SIMPLE HEADSPACE DEVICE FOR DETERMINATION OF VOLATILE COMPOUNDS IN FOOD AND DRUG SAMPLES

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Field of Study	Chemistry
Thesis Advisor	Associate Professor Dr. PAKORN VARANUSUPAKUL

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งานวิจัยนี้เป็นการพัฒนาวิธีที่สามารถเตรียมตัวอย่างและวิเคราะห์สารตัวอย่างที่มีความซับซ้อนได้ในขั้นตอนเดียว โดยการนำเอาอุปกรณ์วิเคราะห์ฐานกระดาษมาใช้ร่วมกับเทคนิคการสกัด/เตรียมตัวอย่างแบบเฮดสเปซ หรือเรียกว่าอุปกรณ์เฮดสเปซ อย่างง่าย โดยขั้นตอนแรกจะทำการเตรียมอุปกรณ์ตรวจวัดฐานกระดาษโดยใช้ปากกามาร์คเกอร์แบบถาวรเพื่อกำหนดขอบเขตในการทำปฏิกิริยา จากนั้นนำชิ้นส่วนของอุปกรณ์ดังกล่าวมายึดติดกับแผ่นรองฝาขวดแก้วขนาดเล็กด้วยกาวสองหน้า ขั้นต่อมาบรรจุสารตัวอย่างที่ต้องการวิเคราะห์ในขวดแก้วขนาดเล็ก แล้วปิดขวดแก้วขนาดเล็กให้สนิทด้วยฝาขวดที่ติดตั้งอุปกรณ์เฮดสเปซอย่างง่ายที่บรรจุสารละลายคัลเลอร์ริเมตริกรีเอนเจนต์ ให้ความร้อนแก่สารละลาย(ที่ 40 องศาเซลเซียสเป็นเวลา 5 นาที สำหรับเอทานอล และ 50 องศาเซลเซียส เป็นเวลา 15 นาที สำหรับไอโอดีน) เพื่อช่วยในการสกัดสารระเหยง่ายให้ขึ้นไปทำปฏิกิริยากับอุปกรณ์ดังกล่าว โดยใช้กระดาษเป็นที่ให้เกิดปฏิกิริยาหรือใช้กระดาษเป็นตัวบ่งชี้สัญญาณ ขั้นตอนสุดท้ายวัดความเข้มของสีที่เกิดขึ้นด้วยโปรแกรมอิมเมจเจ ในโหมดอาร์จีบี เพื่อนำไปหาปริมาณสารที่ต้องการวิเคราะห์ สารตัวแรกคือเอทานอลนั้นถูกใช้เป็นตัวแทนของการตรวจวัดสารระเหยง่ายในยาและเครื่องดื่ม โดยใช้กรดโครมิคเป็นรีเอเจ้นต์ สีของสารละลายบนกระดาษเปลี่ยนจากสีส้มของไดโครเมต (IV) เป็นสีเขียวของโครเมียม (III) ศึกษาปริมาณวิเคราะห์ด้วยการสร้างกราฟมาตราฐานในช่วงความเข้มข้นของเอทานอล(ร้อยละโดยปริมาตร/ปริมาตร) คือ 0 -7% พบว่าสารละลายตัวอย่าง เช่น ยาและเครื่องดื่มแอลกอฮอล์ที่มีการเติมแอลกอฮอล์ที่ความข้มข้น 0.5% (ร้อยละโดยปริมาตร/ปริมาตร)ให้ค่าการกลับคืนอยู่ในช่วง 94-120% และมีค่าคลาดเคลื่อนสัมพัทธ์น้อยกว่า 3% และตัวที่สองคือไอโอดีนซึ่งเป็นตัวแทนของสารกึ่งระเหยง่ายในตัวอย่างที่มีความซับซ้อนมากขึ้นอาทิ เช่น ไข่ จากนั้นเติมไฮโดนเจนเปอร์ออกไซด์เพื่อออกซิไดซ์ไอโอดีนในตัวอย่างไข่ที่กำจัดตะกอนโปรตีนด้วยกรดไตรคลอโรอะซิติกก่อนทำการสกัด โดยใช้น้ำแป้งเป็นรีเอเจ้นต์ของสารละลายบนกระดาษเปลี่ยนจากไม่มีสี เป็นสีเขียวของสีน้ำเงินเข้มของไอโอดีน-น้ำแป้ง ศึกษาปริมาณวิเคราะห์ด้วยการสร้างกราฟมาตราฐานในช่วงความเข้มข้นของไอโอดีน 0-100 ไมโครกรัมต่อลิตร พบว่าสารละลายตัวอย่างไข่ที่มีการเติมสารละลายไอโอไดด์มาตรฐาน 100 มิลลิกรัมต่อลิตร ให้ค่าการกลับคืนอยู่ในช่วง 93% และมีค่าคลาดเคลื่อนสัมพัทธ์น้อยกว่า 2%

สาขาวิชา	เคมี	ลายมือชื่อนิสิต .....
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Kamonwan Meesuwan : SIMPLE HEADSPACE DEVICE FOR DETERMINATION OF VOLATILE COMPOUNDS IN FOOD AND DRUG SAMPLES. Advisor: Assoc. Prof. Dr. PAKORN VARANUSUPAKUL

This work presented a simple alternative method for simultaneous extraction and determination of volatile compounds in complex matrix samples by combining headspace technique with paper based colorimetric method. The paper loaded with colorimetric reagent that was attached to the liner of the vial cap making a simple headspace device for direct detection of the semi-volatile and volatile compounds in headspace extraction mode. A sample was placed in a vial sealed with the paper-based headspace device. The method was tested for determination of volatile compound using ethanol as a model and semi-volatile compound using iodine as a model in complex matrix samples. Ethanol volatilized and reacted with chromic acid reagent, which was generated by mixing sodium or potassium dichromate with sulfuric acid, on the paper. The orange color of solution containing dichromate (VI) change to green solution containing chromium (III) ions. Iodine was volatilized by using oxidizing agent and react with starch reagent on the paper. The colorless of starch solution change to dark blue color of iodine-starch solution. The image of the paper was taken by the scanner for determination of iodine and by digital camera for determination of alcohol. The images were processing by ImageJ software in RGB mode and the blue intensity was taken for quantification. For determination of alcohol, the sample was heated at 40 ^{o} C for 5 min. The method was applied for determination of alcohol content in drug (i.e., stomachic mixtures and Salol et menthol mixture), and beverage sample (i.e., beer). The linear calibration ranged from 0-7 % (v/v) of ethanol with R^2 > 0.97 was obtained. The recovery of 94-120% and the relative standard deviation of less than 3% were achieved. For determination of iodine, the treatment of spiked egg sample with 0.4 g/mL TCA was heated at 50 ^{o}C for 15 min. The linear calibration ranged from 0-100 ppm of iodine with R^2 > 0.98 was obtained. The recovery of 93% and the relative standard deviation of less than 2% were achieved.

Field of Study:	Chemistry	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....

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Kamonwan Meesuwan

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LIST OF ABBREVIATIONS

AU	arbitrary unit
%	percentage
^{o}C	degree Celsius
LOD	limit of detection
M	molar
g	gram
mg	milligram
µg	microgram
mg/L	milligram per liter
g/mL	gram pre milliliter
R^2	correlation coefficient
RSD	relative standard deviation

CHAPTER I

INTRODUCTION

Microfluidic Paper-based analytical device (μPAD or PAD) is an alternative analytical technique widely used for qualitative or semi-quantitative analysis. It is simple to use as portable point-of care testing (POCT) and onsite analysis. It is easy to fabricate, and environmental friendly. Most of paper-based devices are fabricated by using hydrophilic filter paper [1-7]. The channel flow path of liquid sample or reagent was designed by creating a hydrophobic barrier on the filter paper. Several methods have been used for this purpose such as wax printing technology [1, 6, 8].There are several detection methods compatible with paper-based devices [7, 9-12]. A colorimetric method has been wildly used in paper-based assays because it is easily observed by naked eyes, and the reagents are cheap and available [1, 4, 8, 13-16]. The example of colorimetric reagents are such as 2, 4-dinitrophenyl hydrazine for detection of aldehyde group[17], enzyme alkaline phosphatase for detection of p-nitrophenyl phosphate (PNPP) [15, 18], ethylenediaminetetraacetic acid (EDTA) for detection of metal ion such as calcium and magnesium[15], and Cu^{+} complex for detection of nitrogen oxide[19]. The color intensity is directly proportional to the concentration of product compound. Typically, the color image can be scanned by a scanner or taken by either smartphone camera or digital camera and then processed by color analysis software; i.e., ImageJ.

The paper-based device has been used in various applications. For environmental monitoring, Jayewardene, B.M. et al. developed a paper-based device for determination of nitrate and nitrite based on Griess reaction, whereas nitrite reacted with Zn or Cd microparticles on the paper-based device to from red-violet color. However, nitrate cannot be measured directly by the Griess method [8]. It must be reduced to nitrite before analysis. Phansi, P et al. developed the membraneless gas-separation microfluidic paper-based analytical devices (MBL-GS μPADs) for direct determination of ethanol, sulfide, and ammonium in wastewater and fertilizer samples [4]. The analytes were measured using the colorimetric method. Ethanol was determined based on alcohol oxidation reaction using dichromate reagent. The color changed from

orange to green color. Sulfide was determined using N, N-dimethyl-p-phenylenediamine (DMPD), where the color changed from pink to blue color. Ammonium was determined using tetraiodomercurate (II), where the color changed from dark yellow to light yellow. For medical diagnosis, Martinez, A.W et al. developed the paper-based devices for glucose and protein assay. Glucose was determined based on enzymatic oxidation of iodide to iodine, where the color changed from colorless to brown color. Protein assay was determined based on colorimetric method using tetrabromophenol (TBNB) as a reagent where the color changed from yellow to blue [3]. For food analysis, Trifichuk, E. et al. developed a paper-based device for determination of Norfloxacin residues in food samples using iron III nitrate producing a noticeable yellow-orange color coffee ring formation [20].

