DEEP EUTECTIC SOLVENT-COATED PAPER-BASED DEVICE FOR COLORIMETRIC DETECTION OF CHROMIUM(VI)

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อุปกรณ์ฐานกระดาษเคลือบด้วยตัวทำละลายดีปยูเทคติกสำหรับการตรวจวัดเชิงสีของโครเมียม(VI). (DEEP EUTECTIC SOLVENT-COATED PAPER-BASED DEVICE FOR COLORIMETRIC DETECTION OF CHROMIUM(VI)) อ.ที่ปรึกษาหลัก : รศ. ดร.อภิชาติ อิ่มยิ้ม, อ.ที่ปรึกษาร่วม : ผศ. ดร.นครา ภวะเวส

เฮกซาวาเลนท์โครเมียม (Cr(VI)) เป็นหนึ่งในไอออนโลหะหนักที่พบบ่อยในสิ่งแวดล้อม มีพิษสูงและจัดว่าเป็นสารก่อมะเร็ง ในงานนี้ได้พัฒนาวิธีการตรวจวัด Cr(VI) นอกสถานที่ซึ่งสามารถวิเคราะห์ได้ทั้งเชิงคุณภาพและปริมาณ โดยใช้แถบกระดาษเคลือบด้วยตัวทำละลายดีปยูเทกติกซึ่งประกอบด้วย choline chloride และ thymol ซึ่งเป็นตัวทำละลายที่เป็นมิตรกับสิ่งแวดล้อม และใช้ 1,5-Diphenylcarbazide เป็นอินดิเคเตอร์และใช้ในการสร้างสารเชิงซ้อนกับ Cr(VI) โดยใช้สมาร์ทโฟนถ่ายรูปและวิเคราะห์พารามิเตอร์ค่าสี ผลการทดลองแสดงให้เห็นว่าที่ pH 2 แถบกระดาษที่เคลือบด้วยตัวทำละลายดีปยูเทกติกสามารถตรวจวัด Cr(VI) ได้ภายใน 10 นาที กราฟมาตรฐานแสดงความเป็นเส้นตรงในช่วง 250–5000 ไมโครกรัมต่อลิตรโดยมี R^2 เท่ากับ 0.9997 LOD และ LOQ ของวิธีเท่ากับ 103 และ 343 μg/L ตามลำดับ และ %RSD มีค่าน้อยกว่า 7.2% วิธีที่พัฒนาขึ้นนี้มีความจำเพาะสูงต่อ Cr(VI) อย่างไรก็ตาม ไอออนบวกบางชนิดรบกวนการตรวจหากมีความเข้มข้นสูง นอกจากนี้ วิธีการนี้ได้ประยุกต์ใช้เพื่อหาปริมาณของ Cr(VI) ในตัวอย่างน้ำจริงจากสิ่งแวดล้อม แสดงให้เห็นถึงค่าร้อยละการกลับคืนที่ยอมรับได้และน่าพอใจ (82-104%)

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		ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

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KEYWORD: hexavalent chromium (Cr(VI)), deep eutectic solvent (DES), paper-based analytical devices (μPADs)

Fakhry Gripaldi Pandu : DEEP EUTECTIC SOLVENT-COATED PAPER-BASED DEVICE FOR COLORIMETRIC DETECTION OF CHROMIUM(VI). Advisor: Assoc. Prof. Dr. APICHAT IMYIM Co-advisor: Asst. Prof. Dr. NAKARA BHAWAWET

Hexavalent chromium, Cr(VI), is one of the heavy metal ions often found in the environment that is highly toxic and classified as a carcinogen. In this work, rapid screening and onsite monitoring of Cr(VI) using paper-based has been developed. Furthermore, a natural-based deep eutectic solvent, comprising choline chloride and thymol that makes it an environmentally friendly solvent, is applied as a coating medium on the test strips for qualitative and quantitative analysis of Cr(VI) for the first time. 1,5-Diphenylcarbazide was used as an indicator and complexing agent for Cr(VI). The detection was conducted by using a smartphone equipped with an application for color analyzing. The result shows that at pH 2, the DES-fabricated test strip can detect Cr(VI) within 10 min. The calibration curve displays good linearity in the range of 250–5000 μg/L with R^2 of 0.9997. The LOD and LOQ of the method are 103 and 343 μg/L, respectively, and the RSDs are less than 7.2%. This developed method demonstrates high selectivity towards Cr(VI), however some cations may interfere in high concentration. Furthermore, the method has successfully applied for determination of Cr(VI) in real environmental water samples, exhibiting an acceptable and satisfactory recovery (82-104%).

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Student's Signature ....

Advisor's Signature ....

Co-advisor's Signature ....

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The author realized that still many limitations in organizing this thesis. In that case, the author is hoping for a suggestion to improve the thesis. Many thanks.

Fakhry Gripaldi Pandu

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CHAPTER 1 INTRODUCTION

1.1. Background

The increasing of industrial activities in this era is one of the major contributions of pollution that becoming a serious environmental problem. This activity produces wastes, either or both liquid and gaseous waste, which is mostly containing heavy metals that are dangerous to human and other living thing's health. One of the hazardous heavy metals is chromium (Cr).

Hexavalent chromium, Cr(VI), is one of the most stable oxidation forms of chromium and is often found in environment, besides Cr(III). Naturally, Cr(VI) is formed by erosion of natural chromium deposits or produced by industrial processes, such as stainless steel, electroplating, textile manufacturing, leather and paint factories.[1, 2] Interestingly, Cr(VI) attracts high attention because this oxidation form has a higher toxicity than Cr(III) and can easily penetrate in biological fluids. According to the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), the limits for total chromium and Cr(VI) that can be tolerated in drinking water are 100 µg/L and 50 µg/L, respectively. Moreover, Cr(VI) is classified in Group 1, which is carcinogenic to human.[2, 3] Hence, detection and monitoring of Cr(VI) in environment and water is necessary.

There are several methods that have been used for determination of Cr(VI), such as UV-vis spectrophotometry[1, 4], atomic absorption spectrometry (AAS)[2, 5, 6], ion chromatography (IC)[4], inductively coupled plasma-mass spectrometry (ICP-MS)[7], and inductively coupled plasma-optical emission spectrometry (ICP-OES)[8]. Albeit their great sensitivity and selectivity, the methods are time consuming and expensive, and need highly skilled operators. In addition, these instrumental methods are not portable and thus are challenging for on-site analysis.

Recently, microfluidic paper analytical device (μPAD) has become a solution to overcome those weaknesses. Besides being cheap and capable of on-site analysis, μPAD is faster, affordable, easy to fabricate and simple for any users. Qualitative analysis is feasible by simply observing the color change by the naked eye and can be analyzed further quantitatively with simple device, such as digital camera and smartphone equipped with a software.[9] Although the paper is biodegradable and disposable, it is still challenging for colorimetry, where the indicator must be coated with a hydrophobic layer to avoid leakage.[10] Also it is even more challenging, if environmentally friendly material is desirable. Nevertheless, there are several methods for fabricating hydrophobic layer on μPAD that have been reported, such as wax printing, screen printing, inkjet printing and many more[11-14], but these methods require high skills and not all laboratories have equipment for such fabricating methods. Soaking is then a simply desirable way to deal with those limitations because it is simply a single step and does not require complicated equipment. In addition, using hydrophobic deep eutectic solvents (HDESs) is an alternatively green strategy to create a hydrophobic layer for coating the indicator to avoid leaching that can contaminate samples, which could affect the results.[10]

In this work, hydrophobic DES will be prepared from Choline Chloride (ChCl) and Thymol, which will be applied as hydrophobic layer for μPAD for qualitative and quantitative analysis of Cr(VI) in aqueous samples for the first time. 1,5-Diphenylcarbazide (DPC) will be used as an indicator and complexing agent for Cr(VI). The detection will be conducted by using a digital camera or a smartphone, equipped with software or an application.

1.2. Objectives of Research

The objective of this work are to develop a colorimetric paper analytical device soaked with DES for Cr(VI) detection, as well as to apply the device for aqueous environmental water samples.

1.3. Scope of Research

There are several main works of this research:

- Preparation of various hydrophobic DESs with various mole ratios and characterized the selected DES by Fourier Transform Infrared (FT-IR) Spectroscopy and Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy.

- Fabrication of DES paper analytical devices and characterized by FT-IR Spectroscopy.
- Investigation of optimized condition (response time, DPC and pH) and several analytical performances of the method.
- Application of the method for real aqueous environmental water samples.

1.4. Benefits of Research

The benefit of this work is to obtain a developed colorimetric DES paper analytical device as an alternative solution for Cr(VI) detection.

CHAPTER 2 THEORY AND LITERATURE REVIEW

2.1. Hexavalent Chromium (Cr(VI))

Chromium (Cr) is one of transition heavy metals that has an atomic number of 24 with atomic weight of 51.9961 g/mole and it belongs to Group 6B of periodic table. Physically, Cr is a crystalline and white metal, as well as is not too ductile or malleable.[15] It has several oxidation states, start from -2 to +6, however trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) only that are mostly found and stable in the environment.[16] Cr^{3+} is present as a cationic species and plays an important role for living organism's metabolism.[17]

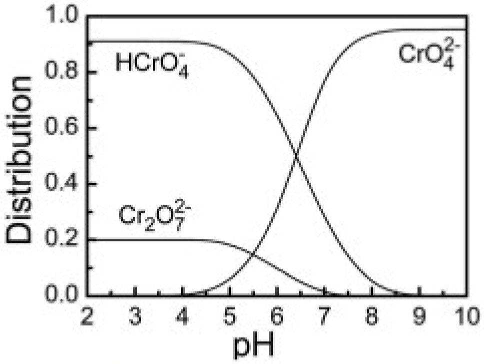


Fig. 2.1. The distribution of Cr(VI) in different pH.[18]

In contrast, Cr(VI) exists as an anionic species of $HCrO_4^-$ and $Cr_2O_7^{2-}$, which is toxic to humans and other living organisms.[17] According to the United States Environmental Protection Agency (EPA), World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), Cr(VI) is classified as a human carcinogen and as a class I human carcinogen, respectively.[2, 3] The distribution and behavior of Cr(VI) species in aqueous media is mainly due to pH and the redox potential, which as presented in Fig. 2.1. Whereas in pH below 1, Cr(VI) form of chromic acid (H_2CrO_4) dominates within these range, hydrogen chromate ($HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$) in range of 1-7 and Cr(VI) species of CrO_4^{2-} dominates in pH above 7.[17]

There are common analytical methods that widely used for determination of Cr(VI), including UV-vis spectrophotometry[1, 4], atomic absorption spectrometry (AAS)[2, 5, 6], inductively coupled plasma-mass spectrometry (ICP-MS)[7] and inductively coupled plasma-optical emission spectrometry (ICP-OES)[8]. Moreover, some papers also have reported some methods used for measuring Cr(VI), such as ion chromatography (IC)[4], high liquid performance chromatography (HPLC), stripping voltammetry and X-ray fluorescence spectrometry. Recently, colorimetric method is widely developed for measuring Cr(VI) due to its simplicity and affordability, such as µPAD.[19,20]

2.2. 1,5 Diphenylcarbazide (DPC)

DPC ($C_{13}H_{14}N_4O$) (Fig. 2.2) is one of chemical of phenylhydrazine group, which has molecular weight of 242.28 g/mole. Physically, DPC is white solid and soluble in organic solvent, including acetone, alcohol and acetic acid glacial and slightly soluble in water. This chemical is highly sensitive toward light, air and incompatible with strong oxidizing agents.[21,22]

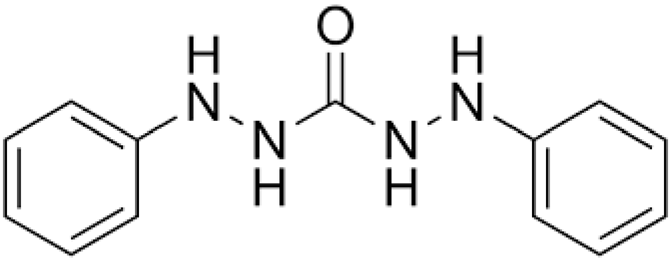


Fig. 2.2. Structure of DPC.

Commonly, DPC is used as colorimetric agent for determination of Cr(VI). The interferences result come from Fe, Mo, V, Cu, and Hg(II), if the concentration is much higher concentrations than the chromium.[23] Moreover, DPC also can be used as an indicator in titrating iron and the detection of cadmium, mercury, magnesium, aldehydes, and emetine.[21] DPC reacts with Cr(VI) in acidic medium and redox reaction occur, whereas DPC is oxidized to diphenylcarbazone (DPCO) and Cr(VI) is reduced to Cr(III)[17] (Fig. 2.3).

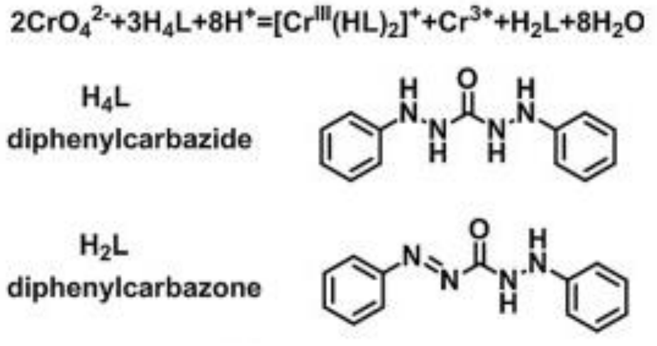


Fig. 2.3. The Complex Formation of Cr(VI) with DPC.[24]

2.3. Deep Eutectic Solvents (DESs)

Deep Eutectic Solvents (DESs) was introduced as a green solvent, which was defined as a mixture of two or more compounds that are typically solid at room temperature and changes into liquid with certain molar ratio at room temperature. DES is synthesized by simply mixing two safe and low-cost components, that are a hydrogen bond acceptor (HBA) (namely, quaternary ammonium halide salts, phosphonium halide salts, or metal chloride) and a hydrogen bond donor (HBD) (namely, carboxylic acids, alcohols, amides, carbohydrates or metal chloride) which associated to each other through hydrogen bonding interaction.[25] Commonly, a DES is prepared with gentle heating to form a liquid at temperatures between room temperature and 70 °C.[26]

This new class of solvent was first studied by Abott *et al* at the beginning of century. They have similar properties to ionic liquids (ILs), such as low volatility, wide liquid range, highly tunable and can dissolve both organic and inorganic compound. Compared with ILs, DES has attracted researcher's attention due to its superior advantages, such as less toxicity, less expensive and simpler synthesis methodology.[26] DES can be applied in a variety of applications, such as bio-catalysis, extraction, organic synthesis, reaction medium, synthesis of nanoparticles and speciation analysis. In addition, DES can also modify on various materials such as graphene, carbon nanotubes, cellulose.[27]

There are several works of Cr(VI) determination using hydrophobic DES. For example, Elahi *et al*. developed ultrasound-assisted liquid-liquid microextraction (UA-LLME) for speciation analysis of chromium by using hydrophilic DES of 1:2 Choline Chloride:Phenol and ammonium pyrrolidine dithiocarbamate as chelating agent for Cr(VI) in tea samples.[5] Shi *et al*. was using hydrophobic DES of trioctylammonium chloride and butyl 4-hydroxybenzoate for Cr(VI) extraction in water samples using DPC chelating agent.[28] Pourmohammad *et al*. developed dispersive liquid-liquid microextraction (DLLME) based on DES of 1:4 benzyltriehylammonium:Phenol with DPC chelating agent for extraction of Cr(VI) in wastewater.[1] However, these methods are time consuming and require expensive equipment. Thus, it is desirable to develop an alternative method that is faster and cheaper, as well as sensitive and selective for determination of Cr(VI).

