SULPHITES ANALYZER IN FRUITS, VEGETABLE AND THEIR PRODUCTS TOWARDS EXPORT PROMOTION STRATEGY

BY

CHUTIKARN KHAMKHAJORN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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DEPARTMENT OF CHEMISTRY
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ENTITLED

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was approved as partial fulfillment of the requirements for
the degree of Master of Science (Chemistry)

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Thesis Title	SULPHITES ANALYZER IN FRUITS, VEGETABLE AND THEIR PRODUCTS TOWARDS EXPORT PROMOTION STRATEGY
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Degree	Master of Science (Chemistry)
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ABSTRACT

In this work, two systems of sulphites analysis were designed and invented based on 3 reactions; firstly, sulphite (SO_3^{2-}) is converted to volatile sulphur dioxide (SO_2) by acidification, then, Fe (III) is reduced to Fe (II) by SO_2, and finally, Fe(II) react with o-phenanthroline to form a red complex. All results of sulphite determination in this study were evaluated in mg L^{-1} as SO_2 following the general regulations of sulphite amounts.

The first system applied the headspace microextraction (HS-ME) incorporating to a lab on cotton swab platform with smartphone detection. A universal clamp sample holder was designed and fabricated for use with a microfluidic cotton swab-based analytical device (μCSAD) on a smartphone. The reactions were performed in 1.5 mL-vial, SO_2 gas was diffused into the cotton head of the swab that hung over the sample solution, then reacted with the colorimetric reagent in the cotton. The red complex was detected by the smartphone. The linear ranges were 0.32–2.40 (R^2 = 0.9997) and 2.4–24.00 mg L^{-1} (R^2 = 0.9936). The limit of detection (LOD) and limit of quantitation (LOQ) was 300 and 600 μg L^{-1}, respectively. Compared to the Ripper method, the developed method showed no significant differences in the amount of SO_2 in 13 bottles of wine samples. This μCSAD was simple, cost-effective, and eco-

friendly**.** Moreover, the designed universal clamp holder and the software application can be used with multiple brands and models of smartphones**.**

The second system, an automatic flow-based analysis via gas diffusion unit was invented, and the light emitting diode (LED 515 nm) and photodiode were used as a detection unit. The extraction time is an important role in controlling the rate of analysis by stopping the pump for 5 min to achieve an appropriate signal. This system presented a non-linear calibration graph, $y = -0.1354x^2 + 14.766x + 21.755$ with $R^2 = 0.9991$, in the range of 1.0-35.0 mg L^{-1}. The LOD and LOQ were 0.32.and 1.08 mg L^{-1}, respectively. The system was successful in the determination of sulphite in wines, coconut juice, and dried fruits. The amounts of sulphite were no significant different from those results analyzed by the iodometric titrimetric method. This system provided eco- and user-friendly with automated control.

Keywords: Headspace Microextraction, Sulphite, Cotton Swab, Smartphone, Gas Diffusion Unit (GDU)

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Finally, I would also like to give special thanks to my parent, for letting me through all the difficulties.

Chutikarn Khamkhajorn

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Symbols/Abbreviations	Terms
%	Percent
µ	Micro
µg	Microgram
µCSAD	Microfluidic cotton swab-based analytical device
Abs	Absorbance
cm	centimeter
et al.	And other
g	Gram
kg	Kilogram
L	Liter
LOD	Limit of detection
LOQ	Limit of quantitation
M	Molar or Molarity
min	Minute
mL	Milliliter
nm	nanometer
Ref.	Reference
s	Second
SO_2	Sulphur dioxide
UV-Vis	Ultraviolet-visible

CHAPTER 1
INTRODUCTION

1.1 Background of sulphite

Sulphites are a group of chemicals substrates used as a preservative in food that inhibits the growth of yeast, mold, and bacteria. It is an antioxidant that inhibits the enzymatic browning reaction and a non-enzymatic browning reaction. Sulphites (E220-E228)(Gómez-Otero, Costas, Lavilla, & Bendicho, 2014) are widely used as additives for food and agricultural product preservation including wines, longan, dried fruit, jam, sugar, palm sugar, syrup, starch products, and frozen food. In addition, they are used in several canned food such as bamboo shoots, mushrooms, coconut milk, and potatoes. However, a high concentration of sulphites caused allergies in sensitive consumers, with asthma and shortness of breath as possible symptoms.

The Information Technology and Communication Center, Ministry of Commerce of Thailand reported that the export value of products that used sulphites as preservative such as longan, dried fruits, and wines in 2019 were 30,000, 8,000, and 1,600 million baht, respectively. Each country sets different maximum levels of sulphites for each product. Under EU allergen labeling regulations, sulphites at concentrations exceeding 10 mg kg^{-1} or mg L^{-1} should be labeled ("Food allergen labelling and information requirements under the EU Food Information for Consumers," 2015). Other countries such as the USA, Canada, Korea, and Thailand also require a warning on the label of foods containing $\geq$ 10 mg kg^{-1} sulphite. In addition, the acceptable daily intake (ADI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974) is 0.7 mg sulfur dioxide/kg body weight. Manufacturers or export companies need to study the maximum limit of sulphites in each country and control the level of sulphites for export their products.

The AOAC method for sulphite analysis in wines uses the optimized Monier−Williams (MW) method ("AOAC official method 990.28-990.31: AOAC (2000) Official Methods of Analysis, 17th edition, 2000.,"), involving SO_2 distillation followed by titration or precipitation, which is complicated, labor- intensive, and time-

consuming. An alternative is the Ripper method using iodometric titration (Ough, 1986), however, there is uncertainty in the visual detection of the titration endpoint, especially in red wines. There are several techniques for sulphites analysis such as spectrophotometry (Bener, Şen, & Apak, 2020), electro-analytical (Norouzi & Parsa, 2018), chromatography (Centonze, Iammarino, Taranto, Nardiello, & Palermo, 2009; K. S. Robbins, R. Shah, S. MacMahon, & L. S. de Jager, 2015), and flow- based methods (Kraikaew et al., 2019). These techniques required high-cost consumption, not only for the imported instrument but also for analysis expenses. However, to avoid interference from the matrix and its color, the sample needs to be pretreated by extraction methods such as headspace single-drop microextraction (Zaruba, Vishnikin, Škrlíková, & Andruch, 2016) or novel paper-based headspace-thin film microextraction (Shahvar, Saraji, Gordan, & Shamsaei, 2019). Headspace microextraction is suitable for extracting volatile analytes and then collected into the acceptor platform. It not only reduces contamination but also uses fewer reagents than the common extraction methods.

