SIMULTANEOUS EXTRACTION AND COLORIMETRIC DETECTION OF IONS USING GEL ELECTROMEMBRANE

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งานวิจัยนี้ได้พัฒนาวิธีการตรวจวัดที่รวมขั้นตอนการสกัดด้วยเจลอิเล็กโทรเมมเบรนและการตรวจวัดความเข้มสีในอุปกรณ์เดียวกัน โดยการผสมสารที่ทำให้เกิดสีกับอะกาโรสเจลก่อนนำไปสกัดและตรวจวัดสาร ได้แก่ ไอโอไดด์ตัวแทนสารไอออนลบและนิกเกิลตัวแทนสารไอออนบวก เมื่อมีการให้ศักย์ไฟฟ้าจากภายนอกสารจะถูกสกัดจากชั้นน้ำหรือเฟสให้ไปในชั้นเจลหรือเฟสรับและเกิดปฏิกิริยากับสารที่ทำให้เกิดสีในชั้นอะกาโรสเจล โดยไอโอไดด์จะเกิดปฏิกิริยากับน้ำแป้งให้สีน้ำเงิน และนิกเกิลจะเกิดปฏิกิริยากับไดเมทิลลิออกซีมให้สีชมพูในชั้นเจลตามลำดับ โดยสีที่เกิดขึ้นสามารถสังเกตเห็นได้ด้วยตาเปล่า ความเข้มของสีของไอโอไดด์สามารถตรวจวัดได้ด้วยเครื่องสเปกโตรมิเตอร์ และความเข้มของสีของนิกเกิลสามารถตรวจวัดได้ด้วยเทคนิคการถ่ายภาพจากสมาร์ทโฟน การตรวจวัดไอโอไดด์ในชั้นเจลประกอบได้ด้วย อะกาโรส 4%(w/v), ไฮโดรเจนเพอร์ออกไซด์ 5% (v/v), น้ำแป้ง 1% (w/v) ในกรดไฮโดรคลอลิก 2 มิลลิโมลาร์ การตรวจวัดนิกเกิลในชั้นเจลประกอบด้วย อะกาโรส 3%(w/v) และสารละลายผสมไดเมทิลลิออกซีมกับ 0.03 มิลลิโมลาร์ แอมโมเนียในอัตราส่วน 1:1 การสกัดใช้ศักย์ไฟฟ้า 50 และ 70 โวลต์ ใช้เวลา 15 และ 5 นาทีสำหรับไอโอไดด์และนิกเกิลตามลำดับ และใช้อัตราในการกวน 600 รอบต่อนาทีสำหรับทั้งไอโอไดด์และนิกเกิล วิธีนี้สามารถใช้ตรวจวัดไอโอไดด์ในช่วงความเข้มข้น 50 ถึง 250 ไมโครกรัมต่อลิตร และมีค่าขีดจำกัดในการตรวจวัดที่ 18 ไมโครกรัมต่อลิตร และวิธีนี้สามารถใช้ตรวจวัดนิกเกิล ในช่วงความเข้มข้น 30 ถึง 750 ไมโครกรัมต่อลิตรและมีค่าขีดจำกัดในการตรวจวัดที่ 1 ไมโครกรัมต่อลิตร วิธีการตรวจวัดนี้สามารถนำไปตรวจวัดปริมาณไอโอไดด์ในอาหารเสริมและตรวจวัดปริมาณนิกเกิลในช็อกโกแลตได้โดยไม่มีการรบกวนจากเมทริกซ์ในสารตัวอย่าง วิธีการนี้มีการใช้งานที่ง่ายและเป็นมิตรต่อสิ่งแวดล้อม และสามารถนำแนวทางนี้ไปพัฒนาสำหรับตรวจวัดสารชนิดอื่นได้

สาขาวิชา	เคมี	ลายมือชื่อนิสิต .....
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Ali Sahragard : SIMULTANEOUS EXTRACTION AND COLORIMETRIC DETECTION OF IONS USING GEL ELECTROMEMBRANE. Advisor: Assoc. Prof. Dr. PAKORN VARANUSUPAKUL

A total integrated electrocolorimetric sensing approach consisting of gel-based electromembrane extraction and colorimetric detection in one-step process was developed. This system was designed using colorimetric reagents preadded to the agarose gel for the determination of the following two model analytes: iodide for negative ions and nickel for positive ions. In this system, when a voltage was applied, the analytes were extracted and transferred from the sample solution (donor phase) to the gel (acceptor phase). The analytes then simultaneously reacted with the colorimetric reagents inside the gel, yielding blue and pink colors for iodide and nickel ions, respectively. These colors were then analyzed using a portable spectrometer for iodide detection and smartphone for nickel detection, which could also be distinguished with the naked eye. Parameters affecting the extraction efficiency were studied and optimized for both analytes. The gel composition for iodide detection was 4% (w/v) agarose, 5% (v/v) H_2O_2, and 1% (w/v) starch in 2 mM HCl. The gel composition for nickel detection was 3% (w/v) agarose and of 50% (%v/v) of 1:1 Dimetylglioxime (0.03 mM): Ammonia (80 mM) in agarose gel. Iodide and nickel were extracted at applied potentials of 50 and 70 V, and extraction time of 15 and 5 min and a stirring rate of 600 rpm, respectively. Under the optimized conditions, the developed systems provided linear responses within 15 min for iodide concentrations ranging from 50 to 250 μg L^{-1} with a detection limit of 18 μg L^{-1} and for nickel concentrations ranging from 30 to 750 μg L^{-1} with a detection limit of 1 μg L^{-1}. Finally, these systems were successfully applied to the determination of iodide in iodide food supplement samples and nickel in chocolate samples, showing a negligible matrix effect. This integration could also be extended to other analytes and detection systems to develop sensitive, simple, and environmentally friendly sensing approaches.

Field of Study:	Chemistry	Student's Signature .....
Academic Year:	2022	Advisor's Signature .....

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Abbreviations

Abbreviation	Definition
AAS	Atomic absorption spectroscopy
AP	Acceptor phase
CPE	cloud point extraction
DC	Direct current
DEHP	Di(2- ethylhexyl) phthalate
DMG	Dimethylglyoxime
DP	Donor phase
DPC	Diphenylcarbazone
EEO	Electroendosmosis
EME	Electromembrane extraction
EOF	Electro-osmotic flow
FAAS	Flame Atomic Absorption Spectroscopy
GC	Gas Chromatography
G-EME	Gel-electromembrane microextraction
HF-LPME	Hollow fiber membrane liquid-phase microextraction
HPLC-UV	High-Performance Liquid Chromatography-Ultraviolet
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ICP–MS	Inductively coupled plasma–mass spectrometry
ID	Internal diameter
IDD	Iodine deficiency disorders
ILs	Ionic liquids
LDR	Linear dynamic range
LED	Light-emitting diode
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification

LPME	liquid phase microextrection
MCL	Maximum contaminant level
NPOE	2-Nitrophenyl octyl ether
µPADs	Microfluidic paper-based analytical devices
PF	Preconcentration factor
PMMA	Polymethylmethacrylate
RGB	Red, green, and blue
RR	Relative recovery
RSD	Relative standard deviation
RTILs	Room-temperature ionic liquids
SCX	Strong cation exchange
SLMs	Supported liquid membranes
SPE	Solid phase extraction
TDI	Tolerable daily intake
UAE	Ultrasound-assisted extraction
VA-DLLME	Vortex-assisted dispersive liquid-liquid microextraction

Chapter 1: Introduction

Introduction

In automatic/integrated analytical methods, all the involved steps, including handling of samples, reagent solutions and organic solvents, and monitoring of the analytical signals, are accomplished in one or only a few steps. Therefore, the sample throughput is maximized using these methods. Also, as the number of steps involving analytical process are decreased, the total analysis time is shortened, and the repeatability and sensitivity of the results can be enhanced [1-3]. Integration in conjunction with colorimetric or spectrometric detection techniques can also significantly simplify the analytical method due to their simplicity and short response time, cost-effectiveness, and on-site detection in which no complex analytical instrument such as high performance liquid chromatography (HPLC), gas chromatography (GC), or inductively coupled plasma (ICP) based methods is needed [4-6].

As colorimetric/spectrometric signals are easily affected by interfering agents, a cleaning or sample preparation step such as solid phase extraction (SPE), solid phase microextraction (SPME), and liquid-liquid extraction (LLE) prior to analysis is predominantly a must. The purpose of sample preparation is to have a processed sample that leads to better analytical results compared to the initial sample. This can be achieved by having a processed sample easier to introduce in the core analytical system with fewer matrix components (cleaner sample for better selectivity), and more concentrated in analytes (for better sensitivity) [7]. Notwithstanding the acceptance of the above sample preparation methods in routine analysis and research settings, practitioners still need to cope with the (i) long synthesis protocols of

customized sorbents in SPE, (ii) high expenses of commercial microfibers in SPME, and (iii) elevated consumption and waste generation of organic solvents in LLE methods [8]. To mitigate the lack of green credentials of LLE, dispersive liquid-liquid microextraction (DLLME) and supported liquid membrane (SLM) based LPME methods, such as hollow fiber liquid phase microextraction (HF-LPME) and electromembrane extraction (EME), have been developed as viable alternatives for extraction of analytes of a broad range of polarity in troublesome biological and environmental samples [9-11]. EME is a variant of HF-LPME in which charged analytes are extracted from sample solutions based on their migration through the SLM into an acceptor solution. Due to the application of a given voltage as a driving force, EME can generally provide higher enrichment factors in shorter extraction times as compared with conventional HF-LPME of neutral species [12]. Integration of sample preparation methods, especially EME, to the spectrometric/colorimetric detection can synergically leverage of all the advantages mentioned for integrated systems and colorimetric detection in addition to addressing sensitivity and selectivity concerns.

However, to the best of our knowledge, there are only a few reports attempting to integrate EME with spectrometric/colorimetric detection, where microfluidic platforms were used for the analysis of Cr(VI), ciprofloxacin, and phenazopyridine [13-15]. The microfluidic chips consisted of two polymethyl methacrylate (PMMA) plates. In each plate, a long channel was craved, where the channel craved at the upper plate was dedicated to the acceptor phase. The other one located in the underneath provided a flow path for the sample solution (For more details, see chapter 2). In each part of the chip, the platinum electrodes were embedded at the bottom of

each channel along the whole length of the channels [13]. During extraction, the channels of sample solution and acceptor phase were separated with a small piece of a porous polypropylene sheet membrane which was impregnated with SLM. In the case of Cr(VI), chromate ions were first reacted with diphenylcarbazide (DPC) to produce a pink color, which were then extracted on the microfluidic device [15]. After extraction, the colored acceptor phase was pumped by air through a strong cation exchange (SCX) material and colored complexes were collected, followed by taking photos using a smartphone. In another study, colorimetric detection of ciprofloxacin was carried out using the same microfluidic platform. However, the obtained acceptor phase was added into a solution containing CdTe quantum dots in this case, and quenching of the quantum dots was tracked by analyzing the main three color components including red, green, and blue using a colorimetric detection [13]. Although these integrated systems were aimed at simplifying the analysis process, reducing the analysis time, and increase the sensitivity in a one step process, the designs of the systems required complex devices such as syringe pumps for injection of the sample and acceptor solutions and solid phase materials or quantum dots in an extra step for colorimetric detection, which added to the number of steps and reduced the repeatability of the results. In addition, the extraction time was lengthy in these studies due to the slow flow rates (sometimes 50 min). Moreover, since they used planar membrane sheets, they needed high amounts of organic solvents and polymer membranes. To tackle the problems associated with these integrated systems such as long extraction time, a two-step process for extraction and detection, use of organic solvents, and sophisticated devices, gel electromembrane extraction (G-EME) was exploited in this work for integration purposes with the strategy of in-gel colorimetric

detection. Practically, colorimetric reagents were added directly to the gel solution in the gel fabrication step. After applying a voltage, model analytes were driven from the donor solution into the gel and reacted with specific reagents to produce colors. To demonstrate the working mechanism of the proposed approach, two different gel compositions for two model analytes, i.e., iodide (negatively charged) and nickel (positively charged) ions were studied. In this work, a portable spectrometer coupled with extraction vial for iodide detection and a smartphone for nickel detection were used to ensure portability and on-site application. After optimization of the controlling parameters, these two systems were utilized to determine the analytes in real samples.

Chapter 2: Theory and literature review

1. Sample preparation

Majority of real samples contain a wide variety of interfering compounds and ions along with target analytes [16]. Such sample matrixes cannot be analyzed directly by most of analytical instruments due to instrumental incompatibility, matrix interferences, and low concentrations of desired analytes [17]. Therefore, these samples should go through some cleanup steps to remove unwanted components, extraction steps to isolate or transfer analytes of interest and preconcentration steps to enrich the target compounds into the final appropriate solvent or solution prior to instrumental analysis [18]. Good sample preparation and extraction methods require less and faster steps, and consequently the total analysis time is not significantly affected by them, which ensures a high-throughput. In addition, a good sample preparation method should provide high reproducibility along with high extraction recovery of target analytes, be easy to use, be environmentally friendly, be economical, and last but not least, should be able to be automated/integrated front end to the detection systems. In the following paragraphs, some of most commonly used sample preparation methods are described.

SPE is the method used for concentration and isolation of target analytes using a solid support or nano sorbents. **Figure 1** [19] shows an example of magnetic sorbents and scheme in SPE. In this system, the magnetic sorbents are added into the sample solution to extract target analytes. Then, an external magnet is placed at the bottom of the tube to isolate the sorbent from the sample solution. Afterwards, the supernatant is decanted and discarded. The extracted analytes are desorbed back to a desired solvent, collected through magnetic separation of the sorbent, and injected into the detection

system for analysis. Even though SPE method can provide good enrichment factors, it needs long synthesis protocols of customized sorbents and so many extraction steps until injection.

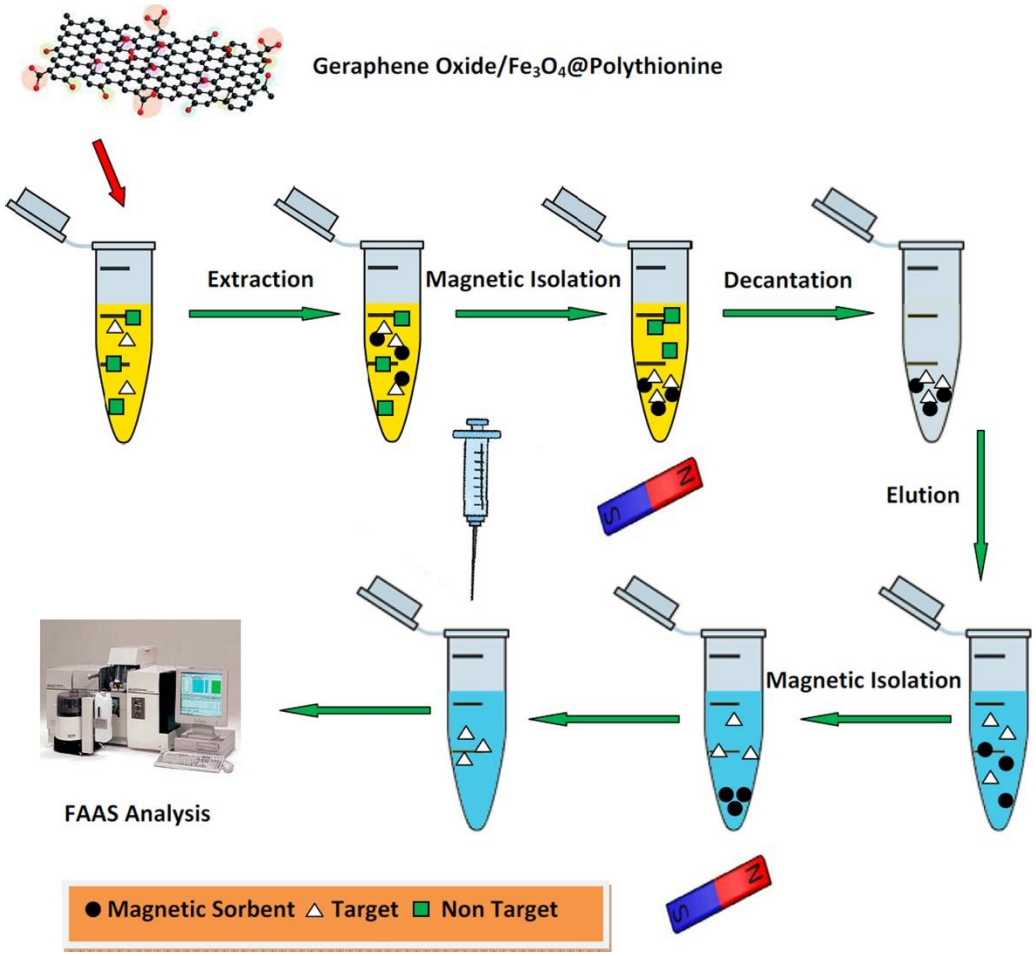


Figure 1 Schematic diagram of SPE for the determination of zinc [19].

Liquid-liquid based extraction methods have been widely exploited as sample preparation techniques in the past decades. In classical LLE, analytes in the samples are distributed between two immiscible liquid phases. To perform LLE, aqueous solvent/sample is mixed with an immiscible organic solvent, where targeted analytes are extracted into the organic phase. Depending on the type of detection instrument, the organic phase might be directly analyzed, or evaporated to dryness and

reconstituted before the detection [20]. Advantages of LLE based methods include large sample capacity and capability of direct analysis of organic phase after extraction. However, some weak points associated with classical LLE are being labor intensive, difficult to automate and using large volume of expensive and environmentally harmful organic solvents [8]. To address these disadvantages, some modern approaches to classical LLE have been developed such as single drop-liquid phase microextrection [21], dispersive liquid-liquid microextrection (DLLME) [22] and supported membrane based extraction methods [23]. Among them, membrane-based extraction techniques provide creative choices over classical methods, since the membranes are stable and easy to set up, use small amounts of organic solvents, and provide high enrichment factors and clean-up capability for complex matrixes. The two most reported setups of membrane-based extraction techniques in the literature are HF-LPME and EME.

In HF-LPME, analytes are extracted and transferred from the sample solution (also called donor solution) across the organic solvent that is held in the porous wall of the polypropylene hollow fiber membrane into the acceptor solution situated in the membrane lumen [11]. The organic solvent is called supported liquid membrane (SLM). In some cases, the organic solvent is mixed with an ionic carrier via the ion-exchange process to enhance the extraction efficiency (**Figure 2**) [24, 25]. However, these processes commonly necessitate quite a long extraction time and generally provide only low enrichment factors. Therefore, a new extraction technique called EME has been introduced to improve the extraction speed.

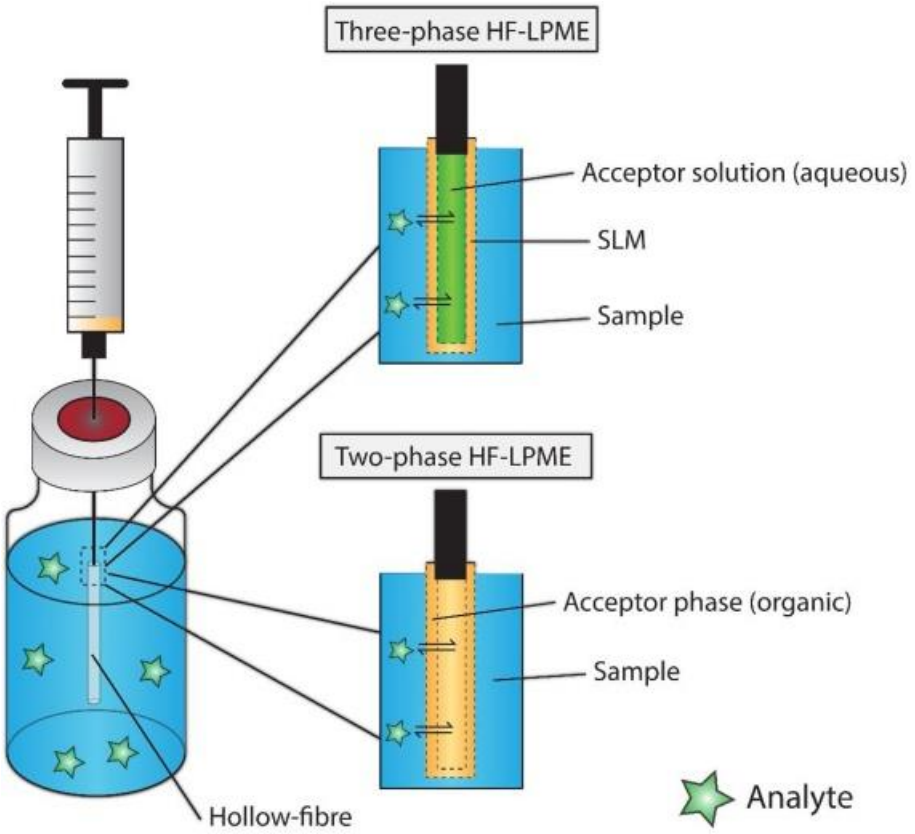


Figure 2 HF-LPME principle [26].