2.4. Microfluidic Paper-based Analytical Devices (μPADs)

Microfluidic Paper Analytical Device (μPAD) has been developed as an alternative method for colorimetric analysis and has become promising and economical tool for environmental monitoring, food safety and on-site diagnosis. Besides, it is suited for low resources settings. The major advantages of this method are simple, light, and portable, highly biocompatible, biodegradable, flexible, as well as chemically and biologically inert.[29]

Filter, chromatography, and nitrocellulose papers are the substrates for µPADs that are mostly used. Moreover, other types of substrates, such as glass fiber, polyester, and polyvinylidene difluoride membranes, have been used. The selection of desired pore size of the substrates should be considered to meet the needs of various applications because the capillary flow rate depends on the size of the pores. For example, What-man® filter paper No. 1 has pores of 11 µm with a medium flow rate and thickness of 180 µm, making it suitable for a wide range of µPAD applications.[17]

There are several detection techniques have been used for the quantitative analysis of µPADs, such as colorimetric, fluorescence, electrochemical, chemiluminescence, electrochemiluminescence, and surface-enhanced Raman spectroscopy techniques. Colorimetry is the most common detection technique for µPADs due to its simplicity, cost-effectiveness, and easiness in operating with minimal training required. The colorimetric quantitative analysis in µPADs requires the capture of images, the analysis of images, and the construction of a calibration curve. Generally, smartphone camera, digital camera, or scanner are required to get the colorimetric images and are then transferred to either a PC or smartphone for further analysis.[17]

There are numerous strategies that have been reported for fabrication of µPAD, including photolithography, wax printing, screen-printing, inkjet printing, laser toner printing, flexography printing, PDMS printing, stamping, wax dipping, drawing, inkjet etching, wet etching, plasma treatment, paper cutting and laser cutting.[11,30]

Nevertheless, DES-fabricated µPAD colorimetric sensors have been reported only few. One of them was the work done by Grazioli *et al.* They fabricated µPAD by soaking the paper disk in a DES containing ChCl and malonic acid for determination of malondialdehyde (MDA) in food samples. Thiobarbituric acid was used as reagent for colorimetric detection, which was already dissolved in the DES and for the detection of MDA in gas phase, a home-made device of headspace was fabricated. Then, the detection of the MDA-TBA pink adduct formed at room temperature was evaluated by reflectance measurements.[29] The only other work that we could find was from Shojaeifard *et al.* They successfully developed µPAD for direct pH detection. A hydrophobic DES was formed from decanoic acid and methyl tri-octyl ammonium bromide. Then, the DES was used to immobilize two sensor indicators, Bromothymol Blue and Bromocresol Purple. The designed paper strip presented homogeneous color, reversibility, and significant leaching stability over months. Moreover, the fabricated strip coupled with multivariate analysis exhibited excellent performance in semi-quantitative and quantitative analysis in almost whole pH value (2.0-12.0) with 0.5 units increments based on their color variations. The DES paper strip was applied in pH detection of the food, biological and environmental samples, which validate the efficiency of the proposed probe for pH detection in complex environments.[10]

Moreover, Jaballah *et al* have successfully developed papers based analytical devices for the colorimetric detection of chromium (VI) and DPC was used as colorimetric agent. In this work, 2 different platform have been designed. The first design, they modified chitosan with DPC (DPC-CS) by crosslinking reaction, which was integrated into a paper to develop the paper-based analytical device (DPC–CS–PAD). This platform demonstrated a linear range of 0.1–5 mg/L with LOD and LOQ about 0.04 and 0.12 mg/L, respectively. The second platform is DPC-Nylon PAD which was prepared by covalent immobilization of DPC directly onto a Nylon paper. The DPC-Nylon-PAD presented a linear response of 0.1–2.5 mg/L with LOD and LOQ of 0.06 and 0.2 mg/L, respectively. Furthermore, these developed platforms were effectively applied for determination of critical concentration of Cr(VI) in water samples.[31]

2.5. Digital Image Colorimetric (DIC) Analysis

DIC is a method for measuring the concentration of analytes based on the color of digital images, which is taken with digital devices. Nowadays, this method is becoming an alternative and powerful tool for on-site and semi-quantitative analysis due to its simplicity, portability and low-cost. Moreover, the utility of digital images could eliminate the inconsistence observation. This method has been developed on many analytical methods for several analytes, such as metal, heavy metal, antibiotics, herbicides, pesticides, biochemical indicators, metabolomics, and pathogens.[32]

The main principles of DIC are demonstrated in Fig. 2.4. Firstly, the analyte was captured by using digital devices, such as camera, scanner, and webcam. The device captures the color and transforms it into an optical and electrical signal using image sensors, such as charge-coupled devices (C-CD), the contact image sensor (CIS), and complementary metal oxide semiconductors (CMOS). The quality of the image depends on the quality of the device, lighting condition, and the photo shooting technique. Finally, quantification of the image can be done by using an image processing software, such as Image J, Adobe photoshop, Matlab, and Pantone studio with appropriate color space. In DIC, there are several color spaces or models that can be used for detection, namely RGB, CMYK, HSV/HSL, CIE-XYZ, L*a*b*, and YUV.[32]

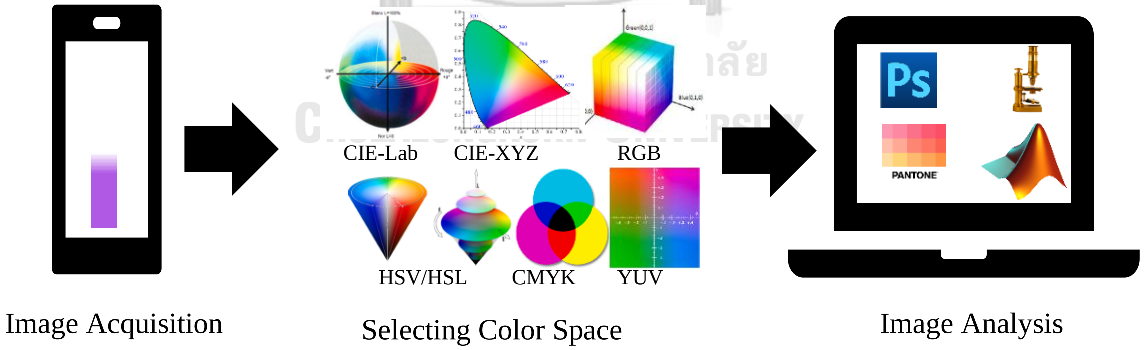


Fig. 2.4. General Procedure of DIC.

The L*a*b* model (Fig. 2.5) is an international standard for color measurements, adopted by the CIE (Commission International d'Eclairag or International Commission on Illumination in English) in 1976. This model is also known as perceptual uniform color space, which means how human eyes observe the differences between two colors. There are 3 components in this color space;

- L*, which is the luminance or lightness component, ranging 0-100 (black-white)
- a*, which represent chromatic component of green and red color, ranging from -120 to 120 (green-red)
- b*, which represent chromatic components of blue and yellow color, ranging from -120 to 120 (blue-yellow)[32]

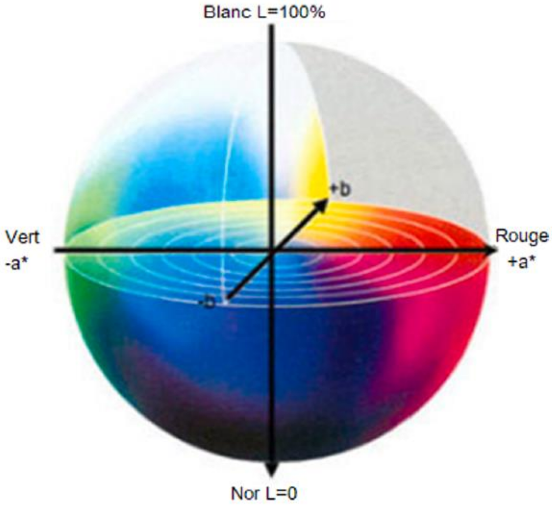


Fig. 2.5. CIE-L*a*b* color space.

This color space has some advantages. It is an independent model that does not depend on either input devices (i.e. camera) or output devices (i.e. monitors and printer). The L*a*b* model includes more color (even more than the human eye can see) than other color spaces.[33]

Color Difference (ΔE)

The difference between the L*, a* and b* values is expressed as Delta E (Δ*E*). The number of Δ*E* is defined as how far apart visually the two samples are in the color space. The criteria of Δ*E* number are shown in Table 1.[34]

Table 2.1. Δ*E* Value Criteria.

Δ*E* Value	Meaning
0-1	A normally invisible difference
1-2	Very small difference, only obvious to a trained eye
2-3.5	Medium difference, also obvious to an untrained eye
3.5-6	An obvious difference
>6	A very obvious difference

Moreover, Δ*E* can be calculated by following equation.

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*_{ab})^2 + (\Delta a^*_{ab})^2 + (\Delta b^*_{ab})^2}$$

where Δ*E* is the value of color difference between before and after immersing the solution, ΔL^*_{ab}, Δa^*_{ab} and Δb^*_{ab} are the difference of each L^*_{ab}, a^*_{ab} and b^*_{ab} values of before and after immersing.[35]

This color space system has been widely used for color-based detection. Baskaran *et al* used this model for determination of Cr(VI) in water samples by using DPC method. In this work, the detection was carried out with camera. According to the results, mobile camera was better than digital camera because it can control the white balance and ISO, except flashlight. The proposed method presents the LOD of 20 ng/mL, which was satisfied with the WHO regulation. In addition, this study also demonstrated LOD of 100 ng/mL based on the mobile camera color strips, which was applied for human naked-eye judgement. This developed method would be helpful for environment monitoring.[36]

CHAPTER 3 EXPERIMENTAL

3.1. Materials and Chemicals

The materials and chemicals used in this research were listed in the table below.

Table 3.1. List of Chemicals.

Chemicals	Manufacturer
Choline Chloride (ChCl)	Fischer
Thymol (Thy)	Tokyo Chemical Industry
Coumarin (Cou)	Tokyo Chemical Industry
Decanoic Acid (DA)	Tokyo Chemical Industry
Menthol (Mt)	Tokyo Chemical Industry
Tetra-n-octylammonium bromide ($N_{8881}Br$)	Tokyo Chemical Industry
$K_2Cr_2O_7$	Fischer
1,5-Diphenylcarbazide (DPC)	Thermo Scientific
$FeCl_3$	Sigma Aldrich
$NiSO_4.6H_2O$	AnalaR
$CaCl_2$ anhydrous	Kemaus
NaCl	Carlo Erba
$CuCl_2.2H_2O$	AnalaR
$Cr(NO_3)_3$	Merck
Nitric Acid (HNO_3)	Merck
Sodium Hydroxide (NaOH)	Sigma Aldrich
Filter Paper	Whatman 42
Printing Paper	Double A

3.2. Analytical Instruments

The instruments used in this research were listed in the table below.

Table 3.2. List of Instruments.

Instruments	Model, manufacturer
Fourier-Transform Infrared Spectroscopy (FTIR) (Transmission Mode)	Nicolet iS50
Fourier-Transform Infrared Spectroscopy (FTIR) (ATR Mode)	Nicolet 6700
Proton Nuclear Magnetic Resonance (^{1}H-NMR)	JNM-ECZR 500-MHz, JEOL
UV-Vis Spectroscopy	Agilent 8453
Hot plate and Stirrer	IKA C-MAG
Sonicator	Mettler Toledo
pH meter	Mettler Toledo
Phone Camera	Samsung Galaxy A50
Image J	1.53k (National Institutes of Health, USA)

3.3. Experimental

3.3.1. Preparation of DESs

In this work, several types of DESs listed in table below were prepared as follows; Briefly, the solid components of HBAs and HBDs with mole ratio indicated in Table 4, were mixed in a scintillation vial and stirred at 70 °C until homogenous liquid was formed.

Table 3.3. List of DES Components.

DES Precursors		Mole Ratio
HBA	HBD	
Choline Chloride (ChCl)	Thymol (Thy)	1:1
		1:2
		1:3
		1:4
Menthol (Mt)		1:1
Coumarine (Cou)		1:2
Thymol (Thy)	Decanoic Acid (DA)	1:1
Menthol (Mt)		1:1
Methyltrioctylammonium bromide (N_{8881}Br)		1:2

3.3.2. Preparation of DES-DPC

Fifteen mg of DPC powder was added into 3 g of DES and dissolved by sonication about 15 min. Finally, a colorless and stable DES-DPC solution was formed and then the selected DES was characterized by FT-IR and ^{1}H-NMR.

3.3.3. Fabrication of DES-Paper Strips

A filter paper (the detection part) was cut into rectangular shape of 0.7×2 cm size, then attached with a piece of 0.7×5 cm printing paper (the holding part) by transparent tape, allowing 1 cm distance between the two parts to prevent liquid diffusing, as illustrated in Fig. 3.1. Then, only the detection part was immersed in the DES-DPC solution for 5 min and let dry naturally. The test strips were freshly prepared before use. Then, the paper-strip was characterized by ATR-FTIR.

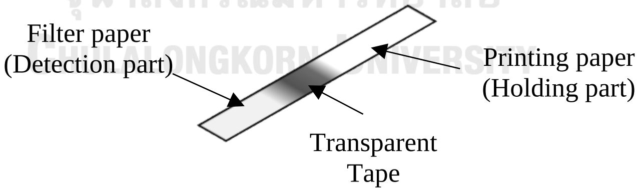


Fig. 3.1. Illustrator of test strip device platform.

3.3.4. Colorimetric Detection of Cr(VI)

For Cr(VI) detection, typically, the paper-strip was dipped into 2 mL of Cr(VI) solution for a certain time. Then, the color change in detection part of the paper strip was recorded by the camera after drying and then analyzed the RGB color by ImageJ software. After that, the RGB color was converted into L*a*b* by using Colormine website (www.colormine.org)

3.3.5. Optimization of DES-Paper Strips

3.3.5.1. Response Time

For the study of response time, DES paper-strip was dipped into 2 mL of 1 mg/L Cr(VI) with variation times of 0.5, 1, 5, 10, 20 and 30 min.

3.3.5.2. Concentration of DPC

In this work, DPC with various concentrations; 1000, 3000 and 5000 μg/g (300, 900 and 1500 μg, respectively) were added into 3 g of DES and dissolve it with the assistance of ultrasound bath about 15 min. After fabricating the DES paper-strips, then they were dipped into 2 mL of Cr(VI) 1 mg/L for the optimized time received from previous section.

3.3.5.3. The effect of pH

The effect of pH for Cr(VI) detection can be done by varying the pH 1-12 of the 1 mg/L Cr(VI) solution (2 mL). The pH adjustment was carried out using HNO_3 or NaOH. Then, the DES paper-strip was dipped into the Cr(VI) solution with the optimized time and concentration of DPC from previous section.

3.3.6. Analytical Performances

In this work, the analytical performances, including linearity, limit of detection (LOD), limit of quantitation (LOQ) and precision, were investigated. Linearity, LOD and LOQ can be done by preparing a calibration curve of a series of standard solution of 10-5000 µg/L Cr(VI), which is diluted with MiliQ water from Cr(VI) stock solution of 1000 mg/L. Then, 2 mL each was taken for the measurement with optimized conditions. For intra-day precision study, the paper-strips and Cr(VI) solutions were prepared for 3 batches, while as for inter-day precision the paper-strips and Cr(VI) solutions were prepared once per day.

3.3.7. Selectivity

Selectivity of the proposed method was evaluated by spiking method. Cr(VI) solution with fixed concentration of 5 mg/L was mixed with the possible interference ions, such as Fe^{3+}, Ni^{2+}, Cr^{3+}, Cu^{2+} Na^{+} and Ca^{2+} with 3 different concentrations. After that, DES paper-strip was dipped into 2 mL of binary mixture of Cr(VI)-interference ions with optimized conditions.

3.3.8. Real Sample Application

In this work, four water samples were used for the application of the proposed method. Drinking water (Aquafina) was purchased from local store in Thailand, tap water was obtained from Mahamakut building, Chulalongkorn University, while ground water and surface water sample were obtained from North of Thailand. The samples were filtered by using filter paper (Whatman 42). Then, 2 mL each was taken and analyzed with DES paper-strips with optimized conditions. For the accuracy study, spike method was used. Three different concentrations of Cr(VI) (0.25, 1 and 3 mg/L) were spiked into filtered water samples, then finally analyzed with DES paper-strips with optimized condition.

3.3.9. Data Analysis

3.3.9.1. Color Difference (ΔE)

Delta E (ΔE) can be calculated by using formula below;

$$\Delta \mathrm{E} = \sqrt{\Delta L^{*}{}_{ab} + \Delta a^{*}{}_{ab} + \Delta b^{*}{}_{ab}}$$

where ΔE is the value of color difference between before and after immersing the solution, ΔL^*_{ab}, Δa^*_{ab} and Δb^*_{ab} are the difference of each L^*_{ab}, a^*_{ab} and b^*_{ab} values before and after immersing.[35] The value of L*a*b* can be obtained from the conversion of RGB value from ImageJ software by using Colormine website (www.colormine.org)

3.3.9.2. Limit of Detection (LOD)

LOD is the lowest concentration that can be detected by the method which can be estimated based on three times of standard error of regression ($S_{y/x}$) divided by slope (m).

$$\mathrm{LOD} = \frac{3S_{y/x}}{\mathrm{m}}$$

3.3.9.3. Limit of Quantitation (LOQ)

LOQ is the lowest concentration that can be measured with reliable accuracy which can be estimated based on ten times of standard error of regression ($S_{y/x}$) divided by slope (m).

$$\mathrm{LOQ} = \frac{10S_{y/x}}{\mathrm{m}}$$

3.3.9.4. Precision

Precision is defined as the closeness of two or more measurements in a set of sample replications. Precision can be determined as relative standard deviation (%RSD), which is calculated by dividing standard deviation (S_d) by the mean of value ($\bar{x}$).

$$\%\mathrm{RSD} = \frac{\mathrm{S_d}}{\bar{\mathrm{x}}} \times 100\%$$

3.3.9.5. Recovery (%R)

Recovery of the spiking method was used to evaluate the performance of the proposed method. This can be calculated by using the formula below;

$$\%\mathrm{R} = \frac{\mathrm{C_{spiked}} - \mathrm{C_{unsipked}}}{\mathrm{C_{added}}} \times 100\%$$

where C_{spiked} is the concentration that found in spiked sample, $C_{unspiked}$ is the concentration that found in unspiked sample and C_{added} is the concentration of the analyte that added into the sample.

