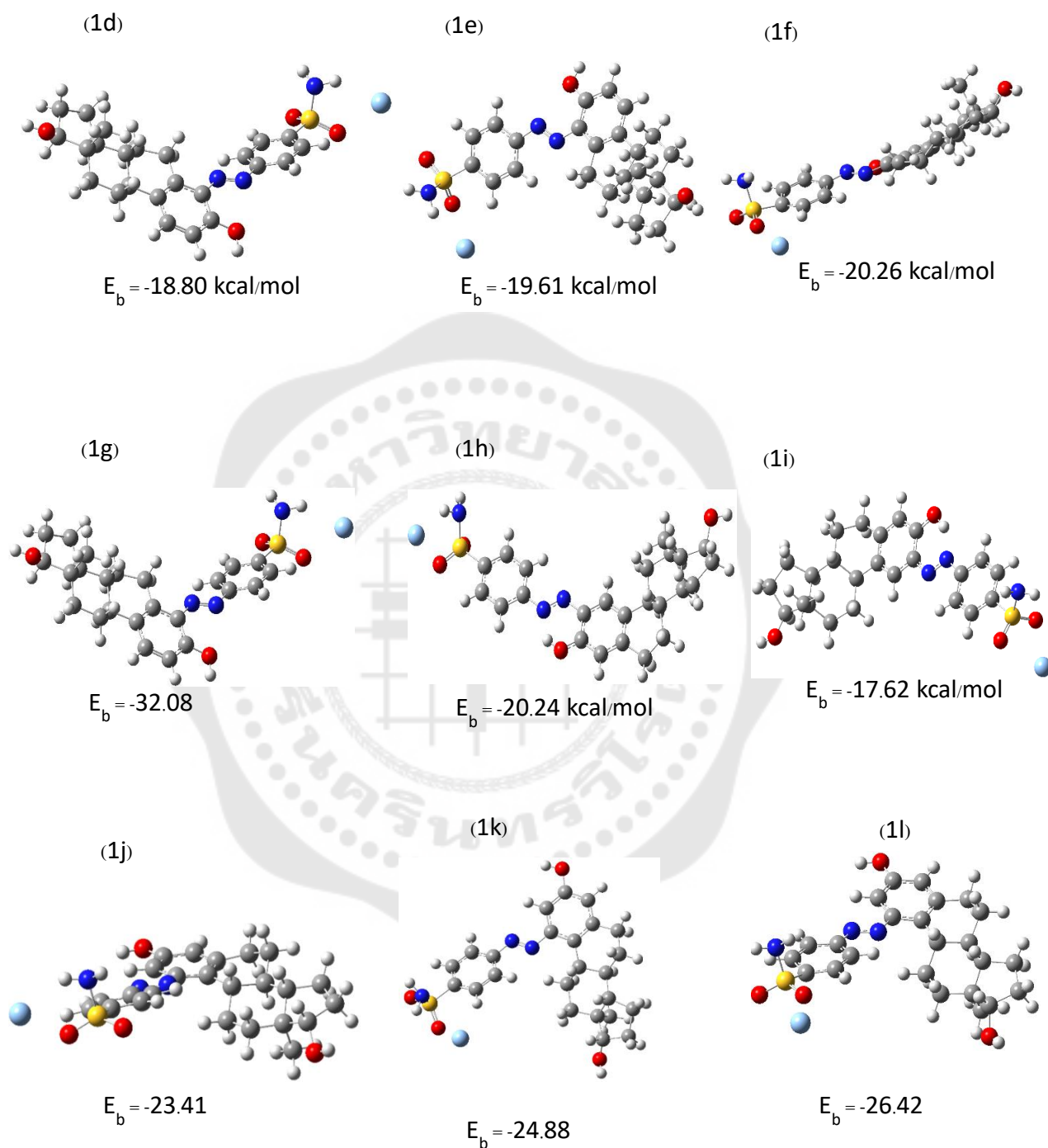


Figure 45 Interaction structure of Azo1 compounds and AgNPs.

Table 6. The optimized structure of complexes between AgNPs and Azo compounds (E2Azo2) for bond length and bond angle

Structures	Angle (deg)	Bond Lengths (Å ^o)		
		Ag-OH	Ag-O ₅₇	Ag-O ₅₈
2d	47.68	3.31	2.59	—
2e	47.97	3.23	—	2.52
2f	54.43	—	2.80	2.71
2g	47.74	3.31	2.59	—
2h	46.84	3.30	—	2.58
2i	40.92	—	3.84	2.52
2j	47.72	3.31	2.61	—
2k	43.49	3.31	—	2.54
2l	46.77	—	3.30	3.10