EME was developed from HF-LPME in 2006 by Pedersen-Bjergaard to overcome the drawbacks of HF-LPME [9, 10, 27]. EME was designed to extract and preconcentrate charged analytes, where the driving force is the electrical potential applied across the SLM [28]. The EME system's configuration is as follows: one electrode is usually placed in the sample/donor solution, whereas the other is placed in the acceptor solution located in the lumen of the hollow fiber (**Figure 3**) [29]. The applied voltage can enhance the mass transportation of charged or ionizable analytes across the SLM into the acceptor solution over an electrokinetic migration mechanism. The resulting steady-state flux of the anionic analyte J_i across the SLM may be calculated by the following equation [30]:

$$J_i = \frac{-D_i}{h}\left(\left(1 + \frac{\nu}{\ln\chi}\right)\right)\left(\frac{\chi - 1}{\chi - \exp(-\nu)}\right)(C_{ih} - C_{i0}\ \exp(-\nu)) \quad (1)$$

where D_i denotes the diffusion coefficient of the analyte, h is the thickness of the membrane, C_{ih} represents the analyte concentration at the SLM/sample interface, and C_{io} is the analyte concentration at the acceptor/SLM interface. ν is a dimensionless driving force defined by

$$\nu = \frac{Z_i}{KT}\ e\Delta\emptyset \quad (2)$$

where z_i is the charge of the analyte, e is the elementary charge, ΔØ is the electrical potential across the SLM, K is the Boltzmann's constant, and T is the absolute temperature.

In an EME system, where both the diffusion coefficient (D_i) and the SLM thickness (h) are constant, equation (1) predicts that the flux of analyte (J_i) can be improved by increasing the potential difference (ΔØ) across the SLM. When no voltage is applied, almost no extraction is observed. The application of an electrical field provides faster extraction of charged species than the typical passive diffusion. EME generally provides a higher preconcentration factor and shorter analysis time in comparison with the HF-LPME due to the application of an electrical driving force [11, 12, 31].

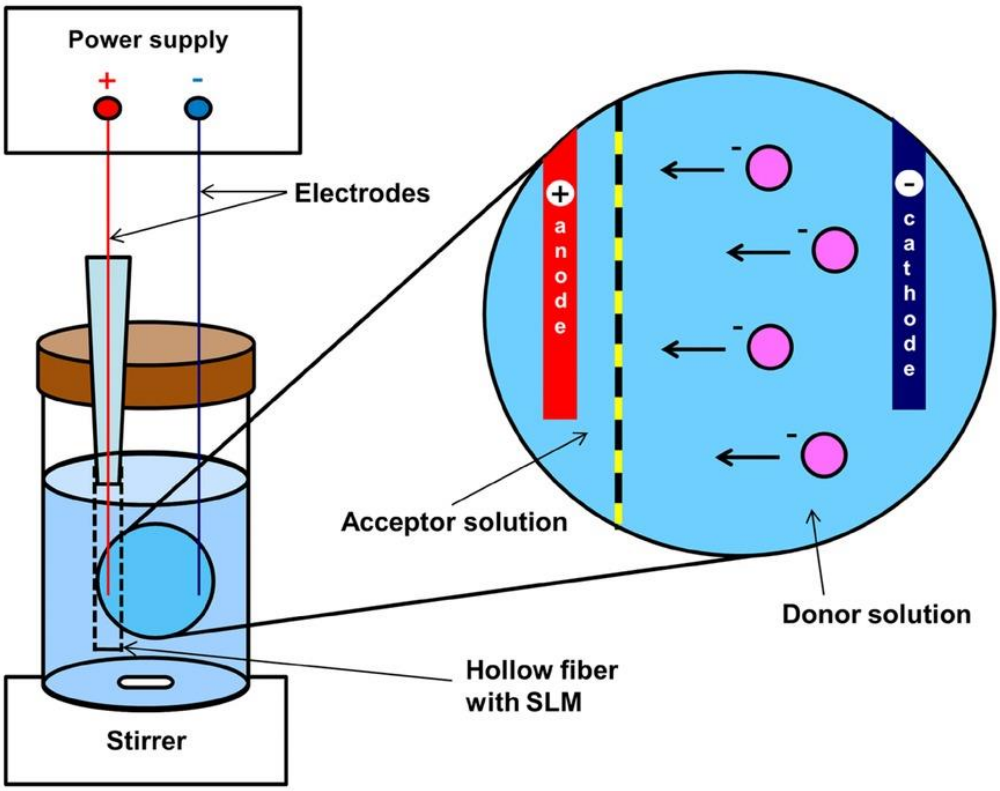


Figure 3 Schematic illustration of the set-up for EME [29].

2. Integrated extraction systems with EME

Zarghampour et al. coupled micro SPE with on-chip EME to extract Cr(VI) in water samples [15]. In this work, Cr(VI) were selectively complexed with DPC prior to the EME step. In this microfluidic device, two polymethylmethacrylate (PMMA) plates were used as substrates, in which spiral channels with the same patterns (with a depth of 500 mm and a width of 2.0 mm) were carved (**Figure 4**). The carved channel on the upper substrate acted as a channel for the acceptor phase, while the lower part was leveraged as a channel for feeding the sample solution. Three holes were drilled in the substrates to place inlet and outlet tubes and insert the electrodes (platinum electrodes). For extraction, after impregnation of the porous polypropylene sheet membrane with 2-Nitrophenyl octyl ether (NPOE) containing 15% (v/v) di(2-

ethylhexyl) phthalate (DEHP), they were sandwiched between the plates and fixed with bolts and nuts. Then, a syringe pump and a microsyringe were used to introduce the sample solution (containing the Cr(VI)-DPC) and acceptor solution (500 µL of 100 mM HCl) into the dedicated channels, respectively. After the extraction, the colored complexes in the acceptor solution were collected by 2 mg of strong cation exchange (SCX) sorbent in a silicon tube. Finally, a smartphone was used to take a photo of the colored solid phase for RGB analysis. Similar methodology has been used for detection of ciprofloxacin and phenazopyridine [13, 14].

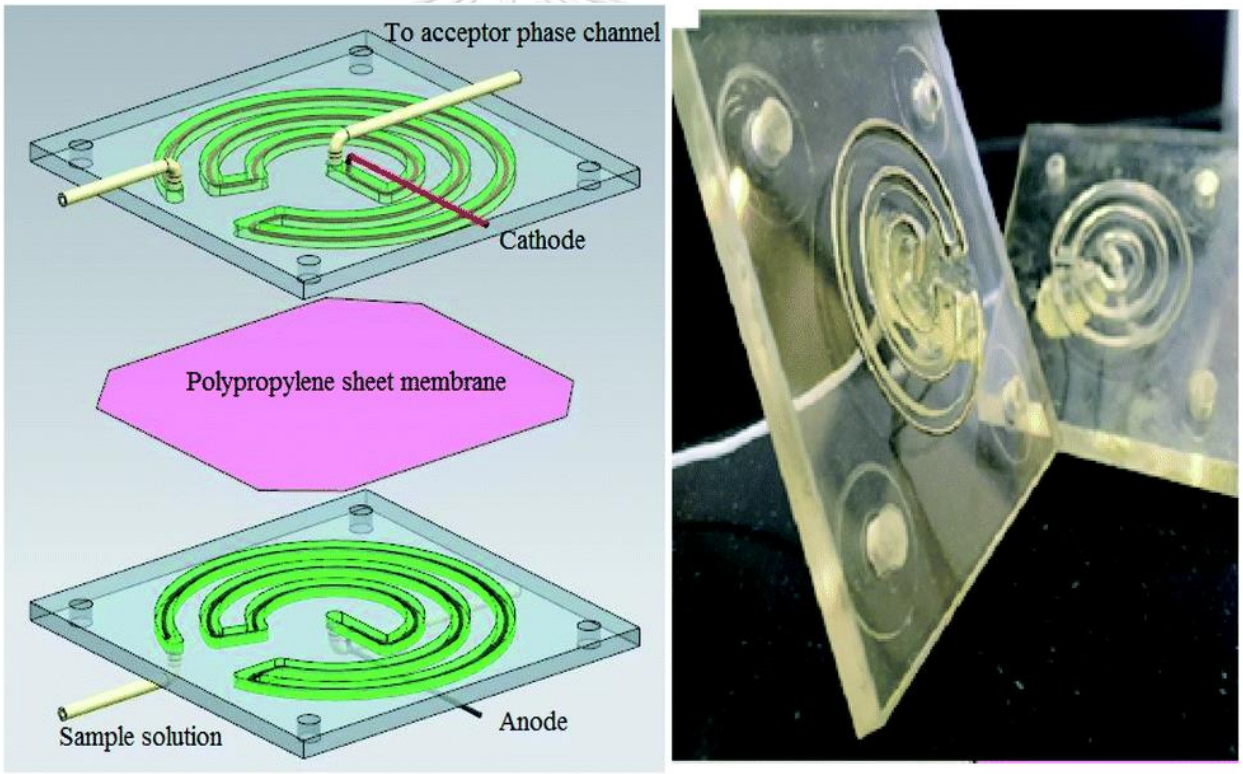


Figure 4 The equipment used in the EME chip device [15].

To address the weak points of these integrated devices as mentioned in the chapter 1, G-EME was proposed in this work for integration purposes [32].

G-EME is a newly developed version of EME as an alternative to organic-based SLM to comply with the green chemistry principles [33]. The early use of agarose gel in EME as a membrane was reported by Hidalgo et al. in 2015 [34]. The configuration and extraction mechanism is similar to EME, where the driving force is the electrical potential applied across the gel membrane (**Figure 5**). G-EME has been widely used recently for extraction and preconcentration of different types of analytes [35, 36]. A comparison between the performance of different extraction systems have been presented in the **Table. 1**. Despite its benefits, G-EME has been reported to be affected by unwanted electroendosmosis (EEO) flow phenomenon, which occurs during the extraction process [37], resulting in changes in the volume of the acceptor and donor phases and consequently affecting the extraction and preconcentration efficiency and repeatability issues.

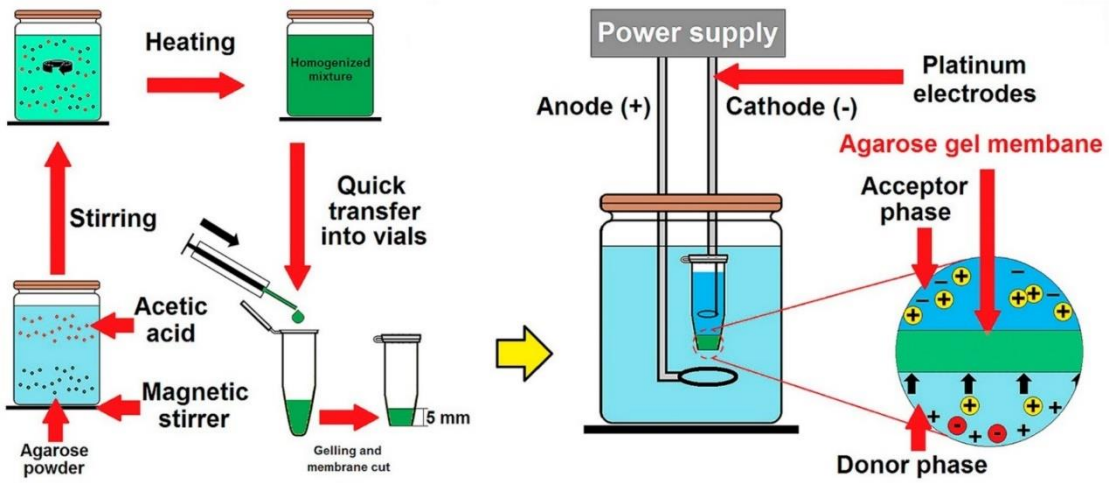


Figure 5 Schematic illustration of the fabrication of gel membrane and G-EME setup [38].

EEO in G-EME, like electroosmotic flow (EOF) in capillary electrophoresis, is created when an electric field is applied to a solution in a capillary (or in the pores in gel) that has fixed charges on its interior wall. Charge is accumulated on the inner

surface of pores when an ion containing solution is placed inside the gel (**Figure 6**). In the gel, sulfate groups attached to the interior wall of the pores are in the negatively charged forms. Attracted to the negatively charged sulfates groups, the positively charged cations present in the gel will form two inner layers of cations (called the diffuse double layer or the electrical double layer in capillary electrophoresis) on the pore wall. The first layer is referred to as the fixed layer because it is held tightly to the sulfate groups. The outer layer, called the mobile layer, is farther from the sulfate groups. The mobile cation layer is pulled in the direction of the negatively charged cathode when an electric field is applied. Since these cations are solvated, the solvent (water) solution migrates with the mobile layer, causing the EEO, and consequently volume changes of acceptor phase. To solve the problem associated with EEO, gel membranes were modified by some additives, including dextrin, chitosan, and xylan, in different concentrations to obtain low-, medium-, and high-EEO levels in a previous study [39]. The results indicated that when agarose gel was mixed with dextrin, a lower EEO level was obtained; however, they could not prevent EEO effects totally and low recoveries were obtained.

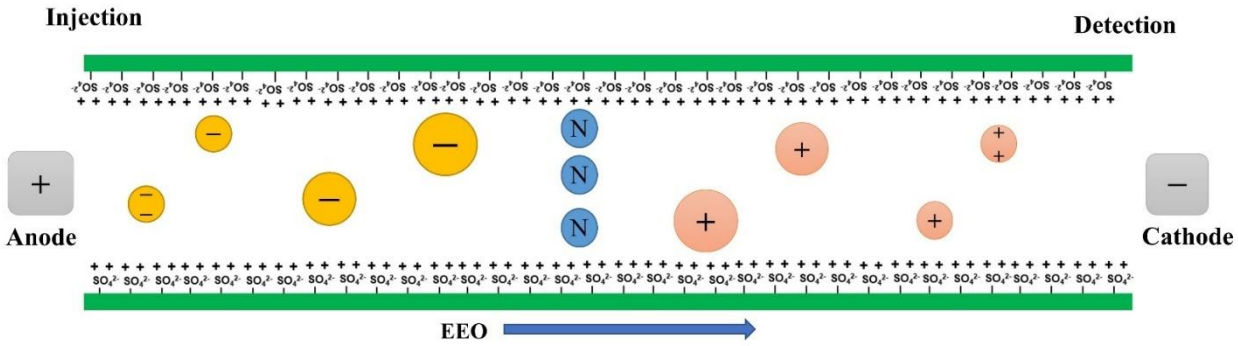


Figure 6 Schematic representation of direction of movement of ions in the gel as well as EEO.

In addition to gel composition modification to offset the EEO issues, a new configuration of G-EME namely as "Two-phase G-EME", was also suggested by Nojavan et al [40]. Compared to classical G-EME, which is based on aqueous-gel-aqueous layout, herein, the gel membrane served as the separation filter (membrane) and acceptor phase as well. Briefly, negative electrode was immersed into the gel and positive electrode into the aqueous donor phase. Based on their results, this simple adjustment could reduce EEO flow phenomenon, which is considered the main issue in G-EME. After extraction, the agarose gel was withdrawn and centrifuged for 5 min with 12000 rpm to disrupt its framework to release the “trapped aqueous acceptor phase” apart from the gel structure. The separated acceptor phase was finally injected into the HPLC-UV for the analysis. Although this approach may have avoided the effect of the EEO phenomenon, this process is complex and time-consuming, and a bulky detection instrument (HPLC-UV) is still required. To address these problems regarding EEO in G-EME and complexity of extraction and analysis with on-chip EME systems, we introduced a new integrated extraction technique called the electrocolorimetric extraction system.

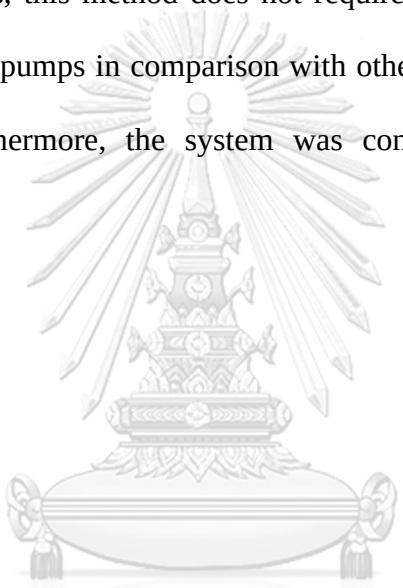
Table 1 Comparison between the different preconcentration techniques [8].

Technique Name	Advantages	Disadvantages
[a]**DLLME**	• Cheap and quick • Requires the use of a small amount of sample • Requires the use of a small amount of organic solvent • The high numerical value of the enrichment coefficient	• Low selectivity • Limited solvent choice
[b]**SPE**	• No need for the organic solvents • The facility of a process for automation • Preconcentrated analytes can be easily stored and transported • Possibility of isolation of analytes with the low volatility	• Sorbent bed clogging, especially in cartridges • Possibility of low recoveries • Sometimes low reproducibility due to differences between sorbent amounts.
[c]**HF-LPME**	• Provides relatively clean acceptor solution • Avoid carry-over from one sample to another • The high numerical value of the enrichment coefficient • High repeatability	• long extraction time • Possibility of fiber pores getting blocked • Usage of organic solvents and polymeric solvents
[d]**EME**	• Very clean extracts from biological fluids like human plasma • The extraction of different basic or acidic analytes • Excellent sample cleanup and analyte enrichment from the complex matrices	• Usage of organic solvents and polymeric fibers • Restricted to charged samples • low repeatability • The high voltage could not be used (not more than 80 V).
[e]**G-EME**	• No ion-pairing reagents are necessary to use • Convenient and green • No organic solvents and polymeric fibers are used	• EEO effect in G-EME • The difficulty of working in a highly acidic medium. • The high voltage could not be used (not more than 80 V).

[a]Dispersive liquid-liquid microextraction (RP-AA-DLLME).
[b]Solid-phase extraction (SPE)
[c]Hollow fiber membrane liquid phase microextraction (HF-LPME).
[d]Electromembrane microextraction (EME).
[e]Gel electromembrane microextraction (G-EME).

In this system, colorimetric reagents were added directly to the gel solution in the gel fabrication step. After applying a voltage between the cathode electrode (negative) in the gel and anode electrode (positive) in the donor solution, the positively charged analytes were driven from the donor solution to the gel, where they reacted with specific reagents and produced colors. This color could then be analyzed in different ways, either with the naked eye, digital cameras, smartphones, or

spectrometric systems. The same mechanism could be applied for electrocolorimetric of negative charged ions. This method minimized the EEO issues, used a lower volume of toxic organic solvents and reduced the number of steps by combining extraction and detection in one place, leading to improved repeatability and sensitivity because of the preconcentration of the analyte in the system and shortening the total analysis time without expensive detection instruments. Moreover, in addition to shorter extraction times, this method does not require any organic solvent, polymer membranes, or syringe pumps in comparison with other integrated and on-chip EME systems [13-15]. Furthermore, the system was convenient to use and possibly portable.



Chapter 3: Electrocolorimetric gel-based sensing approach for simultaneous extraction, preconcentration, and detection of iodide ions

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(Some modifications on the original manuscript have been implemented for better understanding)

1. Introduction

Iodine is one of the essential elements of human nutrition, and it is needed especially for the synthesis of thyroid hormone, which regulates basal metabolism, growth, and development. Low iodine levels can cause goiters, cretinism, and hypothyroidism [41]. It was reported that over two billion people worldwide have insufficient iodine intake and are at risk of developing iodine deficiency disorders (IDDs), whereas IDD is the number one cause of preventable brain damage worldwide. It should be mentioned that excess iodide can produce both hypo- and hyperthyroidism [33, 42, 43].

