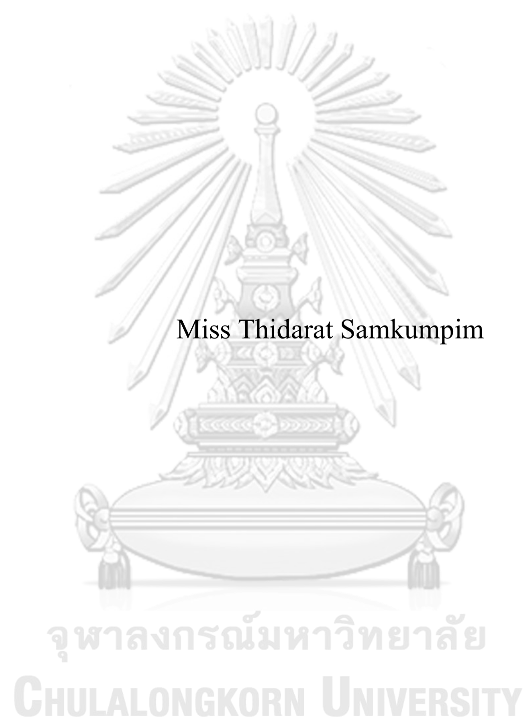
APPLICATION OF OXYGEN SCAVENGER IN GEL ELECTROMEMBRANE EXTRACTION FOR DETERMINATION OF NITRITE AND NITRATE



Miss Thidarat Samkumpim

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Chemistry

Department of Chemistry

FACULTY OF SCIENCE

Chulalongkorn University

Academic Year 2021

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การประยุกต์สารกำจัดออกซิเจนในการสกัดด้วยเจลอิเล็กโทรเมมเบรนสำหรับการตรวจวัดไนไทรต์
และไนเทรต

น.ส.ธิดารัตน์ สามคุ้มพิมพ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	APPLICATION OF OXYGEN SCAVENGER IN GEL ELECTROMEMBRANE EXTRACTION FOR DETERMINATION OF NITRITE AND NITRATE
By	Miss Thidarat Samkumpim
Field of Study	Chemistry
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ธิดารัตน์ สามคุ้มพิมพ์ : การประยุกต์สารกำจัดออกซิเจนในการสกัดด้วยเจลอิเล็กโทรเมมเบรนสำหรับการตรวจวัดไนไทรต์และไนเทรต. (APPLICATION OF OXYGEN SCAVENGER IN GEL ELECTROMEMBRANE EXTRACTION FOR DETERMINATION OF NITRITE AND NITRATE) อ.ที่ปรึกษาหลัก : รศ. ดร.ปกรณ์ วรานุศุภากุล

การสกัดด้วยเจลอิเล็กโตรเมมเบรนเป็นวิธีการเตรียมตัวอย่างที่มีประสิทธิภาพวิธีหนึ่งสำหรับตัวอย่างที่เป็นไอออนการสกัดด้วยเจลอิเล็กโตรเมมเบรนประกอบไปด้วยชั้น donor phase และชั้น acceptor phase ซึ่งทั้งสองชั้นถูกแบ่งด้วยชั้นอะกาโรสเจลเมมเบรน การเคลื่อนที่ของไอออนตัวอย่างเป็นผลมาจากการให้ศักย์ไฟฟ้าจากภายนอก ถึงแม้ว่าการสกัดด้วยเจลอิเล็กโตรเมมเบรนมีข้อดีหลายประการแต่การสกัดด้วยเจลอิเล็กโตรเมมเบรนเมื่อมีการให้ศักย์ไฟฟ้าจากภายนอก ที่ขั้วแอโนดเกิดปฏิกิริยาไฮโดรไลซีสของน้ำทำให้น้ำเกิดการแตกตัวได้ออกซิเจนและไฮโดรเจนไฮโดรเจน ซึ่งมีผลต่อไอออนที่มีความว่องไวในการทำปฏิกิริยากับออกซิเจนเช่น ไนไทรต์ เป็นต้น งานวิจัยชิ้นนี้ได้มีการศึกษาการใช้สารกำจัดออกซิเจนในชั้น acceptor phase เพื่อลดผลของการรบกวนของออกซิเจนในการสกัดไนไทรต์และไนเทรตด้วยเจลอิเล็กโตรเมมเบรน สารกำจัดออกซิเจนที่ใช้ในการศึกษาได้แก่ ผงเหล็ก (Iron), วิตามินซี (ascorbic acid), ซัลไฟต์ (sulfite) และไบซัลไฟต์ (bisulfite) และวิเคราะห์ปริมาณไนไทรต์และไนเทรต ด้วยเทคนิคไอออนโครมาโทกราฟี จากการศึกษาพบว่าสารละลายผสมระหว่างซัลไฟต์ และไบซัลไฟต์ ที่ความเข้มข้น 14 มิลลิกรัมต่อลิตร มีประสิทธิภาพในการช่วยป้องกันการเกิดการออกซิไดส์ของไนไทรต์ เมื่อศึกษาสภาวะที่เหมาะสมสำหรับการสกัดด้วยเจลอิเล็กโทรเมมเบรน สามารถใช้วิธีนี้ในการวิเคราะห์ไนไทรต์และไนเทรตในช่วงความเข้มข้น 10 – 200 ไมโครกรัมต่อลิตร มีค่าขีดจำกัดในการตรวจวัด (LOD) เท่ากับ 12 และ 13 ไมโครกรัมต่อลิตรของไนไทรต์และไนเทรตตามลำดับ นอกจากนี้ยังสามารถนำไปประยุกต์ใช้ในการวิเคราะห์หาปริมาณไนไทรต์และไนเทรตในตัวอย่างไส้กรอกและน้ำได้ในช่วงความเข้มข้นที่ต่ำ

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6370009223 : MAJOR CHEMISTRY

KEYWORD: Gel electromembrane microextraction; Nitrite and Nitrate; Oxygen scavenger; Ion chromatography

Thidarat Samkumpim : APPLICATION OF OXYGEN SCAVENGER IN GEL ELECTROMEMBRANE EXTRACTION FOR DETERMINATION OF NITRITE AND NITRATE . Advisor: Assoc. Prof. Dr. PAKORN VARANUSUPAKUL

Gel electromembrane microextraction (G-EME) have been proven as a promising extraction method due to its effectiveness for extraction and preconcentration of charged analytes and its compliance with the principle of green chemistry. G-EME consists of two phases (donor and acceptor phases), separated by a gel membrane made of agarose. The driving force is the electrical potential applied across the membrane. Despite of all the advantages, the common problem was the oxygen generated in the acceptor solution due to the electrolysis of water that could affect the analysis of easily oxidized analyte species such as nitrite/nitrate species. In this work, application of oxygen scavenger to the acceptor solution has been attempted and studied to prevent or minimize this effect in G-EME for determination of nitrite and nitrate ions. Several oxygen scavengers such as ascorbic acid, iron, sulfite, and bisulfite were tested and examined. Ion chromatography was used for quantitative analysis of nitrite and nitrate ions. The mixture of sulfite and bisulfite (14 ppm) in the acceptor solution could prevent the oxidation of nitrite to nitrate effectively. After optimizing all the parameters, the method was successfully applied for speciation of nitrite and nitrate in water samples giving the linear calibration curves in the range of 10 to 200 µg L^{-1}, the limits of detection were 12 µg L^{-1} and 13 µg L^{-1}, respectively, for both nitrite and nitrate ions. This method was successfully applied to the simultaneous determination of nitrite and nitrate spiked into sausage and water samples at the sub-ppm range.

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Field of Study:	Chemistry	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....

ACKNOWLEDGEMENTS

This thesis could not have been completed without the assistance and input from numerous individuals. I would like to thank my supervisor, Associate Professor Dr. Pakorn Varanusupakul who give my opportunity to join in PV lab and made this work possible. His kind guidance helped me in all the time of research and writing of this thesis.

I also would like to thank my brother Dr.Waleed Alahmad and Mr. Ali Sahragard who support and give me advise for work all the time

Finally, I would like to thank my family and friend for supporting my mental health

Thidarat Samkumpim

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ABBREVIATION

μL	microliter
mL	mililiter
mm	millimetre
rpm	revolutions per minute
M	molar
V	voltage
LOD	limit of detection
LOQ	limit of quantification
IC	Ion chromatography
UV-VIS	UltraViolet-Visible Spectroscopy
EF	Enrichment factor
EE	Extraction recovery
PA	Peak area
μS	Microsiemens
NO_2^-	Nitrite
NO_3^-	Nitrate
G-EME	Gel Electromembrane microextraction
G-EME-IC	Gel Electromembrane microextraction with Ion chromatography
EOF	Electroosmotic flow
SD	Standard deviation
RSD	Relative standard deviation
RR	Relative recovery

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R^2	Coefficient of determination

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Chapter 1 Introduction

1. Introduction

Since the introduction of electromembrane microextraction (EME) in 2006 [1], it has been a significant point of research focus within the analytical chemistry community due to its suitability for selective extraction of ionizable compounds from complex matrices such as biological [2, 3], food [4], and environmental samples [5-7]. In EME, the clean acceptor solution is totally separated from a sample solution by means of a supported liquid membrane (SLM), comprising a water-immiscible (hydrophobic) organic solvent immobilized in the pores of a supporting polymeric membrane, while the driving force is the electrical potential applied across the membrane [8, 9]. Recently, several attempts were reported to replace the organic-based SLM with a green membrane to comply with the principle of green chemistry principles. Gel membranes based on renewable and biodegradable materials have been considered an alternative to petroleum-based materials [10, 11]. Agarose, a natural polymer, is undoubtedly one of the most well-known examples that has been used to prepare a gel membrane [11]. The early use of agarose in EME was proposed by Hidalgo et al. to extract some non-steroidal anti-inflammatory drugs such as salicylic acid [12]. Later on, this technique has been widely used to extract different analytes in various complex matrices [13-17]. The gel electromembrane extraction (G-EME) technique provides high degree of clean-up and selectivity with high enrichment factor. In addition, G-EME uses environmental friendly material with miniaturized setup and requires low volume of chemical consumption, which are complied with green chemistry principles. Despite all the advantages, the common problem was the oxygen generated in the acceptor solution due to the electrolysis of

water that could affect the analysis of easily oxidized analyte species such as iodide species, reported in the previous study [18]. As a result, the oxidization of iodide to iodate led to inaccurate determination of iodide content in samples, and therefore the total iodine (iodide ands iodate) was reported. For this reason, this problem should be resolved, especially for speciation study. Oxygen scavengers have been widely used to help remove or decrease oxygen content in food packages or water systems [19, 20]. Oxygen scavengers could absorb (react with) the dissolved oxygen or oxygen gas in the headspace leading to extended shelf life and retainment of the original food quality. Several types of oxygen scavengers have been used, such as sulfite [21, 22], iron [23, 24], bisulfite [25, 26], and ascorbic acid [27, 28]. Inspired by the above works, along with the need to solve the generated oxygen problem, addition of oxygen scavengers to the acceptor solution was examined to prevent or minimize the oxidization of easily oxidized species during the EME process. Nitrite and nitrate species were chosen as a model for this study. Different oxygen scavengers such as ascorbic acid, iron, sulfite, and bisulfite were tested and examined. Ion chromatography was used for quantitative analysis of nitrite and nitrate ion. Finally, the developed extraction system was evaluated and applied to simultaneously determine nitrite and nitrate in sausage and drinking water samples.