Therefore, in this research, two analytical systems for sulphites analysis were investigated aiming to invent a low-cost device and uncomplicated analysis, and towards green analytical chemistry.

1.2 Objectives

To design and invent 2 analytical systems for the determination of sulphites in wines, juice and dried fruits using;

(1) a headspace microextraction system incorporating a lab on a cotton swab platform and smartphone detection.

(2) a microfluidic system based on gas diffusion unit via flow-based analysis with optical sensor.

1.3 Scope of research

1.3.1 Lab on a cotton swab for sulphites detection

- Design a lab on a cotton base for headspace microextraction and colorimetric detection by smartphone.
- Optimization of the system and study its analytical features.
- Determination of sulphites in some wines.

1.3.2 Microfluidic system for sulphites analysis

- Design and invention of the microfluidic system for gas diffusion unit.
- Optimization of the system and study method validation.
- Application for determination of sulphites in wines, coconut juice and dried fruit.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Regulation of sulphite additives in food and beverages

Sulphites are found in a variety of foods and beverages as preservatives that prevent bacterial growth and oxidation. The ADI established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 0.7 mg sulfur dioxide/kg body weight. Under EU allergen labeling regulations, when sulphites are present at above 10 mg/kg or mg/litre, whether or not they have a technological function, a clear declaration of sulphites or sulphur dioxide is always required ("Food allergen labelling and information requirements under the EU Food Information for Consumers," 2015). Many countries, including the USA, Canada, Korea, and Thailand require a sulphite warning on the label of foods containing concentrations ≥10 mg kg^{-1} sulphites. The maximum levels of sulphites used in foods established by CODEX ("Codex alimentarius," 2021), Australia(Dengate), China("Chinese Standards for Food Additives," 2015) and Thailand("General Standard for Food Additives: GSFA ", 2014) as summarized in table 2.1.

Table 2.1

The maximum level (mg L^{-1}) of sulphites in foods

Producte	Codex (Year)	Australia	China	Thailand (Year)
Dried fruit	1,000 (2011)	300	100	1000 (2006)
Surface-treated fresh fruit	30 (2011)	–	50	30 (2011)
Jam	100 (2008)	–	–	100 (2008)
Candied fruit	100 (2006)	350	350	100 (2006)
Dried vegetables	50 (2006)	500	200	50 (2006)
Fruit juice	50 (2005)	–	50	50 (2006)
Beer and malt beverages	50 (2006)	250	10	50 (2006)
Grape wine	350 (2006)	200	250	350 (2006)
Other (fruits wine)	200 (2006)	–	250	200 (2006)
Potato	50 (2006)	50	–	50 (2006)

2.2 Export value of some Thai food (Thailand Trading Report)

The Thailand Trading Report of the Ministry of Commerce presented the export value of Thai customs in each year. Some food and agricultural products commonly preserved using sulphites are shown in Figure 2.1. China, Vietnam, and Indonesia were the main international markets for Thai fruits such as longan, that was exported in 2019 to each country with the values of 9,698, 7,476, and 2,531 million baht, respectively. The values for dried fruits were 5,889, 1427, and 255 million baht. The export value of some food and agricultural products are shown in Figure 2.1. For wine products, the main export market was Myanmar (797 million baht).

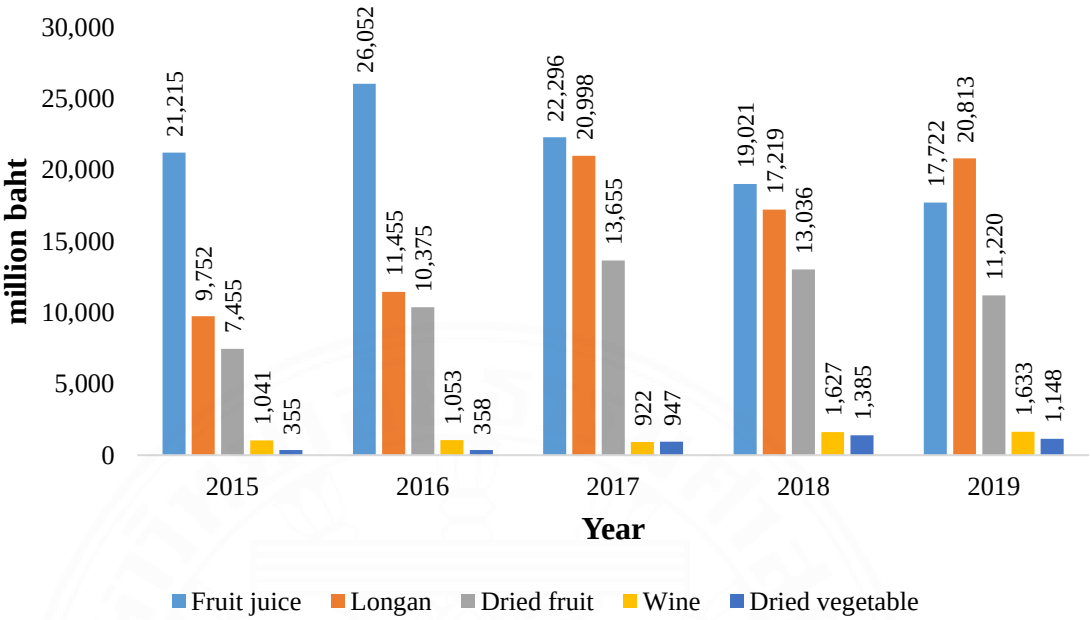

Figure 2.1 The export value some food and agricultural products of Thailand in 2015-2019 (Source: Department of International Trade Promotion, Ministry of Commerce).