In determination of volatile compounds, typically volatile compounds are extracted or sampled using headspace sampling/extraction or via solid phase microextraction (SPME) and directly determined by gas chromatographic method [21-23]. These approaches are clean from unwanted non-volatile compounds and highly sensitive and suitable for trace analysis of volatile compounds. Nonetheless, for samples containing a high concentration of volatile compounds such as alcohol in beverages or drugs, these approaches might not be necessary. For determination of alcohol content in samples, there are several methods have been reported such as gas chromatographic flame ionization method (GC-FID) [24], solid phase microextraction (SPME) coupling with gas chromatographic mass spectrometry (HS-SPME–GC-MS) method [25], Terahertz-time domain spectrometer (THz-TDS) method [26] and paper-based analytical devices based on colorimetric method [4]. For determination of analytes in complex matrices such as food or biological samples, if the analyte can be converted to volatile form, the headspace extraction/sampling may be preferred approach because it would be selective to specific compound and clean from complex matrices. For determination of iodine in food samples, iodine can be isolated/extracted or sample matrix must be treated before analysis. Several methods have been used such as inductively-coupled plasma/mass spectrometry (ICP-MS) method [27], spot–kits

versus titration method [5], solid phase colorimetric, iodometric titration method [28], optical methods using various polymer materials containing polyvinyl pyrrolidone as solid phase extractant [29], spectrophotometric kinetic method [30], and paper-based analytical devices based on colorimetric method [1, 4, 8, 13-16].

Simple paper-based devices have been developed as alternative choices for quick easy and cheap analytical method of such applications. Li, D. et al. developed a paper-based device coupled with headspace sampling method for determination of sulfur dioxide in wine samples [15]. The device was fabricated using cellulose based filter paper immobilized by 4-mercaptopyridine (Mpy)-modified gold nanorods (GNRs)-reduced graphene oxide (rGO) hybrids (rGO/MPy-GNRs), anhydrous methanol, and starch-iodine complex, where the color changed from dark blue to light blue color. Saraji, M. et al. developed a paper-based device for headspace determination of cyanide in water samples [16]. The method was based on chloramine-T pyridine-barbituric acid reaction, where the color changed from red to blue color.

Inspired by the works above and in order to investigate this new method in other matrices, we developed a simple paper-based device for simultaneous extraction and colorimetric detection of volatile compounds in beers, drugs, and food samples. First, the developed device was applied for simultaneous headspace extraction and determination of alcohol content in beer and drug samples based on colorimetric reduction of dichromate reagent. Second, the device was applied for determination of iodine in egg samples using simultaneous headspace extraction of volatile iodine and colorimetric detection based on iodine-starch reaction. The egg samples are considered complex matrix samples, for which sample preparation steps or pretreatments usually are necessary. Therefore, the development of a simple method for direct determination of iodine in eggs.

CHAPTER II

THEORY

2.1 Paper-based analytical device

Since the first paper-based analytical devices (PADs) was introduced by Whitesides and colleagues in 2007, PADs have been developed for the analyses of biomolecules [3], metal ions [31], inorganic anions [4, 8], environmental monitoring [8], and point-of-care medical diagnostics [32]. There are several advantages of PADs including portable point-of care testing (POCT), onsite analysis, cost-effectiveness, easy fabrication and environment friendly. PADs consist of the hydrophilic paper and the hydrophobic barrier as the channel flow path of liquid sample or reagent.

Paper is a thin sheet material produced by mechanically and/or chemically processing from cellulose. The major component of paper is cellulose which made from wood, rags and grasses. The properties of paper such as thickness and pore size depend on the production process. The unique advantages include hydrophilic, lightweight, portability, inexpensive, re-material, biodegradation etc. There are many types of paper which used for PADs such as Whatman paper (filter paper) [33] and Nitrocellulose membrane [34] [35]. As shown in Table 1.1.

Table 2.1 Paper substrates, their characteristics, and applications

Paper substrate	Characteristics	Applications
Whatman paper No. 1	The paper was made from cotton cellulose. The pore size and thickness are 11 and 180 μm, respectively. It has medium retention and flow rate	Apilux A, et. al. have used Whatman paper No.1 for fabricating a device for the measuring of gold and iron [2]
Whatman paper No. 4	The paper was made from cotton cellulose. The pore size and	Li X, et. al. have used Whatman paper No.4 for fabricating

	thickness are 20-25 and 210 μm, respectively. Its properties such as larger pore size and higher retention rate were utilized in making paper-based devices	paper-based microfluidic sensors by inkjet printing [33].
Nitrocellulose membrane	Nitrocellulose making from cellulose in cotton linter and mixture of sulfuric acid and nitric acid based on nitration reaction. The pore size and thickness are 20-25 and 210 μm, respectively. It has very smooth and uniform pores.	Tung R, et al. have developed Chitosan modification of nitrocellulose membrane to enhance biomolecule immobilization for paper-based point of care testing [34].
Bioactive paper	The modification of paper matrix with biomolecules was used to produce the bioactive paper. And its advantage is it does not need complicated equipment for operation.	Bioactive paper is used in many analytical applications such as pesticides residues detection [36] and pathogenic bacterial detection [35].

The advantages of these paper as substates are applied to compatibility with the analyte. The most useful type of PADs application is Whatman paper No. 1. Due to its compatibility with the majority of fabrication methods, many works reported the use of Whatman paper No.1 for making a device for different type of analytes.

Several techniques to produce PADs channel (hydrophobic barriers) are photolithography, plotter, and ink jet etching, etc. However, the main disadvantage of these techniques is using the complicated instrument for production of the hydrophobic barriers that may cause the high cost. Hence, in this work, we used the permanent marker for hydrophobic barrier because it is easy, portable and low cost.

The most widely used detection methods of PADs is colorimetric detection because of the visual readout of qualitative or semi-quantitative by comparing the color intensity of a sample with those of standards. Colorimetric detection involves with the color changing of colorimetric reagent, when it reacts analyte solution (e.g., biomolecules and toxic ions). The commonly image of PADs were taken by cell phone cameras, or digital/CMOS cameras and are then transferred to a PC or cell phone for analysis purposes.

2.2 Headspace sampling/extraction (HS)

Headspace sampling/extraction (HS) has been the method of choice for determination of volatile compounds in solid and liquid samples, because it is simple and relatively clean from the matrix interference. This technique has been developed and integrated with gas chromatography for analysis of volatile analytes [37-39]. Generally, the static headspace sampling/extraction is the simplest method. As shown in Figure 2.1, the sample is placed in a tightly sealed vial and the volatile component diffuses from sample phase (liquid or solid) into gas (headspace) phase till it reaches equilibrium.

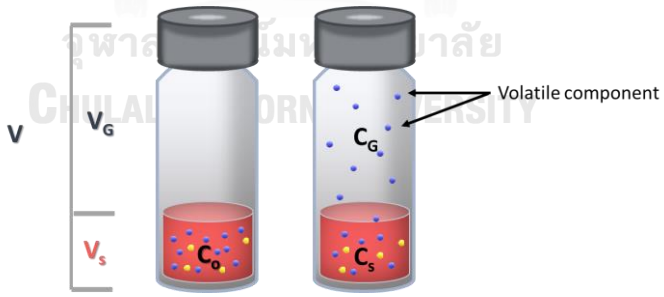


Figure 2.1 Schematic of static headspace sampling/extraction; on the left, the concentration of analyte (C_O) in the sample before equilibrium; on the right, the distribution of analytes between sample phase (C_S) and gas (headspace) phase (C_G)

There are two parameters affecting the sensitivity of headspace analysis, which are the distribution constant (K) and the phase ratio (β). The distribution ratio, K, is defined as the ratio of concentration of gas phase (C_G) and sample phase (C_S) at equilibrium [40].

$$K = \frac{C_S}{C_G} \qquad \text{Equation 2.1}$$

The analyte at high value of K favors the liquid phase while that at low value of K favors the headspace phase.

The phase ratio, β, is defined as the ratio of the volume of gas phase (V_G) to the volume of liquid phase (V_S.)

$$\beta = \frac{V_G}{V_S} \qquad \text{Equation 2.2}$$

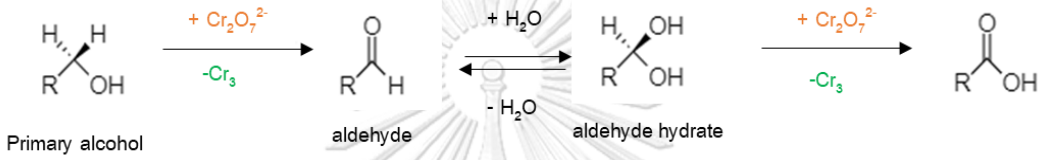
The concentration of analyte in headspace (C_G) is directly proportional to the concentration of analyte originally present in the sample (C_O) and can be expressed as a relationship between the distribution constant and phase ratio by the following equation

$$C_G = \frac{C_0}{(K+\beta)} \qquad \text{Equation 2.3}$$

From the equation 2.3, the sensitivity of headspace or the concentration of analyte in headspace (C_G) could be improved by decreasing the phase ratio (β) and partition coefficient (K). The phase ratio (β) will be reduced by increasing of the sample volume (V_S), so the headspace volume (V_G) will be decreased, in the same vial. In term of partition coefficient (K), it can be decreased by increased temperature and salting out effect; subsequently, more volatile component will diffuse into the headspace [41].