Among all existing iodine-containing species, iodide is one of the most dominant and important forms of iodine in waters and some other samples such as food samples due to its mobility and bioavailability. This makes it important to develop analytical methods for simple, selective and sensitive determination of iodide in various matrices and in presence of other iodine-containing species. To date, a number of methods for the determination of iodide have been reported, including chemiluminescence[44], colorimetric[45], electrochemistry [46], liquid chromatography[47], HPLC-tandem mass spectrometry[48], and ion chromatography–inductively coupled plasma mass spectrometry[49]. Although some of these methods have very high sensitivity, they require complex multistep sample

preparation. In addition, some of them need sophisticated and expansive equipment. Therefore, from this list, spectrophotometry/colorimetry seems to be one of the simplest, most rapid, and cheapest approaches. This makes it attractive for large scale routine and field analyses, which in some cases can be performed even by naked-eye detection. Main disadvantages of classical spectrophotometry are limited sensitivity and low selectivity.

Some extraction methods have been applied prior to the mentioned detection techniques, such as vortex-assisted DLLME (VA-DLLME)[50], salt-assisted DLLME [51], in-syringe DLLME [52], liquid phase microextraction[53], and dynamic gas extraction for detection of iodide [54] to increase sensitivity and selectivity. As explained in Chapter two (Section 2), a gel-electromembrane technique coupled with colorimetric detection approach has been used for iodide detection using starch as the host in this thesis. In here, Amylose in starch is responsible for the formation of a deep blue color in the presence of iodine and iodide. The iodine molecules are formed by oxidation of some iodide ions using oxidizing agents or acids. Iodine is not very soluble in water; therefore, the iodine molecules react with iodide ions, forming triiodide ions. This makes a linear triiodide ion complex that slips into the coil of the starch causing an intense blue-black color. A comparison between the results of previous extraction methods and proposed method will be presented in the section 3.5.

2. Experimental section

2.1. Chemicals

All chemicals used in this study were of analytical reagent grade, and solutions were prepared in Milli-Q water (Millipore, USA). Hydrogen peroxide (H_2O_2, 30%), potassium iodide, potassium dichromate (99%), sodium hydroxide (NaOH), sulfuric

acid (99%), nitric acid (65%), and hydrochloric acid (HCl) were obtained from Merck (Germany). Agarose was purchased from SeaKem LE Agarose (low EEO: 0.09–0.13; gel point [°C]: 36±1.5 at 1.5%; gel strength [g cm^{-2}]: >1200 at 1.0%), and starch was supplied by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Apparatus and detection system

For the detection and extraction systems, a 5 V battery and 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 were used. A platinum wire (0.2 mm diameter) was obtained from Sigma-Aldrich (St. Louis, MO, USA). For the extraction process, the solution in the extraction cell was stirred with a stirring rate of 100–800 rpm. A benchtop pH meter with a glass electrode (Mettler Toledo, USA) was used to measure the pH of the solutions.

A portable USB2000 spectrometer (Ocean Optics, Ottawa, Canada) was used to record the signals, and SpectraSuite software (Ocean Optics, Ottawa, Canada) was used for data acquisition and method validation. In addition, a locally purchased LED light source, with a wavelength of 609 nm, was used for the detection of iodine-starch. In these experiments, a resistor (100 Ω) was attached to the positive poles of LEDs to limit the electric current, avoiding burning of the LED.

2.3. Gel preparation

The agarose membrane was fabricated according to previous studies, with some additional materials and modifications [33, 37]. In the case of iodide detection, a solution with optimized amounts of agarose (4% w/v), starch (1% w/v), HCl (2 mM), and H_2O_2 (5% w/v) was prepared in the first step. Then, this solution was put into an 800 W microwave (Electrolux, Sweden) for 3 min to completely dissolve all

components, producing a liquid gel. Hand stirring was used to ensure that the gel solution was homogenized. In the next step, 450 μL of the obtained hot mixture was transferred into a 1.5 mL Eppendorf tube (Hamburg, Germany) using a micropipette and placed in a refrigerator at 4°C for 24 h. The gel was formed in the lower half of the Eppendorf tube, while the upper half of the tube was empty. Finally, the bottom conical part of the tube was cut with a razor blade to give a sheet membrane of 11 mm in thickness (optimized value).

2.4. Extraction steps and detection procedure in the electrocolorimetric sensing approach

Figure 7 represents the setup for the proposed approach. To set up the extraction system, as-prepared vials with a gel thickness of 11 mm (optimized thickness) were first analyzed before the extraction step to obtain the blank intensity in each experiment. For this, the vial containing the gel was placed in a homemade holder and irradiated by an LED light source with a λ_{max} of 609 nm for iodide complexes, and the intensities were recorded. Then, 10 mL of the standard solution (containing 500 μg L^{-1} iodide) was introduced into the extraction beaker using a pipette. Then, the vial containing gel was fixed in a polypropylene stopper as shown in **Figure 8.** As both the anode and the cathode, platinum electrodes connected to a DC power supply were placed in the donor phase and in the gel, respectively (**Figure 7**).

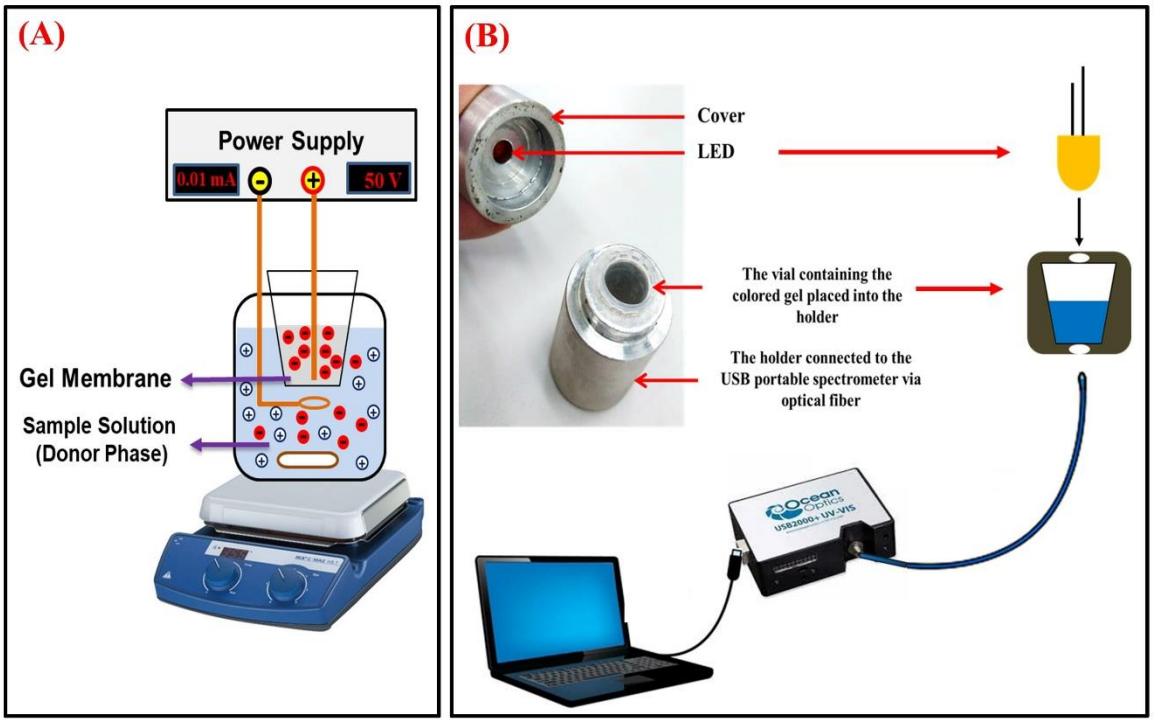

Figure 7 (A) The schematic of working principles of the proposed electro-colorimetric sensing approach. (B) A representation of the home-made holder used in the detection part and its different components.

The distance between the electrode (inside the gel) and the tip of the gel was kept constant at 3 mm in all experiments. The whole extraction cell was placed on a stirrer, 50 V (optimized value) was applied, and the extraction was carried out for 15 min (optimized value). In this stage, the model analytes, preconcentrated in the gel, reacted with the reagents and formed colors [blue for iodide complexes that were visible to the naked eye]. When the extraction time was finished, the power supply and the stirrer were turned off, the vial containing the colored gel was placed in the homemade holder again and irradiated by LED light sources, and the intensities were recorded with a portable spectrometer, wherein data were acquired using SpectraSuite desktop software. The difference between the amount of intensity before the extraction (gel with no color) and the amount of intensity after extraction (gel with

color) gives the amount of intensity absorbed by the colored complexes which is directly correlated with the concentration of the analytes (ΔI = Blank Intensity - Standard Intensity). Parameters that could affect the extraction efficiency such as the pH of the gel (type and concentration of acid), concentration of agarose, thickness of the gel, applied voltage, stirring rate, pH of the sample solution, and extraction time were investigated to obtain the best results.

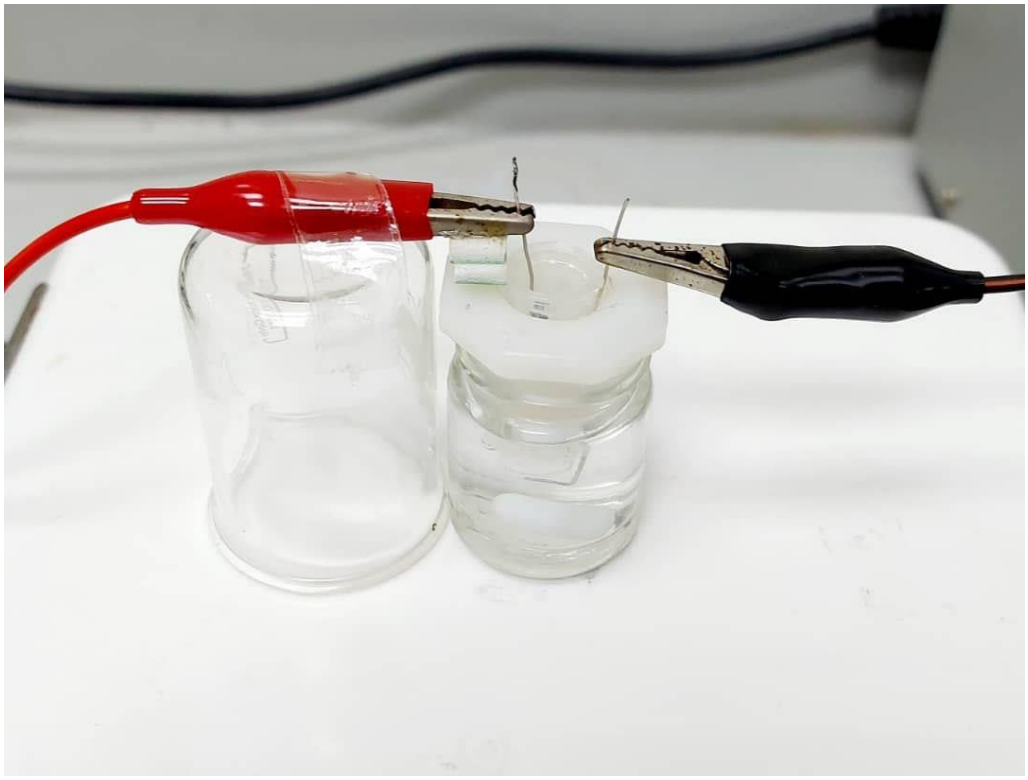

Figure 8 A photo of the extraction cell.

2.5. Standards and sample solutions

Stock solution of 1000 ppm iodide ions was prepared from potassium iodide in Milli-Q water and stored at 4.0°C. Then, the required working standard solutions were freshly prepared by diluting the appropriate volume of the stock solution with Milli-Q water. Real samples of iodide were dietary supplement capsules and mineral supplement tablets. To prepare the sample from each supplement, one tablet (1 g) was

dissolved in 40 mL of Milli-Q water and ultrasonicated for 30 min. Then, the suspension was removed by centrifugation at 3000 rpm for 20 min. The supplement stock solution was stored in a refrigerator at 4°C. Each supplement solution was prepared by diluting the supplement stock solution with Milli-Q water at a 1:5 ratio, and then a 10 mL was taken for extraction. The pH was adjusted to 6.0 (the optimized value) by the addition of 0.1 M NaOH or HCl solutions prior to the extraction. After that, the extraction system was run as mentioned in section 2.4. When the extraction time was finished, the gel containing the color was analyzed by a portable spectrometer. The spiked supplement solution at 50 μg L^{-1} of iodide was prepared and proceeded for extraction and analysis in the same way.

3. Results and discussion

In the proposed approach, desired analytes (iodide ions) migrate from the sample solution/matrix toward the agarose gel as a result of the external electric potential. In fact, during extraction, when desired analytes reach the gel because of the force of the applied voltage, they penetrate the gel. Afterwards, due to the presence of the pre-existing reagents, these electropreconcentrated analytes react with those reagents and produce different colors. Since the color change strategy can be applied for a large number of analytes through different mechanisms, this system shows a wide variety of promising applications in the development of sample preparation methods. In this chapter, application of this new approach has been shown for the extraction of iodide. In the following sections, the effects of different parameters on the extraction efficiency were studied in order to obtain the best results. Herein, agarose with low EEO was used to suppress the effect of EEO on extraction [37], which is explained in the following sections.

3.1. Effect of the type of acids and their concentrations in the gel preparation

According to the mechanism proposed for color formation in the starch–iodide reaction, the addition of acids and some oxidizing agents, such as H_2O_2, can cause iodide ions to be oxidized to iodine molecules inside the gel as they have been commonly used in the determination of iodide [55, 56]. After the reaction of iodide with iodine molecules, triiodide ions (I_3^-) are formed and trapped in the helix parts of the starch structure and produce a blue color. To optimize this process, the effect of the type of acid on the extraction was first studied by four different acids, including nitric acid (2 mM), sulfuric acid (2 mM), HCl (2 mM), and a mixture of H_2O_2 (5% w/v) and HCl (2 mM). Milli-Q water (H_2O) was also studied (**Figure 9**). In fact, the pH of the gel can be controlled by using these acids during the gel fabrication stage. In these experiments, sulfuric acid showed the lowest level of extraction. Likewise, nitric acid, H_2O, and HCl exhibited similar extraction levels, which were nearly twice as high as those of sulfuric acid. Although the presence of acid is important in the color formation process due to the conversion of iodide to iodine, in these experiments, the acids exhibited similar extraction levels similar to those of water. It can be considered that the hydrogen ions produced in the electrolysis of water can fulfil the acidic medium required. We observed a slight increase in acidity tested with paper strips inserted into the gel after extraction. However, when sulfuric acid was used, the gel was not well solidified, and agarose could be decomposed on the surface of the platinum electrode in the presence of sulfuric acid [57]. The best result was obtained with H_2O_2/HCl; therefore, it was selected for subsequent experiments.

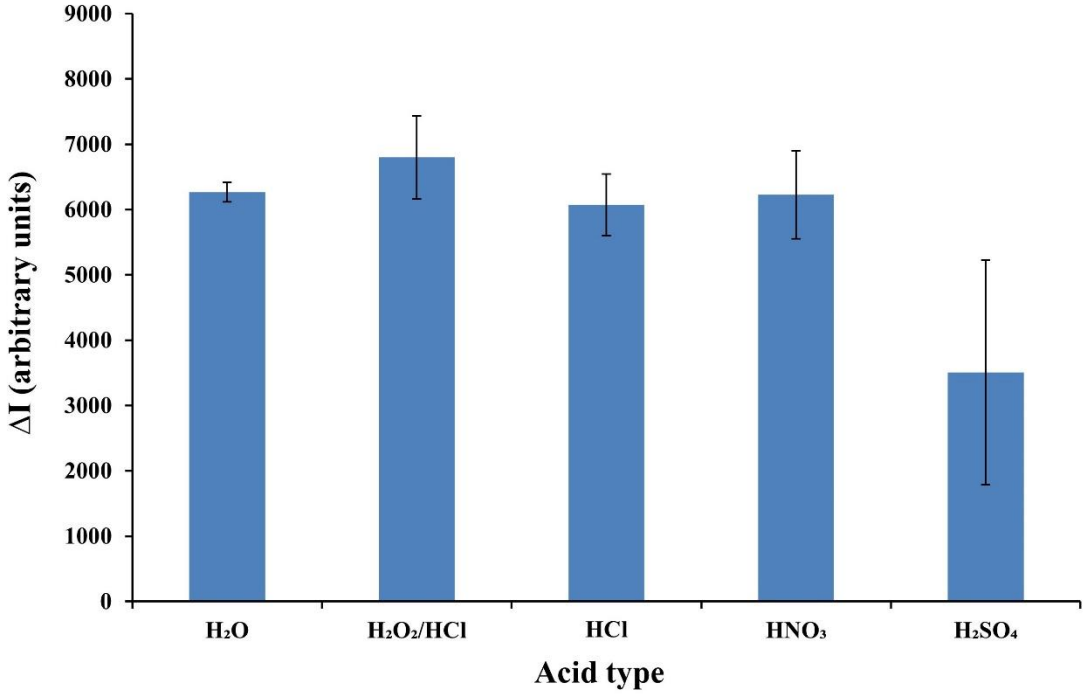

Figure 9 Effect of the type of acids (nitric acid (2 mM), sulfuric acid (2 mM), HCl (2 mM), and a mixture of H_2O_2 (5 % w/v) and HCl (2 mM)) in the gel on the extraction of iodide. Other extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 10 min, stirring rate: 600 rpm, gel thickness: 9 mm, concentrations of iodide: 500 µg L^{-1}, starch: 1 % (w/v), and agarose: 2 % (w/v). Error bars were obtained based on three replicates.

Furthermore, several experiments were carried out with different concentrations of H_2O_2/HCl in the gel ranging from 1 to 7%. As shown in **Figure S1**, an increase in the concentration of H_2O_2 led to an increase in the extraction from 1 to 5%, which became almost constant between 5 and 7%. These results indicate that color formation would be complete at H_2O_2 concentrations exceeding 5%. Therefore, the optimum concentration of 5% H_2O_2 prepared in HCl was selected.

3.2. Effect of the agarose concentration

In the agarose gel, the pore size depends on the concentration of agarose used in the gel fabrication process [58]. Control of the pore size is important because it can affect the EEO level in the experiments and change the extraction efficiency. In fact, when EEO increases, analytes would move along the gel with the solution and would not stay in the gel to produce color. Therefore, in the following optimization process, the effect of the agarose concentration on the extraction was examined in the range of 1 to 5%. At low concentrations of agarose (under 1%), the gel was not so solidified, and thus it was difficult to place the electrode in the gel. As seen in **Figure 10**, as the concentration of agarose increases, the extraction level increases until reaching a concentration of 5%. This could be due to low levels of EEO through the small pores in the higher concentrations of agarose [58]. Here, we selected 4% agarose due to the difficulty of working with the 5% gel in the preparation step as a result of its very short solidification time.

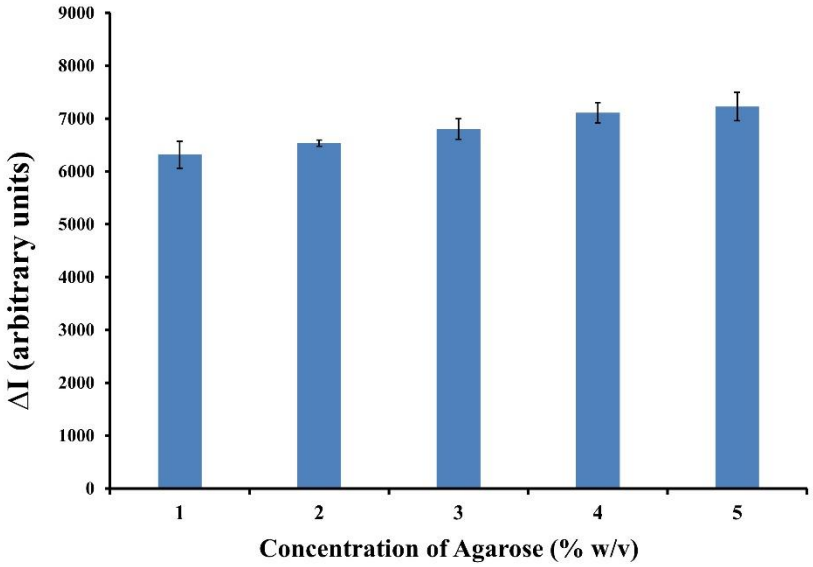

Figure 10 Effect of the agarose concentration on the extraction of iodide. Other extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 10 min, stirring rate: 600 rpm, gel thickness: 9 mm, concentrations of iodide: 500 µg L^{-1}, H_2O_2: 5 % (w/v), HCl: 2 mM, and starch: 1 % (w/v). Error bars were obtained based on three replicates.