An example of the export's problem is the case of which loss of the Chinese export value of longan by some Thai companies because sulphurdioxide was detected at higher than maximum levels (50 mg kg^{-1} SO_2) in 2013. Therefore, to prevent the export value decreasing, the Plant Standard and Certification Division, Ministry of Agriculture and Cooperatives of Thailand, improved the quality control system of exported longan to investigate the safety and create the partner confidence.

2.3 Techniques for sulphite determination

Determination of sulphites by Association of Official Agricultural Chemists (AOAC, 2000) uses the optimized Monier−Williams (MW) method ("AOAC official method 990.28-990.31: AOAC (2000) Official Methods of Analysis, 17th edition, 2000.,"), involving SO_2 distillation followed by titration or precipitation. However, this method is complicated, labor-intensive, and lengthy. Other techniques

for determination of sulphites include spectroscopy (Bener et al., 2020), electro-analysis (Norouzi & Parsa, 2018) and chromatography (Katherine S. Robbins, Romina Shah, Shaun MacMahon, & Lowri S. de Jager, 2015). These are capital-intensive require expertise, making them unsuitable for small companies or industries. Some of these techniques were presented in Table 2.2.

Headspace analysis involves the direct analysis of the volatiles in the gas phase above a sample. It is an simple technique that numerous advantages over more traditional sample preparation techniques such as extraction, adsorption, precipitation, distillation, etc.(Rouseff & Cadwallader). Adsorbent extraction technique for the vapor extraction of the interested substance, the extraction characteristics can be divided into 2 forms: Static extraction, which uses the diffusion of the interested substance to adsorb on the surface of the adsorbent, such as solid phase micro extraction technique, SPME and dynamic will use an inert gas to transport the vapor of the interested substance through the absorber all the time, such as thermal desorption, TD or purge and trap techniques or in-tube extraction techniques, ITEX etc. Normally, determination of sulphite, it is popular to convert to sulfur dioxide by diffusion through a gas diffusion unit to react with the colorimetric reagent. It was presented in table 2.3.

Table 2.2

Some techniques for sulphites determination.

Author (Year)	**Sample**	**Detection method**	**Linear range** ($mg\ L^{-1}\ SO_2$)	**LOD**
(Bener et al., 2020)	Wine and Vinegar	UV-vis spectrophotometry	0.32-19.08	0.084
(Kraikaew et al., 2019)	White wine	On-line cone reservoirs membrane less gas-liquid separation flow system with capacitively coupled conductive detector (C4D).	8-160	–
(Norouzi & Parsa, 2018)	Weak liquor	Electrochemical Sensor Based on Ni/Poly (4-Aminobenzoic Acid)/Sodium Dodecyl sulfate/Carbon Paste Electrode	0.08–0.8 and 0.8-8*	0.04
(Zaruba et al., 2016)	Wine, Jam, and Juice.	Optical Probe as the Microdrop Holder in Headspace Single Drop Microextraction	0.032−0.320	0.008
(Katherine S. Robbins et al., 2015)	dried fruits, vegetables, frozen seafood, sweeteners, and juices.	Liquid Chromatography−Tandem Mass Spectrometry	0.25−114	–

Table 2.2

(Continued)

Author (Year)	Sample	Detection method	Linear range (mg L^{-1} SO_2)	LOD
(Centonze et al., 2009)	Fresh meats and shrimps	Ion-exchange chromatography with conductivity detection	8.2–160**	2.7**
(Jankovskiene, Daunoravicius, & Padarauskas, 2001)	Wine	Capillary electrophoretic method with UV-Vis spectrophotometry	0.8-6.4*	0.16
(Lowinsohn & Bertotti, 2001)	Wine	Coulometric titration	25.6-76.8	0.64
(Yamada, Nakada, & Suzuki, 1983)	–	Flow injection system with chemiluminescent detection	0.9-35***	0.9***

*SO_3^{2-}

** mg Kg^{-1}

*** ng SO_3^{2-}

Table 2.3

Review flow analysis with gas diffusion unit and spectrometry for sulfites analysis.

Author (Year)	**Colorimetric Reagent (method)**	**Linear range** (mg L^{-1} of SO_2)	**LOD** (mg L^{-1})	**Sample**
(Sullivan et al.)	Malachite green (FIA)	n.a.	n.a.	wine
(Bartroli, Escalada, Jimenez Jorquera, & Alonso, 1991)	P-Aminoazobenzene (FIA)	5-300 2-35	2 0.2	wine
(Decnop-Weever & Kraak, 1997)	Bromocresol green (FIA)	1-20	0.1	red wine, white wine and rose wines.
(Silva, Silva, Nóbrega, & Neves, 1998)	Oxidation of Mn(II) and react with iodide	1-26	1.0	white wine, red wine
(Segundo & Rangel, 2001)	Formaldehyde and pararosaniline (SIA)	25-250 2-40	0.6 0.1	white wine, red wine
(Oliveira, Lopes, Tóth, & Rangel, 2009)	Malachite green (FIA) Pararosaniline (FIA)	1-40 25.0-250	0.3 0.7 0.6 0.8	white wine, red wine
(Reanpang, Pun-uam, Jakmunee, & Khonyoung, 2021)	Roselle extract (FIA)	5-100	2.0	Sparkling wine, white wine, red wine

2.4 Application of smartphone as an analytical detection

Smartphones are widely spread, and their usage does not require training. Recently, smartphones have been successfully used in analytical chemistry as a simple detection tool for applications based on color measurement. Some applications are listed in Table 2.4.

Table 2.4

Smartphone for chemical analysis.

Author (Year)	Analyte	Method	LOD
(Siangdee & Youngvises, 2019)	TNT	Reaction of TNT and NaOH was analyzed on cotton swab platform and smartphone and detected the red color by ImageJ application.	0.11 μg-TNT
(Shahvar et al., 2019)	Sulphite in food samples	SO_3^{2-}/ HSO_3^- + H^+ → SO_2(g) SO_2 + Fe (III) → Fe (II) Fe (II) +3 o-phen →$Fe(phen)_3^{2+}$	0.04 μg L^{-1}.
(Wang et al., 2019)	Lead ion	A smartphone was used to capture digital images and show RGB values A smartphone was used to capture digital images showing RGB values of the fluorescent probe of carbon dots corresponding to the Pb^{2+}	0.59 μg L^{-1}.