2.3 Colorimetric determination of alcohol

The common colorimetric method for determination of alcohol is the oxidation reaction of alcohol with dichromate in acidic solution. As shown in Equation 2.4, primary alcohol can be oxidized to form aldehyde and carboxylic acid whereas dichromate (Cr (VI)) is reduced to chromic acid (Cr (III)). The orange color of solution containing dichromate changes to green solution containing chromium (III) ion.



Equation 2.4

2.4 Colorimetric determination of iodine

The common colorimetric method for determination of iodine is iodine-starch reaction. Iodine that is present as iodide ion (I^-) in the sample is first oxidized to iodine (I_2), then combines with iodide ion to become triiodide ion (I_3^-), which can be bound with amylose chains in starch solution giving a dark blue color as shown in Equation 2.5.

$$2I^- + 2H_2O_2 + 2H^+ \longrightarrow I_2 + H_2O$$

$$I^- + I_2 \longrightarrow I_3^-$$

$$I_3^- + \text{starch} \longrightarrow \text{Dark blue complex}$$

Equation 2.5

After the volatile component were extracted. The analytes will adsorb on paper-based analytical device that contain the colorimetric reagent to detect the volatile compound. As shown in Figure 2.2.

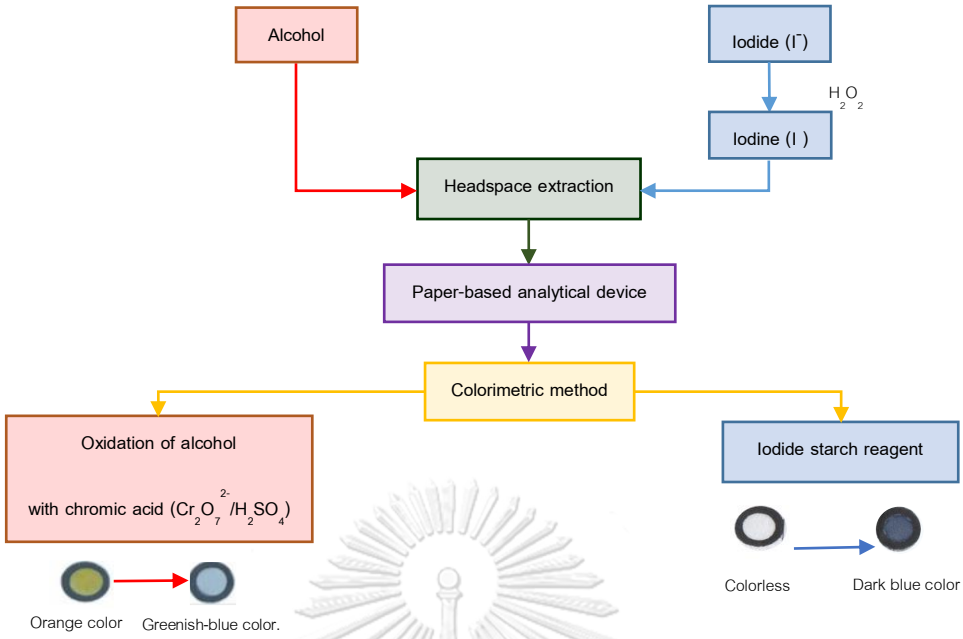


Figure 2.2 Schematic diagram of headspace extraction and colorimetric determination of volatile compounds on paper based analytical device

CHAPTER III

EXPERIMENTAL

3.1 Instrument and Equipment

Scanner (Epson L360, Japan)

Digital Camera (Fuji X-A3, Japan)

Filter paper (Whatman No. 1, MERCK, Germany)

25 mL headspace vial with Teflon lined cap

3.2 Chemicals and Reagents

3.2.1 Standard Iodine solutions

A 0.1 M stock standard iodide solution was prepared by appropriate dissolution of 0.415 g of potassium iodide (Carlo-Elba, France) with 25 mL Milli-Q water.

Working iodide standard solutions of 5, 10, 25, 50, 100 mg iodide/L were prepared by pipetting 4, 8, 20, 40, and 80 µL, respectively from 0.1 M stock standard iodide solution and diluting in 10 mL of Milli-Q water.

3.2.2 Starch solution

A 1% Starch solution was prepared by dissolving 1.0 g of potato starch (Mcgarrett, Thailand) in Milli-Q water and heating on the hotplate. After cooled down at room temperature the solution was diluted with Milli-Q water in 100 mL volumetric flask.

3.2.3 Oxidizing agents

The oxidizing agents including hydrogen peroxide, sulfuric acid, nitric acid, and perchloric acid purchased from MERCK, Germany were used to oxidize iodide solution to iodine gas, which subsequently reacted with starch solution on the paper-based device to from dark blue color.

A 2% hydrogen peroxide were prepared by appropriate dilution of 30% hydrogen peroxide (MERCK, Germany) in 25 mL of 2 mM hydrochloric acid (MERCK, Germany)

3.2.4 Standard alcohol solutions

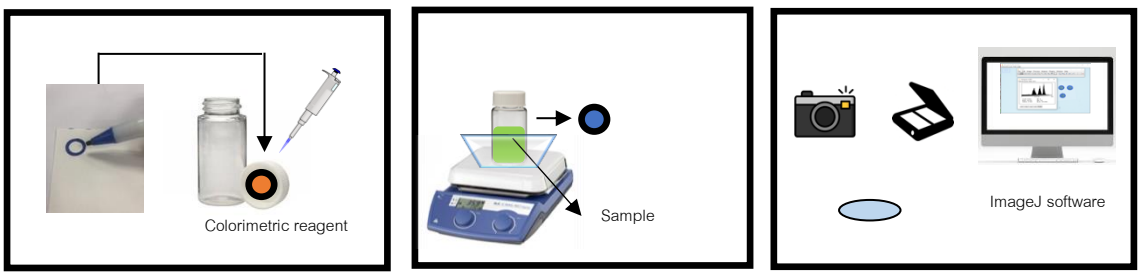
Standard ethanol solutions in the range of 0 to 9% (v/v) were freshly prepared by appropriate dilution of 99.5% (v/v) ethanol (MERCK, Germany) with Milli-Q water. The solutions were kept in tightly sealed containers to prevent loss of ethanol.

3.2.5 Potassium dichromate

A 0.4 M potassium dichromate was prepared by dissolving 2.9 g of potassium dichromate crystal (MERCK, Germany) in 25 mL of 4 M sulfuric acid.

3.3 Headspace paper-based device for colorimetric detection

A piece of filter paper (Whatman filter paper No. 1) was circled by a permanent marker (12 mm i.d.) making a hydrophobic barrier to contain a drop of 10 µL colorimetric reagents; i.e., starch for determination of iodine and potassium dichromate in sulfuric acid for determination of alcohol. The paper was attached to the liner of the vial cap making a headspace paper-based device. The scheme of the headspace paper-based device for colorimetric determination of volatile compounds is shown in Figure 3.1 The sample was heated. The analyte vaporized and reacted with the colorimetric reagents on the headspace paper-based device to either form a color (colorless to blue for determination of iodine) or change the color (orange to light blue for determination of alcohol). The images of the color papers were taken by a scanner or by a preset digital camera in a dark box where the light conditions had been controlled. Finally, the color images were analyzed by ImageJ software.



a) Making hydrophobic barrier

b) Headspace paper-based device for colorimetric detection

c) Color image processing

Figure 3.1 Schematic process of headspace paper-based device for colorimetric determination of volatile compounds

3.4 Color image processing

Firstly, the colored paper was taken off from the liner cap. The image was taken by scanner for determination of iodine and digital camera for determination of alcohol. After that, the color image was analyzed by ImageJ software in RGB mode. RGB has 3 primary colors i.e. red, green, and blue. The intensity values range from 0 (black) to 255 (white) for each of the RGB color. One color was chosen based on the change of color intensity that corresponding to the change of the concentration of the analyte. The intensity value was used for quantitative analysis. For determination of iodine, the color changes from colorless (R255, G255, B255) to dark Blue (R0, G0, B139), as shown in Figure. 3.2A. The difference of blue intensity was higher than other color intensity. As amount of iodine increased, the blue intensity value decreased, so the blue intensity value was chosen. A 10uL of starch reagent was dropped onto the headspace paper-based device and waited it was dry prior to use. The image of color paper was taken immediately by a scanner. For determination of alcohol, the color changes from orange (R255, G127, B0) to light blue (R173, G216, B240), as shown in Figure. 3.2B. The difference of blue intensity was higher than other color intensity. As amount of alcohol increased, the blue intensity value increased, so the blue intensity value was chosen. A 10uL of dichromate reagent was dropped onto the headspace paper-based device. The device was applied for determination of alcohol immediately. The image of color paper was taken immediately by a digital camera.

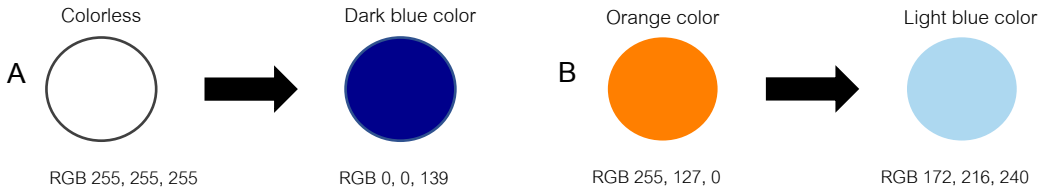


Figure 3.2 Comparison of intensity in RGB mode; a) orange color to light blue color, and b) colorless to dark blue color.

3.5 Headspace paper-based device for colorimetric determination of alcohol

The ethanol was extracted or isolated into headspace and reacted with colorimetric reagents on the paper via alcohol oxidation with potassium dichromate. The orange color turned to greenish-blue color.