CHAPTER 4 RESULTS AND DISCUSSION

4.1. Selection of DES

In this work, DES was used to trap the indicator and coat on the paper-strips. In order to do so, DES needs to have hydrophobic properties to minimize or avoid leaching of the indicator from paper-strips into the sample. Table 4.1 shows the summary of hydrophobic DESs that were synthesized with various HBDs, HBAs and molar ratios.

Table 4.1. The summary of synthesis of several DESs.

DES Precursors		Mole Ratio	Physical Appearance
HBA	HBD		
Choline Chloride (ChCl)	Thymol (Thy)	1:1	Solid
		1:2	Solid
		1:3	Solid
		1:4	Liquid
Menthol (Mt)		1:1	Liquid
Coumarine (Cou)		1:2	Liquid

Thymol (Thy)	Decanoic Acid (DA)	1:1	Liquid
Menthol (Mt)		1:1	Liquid
Methyltrioctylammonium bromide ($N_{8881}Br$)		1:2	Liquid

From the results, all types of DESs except ChCl:Thy with mole ratio of 1:1,1:2 and 1:3 were liquid, which indicated that the DESs were successfully prepared. After that, the successful DESs were further investigated by dissolving 5 mg of DPC in 3 g of DESs.

Table 4.2. Summary of dissolution of DPC in DESs.

DES Precursors		Mole Ratio	Physical Appearance	Picture
HBA	HBD			
Choline Chloride (ChCl)	Thymol (Thy)	1:4	Clear Liquid	
Menthol (Mt)		1:1	Pale Pink Liquid	
Coumarine (Cou)		1:2	Pale Pink Liquid	

Thymol (Thy)	Decanoic Acid (DA)	1:1	Purple Liquid	
Menthol (Mt)		1:1	Pink Liquid	
Methyltrioctylammonium bromide ($N_{8881}Br$)		1:2	Precipitated	

From Fig 4.1, only $N_{8881}Br$:DA 1:2 cannot dissolve DPC well, which is probably due to high viscosity of the DES. DPC immediately changed color to purple or pink color in some DESs, including Thy:DA, Mt:DA, Mt:Thy and Cou:Thy. This indicated that DPC was less stable in those DESs, which was probably due to pH of each DESs. In these DES, the DPC was probably deprotonated and oxidized into purple or pink color of DPCO due to the OH-group dominant of DES precursors. While in ChCl:Thy, DPC is more stable than the other DESs, which is proven that DPC was dissolved well and did not affect the DPC color. Thus, 1:4 ChCl:Thy was selected for further analysis. However, the color of DPC still changed into pink the next day, so it needs to be freshly prepared each day. This was probably due to the reaction of DPC with oxygen, which making the oxidation process into diphenylcarbazone (DPCO) and the DES solution became pink color.

4.2. Characterization of Selected DES

4.2.1. Characterization of DES ChCl:Thy (1:4)

To prove that it is formed through hydrogen bond interaction, the DES was characterized by FT-IR and ^{1}H-NMR. From the FT-IR spectrum of ChCl:Thy in Fig. 4.1, it shows that there is a shifting peak of -OH stretching band of thymol (3231.147 cm^{-1}) and choline chloride (3431.707 cm^{-1}) to 3262.484 cm^{-1} in DES spectra. In addition, a peak at 1083.798 cm^{-1} in choline chloride spectra, which is corresponding to C-N band, is sharper and shifted to 1090.066 cm^{-1} in DES spectra. This indicates the possibility the hydrogen bond interaction occurs between -OH of thymol with C-N, -OH and Cl$^-$ in choline chloride.

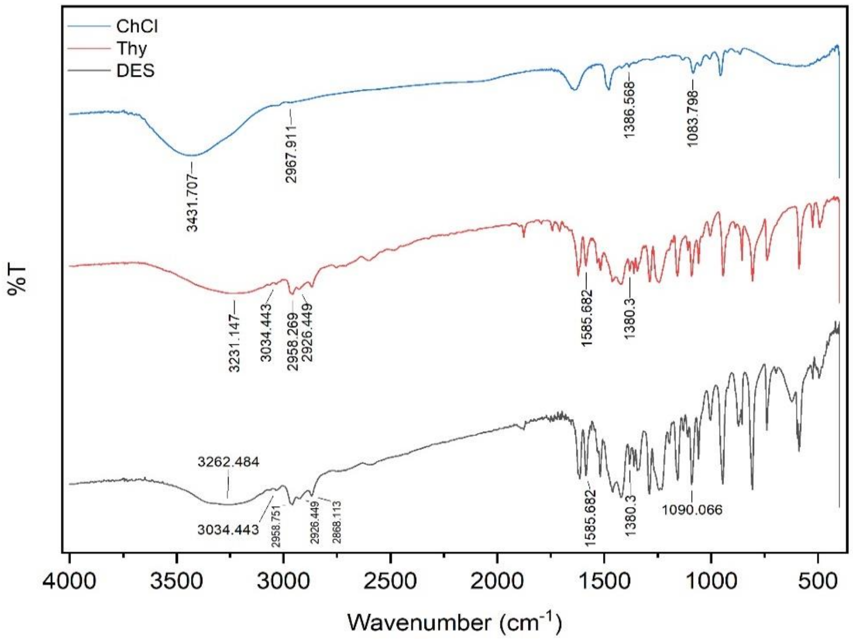


Fig. 4.1. FTIR Spectra of DES (black line), ChCl (blue line) and Thy (red line).

The hydrogen bond interaction between ChCl and Thy can be proven even more by ^{1}H-NMR, which is presented in Fig. 4.2. The chemical shift of -OH group of thymol and choline chloride were observed at δ 9.04 ppm (s,0.78H) and δ 5.49 ppm (s, 0.29H), respectively. In DES spectra, both chemical shift of -OH group were shifted to δ 9.12 ppm (s, 0.74H), which indicates the hydrogen bond interaction between -OH of thymol and choline chloride.

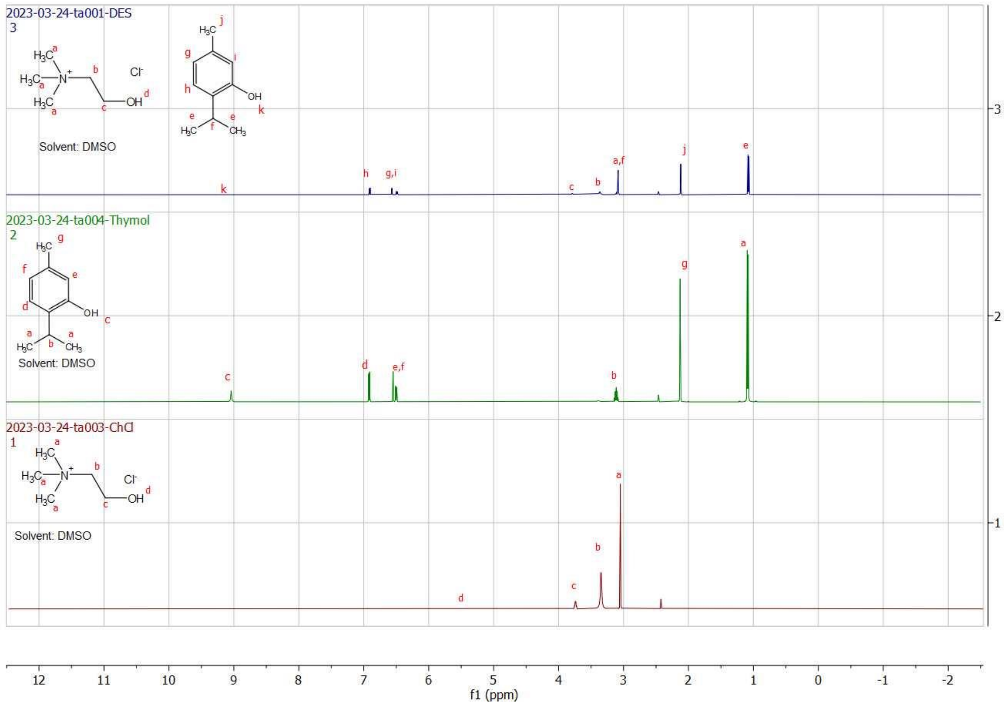


Fig. 4.2. NMR spectra of DES (top), Thy (middle) and ChCl (bottom).

Moreover, the NMR spectra can give information the mole ratio of ChCl and Thy by seeing the integration. As can be seen in Fig. 4.3, the chemical shift at position *e* (δ 1.08 ppm, *d*) shows C-H group of thymol. The integration of this peak represents the number of protons of that molecule, which is 24 protons. For 1 mole of thymol of this peak, it has 6 protons (1 mole: 4H), so there are 4 moles of thymol, which has 24 protons (4 moles: 24 H). On the other hand, at position *c* (δ 3.79 ppm, *m*) represents the chemical shift of C-H group of choline chloride, which has 1.87 protons (rounded into 2 protons). For 1 mole of choline chloride, it has 2 protons, so there is 1 mole of choline chloride. Thus, it can be concluded that the selected DES of ChCl:Thy has the mole ratio exactly 1:4.

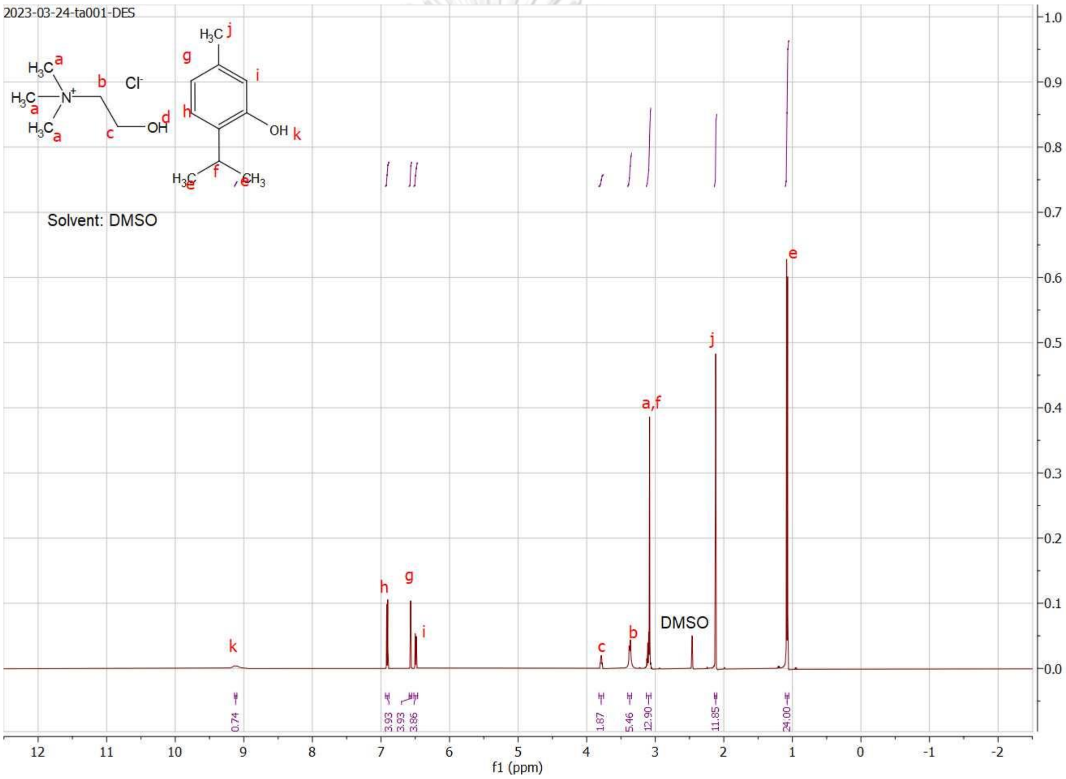


Fig. 4.3. NMR Spectra Details of DES ChCl:Thy.

4.2.2. Characterization of DES-DPC

As shown the FT-IR spectrum of DES-DPC in Fig. 4.4, it was observed that both spectra (with and without DPC) were almost identical, but the stretching absorption peak of C-H aromatic rings in DES-DPC spectra was shifted from 3034.443 cm^{-1} to 3029.14 cm^{-1}. In addition, there was also a shifting peak from -OH group (3262 cm^{-1}), C=C aromatic ring (1585.682 cm^{-1}), C-N (1090.066 cm^{-1}) to 3312.625 cm^{-1}, 1584.236 cm^{-1}, 1088.62 cm^{-1}, respectively. Moreover, NMR spectra (Fig. 4.5) showed that both spectra (with and without DPC) were also almost identical and chemical shift of C-H aromatic rings (δ 6.57 ppm) and O-H (δ 9.12 ppm) were shifted to δ 6.58 ppm and δ 9.13 ppm, respectively. From these results, there are possibility several interactions between DES and DPC, such as hydrogen bond between -OH group of DES precursors and DPC, phi-stacking of thymol with DPC and electrostatic interaction of cholinium ion of ChCl with DPC.

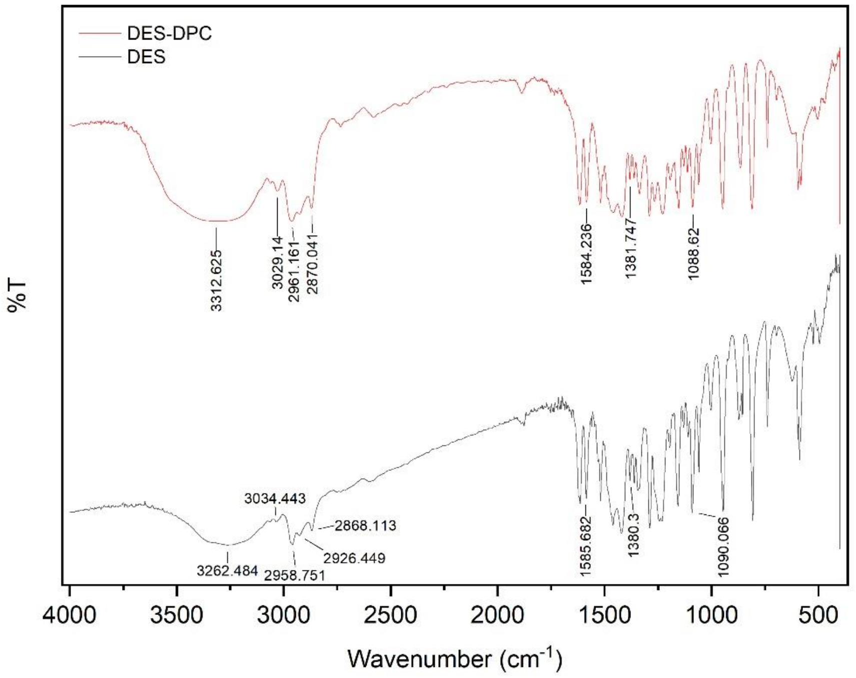


Fig. 4.4. FT-IR spectra of DES (black line) and DES-DPC (red line).

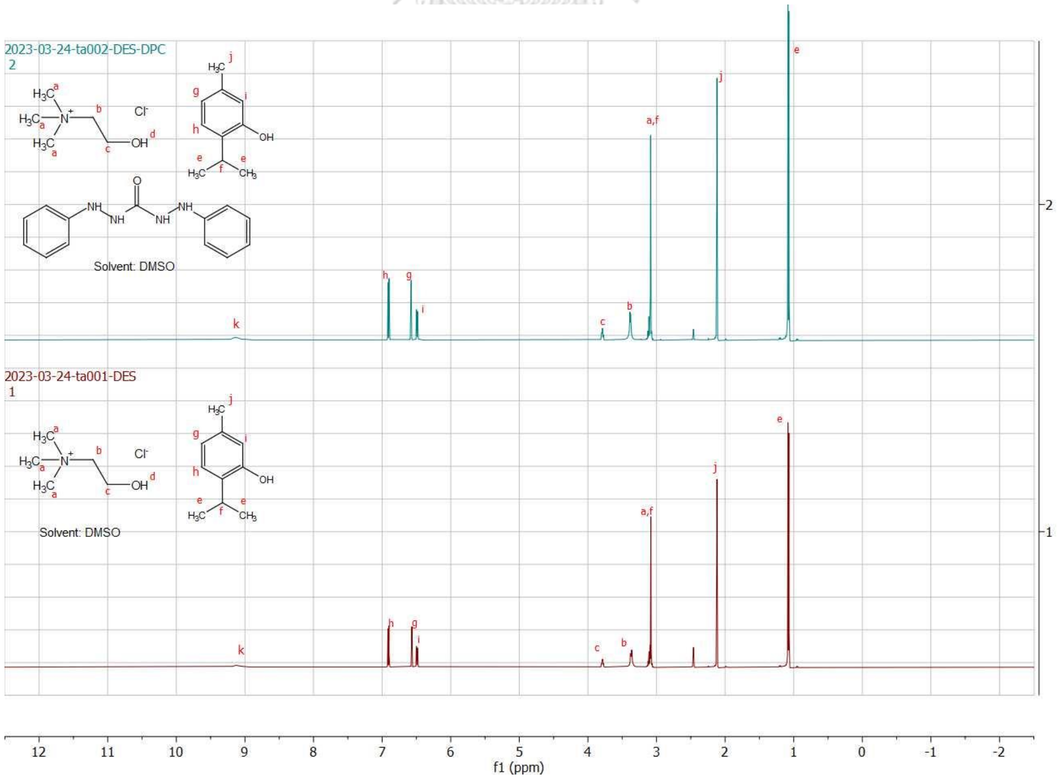


Fig. 4.5. NMR spectra of DES (bottom) and DES-DPC (above).