Table 2.4

(Continued)

Author (Year)	Analyte	Method	LOD
(Mutlu & Kilic, 2018)	Harmful Dyes Detection in Water	The proposed system used a custom cradle to transmit built-in flashlight via fiber cable, and to capture the visible absorbance spectroscopy by camera of smartphone.	500 μg L^{-1}
(Ait Errayess, Idrissi, & Amine, 2018)	Sulfonamide in Pharmaceutical	A software program was developed to be used for analyzing. Various parameters for digital colorimetric detection	110 μg L^{-1}.
(Kostelník, Cegan, & Pohanka, 2017)	Galanthamine and Donepezil	Smartphone served as a tool for measurement of indicator color change from red to orange. Digital photography was evaluated using RGB channels.	42.8 μg L^{-1} Galantamine 8.46 μg L^{-1} Donepezil
(Intaravanne & Sumriddetchkajorn , 2015)	The amount of N fertilizer for rice field.	Photography by ‘‘BaiKhao’’ application. The color level of the rice leaf corresponds to the nitrogen status of rice in the field	–

2.5 Lab on cotton swab

Lab on cotton swab or a microfluidic cotton swab-based analytical device (μCSAD) is one of the platforms of microfluidic systems using a cotton head for the reaction and detection zone, as well as for swabbing the sample. The cotton is cellulose-based that can adsorb the colorimetric reagents and analytes in microliter volumes to minimize reagent consumption and waste generation. Some methods used in this platform are presented in Table 2.5. The platform of lab on cotton swab is simple and cheap and is suitable for use in field analysis.

Table 2.5

The recent lab on cotton swab

Author (Year)	Analyte	Details	LOD
(Siangdee & Youngvises, 2019)	2,4,6-trinitrotoluene (TNT)	Naked-eye observation and smartphone detection of color change and evaluated by ImageJ	0.11 μg-TNT·
(Alamer, Eissa, Chinnappan, Herron, & Zourob, 2018)	Foodborne pathogenic bacteria.	The images of the cotton swab were captured using a smartphone. The intensity of the color was determined by using ImageJ	10 cfu/ml Salmonella typhimurium, 100 cfu/ml Salmonella enteritidis, and 100 cfu/ml Staphylococcus
(Serra-Mora et al., 2018)	Quaternary ammonium compounds (QACs)	Naked-eye observation of color change	0.8 mg L^{-1}

Table 2.5

(Continued)

Author (Year)	Analyte	Details	LOD
(Schaude et al., 2017)	pH of Wounds	Naked-eye observation of color change	–
(Kanehira et al., 2012)	Hydrogen sulfide on the tongue dorsum	The color density was analyzed using an optical device containing a light-emitting diode (LED) at a wavelength of 660 nm and a photodetector	0.58 mg L^{-1}

2.6 Colorimetric reagent of sulphites analysis

The colorimetry is a user-friendly method, and the equipment is available in most laboratory, therefore, many researchers are interested to develop the colorimetric reagents for sulphite analysis. Some of them are presented in Table 2.6.

Table 2.6

Colorimetric reagent for sulphite determination

Author (Year)	**Analyte**	**Reaction**	**Wavelength (nm)**	**LOD ($\mu g\ L^{-1}\ SO_2$)**
(Bener et al., 2020)	Sulphite	Reaction with p-rosaniline and formaldehyde	588	84
(Zaruba et al., 2016)	Sulphites	HS-SDME Spectrophotometry $SO_3^{2-}/ HSO_3^- + H^+ \rightarrow SO_2(g)$ $SO_2 + Fe\ (III) \rightarrow Fe\ (II)$ $Fe\ (II) + 3\ o\text{-phen} \rightarrow Fe(Phen)_3^{2+}$	510	8
(Gómez-Otero et al., 2014)	Sulphites	HS-SDME-Spectrophotometry $SO_3^{2-}/ HSO_3^- + H^+ \rightarrow SO_2(g)$ then react medication with 5,5′dithiobis (2-nitrobenzoic acid) (DTNB)	410	3.8

Table 2.6

(Continued)

Author (Year)	**Analyte**	**Reaction**	**Wavelength (nm)**	**LOD ($\mu g\ L^{-1}\ SO_2$)**
(Li & Zhao, 2006)	Sulphites	1) Reaction with p-rosaniline and formaldehyde, measurement of light absorption at 575 nm	575	50
		2) Reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB)	412	50
(Bartroli, Escalada, Jorquera, & Alonso, 1991)	Free SO_2	FIA + Gas diffusion + Spectrophotometry 1) Free SO_2 method: acidify and then convert to gas and separate through the membrane and react with p-amino azobenzene and formaldehyde	520	200
	Total SO_2	2) Total SO_2 method: The same procedure as 1), however, the sample needed pre-hydrolysis prior to acidification		2,000

2.7 Portable equipment and test kit

As noted above, Liquid Chromatography−Tandem Mass Spectrometry and UV-Vis spectrophotometry are generally used for the determination of sulphite. It is accurate and is accepted for legal verification, but expensive and unsuitable for on-site analysis. Therefore, the equipment has been developed that can be portable and simple. The test kit and some analyzers were reviewed as shown in Table 2.7.