3.5.1 The effect of time and temperature on the blue intensity

Since the concentration of volatile compound in headspace phase can be enhanced by increased temperature; subsequently, the color intensity on the paper-based lined cap would be increased, the sample for determination of ethanol was heated in water bath. The temperature and the time were studied.

A 10 mL of 3% ethanol standard solutions (%v/v) placed in the 25 mL headspace vial sealed with the paper-based lined cap loaded with 0.4 M dichromate reagent. The concentration of dichromate reagent adopted from previous paper [4]. The vial was heated in water bath at various temperatures and time. The images of colored paper were taken by the digital camera. The blue intensity was measured and compared.

3.5. Method Evaluation

The calibration curve was plotted between blue intensity and various concentration of ethanol standard solutions (0-5% v/v) at the optimized temperature and time (according to section 3.5.1). The linear regression method was used to obtain slope, intercept and R^2 and LOD. The recovery and the relative standard deviation (n=3) were evaluated by spiking beverage and drug samples with 0.5 (%v/v) of the ethanol standard.

3.5.3 Samples for determination of alcohol

The headspace paper-based device was applied for determination of alcohol content in alcohol contained beverages and drugs such as beer purchased from a local supermarket and gastrointestinal drugs purchased from local drug store. All samples were stored in a refrigerator prior to use.

3.6 Headspace paper-based device for colorimetric determination of iodine

The headspace paper-based device has been developed for determination of analyte in complex matrices such as food samples by converting the analyte into volatile species, headspace extraction and direct detection onto the paper-based device based on colorimetric method. The headspace paper-based device was applied for determination of iodine in egg samples by oxidizing iodine that is present as iodide ion (I^-) in the sample to iodine (I_2) species, which is more volatile for headspace extraction and colorimetric detection based on iodine-starch reaction.

3.6.1 Headspace paper-based device for colorimetric determination of iodine of in water sample

The headspace paper-based device for colorimetric determination of iodine based on iodine-starch reaction was preliminarily studied using iodide solution.

3.6.1.1 Oxidizing agent

Oxidizing agent plays important role in oxidizing activity and the efficiency in converting iodide (I^-) species into more volatile iodine (I_2) species, and subsequently, affect the intensity of the dark blue color of iodine-starch reaction. Type of oxidizing agents such as 2% hydrogen peroxide in 2 mM hydrochloric acid, nitric acid, sulfuric acid and perchloric acid were studied. In addition, the amount of oxidizing agent should be enough to oxidize iodide to iodine, therefore, concentrations of oxidizing agent were also studied. A 1 mL of oxidizing agent was added to 10 mL of 100 ppm iodide solutions placed in the 25 mL headspace vial and sealed with the headspace paper-based lined cap loaded with starch solution. The vial was heated at 40 ^{o} C in water bath for 5 min. The blue intensity was measured and compared.

3.6.2 Headspace paper-based device for colorimetric determination of iodine in egg samples

The headspace paper-based device was applied for colorimetric determination of iodine in egg samples using conditions from previous study. A 10 g of homogenized egg spiked with 100 ppm iodide was used in this study.

3.6.2.1 Effect of egg matrix on oxidation efficiency

The egg matrix could have affected the oxidation efficiency converting iodide (I^-) to iodine (I_2). Types of oxidizing agents and concentrations of oxidizing agent i.e., 2% hydrogen peroxide in 2 mM hydrochloric acid, nitric acid, sulfuric acid and perchloric acid were restudied using egg samples. A 1 mL of oxidizing agent was added to 10 g of homogenized egg samples spiked with 100 ppm iodide placed in the 25 mL headspace vial and sealed with the headspace paper-based lined cap loaded with starch solution. The vial was heated at 40 $^{\circ}$ C in water bath for 5 min. The blue intensity was measured and compared.

3.6.2.2 Removal of egg matrix by protein precipitation method

Since the egg matrix has significantly affected the analytical process that could interfere the oxidation and volatilization of iodine (I_2), the egg sample must be treated to remove complicated components such as proteins prior to analytical process. Protein precipitation method is considered. Trichloroacetic acid (TCA) is a common reagent for protein precipitation that had been used in analytical methods for food samples [42]. The effect of TCA was studied. According to previous studies that have been using TCA for treating samples as protein precipitation method, a 10 mL of 1 g/mL TCA was added to 10 g of homogenized egg samples spiked with 100 ppm iodide. Precipitate was removed by vacuum filtration with Whatman filter paper No1. The filtrate was placed in the 25 mL headspace vial. A 1 mL of various concentrations of hydrogen peroxide was added. The vial was sealed with the headspace paper-based lined cap loaded with starch solution and heated at 40 $^{\circ}$ C in water bath for 5 min. The blue intensity was measured and compared.

3.6.2.3 Salting out effect

Salting out is one approach that can enhance volatilization of less polar volatile compounds by increasing the ionic strength or polarity of the solution resulting in reducing the solubility of the less polar volatile compounds and driving them to the headspace. A 10 g of homogenized egg samples spiked with 100 ppm iodide was treated with 10 mL of 1 g/mL TCA. A 1 g of magnesium sulfate was added to the filtrate placed in the 25 mL headspace vial after removal of precipitate by vacuum filtration with Whatman filter paper No1. A 1 mL of 5% hydrogen peroxide was added to the sample. The vial was sealed with the headspace paper-based lined cap loaded with starch solution and heated at 40 ^{o} C in water bath for 5 min. The blue intensity was measured and compared.

3.6.2.4 Effect of TCA on the blue intensity of iodine-starch reaction

The effect of TCA on the blue intensity of iodine-starch reaction on the paper-based device was investigated. The blue intensities of iodine-starch reaction were obtained and compared between a) 10 mL of 100 ppm iodide solution; b) 5 mL of 200 ppm iodide solution mixed with 5 mL of 1g/mL TCA; c) 5 mL of 200 ppm iodide solution mixed with 5 mL of 1g/mL TCA and 1 g magnesium sulfate; d) 10 g egg sample treated with 10 mL 1g/mL TCA and e) 10 g egg sample treated with 10 mL 1g/mL TCA and 1 g magnesium sulfate. Treated egg samples were filtered by vacuum filtration with Whatman filter paper No. 1. A 1 mL of 5% hydrogen peroxide was added to each sample, placed in the 25 mL headspace vial sealed with the headspace paper-based lined cap loaded with starch solution. The vial was heated at 40 ^{o} C in water bath for 5 min.

The concentration of TCA was also investigated. TCA concentrations were varied from 0.01-1 g/mL. A 10 mL of TCA was added to 10 g of homogenized egg sample spiked with 100 ppm iodide. Precipitate was removed by vacuum filtration with Whatman filter paper No1. The filtrate was placed in the 25 mL headspace vial. A 1 mL

of 5% hydrogen peroxide and 1 g of magnesium sulfate was added to each sample. The vial was sealed with the headspace paper-based lined cap loaded with starch solution and heated at 40 $^{\circ}$ C in water bath for 5 min. The blue intensity was measured and compared.

3.6.2.5 Optimization of TCA and egg sample

Since the amount of egg sample would correspond to the amount of iodine determined and subsequently, the sensitivity of the method, the amount of TCA should be appropriately optimized not only to effectively remove the egg matrix but also not affect the blue intensity on the paper-based device. Therefore, the concentration of TCA and the amount of egg sample were optimized. The optimum condition would be the highest amount of egg treated with the minimum concentration of TCA that yield the most effective protein precipitation with clear in appearance. Furthermore, the volume of filtrate should provide low headspace volume to achieve the most concentrated volatile iodine (I_2).

3.6.2.6 Effect of time and temperature on the blue intensity.

The effect of time and temperature on the blue intensity on the paper-based device were studied using iodide solution. A 5 mL of 200 ppm iodide was mixed with 5 mL of 1g/L TCA. A 1 g of magnesium sulfate and 1 mL of 5% hydrogen peroxide was added to the solution placed in the vial sealed with the headspace paper-based lined cap loaded with starch solution. The extraction time profiles from 1-30 min at various heating temperatures from room temperature 25 – 60 $^{\circ}$C were established.

3.6.2.7 Method Evaluation

The calibration curve was established between blue intensity and various concentration of iodide 0-100 mg iodide/L in egg samples. Egg samples were treated with TCA at the optimum condition obtained form 3.6.2.5 and 3.6.2.6. The linear regression method was used to obtain slope, intercept and R^2. The recovery and the relative standard deviation (n=3) were evaluated by spiking egg samples with 100 mg of iodide/L.

CHAPTER IV

RESULT AND DISCUSSION

4.1 Development of headspace paper-based device for determination of alcohol in beverages and drugs

4.1.1 The effect of time and temperature on the blue intensity

The ethanol standard solution was determined by headspace paper-based device based on colorimetric reaction. The extraction time were studied to provide the best condition for analysis of ethanol. In this study, a 10 mL of 3% of ethanol standard solution was placed in vial. Then, the vial was sealed the paper based lined cap loaded with the 0.4 M of dichromate reagent [4]. The vial was heated at various time (1, 3, 5, 7, and 10 min) and various temperature (25, 40, 50 ,60 and 70). The results are shown in Figure 4.1. As we expected, the blue intensity value was increased by increasing both of temperature and time till got a peak at 5 min, and then it remained stable until 10 min at temperatures 50, 60, and 70 ^{O}C. At high temperature, despite the more volatile compound being extracted, the colors on device were affected by the moisture from the samples that may cause alteration in color reading. Figure 4.2 demonstrated the inconsistent blue color on the paper devices obtained at high operating temperature of 50 ^{O}C. The same effect was observed at the operating temperatures of 60 and 70 ^{O}C resulting in high %RSD. At low temperature; i.e., 25 ^{O}C, the blue color on the paper device was relatively less intense and took long time for the color being developed. In addition, the extraction time profiles of 40 and 50 ^{O}C showed that the relatively intense blue color on the paper devices were observed at 7 min. However, to avoid the moisture effect and the extraction temperature of 40 ^{O}C at 5 min was chosen for further study.