4.3. Characterization of DES Paper-strips

The DES paper-strip was characterized by using ATR-FTIR to observe the successfulness of DES coating on the paper-strip and the result was presented in Fig. 4.6. It was shown that there is no significant difference between before and after modifying with DES. The main functional group of DES, including -OH and C-H aliphatic, were overlapping with cellulose of the paper-strip. However, there is a peak at 1583.271 cm^{-1} in DES paper-strip spectra, which represent C=C aromatic rings of thymol and DPC. This indicates that the DES paper-strip was successfully fabricated.

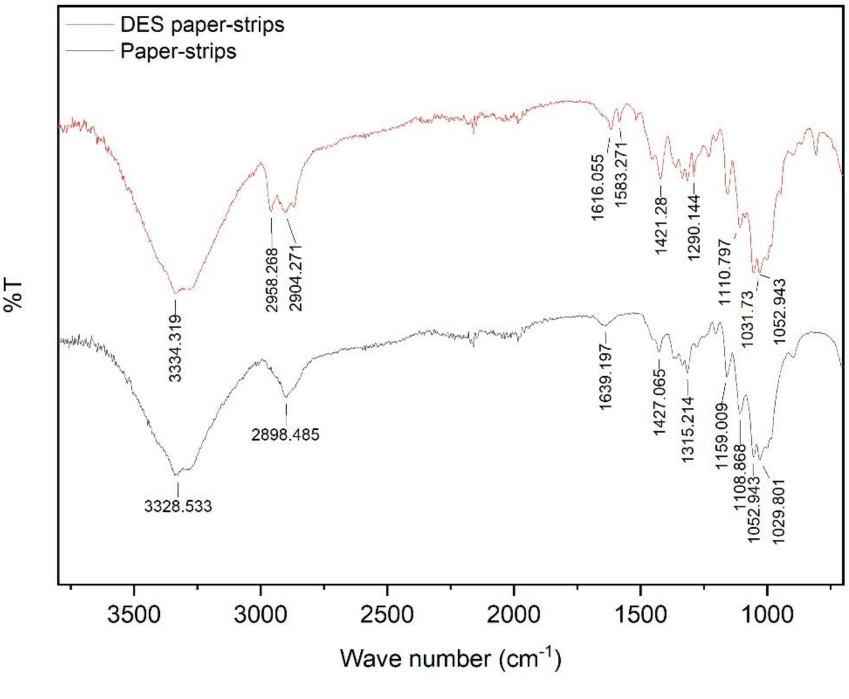


Fig. 4.6. ATR-FTIR spectra of paper-strips (bottom) and DES paper-strips (above).

4.4. Preparation and Colorimetric Detection of DES Paper-strips

Fig 4.7 presented the DES paper-strips. The paper-strip was prepared by immersing the detection part into a DES-DPC solution. After drying, the paper-strip was ready to use. As the strips made contact with the aqueous sample solution, DES-DPC could leak from paper-strip and dissolved in solution and the sample solution changed into pink-purple color of complex Cr(VI)-DPC.

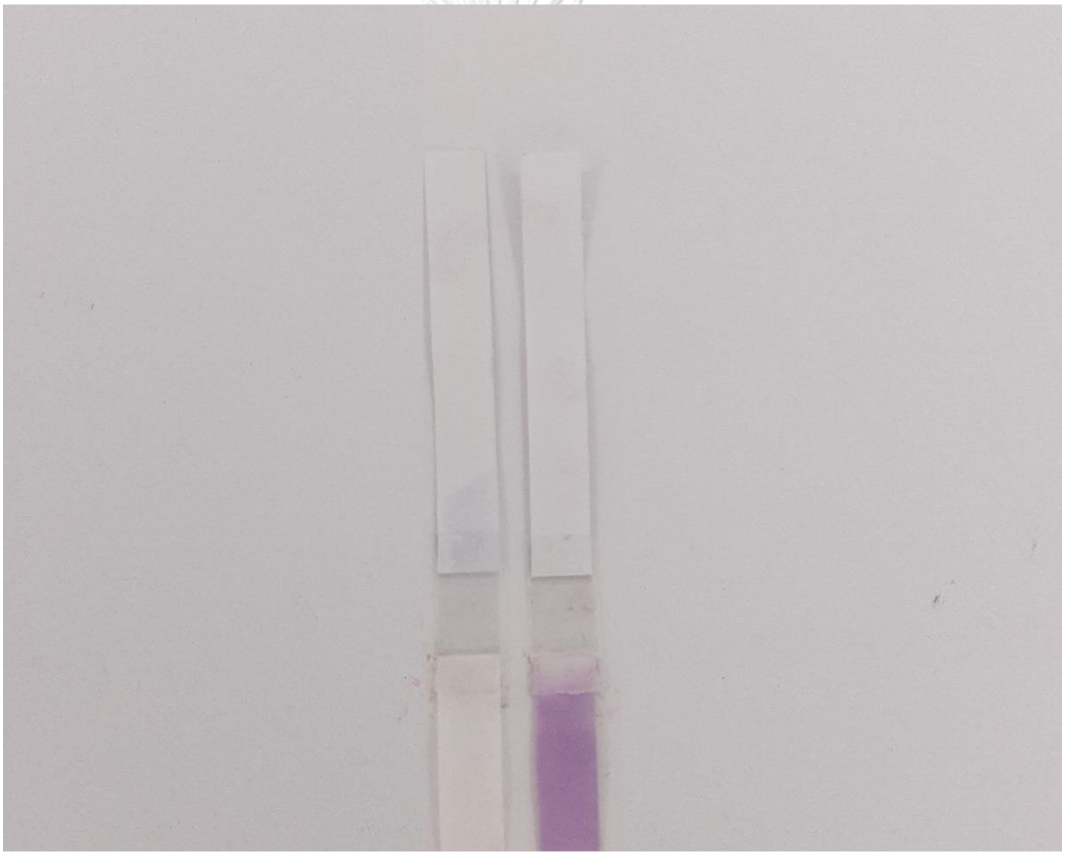


Fig. 4.7. The DES Paper-strips before (left) and after (right) dipping in 5 mg/L of Cr(VI) solution.

Fig. 4.8. shows the extraction schematic of the proposed method. DES was coated on the paper-strip, which contained DPC as Cr(VI) chelating agent, and have a role as extractant. When dipping into Cr(VI) solution, the sample contacts the detection part and the paper changes into homogenous pink-purple color, which indicates complex reaction between Cr(VI) and DPC. The Cr(VI) of aqueous phase was extracted into DES phase, where it reacts to form Cr(VI)-DPC complex by redox reaction. In this part, Cr(VI) in form of $HCrO_4^-$ was reduced into Cr(III) and DPC is oxidized to DPCO and the complex formation between the reduced Cr(III) and oxidized DPC occur.

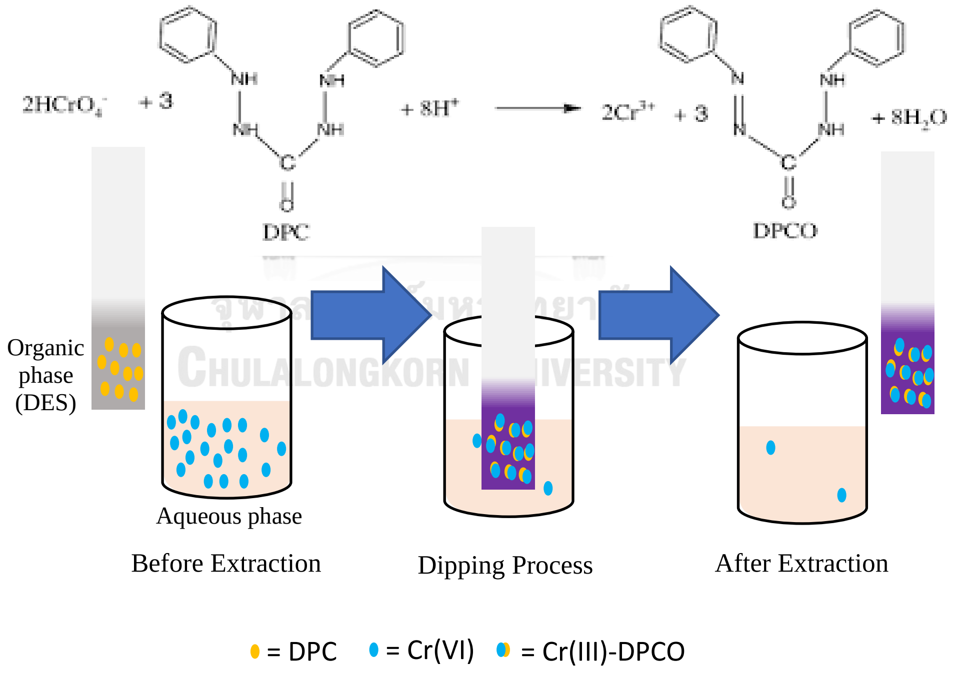


Fig. 4.8. The Schematic of Cr(VI) extraction by DES Paper-strip.

4.5. Optimization of DES Paper-strips

4.5.1. Response Time

The response time was investigated to determine the duration for the paper-strip to detect Cr(VI) in the sample. As shown in Fig. 4.10, the time of 20 min gave the highest ΔE, implying that the reaction between Cr(VI) and DPC reached its equilibrium. Besides, dipping the test strips for longer than 20 min resulted in decreasing ΔE, which was probably due to the leakage of DPC from the paper strips, as indicated by solution color changing to pale purple.

Nevertheless, considering aiming for fast detection of the method, the time of 10 min did not yield ΔE too far below that of 20 min. The student *t*-test was further proved that there was no significant difference at the confidence level of 95% between them. As such, the response time of 10 min was selected for further experiments. Also, the investigated concentration of Cr(VI) would be 10-fold lower onwards as color corresponding to ΔE value of about six can be differentiated by naked-eye, and the Cr(VI) concentration is of the same order of magnitude as the standard method.[37]

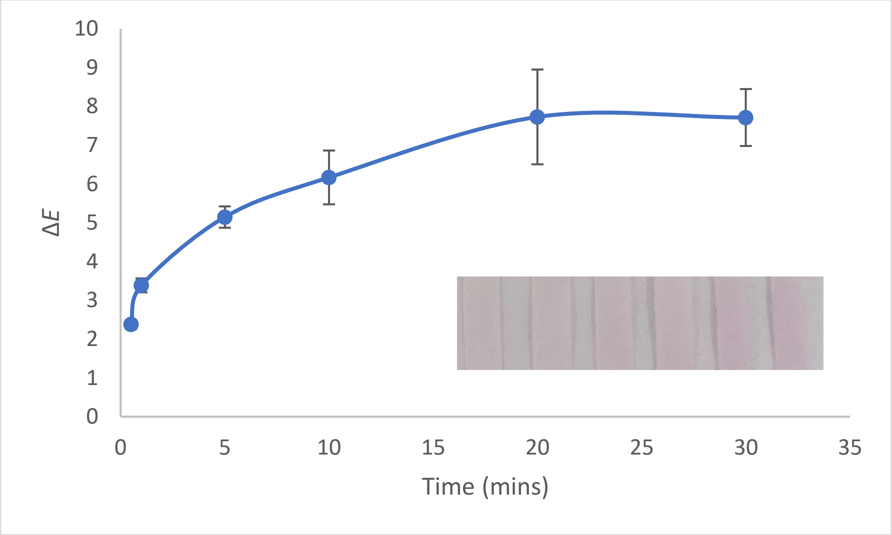


Fig. 4.9. Effect of response time to the Δ*E*. The test strips prepared using 1000 mg/L DPC were tested with 1 mg/L Cr(VI) at pH 2.

For study of leakage of DPC from the paper strip, the DES-saturated pale purple solutions were evaluated by UV-Vis spectrophotometer and the result is shown in Fig. 4.10. The graph showed that there is a peak at 540 nm, which corresponds to the Cr(VI)-DPC complex. This indicates that the pale purple solution was come from the Cr-DPC complex leaked from the paper strips and make a complex reaction with Cr(VI) left in the solution.

To know the amount of DES-DPC, paper-strip before and after soaked in DES was weighted by using balance. The amount of DES was calculated by mass of paper-strip after soaking in DES subtracted with before soaking in DES, revealing that the mass of DES coated on paper strip was 15.37±0.15 mg.

Table 4.3 shows the percentage of DPC leakage from the paper-strips. It can be calculated by the ratio of mass of DPC on solution and on paper-strip. The mass of DPC on paper-strip can be found by multiplication of concentration of DPC with mass of DES coated on paper-strip, while mass of DPC on solution can be calculated by plotting calibration curve of concentration of DPC vs absorbance in UV-Vis. The calculation exhibits the leakage percentage of 14-29%. From this results, it indicates that DPC was still leaking from the DES ChCl:Thy.

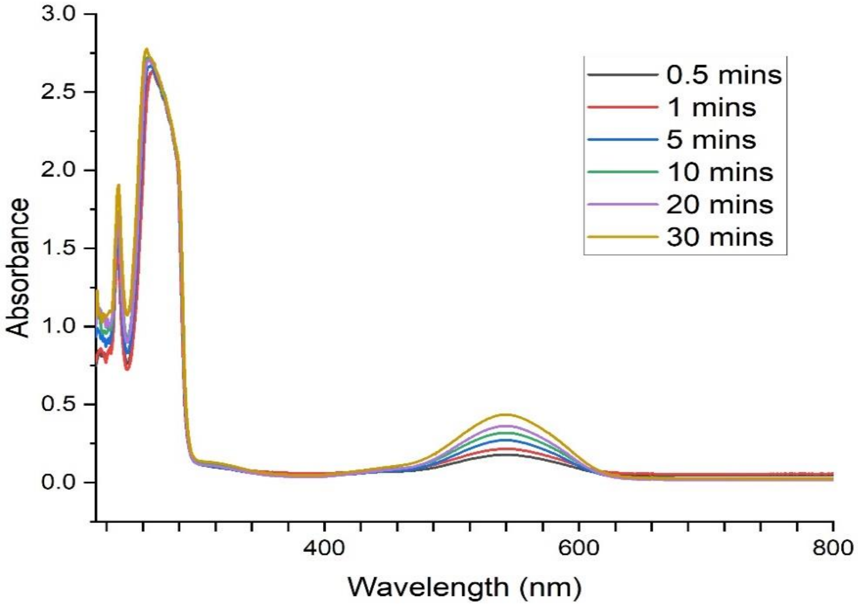


Fig. 4.10. UV-Vis Spectra of DES-saturated solution after the paper-strips immersed with various times.

Table 4.3. Percentage of DPC Leakage in different times.

Time (mins)	Leak of DPC (mg)	%Leak
0.5	0.010	14
1	0.013	16
5	0.014	19
10	0.016	21
20	0.018	23
30	0.022	29

4.5.2. Concentration of DPC

DPC is a colorimetric reagent that can form a complex reaction in acidic medium. Hence, the amount of DPC is important for effective and maximizing Cr(VI) detection. Thus, the effect of concentration of DPC (1000, 3000 and 5000 μg/g) in 3 g of DES was studied. The fabricated DPC-DES paper-strips were dipped into 1 mg/L Cr(VI) for 10 min. ΔE values (Fig. 4.11) exhibited that they increased with concentration of DPC. This is probably due to the increasing of mass transfer ability from DES-DPC to extract Cr(VI). As the concentration of DPC increased, the rate of extraction of Cr(VI) from aqueous phase into DES phase increased, which is proven from more intense color. Therefore, 5000 μg/g of DPC was chosen for further investigations. The concentration of higher than 5000 μg/g was not further studied because at higher concentration, there would probably be a leakage of DPC from paper-strip into the solution. In addition, this chosen concentration is the same as one suggested by the standard method.[37]

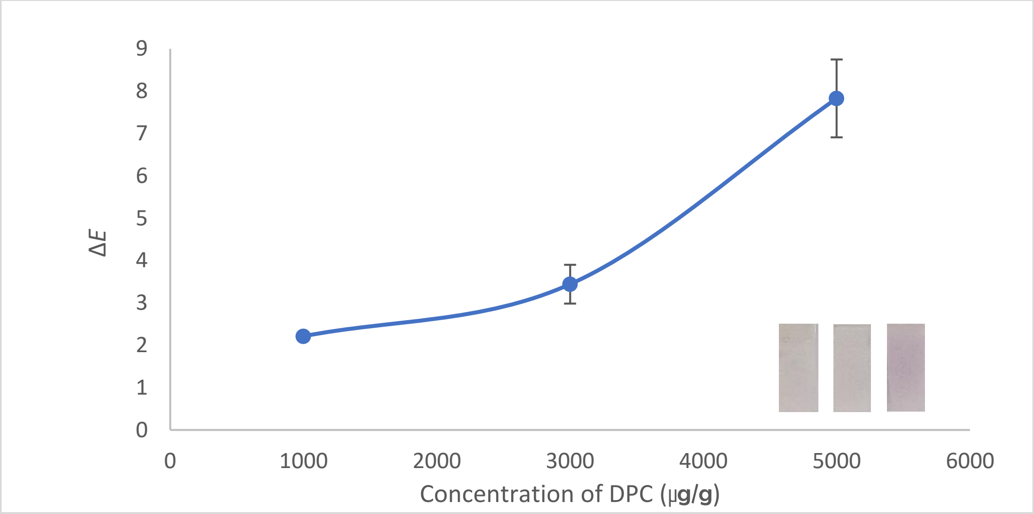


Fig. 4.11. Effect of concentration of DPC to the Δ*E*. The test strips were tested with 1 mg/L Cr(VI) at pH 2 for 10 min dipping time.