3.3. Influence of the gel thickness

The influence of gel thickness on the extraction was studied in the range of 5 mm to 13 mm, while the tip of the electrode was always kept in the middle and at a distance of 3 mm above the tip of the gel. Herein, with an increase in the gel thickness, extraction increased from 5 to 11 mm before starting to decrease at higher thicknesses (13 mm). At gel thicknesses lower than 5 mm, it was difficult to place the electrode in the gel, and the gel was easily detached from the wall of the vial. From 5 to 11 mm, an increase in the thickness of the membrane could reduce the transfer of analytes and reagents across the gel due to a decrease in EEO (**Figure S2**). However, when the thicknesses of agarose are higher than 11 mm, the intensity of incident reaching the color formed in the gel drops due to the attenuation of incident probably through scattering/reflecting/absorbing by the agarose and/or starch. As a result, the

absorbance decreases. Therefore, a gel thickness of 11 mm was chosen as the optimum thickness in the subsequent experiments.

3.4. Effect of the applied voltage

In EME, the electrical voltage is the main driving force for transferring the analytes from the donor to the acceptor solutions through the membranes [59]. As a result, a series of experiments with various extraction voltages between 0 and 90 V were conducted to determine the most efficient voltage. As seen in **Figure 11**, when no voltage was applied, no extraction was obtained, showing that no analyte could diffuse from the donor phase to the gel. With an increase in the applied voltage from 10 to 50 V, the extraction increased dramatically which was due to an improvement in the flux of analytes with an increase in the driving force between the electrodes. However, from 50 to 90 V, a slight decrease was observed. At voltages above 50 V; the extraction decreased, probably due to the increased electrical current (greater than 300 µA), which could be a result of the aqueous nature of the agarose membrane and the lack of resistance. Higher EEO was also observed at higher voltages, resulting in decreased extraction efficiency. Moreover, at high voltages, bubble formation might have caused some negative effects on extraction efficiency by detachment of the gel from the vial wall as well as prevention of analytes from reaching the gel and being extracted. According to these results, 50 V was selected as the applied voltage between the donor solution and the gel, creating an electrical current of less than 100 µA.

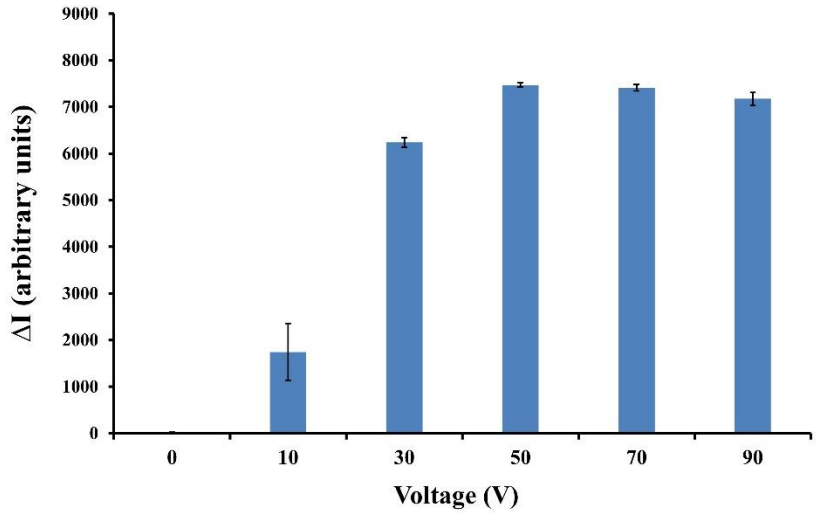

Figure 11 Effect of extraction voltage on the extraction of iodide. Other extraction conditions: donor pH: 6.0, extraction time: 10 min, stirring rate: 600 rpm, gel thickness: 11 mm, concentrations of iodide: 500 µg L^{-1}, H_2O_2: 5 % (w/v), HCl: 2 mM, agarose: 4% (w/v) and starch: 1 % (w/v). Error bars were obtained based on three replicates.

3.5. Effect of stirring rate

The stirring rate can also play a vital role in the intensity enhancement of the gel color by decreasing the double layer thickness at the interface of the donor phase and the gel as well as increasing the mass transfer of the analytes (ensuring constant contact of analyte ions in the donor solution with the gel surface). In this experiment, the stirring rate was investigated within the range of 0 to 800 rpm (**Figure S3**). By increasing the stirring speed from 0 to 600 rpm, the extraction increased because of convection effects, whereas the extraction decreased at very high stirring rates (800 rpm). Due to the formation of vortices and violent convection, a high stirring rate can cause bubble formation in addition to making the donor phase, vial, and electrodes unstable by moving them mechanically, resulting in the separation of gel from the vial wall. Therefore, a high stirring rate decreases the extraction level and the repeatability

of the extraction, and 600 rpm was selected as the optimal stirring rate for subsequent experiments.

3.6. Effect of the pH of the donor phase

In EME systems, analytes should be converted to their suitable ionic form for efficient extraction under the application of an external voltage. To obtain maximum extraction for iodide, in this experiment, the pH of the donor phase was investigated to be between 4.0 and 9.0 (the pH of Milli-Q water was determined to be 6.0). NaOH and HCl solutions (0.1 M) were used for the pH adjustments of the donor phase. The results are summarized in **Figure S4**. To achieve electrokinetic migration, it is necessary to convert the analytes into their ionized forms. In these experiments, the extraction increased from 4.0 to 6.0 possibly because iodide ions are more available in a neutral environment than in an acidic solution. On the other hand, at higher pH values (>6.0), the extraction exhibited a gradual decline, which could be due to the competition of iodide ions with hydroxyl ions for migration from the donor phase toward the gel. In addition, a basic donor solution might result in neutralization of the gel acidity and reduce the efficiency of the color formation reaction. Consequently, a pH of 6.0 was used as the optimum value for the donor phase.

3.7. Effect of extraction time

Extraction time is an important parameter that determines the total amount of analytes transferred from the donor phase to the gel; therefore, it can affect the sensitivity of the developed approach. A set of extractions was carried out at various extraction times ranging from 5 to 20 min (**Figure S5**). The extraction increased as a function of time up to 15 min and decreased slightly for longer extraction times. This decrease could be assigned to the detachment of the gel from the vial wall and instability of the

agarose gel at longer extraction times due to bubbles resulting from the electrolytic reactions and the occurrence of unwanted electrolytic reactions such as water electrolysis, which can change the pH due to the formation of H^+ ions. These probabilities have been given due to the observation of some water on the top of the gel for longer times. Hence, the extraction time of 15 min was selected as the optimum value.

3.8. Method validation

To evaluate the analytical performance of the system under the optimized conditions, the figures of merit for both systems, including the repeatability, preconcentration factor (PF), limit of detection (LOD), limit of quantification (LOQ), extraction recovery (ER%) and linear dynamic range (LDR), were investigated and summarized in **Table 2**. Under the optimal conditions, the calibration curves were linear in the range of 50-250 µg L^{-1} for iodide, as shown in **Figure 12** (A. B). In contrast to gel electrophoresis in which a concentration gradient is observed along the migration path, in our experiments, the results showed that when analytes move into the gel, they form complexes with their selective reagents and are slowed down. This can be confirmed by the images of the gel after extraction, which showed that there was almost no gradient along the gel. Even if it exits, since light passes all through the gel because of the source angle, it will interact with all the formed colors and there will not be any problem with the gradient resulting from the difference in the longitudinal movement. Moreover, **Figure 12** (A) shows the real photos of the colored gel after extraction of these concentrations, where the color between the concentrations could be distinguished easily with the naked eye.

Table 2 Analytical features of the proposed sensing approaches.

Figures of merit	**Iodide**
Limit of detection (μg L^{-1})	18
Limit of quantification (μg L^{-1})	54
Slope of calibration curve	0.170±0.006
Coefficient of determination (r^2)	0.996±0.10
Linear dynamic range (μg L^{-1})	50-250
RSD (%),	
Intra-day (n=5)[a]	<2 %
Inter-day (n=5x3)[b]	<3 %
Preconcentration factor	69
Extraction recovery (%)	76

[a] Intra-day precision was calculated for five replicates at the concentration of 50 μg L^{-1} for iodide.
[b] Inter-day precision was obtained for three days at the concentration of 50 μg L^{-1} for iodide.

In **Figure 12** (A), for the iodide system, the color is formed inside the gel; therefore, photos were not clear if they were taken from the top view of the gel. Accordingly, the photos were taken from the side view of vials. The sensitivity and coefficient of determination (r^2) were calculated from the slopes of the calibration curves obtained on three different working days. The results showed that the calibration curves had reproducible sensitivities between different days.

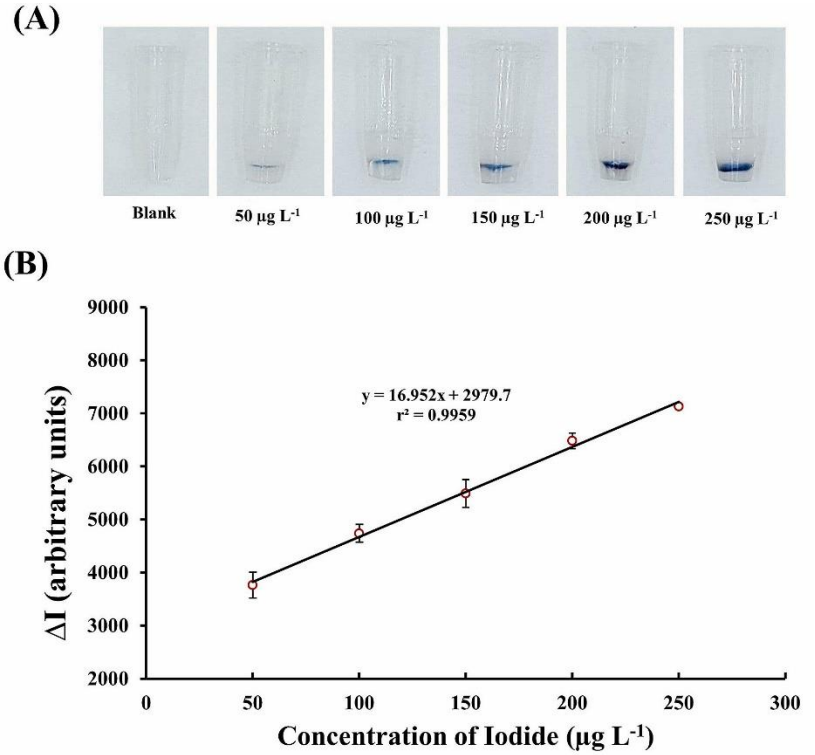

Figure 12 (**A**) Images of gel and **(B)** calibration curve obtained from extraction of different concentrations of iodide (50-250 µg L^{-1}). Extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 15 min, gel thickness: 11 mm, stirring rate: 600 rpm, concentrations of H_2O_2: 5 % (w/v), HCl: 2 mM, agarose: 4% (w/v), and starch: 1 % (w/v).

Additionally, the LODs were obtained on the basis of 3.3 σ/m, where σ is the standard deviation of the absorbance obtained from the blank solution (n=5) and m is the slope of the regression line. Relatively low LOD values of 18 µg L^{-1} for iodide was obtained. PF for iodide ions was calculated according to equation (3).

$$PF = \frac{C_{a,final}}{C_{s,initial}} \quad (3)$$

where $C_{a,final}$ is the final concentration of analyte in the gel (obtained from the calibration curve of known concentrations) and $C_{s,initial}$ is the initial analyte concentration in the donor solution. In this study, a PF of 69 was achieved for 500 µg L^{-1} iodide ions under the optimal conditions. Moreover, the interday and intraday

precision (representing the reproducibility) were 3% and 2%, respectively, for iodide detection,

3.9. Analysis of real samples

To indicate the practicality of the proposed approach, it was applied to determine the concentration of iodide ions in the iodide supplements. **Figure S6** shows the intensity profiles obtained from application of our integrated G-EME colorimetric system for detection of (A) iodide as an iodine-starch complex at 609 nm after G-EME of iodine-containing supplement samples. The decrease in intensity shows absorption of the extracted colored complex at a specific wavelength. The extraction relative recovery (RR%) was determined from the spiked samples calculated by the following equation (4):

$$RR\% = [(C_{found} - C_{real})/C_{added}] \times 100 \quad (4)$$

where C_{found} is the concentration of analyte found in the spiked sample, C_{real} is the concentration of analyte present in the real sample and C_{added} is the concentration of spiked analyte in the real sample. For iodide detection, the corresponding relative recoveries (RR%), relative standard deviations (RSDs%), and the values written on the label of products are summarized in **Table 3**. The RR% for the supplement samples was between 93% and 96% with RSD% ≤3%. These results demonstrated that the matrices of the analyzed supplement samples had a negligible effect on the performance of the proposed method, and good sample clean-up was obtained. On the other hand, there was an insignificant difference between the amounts obtained using the proposed system and those reported by the manufacturers labeled as the total iodine (accuracies were between 90 and 94%) because these samples contain iodate

from sea plants and potassium iodide that was added to achieve their desired concentrations.

Table 3 Determination of iodide ions in supplement samples

Supplement samples	Added (µg L^{-1})	Found (µg L^{-1}) (±SD)	RSD (%)	RR (%) (±SD)	Total amount obtained	Label	Accuracy (%)
Dietary supplement capsule	0	126.8±0.9	0.7	-	203µg /tablet	225 µg /tablet	90
	50	175±1	0.6	96±1			
Mineral supplement tablet	0	137±4	3	-	935 µg /tablet	1000 µg /tablet	94
	50	183.6±0.4	0.2	93±1			

3.10. Comparison of the proposed method with other methods

A comparison between the presented approach and the other previously reported methods for the preconcentration and detection of iodide ions in terms of the organic solvent used, LOD, LDR, and RSD (%) is summarized in **Table. 4**. Our method uses no organic solvent with lower RSDs while LOD and LDR values are comparably achieved. In contrast to other methods that contain many steps [47], our approach combines preconcentration and detection steps that are easily carried out in one step.

Table 4 Comparison of previous extraction methods with the proposed method for determination of iodide ions.

Analyte	Extraction technique	Extraction solvent (Volume(µL))	Linear Dynamic Range (µg L^{-1})	LOD (µg L^{-1})	RSD (%)	Detection technique	Sample	Ref
Iodide	VA-LLME[a]	Amyl acetate:CCl4 (250 µL)	16.9-169	1.75	2-6	UV–vis	Mineral waters	[50]
Iodide	SALLME[b]	1-octanol (4 µL)	1-2000	0.5	5	GC-ECD	Milk samples	[51]
Iodide	IS-DLLME[c]	Toluene (175 µL) and acetone (700 µL)	330-13300	10.5	6-7	UV–vis	Waters	[52]
Iodide	LPME[d]	n-hexane	25-750	10	3-6	UV–vis	Table salt, seawater, milk powder water	[53]
Iodide	DGE[e]	-	30-300	10	3-4	Colorimetric	Food samples	[54]
Iodide	EC-GSA[f]	-	50-250	18	≤3	UV–vis	Food supplements	This work

[a] Vortex-assisted liquid–liquid microextraction, [b] Salt-assisted liquid–liquid microextraction, [c] In-syringe dispersive liquid–liquid microextraction, [d] Liquid phase microextraction, [e] Dynamic gas extraction, [f] Electro colorimetric-gel sensing approach.

4. Conclusion

A sensing approach of integrating G-EME and the color change of the gel as a colorimetric signal into one-step process was successfully developed in this study for the first time. In this approach, analytes were able to be extracted, preconcentrated and detected in the agarose gel where no acceptor was required; therefore, the effect of EEO that is typically encountered in G-EME could be eliminated. The system was tested, optimized and validated using iodide as a model analyte by addition of their specific colorimetric reagents to the gel. This analyte could be detected with the naked eye at the µg L^{-1} level within 15 min. The analytical performances were comparable

with previously reported extraction methods accompanied by better repeatability, more simplicity, and shorter total analysis time. The system was successfully applied for the determination of iodide in supplement tablets. The results were in good agreement with those on the labels. The total integrated electrocolorimetric sensing approach does not require fabrication of complex microfluidic chips, synthesis of sorbents or use of organic solvents. Furthermore, the agarose gel used in this work is an environmentally friendly and colorless material that is compatible with both the extraction mechanism and colorimetric detection. Therefore, our approach of integrating G-EME with colorimetric sensors has complied with the green chemistry principles. This approach can also be easily extended to other analytes and detection systems.

Chapter 4: Application of electrocolorimetric extraction for the determination of Ni(II) ions in chocolate samples: A green methodology for food analysis

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(Some modifications on the original manuscript have been implemented for better understanding)

1. Introduction

Cocoa powder and chocolate are produced from cocoa beans (Theobroma cacao) and largely consumed worldwide, especially by children [60]. However, depending on factors such as the variety of crop sources, fertilization, and environmental pollution, cocoa can contain nonessential heavy metals such as lead (Pb) and cadmium (Cd) at trace levels [61]. Nickel is another one of the known primary contaminants resulting from the manufacturing process of chocolate. Nickel is used as a catalyst to harden chocolates by hydrogenation of unsaturated fats [62]. Nickel is known to cause various adverse effects on human health, such as allergies, cardiovascular and kidney diseases, oxidative stress, and lung and nasal cancer [63]. These threats increase in line with the low age and bodyweight of the potential consumer. As a result, EFSA's Scientific Panel on Contaminants in the Food Chain (CONTAM) established a tolerable daily intake (TDI) of nickel at 2.8 µg (Ni)/kg body weight per day [64, 65]. Therefore, the development of accurate, sensitive, selective, and rapid analytical methods for the determination of nickel in different samples, especially chocolates, is necessary for both manufacturers and consumers.

Various detection techniques are available to determine nickel in various samples, such as inductively coupled plasma–mass spectrometry (ICP–MS) [66, 67], atomic absorption spectroscopy (AAS) [62, 68], and inductively coupled plasma

optical emission spectroscopy (ICP–OES) [69-71]. These methods can provide low detection limits and high sensitivities. However, most of these detection methods require bulky and high-cost instruments and skilled users [72]. In addition, separation and preconcentration steps are still required in trace analysis due to low sensitivity and matrix effects on the direct analysis of real samples [73].

To date, many materials have been reported for the construction of sensors, such as anionic and cationic dyes [74], Schiff base chemosensors [75], and nanoparticles [76], for the selective detection of nickel. However, some of these materials showed low sensitivity and required time-consuming synthesis methods; in addition, they were not environmentally friendly and expensive. Moreover, some sample preparation methods, including cloud point extraction (CPE) [77, 78], LLE [79], ultrasound-assisted extraction (UAE) [80, 81], SPME [82], and VA-DLLME [83], have been developed for the separation and preconcentration of nickel from various samples. However, some of these extraction methods are expensive and still use a relatively large volume of toxic chemicals (surfactants and organic solvents) as extraction solvents. Furthermore, most extraction processes were slow and involved several steps, elongating the total analysis time. Therefore, analytical chemists are placing a significant emphasis on developing simpler, greener, and faster extraction techniques.

G-EME is an extraction method recently developed and expanded by Tabani et al., in which an electrical field is used as a propelling force to transfer ions among different phases, i.e., from donor solution across the membrane (Gel) to acceptor solution [84]. This technique uses a new solvent-free membrane and a green alternative to polypropylene membranes impregnated with organic solvents in traditional DLLME and EME techniques. However, the major problem during

agarose G-EME is the EEO flow phenomenon, which can occur within the gel membrane due to the presence of anionic groups (e.g., sulfates). This phenomenon can change the volume of acceptor solutions during the extraction [39].