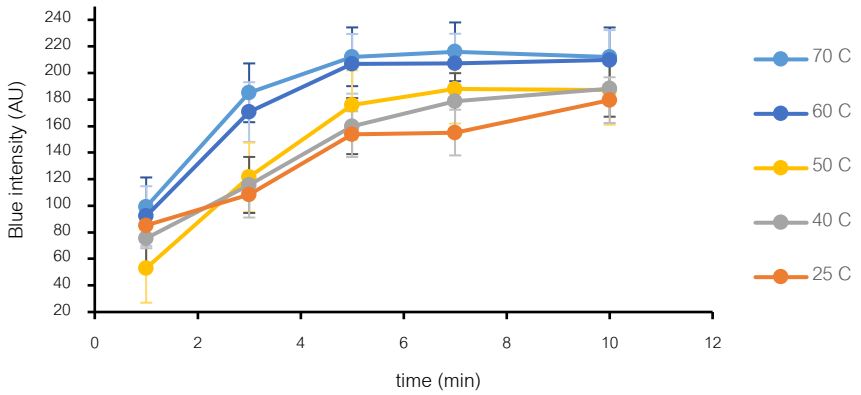


Figure 4.1 The blue color of 3% of ethanol standard solution using paper-based device for colorimetric determination

Figure 4.2 The inconsistent blue color observed on triplicate paper devices at operating temperature of 50° C, 7 min

4.1.2 Method evaluation

Each of standard ethanol solution of 0 to 7 (%v/v) was placed in vial sealed with the headspace paper-based lined cap loaded with dichromate reagent extracted at 40 °C for 5 min. The calibration curve was established as shown in Figure 4.2. The linear relationship was achieved with coefficient of determination (R^2) greater than 0.96.

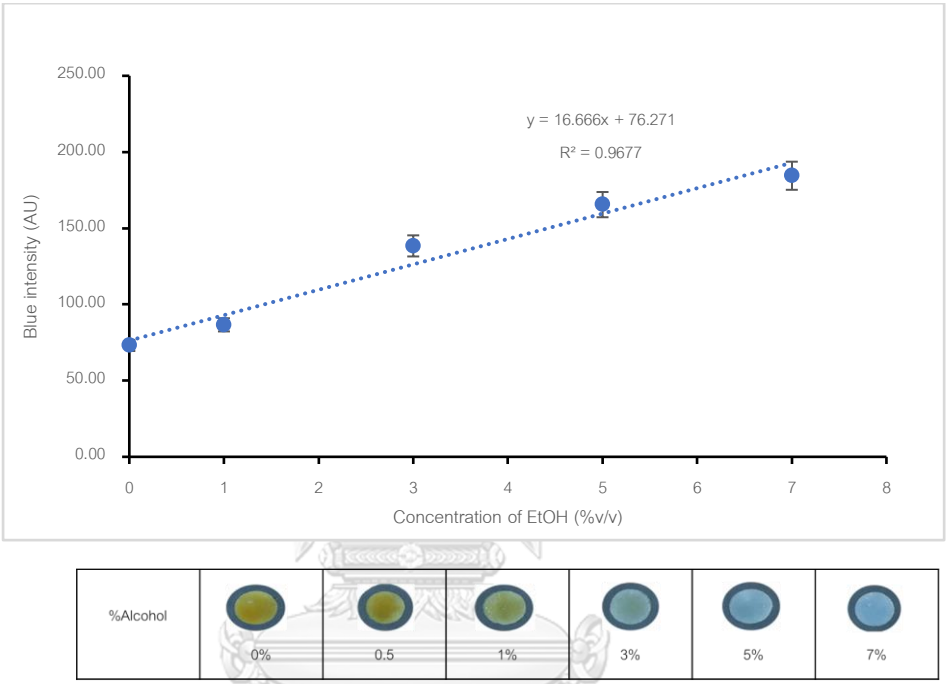


Figure 4.3 Calibration curve between blue intensity and concentrations of ethanol using headspace paper-based device for colorimetric determination

The method was tested and evaluated for their accuracy and precision by applying to the real samples and spiked samples. The method was applied for determination of alcohol content in stomachic mixture, salol et menthol mixture and beer sample. The stomachic mixture is red-brown colored liquid while the salol et menthol mixture is white suspension. Beer sample is yellow color and it also contains beer foam. The method was directly applied to the samples without any pretreatment. The results were summarized in Table 4.1. In general, the method performances (% RSD and recovery) depend on types of samples. The alcohol content in stomachic mixture was labeled as 6.65%. The sample was diluted with MilliQ water (1:1) prior to use. Our method showed the alcohol content of 3.4% with the relative error of 2.3% in stomachic mixture. The recovery of sample spiked with 0.5% ethanol was 94% with %RSD below than 2%.

The alcohol content in salol et menthol mixture analyzed by using our method was 1.2% compared to its label of 1% resulting in the relative error of 20%. The recovery of 0.5% ethanol spiked into the salol et menthol sample was as high as 120% with %RSD less than 3% while the recoveries of ethanol from the other samples were acceptable (94-96%). One reason might be attributed to that the salol et menthol mixture contains phenyl salicylate and menthol as labeled. These compounds have hydroxyl group, which can also react with dichromate reagent increasing the intensity of blue color. So, the salol et menthol gave the higher recovery than the other samples.

The alcohol content in beer sample was labeled as 5.0%. The samples were diluted with MilliQ water (1:1) prior to use. This method showed the alcohol content of 2.4% with the relative error of 4% in beer sample. The recovery of sample spiked with 0.5% ethanol was 96% with %RSD below 2%.

Table 4.1 Determination of ethanol in drug and beverage samples by using headspace paper-based device

Sample	Spike EtOH (%)	% EtOH	%RSD	%Relative error	% Recovery
Stomachic mixture (6.65%)		3.4*	1.8	2.3	
Spiked Stomachic mixture	0.5	3.8*	0.5		94
Salol et Menthol Mixture (1%)		ND			
Spiked Salol et Menthol Mixture	0.5	1.8	0.6		120
Beer (A) (5%)		2.4*	2.0	6	
Spiked beer (A)	0.5	2.9*	1.4		96

*Sample dilutes with Milliq water (1:1)

4.2 Development of headspace paper-based device for determination of iodine in water sample

4.2.1 Investigation of method for iodide determination in water

4.2.1.1 Type of oxidizing agent

The iodine was determined based on iodine-starch reaction. The oxidizing agent plays important role in converting other forms of iodine such as iodide (I^-) to iodine (I_2) to achieve the blue intensity on the paper device for quantitative analysis. So, type of oxidizing agent was investigated. The blue intensity was decreasing when the blue color was getting darker. Figure 4.3 showed that the blue intensity obtained from hydrogen peroxide gave lower intensity compared with the other oxidizing agents, probably because the hydrogen peroxide has the highest E^0 reduction value. As the result, the hydrogen peroxide was chosen.

The concentration of hydrogen peroxide was investigated. The results are shown in Figure 4.3. The concentration of hydrogen peroxide has a direct effect on blue intensity value. The blue intensity value decreased as the concentration of hydrogen peroxide was increased up to 5%. The blue intensity was increased at the concentration of hydrogen peroxide was 10% and 15%. Because vaporized hydrogen peroxide can hydrolyze the starch solution on the headspace paper-based device that attached on the lined cap. This may cause an increase in the blue intensity at 10 and 15% of hydrogen peroxide concentration. As the result, 5% of hydrogen peroxide was chosen for determination of iodine in water sample

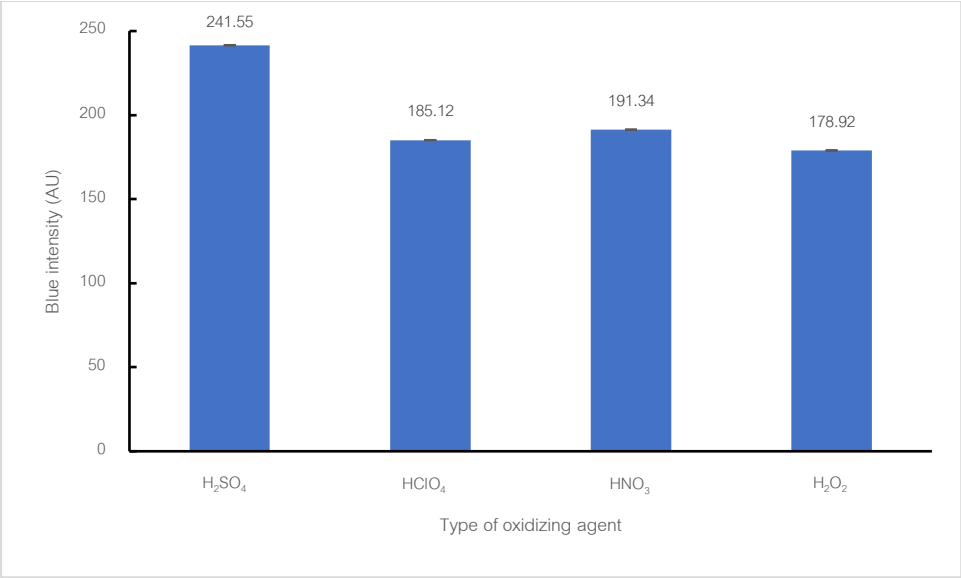


Figure 4.4 The blue intensities obtained from iodide solution by various type of oxidizing agents

4.2.2 Development of headspace paper-based device for colorimetric determination of iodine in egg samples

Types of oxidizing agents and concentrations of oxidizing agent i.e., 2% hydrogen peroxide in 2 mM hydrochloric acid, nitric acid, sulfuric acid and perchloric acid were restudied using spiked egg samples. The blue color on the paper device was not observed from any egg sample. However, the matrix of egg sample may affect the oxidizing activity and the efficiency in converting iodide to iodine, and subsequently, the intensity of the dark blue color.