The stoichiometry of the Cr(VI)-DPC complexion was also investigated, whereas 2 mole of $HCrO_4^-$ and 3 mole of DPC are needed to form Cr(VI)-DPC complex in acidic condition, revealing that in this experiment Cr(VI) is the limiting agent under this condition. However, from the calculation, 5.5 mg/L of Cr(VI) is the highest concentration that could be used, since 5000 µg/g of DPC would become a limiting reagent.

Mole of DPC $= \frac{5000\ \mathrm{mg}}{1\ \mathrm{kg}} \times 1.53 \times 10^{-5}\ \mathrm{kg} \times \frac{1\ \mathrm{mmol}}{242.28\ \mathrm{mg}}$

$= 3.17\times10^{-4}$ mmol

- 2 mole of Cr^{6+} ≈ 3 mole of DPC

Moles of Cr^{6+} = 3.17×10^{-4} mmol of DPC $\times \frac{2\ moles\ of\ Cr^{6+}}{3\ moles\ of\ DPC}$

$= 2.11\times10^{-4}$ mmol of Cr^{6+}

Concentration of Cr^{6+} = 2.11×10^{-4} mmol $\times \frac{51.99\ \mathrm{mg}}{1\ \mathrm{mmol}} \times \frac{1}{2\times10^{-3}\ \mathrm{L}}$

= 5.5 mg/L of Cr^{6+}

4.5.3. pH

The pH of the solution also plays a crucial role for a complex formation between Cr(VI) and DPC. Thus, the effect of pH was evaluated by varying the pH of the solution in the range of 1–12 using NaOH and HNO_3. According to Fig. 4.12, pH 2 gave the highest ΔE and thus most optimal pH for Cr(VI) detection. This might be due to in these acidic conditions, $HCrO_4^-$ and $Cr_2O_7^{2-}$ species are dominant. These two species are mostly found in pH 2–6, the lower the pH of the solution, the more dominant of the species. While for the pH above 3, the value of ΔE significantly decreased due to the decreasing of existence of Cr(VI) from these two species.[17] However, the value of ΔE was increased at pH higher than 7. This was probably owing to the oxidation of DPC to DPCO, which was proven by the control test that the value of ΔE increased and red color of paper-strips was observed.[38] In this work, Δ*E* of control is higher than the Cr(VI) solution because all DPC in control solution were oxidized to DPCO. In basic condition, DPC on control solution, which did not contain any ions, reacted with OH$^-$ and the deprotonation of DPC into DPCO could occur.

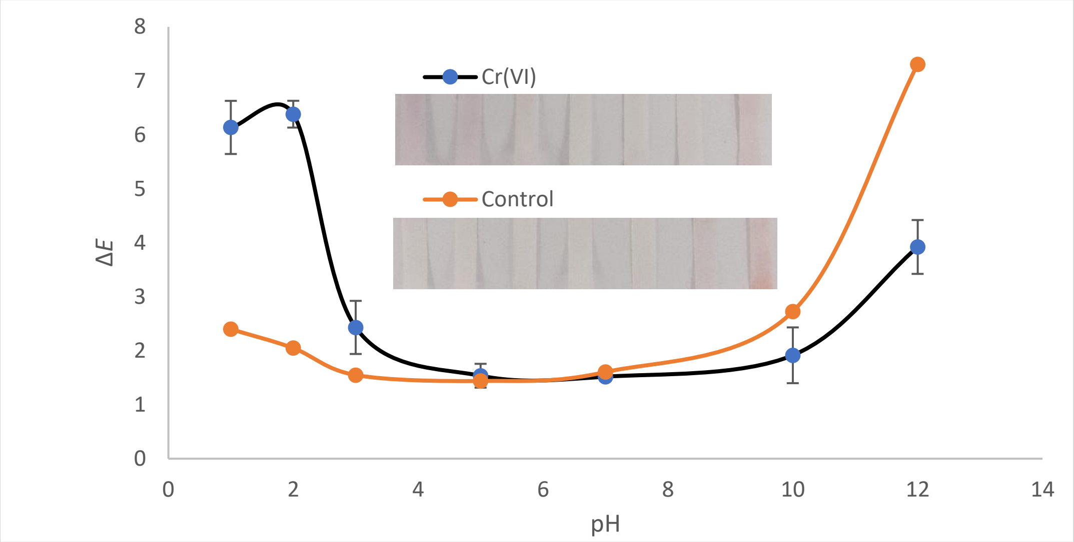


Fig. 4.12 Effect of pH to the Δ*E*. The test strips prepared using 5000 mg/L DPC were tested with 1 mg/L Cr(VI) at pH 2 for 10 min dipping time.

4.6. Analytical Performances

Under the optimal conditions, the analytical performances of the proposed method were evaluated. A series of Cr(VI) standard solution with concentrations ranging from 10 to 5000 μg/L were determined under the optimized conditions. A calibration curve of concentration of Cr(VI) *vs* Δ*E* was constructed and the result showed good linearity with R^2 of 0.9997 (Fig. 4.13). Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using standard error of regression, which were mentioned at section 3.3.9.2 and 3.3.9.3, respectively, exhibiting the LOD and LOQ of the method were 103 and 343 μg/L, respectively.

Furthermore, the repeatability and reproducibility of the developed method were evaluated by determining the relative standard deviation (%RSD) of standard solutions of 1 and 5 mg/L. As shown in Table 4.4, %RSD for both intra-day (n=3) and inter-day (n=3×3) were lower than 7.2%, which was acceptable for practical use according to AOAC guidelines.[39]

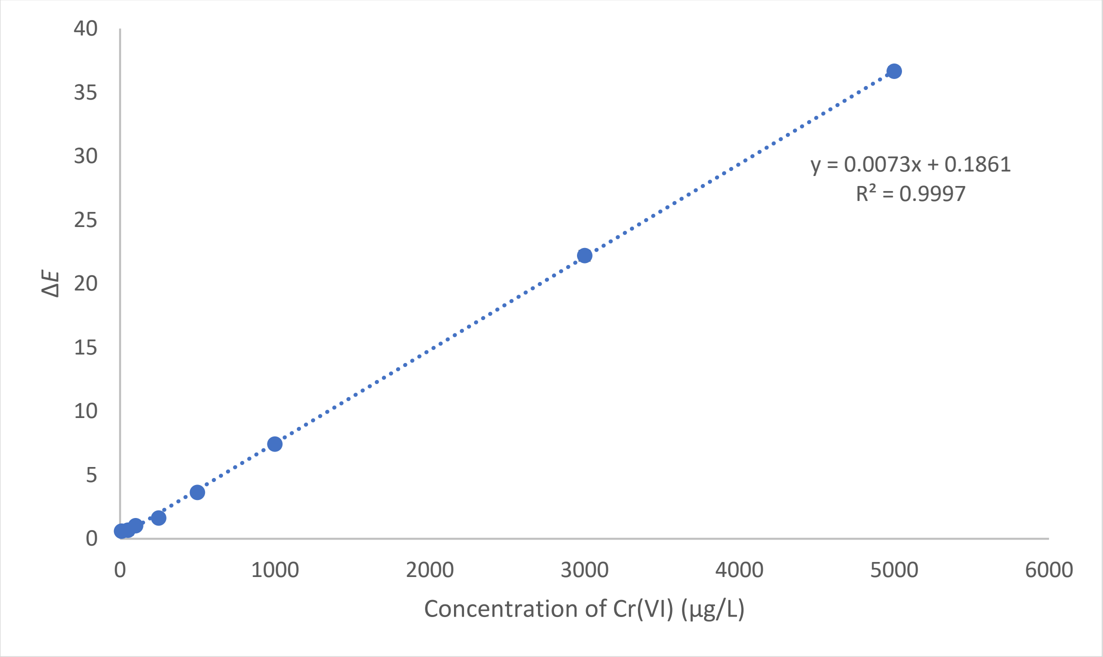


Fig. 4.13. Calibration curve of Cr(VI) concentration and ΔE, constructing under the optimized condition.

Table 4.4. Analytical Performances of the Method.

Parameter	**Value**
Linear range (μg/L)	250-5000
R^2	0.9997
LOD (μg/L)	103
LOQ (μg/L)	343
%RSD (Intra-day)	
- 1 mg/L	1.2–2.6
- 5 mg/L	1.3–3.6
%RSD (Inter-day)	
- 1 mg/L	6.5–7.0
- 5 mg/L	1.1–7.2

4.7. Selectivity

In the environment, Cr(VI) inevitably coexists with several cations and anions, which may interfere Cr(VI) determination. In this work, several ions coexisting with Cr(VI), such as Fe^{3+}, Ni^{2+}, Cr^{3+}, Cu^{2+}, Na^+ and Ca^{2+} were investigated. The ΔE of Cr(VI) binary mixtures will compare statistically with the single Cr(VI) solution. Single interfering ion solutions were used as control sets.

According to the Fig. 4.14, the ΔE of binary mixture Fe(III)-Cr(VI) showed decreasing with the increasing of concentration of Fe(III), which is probably due to the increasing of ionic strength of binary mixture system. In general liquid-liquid extraction process, the extraction of metal decreases when the ionic strength increases.[40] As the ionic strength increased, it may reduce the mass transfer of Cr(VI) from aqueous phase to DES phase onto the paper resulting in decreasing in complex formation of Cr(VI)-DPC. Moreover, there is possibility that Fe(III) also make a complex with DPC and choline chloride in DES phase[41], so the interruption of Cr(VI)-DPC complex formation occurred. From the Student *t*-test, the ΔE of 10 mg/L of Fe(III) gave significant difference with the single Cr(VI) solution, which means that Fe(III) with the concentration of 10 mg/L or more may interfere the Cr(VI) analysis. This can be proven also from the control set that DPC make a complex reaction with Fe(III) 10 mg/L and the increasing of ΔE was observed. Thus, if contain Fe(III) with that concentration, masking agent is needed before the analysis, such as ethylenediaminetetraacetate (EDTA) or phosphoric acid (H_3PO_4).[23]

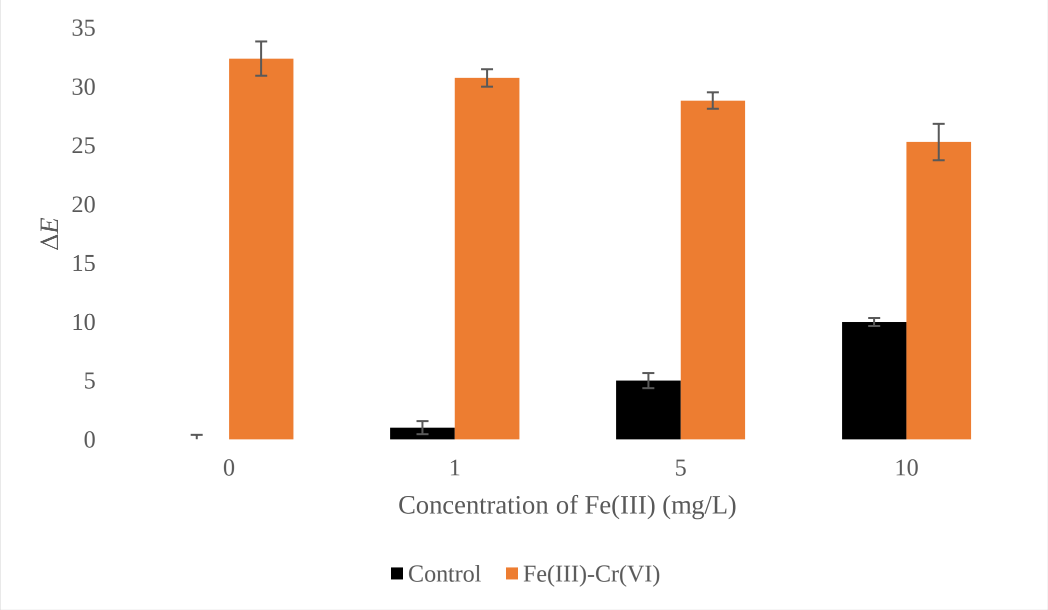


Fig. 4.14. ΔE of binary mixture Fe(III)-Cr(VI) (orange bar) and control of (black bar).

Fig. 4.15 reveals the ΔE of binary mixture Ni(II)-Cr(VI), exhibiting decrease with the increasing of concentration of Ni(II). This is probably due to the increasing of ionic strength of binary mixture system that may affect towards the complex formation of Cr(VI) with DPC as mentioned above in the case of Fe(III). Based on Student t-test calculation, there is no significant difference from the results obtained between binary mixture of Ni(II)-Cr(VI) and single Cr(VI) solution. In addition, Cr(III) (Fig. 4.16) also showed no significant difference between binary mixture and single Cr(VI) solution in those concentrations. Thus, Ni(II) and Cr(III) did not interfere the Cr(VI) in this work.

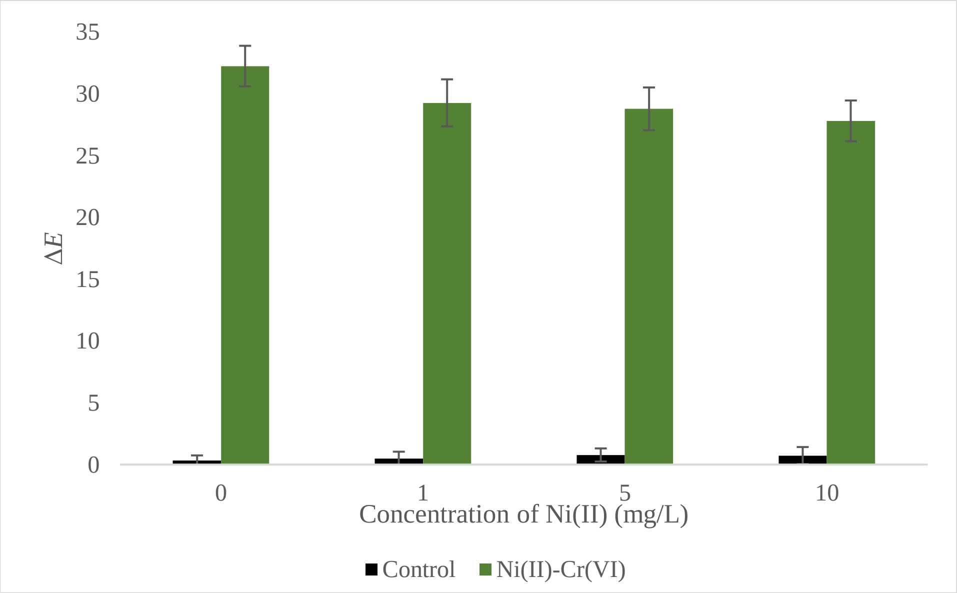


Fig. 4.15. ΔE of binary mixture Ni(II)-Cr(VI) (green bar and control (black bar).

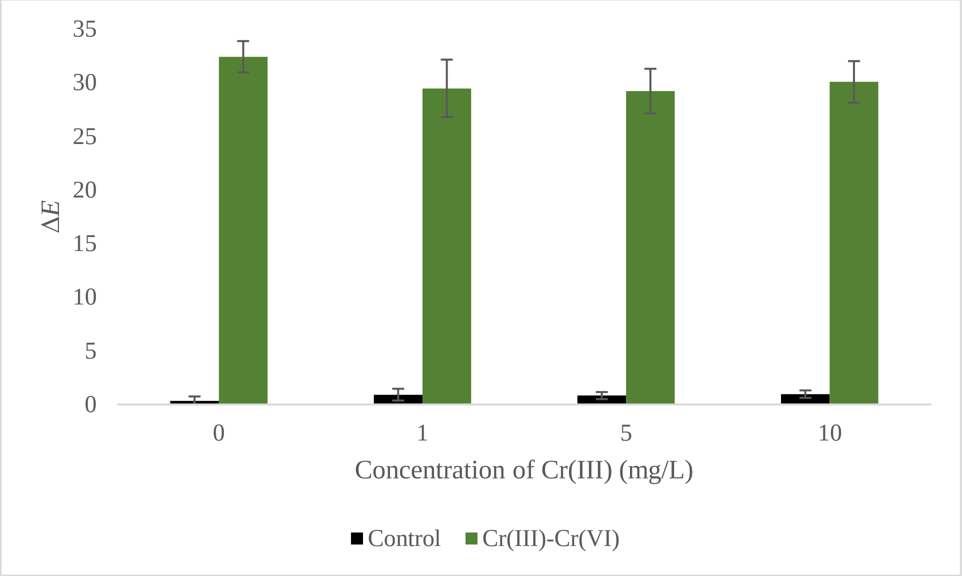


Fig. 4.16. Δ*E* of binary mixture Cr(III)-Cr(VI) (green bar) and control (black bar).