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2. Objectives

1. Study the application of oxygen scavengers in gel electromembrane extraction for the analysis of easily oxidized species such as nitrite and nitrate

2. Apply the developed method for determination of nitrite and nitrate in food samples

3. Assumptions

Gel electromembrane extraction (G-EME) technique with oxygen scavenger in acceptor phase can reduce the effect of generated oxygen in extraction and determination of nitrite and nitrate ion. The developed method also can be applied to the determination of nitrite and nitrate ion in real samples (sausage and drinking water).

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Chapter 2 Theory and literature review

1.Sample preparation

Sample preparation is one of the most important steps in an analytical method prior to determination by an analytical technique or instrument because the matrix components and interferences may alter the signal of the analyte of interest either suppression or enhancement the signal leading to false negative or false positive results. It is important to report the results with confidence in terms of accuracy and precision with a certain and desired limit of detection [29]. Sample preparation may generally involve pretreatment and extraction steps. Pretreatment is the step that analytes are separated from the sample matrix components while extraction is the step that analytes are transferred or isolated or even preconcentrated into a solvent or solution that is compatible and detectable with an analytical instrument.

2. Extraction techniques

Extraction is the most common strategy in analytical sample preparation procedures to isolate, clean up, and preconcentrate analytes of interest from the sample matrix into the final solvent or solution prior to proceeding to analytical separation and/or detection instrumentation. Typically, in extraction techniques, the extracting phase (extractant) comes into contact with the sample matrix where the analytes could be transferred into the extracting phases. [30]

2.1. Microextraction techniques

Conventional sample preparation techniques such as liquid-liquid extraction, cloud point extraction, soxhlet extraction, coprecipitation, and solid phase extraction usually involve many experimental processes. These techniques have lost their popularity due to their disadvantages, such as expensive laboratory equipment, time consuming processes, use of chemicals that are harmful to human health and the

environment and need a trained technician [31]. One of the most important efforts is the development of sustainable micro sized sample preparation techniques that use less harmful or harmless chemicals, reduce energy consumption and working time, and are environmentally friendly. Sample treatment has evolved following six general trends, namely, simplification, automation, miniaturization, expeditiousness, economical aspects, and safety aspects [32]. Solid phase microextraction techniques (SPME) that is miniaturized solid phase extraction (SPE) methods, and liquid phase microextraction techniques (LPME) that is miniaturized liquid phase extraction (LPE) methods have been introduced and well responded to those requirements that in some cases are unattainable with classical techniques and have become more popular these days.

2.1.1. Hollow-fiber microextraction (HF-LPME)

Hollow fiber microextraction (HF-LPME) was developed to improve stability of the drop in single drop microextraction (SDME). The microvolume of the extracting solvent is impregnated in the pores of the membrane known as a supported liquid membrane (SLM) where the transfer of analyte takes place and also filled in the lumen of a microporous hollow fiber as an acceptor phase. HF-LPME has variety of configurations (rod like, u-shaped, hollow-fiber solvent bar, and knotted hollow-fiber) depending on the physicochemical characteristics of analytes and levels of complexity of the sample.[33] HF-LPME can be categorized into two phase and three-phase mode (Fig. 1). In two phase mode, the extracting phase is impregnated in the pores and the same solvent is filled in the lumen of the fiber as the acceptor phase. In three-phase mode, the extracting solvent is impregnated in the pores and the different acceptor phase is filled in the lumen of the fiber. The two-phase HF-LPME is usually

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applied to volatile and semi volatile organic analytes that can be analyzed with gas chromatography (GC) while the three-phase HF-LPME is usually applied to less volatile and relatively large organic compounds, dissociated compounds (acidic and basic compounds) and ionic compounds including metal ions that can be analyzed with High Performance Liquid Chromatography (HPLC), Capillary electrophoresis (CE), electrochemical methods and spectrometric methods.

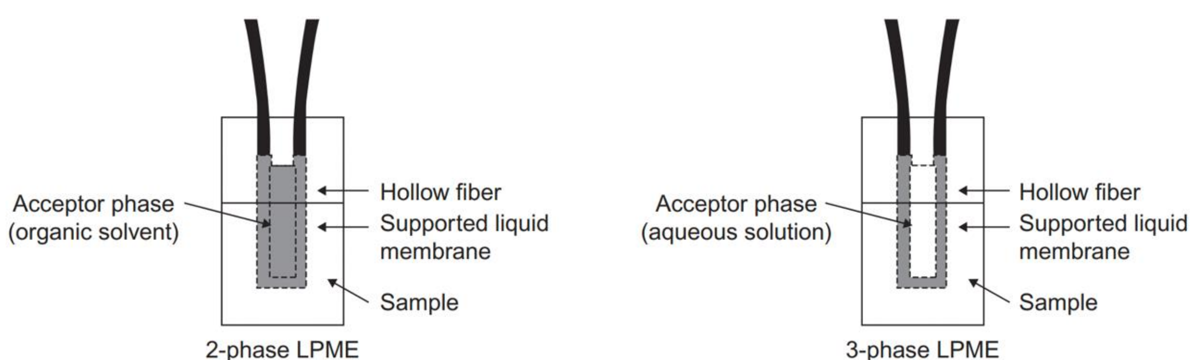

Figure 1 Schematic diagram of two-phase and three-phase HF-LPME [31]

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2.2. Electromembrane extraction (EME)

The disadvantage of HF-LPME is the relatively slow extraction because the mass transfer typically is based on passive diffusion due to concentration gradient. Electromembrane extraction (EME) was developed to improve mass transfer problem by Pedersen-Bjergaard in 2006. The movement of analytes is based on electrokinetic migration of charged analytes from the sample solution (donor phase) passing through the SLM into an acceptor solution [34]. In EME, analytes must be in charged form or ionized species. Sometimes, the pH of both donor phase and acceptor phase must be properly adjusted to ensure the ionization of charged analytes. The analytes are extracted selectively with their charged forms migrating through SLM towards their respective electrode of opposite charge where the mass transfer can be enhanced by the influence of an electrical field. The general setup for EME consists of two

electrodes (usually platinum wires) that connected to a direct current (DC) power supply; one electrode is placed in the acceptor phase into the hollow fiber lumen and the second electrode is inserted into the sample solution (Fig.2) [33]. The advantages of EME are high selectivity, rapid extraction process, low costs, high extraction efficiency, less consumption of organic solvents, and low sample volumes. The disadvantages of EME are that two (or more) chemically inert electrodes and an external source of high voltage are required, which increase instrumental complexity of the EME set-up. Moreover, since a high voltage is applied to the electrodes located directly in the donor and the acceptor solutions, electrolysis of water could play a significant role in EME process. Bubble formation, electrolytically-induced changes in pH of operational solutions and double layer build-up at phase interfaces could have direct effect on EME performance and eventually result in collapse of the EME system.

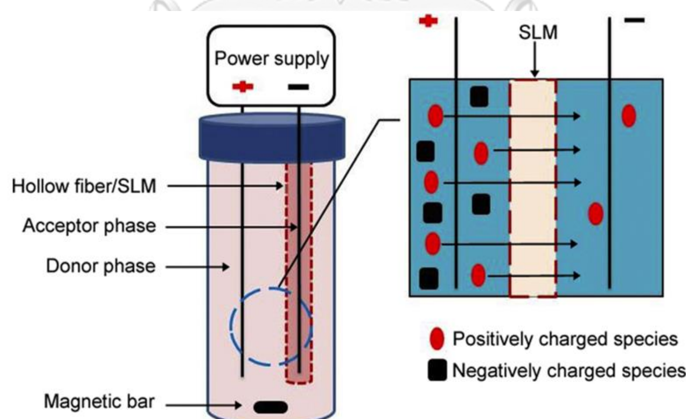


Figure 2 Schematic mechanism for electromembrane extraction [33]

2.3. Gel electromembrane extraction (G-EME)

To improve efficiency of EME, alternative SLM have been developed. Recently, several attempts were reported to replace the organic-based SLM with a green membrane to comply with the principle of green chemistry. New EME using

biopolymeric gel membrane has been introduced so called gel-EME (G-EME) has been introduced. G-EME approach is considered green analytical technique because the gel could be prepared from renewable and biodegradable resources and no need to use organic solvent. Agarose gel is commonly used in EME. Agarose is polysaccharide that consists of a hydrophilic polymeric network with a flexible pore-size [35]. Agarose is suitable for EME because it is stable in wide range of pH and temperatures. Agarose is easy to prepare and easy to change structure after solidified [36]. Similarly to EME, the setup of G-EME consists of two phases (donor and acceptor phases), separated by a gel membrane made of agarose (Fig. 3). The driving force is the electrical potential applied across the membrane. G-EME technique has several advantages including using environmental-friendly material, providing high enrichment factor with high degree of clean-up and selectivity, requiring low sample volumes, and a miniaturized extraction setup.

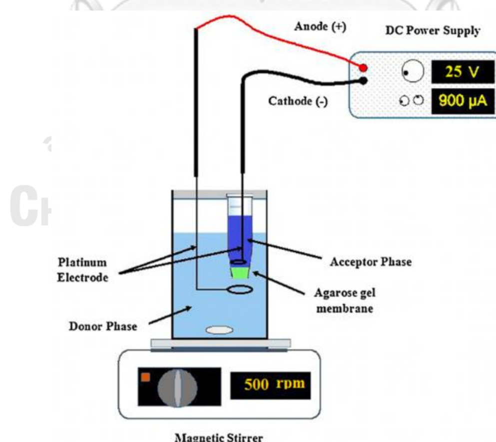


Figure 3 Schematic illustration of EME setup [36]

2.3.1. Electroosmotic flow (EOF) problem in Gel electromembrane extraction

Electroosmotic flow (EOF) is a phenomenon that occurs when an electrical potential is applied to a solution inside a capillary column (in CE) or in this case,

inside the pores of the gel membrane (in G-EME). After applied voltage, charged ions will move towards an electrode with opposite charge. Inside the agarose gel that contains negatively charged sulfate group, positively charged ions in the aqueous solution would accumulate on the negatively charged inner surface inside the pores of the gel forming a layer of cations (Fig.4) on the pore wall [37]. The bulk positive layer could move towards the cathode (negative electrode) and the negative ions could move towards the anode (positive electrode). The water that solvates the layer of positive ion could also move with the same direction of the bulk positive layer resulting in increase of the volume of the solution (dilution) where the cathode is inserted while the volume of the solution where the anode is inserted would be decreased (Fig.5).