To address this problem, Sahragard et al. recently introduced a new integrated extraction technique called the electrocolorimetric extraction system [85]. In this system, colorimetric reagents are added directly to the gel solution in the gel fabrication step. After applying a voltage between the cathode electrode (negative) in the gel and anode electrode (positive) in the donor solution, the positively charged analytes were driven from the donor solution to the gel, where they reacted with specific reagents and produced colors. This color could then be analyzed in different ways, either with the naked eye, digital cameras, smartphones, or spectrometric systems. This method used a lower volume of toxic organic solvents and reduced the number of steps by combining extraction and detection in one place, leading to improved repeatability and sensitivity because of the preconcentration of the analyte in the system and shortening the total analysis time without expensive detection instruments. Moreover, in addition to shorter extraction times, this method does not require any organic solvent, polymer membranes, or syringe pumps in comparison with other integrated and on-chip electromembrane extraction systems [15]. Furthermore, the system was convenient to use and possibly portable. However, despite all these advantages, the electrocolorimetric extraction system in previous research [85] was only applied for anionic analytes in the sample, which has simple matrices. Therefore, our next challenge is to extend the application of the system to cationic detection, such as Ni(II), in food samples that contain complex matrices.

In this work, an electrocolorimetric extraction system was developed to determine Ni(II) using gel modified with dimethylglyoxime (DMG) and ammonia. When a voltage was applied between the gel and donor solution, Ni(II) ions were extracted, and the pink color of the Ni(II)-DMG complex was formed in the gel. Digital images of the gels taken before and after extraction by smartphones were used for quantitative colorimetric analysis. In this study, the main goal was to investigate the potential of the electrocolorimetric extraction system for the detection and determination of Ni(II) as a positive ion in a complex food sample. The potential factors affecting the system efficiency, such as the pH of the gel, the applied voltage, and the extraction time, were optimized and discussed in detail. Finally, the optimized procedure was used to determine Ni(II) ions in chocolate samples.

2. Experimental section

2.1. Chemicals and samples.

All chemicals used were of analytical reagent grade and high purity (99.9%). DMG, ammonia, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3{\times}9H_2O$, $Pb(NO_3)_2$, $Co(NO_3)_2$, $Hg(NO_3)_2$, $Cr(NO_3)_3$, $Mn(Cl)_2$, KNO_3, $MgCl_2$, $CaCl_2$, NaOH, nitric acid, and hydrochloric acid were used without further purification (Merck, Germany). A stock standard solution containing 1000 mg L^{-1} Ni(II) was prepared by dissolving an appropriate amount of $Ni(NO_3)_2$ in Milli-Q water, which was obtained through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions. Agarose was purchased from SeaKem LE Agarose (low EEO: 0.09-0.13; gel point [°C]: 36±1.5 at 1.5%; gel strength [g cm^{-2}]: >1200 at 1.0%). Different kinds of chocolate samples, such as bars, nuggets, and dark samples, were purchased from supermarkets

(Bangkok, Thailand). The pH values of all samples and standards were adjusted by adding sodium hydroxide/hydrochloric acid solutions before extraction.

2.2. Instrumentation

For the electrocolorimetric extraction system, a GPR-11H30D 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. A platinum wire (0.5 mm diameter) was obtained from Sigma–Aldrich (St. Louis, MO, USA). The image of the colored gel was taken by an A71 Samsung smartphone. It is worth mentioning here that other smartphones can be used as well. However, the validation step should be carried out again. A light-tight black box with LEDs was used to control the illumination conditions when taking the image of the colored gel. The blue color intensity was measured and processed using ImageJ software. A benchtop pH meter with a glass electrode (Mettler Toledo, USA) was used to measure the pH of the solutions. A Thermo Scientific ICAP6500 Duo inductively coupled plasma–optical emission spectrometry (ICP–OES) system was used to quantify Ni(II) in chocolate samples as a standard verification method **(Table. 5)**. Agarose gel solution and chocolate samples were dissolved using a microwave at 800 W (Electrolux, Sweden).

Table 5 Operating conditions of ICP-OES.

Plasma gas flow rate	12 L min^{-1}
Auxiliary gas flow rate	0.5 L min^{-1}
Nebulizer gas flow rate	0.5 L min^{-1}
Coolant gas flow rate	12 L min^{-1}
RF power	1150 watts
Plasma view	Axial
Read delay	30 sec
Spray chamber	Cyclonic
Pump speed	50 rpm
Injector	Quartz tapered 1.0 mm bore
Nebulizer	Glass concentric
Sample tubing	Pump tube aqueous sample 0.64 mm i.d.
Drain tubing	Aqueous drain pump tube 1.02 mm i.d.
Torch	Duo Torch
Replicates	3

For the spectrophotometric measurement, a portable USB4000 spectrometer (Ocean Optics, Ottawa, Canada) was used to record the UV–Vis signals, and SpectraSuite software (Ocean Optics, Ottawa, Canada) was used for data acquisition. In addition, a locally purchased LED light source with a wavelength of 550 nm was used for the spectrometric detection of Ni(II)-DMG complexes [86].

2.3. Fabrication of agarose gel membrane

The agarose membrane was fabricated in a similar method to that of our previous works [33, 85]. First, the reagent solution was prepared by adding 2.5 mL of 80 mM DMG and 2.5 mL of 0.03 M ammonia to 20 mL of Milli-Q water (the final volume solution equals 25 mL). Then, 0.75 g of agarose powder (3%) was weighed and mixed with the reagent solution in a beaker. After that, this solution was put into a microwave for 3 min to dissolve all components completely, producing a liquid gel. After that, 425 μL of the hot mixture was quickly transferred into a 1-mL Eppendorf tube (Hamburg, Germany) and placed in the refrigerator at 4 °C for 24 h. The gel was formed at the lower half of the Eppendorf tube. Finally, the conical bottom part of the vial was cut with a razor blade, giving the optimized gel thickness (11 mm). After studying all parameters, optimized conditions, including membrane thickness (11 mm), electrode distance (3 mm), extraction time (5 min), stirring rate (600 rpm), pH of donor solution (6.0), and applied voltage (70 V), were used for analytical determinations. The average current during extractions was ~ 200 μA.

2.4. Extraction procedure

The electrocolorimetric extraction system was set up as illustrated in **Figure 13**. A prepared agarose gel membrane with optimized thickness was placed into the standard/sample solution (10.0 mL). The platinum electrodes connected to a DC power supply were positioned in the donor solution as an anode and in the gel as a cathode. The distance between the cathode inside the gel and the tip of the gel was optimized. The whole extraction cell was placed on the stirrer, and the extraction was carried out for a predetermined time after applying the electrical potential. In this stage, nickel ions were extracted into the gel and reacted with the reagents, forming a

pink color inside the gel. Then, the tube containing the colored gel was placed at a fixed position in the homemade light-controlled box. The image of the bottom surface of the colored gel was taken by a smartphone mounted 15.0 cm above the gel. The image was processed by ImageJ software using the blue intensity of RGB mode. The concentration-related signals were calculated by subtracting the intensity obtained for the colored gel after extraction of standard/sample from the intensity obtained for the gel after extraction of blank solution (Δ blue intensity = Blank Intensity - Standard Intensity).

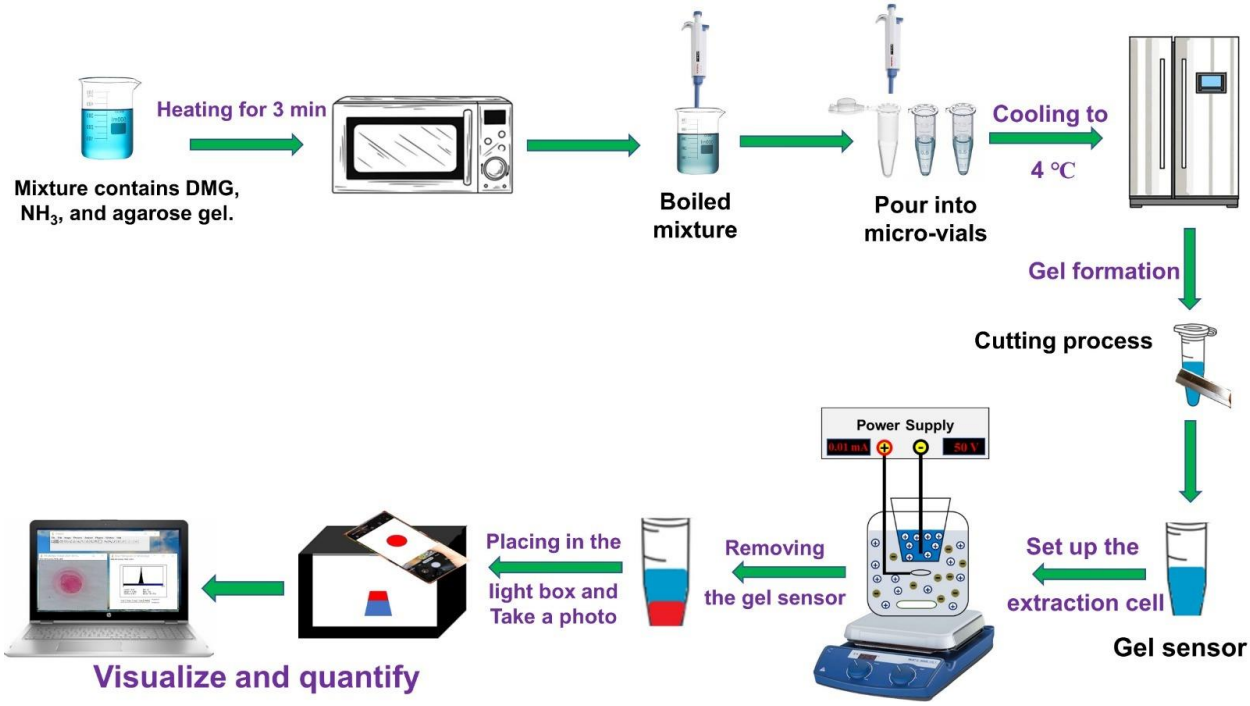

Figure 13 Schematic of the working principles of the proposed electrocolorimetric system for Ni(II) detection.

2.5. Preparation of the samples

Sample pretreatment steps were carried out based on previous studies with some modifications [33, 60, 87]. First, the chocolate sample was ground and thoroughly homogenized by a laboratory blender. Next, 2.0 g of sample was weighed and

transferred to a beaker. A total of 1.0 mL of concentrated HNO_3 and 3.0 mL of water were added to the sample. The beaker was closed and heated for acid digestion in a microwave operated at 800 W for 6 min. After cooling to room temperature, the volume was brought to just under 10.0 mL with Milli-Q water for extraction. Then, the pH values of the digested solutions were adjusted to 6.0 with a 6 mol L^{-1} NaOH solution and filtered, and then the volume was adjusted to 10.0 mL. The final solutions were used in the electrocolorimetric extraction system. The same steps were followed for ICP analysis in a final volume of 40.0 mL. Since the samples were filtered, the color of the sample did not affect the color of the gel in the extraction experiments.

2.6. Calculations and anti-contamination procedure

The preconcentration factor (PF) and relative recovery percentage (RR%) were calculated based on equations (3) and (4), respectively.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated experimentally by $3.3\sigma/m$, and $10\sigma/m$, respectively, where σ is the standard deviation of the Y-intercept and m is the slope of the regression line.

The extraction glass vial, platinum electrodes, and other glassware were washed first with HNO_3 (1.0 M) and then twice with Milli-Q water before each extraction to avoid contamination, especially contamination of nickel. In addition, the laboratory practices, the use of personal protective equipment, and the method of waste disposal complied with the protocols provided by the Department of Chemistry and the Centre for Safety, Health, and Environment of Chulalongkorn University.

3. Results and discussion

In this work, the method was developed to determine a cationic analyte. The electrical potential was applied in the opposite direction compared to our previous work (anion) [85], which could affect the direction of EEO flow. Furthermore, from our previous work, a colored complex could form at the middle or at the tip of the gel. Therefore, all related parameters were reinvestigated and optimized for better understanding and observation.

3.1. Optimization of physical and chemical parameters in the detection system

3.1.1. Optimization of physical parameters

3.1.1.1. Quantitative analysis of the Ni-DMG colored-gel images using ImageJ software

One of the most common methods for colored image processing using ImageJ software is measuring red, green, and blue intensities in RGB mode. Herein, four plots between concentration and blank subtracted intensity (ΔI = Blank Intensity - Standard Intensity) were obtained by analyzing RGB values using the histogram in ImageJ. As shown in **Figure S1**, the blank subtracted intensities (ΔI) of red, green, blue, and gray increased with an increase in Ni(II) concentrations, and the highest sensitivity (steepest slope) was observed for the blue intensity. Therefore, the blue intensity in RGB mode was chosen to determine Ni in Ni-DMG colored-gel images.

3.1.2. Optimization of chemical parameters

3.1.2.1. Composition of calorimetric reagents in agarose gel

The composition of colorimetric reagents in agarose gel was studied to obtain the highest color intensity. Agarose gel was prepared by 3% w/v agarose and equal volumes ($V_{ammonia}$=V_{DMG}) of 80 mM DMG and 0.03 M ammonia, producing total %v/v concentrations (($V_{ammonia}$+V_{DMG})/ $V_{total\ gel\ solution}$) ×100) of 2, 10, 20, 30, and

40%v/v. The results in **Figure 14** show that as %v/v of 1:1 DMG:ammonia was increased from 2 to 20%v/v, the color intensities increased since more Ni-DMG complexes could be formed. It was observed that the colored Ni-DMG complex was formed at the bottom side of the gel. Apparently, it is where the positively charged Ni ion that migrated toward the negative electrode inside the gel meets the negatively charged DMG and produces the color. As the %v/v of 1:1 DMG:ammonia increased from 20 to 40%v/v, the intensities did not increase but somewhat dropped. This phenomenon could be attributed to leakage of ammonia into the donor solution in very high concentrations of reagents, changing the pH to basic values, which was observed experimentally. In the basic condition, hydroxyl ions can react with Ni(II) ions and produce $Ni(OH)_2$. Since this species is neutral, it cannot migrate toward the gel under the application of voltage and cannot be extracted, resulting in a decrease in the extraction efficiency. Therefore, 20% v/v 1:1 DMG:ammonia was chosen as the optimal volume for further studies.

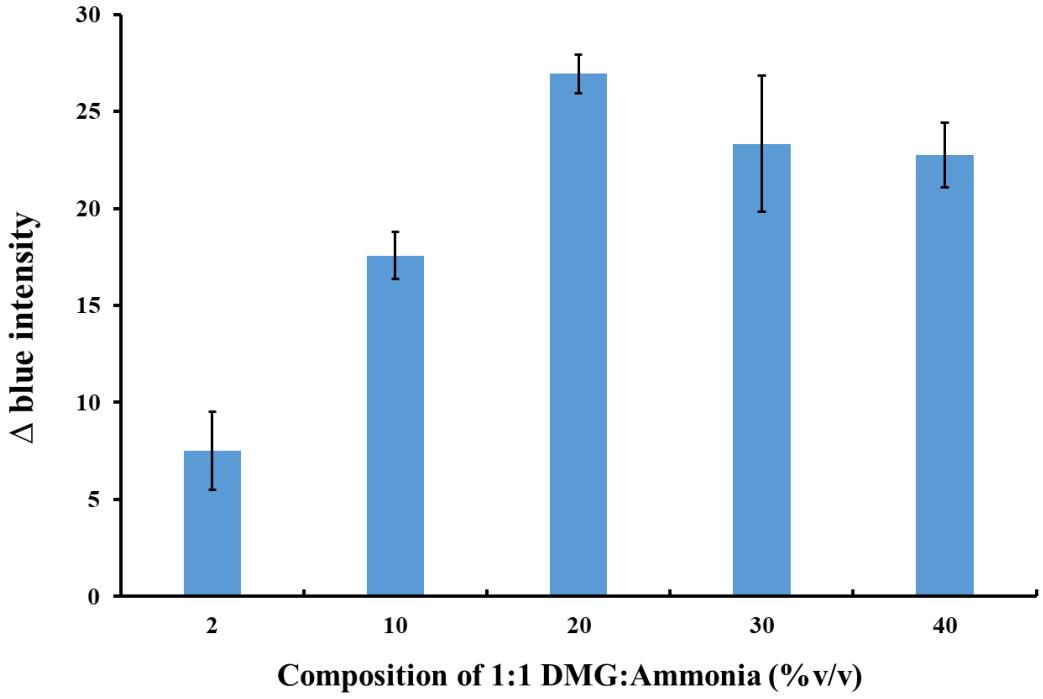

Figure 14 Effect of the composition of 1:1 DMG:Ammonia (%v/v) on the intensity (2, 10, 20, 30, and 40 (%v/v)). Other extraction conditions: concentration of Ni(II): 700 µg L^{-1}, voltage: 50 V, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, extraction time: 10 min, stirring rate: 600 rpm, (w/v), gel thickness: 9 mm, concentrations of agarose: 2%, DMG: 80 mM and agarose: 2 % (w/v). Error bars were obtained based on three replicates.

3.1.2.2. Agarose gel concentration (%w/v agarose)

Agarose gel concentration (%w/v agarose) could affect the physical stability of the gel in holding the colorimetric reagents, the migration of Ni ions into the gel, the formation, and the color intensity of the colored Ni-DMG complex in the gel. Therefore, it is necessary to optimize the agarose gel concentration. In this experiment, different concentrations of agarose gel from 1 to 5% w/v were examined. At agarose concentrations less than 1% w/v, the gel was not well solidified, and the electrode could not be steadily placed. As shown in **Figure S2**, the color intensities increased to the maximum when the agarose concentration was 3% and became almost constant at agarose concentrations between 3-5% w/v. This could be due to the

relationship between the pore sizes of the gel and the EEO level [37]. When the agarose concentration increased from 1 to 3%w/v, the pore sizes decreased. This could result in lower levels of EEO and, consequently, less dispersion of the color. When EEO increases, the analyte moves along the gel with the solution, causing band dispersion of the color. Consequently, 3% agarose was chosen for subsequent experiments.

3.1.2.3. Electrode distance and gel thickness

The effects of electrode distance (anode and cathode) and gel thickness on the color intensity were investigated. The distance between the electrodes influences the electrical current and the occurrence of electrolysis in the system, which could affect the transfer of Ni ions into the reaction zone in the gel (**Figure S3**). Based on observed data, 3 mm was chosen for the distance between electrodes. The gel thickness is also attributed to the color dispersion because of the EEO effect in the gel.

The gel thickness ranged from 7 to 13 mm, while the electrode was fixed at 3 mm above the bottom surface of the gel. For gel thicknesses less than 7 mm, the electrode was not able to be firmly placed inside the gel. As the gel thickness increased from 7 to 11 mm, the mean color intensity increased and reached a plateau at a thickness of 13 mm, as shown in **Figure S4**. It is worth mentioning that the current decreased by increasing the gel thickness (from 300 to 50 μA). Observably, color dispersion gradually developed for the thinner gel thickness because of the effect of EEO flow due to the relatively low electrical resistance of the gel. Since the colored image was taken at the bottom side of the gel, the color dispersion in the thinner gel thickness could significantly decrease the color intensity. For the thicker

gel thickness, despite the low level of EEO flow, the formation of the colored Ni-DMG complex may be restricted by the ion transferability inside the gel. Therefore, a gel thickness of 11 mm with a current level of 175 µA was chosen for the subsequent experiments.

3.1.2.4. Effect of Stirring Rate

The stirring of the donor solution plays a critical role in enhancing the extraction efficiency by ensuring constant contact of analyte ions in the donor solution with the gel surface. In this study, the stirring of the donor solution was examined between 0-1000 rpm, and the results are shown in **Figure S5**. By increasing the stirring speed from 0 to 600 rpm, the extraction efficiency increased because of an increase in the diffusion of the analyte into the gel membrane. However, higher stirring rates (800-1000 rpm) can cause bubble formation, making the sample solution, vial, and electrodes unstable and detaching the gel from the vial wall; moreover, poor repeatability results. Therefore, 600 rpm was selected as the optimum condition.