Table 2.7

Portable sulphites detection

Invention Type	Details	Reference
Test kit	Hanna, HI3822 sulphite test kit resolution 0-200 ppm (2 ppm) 0-20 ppm (0.2 ppm) 110 tests 5,000 baht	(Neonics)
Test kit	Hach 148002 Sulphite Test Kit, Model SU-5 10 - 200 mg/L SO_3^{2-} 100 tests 3,300 baht	(Merck)
Test kit	Alert® for Sulphites Sensitivity: < 10 ppm 10 - 100 ppm > 100 ppm Testing time: 1 minute Tests per kit: 200	(Neogen)

Table 2.7

(Continued)

Invention Type	Details	Reference
Test strip	1.10013 EMD Millipore Sulphite Test colorimetric with test strips 10 - 40 - 80 - 180 - 400 mg/l SO_3^{2-} MQuant™ 100 tests	(Amazon)
Analyzer	Vinmetrica SC-100A Sulphite Analyzer 204*32.40 Titration: Used to find an endpoint Sensitivity: detects less than 2 ppm Free or Total SO_2 in a 25 mL sample Accuracy: +/- 2 ppm Free or Total SO2	(Vinmetrica)
Method	Rapid determination of concentration of the total sulphur dioxide and lignosulphonate in sulphite pulping process liquors	(US4889593A)

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Chemicals

All chemicals used in this research were of analytical reagent (AR) grade. Chemicals and their manufacturer are listed in Table 3.1.

Table 3.1
List of the chemicals used in this research.

Chemicals	Formula	Manufacturer
Glacial acetic acid	CH_3COOH	QRec, New zealand
Deionized water (DI)	H_2O	ELGA, America
Ethanol	C_2H_5OH	Merck, Germany
Ethylene diamine tetra-acetic acid	EDTA	CARLO ERBA, Germany
Hydrochloric acid, 37 % (w/w)	HCl	QRec, New zealand
Iron (III) nitrate nonahydrate	$Fe(NO_3)_3.9H_2O$	QRec, New zealand
1,10-phenanthroline	$C_{12}H_8N_2.H_2O$	Kemaus, Australia
Sodium hydroxide	NaOH	QRec, New zealand
Sodium sulfite	Na_2SO_3	QRec, New zealand

3.2 Lab on a cotton swab for sulphites determination

The method is based on 3 reactions: 1) conversion of sulphite to volatile sulphur dioxide (SO_2) by acidification 2) reduction of iron (III) to iron (II) by SO_2 and 3) complex formation of iron (II)-phenanthroline, producing a red color. The two last reactions were demonstrated on the cotton swab and the red color was observed and detected by a smartphone. The Colorimetric sensor application was used to evaluate the amount of sulphite.

a) $SO_3^{2-}(aq) + 2H^+(aq) \longrightarrow SO_2(g) + H_2O(l)$ …(1)

b) $SO_2(g) + 2H_2O(l) + 2Fe^{3+}(aq) \longrightarrow SO_4^{2-}(aq) + 4H^+(aq) + 2Fe^{2+}(aq)$ …(2)

c) $Fe^{2+}(aq) + 3phen(aq) \longrightarrow Fe(phen)_3^{2+}(aq)$ (red color) …(3)

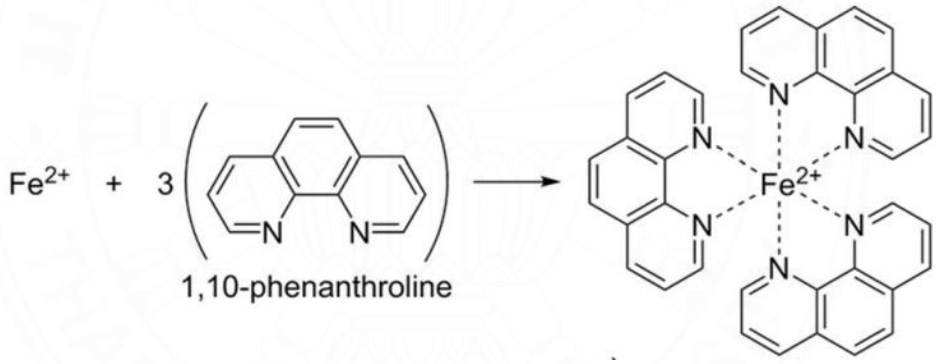

Figure 3.1 Chemical reaction of Fe^{2+} and 1,10-phenanthroline.
Note. From "Simple and Precise Quantification of Iron Catalyst Content in Carbon Nanotubes Using UV/Visible Spectroscopy" by (Agustina et al., 2015)

3.2.1 Preparation of standard sulphite and colorimetric reagent

3.2.1.1 Standard solutions

Stock standard solution of sulphite 1,000 mg L^{-1} was prepared by dissolving 0.1575 g of sodium sulphite and the final volume was adjusted to 100 mL with 1 mmol L^{-1} of ethylenediaminetetraacetic acid (EDTA). The EDTA solution at the

concentration level of 0.01 mol L^{-1} was obtained by dissolving 0.3722 g of EDTA and the final volume was adjusted to 100 mL with deionized water.

3.2.1.2 Colorimetric reagents

A stock solution of 0.05 mol L^{-1} iron (III) nitrate was prepared by dissolving 0.2020 g of $Fe(NO_3)_3$ in 10 mL of 0.3 mol L^{-1} hydrochloric acid. The solution of 1,10-phenanthroline (Phen) 0.25 mol L^{-1} was prepared by dissolving 0.4956 g in 10 mL of DI water. The acetate buffer pH 5.5 was prepared by mixing 35 mL of 1 mol L^{-1} acetic acid with 15 mL of 2 mol L^{-1} sodium hydroxide. A colorimetric reagent was prepared by mixing an 80 µL of 0.05 mol L^{-1} iron (III) nitrate, 0.5 mL of acetate buffer solution (pH 5.5), and 300 µL of 0.25 mol L^{-1} Phen. Then, the mixing solution was diluted to 5 mL with DI water.

3.2.2 Clamp for detection by smartphone

The 3D-printed sample holder was made of a standard resin (eSUN, China). As shown in Figure 3.2, it consisted of a clip for attachment to the smartphone, a holder for the cotton swab, a convex lens, and a white light-emitting diode (LED, 3 mW). The colorimetric sensor application was designed to capture the images, evaluate the RGB values, create calibration graphs, and analyze the amounts of sulphites as SO_2 in units of mg L^{-1}.