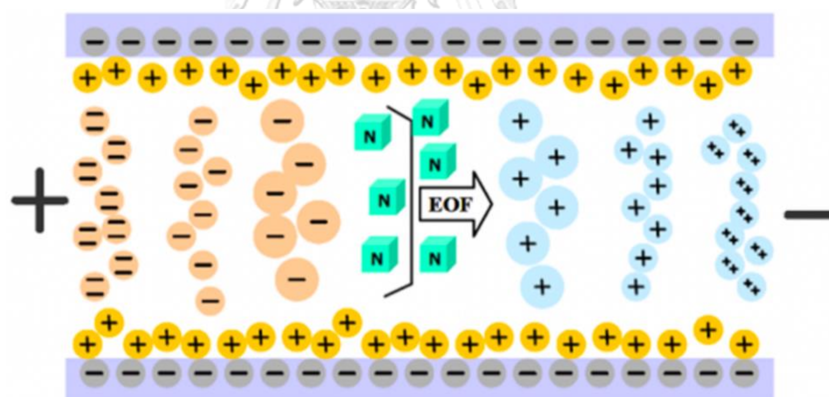


Figure 4 Schematic illustration of Electroosmotic flow (EOF) formation [37]

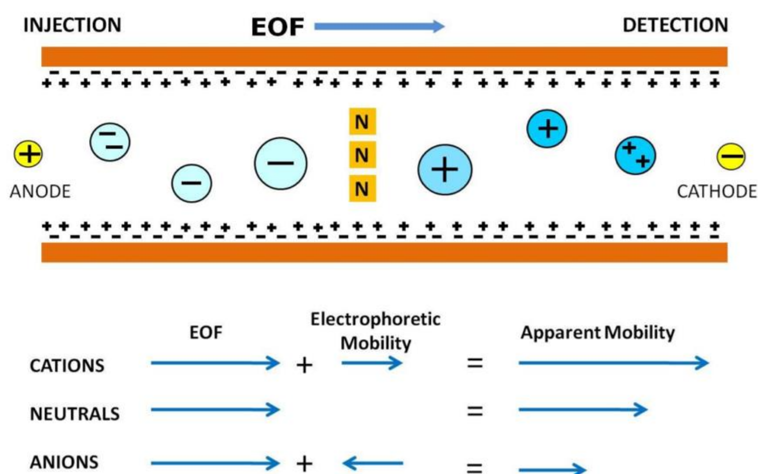
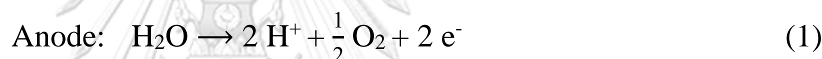


Figure 5 Schematic illustration of direction of Electroosmotic flow (EOF) [38]

2.3.2. Electrolysis problem in Gel electromembrane extraction

In G-EME, when an electrical potential is applied to the aqueous solution, electrolysis of water could occur at both electrodes (eq.1-2) resulting in the occurrence of bubble gas.

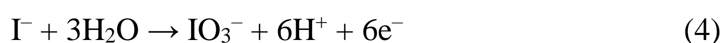


$$\text{Anode:}\quad H_2O \longrightarrow 2\,H^+ + \frac{1}{2}\,O_2 + 2\,e^- \qquad (1)$$

$$\text{Cathode:}\ 2\,H^+ + 2\,e^- \longrightarrow H_2 \qquad (2)$$

The formation of bubble gas (hydrogen gas at cathode and oxygen gas at anode) may affect the mass transfer of analytes as well as the collection of the acceptor solution for further analysis. Moreover, the generation of oxygen from electrolysis could affect the determination of easily oxidized species such as nitrite (eq.3) and iodide ions (eq.4) due to the oxidation process.

$$NO_2^- + O_2 \rightarrow 2NO_3^- \qquad (3)$$



$$I^- + 3H_2O \rightarrow IO_3^- + 6H^+ + 6e^- \qquad (4)$$

3. Oxygen scavenger

Oxygen scavenger also known as an oxygen absorber have been widely used to help remove or decrease oxygen content in food packages or water systems (Fig. 6). Oxygen scavenger is a chemical substance that is used to reduce or remove oxygen in fluids and enclosed spaces. Oxygen scavengers are categorized as metallic, non-

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metallic and enzymatic oxygen scavenger such as iron powder, ascorbic acid, photosensitive polymers, enzymes, etc. The main purpose of oxygen scavenger is to limit the amount of oxygen that can lead to reduced functionality of many types of products. For industry, oxygen scavenger is used to prevent oxygen-induced corrosion in oil and gas production installations. For pharmaceutical products and many foods, oxygen scavenger is used to extended shelf life and retainment of the original food quality by reducing the rate of lipid oxidation, aerobic microorganism growth and enzymatic browning [19, 20].

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Figure 6 Schematic illustration of oxygen scavengers

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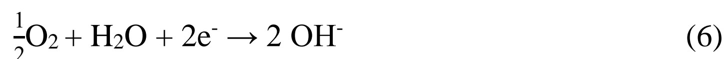
3.1. Iron

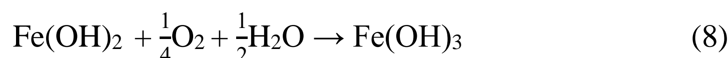
Iron (Fe) is one of common oxygen scavengers that use in food and pharmaceuticals industry. Iron could react with oxygen in the presence of water as summarized in eq. 5-8 [39]. The oxidation reaction of iron is slow. Water is one of catalyst that can accelerate these reactions.

$$Fe \rightarrow Fe^{2+} + 2e^- \quad (5)$$



$$\frac{1}{2}O_2 + H_2O + 2e^- \rightarrow 2\ OH^- \quad (6)$$

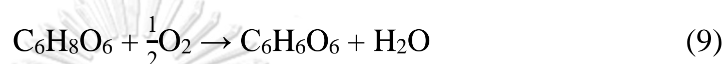
$$Fe^{2+} + 2\ OH^- \rightarrow Fe(OH)_2 \quad (7)$$



$$Fe(OH)_2 + \frac{1}{4}O_2 + \frac{1}{2}H_2O \rightarrow Fe(OH)_3 \quad (8)$$

3.2. Ascorbic acid

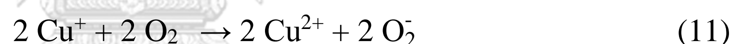
Ascorbic acid is a natural oxygen scavenging compound and use in food industry. Ascorbic acid is an antioxidant having relatively low reduction potential [40]. Ascorbic acid is readily oxidized by oxygen to dehydroascorbic acid as shown in eq 9.



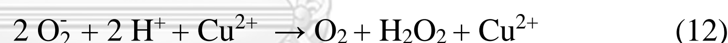
$$C_6H_8O_6 + \frac{1}{2}O_2 \rightarrow C_6H_6O_6 + H_2O \quad (9)$$

Oxidation reaction of ascorbic acid is slow [41]. To accelerate this reaction, transition metal can be used to catalyed this reaction. Copper is a common metal for accelerating oxidation reaction of ascorbic acid as summarized in eq. 10-13.

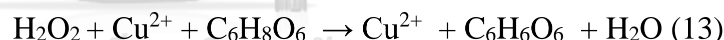
$$C_6H_8O_6 + 2\ Cu^{2+} \rightarrow C_6H_6O_6 + 2\ Cu^{+} + 2\ H^{+} \quad (10)$$



$$2\ Cu^{+} + 2\ O_2 \rightarrow 2\ Cu^{2+} + 2\ O_2^{-} \quad (11)$$



$$2\ O_2^{-} + 2\ H^{+} + Cu^{2+} \rightarrow O_2 + H_2O_2 + Cu^{2+} \quad (12)$$



$$H_2O_2 + Cu^{2+} + C_6H_8O_6 \rightarrow Cu^{2+} + C_6H_6O_6 + H_2O \quad (13)$$

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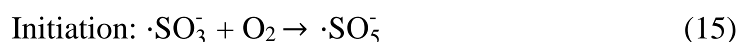
3.3. Sulfite and Bisulfite

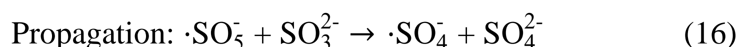
Sulfite is used to consume dissolved oxygen in water systems by reacting with dissolved oxygen to form sulfate as shown in eq. 14. [42] Sulfite use for reduce corrosion of steel in industry.

$$2SO_3^{2-} + O_2 \leftrightarrow 2SO_4^{2-} \quad (14)$$

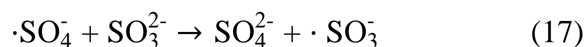
The sulfite oxidation is free radical reaction involving 3 steps [43].



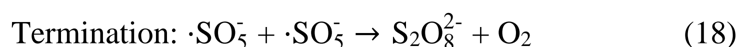
$$\text{Initiation: } \cdot SO_3^{-} + O_2 \rightarrow \cdot SO_5^{-} \quad (15)$$



$$\text{Propagation: } \cdot SO_5^{-} + SO_3^{2-} \rightarrow \cdot SO_4^{-} + SO_4^{2-} \quad (16)$$



$$\cdot SO_4^{-} + SO_3^{2-} \rightarrow SO_4^{2-} + \cdot SO_3^{-} \quad (17)$$



$$\text{Termination: } \cdot SO_5^{-} + \cdot SO_5^{-} \rightarrow S_2O_8^{2-} + O_2 \quad (18)$$

Bisulfite also effectively reduce amount of oxygen in s solution. The reaction is similar to sulfite as shown in eq. 19. [44]

$$HSO_3^{-} + O_2 \rightarrow 2SO_4^{2-} + H^{+} \quad (19)$$

4. Nitrite and Nitrate

Nitrate is a stable form of oxidized nitrogen which means it is stable and unlikely to change and cause harm. Although nitrate is stable, nitrate can be converted to nitrites by bacteria or enzymes that could be harmful [45]. Nitrite is relatively unstable and reactive [46]. Nitrite can react with secondary amines and amides forming nitrosamines (n-nitrosamines), some of which are considered carcinogenic compounds increasing the risk for cancer. High absorption of nitrite and nitrate leads to gastric cancer, thyroid cancer, and colorectal cancer. In pregnant women, nitrite can reduce ability of hemoglobin to exchange oxygen in the body tissues which can lead to methemoglobinemia (blue baby syndrome) and increase the risk of maim and miscarriage.

Sodium nitrate (E251) or potassium nitrate (252) and sodium nitrite (E250) or potassium nitrite (E249) are used in processed meat to inhibit the growth of microorganisms and also gives reddish color and flavor [47]. The permissible limit of nitrate and nitrite in meat products in Thailand are 500 and 125 mg kg^{-1}, respectively [48] and in drinking water are 45 and 3 mg L^{-1} , respectively [49].

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5. Ion Chromatography

Ion chromatography is used for analysis of common anions (such as fluoride, chloride, nitrite, nitrate, and sulphate) and common cations (lithium, sodium, ammonium, potassium, magnesium and calcium) in aqueous samples at ppm level. Ion chromatography separates ions based on electrostatic interaction of ions (charges and sizes) with ion exchange column (stationary phase) and the eluent (mobile phase) (Fig. 7). The conductivity detector is a common detector used in IC. It measures conductance that is proportional to the concentration of ions.

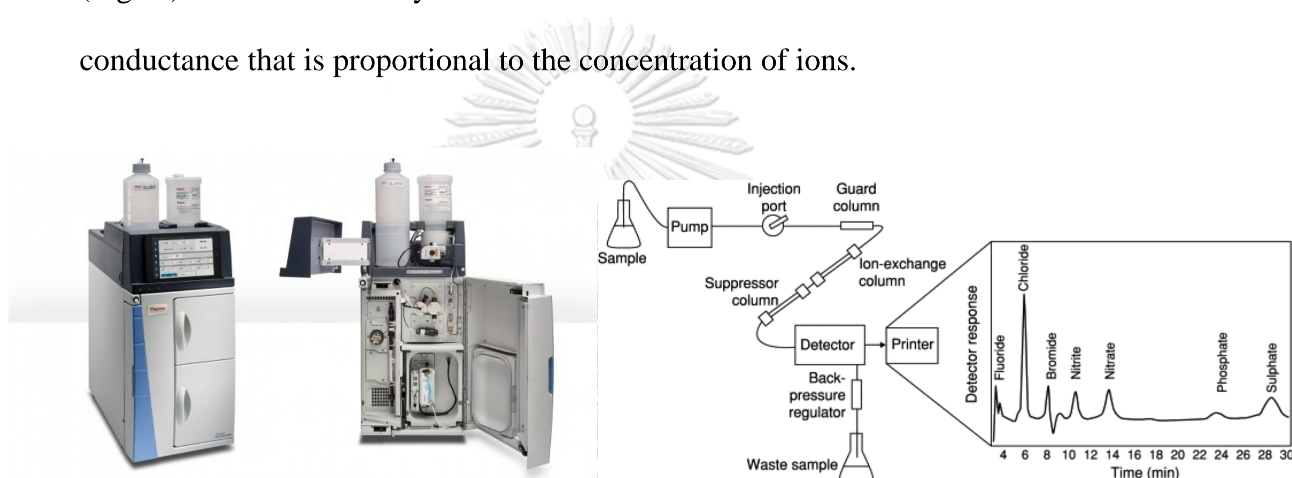

Figure 7 Schematic illustration of Ion Chromatography and Ion Chromatography diagram [50]

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6. Griess reaction

Griess Reagent is a classic protocol for the estimation of nitrite in biological samples. Nitrite is derivatized with sulfanilamide and N-(1-naphthyl)ethylenediamine (NED), resulting in formation of a redpink azo dye. In the case of nitrate, nitrate must be reduced to nitrite by cadmium (Cd) or zinc (Zn) , and then the Griess reaction can proceed. Griess reaction sequence begins with aromatic amine (e.g. sulfanilamide) that undergoes diazotisation in the presence of nitrite and an acid catalyst resulting in the formation of a reactive diazonium salt. Upon the addition of an electron rich

coupling agent (e.g. N-(1-naphthyl) ethylenediamine, NED), a highly coloured diazo dye is formed (Fig. 8). The absorption intensity of this dye is directly proportional to the nitrite concentration in the sample.