3.1.2.5. The pH of the donor solution

Depending on the pH of the solution, cations can be present in different forms. Therefore, this experiment was conducted to study the pH effect in the range of 2.0 to 9.0 on the sensitivity of Ni(II) detection **(Figure 15).** The results showed that the color intensity changed from pH 2.0 to 6.0. At low pH values (2.0 and 3.0), the electrical current was so high because of the participation of H^+ ions in the electrolysis. Moreover, the high concentration of hydrogen ions can compete with Ni ions in reaching the negative electrode. Additionally, a high level of bubbles formed in the electrolysis process can create a barrier between the donor solution and gel. As the pH value was increased under acidic conditions, the effect of electrolysis and

bubble formation was decreased, while Ni(II) ions were still able to migrate from the solution into the gel and form a colored Ni-DMG complex, yielding a pink color in the gel. However, when the pH was increased to a less acidic condition from pH 6.0 to 9.0, the color intensity decreased. According to the $Ni(OH)_2$ reaction ($Ni^{2+}_{(aq)} + 2OH^-_{(aq)} \rightleftharpoons Ni(OH)_{2\ (s)}$), an increase in hydroxide ions increases the shift of the equilibrium toward $Ni(OH)_2$, resulting in less Ni(II) being electromigrated into the gel. In addition, additional Na^+ could compete with Ni(II) in electromigration into the gel. Therefore, the optimum pH was chosen to be 6.0.

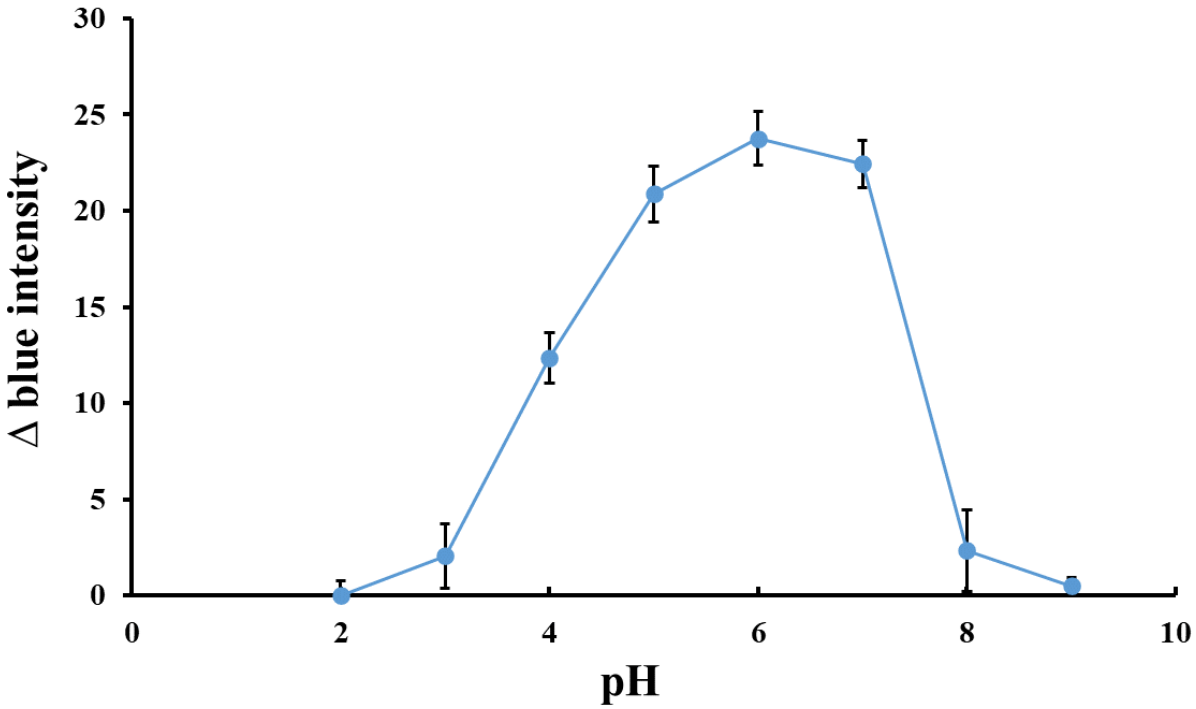

Figure 15 Effect of pH in the donor phase on the intensity. Other extraction conditions: concentration of Ni(II): 500 µg L^{-1}, voltage: 50 V, electrode distance from the gel tip: 3 mm, extraction time: 10 min, gel thickness: 11 mm, stirring rate: 600 rpm, and concentration of agarose: 3% (w/v), and concentration of Ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates.

3.1.2.6. Applied voltage

In electro-driven extraction systems, electrophoresis is the main driving force for transferring the analytes from the donor to the acceptor phases [88, 89]. As a result, a

series of experiments with various extraction voltages between 0 and 90 V was conducted to determine the most efficient voltage for nickel extraction. As shown in **Figure 16**, a tiny amount of color was observed when no voltage was applied, showing that the analyte could only barely diffuse from the donor phase to the gel. The color intensity increased dramatically with an increase in the applied voltage from 10 to 70 V. However, a gradual decrease was observed from 70 to 90 V. At voltages above 70 V, we observed the formation of bubbles at both electrodes because of electrolysis, which could create a barrier in the movement of ions toward the gel and negative electrode. At these voltages, some heat may also be generated during electrophoretic extraction, destabilizing the gel. In addition, an increase in electric current and EEO flow can also result in color dispersion and a decrease in sensitivity. The magnitude of the currents was observed in the range of 80-300 μA. Therefore, 70 V was chosen for the subsequent experiments, providing an electrical current of ~ 200 μA.

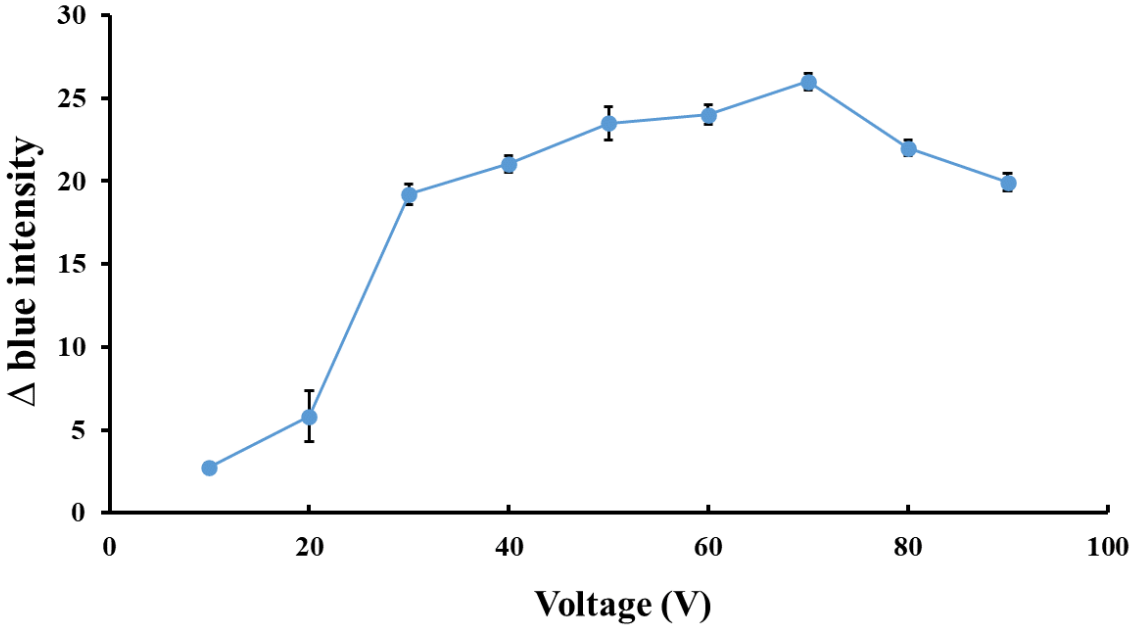

Figure 16 Effect of extraction voltage on the intensity. Other extraction conditions: concentration of Ni(II): 500 µg L^{-1}, pH of donor solution: 6.0, extraction time: 10 min, electrode distance from the gel tip: 3 mm, stirring rate: 600 rpm, gel thickness: 11 mm, concentration of agarose: 3% (w/v) and concentration of Ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates.

3.1.2.7. Extraction time

In extraction systems, the migration of analytes across the gel is also directly dependent upon extraction time [90]. Therefore, a series of experiments with different extraction times between 2.5 and 15 min were investigated and optimized using three different Ni concentrations of 300, 500, and 700 µg L^{-1}. The results are shown in **Figure 17.** For all Ni concentrations, the color intensity increased and reached a maximum at an extraction time of 5 min. After 5 min, the color intensity somewhat decreased. A longer extraction time increases the chance of color dispersion due to the effect of EEO flow. Therefore, an extraction time of 5 min was selected for this method.

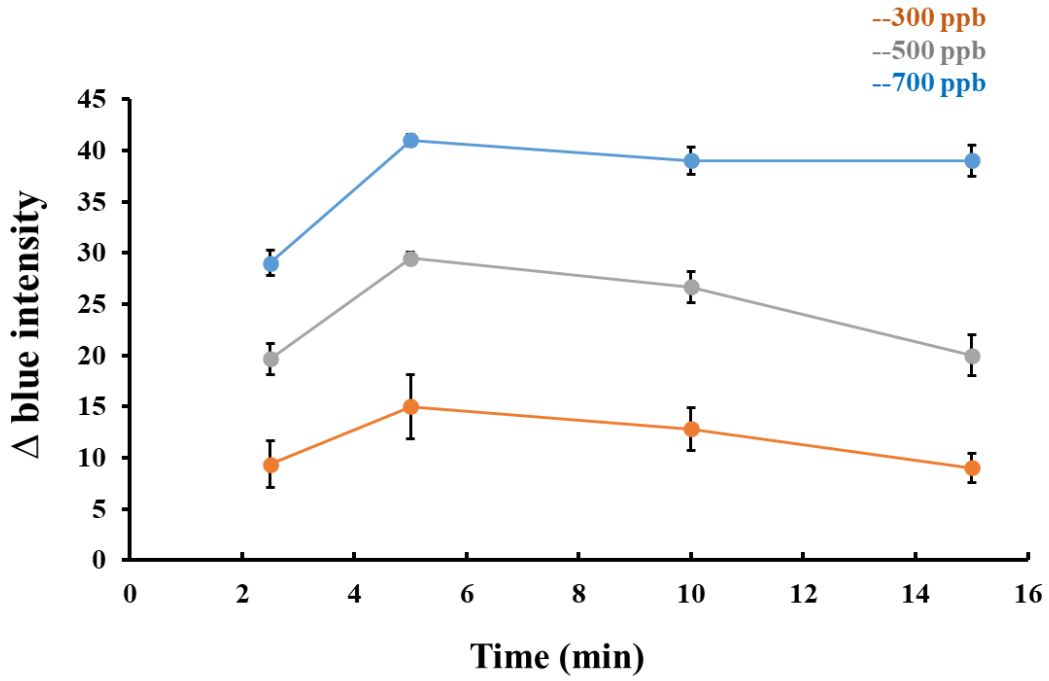

Figure 17 Effect of extraction time on the intensity. Other extraction conditions: concentration of Ni(II): 500 µg L^{-1}, voltage: 70 V, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, stirring rate: 600 rpm, gel thickness: 11 mm, concentration of agarose: 3% (w/v) and concentration of ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates (standard deviations were added to figures).

3.1.2.8. Ionic strength

The effect of ionic strength in the donor solution on extraction efficiency has been widely studied. Some studies reported that ionic strength in the donor solution could suppress the extraction of the target analyte [91]. Therefore, in this study, donor solutions with various ionic strengths were investigated using various concentrations of NaCl in the range of 0-5 mM **(Figure 18)**. The results indicated that the donor solution with more than 2.7 mM ionic strength had a negative effect on the signal intensity. Solutions with high ionic strength typically contain a large number of ions in the sample, leading to an increase in electrical current due to electrolysis resulting in the formation of bubbles near the solution-gel interface.

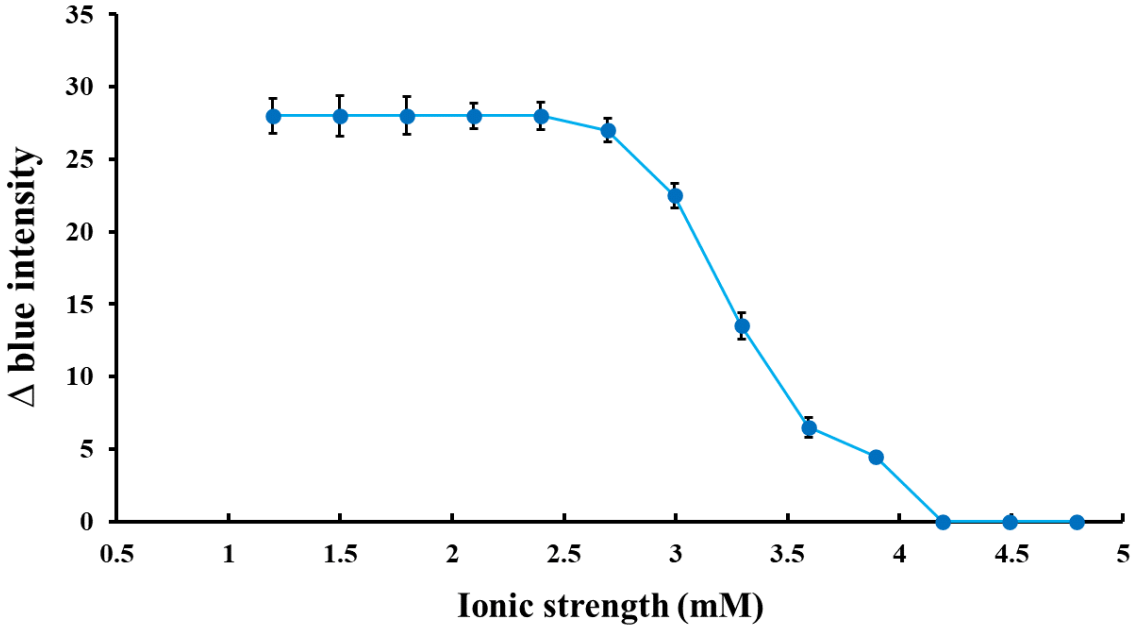

Figure 18 Effect of ionic strength on the intensity (0-5 mM NaCl). Other extraction conditions: concentration of Ni(II): 500 µg L^{-1}, voltage: 70 V, extraction time: 5 min, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, stirring rate: 600 rpm, gel thickness: 11 mm, concentration of agarose: 3% (w/v) and concentration of ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates (standard deviations were added to figures).

Moreover, many cationic species, i.e., Na^+, could compete with Ni(II) in electromigration to the gel interface. As the ionic mobility of Na^+ is relatively larger than that of Ni(II) (Dean, 1990), Na^+ could form a positive layer at the gel interface and prevent Ni(II) from migrating into the gel, resulting in no colored Ni-DMG complex being formed. This result suggested that our electrocolorimetric extraction system could be applied to samples with ionic strength up to 2.7 mM without a drop in its sensitivity. 5 and 50 µg L^{-1} of Ni(II) were also tested in this experiment in the presence and absence of 2.7 mM ionic strength, and no significant difference was observed. We also studied the effect of adding salt to the gel. The results showed that there is no effect until 3.4 mM as shown in **Figure S6.**

3.2. Analytical figures of merit

The analytical performance data for the electrocolorimetric system were evaluated in this section (**Table. 6**). Under the optimized conditions described above, the calibration curve for Ni(II) was linear over the concentration range of 30-750 µg L^{-1} with a coefficient of determination (R^2) of 0.9982, giving a linear equation of y = 0.0593x - 1.3727 (R^2 = 0.998) (**Figure 19**). The LOD of this method was obtained to be 1 µg L^{-1}, while LOQ was 4 µg L^{-1}. After that, this concentration was prepared, preconcentrated, and detected. The obtained color intensity was greater than that of the blank.

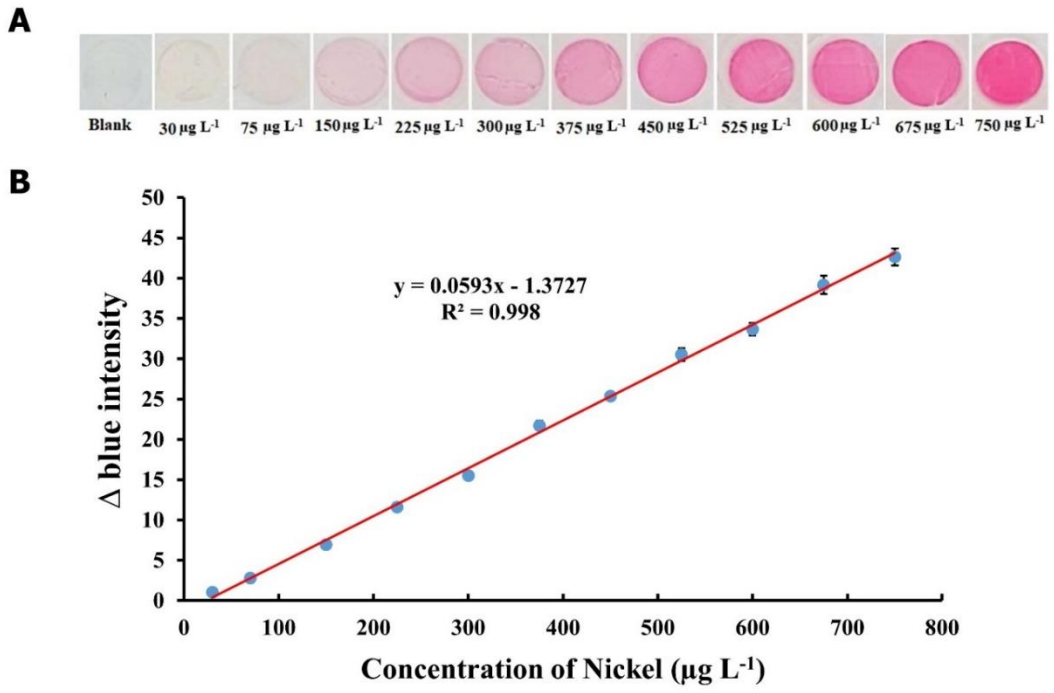

Figure 19 (A) Images of the electrocolorimetric system used for the detection of Ni(II) at different concentrations (30, 75, 150, 225, 300, 375, 450, 525, 600, 675 and 750 µg L^{-1}). (B) Analytical calibration curve obtained at different concentrations of Ni(II).

In addition, the detection method using color image analysis was compared to that using a spectrometer, as described in our previous research [85]. The results

showed that using a spectrometer gave a narrower linear range and lower sensitivity. Since the spectrometer measures transmitted light intensity, uneven color distribution and accumulation at the bottom of the gel could have been attributed to the low intensity of transmitted light. This suggests the advantage of camera-based detection over spectrometers as an alternative detection tool for our novel developed system.

Table 6 Analytical features of the electrocolorimetric extraction system.

Figures of merit	Ni (II)
Limit of detection (μg L^{-1})	1
Limit of quantification (μg L^{-1})	4
The slope of the calibration curve	0.0593
Coefficient of determination (R^2)	0.998
Linear dynamic range (μg L^{-1})	30 -750
RSD (%),	
Intraday (n=7)[a]	
70	1.4 %
375	1.2 %
675	1.9 %
Interday (n=7x3)[b]	
70	2.3 %
375	2.1 %
675	2.6 %
Preconcentration factor	42

[a] Intra-day precision was calculated for seven replicates at different concentrations of Ni(II).

[b] Inter-day precision was obtained for three days at different concentrations of Ni(II) (seven replicates).

The PF for Ni(II) was calculated to be 42 according to equation (3). Moreover, the intraday (n=7) and interday (n=3x7) relative standard deviation (%RSD) (representing the repeatability) for Ni(II) standard solutions at concentrations of 70, 375, and 675 µg L^{-1} were < 2.6% and < 1.9%, respectively, which are acceptable and considered practical.

3.3. Selectivity of the developed system

Selectivity is one of the most important factors for detecting ions due to coexisting ions in real samples along with the desired analytes, which may interfere with their signals. **Table. 7** shows the effects of some metal ions such as Cd^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Na^{+}, Cr^{3+} and Fe^{3+} on the responses of the Ni(II)-DMG complex in the extraction setup. As far as colorimetric detection is concerned, different interfering cations exhibit different effects. Cr^{3+}, Hg^{2+}, Pb^{2+}, Mn^{2+}, and Cd^{2+} did not form any color with the reagents inside the gel at any concentration. In fact, due to their positively charged ion, they could compete with Ni(II) in electromigration to the gel interface. The color intensity of Ni-DMG in the gel started to become affected at a concentration of 15 mg L^{-1}. The presence of Fe^{3+}, Cu^{2+}, and Co^{2+} at low concentrations did not affect the color of the gel. However, at concentrations higher than 10 mg L^{-1}, Fe^{3+} could form a yellow-brownish layer on the surface of the gel [92], Cu^{2+} could deeply penetrate the gel, forming a greenish color inside the gel, and Co^{2+} could turn the gel into a dark reddish color.