As shown in Fig. 4.17, the Δ*E* of binary mixture Cu(II)-Cr(VI) decrease with the increasing of concentration of Cu(II). This is probably due to the ionic strength of binary mixture system increased, which may reduce the complex interaction between Cr(VI) with DPC. In addition, similar to Fe(III) there is possibility that Cu(II) could form a complex with DPC and choline chloride in DES phase[41] and this may also interrupt complex formation of Cr(VI)-DPC. Student t-test calculation suggest that from 5 mg/L of Cu(II) yield Δ*E* too far from the single Cr(VI). Moreover, the increasing of Δ*E* from control was observed in such concentration. In conclusion, 5 mg/L or above of Cu(II) interfere the Cr(VI) and need to use masking agent before analysis, such as EDTA.[23]

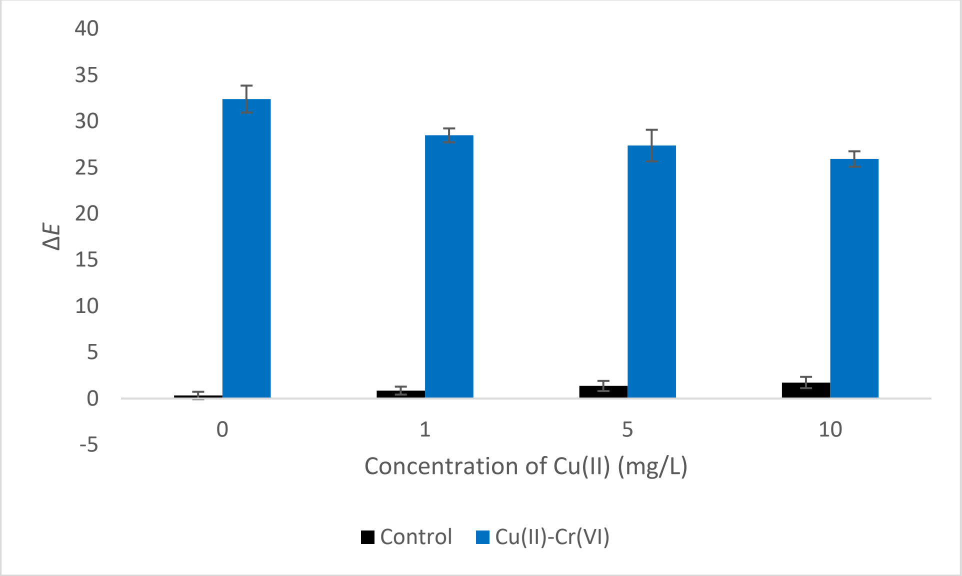


Fig. 4.17. Δ*E* of binary mixture Cu(II)-Cr(VI) (blue bar) and control (black bar).

The Δ*E* of binary mixture Na^+-Cr(VI) and Ca^{2+}-Cr(VI) are shown in Fig. 4.18 and Fig. 4.19, respectively. Both graphs show that the value of Δ*E* decrease with the increasing of concentration of each interference ions, which is probably due to with the increasing of ionic strength of each binary mixture system and the electrostatic interaction of cations and dichromate anion could occur.[40] According to Student t-test calculation, there is a significant difference between 1000 mg/L of Na^+ and 100 mg/L of Ca^{2+}, respectively. Thus, Na^+ and Ca^{2+} may interfere the Cr(VI) analysis in high concentration.

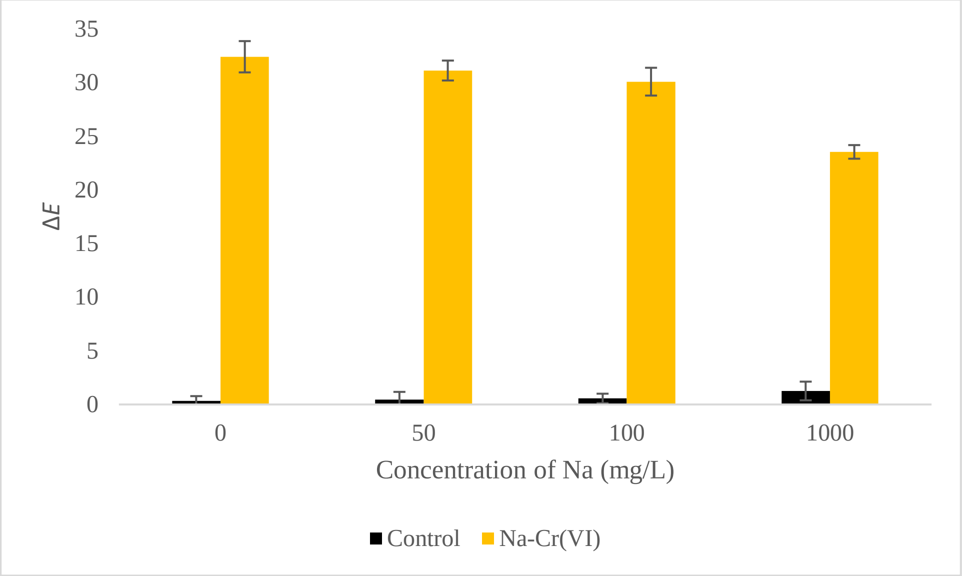


Fig. 4.18. Δ*E* of binary mixture Na^+-Cr(VI) (yellow bar) and control (black bar).

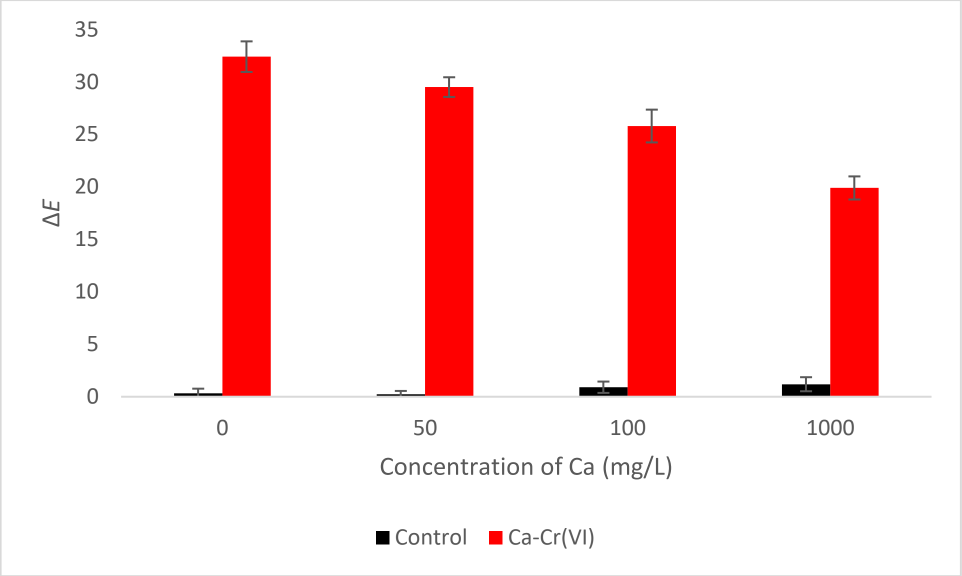


Fig. 4.19. Δ*E* of binary mixture Ca^{2+}-Cr(VI) (red line) and control (orange line).

4.8. Real Sample Application

The proposed method was applied for determination of Cr(VI) in environmental water samples, such as drinking water, tap water, ground water and surface water. The results are shown in Table 4.5. Based on the results, the concentration of Cr(VI) in all water samples were found below the LOD of the method. Thus, a recovery study was carried out by spiking method, whereas Cr(VI) with three different concentrations were added into the water samples to achieve the feasibility and accuracy of the method. The results show that the recovery of tap water, drinking water, surface water and ground water were obtained in the range of 88-104%, 100-104%, 82-88% and 86-100%, respectively with RSD of 2-15%, 5-12%, 4-16% and 2-10%, respectively. From these results, it can be concluded that the proposed method has good and acceptable recovery and precision. In addition, this method also follow the AOAC guideline.[39]

Table 4.5. Determination of Cr(VI) in Several Samples.

Sample	Spike (mg/L)	Found (mg/L)	%recovery	%RSD
Tap water	0	<LOD	-	-
	0.25	0.30 ± 0.01	104	2%
	1	0.92 ± 0.13	88	15%
	3	2.76 ± 0.16	91	6%

Drinking water	0	<LOD	-	-
	0.25	0.30 ± 0.02	101	6%
	1	1.04 ± 0.13	100	12%
	3	3.16 ±0.14	104	5%
Surface water	0	<LOD	-	-
	0.25	0.26 ± 0.04	86	16%
	1	0.86 ± 0.04	82	5%
	3	2.87 ± 0.10	88	4%
Ground water	0	<LOD	-	-
	0.25	0.26 ± 0.03	86	10%
	1	1.04 ± 0.04	100	3%
	3	2.98 ± 0.06	98	2%

In addition, the proposed method was compared with the previous reports on the determination of Cr(VI) (Table 4.6). Although the LOD is still higher than some existing methods that have better LOD, this proposed method still demonstrates low LOD and better than the common methods. This developed method provides a wider linear range, making it possible to determine Cr(VI) at various concentrations. Moreover, this method displays acceptable and good %RSD and recovery. This proposed method display more environmental friendly, lower cost, less time-consuming and simpler detection of Cr(VI), which is more suitable for on-site analysis.

Compared with the other μPADs methods, this proposed method uses DES, which modified with DPC as indicator to improve selectivity, as extractant and as hydrophobic layer to minimize the leakage of indicator. Moreover, this method also can provide pre-concentration that improves analytical performance. This developed dip-typed device demonstrates more user-friendly and does not require any equipment for sample introduction, which is the novelty of this method. However, this method relies on dipping directly contact with the sample, so leaking still may occur.

Table 4.6. Comparison of proposed method with existed methods.

Analytes	Analytical Method	Detection Method	Linear Range	LOD	%RSD	%Recovery	Ref.
Cr(VI)	DLLME	UV-Vis	5-200 µg/L	1.5 µg/L	<6%	90-98.2%	1
Total Cr	UA-DES-LLME	FAAS	3.2-70 µg/L	0.8 µg/L	<4.8%	94-104%	5
Cr(VI)	HF-LPME	Colorimetric µPADs (Wax Printing)	10-90 µg/L	3 µg/L	<6%	93-112%	42
Total Cr	µPADs (Wax Printing)	Colorimetric	6-94 mg/L	3 mg/L	<4.9%	NR	20
Cr(VI)	µPADs (Photolithographic)	Colorimetric	40-400 mg/L	30 mg/L	<8.7%	94-109%	19
Cr(VI)	DES-µPADs (Soaking)	Colorimetric	250-5000 µg/L	103 µg/L	<7.2%	82-104%	This work

NR=Not reported; DLLME=Dispersive Liquid-liquid Microextraction, UA-DES-LLME= Ultrasound-assisted Deep Eutectic Solvent-Liquid-liquid Microextraction; HF-LPME= Hollow Fiber membrane-Liquid Phase Microextraction, FAAS= Flame

CHAPTER 5 CONCLUSION

5.1. Conclusion

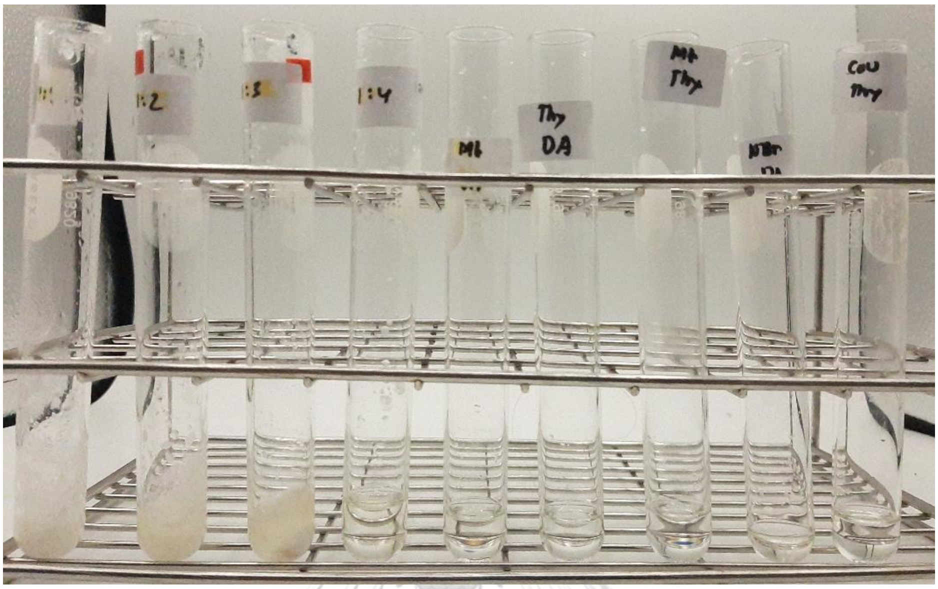
This research has successfully developed a rapid, simple and inexpensive detection of Cr(VI) using paper test strip device based on an environmentally friendly solvent. In this work, a DES comprising of choline chloride and thymol with molar ratio of 1:4 was applied as a hydrophobic layer on the test strip and DPC was used as an indicator and complexing agent for Cr(VI). The optimized conditions: 5000 mg/L DPC, 10 min dipping time in a pH 2 solution, were exploited to construct a calibration curve showing good linearity. The method revealed LOD and LOQ of 103 and 343 μg/L, respectively, and acceptable RSDs (<7.2%). This developed method demonstrates high selectivity towards Cr(VI), however some cation, such as Fe(III), Cu(II), Na^+ and Ca^{2+} may interfere in high concentration. The method has successfully applied for determination of Cr(VI) in real environmental water samples, exhibiting an acceptable and satisfactory recovery (82-104%). Furthermore, this developed method also can be applied for determination of Cr(VI) in industrial, environmental water and wastewater samples in other country, including Indonesia because this method was appropriate with Indonesia regulations that the limit of Cr(VI) in environment, industrials and waste waters is not greater than 0.1 ppm.[43]

5.2. Suggestion for Future Work

The stability of DPC in various DES precursors should be added and investigated in detail. Moreover, the extraction recovery (%EE) of DES should be investigated in selecting the suitable hydrophobic layer for the paper-strip.

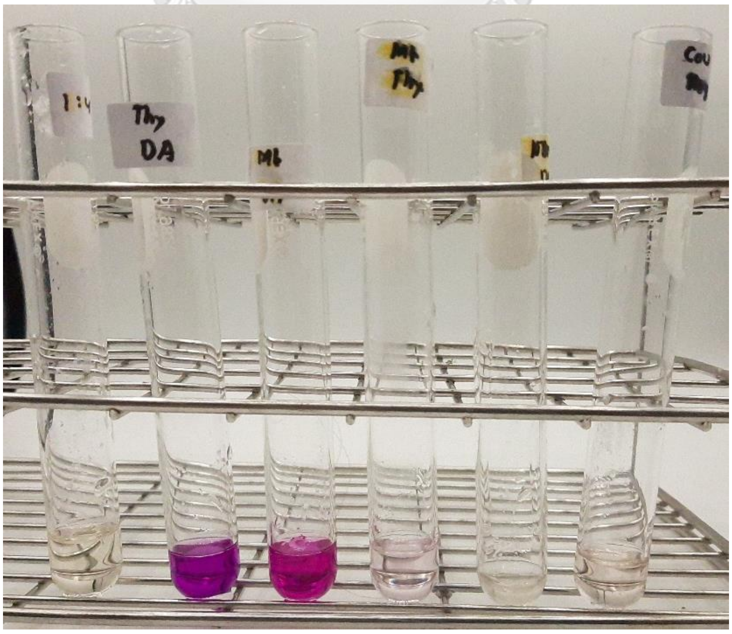
APPENDIX

- Selection of DES



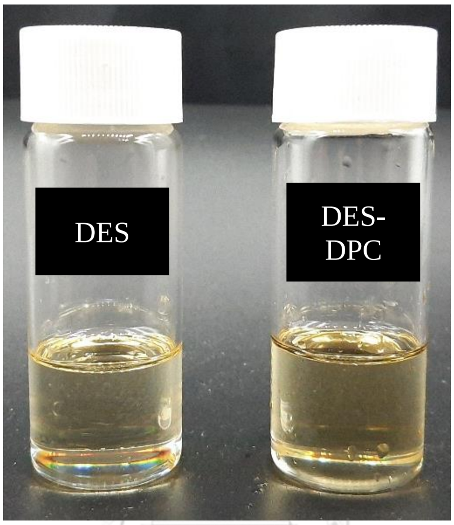
From left: ChCl:Thy (1:1); ChCl:Thy(1:2); ChCl:Thy (1:3); ChCl:Thy (1:4); Mt:DA (1:1); Thy:DA (1:1); Mt:Thy (1:1); N_{8881}Br:DA (1:2); Cou:Thy (1:2).