4.2.2.1 Effect of egg matrix on oxidation efficiency

Oxidizing agent could be converted the iodide (I^-) in egg sample to iodine (I_2) to achieve the blue intensity. Concentrations of hydrogen peroxide i.e., 5,10, and 15% was studied using spiked egg samples for increasing oxidation efficiency. The blue color on the paper device was not observed from any egg sample. As the result, the egg matrix could have affected the oxidation efficiency converting iodide (I^-) to iodine (I_2).

4.2.2.2 Removal of egg matrix by protein precipitation method

The egg matrix has an enormous impact on the analytical process that could interfere the oxidation and volatilization of iodine (I_2). The egg sample was treated by using trichloroacetic acid (TCA) for protein precipitation prior to analytical process. A 1 g/mL TCA was added to spiked egg samples. The precipitate was filtered. The filtrate was collected and placed in 25 mL vial and proceeded through oxidation and detection process. The results showed that the blue color was not obtained on the paper device. TCA definitely affected the analytical process. Possible explanations might be attributed to the high concentration of TCA that might have 1) inhibited the oxidation process of hydrogen peroxide; 2) inhibited the volatilization of iodine (I_2) so that I_2 could not react with the starch on the paper; so that the blue color was not developed. These hypotheses were tested. To enhance the volatilization of I_2, salting out effect was studied. The effect of TCA on oxidation process was investigated using iodide solution.

4.2.2.3 Salting out effect.

Salting out has an effect based on the electrolyte–non-electrolyte interaction. Addition of salt could enhance the vaporization of iodine (I_2). Despite the egg sample was treated with TCA to remove proteins, the solution might still contain other dissolved compounds that interact with iodine (I_2). After the spiked egg sample was treated with 1 g/mL of TCA and precipitate was removed, 1 g of magnesium sulfate was added to the filtrate, placed in the 25 mL headspace vial. The results are shown in Figure 4.4. The blue color was observed more intensely on the headspace paper-based device. Therefore, addition of salt could help driving iodine (I_2) from the sample phase to the headspace phase; consequently, the blue color was developed. Furthermore, the increased ionic strength might affect the increase in dissociation of TCA resulting in less volatile TCA and the effect of TCA to the starch on the paper would be subsided.

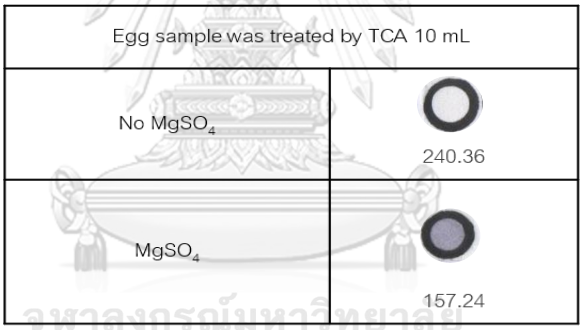


Figure 4.5 The blue colors on the headspace paper-based device observed with addition of magnesium sulfate to the TCA treated egg samples

4.2.2.4 Effect of TCA on the blue intensity of iodine-starch reaction.

The effect of TCA on oxidation process and volatilization of generated iodine (I_2) were investigated using iodide solution. The blue intensities on the headspace paper-based devices were obtained and compared to those using spiked egg samples. The results are shown in Figure 4.5. The blue intensities obtained from iodide solutions (Figure 4.5 a-c) were not much different between with addition of TCA (Figure 4.5 b), without addition of TCA (Figure 4.5 a) and with addition of TCA and magnesium sulfate (Figure 4.5 c). It means that TCA has no effect on oxidation process in generating iodine (I_2). Addition of salt to iodide solution had insignificant effect to volatile iodine (I_2) in aqueous matrix. The blue intensities obtained from spiked egg samples (Figure 4.5 d) were higher (less dark) than those obtained from iodide solutions. Apparently, the egg matrix, even though it had been treated and removed with TCA, would still have affected the blue color. However, the blue intensity obtained from the spiked egg sample treated with TCA was improved (darker) when magnesium sulfate was added (Figure 4.5 e). It means that treated egg matrix has significant effect to volatile iodine (I_2), which can be improved by addition of salt.

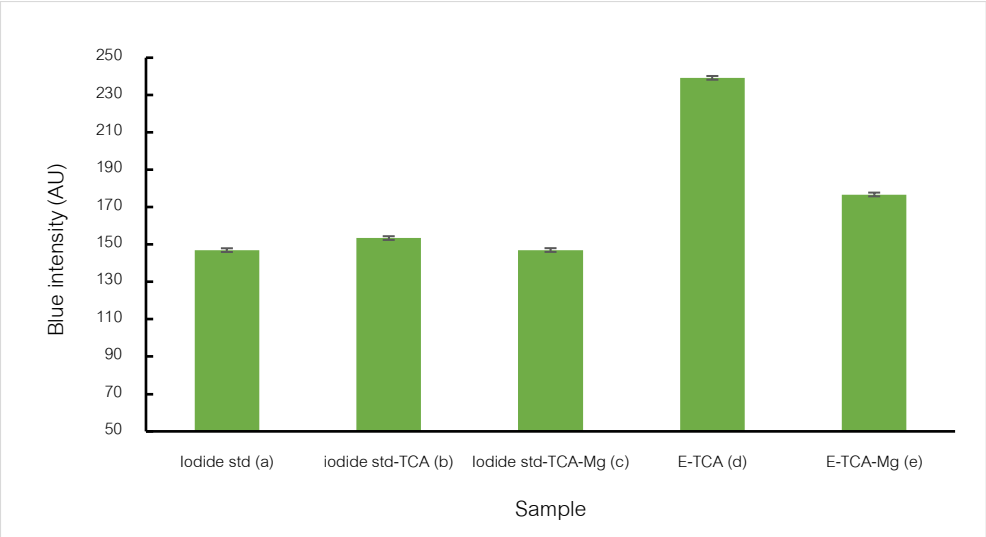
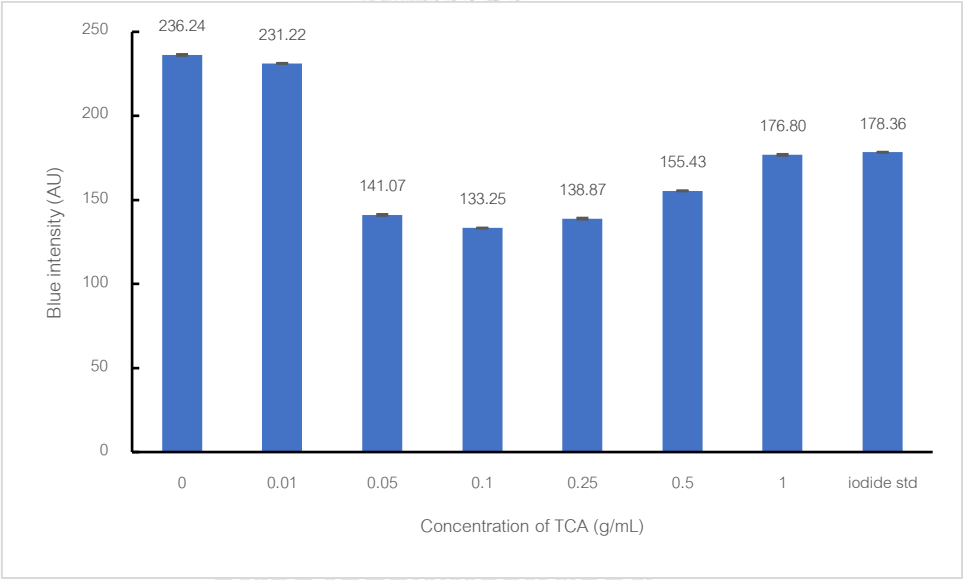


Figure 4.6 The blue intensities obtained from a) iodide solution; b) iodide solution mixed with TCA; c) iodide solution mixed with TCA and magnesium sulfate; d) spiked egg sample treated with TCA and e) spiked egg sample treated with TCA and magnesium sulfate.

The concentration of TCA was also investigated at different concentrations; 0, 0.01, 0.05, 0.10, 0.25, 0.50, and 1 g/mL. The results are shown in Figure 4.6. The higher concentrations of TCA were added, the more aggregation or protein precipitation were obtained and the less blue intensities (darker blue color) were obtained. Nonetheless, TCA has a significant effect on volatilization of iodine (I_2) resulting in increased blue intensities (less intense blue color) at high concentration of TCA. According to the results, the optimized concentration of TCA to treat 10 g of egg sample was 0.1 g/mL TCA.



Concentration of TCA g/mL	0	0.01	0.05	0.1	0.25	0.5	1
	236.24	231.22	141.07	138.25	138.87	155.43	176.80

Figure 4.7 The blue intensities and blue colors observed from treated egg sample by various concentrations of TCA (g/mL)

4.2.5 Optimization of TCA and egg sample

Since the amount of egg sample corresponds to the amount of iodine determined and subsequently, the sensitivity of the method, the amount of TCA should be appropriately optimized not only to effectively remove the egg matrix but also not affect the blue intensity on the headspace paper-based device. Therefore, the concentration of TCA and the amount of egg sample were optimized. The various amounts of spiked egg samples; i.e.,10, 20, 30, 40 g were treated by various concentration of TCA i.e.,0.1, 0.2, 0.3 and 0.4 g/mL. The effectiveness of TCA in removal of the egg matrices could be considered by their physical observations which were summarized in Table 6.5 in Appendix. The concentration of TCA should effectively remove egg matrix, where clear filtrate should be observed. At the fix volume of TCA (10 mL), the more amount of egg requires more concentration of TCA. Too high concentration of TCA would affect the blue color on the paper device.