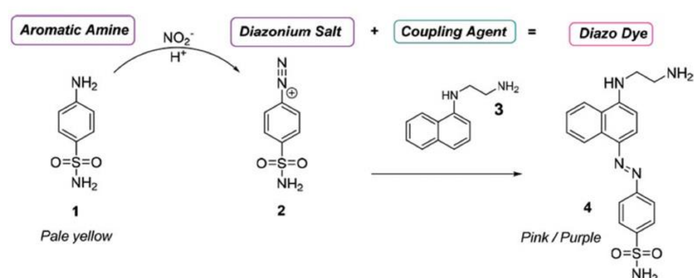

Figure 8 Schematic illustration of Griess reaction for the detection of nitrite in aqueous media [51]

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Chapter 3 Methodology

1. Materials and methods

1.1. Chemicals, samples, and instruments

All chemicals used in this study were analytical reagent grade. All solutions were prepared in Milli-Q water (Millipore, USA). Sodium nitrite, potassium nitrate, sodium sulfite and sodium bisulfite, chromotropic acid were purchased from Merck (Germany). Ceftazidime was purchased from TCI (Japan). Agarose gel was prepared from SeaKem LE Agarose (low electroendosmosis: 0.09 - 0.13; gel point [◦C]: 36 ± 1.5 at 1.5%; gel strength [g cm^{-2}]: >1200 at 1.0%). A benchtop pH meter with a glass electrode (Mettler Toledo, USA) was used to measure the pH of the solutions. GPR-11H30D benchtop DC power supply (GwInstek, China) with adjustable voltage within the range of 0 -110 V and a current range of 0-3 A was used to provide the necessary voltage. Aluminum sheet cut into strips with different widths were used as electrodes. Drinking water samples were purchased from supermarkets (Bangkok, Thailand). The pH samples and standards were adjusted by adding 0.1 mol L^{-1} sodium hydroxide and 0.1 mol L^{-1} hydrochloric acid solutions prior to extraction.

1.2. Fabrication of the agarose gel membrane

The agarose membrane was fabricated according to our previous works [18]. Briefly, 0.20 g of agarose powder was dissolved in 10 mL of Milli-Q water. The solution was heated in a microwave oven at 90 °C for 1 min. After that, 400 µL of the hot mixture was quickly transferred to a 1.5-mL Eppendorf tube (Hamburg, Germany) and placed in a refrigerator at 4 °C for 4 h. The gel was solidified in the lower half of the tube. The bottom conical part of the tube was cut with a razor blade to give the membrane thickness of 7 mm (optimized value). The upper empty half of the tube could be filled with the acceptor solution.

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1.3. Gel electromembrane extraction (G-EME) procedure

Fig. 9 shows the schematic setup for the gel electromembrane extraction system. First, 10 mL of the donor solution; standard/sample solution, adjusted to pH 7 was added to a 12 mL glass vial. The Eppendorf tube containing agarose gel membrane was placed having the bottom part in contact with the donor solution. The upper half of the Eppendorf tube was filled with 500 µL of the acceptor solution containing a certain concentration of oxygen scavenger. Next, the positive electrode (anode) was placed into acceptor solution at the upper part of the Eppendorf tube, while the negative electrode (cathode) was placed into the sample solution. Both were connected to the power supply to obtain the optimized voltage (30 V). During the extraction, the solution was stirred at a predetermined speed (600 rpm) for a specified amount of time (10 min). After the extraction was complete, the acceptor phase was collected using a micropipette and analyzed by an ion chromatography instrument. This procedure was repeated three times for each standard/sample, and each gel membrane was used only once to avoid memory effects.

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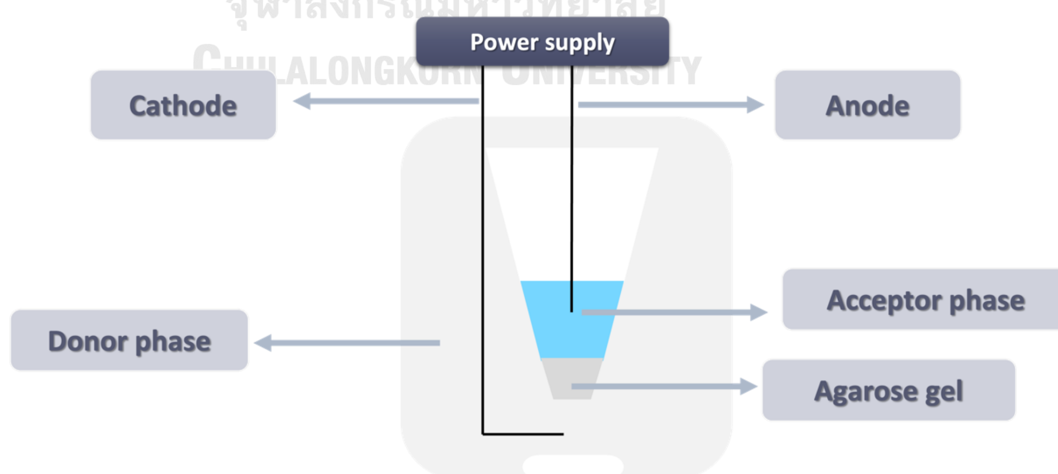

Figure 9 Schematic illustration of setup for the gel electromembrane extraction system

1.4. Ion chromatography conditions

A reagent-free ion chromatography system with eluent generation (RFIC-EG) (Dionex™ Integrion™ HPIC™ System, Thermo Fisher Scientific, USA) equipped with a conductivity detector was employed for the analysis of nitrite and nitrate ions. Potassium hydroxide (KOH) was used as an eluent, and its concentration was automatically generated and adjusted by the eluent generator system (Dionex EGC 500 KOH, /, USA). The gradient elution started with 10 mM KOH for 5 min, then increased to 50 mM KOH for 20 min, and maintained at 50 mM KOH for 7 min. The anion exchange column was Dionex IonPac AS19; 250 mm × 4 mm; 7.5 µm particle size (Thermo Fisher Scientific, USA) with guard column; Dionex IonPac AG19; 50 mm × 4 mm; 11 µm particle size (Thermo Fisher Scientific, USA). The electrolytically regenerated suppressor (Dionex AERS 500, Thermo Fisher Scientific, USA) was set at 124 mA. The injection volume was 25 µL, the flow rate was 1.0 mL min^{-1}, and column temperature was set at 30 °C. The total runtime was 27 min for each sample, including 5 min equilibration time. Chromeleon (Ver. 7) data processing software was used for data acquisition and quantification.

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1.5. Sample preparation for G-EME analysis

1.5.1. Sausage sample

Five grams of blend homogenized sausage sample were mixed with 100 mL of Milli-Q water and placed in water bath at 70 ◦C (± 5 ◦C) for 5 min. After cooling, the supernatant was filtered syringe nylon filter (13 mm; 0.45 µm; vertical). Finally, sample solution was ready for G-EME as described in section 2.3

1.6 Sample preparation for UV-Vis validation method (modified griess reaction)

In this work, modified griess reaction was used in this work [52]. Ceftazidime (aroma tic amine, 1000 mg L^{-}) was mixed with sample solution or nitrite solution in acidic condition (3 mol L^{-} HCI solution) to get yellow solution. After that, chromotropic acid as the coupling reagent was added. A pink solution was formed after 10 minutes and measured for nitrite concentration by absorption intensity from uv-vis spectrometer at wavelength of 513 nm.

For detection nitrate, Zn dust was mixed with sample solution to reduce nitrate to nitrite before using modified Griess reaction.

2. Calculations

Enrichment factor (EF) was calculated based on the following equation (20):

$$EF = \frac{C_{a,\ final}}{C_{d,\ initial}} \qquad (20)$$

where $C_{a,\ final}$ is the final concentration of analyte in the acceptor (obtained from the calibration curve), and $C_{d,\ initial}$ is the initial analyte concentration in the donor solution.The value of ER% was calculated according to the following equation for each analyte:

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$$ER = \frac{n_{a,\ final}}{n_{d,\ initial}} \times 100 = \left(\frac{Va}{Vd}\right)\left(\frac{C_{a,\ final}}{C_{d,\ initial}}\right) \times 100 \qquad (21)$$

where $n_{d,i}$ and $n_{a,f}$ are the mass (moles) of the analyte initially present in the DP, and the mass (moles) of the analyte finally collected in the AP, respectively. V_a is the volume of AP and V_d is the volume of DP.

The relative recovery percentage (RR%) was calculated based on the following equation:

$$RR\% = \left[\frac{(C_{found} - C_{real})}{C_{added}}\right] \times 100 \qquad (22)$$

where C_{found} is the concentration of the analyte found in the spiked sample, C_{real} is the concentration of the analyte found in the sample, and C_{added} is the concentration of the spiked analyte in the sample. The limit of detection (LOD) and limit of quantification (LOQ) were determined experimentally based on standard error of linear regression line ($s_{y/x}$). LOD was calculated by $3s_{y/x}/m$ and LOQ was calculated by $10s_{y/m}/m$, where m was the slope of the regression line.