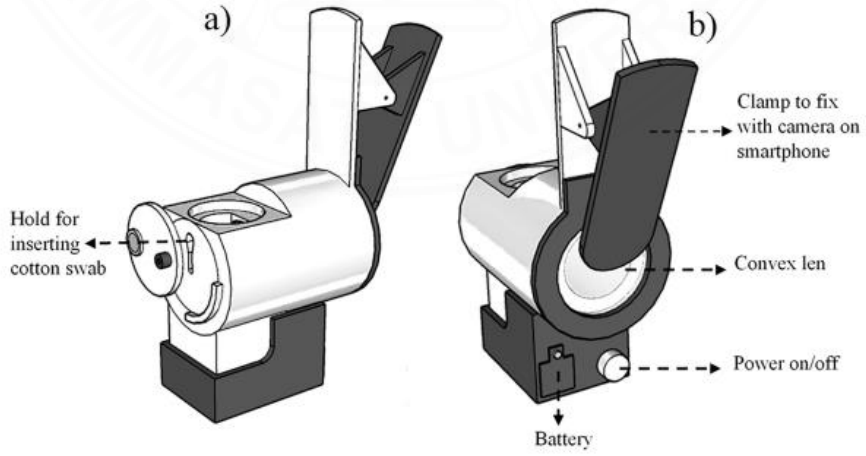

Figure 3.2 Configuration and dimensions of the sample holder: a) side view, b) back view, using 3D drawing of the equipment by the Sketchup program.

3.2.3 Cotton sensor preparation and detection process

Colorimetric reagents consist of 0.8 mmol L^{-1} $Fe(NO_3)_3$, acetic acid pH 5.5, and 15 mmol L^{-1} of phen. Pipet sample/standard 1.0 mL and then, add EDTA 100 µL into a vial to prevent the catalytic action of trace amount of heavy metals. Pipet reagent at a suitable volume to cotton head and insert it into the septum of a cap (Figure3.2. a). After that 200 µL of hydrochloric acid was pipetted into a vial that contains the sample, and suddenly closed the vial by cap and septum as shown in (Figure3.2. c). Wait for the reaction to complete (Figure3.2. d) and inserted it into the sample clamp holder fixed to smartphone. Colorimetric app named "Canal" developed by Dr. Somkid Pencharee; Ubon Ratchathani University) was used to evaluate the amount of sulphite (Figure3.2. f). The value is calculated by subtraction from the intensity of a colorimetric reagent as shown in equation (4).

$$\Delta I = I_b - I_s \quad \ldots(4)$$

Whereas I_b and I_s is the intensity value of blank and standard or sample, respectively, value is from Canal app.

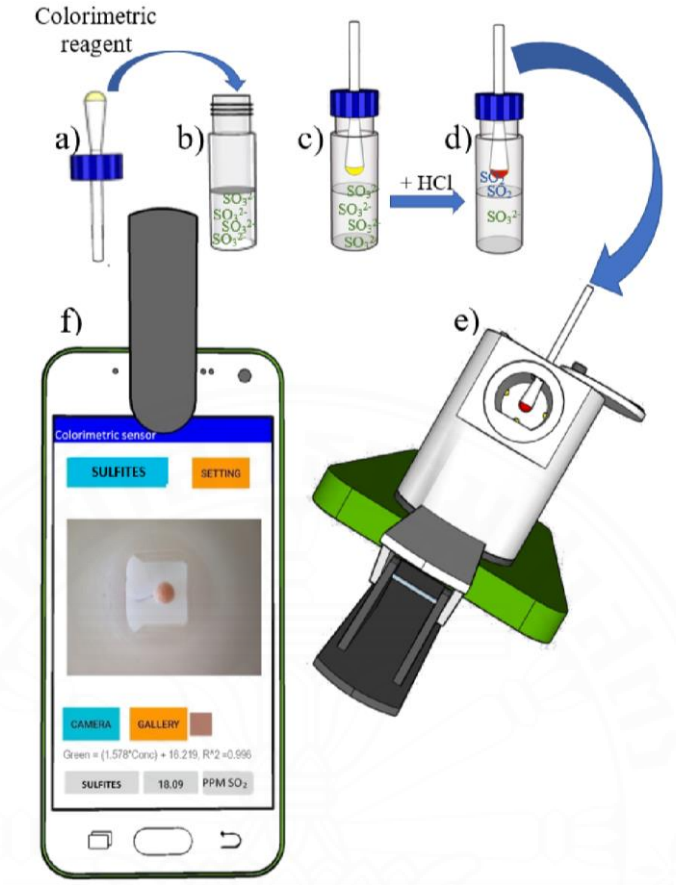

Figure 3.3 Analysis of sulphites based on head space microextraction and smartphone detection. a) The reagent was pre-adsorbed on the cotton swab head; b) The standard or sample solution was pipetted into a 1.5 mL vial; c) The liberated SO_2 reacted with reagent on cotton head; d) Wait for reaction to complete; e) The cotton swab was inserted into the sample clamp holder fixed to smartphone(top view), and f) Colorimetric sensor application was displayed.

3.2.4 Ripper titration

Pipette 10.0 mL of standard sulphite into a 250 mL conical flask. Then, add 5 mL of 25% H_2SO_4. Rinse and fill the burette with 0.001 mol L^{-1} iodine solution by standardizing 0.001 mol L^{-1} iodine solution with 0.001 mol L^{-1} $Na_2S_2O_3$ solution. Next, add about 0.2 g of $NaHCO_3$ and starch 0.5 ml to the flask and commence the titration immediately. Titrate rapidly until the solution turns a blue color which persists for 30 seconds. Calculate the free SO_2 concentration in mg L^{-1} using the following equation.

$$\mathbf{SO_2\ mg\ L^{-1}} = \frac{(\mathbf{mL\ iodine})\times(\mathbf{M\ iodine})\times(\mathbf{64})\times\mathbf{1000}}{\mathbf{mL\ wine}} \quad \ldots(5)$$

3.2.5 Optimization

Due to the system based on headspace microextraction and colorimetric reaction. Parameters that need to be optimized include extraction time, the volume of reagent, the concentration of Fe $(NO_3)_3$, the pH of reagent, the concentration of phen (1,10-phenanthroline), the concentration of HCl, and the color of analysis detection mode. The univariate optimization was proceed using 10 mg L^{-1} of sulphite, 0.75 mmol L^{-1} $Fe(NO_3)_3$, 15 mmol L^{-1} Phen, pH 5.6, and 2 mol L^{-1} HCl 2 mol L^{-1} with three replications for each condition.