The optimum condition would be the highest amount of egg treated with the minimum concentration of TCA that yield the most effective protein precipitation with clear in appearance. Furthermore, the volume of filtrate should provide low headspace volume to achieve the most concentrated volatile iodine (I_2). Figure 4.10 showed the volume of filtrate Therefore, 40 g of spiked egg sample treated with 0.2 g/mL of TCA was chosen.

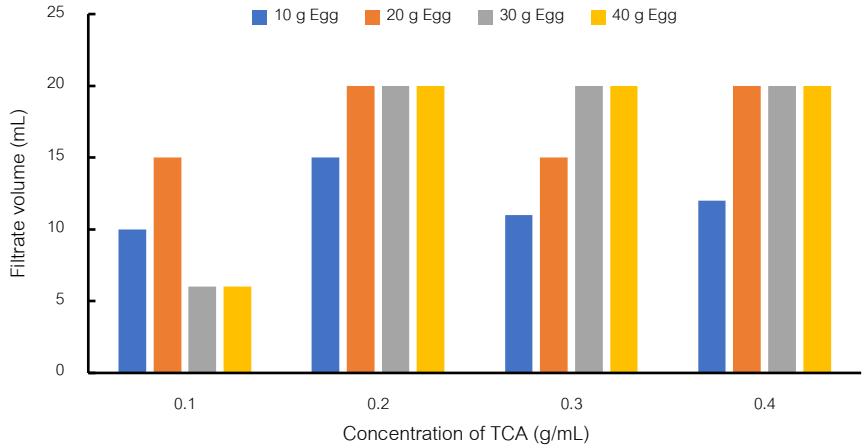


Figure 4.8 Comparison of filtrate volume after treating various amount of egg sample (g) with 10 mL various concentration of TCA (g/mL)

4.2.6 The effect of time and temperature on the blue intensity

The effect of time and temperature on the blue intensity on the paper-based device were studied using iodide solution. A 5 mL of 200 ppm iodide was mixed with 5 mL of 1g/mL TCA. A 1 g of magnesium sulfate and 1 mL of 5% hydrogen peroxide was added to the solution placed in the vial sealed with the headspace paper-based lined cap loaded with starch solution. The extraction time profiles from 1-30 min at various heating temperatures from room temperature 25 – 60 ^{o}C were established. The results are shown in Figure 4.9. As expected, the blue intensity value was decreased (darker) by increasing both temperature and time. The blue intensity was the lowest (darkest) for the temperature of 50 ^{o}C and 15 min. At high temperature, despite the more iodine (I_2) was driven into the headspace, the blue intensity on the paper device was increased (less dark), probably because of thermal decolorization phenomenon. For these reasons, the heating temperature of 50 ^{o}C for 15 min was chosen for further study.

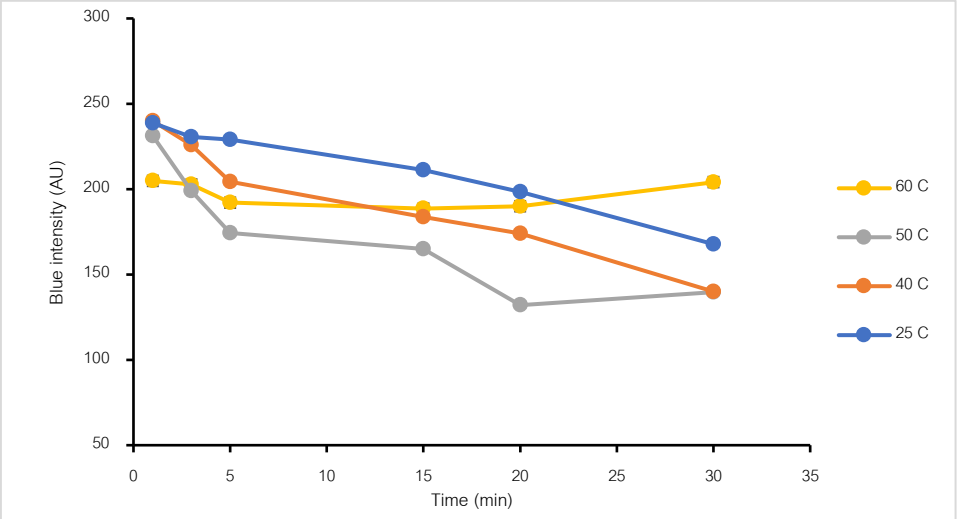


Figure 4.9 The blue intensity obtained from headspace paper-based device for colorimetric determination of iodine at varied temperature and time; 100 ppm iodide solution mixed with 1g/mL TCA and 1 g magnesium sulfate

4.2.7 Method Evaluation

The calibration curve was established using 40 g of homogenized egg samples labeled to contain 50 µg iodine/g spiked with various concentration of iodide ranging from 0, 25, 50, 75, and 100 mg/L. The egg samples were treated by 10 mL of 0.2 g/mL TCA. The precipitate was removed by vacuum filtration. A 1 g of magnesium sulfate and 1 mL of 5% hydrogen peroxide were added to the filtrates, placed in 25 mL vials sealed the paper based lined cap loaded with starch solution. The vial was heated at 50 ^{o} C in water bath for 15 min. The linear calibration curve for determination of iodine was obtained with coefficient of determination (R^2) greater than 0.98 shown in Figure 4.10. The recovery from spiked egg sample at 100 mg/L was 92.8% with relative standard deviation (RSD) of less than 2% (n=3). The limit of detection (LOD) calculated from linear regression method was 17.7 mg/L.

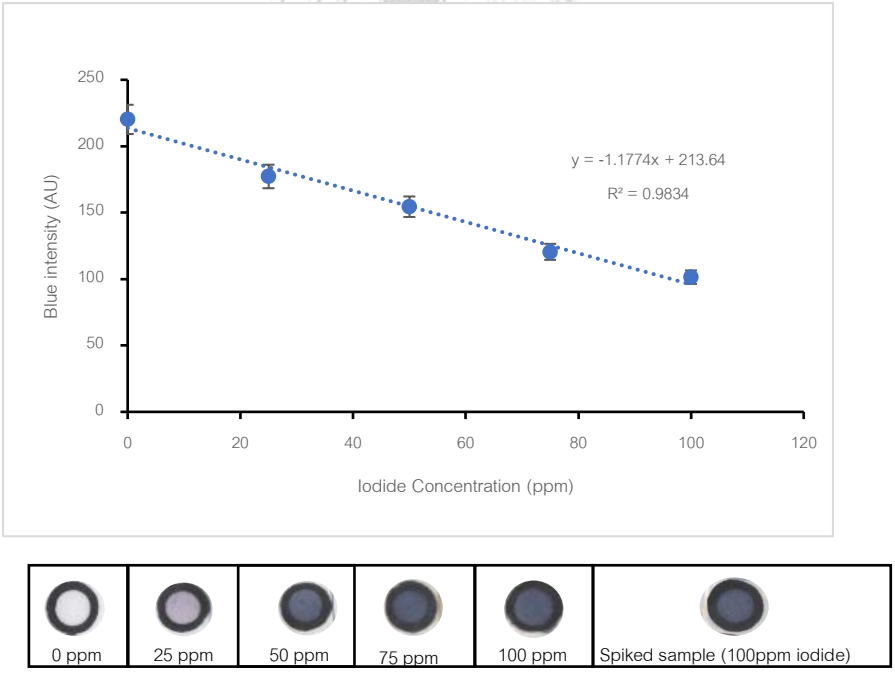


Figure 4.10 Calibration curve between blue intensity and concentrations of iodide in spiked egg samples using headspace paper-based device. (40 g of egg sample treated with 0.2 g/mL TCA)

CHAPTER V

CONCLUSION AND SUGGESTTION OF FUTURE WORK

5.1 Conclusion

The work of this thesis focused on the development of paper-based approach for colorimetric detection of volatile and semi-volatile compounds in headspaces of complex samples. The approach applied different colorimetric reagent of chromic acid or starch solution onto paper attached onto the liner part of a vial cap. This allows selective detection of ethanol and iodine, respectively, as observed by the blue and dark blue color of the detection zones. To this end, a sample can be placed in a vial closed with the paper modified cap. The sample was then heated, and the target analytes were extracted. The detection zone images were recorded by a digital camera with the color intensity further analysed using ImageJ software under RGB mode. The analysis performance depends on several experimental conditions including sample volume, sample concentration matrix sample, extraction time and extraction temperature. The optimized extraction conditions for ethanol and iodine were at 40 o C for 5 min and at 50 o C for 15 min, respectively. This is successfully demonstrated for selective analysis of ethanol in drug and beverage samples and iodine in egg samples. Under these conditions with well controlled photographic conditions and the light box setup, the developed approach offers simultaneous headspace extraction and colorimetric determination methods of volatile compounds, which is simple, relatively clean with less interferences and uses small sample volume. Despite this method is not capable for trace analysis, it is applicable for samples containing high concentration of analytes or for screening or semi-quantitative analysis purposes.