Chapter 4 Results and discussions

1. Effect of generated oxygen in EME to determination of nitrite

During the extraction with EME, water in donor phase (positive electrode) was electrolyzed and then generated oxygen and hydrogen gas. When nitrite ion was transferred to the acceptor phase, nitrite ion would get oxidized by the generated oxygen to nitrate ion (eq.23). As shown in the ion chromatogram (Fig 10), after G-EME of nitrite with the typical acceptor solution, the peak of nitrite (t_R = 8.16 min, Fig. 10a) was diminished (t_R = 8.25 min, Fig. 10c), and the peak of nitrate (t_R = 10.30 min, Fig. 10b) was appeared (t_R = 10.37 min, Fig. 10c). To eliminate or minimize the effect of the generated oxygen in oxidation of nitrite ion, application of oxygen scavenger in the acceptor solution was attempted and studied.

$$NO_2^- + O_2 \rightarrow 2NO_3^- \quad (23)$$

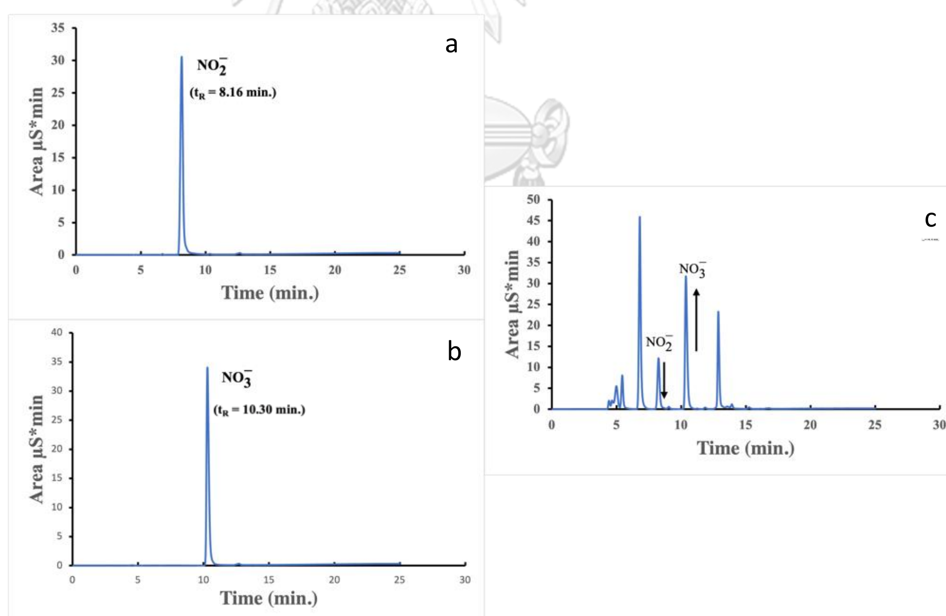

Figure 10 Chromatogram of standard nitrite solution (100 mg L^{-1}) (a); Chromatogram of standard nitrate solution (100 mg L^{-1}) (b); and Chromatogram of standard nitrite solution after extract with G-EME (c), nitrite concentration, 1 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water; extraction time, 10 min; stirring rate, 600 rpm

2. Oxygen scavenger for diminution effect of generated oxygen in oxidation of nitrite

2.1. Types of oxygen scavengers

In this work, ascorbic acid, iron, sulfite, and bisulfite were studied as oxygen scavenger. Oxygen scavenger solution was mixed with the acceptor phase solution before the extraction with G-EME. Herein, when the applied voltage turned on, electrolysis of water occurred, and oxygen was generating. Oxygen scavenger could react with the generated oxygen and therefore reduces or controls the amount of the generated oxygen in the system. Each oxygen scavenger has different efficiency depends on the ability to react with the oxygen. After the G-EME extraction process was done, the acceptor solution was analyzed with ion chromatography (IC). To evaluate the efficiency of oxygen scavengers, the peak of nitrite would be efficiently maintained or less diminished while the peak of nitrate was not shown or slightly observed. Figure 11 shows the ratio between nitrite and nitrate peak areas representing the efficiency of oxygen scavenger for various types and concentrations (mole L^{-1}) of oxygen scavengers. Apparently, sulfite and bisulfite showed satisfactory efficiency in reduction of the generated oxygen in oxidation of nitrite because sulfite and bisulfite are very active to react with dissolved oxygen in water. Ascorbic acid and iron ion did not show good efficiency. Therefore, sulfite and bisulfite were chosen for next study.

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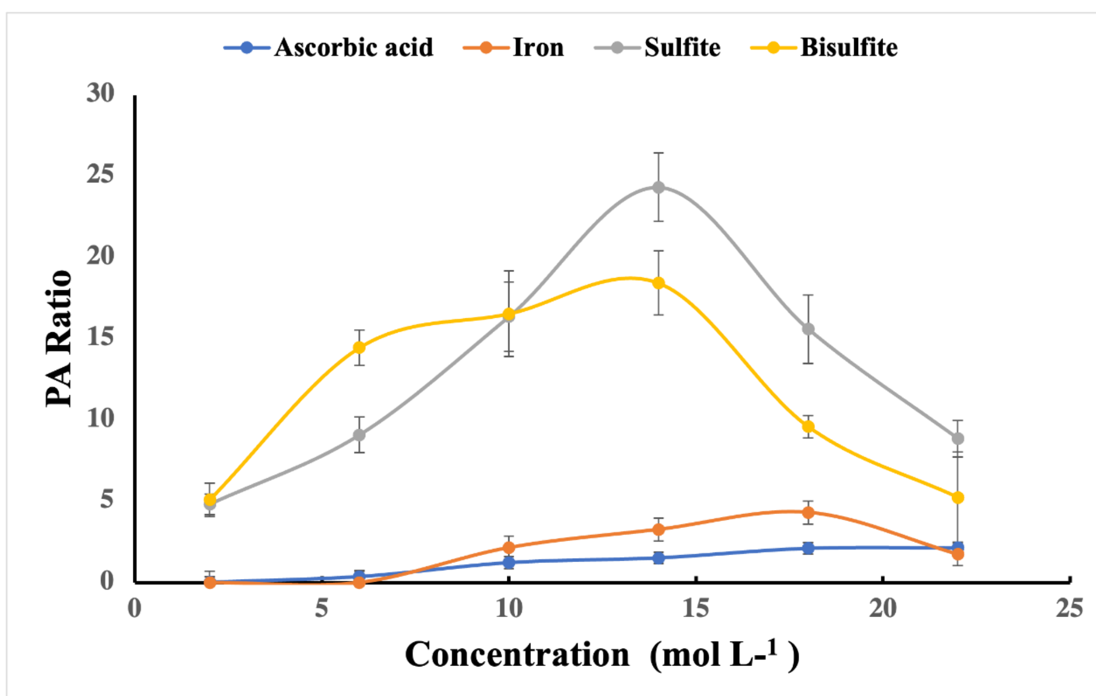

Figure 11 Peak area ratio between nitrite and nitrate peaks for various types and concentrations of oxygen scavengers (mol L^{-1}) in the acceptor solution. nitrite concentration, 1 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water; extraction time, 10 min; stirring rate, 600 rpm

2.2. Concentration of oxygen scavenger

The concentrations of sulfite and bisulfite in the acceptor solution were studied to diminish effect of generated oxygen to the oxidation of nitrite. Fig. 12 shows the peak areas of nitrite and nitrate obtained from G-EME of spiked nitrite sample at various concentrations of sulfite (Fig. 12a) and bisulfite (Fig. 12b) in the acceptor solution. At no oxygen scavenger (zero concentration), the peak area of nitrite was found small while the peak area of nitrate was significantly appeared. With addition of oxygen scavenger (both sulfite and bisulfite), the peak area of nitrite was increased while the peak area of nitrate was significantly diminished. As concentration of oxygen scavenger was increased, the peak of nitrite increased despite the peak of nitrate was regardless small. Increase in nitrite peak means more mass

transfer of nitrite into the acceptor solution. According to the Nernst-Planck equation (eq.24), flux of anion (J_i) towards acceptor solution could be influenced by the total anionic concentration in the acceptor solution as described by ion balance (χ) term defined as the ratio of the total ionic concentration in the donor phase to that in the acceptor phase (eq.25).

$$J_i = -\frac{D_i}{h}\left(1 + \frac{v}{\ln \chi}\right)\left(\frac{\chi - 1}{\chi - \exp(-\upsilon)}\right)\left(c_{ih} - c_{i0}\exp(-\upsilon)\right) \quad (24)$$

$$\chi = \frac{\sum_i c_{ih} + \sum_i c^*_{kh}}{\sum_i c_{i0} + \sum_i c^*_{k0}} \quad (25)$$

Where J_i is flux of ions; D_i is the diffusion coefficient for the ion in the SLM; h is the thickness of the SLM; υ is a dimensionless driving force proportional to the applied voltage; χ is ion balance; $\sum_i c_{ih} + \sum_i c^*_{kh}$ are total anionic concentration in the sample and at the sample/SLM interface; and $\sum_i c_{i0} + \sum_i c^*_{k0}$ are total anionic concentration in the acceptor solution and at the SLM/acceptor interface [53].

The peak area of nitrite was stable at the concentration of sulfite or bisulfite more than 10 mg L^{-1}. Noted that at the concentration of bisulfite greater than 14 mg L^{-1}, the peak area of nitrite was dropped. Despite the mass transfer of nitrite could be enhanced by the total concentration of anionic species in the acceptor solution, it could be limited by the counter mass transfer of the cationic species from sulfite and bisulfite salts towards the opposite direction.

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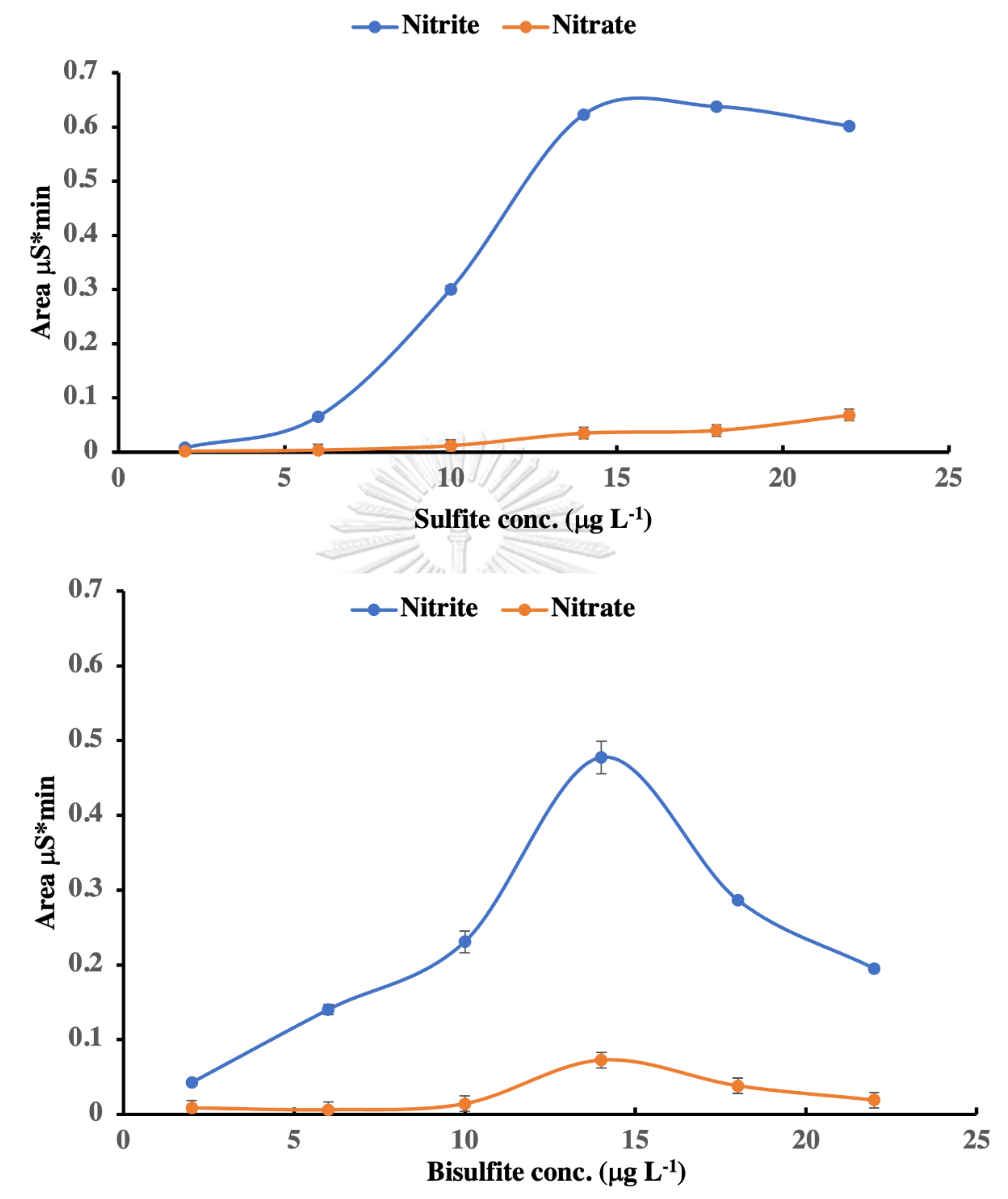

Figure 12 The relation between peak area and concentration of sulfite (a) and bisulfite (b). nitrite concentration, 100 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water; extraction time, 10 min; stirring rate, 600 rpm

Sulfite (14 mg L^{-1}) provided extraction efficiency of 15 % and bisulfite (14 mg L^{-1}) provided extraction efficiency of 11 %. To improve efficiency of oxygen scavenger, mixture of sulfite and bisulfite at 1:1 ratio (14 mg L^{-1} of each) was studied.