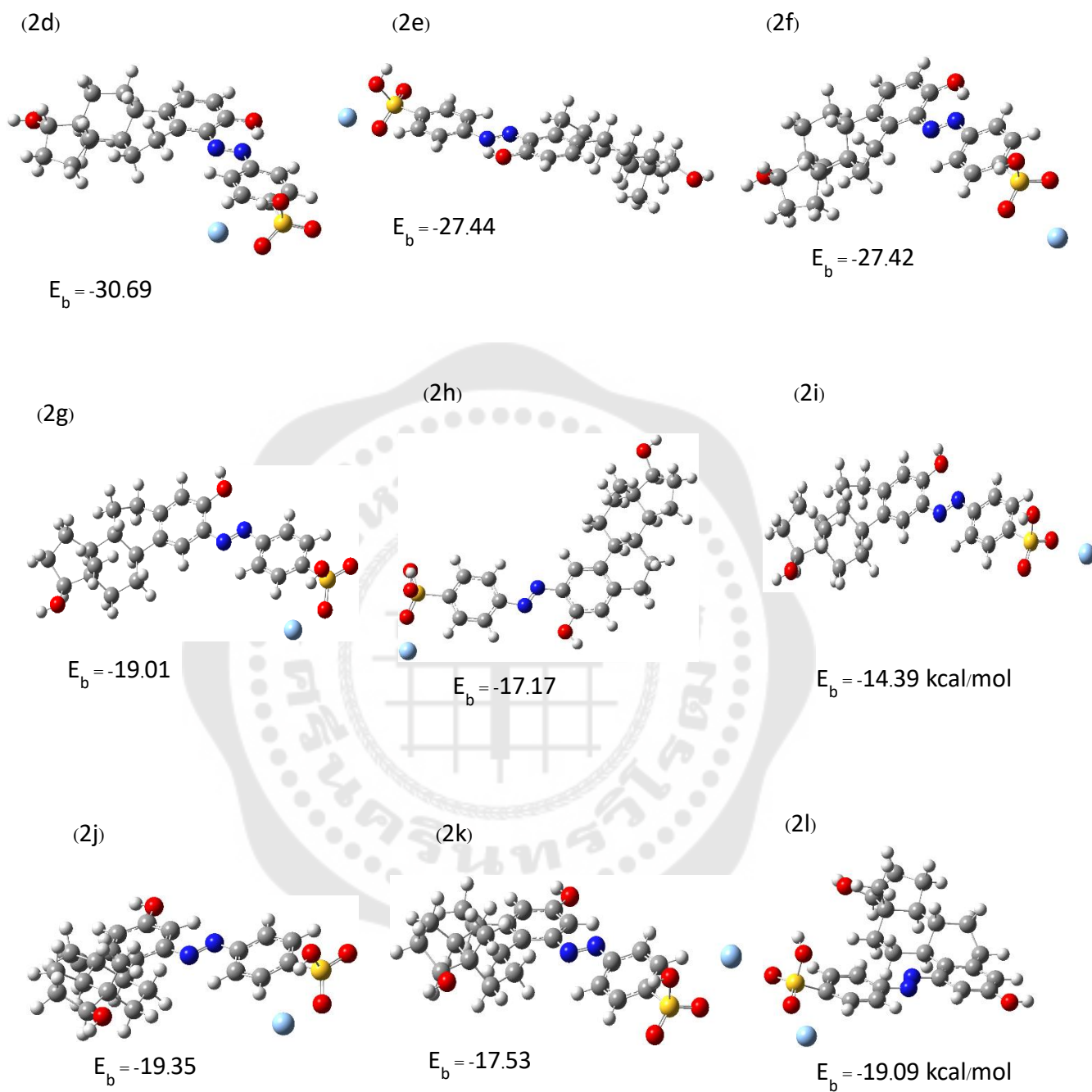


Figure 46 interaction structure of Azo2 compounds and AgNPs.

CHAPTER 5

SUMMARY DISCUSSION AND SUGGESTION.

In this study, the modified synthesis of AgNPs by silver nitrate using sodium borohydride were used. It showed strong absorption peak at 395 nm corresponding to the size of AgNPs 10-15 nm. In addition, both of synthesized Diazonium salt solution Azo1 and Azo2 have strong peak at 350 nm and they were stable in acid condition. Interaction of AgNPs with Azo1 was observed red shift with 1:1 ratio of M:L but interaction of AgNPs with Azo2 was observed blue shift with 1:2 ratio of M:L. This information was useful for application as phenol group and hormone detection.

Studies of 17β -estradiol interactions were compared between diazonium ligands, mixture of silver ion with diazonium and modified AgNPs with diazonium ligands. They were shown both of Azo1 and Azo2 react with 17β -estradiol, giving a yellowish color and changing the absorbance spectrum. But in the experimental mixture of silver ion and diazonium solution, it was found that the experimental results were the same as experiment with only diazonium solution. That was assumed for adding silver ions to the solution had no effect on the reaction between diazonium and 17β -estradiol.

In interaction test between AgAzo1 and 17β -estradiol, it was found precipitation when adding more 17β -estradiol concentration at high level. After measuring the FT-IR spectrum of the result showed the coupling reaction of diazonium and estrogens of azo compounds. When considering the absorption spectra, it was observed blue shifted of modified AgNPs with diazonium salt (AgAzo1) absorption spectra for 405 nm to 395 nm. It was decreased absorption when adding the increased concentration of 17β -estradiol was increased. After 17β -estradiol solution were added to AgAzo1 for 30 minute, it was observed the precipitation in mixture form as shown in figure 37. After adding standard 25 ppm of 17β -estradiol. From this, it indicated that after 17β -estradiol solution was added to the AgAzo1 solution, the diazonium ligand on the surface of the silver nanoparticles were released and reacted with phenol group of 17β -estradiol.