5.2 Suggestion of future work

The established approach is expected to be applicable for low cost small-scale monitoring of volatile and semi-volatile compounds in samples with complex matrices such as food drug and beverage sample in the future

APPENDIX

Table 6.1 The blue intensities of 3% of ethanol standard solution using paper-based device for colorimetric determination

Temperature (° C)	Time (min)	Blue intensity (AU) 1	2	3	Average	SD	RSD
Room Temperature	1	84.72	82.89	88.15	85.25	2.67	3.1
	3	107.29	109.51	108.24	108.35	1.11	1.0
	5	153.27	153.48	155.08	153.94	0.99	0.6
	7	157.16	154.38	153.9	155.15	1.76	1.1
	10	179.34	180.5	178.74	179.53	0.89	0.5
40	1	77.35	75.63	73.56	75.51	1.90	2.5
	3	114.62	116.52	115.85	115.66	0.96	0.8
	5	160	157.85	162.11	159.99	2.13	1.3
	7	179.35	177.43	179.41	178.73	1.13	0.6
	10	188.53	183.9	192.05	188.16	4.09	2.2
50	1	52.64	53.63	52.96	53.08	0.51	1.0
	3	122.27	120.86	121.28	121.47	0.72	0.6
	5	173.2	176.71	177.85	175.92	2.42	1.4
	7	188.77	221.99	187.88	199.55	19.44	9.8
	10	186.94	187.20	187.03	187.06	0.13	0.1
60	1 min	91.51	92.11	93.14	92.25	0.82	0.9
	3 min	172.16	223.19	169.57	188.31	30.24	16.1
	5min	185.27	208.99	189.16	194.47	12.72	6.6
	7min	205.66	207.88	207.60	207.05	1.21	0.6
	10 min	212.03	209.11	208.23	209.79	1.99	1.0
70	1 min	97.39	100.98	99.26	99.21	1.80	1.8
	3 min	184.36	224.01	184.67	197.68	22.80	11.5
	5min	210.75	215.1	210.68	212.18	2.53	1.2
	7min	218.41	214.45	214.86	215.91	2.18	1.0
	10 min	211.42	213.53	211.45	212.13	1.21	0.6

Table 6.2 The blue intensities observed from various concentration of alcohol solution (%)

Concentration of alcohol (%)	Blue intensity (AU)			Average	SD	%RSD
	1	2	3			
0	50.17	53.20	52.36	51.91	1.56	3.0
0.5	60.79	60.47	60.17	60.48	0.31	0.5
1	88.17	85.36	89.63	87.72	2.17	2.5
3	155.36	154.88	155.91	155.38	0.52	0.3
5	180.35	178.92	181.83	180.37	1.46	0.8
7	187.10	189.01	191.56	189.22	2.24	1.2

Table 6.3 Determination of ethanol in drug and beverage samples by using headspace paper-based device

Sample	Spiked EtOH	%EtOH	Blue intensity (AU)			Average	SD	%RSD
			1	2	3			
Stomachic mixture		3.4	145.23	146.65	150.32	147.40	2.6	1.8
Spiked Stomachic mixture	0.5	3.8	160.2	160.94	159.18	160.11	0.9	0.6
Salol et Menthol Mixture		1.2	84.28	89.55	89.05	87.63	2.9	3.3
Spiked Salol et Menthol Mixture	0.5	1.8	104	105.12	104.12	104.41	0.6	0.6
Beer (A)		2.4	122.77	121.43	118.00	120.73	2.5	2.0
Spiked beer (A)	0.5	2.9	135.33	134.88	131.91	134.04	1.9	1.4

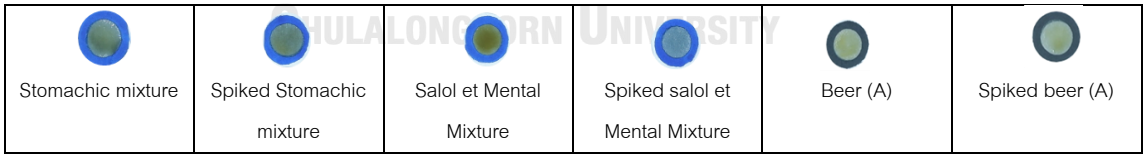


Figure 6.1 The blue colors observed from drug and beverage samples

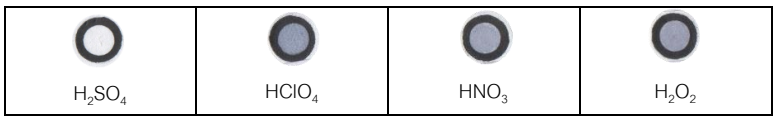


Figure 6.2 The blue intensities and blue colors observed from water sample by various type of oxidizing agent

Sample 10 mL	H_2O 1mL	1 % H_2O_2 1 mL	2 % H_2O_2 1 mL	5 % H_2O_2 1 mL	10 % H_2O_2 1 mL	15 % H_2O_2 1 mL
H_2O (100 ppm I^-)	 239.39	 176.76	 179.79	 178.36	 195.80	 195.09

Figure 6.3 The blue intensities and blue colors observed from water sample by various concentrations of hydrogen peroxide (%)

H_2O 1mL	1 % H_2O_2 1 mL	2 % H_2O_2 1 mL	5 % H_2O_2 1 mL	10 % H_2O_2 1 mL	15 % H_2O_2 1 mL
 240.29	 238.85	 239.69	 239.14	 239.75	 240.00

Figure 6.4 The blue intensities and blue colors observed from egg sample by various concentrations of hydrogen peroxide (%)

H_2O 1mL	1 % H_2O_2 1 mL	2 % H_2O_2 1 mL	5 % H_2O_2 1 mL	10 % H_2O_2 1 mL	15 % H_2O_2 1 mL
 239.97	 238.80	 240.16	 239.34	 239.85	 240.11

Figure 6.5 The blue intensities and blue colors observed from treated egg sample by various concentrations of concentrations of hydrogen peroxide (%)

Sample	H_2O 1mL	1 % H_2O_2 1 mL	2 % H_2O_2 1 mL	5 % H_2O_2 1 mL	10 % H_2O_2 1 mL	15 % H_2O_2 1 mL
Treatment of egg sample mixed with TCA 1g/mL	 240.29	 238.85	 239.69	 239.14	 239.75	 240.00
Iodide standard solution mixed with TCA 1g/mL	 238.38	 201.77	 197.24	 193.10	 197.80	 183.56

Figure 6.6 Comparison of blue intensities and blue colors between treated egg sample with TCA and iodide solution mixed with TCA by various concentrations of hydrogen peroxide (%)

Table 6.4 The blue intensities of iodide solution mixed with 1g/mL TCA, 1 g of magnesium sulfate and 1 mL of 5% hydrogen peroxide using paper-based device for colorimetric determination

Temperature		Blue intensity (AU)					
(o C)	Time (min)	1	2	3	Average	SD	% RSD
Room temperature	1	238.79	239.11	237.44	238.45	0.89	0.4
	3	230.53	229.12	232.11	230.59	1.50	0.7
	5	228.98	227.33	226.99	227.77	1.06	0.5
	15	211.35	210.00	209.49	210.28	0.96	0.5
	20	198.37	199.32	198.03	198.57	0.67	0.3
	30	167.78	166.44	166.74	166.99	0.70	0.4
40	1	240.00	237.11	238.44	238.52	1.45	0.6
	3	225.92	223.86	226.12	225.30	1.25	0.6
	5	204.28	204.13	204.55	204.32	0.21	0.1
	15	183.84	183.56	184.08	183.83	0.26	0.1
	20	174.04	176.10	175.27	175.14	1.04	0.6
	30	140.02	139.88	142.47	140.79	1.46	1.0
50	1	231.18	230.45	229.89	230.51	0.65	0.3
	3	198.89	199.05	199.55	199.16	0.34	0.2
	5	174.45	173.12	174.87	174.15	0.91	0.5
	15	164.87	164.22	165.78	164.96	0.78	0.5
	20	132.04	131.04	134.67	132.58	1.88	1.4
	30	139.78	140.35	138.22	139.45	1.10	0.8
60	1	204.85	206.11	204.44	205.13	0.87	0.4
	3	202.74	202.00	204.07	202.94	1.05	0.5
	5	192.07	192.33	192.78	192.39	0.36	0.2
	15	188.61	189.46	188.67	188.91	0.47	0.3
	20	190.00	190.45	190.67	190.37	0.34	0.2
	30	204.14	206.57	203.33	204.68	1.69	0.8

Table 6.5 Physical properties of treated egg sample

TCA (g/mL) 10 mL	Egg (g)	TCA in egg (g/mL)	Supernatant (mL)	Precipitation		Filtrated sample		
				Cream	Suspensions	Clear	Slightly cloudy	Cloudy white
0.1	10	0.50	10	✓		✓		
	20	0.33	15	✓		✓		
	30	0.25	6	✓				✓
	40	0.20	6	✓				✓
0.2	10	0.67	20		✓	✓		
	20	0.50	15		✓	✓		
	30	0.40	20	✓		✓		
	40	0.33	25	✓		✓		
0.3	10	0.75	11		✓	✓		
	20	0.60	15		✓		✓	
	30	0.50	20	✓		✓		
	40	0.43	20	✓			✓	
0.4	10	0.80	12		✓		✓	
	20	0.67	20		✓		✓	
	30	0.57	20	✓		✓		
	40	0.50	20	✓		✓		

Table 6.6 Determination of iodine in treated egg samples by various iodide concentration (*mg iodide/L*) by using headspace paper-based device

Concentration. of iodide (mg iodide/L)	Blue intensity (AU)			Average	SD	%RSD
	1	2	3			
0	219.00	220.11	221.55	220.22	1.04	0.4
25	174.73	178.77	178.23	177.24	1.79	1.0
50	152.67	154.44	156.33	154.48	1.49	1.0
75	119.87	122.03	119.55	120.48	1.10	0.9
100	100.27	101.45	102.56	101.423	0.94	0.9
Spiked sample	104.11	105.37	103.56	104.35	0.76	0.7

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