The mixture of sulfite and bisulfite can improve extraction efficiency to 17%. Mixture of sulfite and bisulfite at 14 mg L^{-1} of each was selected for testing nitrite and nitrate with G-EME.

3. Optimization of the EME procedure

3.1. Applied voltage

The standard nitrite solution was used for the optimization study. A pH of 7 was chosen for the donor based on our preliminary results. Gel concentration (2%), pH of the gel (7), and stirring rate (600 rpm) were adapted from our previous work [18]. First of all, the effect of DC voltage on the extraction of nitrite was studied in the range of 10-50 V. As expected, the increase in the applied voltage up to 30 V led to an increase in the nitrite peak area while the nitrate peak remained unchanged. However, at 40V, the nitrite peak decreased almost 40%, while the nitrate peak increased 2.5 fold (Fig.13) In addition, the effect of electroosmotic flow (EOF) could be more critical at higher voltage. The direction of EOF is opposite to the direction of nitrite. EOF is the movement of water from acceptor phase to donor phase while nitrite move from donor phase to acceptor phase. The repulsion force between different charge ion may lead to less nitrite in acceptor phase. When EOF is high, the acceptor phase volume was decreased. At highest current (50V), water in acceptor phase is empty. Therefore, 30V was chosen as the optimal condition for further study.

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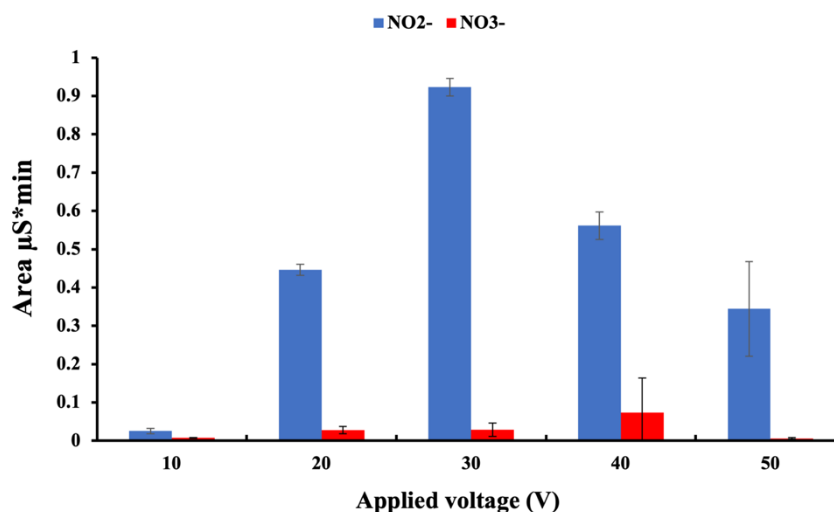

Figure 13 Effect of the applied voltage on the gel electromembrane extraction of nitrite. Analyte concentration, 0.5 mg L^{-1}; gel thickness, 7 mm; pH of gel and donor solutions, 7; acceptor solution, a mixture of sulfite and bisulfite (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm; electrode, Aluminium; and electrode width, 1 mm; n = 3.

3.2. Gel thickness

In G-EME, gel thickness plays an important role in the permeability of the analyte into the acceptor solution. The analytes are transferred from the donor solution to the acceptor solutions through the gel membrane. Thus, gel thickness will determine the number of analytes that reach the acceptor solution. Herein, the effect of gel thickness in the range between 5 and 11 mm on the extraction efficiency of nitrite ion was examined. The peak area of nitrite was decreased when the agarose gel was thicker than 7 mm. At thicker agarose gel, nitrite ion could have stuck in agarose gel pore and required longer time travelling through whereas at thinner gel, the gel could have easily detached from the wall of the vial. As shown in Fig.14, the highest nitrite peak area was observed at gel thickness of 7 mm, and thus, it was chosen for consequent experiments.

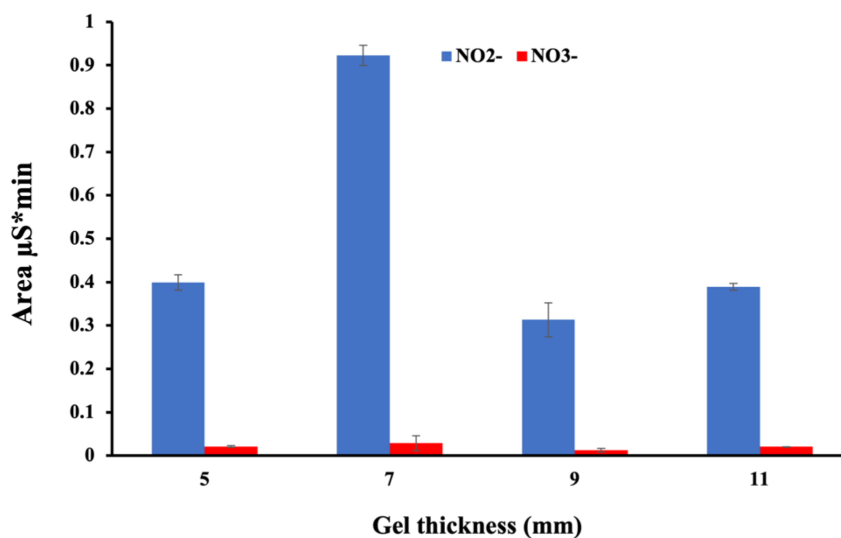

Figure 14 Effect of the gel thickness on the gel electromembrane extraction of nitrite. Analyte concentration, 0.5 mg L^{-1}; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, a mixture of sulfite and bisulfite (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm; electrode, Aluminium; and electrode width, 1 mm; n = 3.

3.3. Extraction Time

Extraction time is one of the most important parameters to be optimized in the G-EME system because of its direct relation to the sensitivity. The extraction time in this study was investigated in the range between 5 and 20 min at 5-min intervals. As shown in Fig.15, the nitrite peak area increased with increasing extraction time. The peak area of nitrite was highest at 10 min, and decreasing after 15 min. The decrease in mass transfer of nitrite after 15 min could be attributed to the effect of an electroosmotic flow (EOF) where the acceptor moved in the opposite direction. Hence, 10 min was chosen as the optimum extraction time for further experiments.

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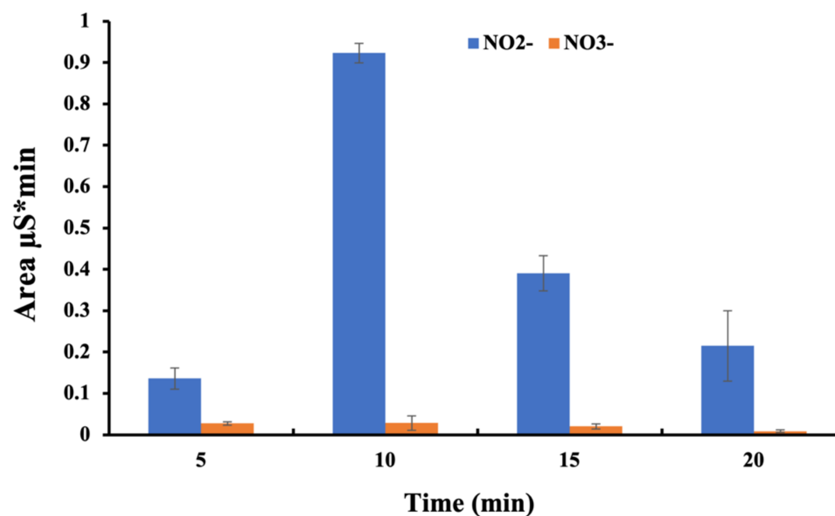
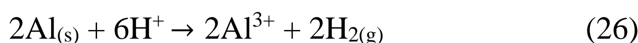


Figure 15 Effect of Time on the gel electromembrane extraction of nitrite. Analyte concentration, 0.5 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, a mixture of sulfite and bisulfite (14 mg L^{-1}); stirring rate, 600 rpm; electrode, Aluminium; and electrode width, 1 mm; n = 3.

3.4. Aluminum strip electrode

Aluminum electrode may have helped prevent oxidation of nitrite because aluminum electrode could react with oxygen and get oxidized to aluminium ion in acidic condition (eq.26). Oxidation reaction of aluminum can reduce amount of hydronium ion in acceptor phase solution. The oxidation of nitrite was reduced at low concentration of hydronium ion. The aluminum anodic electrode size (width) was examined in the range of 1-5 mm. For anode, aluminum was inserted in small Eppendorf tube that can support For anode, aluminum was inserted in small eppendorf tube that can support only 5 mm of anode. The results showed that the nitrite peak increased by increasing the electrode size (Fig.16). As the aluminum electrode surface was increased, it allowed the reaction between aluminum surface and hydronium ion more preferable than the oxidization process of nitrite. Moreover, the larger surface area could enhance ion transportation and thus enhance the extraction efficiency. However, the nitrate peaks at 4 and 5 mm of the electrode width were similar to blank

because the oxidation of aluminum electrode reduced amount of hydronium ion in system. Consequently, 5 mm electrode width was chosen for the subsequent experiments.



$$2Al_{(s)} + 6H^{+} \rightarrow 2Al^{3+} + 2H_{2(g)} \qquad (26)$$

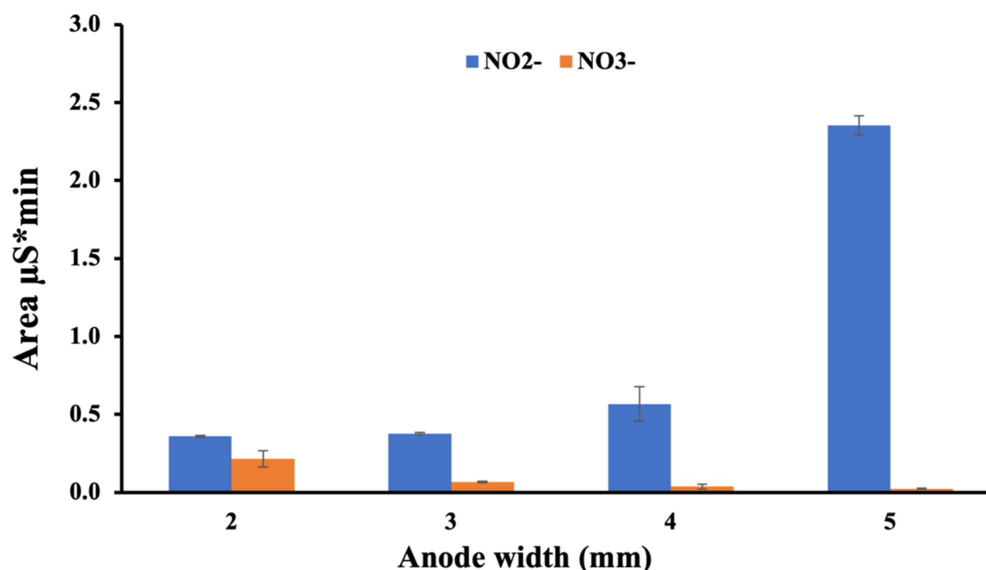


Figure 16 Effect of the width of Aluminum anode electrode on the gel electromembrane extraction of nitrite. Analyte concentration, 0.5 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, a mixture of sulfite and bisulfite (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm; electrode, Aluminium

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4. Simultaneous determination of nitrite and nitrate.