3.2.5.1 Influence of reaction time

The reaction time, including extraction, reduction, and complex formation time, were studied. The extraction time is an important, and it controls the rate of the whole reaction because its rate was slower than others. After adding acid in the sulphite solution in closed system and set-up smartphone for capturing color on cotton head every minute from 1- 24 min.

3.2.5.2 Effect concentration of hydrochloric acid

Sulphite was converted to sulphur dioxide by acidification, so the effect of hydrochloric acid concentration was examined by injecting 200 µL of various concentrations of hydrochloric acid 0.32, 0.65, 0.98, 1.3, 1.6, and 2.3 mol L^{-1}.

3.2.5.3 Volume of reagent on a cotton swab

Colorimetric Reagent was loaded at the center head of the cotton swab with 20, 30, 40, and 50 µL by pipette to determine optimal color detection**.**

3.2.5.4 Concentration of Fe(III) nitrate

The concentrations of $Fe(NO_3)_3$ 0.2, 0.5, 0.8, and 0.10 mmol L^{-1} were studied.

3.2.5.5 Concentration of 1,10-phenanthroline

The concentration of reagent onto a cotton swab. Colorimetric reagents were mixed at concentrations of phen 3, 5, 10, 15, and 20 mmol L^{-1}.

3.2.5.6 pH of colorimetric reagent

The acetate buffer was prepared by mixing 1.0 mol L^{-1} acetic acid with 2.0 mol L^{-1} sodium hydroxide and was measured by pH meter. The colorimetric reagent at pH 3.0, 4.0, 5.2, 5.5, 5.8, 6.0, and 6.8 were studied.

3.2.6 Detection of sulphites in wine

13 bottled of wine samples were studied including 7 bottles of white wine; 3 bottles of red wines and rose wine; and 3 bottles of sparkling wine. The amount of sulphite of these samples was analyzed and calculated in form of SO_2 by the proposed method compared to the ripper method.

3.2.6.1 Sample preparation

All samples were analyzed immediately after opening the wine bottles. After transferring the 0.5 mL sample into a 1.5 mL vial. To test the accuracy of the proposed method, the samples were also analyzed by the Ripper method using iodometric titration.

3.2.7 Some analytical features

3.2.7.1 Linearity

The experiments were carried out using sulphite (mg L^{-1}) standard at concentrations 0.07, 0.15, 0.30, 0.45, 0.60, 0.75, 1.13, 1.50, 1.88, 2.25, 3.75, 7.50, 15.00, 18.75, and 22.50 mg L^{-1} with three replicates for each concentration. Linearity was investigated using sulphite concentration on the x-axis and ΔI on the y-axis and calculated the intercept and R_2 from the equation of the calibration curve.

3.2.7.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using three and ten times the blank signal, respectively.

3.2.7.3 Selectivity

The selectivity was performed based on the addition of some ingredients in wines (100-1000 mg L^{-1}) into sulphite solution 10 mg L^{-1} as SO_2, compared to the control solution sulphite 10 mg L^{-1}(as SO_2). Ascorbic acid, sodium nitrite, sodium sulphide, sodium bicarbonate, tartaric acid, boric acid, sucrose, fructose, and glucose were among the examined chemicals that were frequently present in beverages.

3.2.7.4 Recovery

The recoveries were performed by spiking sulphite at the concentration of 3.75, 7.5, and 15.0 mg L^{-1} (as SO_2) in wine samples such as sparkling, white, red, and rose wines with 3 replicate measurements, then percentage recoveries were calculated.

3.2.7.5 Determination of sulphite in sample with Ripper titration

Pipette 10.0mL of wine into a 250 mL conical flask. Add 5.0 mL of 25% H_2SO_4. Rinse and fill the burette with 0.001 M iodine solution. Add about 0.2 g of $NaHCO_3$ and starch 0.5 ml to the flask and commence the titration immediately. Titrate rapidly until the solution turns a color change which persists for 30 seconds. Calculate the free SO_2 concentration in mg/L.

3.2.7.6 Determination of sulphite in samples with smartphone

Pipet sample/standard 0.5 mL and DI water 0.5 mL then, add EDTA 100 µL into the vial. Pipet reagent at a suitable volume to the cotton head and insert it into the septum of the cap (Figure 3.2. a). After that pipet 200 µL of hydrochloric acid in a vial that contains the sample, and suddenly closes the vial by cap and septum as shown in (Figure 3.2. b). Wait for the reaction to complete (Figure 3.2. d) and inserted it into the sample clamp holder fixed to the smartphone. Colorimetric app (designedby Dr. Somkid Pencharee; Ubon Ratchathani University) was used to evaluate the amount of sulphite. (Figure 3.2. f). The value is calculated by subtraction from the intensity of a colorimetric reagent.

3.3 Flow-based analysis via gas diffusion unit

3.3.1 Standard

Stock standard solutions of sulphite 1,000 mg L^{-1} was prepared by dissolving 0.1970 g of sodium sulphite and the final volume was adjusted to 100 mL with 1 mmol $L^{-1}$of ethylenediaminetetraacetic acid (EDTA).

3.3.2 Colorimetric reagent

A stock solution of 0.05 mol L^{-1} iron (III) nitrate was prepared in 0.3 mol L^{-1} hydrochloric acid. A solution of 0.25 mol L^{-1} 1,10-phenanthroline (Phen) was prepared in EtOH. An acetate buffer (pH 5.5) was prepared by mixing 35 ml of 1 mol L^{-1} acetic acid and 15.0 mL of 2.0 mol L^{-1} sodium hydroxide solution. Then, the colorimetric reagent was mixed with 3.0 mL of iron (III) nitrate solution, 22.0 mL Phen solution, and 25.0 mL of acetate buffer solution, and the mixture was diluted to 250.0 mL using DI water. The reagent solution was prepared daily and stored in an amber-colored glass bottle.