In interaction test between AgAzo2 and 17 β -estradiol. It was different from changed like AgAzo1. The spectrum was observed new absorption peak at 350 nm. From results, absorbance is assumed to be a result of the coupling reaction between the diazonium compounds and 17 β -estradiol. From computational calculations, it was found that the energies of the complexes AgAzo2 were lower than AgAzo1 indicated the reaction coupling reaction with 17 β -estradiol of AgAzo2 were more difficult than that of AgAzo1.

Structures of azo compounds were performed by coupling reaction between diazonium compounds and 17 β -estradiol. The binding energy were investigated by the density functional theory method using B3LYP functional with the 6-311g (d,p) basis set. The results of the calculation showed that the substitution reaction on the 2nd position of the estrogen phenyl gave the lowest energy of both Azo1 and Azo2. Calculation energy of structure were -194.79 and -189.36 kcal/mol, respectively. Because the 2nd position has effects from the substituent group including phenol group and CH₂ at 6th position. which affected to the 2nd position react easily. Moreover, the steric effect form 17 β -estradiol is lower than other positions.

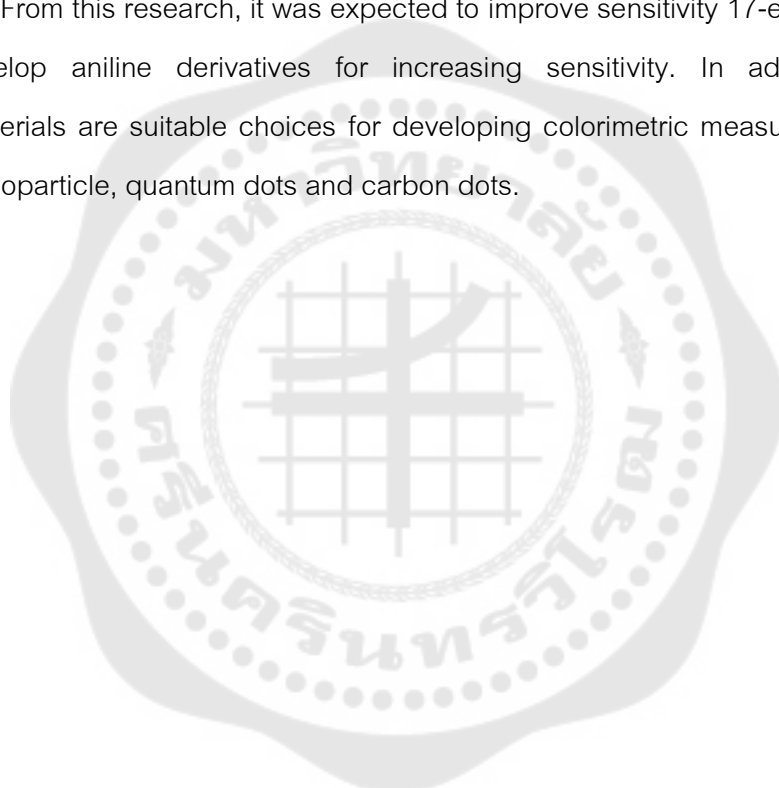
Complexes structures of Azo1 and AgNPs was showed changing of all complexes to 3rd position and calculation energy of three structures were -45.24 kcal/mol. That was indicated stable complex structures between AgNPs and Azo1. Complexes structures of Azo2 and AgNPs was not changing like Azo1 complexes. Calculation energy of Azo2 complexes were -202.23 to -202.25 kcal/mol. The results indicate that the Azo2 complexes structures can occur in all three positions.

Interaction between AgNPs and Azo1 compounds were showed binding energy value in the range of -17.62 to -32.08 kcal/mol. The binding energy value were compared with complexes of AgNPs and Azo1. It was found increasing of binding energy in complexes of AgNPs and Azo1 compounds. That results of this experiment indicated structure of AgNPs and Azo1 compounds can be difficult to form. Interaction between AgNPs and Azo2 compounds were showed binding energy value in the range of -14.39 - -30.69 kcal/mol. The binding energy value were compared with complexes of

AgNPs and Azo2. It was similaraly as the structure of AgNPs and Azo1 compounds. Therefore, UV titration and the computational chemistry results were confirmed about the possible structures but it cannot be applied to detection estrogens because the interaction between AgNPs and Diazonium compounds were stronger than interaction between AgNPs and Azo compounds. As a result, interactions between AgNPs and Azo compounds were difficult to occur.

Suggestion

From this research, it was expected to improve sensitivity 17-estradiol detection by develop aniline derivatives for increasing sensitivity. In addition, changing nanomaterials are suitable choices for developing colorimetric measurements such as gold nanoparticle, quantum dots and carbon dots.



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