To prove that our method could determine nitrite and nitrate simultaneously, simultaneous determination of nitrite and nitrate has been demonstrated. Three calibrations curves were plotted using G-EME at the optimal conditions obtained from the previous sections (3.1 & 3.2). Fig.17a and Fig.17b showed calibration curves of nitrite and nitrate ion, respectively, while Fig.17c showed calibration curve of nitrite and nitrate ion obtained from a mixture of nitrite and nitrate (14 mg L^{-1}). The slopes were not significantly different (t-test; $P > 0.05$, Fig.17c). From these

observations, the speciation of nitrite and nitrate in one sample with high accuracy could be possibly obtained. The error bar from three calibrations curves are high because of the limitation of gel electromembrane system such as the position of electrode, the uncertainty of power supply etc.

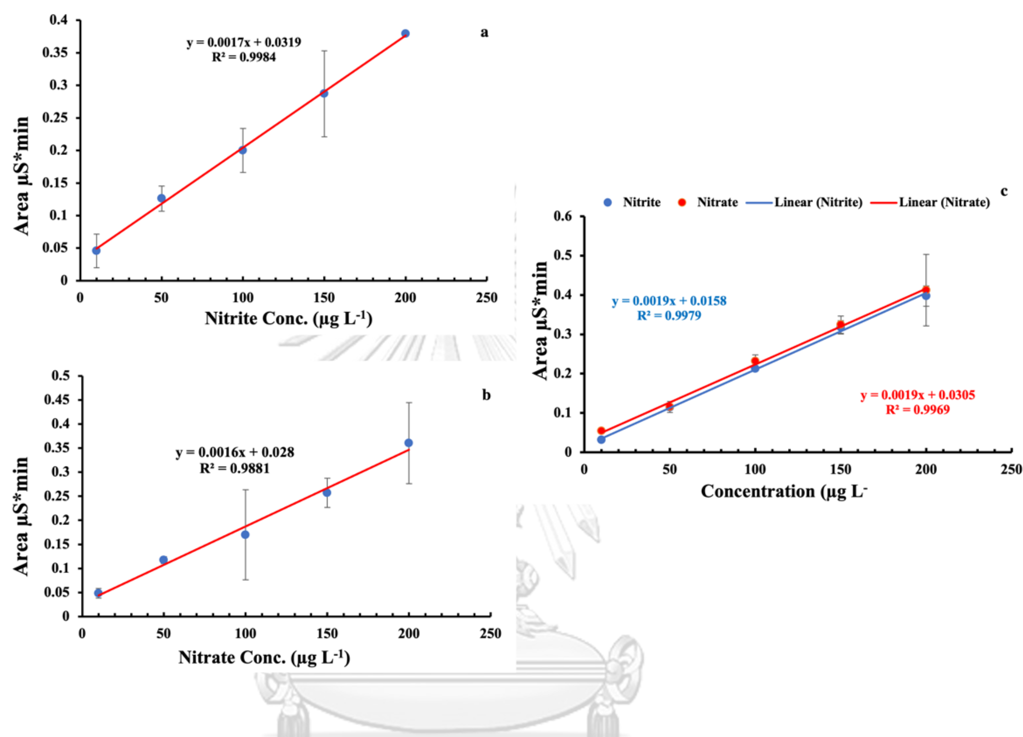

Figure 17 Calibrations curves, Analyte concentration (nitrite (a), nitrate (b), mixture nitrite and nitrate (c)) 10 - 200 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution,; extraction time, 10 min; stirring rate, 600 rpm; electrode, Aluminium; n = 3

5. Analytical features

The analytical merits of our developed method (G-EME-IC) were examined and summarized in Table 1. The experimental conditions for G-EME were as follows: type of gel, agarose; gel concentration, 2% w/v; gel thickness, 7 mm; pH of gel and donor solutions, 7; acceptor solution composition, a mixture of sulfite and bisulfite (14 mg L^{-1}); stirring speed, 600 rpm; applied voltage, 30 V; extraction time, 10 min; electrode, Aluminium; and electrode width, 5 mm. Under these optimal conditions,

the calibration curves were linear in the range of 45 to 200 µg L^{-1} for both nitrite and nitrate ions. The limit of detection (LOD) and limit of quantification (LOQ) were calculated experimentally based on the standard error of regression line, where LOD was $3s_{y/x}$/slope and LOQ was $10s_{y/x}$/slope [54]. The LODs were 12 µg L^{-1} and 13 µg L^{-1} for nitrite and nitrate ions, respectively. The LOQs were 41 µg L^{-1} and 45 µg L^{-1} for nitrite and nitrate ions, respectively. The limit of detection (LOD) of ion chromatography is around 100 µg L^{-1} without preconcentration. This work can improve sample preparation method before detecting the amount of nitrite and nitrate by ion chromatography at lower than 100 µg L^{-1} level. The maximum permitted added level of nitrate and nitrite salts in comminuted meats in Thailand, based on Thailand Industrial Standards Institute rules, are 500 and 125 mg kg^{-1}, respectively and in drinking water are 45 and 3 mg L^{-1}, respectively. The limit of detection (LOD) and limit of quantification (LOQ) of nitrite and nitrate in this work are lower than the maximum permitted added level of nitrate and nitrite in food sample. G-EME-IC method can be used to determine amount of nitrite and nitrate in real sample that has low level of nitrite and nitrate. Furthermore, the repeatability in this study was evaluated using the intra-day ($n = 6$) and inter-day ($n = 3 \times 6$) relative standard deviation (%RSD) for a standard solution at a concentration of 50 µg L^{-1}. The %RSD value was <8% for intra-day and < 9% for inter-day, which is acceptable and considered practical. Moreover, the enrichment factor (EF) determined at 50 µg L^{-1} of nitrite and nitrate and calculated as described in Ch 2, eq 21 was around 3.4 - 4 and the corresponding extraction efficiency (%EE) calculated as described in Ch 2, eq 22 was 17 - 20%.

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Table 1 Analytical merits of G-EME-IC.

Method analytical merits	Nitrite (NO_2^-)	Nitrate (NO_3^-)
Limit of detection (µg L^{-1})	12	13
Limit of quantification (µg L^{-1})	41	45
The slope of the calibration curve	0.0019	0.0019
Coefficient of determination (R^2)	0.9979	0.9969
Linear dynamic range (µg L^{-1})	41 -200	45 -200
RSD (%),		
Intra-day (n=5)[a]	<8	<8
Inter-day (n=5x3)[b]	< 9	< 9
Enrichment factor	3.4	4
Extraction Efficiency (%EE)	17	20

6. Analysis of real samples

The application of the developed method (G-EME-IC) for simultaneous determination of nitrite and nitrate ions was tested on sausage and drinking water samples. The assessment of the method recoveries (relative recoveries, %RR) was conducted by spiking the samples with different concentrations of standards, as shown in Table 2. The RR% were in the range of 95–110 and 96–104 for drinking water, and sausage, respectively. The maximum permitted added level of nitrate and nitrite salts in comminuted meats in Thailand, based on Thailand Industrial Standards Institute rules, are 500 and 125 mg kg^{-1}, respectively.[55] From table 2, level of sodium nitrite and sodium nitrate in sausage sample from our method are 1.41 and 0.79 mg kg^{-1}, respectively and in drinking water are 45 and 3 mg L^{-1},

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respectively. All of samples has lower level of nitrite and nitrate than the permission. This method can be used for determination of nitrite and nitrate at low level in sausage samples. In addition, the results obtained by our developed method were not significantly different from those obtained from the modified Griess reaction (paired t-test; $t_{observed} = 0.97$ and $t_{critical}$ (two tailed) = 4.30; $p > 0.05$, N=3). Noted that the calibration curve of modified Griess reaction ranged from 50 to 250 µg L^{-1} for both nitrite and nitrate ions. Despite the relative recovery of our proposed method was better than the modified Griess reaction, it could be noted that the reaction time of ceftazidime in our modified Griess reaction might not be sufficient. Ceftazidime is relatively large molecule that may need more time for the reaction to complete.

In comparison to the previous work reported by Berardi et al., 2021 for the determination of nitrite and nitrate in different types of meat products (sausage, ham, bacon etc.) using the ion chromatography without preconcentration step, the LODs were 1.5 mg L^{-1} and 3.2 g L^{-1} for nitrite and nitrate ions, respectively and the LOQs were 4.9 mg L^{-1} and 5.5 g L^{-1} for nitrite and nitrate ions, respectively, which were much higher than our method. The proposed G-EME method has improved sensitivity for determination of trace levels of nitrite and nitrate in meat products that usually are in ppb level.

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Table 2 Concentrations of nitrite and nitrate in samples obtained by the proposed method and modified Griess reaction

	NO_2/ NO_3 Added (µg L^{-1})[a]	This Work						Modified Griess reaction					
		Nitrite			Nitrate			Nitrite			Nitrate		
		Found (%RSD) N=3 (µg L^{-1})	RR%	Found (mg kg^{-1})	Found (%RSD) N=3 (µg L^{-1})	RR%	Found (mg kg^{-1})	Found (%RSD) N=3 (µg L^{-1})	RR%	Found (mg kg^{-1})	Found (%RSD) N=3 (µg L^{-1})	RR%	Found (mg kg^{-1})
Sausage (5 g)	0	46 (33)	-	0.9	30 (27)	-	0.6	ND*	-	-	ND*	-	-
	50	98 (11)	102		77 (4)	95							
	100	151 (7)	104		132 (7)	102		82 (29)	82		110 (7)	110	
	200							213 (3)	107		190 (19)	95	
Drinking Water	0	ND[b]	-		ND[b]	-		ND[c]	-		ND[c]	-	
	50	48 (17)	95		48 (22)	96							
	100	111 (9)	110		105 (13)	103		84 (5)	84		71 (23)	71	
	200							196 (3)	98		209 (18)	105	
Drinking Water	0	ND[b]	-		ND[b]	-		ND[c]	-		ND[c]	-	
	50	51 (14)[d]	101		44 (7)	88							
	100	96 (10)[d]	96		104 (11)	104		109 (6)	109		83 (13)	83	
	200							212 (2)	106		181 (8)	91	

[a]Nitrite and nitrate were added to sample separately and analyzed for nitrite and nitrate separately.
[b]Not detected (LOD of G-EME-IC method were 12 and 13 µg L^{-1} for nitrite and nitrate ions, respectively
[c]Not detected (LOD of modified Griess method were 38 and 47 µg L^{-1} for nitrite and nitrate ions, respectively
[d]N=2

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Chapter 5 Conclusion

In this work, the effect of generated oxygen to the determination of easily oxidized species in gel electromembrane extraction was controlled by adding oxygen scavengers to the acceptor solution. Several types of oxygen scavengers were examined and evaluated to suppress the oxidization of nitrite to nitrate (model analyte in this study). The results showed that the mixture that contained sulfite and bisulfite (14 mg L^{-1}) had the best performance in preventing the oxidation of nitrite to nitrate by reacting with the generated oxygen. The calibration curves for both nitrite and nitrate were linear between 45 and 200 μg L^{-1}, while the LOD were 12 μg L^{-1} and 13 μg L^{-1} and LOQ were 41 μg L^{-1} and 45 μg L^{-1}, respectively, for both nitrite and nitrate ions. Furthermore, this developed method was successfully applied to determine nitrite and nitrate simultaneously in sausage and water samples. The results were in good agreement with those obtained using the conventional UV-Vis method (modified Griess reaction). The detection limit of this method is lower than the maximum permitted added level of nitrate and nitrite in food. This method can be used for determining nitrite in food sample without interruption of oxygen while extraction. Nitrite can extract directly by using G-EME with oxygen scavenger. This work shows better reproducibility than the modified griess method because this method can detect nitrite and nitrate directly and no need reduction reaction of nitrate. To the best of our knowledge, this is the first study to use oxygen scavangers in acceptor phase to solve the oxidization problem in G-EME.