Nevertheless, these problems did not significantly interfere with Ni(II) detection at concentrations lower than 10 mg L^{-1}. It is worth mentioning that there is no report on the high concentration of Fe^{3+}, Cu^{2+}, and Co^{2+} ions in the chocolate samples, even at the level of 7.5 mg L^{-1}. In addition, no difference was observed for K^{+}, Mg^{2+}, or

Ca^{2+} ions until a very high concentration of them. There was no effect of anions in the present system because they are not drawn toward the negative electrode inside the gel due to their charge.

Table 7 Effect of some co-existing ions on the recoveries, based on triplicate measurements of 500 µg L^{-1} Ni(II).

Ions	Added as	Tolerance limit (mg L^{-1})[a]	RSD (%)	Recovery (%)
Na^{+}	NaCl	157	1.8	98
Zn^{2+}	$Zn(NO_3)_2$	12.5	2.1	98
Cd^{2+}	$Cd(NO_3)_2$	15	1.9	97
Fe^{3+}	$Fe(NO_3)_3 \times 9H_2O$	10	2.5	103
Pb^{2+}	$Pb(NO_3)_2$	15	2.1	98
Cu^{2+}	$Cu(NO_3)_2$	10	2.7	102
Co^{2+}	$Co(NO_3)_2$	10	2.2	102
Hg^{2+}	$Hg(NO_3)_2$	15	1.4	97
Cr^{3+}	$Cr(NO_3)_3$	15	1.7	98
Mn^{2+}	$Mn(Cl)_2$	15	1.8	99

[a] An ion was considered as interference when it caused a change greater than ± 3 % in the analytical signal of the Ni(II).

3.4. Real sample analysis

The applicability of the proposed system for preconcentration and detection of trace levels of Ni(II) ions was tested using eight chocolate samples from eight different brands. The samples were pretreated and adjusted to pH as described in Section 2.5 before extraction. Samples spiked with 0.25, 0.75, 1.50, and 2.25 µg/g Ni(II) ions were used for the assessment of the method recovery (relative recoveries, %RR) and %RSD. The concentration of Ni(II) found in chocolate samples, %RR, and %RSD, are summarized in **Table. 8**. The concentration of nickel in each sample could vary

and be directly related to chocolate production. The %RSDs % and %RRs were in the range of 0.9-5.8% and 91-109%, respectively. In addition, the results obtained from our proposed system were not significantly different from those obtained from the ICP–OES method ($t_{observed}$= 0.46 and $t_{critical}$ =1.76 at p= 0.05).

Table 8 Determination of Ni(II) ions in chocolate samples.

	Proposed method				ICP–OES			
Sample No.	**Added (µg/g)**	**Found (µg/g)**	**RSDs (%)**	**RR (%)**	**Added (µg/g)**	**Found (µg/g)**	**RSDs (%)**	**RR (%)**
1	0.00	0.16	1.1	-	0.00	0.18	0.92	-
	0.25	0.39	3.1	92				
	0.75	0.88	4.9	95				
	1.50	1.59	2.2	95	20.00	19.18	1.0	95
	2.25	2.47	5.8	103				
2	0.00	0.55	0.98	-	0.00	0.62	0.73	-
	0.25	0.82	3.1	106				
	0.75	1.28	2.3	97				
	1.50	1.95	3.6	93	20.00	19.46	0.84	94
	2.25	2.61	4.9	91				
3	0.00	0.00	-	-	0.00	0.00	1.1	-
	0.25	0.23	3.6	94				
	0.75	0.74	4.1	99				
	1.50	1.45	2.4	97	20.00	19.60	0.91	98
	2.25	2.45	2.0	109				
4	0.00	0.57	1.7	-	0.00	0.62	0.92	-
	0.25	0.82	2.9	100				
	0.75	1.31	4.9	99				
	1.50	2.00	3.2	96	20.00	21.02	1.1	102
	2.25	2.64	3.2	92				
5	0.00	0.34	1.4	-	0.00	0.40	0.63	-
	0.25	0.60	4.0	104				

	0.75	1.03	2.4	91				
	1.50	1.89	2.9	103	20.00	20.10	1.2	98
	2.25	2.53	1.9	97				
6	0.00	0.76	2.0	-	0.00	0.84	0.93	-
	0.25	1.00	4.2	96				
	0.75	1.49	3.4	97				
	1.50	2.18	2.7	95	20.00	19.94	1.1	95
	2.25	2.84	3.4	92				
7	0.00	2.66	1.5	-	0.00	3	1.0	-
	0.25	2.92	5.3	104				
	0.75	3.41	2.7	100				
	1.50	4.13	2.7	98	20.00	21.3	1.2	91
	2.25	4.76	3.7	93				
8	0.00	0.20	1.2	-	0.00	0.24	0.84	-
	0.25	0.44	5.5	96				
	0.75	1.01	4.8	109				
	1.50	1.79	2.2	106	20.00	20.34	1.1	100
	2.25	2.61	1.9	107				

3.5. Comparison of the proposed method with other methods

A comparison between our method and the previously reported methods for Ni(II) preconcentration and detection in terms of several parameters, such as using an organic solvent, LOD, %RSDs, and linear dynamic range (LDR), is summarized in **Table. 9**. It is evident that our method uses no organic solvent at all, while the previous extraction methods mostly needed a high amount of solvents, especially liquid extraction-based systems. In addition, the extraction time and total analysis time of our method are within 6 min, which is significantly short since the extraction and detection steps are integrated with comparable LOD, LDR, and repeatability (as %RSDs). Moreover, our colorimetric detection method uses only a smartphone,

which is fast, easy, low-cost, and user-friendly. No expensive and bulky instruments are required. In addition, there is no need for synthesized materials, which are difficult to synthesize, expensive, and not environmentally friendly.

Table 9 Comparison of previous methods with the proposed method for determination of Ni(II).

Extraction technique	Extraction solvent	Extraction solvent Volume (µL)	Linear dynamic range (µg L^{-1})	Time (min)	Volume of aqueous phase (mL)	LOD (µg L^{-1})	RSD (%)	EF	Detection technique	Sample	Ref
Quinoxaline	CH_3CN–HEPES	-	-	-	-	86	-		Colorimetric	Water	[93]
Schiff base	Water	-	-	-	-	59	-		Colorimetric	Water	[94]
-	Water	-	0.059-58690	-	-	138	-		Electrochemical	Water	[95]
LL-SHS-LPME[a]	1-EP and water	800	17-500		10	5.2	6	10	FAAS	Food and cigarette	[96]
USAEME[b]	Trichloroethylene	100	-	-	5	0.23	2.3–4.1	190	FAAS	Water	[97]
AA-HLLME[c]	$CHCl_3$	150	5-600	5	20	3.6	1.6	340	FAAS	Water	[98]
VA-IL-DLLM[d]	Ninhydrin [C6mim][FAP] Ethanol	500	1-350	2	125	0.3	2.5–3.6	75	FAAS	Chocolate	[61]
PV-IS-DLLME[e]	[C4MIM][PF6]	120	6.7-100	1	5	2	4.8	17	FAAS	Chocolate	[99]
ECGS[f]	-	0	30-750	5	10	1	1.1-3.7	35	Colorimetric	Chocolate	This work

[a]Ligandless switchable solvent-based liquid-phase microextraction method (LL-SHS-LPME)
[b]Ultrasound-assisted emulsification microextr ion (USAEME)
[c]Aeration-assisted homogeneous liquid-liquid microextraction (AA-HLLME)
[d]Vortex assisted-ionic liquid-based dispersive liquid-liquid microextraction
[e]Pressure variation in-syringe dispersive liquid-liquid microextraction (PV-IS-DLLME)
[f] Electrocolorimetric gel sensing approach.

4. Conclusion

In this work, an electrocolorimetric extraction system was used to determine Ni(II) in chocolate samples for the first time. The developed method combined clean-up, preconcentration, and detection in only one step, providing higher repeatability, simplicity, and shorter total analysis time with an LOD and LDR that are comparable with previously reported extraction methods. Moreover, the agarose gel used in this work is an environmentally friendly material that is colorless and, more importantly, compatible with the extraction mechanism and colorimetric detection. The proposed method obeys green chemistry principles since no organic solvent is required. From a technical aspect, there was no need to fabricate complex microfluidic chips and sorbents typically required in integrated systems. Furthermore, this system was successfully applied to determine Ni(II) in chocolate samples, and the results were in good agreement with those obtained using the conventional ICP–OES method.

Chapter 5: Conclusions

In this thesis, we focused on the development of a new extraction system, called gel based electrocolorimetric extraction, which is categorized in the scope of sample preparation methods prior to analysis. This system was designed using colorimetric reagents pre-added to the agarose gel for the determination of the following two model analytes: iodide and nickel ions. Herein, starch, HCl, and H_2O_2 were used as colorimetric reagents for color formation in iodide detection, while DMG and ammonia were used as colorimetric reagents for nickel detection. In these systems, when a voltage is applied, the analytes are extracted and transferred from the sample solution (donor phase) to the gel (acceptor phase) under application of an appropriate voltage. The analytes then simultaneously reacted with the colorimetric reagents inside the gel, yielding blue and pink colors for iodide and nickel complexes, which were then analyzed by a portable spectrometer, and a smartphone, respectively. Parameters that could affect the extraction efficiency such as the pH of the gel (type and concentration of acid), concentration of agarose, thickness of the gel, applied voltage, stirring rate, pH of the sample solution, and extraction time were investigated to obtain the best extraction efficiencies. Finally, the optimal systems were employed to determination of iodide ions in the food supplements and nickel ions in chocolate samples. Possibility of direct extraction from the complex matrices, providing considerable sample clean-up and acceptable LODs, and capability of running a low-cost and on-site analysis are among the advantages of the proposed method. This efficient method can be expanded as a simple, portable, green, and useful method for the analysis of a wide variety of analytes in various types of real samples. In brief, highlights of this thesis include: (A) agarose gel as a green material is used for the

fabrication of extraction systems, (B) extraction, preconcentration, and detection steps are achieved in one step by these platforms, (C) in-situ detection of analytes at ppb levels by the naked eye, and (D) this sensing methodology can be exploited to analyze a wide range of analytes and real samples using different detection systems.

Appendix A: Chapter 3 Supplementary Materials

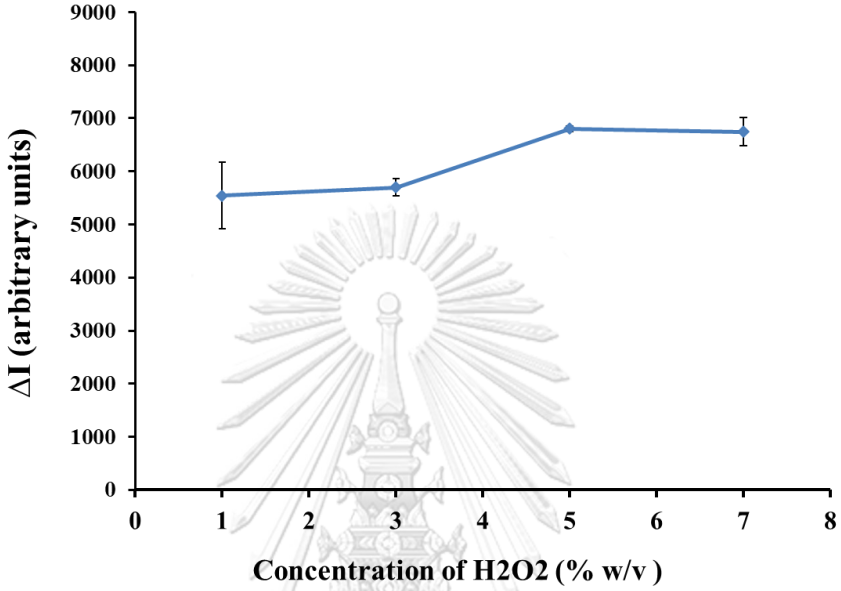

Figure S1. Effect of concentration of H_2O_2 in the gel on the extraction of iodide. Other extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 10 min, stirring rate: 600 rpm, gel thickness: 9 mm, concentrations of iodide: 500 µg L^{-1}, HCl: 2 mM, starch: 1 % (w/v), and agarose: 2 % (w/v). Error bars were obtained based on three replicates.

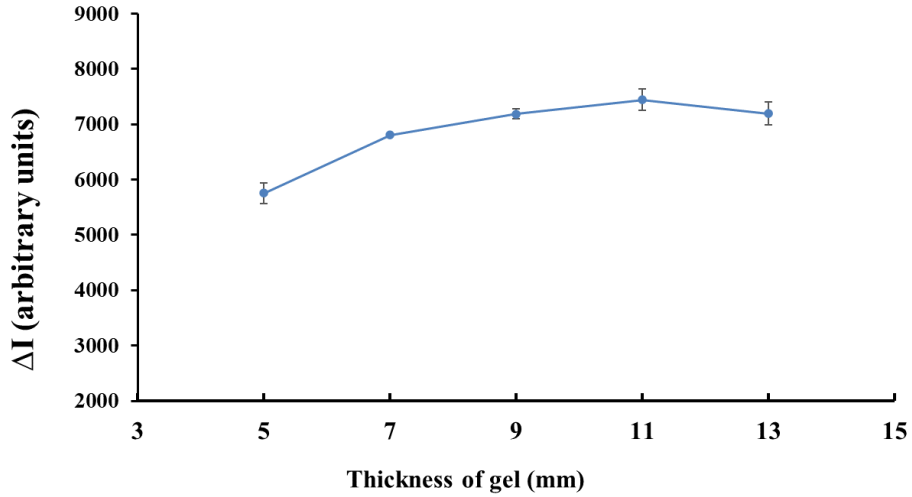

Figure S2. Effect of gel thickness on the extraction of iodide. Other extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 10 min, stirring rate: 600 rpm, concentrations of iodide: 500 µg L^{-1}, H_2O_2: 5 % (w/v), HCl: 2 mM, agarose: 4% (w/v), and starch: 1 % (w/v). Error bars were obtained based on three replicates.

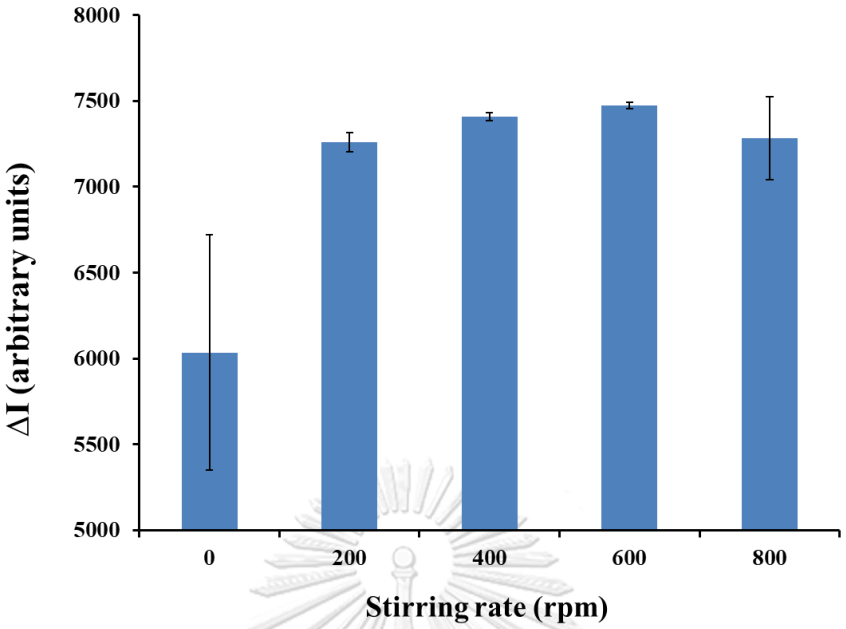

Figure S3. Effect of stirring rate on the extraction of iodide. Other extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 10 min, gel thickness: 11 mm, concentrations of iodide: 500 µg L^{-1}, H_2O_2: 5 % (w/v), HCl: 2 mM, agarose: 4% (w/v), and starch: 1 % (w/v). Error bars were obtained based on three replicates.

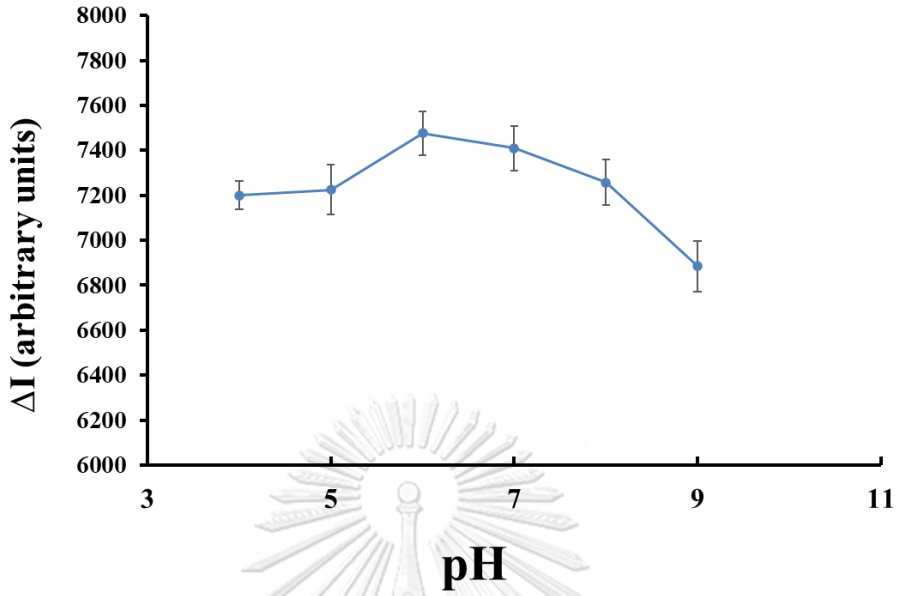

Figure S4. Effect of pH in the donor phase on the extraction of iodide. Other extraction conditions: voltage: 50 V, extraction time: 10 min, gel thickness: 11 mm, stirring rate: 600 rpm, concentrations of iodide: 500 µg L^{-1}, H_2O_2: 5 % (w/v), HCl: 2 mM, and agarose: 4% (w/v), and starch: 1 % (w/v). Error bars were obtained based on three replicates.

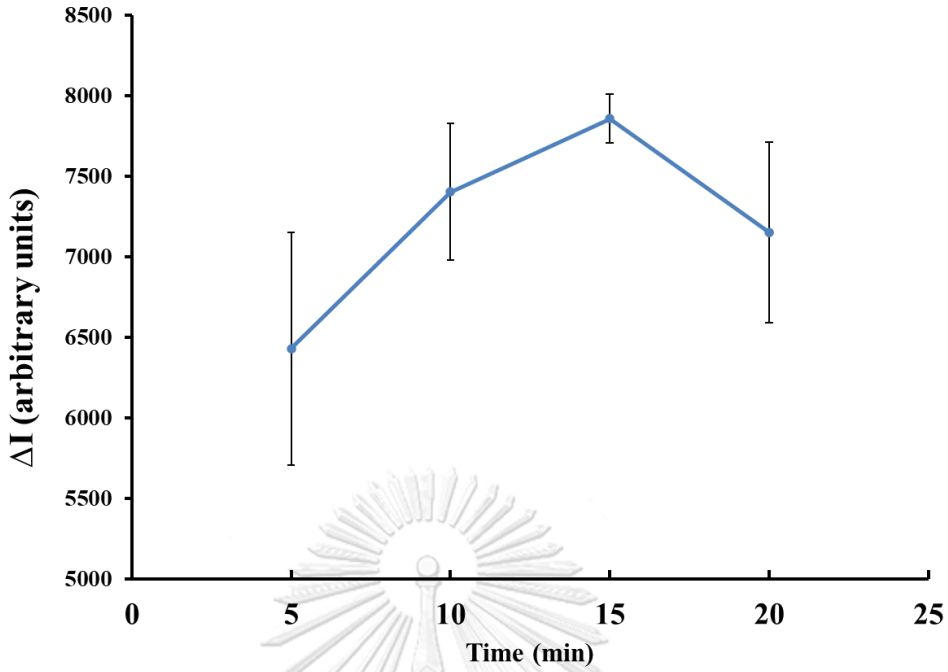

Figure S5. Effect of extraction time on the extraction of iodide. Other extraction conditions: voltage: 50 V, donor phase pH: 6.0, stirring rate: 600 rpm, gel thickness: 11 mm, concentrations of iodide: 500 µg L^{-1}, H_2O_2: 5 % (w/v), HCl: 2 mM, and agarose: 4% (w/v), and starch: 1 % (w/v). Error bars were obtained based on three replicates.