  - When adding DPC

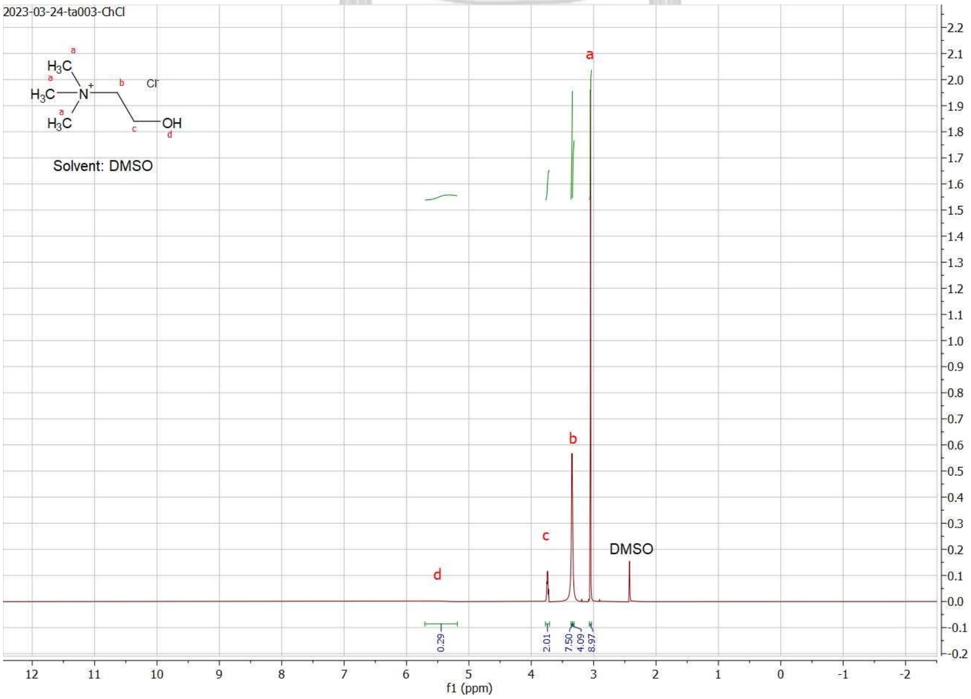


From left: ChCl:Thy (1:4); Mt:DA (1:1); Thy:DA (1:1); Mt:Thy (1:1); N_{8881}Br:DA (1:2); Cou:Thy (1:2).

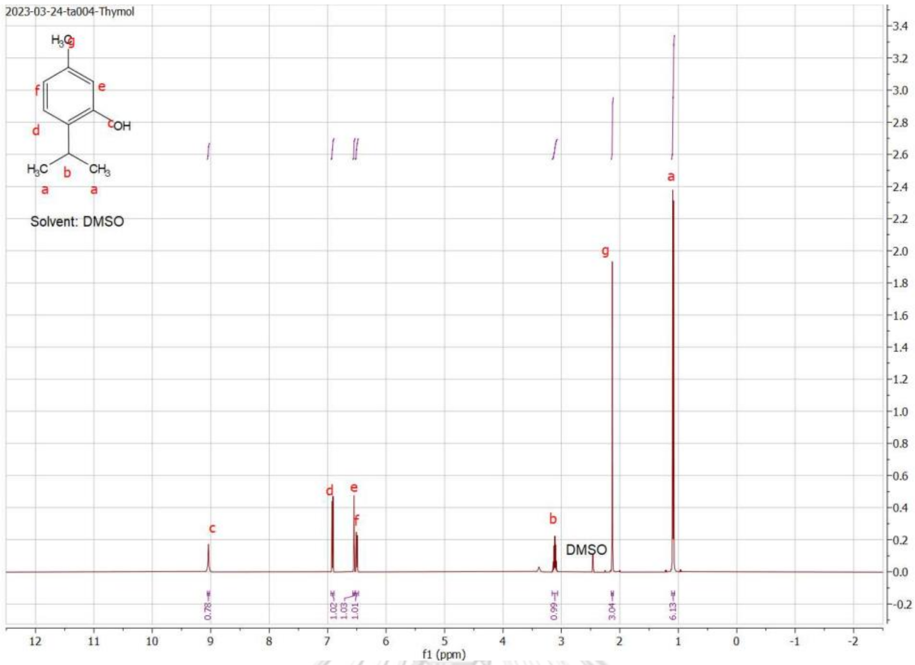
- DES and DES-DPC Solution



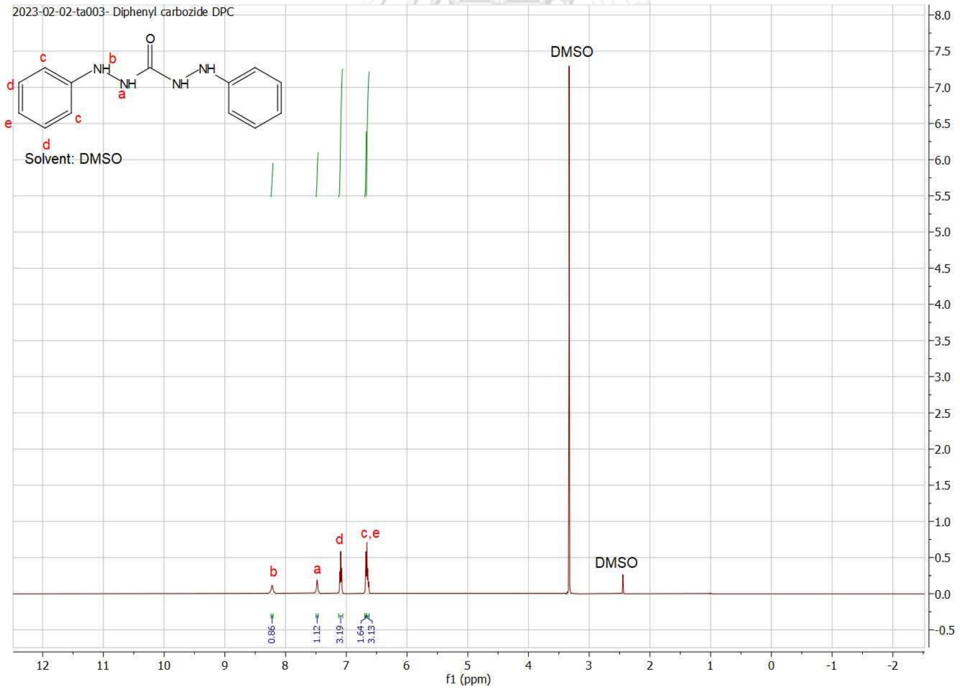
- Full details of NMR Spectra
    - ○ Choline Chloride



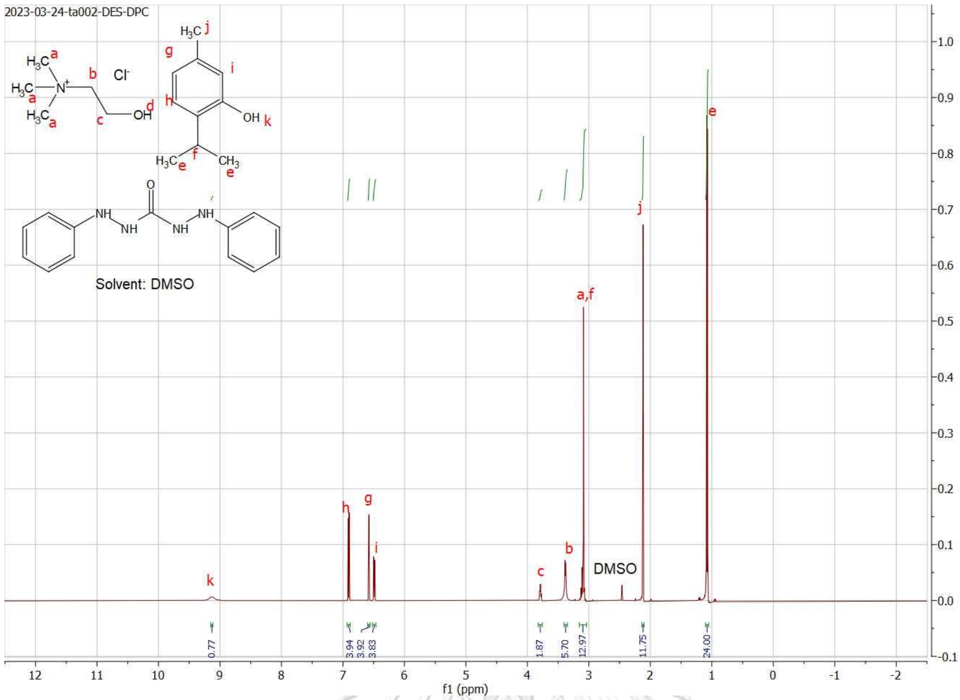
○ Thymol



○ Diiphenylcarbazide



○ DES-DPC



– RGB and Lab Value

○ Response Time

Time (min)	R	G	B	L	a	b	Δ*E*
Reference	192.62±1.45	186.16±1.89	182.81±1.35	75.99±1.02	1.67±0.65	2.49±0.34	0
0.5	192.16±1.30	182.61±1.27	182.75±1.27	75.04±0.47	3.40±0.04	1.13±0.13	2.38±0.12
1	190.13±0.45	179.82±0.69	180.93±0.69	74.10±0.23	3.86±0.12	0.74±0.05	3.39±0.18
5	188.42±0.33	176.04±0.42	179.05±0.14	72.94±0.13	4.97±0.13	-0.01±0.23	5.15±0.28
10	188.94±1.12	175.07±1.73	179.94±1.05	72.75±0.56	5.83±0.39	-0.70±0.27	6.17±0.69
20	187.79±0.94	172.62±1.82	179.91±0.64	72.02±0.53	6.75±0.83	-1.77±0.79	7.73±1.22
30	188.61±1.25	173.54±0.98	181.23±20.03	72.37±0.38	6.77±0.55	-1.98±0.73	7.71±0.73

○ Concentration of DPC

[DPC] (mg/L)	R	G	B	L	a	b	Δ*E*
1000	195.72±2.21	188.95±2.45	185.88±2.61	76.95±0.71	1.41±0.81	2.24±0.38	2.21±0.07
3000	192.79±1.93	183.56±2.46	182.29±2.51	75.33±0.86	3.035±0.22	1.80±0.10	3.44±0.46

5000	187.90±1.18	174.77±1.92	179.85±1.09	72.59±0.62	5.60±0.50	-0.91±0.55	7.83±0.92

- References

[DPC] (mg/L)	R	G	B	L	a	b
1000	199.39±1.31	192.37±1.54	186.59±1.21	78.24±0.20	1.44±0.50	3.77±0.11
3000	195.12±0.98	189.48±1.30	183.41±1.32	77.07±0.32	0.90±0.21	3.75±0.06
5000	193.98±1.31	189.03±0.90	183.38±1.44	76.87±0.23	0.72±0.09	3.45±0.23

- pH

pH	R	G	B	L	a	b	ΔE
References	195.42±2.87	189.35±2.43	183.00±2.19	77.06±1.01	1.00±0.92	3.95±1.23	0
1	191.40±2.49	178.46±2.02	180.54±1.63	73.85±0.77	4.98±0.09	0.59±0.28	6.14±0.49
2	186.80±1.50	175.48±1.53	176.71±2.47	72.59±0.59	4.26±0.22	0.81±0.52	6.38±0.25
3	192.76±1.71	185.05±1.70	182.27±1.38	75.71±0.61	2.22±0.11	2.37±0.16	2.43±0.49
5	194.85±1.97	188.87±2.21	185.12±1.91	76.94±0.77	1.43±0.28	2.63±0.11	1.54±0.22
7	196.73±1.23	190.95±0.78	187.10±0.63	77.68±0.30	1.33±0.23	2.65±0.22	1.52±0.08
10	197.79±2.46	191.91±1.79	188.49±2.12	78.05±0.71	1.45±0.29	2.44±0.11	1.92±0.52
12	200.08±1.45	186.03±2.43	184.18±2.35	76.61±0.79	4.64±0.5	2.72±0.11	3.92±0.50

- Control

pH	R	G	B	L	a	b	ΔE
References	195.42±2.87	189.35±2.43	183.00±2.19	77.06±1.01	1.00±0.92	3.95±1.23	0
1	200.95±2.76	193.16±2.01	189.94±1.41	78.66±1.66	2.15±0.23	2.58±0.10	2.40±0.30
2	199.85±2.50	192.61±1.87	189.03±2.03	78.40±1.00	1.90±0.12	2.69±0.21	2.05±0.16
3	197.40±1.98	190.96±1.56	187.21±2.09	77.74±0.92	1.58±0.21	2.68±0.22	1.55±0.43
5	196.26±2.22	190.18±2.23	186.44±1.67	77.42±1.01	1.46±0.24	2.63±0.08	1.44±0.90
7	196.99±2.54	190.75±2.03	187.19±1.73	77.65±1.43	1.55±0.43	2.56±0.04	1.61±1.32
10	198.00±1.45	189.04±2.13	187.35±1.70	77.29±0.78	2.86±0.09	1.96±0.03	2.73±0.85
12	198.29±1.66	176.43±1.73	174.55±1.88	73.77±0.89	7.53±0.51	3.82±0.32	7.31±0.98

- Calibration Curve

[Cr(VI)] (ppb)	R	G	B	L	a	B	ΔE
References	194.24±1.30	189.256	185.253	76.99319	1.029689	2.62883	0
0	195.05±1.31	189.06±1.07	184.76±-0.96	76.99±0.41	1.330.06	2.91±0.08	0.52±0.15
10	192.80±0.89	186.51±2.24	182.85±2.10	76.49±0.38	1.24±0.14	2.66±0.19	0.59±0.06
50	195.24±1.79	189.84±1.72	186.47±1.92	76.95±0.37	1.45±0.18	2.60±0.09	0.69±0.17
100	192.71±0.83	186.93±0.84	183.64±0.96	76.36±0.53	1.6**2±0.33**	2.47±0.15	1.02±0.13
250	193.18±0.41	186.15±0.83	183.65±0.33	76.02±0.13	2.26±0.18	2.21±0.29	1.63±0.25
500	192.15±1.64	182.42±1.72	182.91±1.92	74.99±0.63	3.53±0.07	0.97±0.17	3.63±0.30
1000	187.76±0.38	173.81±0.35	178.37±0.43	72.29±0.08	5.81±0.35	-0.53±0.29	7.42±0.38
3000	170.91±1.99	143.64±1.39	166.22±1.35	62.72±0.58	14.23±0.18	-8.05±0.18	22.19±0.32
5000	150.56±3.22	114.47±1.85	154.37±5.05	52.98±0.98	21.39±1.07	-16.08±1.07	36.66±0.77

- Intraday Precision
  - 1st Batch

[Cr(VI)] (mg/L)	R	G	B	L	a	b	ΔE	%RSD
Reference	194.22±2.03	186.36±2.11	180.22±2.09	76.82±1.12	1.54±0.32	3.77±0.56	0	0
1	189.94±1.08	177.41±0.27	180.40±0.23	73.45±0.38	4.99±0.27	0.08±0.23	6.08±0.16	2.59
5	158.44±0.13	127.82±0.33	158.69±0.27	57.27±0.23	17.33±0.32	-12.02±0.26	29.68±0.39	1.31

- 2nd Batch

[Cr(VI)] (mg/L)	R	G	B	L	a	b	ΔE	%RSD
Reference	194.22±2.03	186.36±2.11	180.22±2.09	76.82±1.12	1.54±0.32	3.77±0.56	0	0
1	188.51±0.75	174.98±0.17	179.25±0.16	72.68±0.24	5.60±0.17	-0.45±0.16	7.17±0.09	1.23
5	156.80±3.28	124.63±0.63	157.15±0.73	59.75±1.77	15.31±0.63	-9.49±0.73	25.63±1.89	7.39

- 3rd Batch

[Cr(VI)] (mg/L)	R	G	B	L	a	b	ΔE	%RSD
Reference	194.22±2.03	186.36±2.11	180.22±2.09	76.82±1.12	1.54±0.32	3.77±0.56	0	0
1	189.80±2.46	176.69±0.21	180.43±0.64	73.26±0.96	5.34±0.21	-0.23±0.64	6.63±0.15	2.19
5	157.83±2.71	125.18±0.33	156.99±1.18	56.48±0.79	18.31±0.33	-12.23±1.18	30.85±0.56	1.83