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Appendix

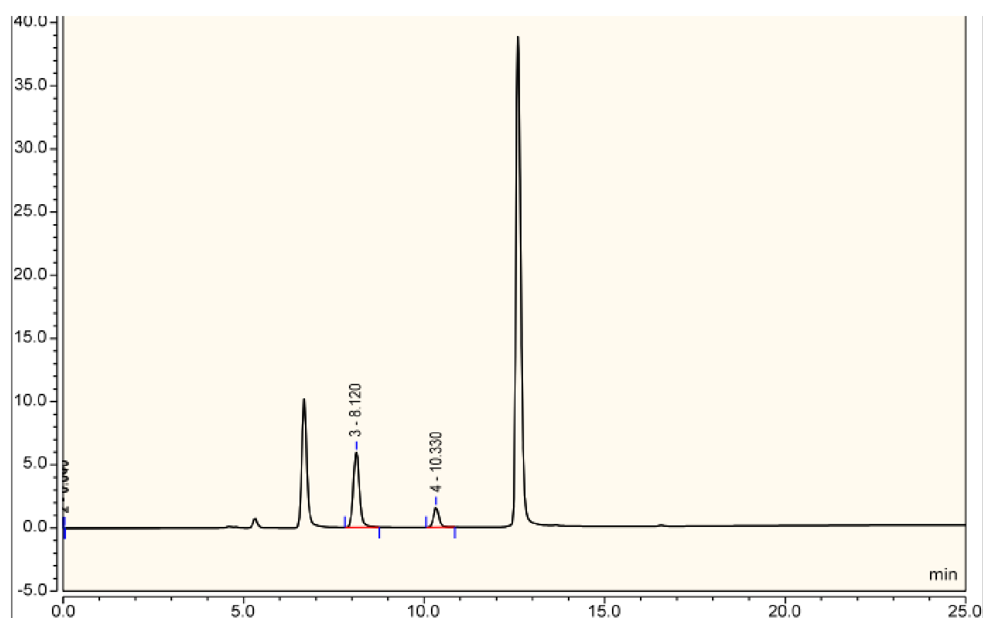

Figure 18 Chromatogram of standard nitrite solution after extract with G-EME (c), nitrite concentration, 1 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water and sulfite (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm

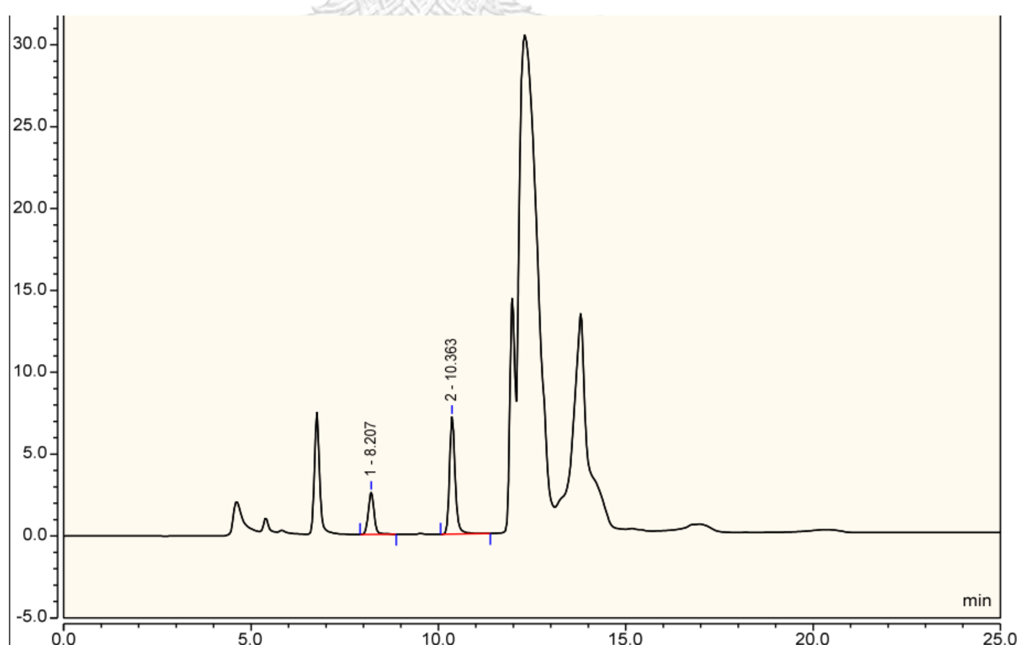

Figure 19 Chromatogram of standard nitrite solution after extract with G-EME (c), nitrite concentration, 1 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water and ascorbic acid (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm

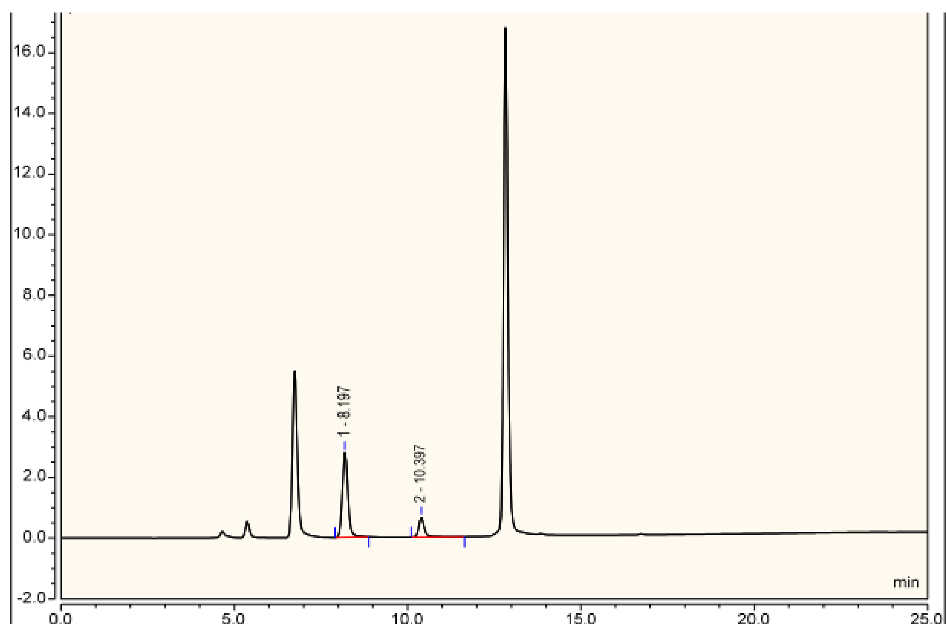

Figure 20 Chromatogram of standard nitrite solution after extract with G-EME (c), nitrite concentration, 1 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water and iron (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm

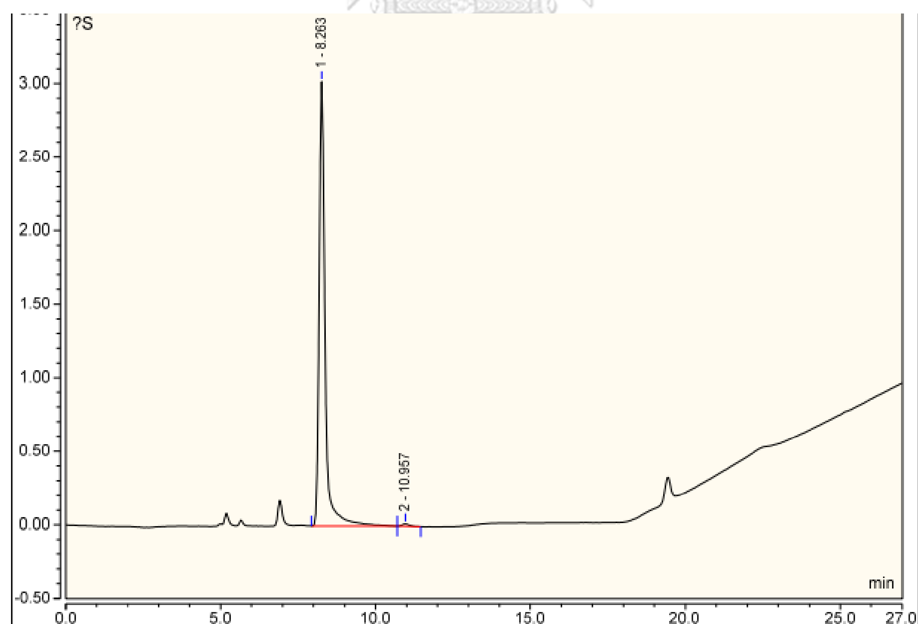

Figure 21 Chromatogram of standard nitrite solution after extract with G-EME (c), nitrite concentration, 1 mg L^{-1}; gel thickness, 7 mm; applied voltage, 30 V; pH of gel and donor solutions, 7; acceptor solution, Milli Q water and bisulfite (14 mg L^{-1}); extraction time, 10 min; stirring rate, 600 rpm

Figure 22 Agarose gel

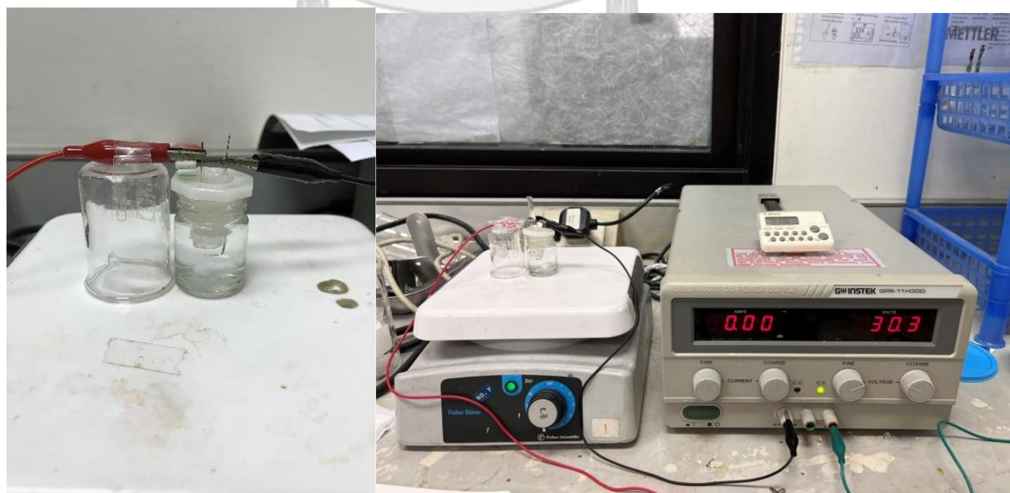

Figure 23 G-EME set up

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