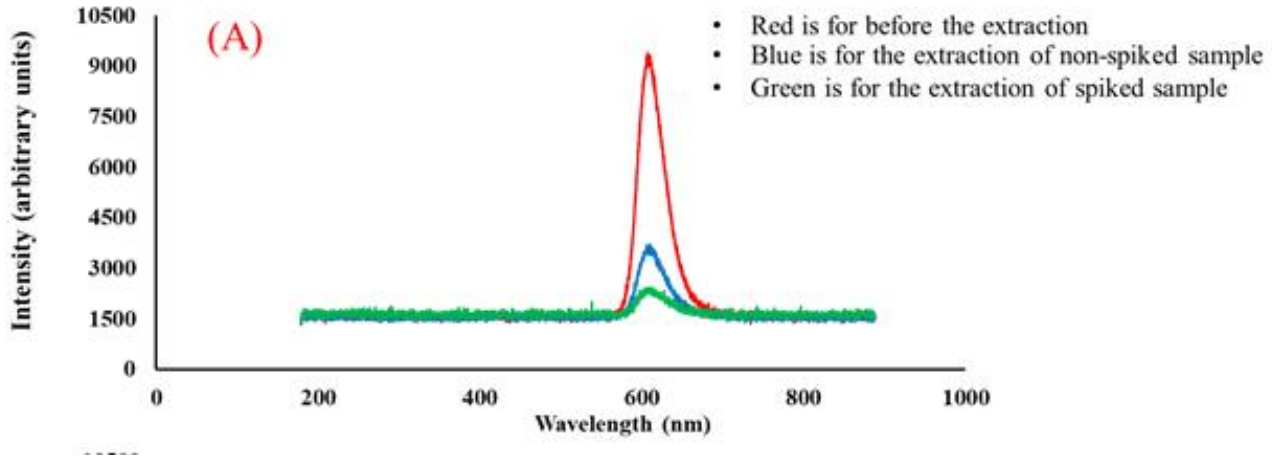

Figure S6. Intensity profiles obtained from integrated G-EME colorimetric system (A) at 609 nm for iodine-starch complex; extraction conditions: voltage: 50 V, donor phase pH: 6.0, extraction time: 15 min, gel thickness: 11 mm, stirring rate: 600 rpm, concentrations of H_2O_2: 5 % (w/v), HCl: 2 mM, agarose: 4% (w/v), and starch: 1 % (w/v). Red: before extraction; Blue: after extraction of real samples; Green: after extraction of spiked samples (50 µg L^{-1} for iodide).

Appendix B: Chapter 4 Supplementary Materials

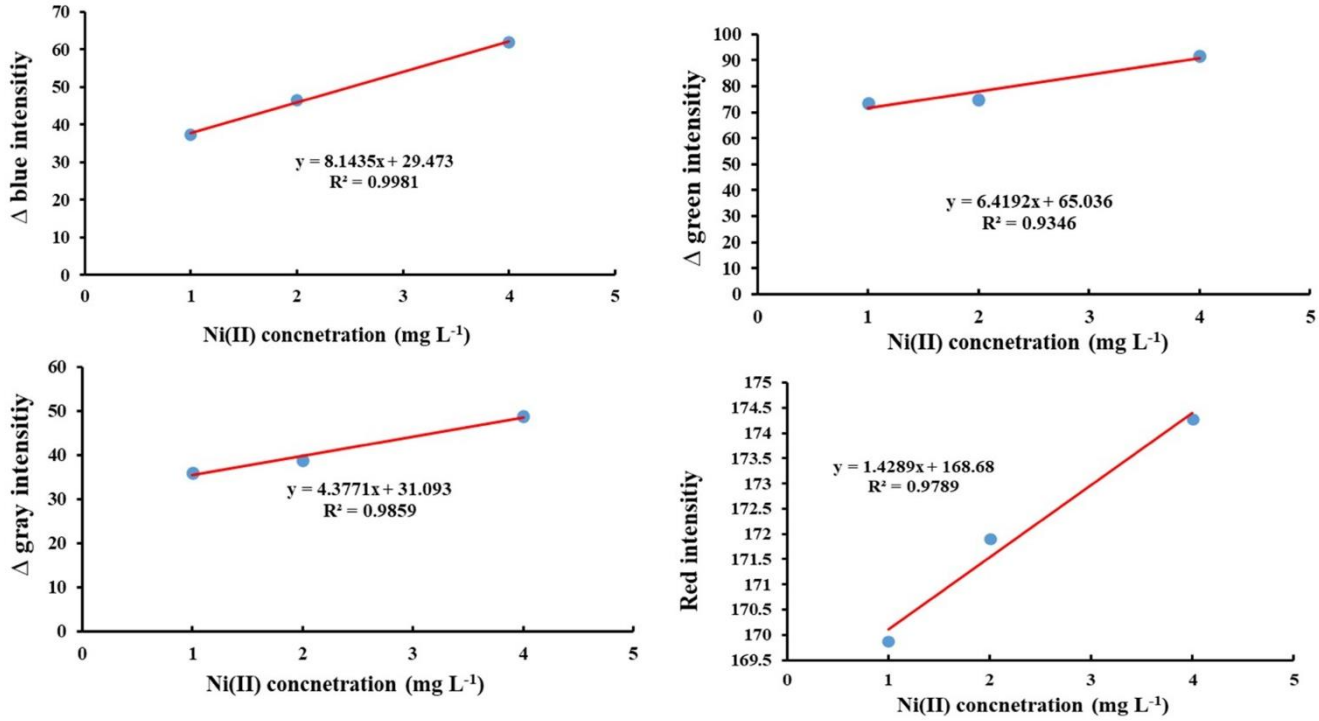

Figure S1. Effect of the color component on the intensity at different nickel concentrations.

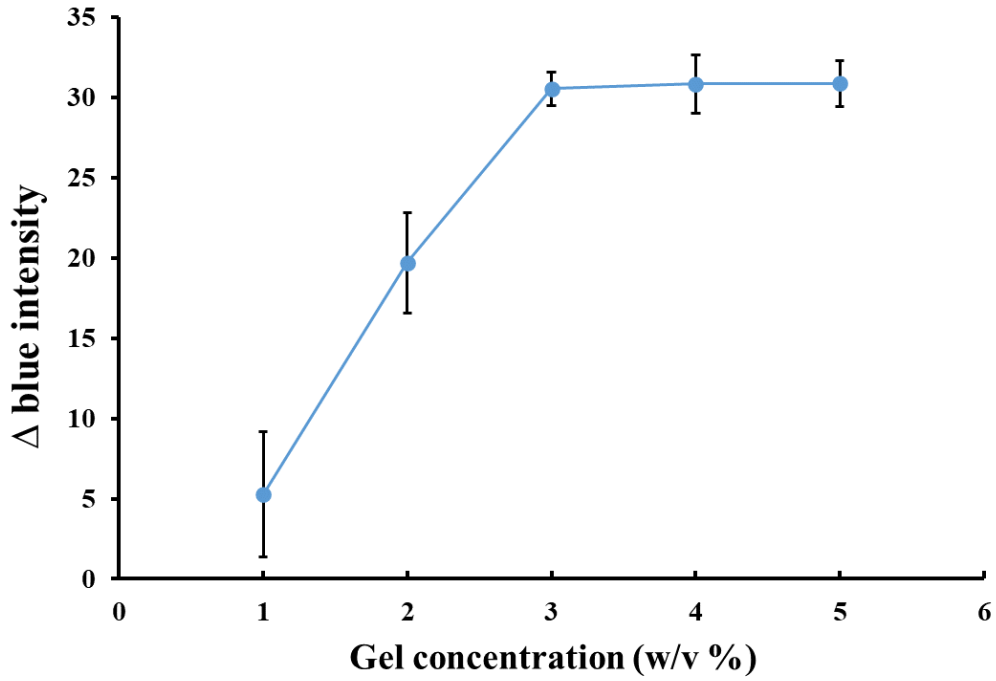

Figure S2. Effect of the agarose concentration on the intensity. Other extraction conditions: concentration of Ni(II): 700 µg L^{-1}, voltage: 50 V, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, extraction time: 10 min, stirring rate: 600 rpm, gel thickness: 9 mm, and concentration of Ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates.

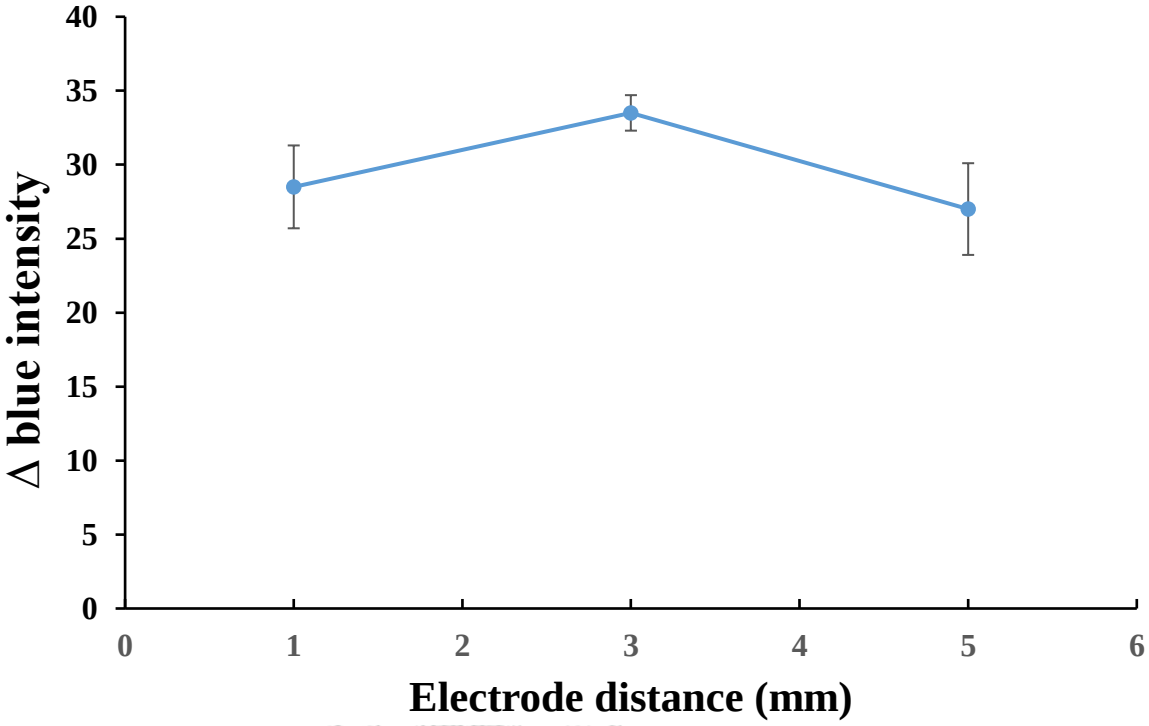

Figure S3. Effect of electrode distance (mm) on the intensity. Other extraction conditions: concentration of Ni(II): 700 µg L^{-1}, voltage: 50 V, gel thickness: 11 mm, pH of donor solution: 6.0, extraction time: 10 min, stirring rate: 600 rpm, concentration of Ammonia (0.03 M), and DMG: 80 Mm (20% v/v) and concentration of agarose: 3% (w/v). Error bars were obtained based on three replicates.

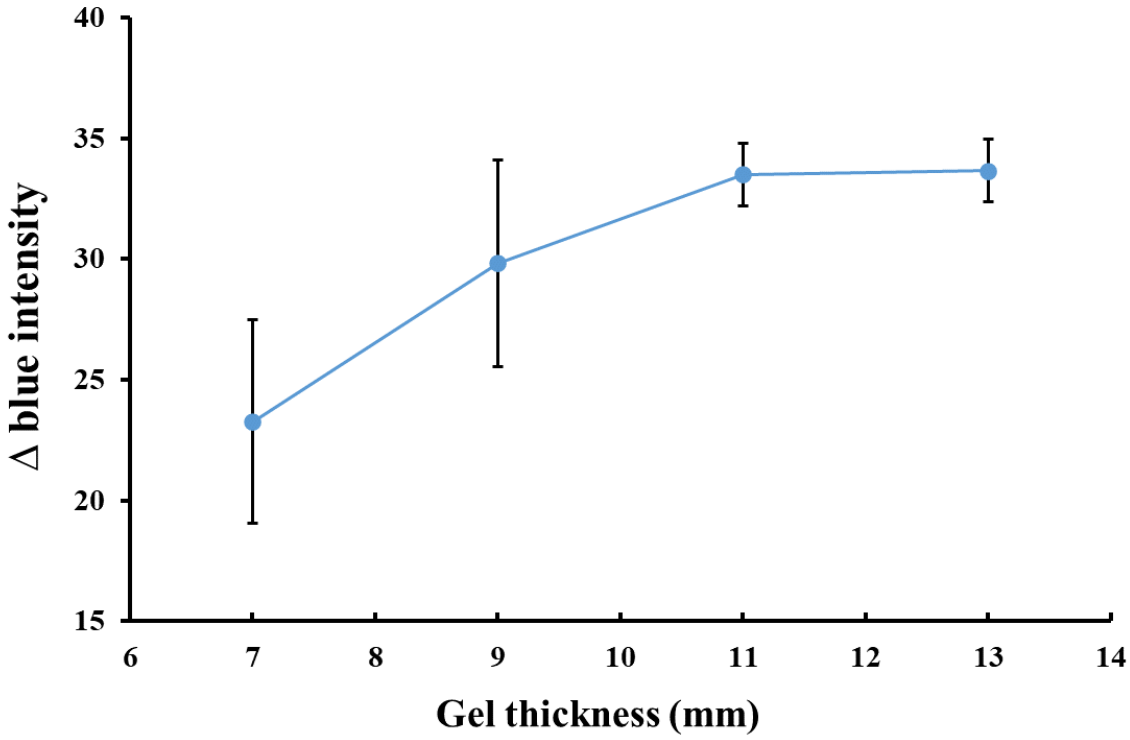

Figure S4. Effect of gel thickness (mm) on the intensity. Other extraction conditions: concentration of Ni(II): 700 µg L^{-1}, voltage: 50 V, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, extraction time: 10 min, stirring rate: 600 rpm, concentration of Ammonia (0.03 M), and DMG: 80 Mm (20% v/v) and concentration of agarose: 3% (w/v). Error bars were obtained based on three replicates.

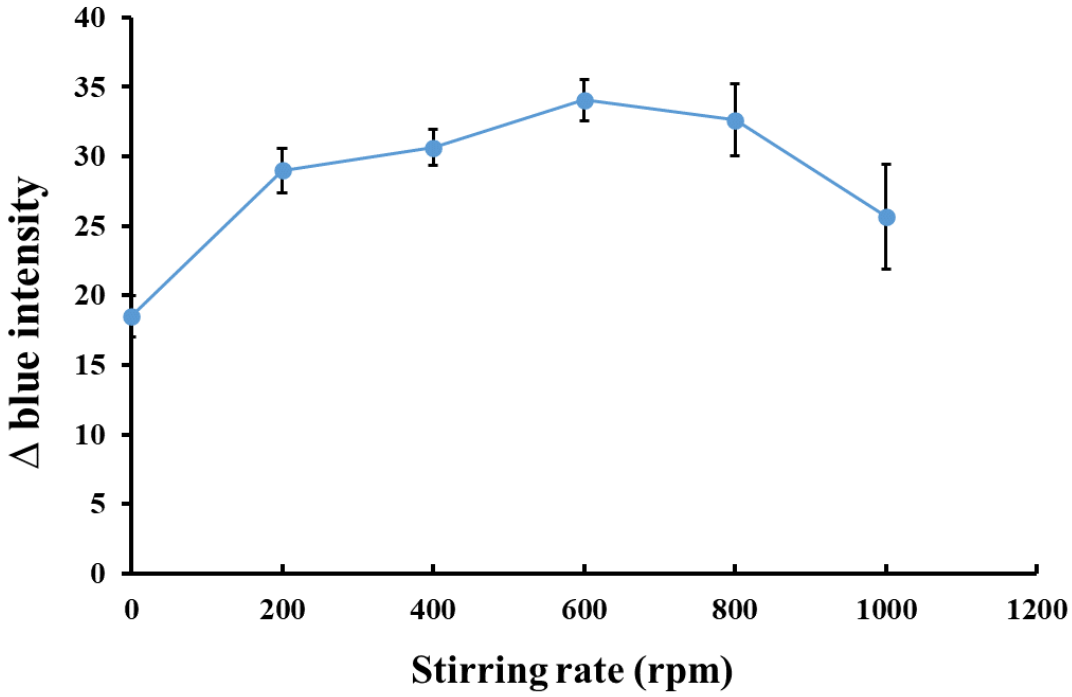

Figure S5. Effect of stirring rate on the intensity. Other extraction conditions: concentration of Ni(II): 700 µg L^{-1}, voltage: 50 V, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, extraction time: 10 min, gel thickness: 11 mm, concentration of agarose: 3% (w/v), and concentration of Ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates.

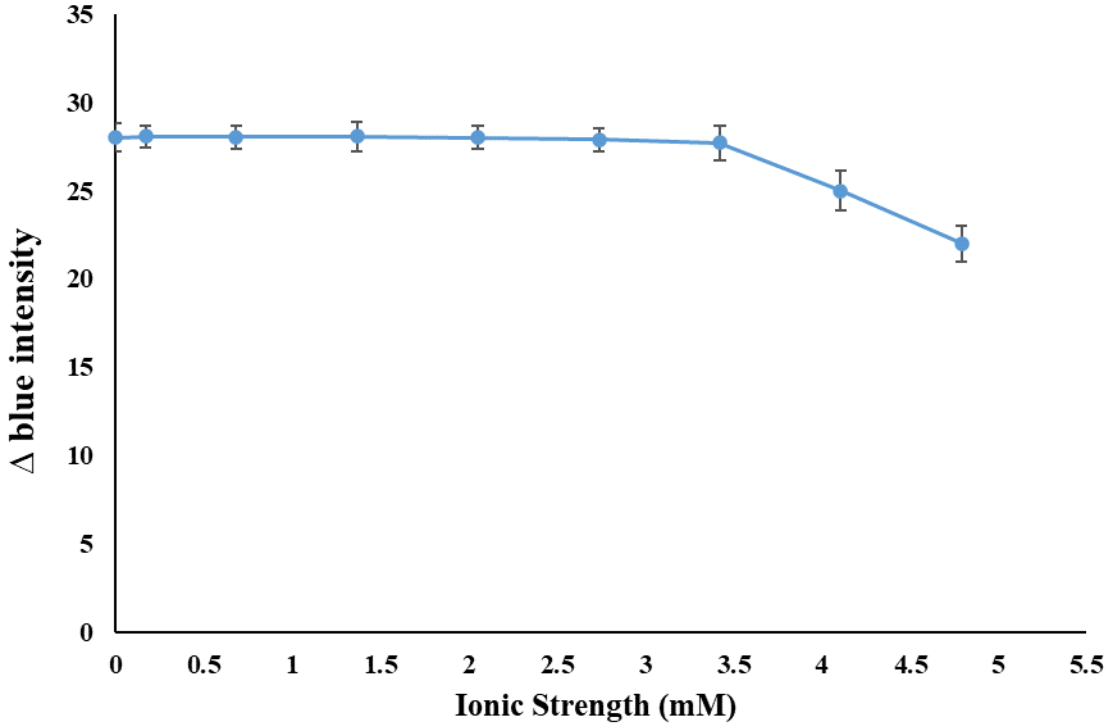

Figure S6. Effect of ionic strength inside gel on the intensity (0-4.8 mM NaCl). Other extraction conditions: concentration of Ni(II): 500 µg L^{-1}, voltage: 70 V, extraction time: 5 min, electrode distance from the gel tip: 3 mm, pH of donor solution: 6.0, stirring rate: 600 rpm, gel thickness: 11 mm, concentration of agarose: 3% (w/v) and concentration of ammonia (0.03 M), and DMG: 80 mM (20% v/v). Error bars were obtained based on three replicates (standard deviations were added to figures).

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