- Interday Precision
  - 1st Day

[Cr(VI)] (mg/L)	R	G	B	L	a	b	ΔE	%RSD
Reference	194.10±1.53	188.38±2.03	182.43±2.45	76.68±1.22	0.95±0.09	3.71±0.31	0	0
1	191.48±1.72	181.23±0.33	181.99±0.25	74.61±0.63	3.77±0.33	0.91±0.25	4.52±0.32	7.04
5	166.05±0.59	138.47±0.21	162.55±0.27	60.83±0.20	14.72±0.21	-8.83±0.27	24.46±0.28	1.13

- 2nd Day

[Cr(VI)] (mg/L)	R	G	B	L	a	b	ΔE	%RSD
Reference	193.71±1.80	187.86±1.23	181.26±2.05	76.49±0.59	0.88±0.12	4.05±0.65	0	0
1	191.04±1.13	180.66±0.21	181.86±0.02	74.42±0.53	3.89±0.21	0.70±0.02	4.97±0.34	6.77
5	163.64±4.25	135.35±0.63	160.84±0.73	59.75±1.77	15.31±0.63	-9.49±0.73	25.92±1.87	7.23

- 3rd Day

[Cr(VI)] (mg/L)	R	G	B	L	a	b	ΔE	%RSD
Reference	193.71±1.80	187.86±1.23	181.26±2.05	76.49±0.59	0.88±0.12	4.05±0.65	0	0
1	190.92±1.26	179.98±0.15	181.33±0.04	74.22±0.55	4.13±0.15	0.69±0.04	5.25±0.34	6.55
5	163.86±1.51	134.88±0.26	159.41±0.39	59.61±0.56	15.38±0.26	-8.88±0.39	25.74±0.51	1.97

- Selectivity
    - Fe(III)

[Fe(III)] (mg/L)	R	G	B	L	a	b	ΔE
Reference	194.57±2.11	189.15±2.22	185.92±1.78	77.01±1.03	1.32±0.32	2.29±0.12	0
0	154.58±3.01	121.02±3.42	155.30±2.36	55.05±1.23	19.21±0.80	-13.44±0.65	32.40±1.46
1	160.26±1.79	125.72±1.65	158.86±1.05	56.93±0.62	19.21±0.15	-12.62±0.59	30.76±0.74
5	163.79±0.65	130.39±1.10	161.81±0.19	59.71±1.70	17.56±0.94	-11.06±0.99	28.83±0.70
10	169.02±2.53	138.30±3.66	166.04±2.31	61.18±1.23	16.55±0.77	-10.26±0.71	25.30±1.55

- Ni(II)

[Ni(II)] (mg/L)	R	G	B	L	a	b	ΔE
Reference	194.57±2.11	189.15±2.22	185.92±1.78	77.01±1.03	1.32±0.32	2.29±0.12	0
0	154.58±3.01	121.02±3.42	155.30±2.36	55.05±1.23	19.21±0.80	-13.44)±0.65	32.40±1.46
1	162.30±3.83	128.73±4.40	160.06±3.33	57.92±1.58	18.45±0.91	-11.79±1.11	29.27±1.90
5	163.54±3.50	130.02±3.69	161.37±3.53	58.41±1.37	18.41±0.75	-11.79±1.09	28.79±1.73
10	166.73±2.47	133.40±3.10	164.15±1.69	59.67±1.05	18.15±0.89	-11.47±1.11	27.81±1.65

- Cr(III)

[Cr(III)] (mg/L)	R	G	B	L	a	b	ΔE
Reference	194.57±2.11	189.15±2.22	185.92±1.78	77.01±1.03	1.32±0.32	2.29±0.12	0
0	154.58±3.01	121.02±3.42	155.30±2.36	55.05±1.23	19.21±0.80	-13.44±0.65	32.40±1.46
1	166.38±2.17	131.71±4.05	166.29±0.37	59.28±1.21	19.41±1.59	-13.27±1.99	29.46±2.68
5	163.68±1.41	129.42±2.97	164.67±0.49	58.39±0.86	19.45±1.29	-13.72±1.58	30.22±2.07
10	161.51±0.73	126.87±2.25	163.32±1.45	57.49±0.59	19.88±1.29	-14.32±1.70	31.33±1.94

- Cu(II)

[Cu(II)] (mg/L)	R	G	B	L	a	b	ΔE
Reference	194.57±2.11	189.15±2.22	185.92±1.78	77.01±1.03	1.32±0.32	2.29±0.12	0
0	154.58±3.01	121.02±3.42	155.30±2.36	55.05±1.23	19.21±0.80	-13.44±0.65	32.40±1.46
1	164.12±1.29	130.86±1.52	161.63±0.81	58.69±0.53	18.19±0.35	-11.52±0.50	28.48±0.76
5	166.89±3.47	133.79±4.76	163.59±3.67	59.76±1.65	17.89±0.78	-11.01±0.41	27.37±1.71
10	169.88±1.87	137.88±1.79	166.77±2.74	61.18±0.65	17.23±0.90	-10.66±0.72	25.91±0.84

- Na^+

[Na] (mg/L)	R	G	B	L	a	b	ΔE
Reference	194.57±2.11	189.15±2.22	185.92±1.78	77.01±1.03	1.32±0.32	2.29±0.12	0
0	154.58±3.01	121.02±3.42	155.30±2.36	55.05±1.23	19.21±0.80	-13.44±0.65	32.40±1.46
1	157.25±0.89	123.63±1.63	156.31±0.91	56.02±0.49	18.85±0.75	-12.55±0.49	31.72±0.93

5	161.25±3.20	127.56±2.75	160.31±1.58	57.53±1.05	18.80±0.29	-12.53±1.05	30.08±1,30
10	171.71±1.71	142.20±1.85	168.02±1.44	62.48±0.67	15.67±0.17	-9.41±0.28	23.53±0.63

- Ca^{2+}

[Ca] (mg/L)	R	G	B	L	a	b	Δ*E*
Reference	194.57±2.11	189.15±2.22	185.92±1.78	77.01±1.03	1.32±0.32	2.29±0.12	0
0	154.58±3.01	121.02±3.42	155.30±2.36	55.05±1.23	19.21±0.80	-13.44±0.65	32.40±1.46
1	160.99±0.52	127.87±1.45	159.33±0.05	57.56±0.64	18.34±0.64	-11.94±0.61	29.50±0.94
5	169.40±2.67	137.31±3.84	165.43±2.85	60.95±0.77	17.13±0.77	-10.25±0.87	25.79±1.56
10	177.49±2.17	150.73±2.75	172.83±1.66	65.33±0.94	13.85±0.50	-7.82±0.49	19.90±1.10

- Sample Application
  - Tap Water

[Cr(VI)] (mg/L)	R	G	B	L	a	b	Δ*E*
Reference	192.74±2.41	187.97±2.10	184.31±1.98	76.51±0.97	1.02±0.08	2.43±0.11	0
0	193.47±1.21	189.76±1.09	184.82±0.94	76.59±0.40	1.35±0.12	2.28±0.33	0.54±0.07
0.25	192.95±2.13	187.75±1.66	185.33±1.85	75.86±0.65	2.72±0.21	0.92±0.14	2.44±0.05
1	188.46±0.84	179.09±1.48	179.43±1.48	72.59±0.44	5.80±0.65	-0.68±0.80	6.94±0.98
3	174.28±2.82	156.36±2.49	168.79±1.49	64.07±1.11	13.80±0.67	-7.45±0.69	20.41±1.14

  - Drinking Water

[Cr(VI)] (mg/L)	R	G	B	L	a	b	Δ*E*
Reference	192.74±2.41	187.97±2.10	184.31±1.98	76.51±0.97	1.02±0.08	2.43±0.11	0
0	193.03±0.97	189.10±0.94	184.09±0.27	76.36±0.27	1.43±0.12	2.33±0.33	0.57±0.09
0.25	194.72±2.72	187.16±2.09	187.33±0.86	76.54±0.86	2.69±0.21	0.85±0.14	2.41±0.12
1	186.45±0.94	171.96±1.28	177.50±1.28	71.67±0.40	6.19±0.65	-0.96±0.80	7.86±0.94
3	168.76±1.77	139.84±2.07	163.65±2.07	61.47±0.79	15.13±0.67	-8.47±0.69	23.34±1.05

  - Surface Water

[Cr(VI)] (mg/L)	R	G	B	L	a	b	Δ*E*
Reference	192.74±2.41	187.97±2.10	184.31±1.98	76.51±0.97	1.02±0.08	2.43±0.11	0
0	194.96±0.49	189.94±0.51	186.77±0.42	77.27±0.18	1.19±0.02	2.21±0.05	0.59±0.11
0.25	194.29±0.36	188.61±0.51	186.10±0.37	76.85±0.15	1.54±0.26	1.96±0.24	2.16±0.31
1	190.43±2.58	179.19±2.18	182.60±2.94	74.01±1.11	4.61±0.34	-0.31±0.19	6.54±0.28
3	179.46±2.74	155.58±3.57	173.94±1.70	66.77±1.20	12.06±0.69	-6.28±0.86	19.76±0.73

  - Ground Water

[Cr(VI)] (mg/L)	R	G	B	L	a	b	Δ*E*
Reference	192.74±2.41	187.97±2.10	184.31±1.98	76.51±0.97	1.02±0.08	2.43±0.11	0
0	193.87±0.35	188.86±0.37	185.95±0.43	76.88±0.13	1.23±0.01	2.08±0.04	0.55±0.11
0.25	193.03±0.53	186.77±1.04	186.01±0.75	76.27±0.33	2.06±0.24	1.16±0.12	2.12±0.19
1	189.01±0.95	177.53±0.95	181.23±0.81	71.93±0.09	5.78±0.25	-1.79±0.26	7.85±0.26

3	175.19±2.42	150.50±2.82	170.99±2.23	62.05±0.67	13.89±0.26	-8.05±0.06	22.01±0.44

- The amount of DPC on paper-strip calculation

m_{paper} = 57.33±3.37 mg

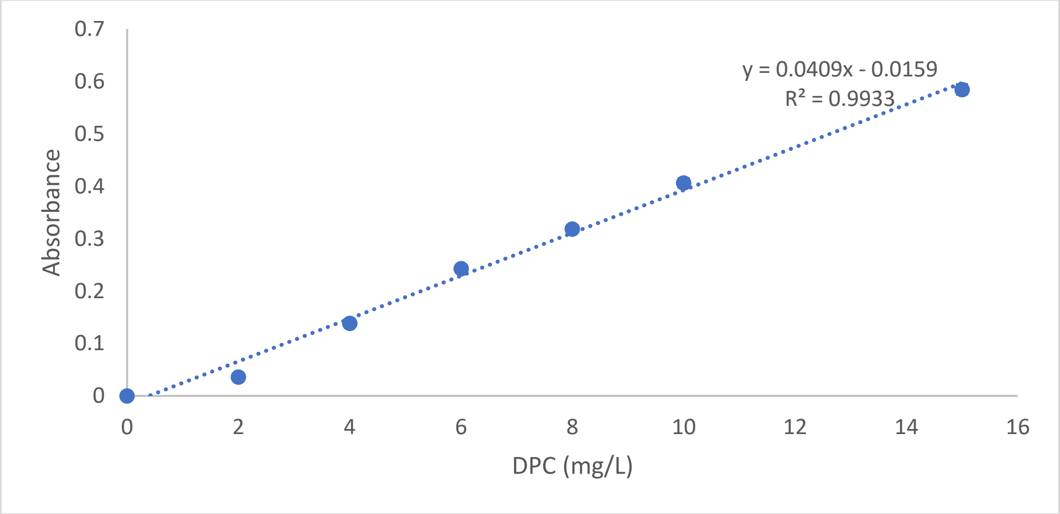
$m_{paper+DES}$ = 72.7±4.16 mg

m_{DES} = $m_{paper+DES}$ - m_{paper}

= 72.70 mg – 57.33 mg

= 15.37±0.15 mg

- %DPC Leakage
  - Calibration Curve



Time (mins)	[DPC] (mg/L)	SD
0.5	5.225	0.016
1	6.303	0.029
5	7.119	0.028
10	8.177	0.013
20	8.968	0.019
30	11.029	0.002

- ○ % Leak Calculation

m_{DPC} $= [DPC] \times V_{sample}$

$= \frac{5.225\ \mathrm{mg}}{\mathrm{L}} \times 2 \times 10^{-3}\ \mathrm{L}$

$= 0.010\ \mathrm{mg}$

$m_{DPC(paper\text{-}strips)}$ $= [DPC] \times m_{DES}$

$= \frac{5000\ \mathrm{mg}}{\mathrm{kg}} \times 1.53 \times 10^{-5}\ \mathrm{kg}$

$= 0.077\ \mathrm{mg}$

%Leak $= \frac{\mathrm{m_{DPC}}}{\mathrm{m_{DPC\ (paper\ strip)}}} \times 100\%$

$= \frac{0.010}{0.077} \times 100\%$

$= 13.6\%$

– Stoichiometry calculation

$n_{Cr^{6+}} = \frac{\frac{1\ mg}{L} \times 2 \times 10^{-3} L}{51.99\ \ \mathrm{g/mole}}$

$= 3.84\times10^{-5}$ mmol

$n_{DPC} = \frac{\frac{5000\ mg}{kg} \times 1.53 \times 10^{-5}\ kg}{242.28\ \ \mathrm{g/mole}}$

$= 3.17\times10^{-4}$ mmol

$2HCrO_4^-$	$+ 3C_{13}H_{14}N_4O$	$+ 8H+$	→	$2Cr^{3+}$	$+ 3C_{13}H_{12}N_4O$	$+ 8H_2O$
3.8410^{-5} mmol	$3.17.10^{-4}$ mmol					
$-3.84.10^{-5}$ mmol	$-5.76.10^{-5}$ mmol				$+3.84.10^{-5}$ mmol	$+5.76.10^{-5}$ mmol
-	$2.59.10^{-4}$ mmol				$3.84.10^{-5}$ mmol	$5.76.10^{-5}$ mmol

- Student T-test
  - ○ Response Time

t-Test: Paired Two Sample for Means

	10 mins	20 mins
Mean	6.169967	7.729608
Variance	0.480708	1.492965
Observations	3	3
Pearson Correlation	-0.17253	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.79455	
P(T<=t) one-tail	0.107288	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.214577	
t Critical two-tail	4.302653	

- ○ Selectivity
  - ■ Fe(III)

t-Test: Paired Two Sample for Means

	Cr(VI) 5 mg/L	Fe(III) 10 mg/L-Cr(VI) 5 mg/L
Mean	32.40362053	25.3013311
Variance	2.126046487	2.40579762
Observations	3	3
Pearson Correlation	-0.55080389	

Hypothesized Mean Difference	0
df	2
t Stat	4.641840438
P(T<=t) one-tail	0.021705544
t Critical one-tail	2.91998558
P(T<=t) two-tail	0.043411089
t Critical two-tail	4.30265273

- Ni(II)

t-Test: Paired Two Sample for Means

	Cr(VI) 5 mg/L	Ni(II) 10 mg/L – Cr(VI) 5 mg/L
Mean	32.24839568	27.8143613
Variance	2.697959776	2.72066304
Observations	3	3
Pearson Correlation	-0.98109176	
Hypothesized Mean Difference	0	
df	2	
t Stat	2.344033812	
P(T<=t) one-tail	0.071882773	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.143765547	
t Critical two-tail	4.30265273	

- Cr(III)

t-Test: Paired Two Sample for Means

	Cr(VI) 5 mg/L	Cr(III) 1 mg/L – Cr(VI) 5 mg/L
Mean	32.40362	29.45572
Variance	2.126046	7.192267
Observations	3	3
Pearson Correlation	-0.14038	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.582045	
P(T<=t) one-tail	0.127227	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.254454	
t Critical two-tail	4.302653	

- Cu(II)

t-Test: Paired Two Sample for Means

	Cr(VI) 5 mg/L	Cu(II) 5 mg/L – Cr(VI) 5 mg/L
Mean	32.40362	27.88626
Variance	2.126046	0.931055
Observations	3	3
Pearson Correlation	0.996841	
Hypothesized Mean Difference	0	
df	2	

t Stat	15.58265
P(T<=t) one-tail	0.002047
t Critical one-tail	2.919986
P(T<=t) two-tail	0.004093
t Critical two-tail	4.302653

- Na^+

t-Test: Paired Two Sample for Means

	Cr(VI) 5 mg/L	Na 1000 mg/L-Cr(VI) 5 mg/L
Mean	32.40362	23.53589
Variance	2.126046	0.402041
Observations	3	3
Pearson Correlation	0.695956	
Hypothesized Mean Difference	0	
df	2	
t Stat	13.78633	
P(T<=t) one-tail	0.00261	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.00522	
t Critical two-tail	4.302653	

- Ca^{2+}

t-Test: Paired Two Sample for Means

	Cr(VI) 5 mg/L	Ca 100 mg/L-Cr(VI) 5 mg/L
Mean	32.40362	25.79494
Variance	2.126046	2.43646

Observations	3	3
Pearson Correlation	0.08133	
Hypothesized Mean Difference	0	
df	2	
t Stat	5.590484	
P(T<=t) one-tail	0.015269	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.030538	
t Critical two-tail	4.302653	

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