3.3.3 Design and invent microfluidic system

Flow-based analysis via gas diffusion unit was designed based on three reactions in accordance with 3.2 (Figure 3.1) by injecting sulphite into HCl stream (donor), generating SO_2 (g), and then diffusion into the acceptor stream (colorimetric reagent, Fe^{3+} mixed to Phen). Finally, the red color of $Fe(Phen)_3^{2+}$ was detected by the optical sensor.

The system was designed in accordance with Figure 3.4. Two peristaltic pumps (Leadfluid BT50s, China), (Kamore, DC12V) were used to propel the system with tubing of a diameter of 1.0 mm, a six-port injection valve (Idex V-24, USA), a mixing coil (PTEF, i.d. 1.0 mm), a homemade flow-through cell and a homemade optical sensor. The system was controlled by a personal computer using a LabVIEW software program written in-house. In the preliminary study, we found that reaction in the gas diffusion unit needs to stop the donor stream. The analysis step was presented in Table. 3.2.

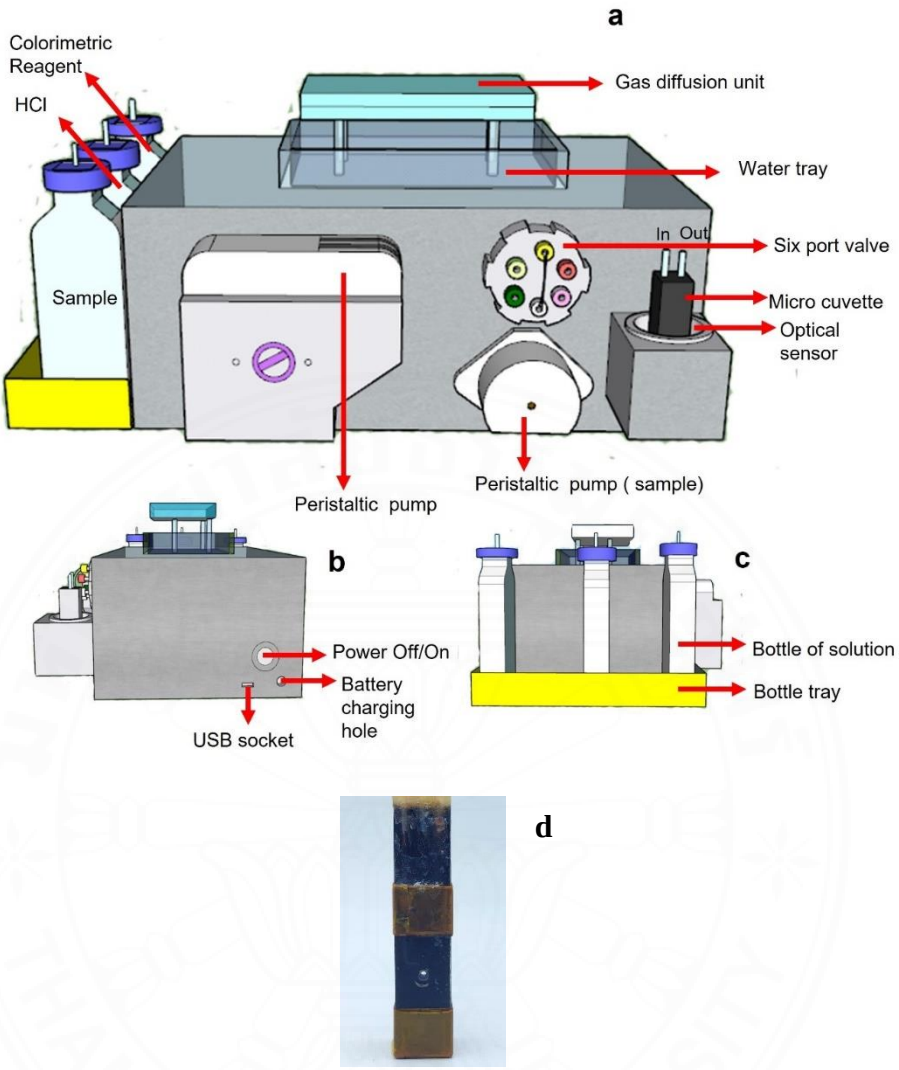

Figure 3.4 Automatic microflow gas diffusion unit system design, the apparatus consists of a peristaltic pump for suctioning the colorimetric solution and HCl solution, a peristaltic pump for sucking the standard solution into the injection valve, a six-port valve, a gas diffusion unit, micro-cuvette, and optical sensor at wavelength 515 nm (a), The right side of the automatic analyzer contains a power off/on, battery charging hole, and USB socket (b), the left side of the automatic analyzer contains a bottle tray (c), and a flow cell (d).

Table 3.2

Steps of automatic sulphite analyzer

Steps	Flow rate (rpm)	Time (s)	Description
Set pump CW	–	–	Washing
Run pump	20	–	
Time delay	–	60	
Stop pump	–	–	Set zero Abs
Zero Absorbance	–	–	
Time delay	–	5	
Run pump	20	–	Load sample
Load sample	–	–	
Inject sample	–	–	
Time delay	–	40	
Stop pump	–	–	Extraction
Time delay	–	300	
Run pump	20	–	Flux color solution
Time delay	–	250	
Stop pump	–	–	Show Abs
Pause measure	–	–	
Absorbance	–	–	

3.3.3.1 Gas Diffusion unit (GDU)

The GDU consisted of two symmetric acrylic blocks, colorless with a rectangular shape. The microchannels were engraved onto two pieces of polymethylmethacrylate (2.5 mm wide×208 mm long×0.8 mm thick) by laser etching (CNCBro, China). The two blocks were placed in mirror images and the PTFE membrane size 19.0 mm wide×70.0 mm long×0.1 mm thick (Towai brand, Thailand) was placed between two blocks. Using the inner diameter of 1.00 mm PTFE (VICI, Canada) tubing was connected with tube fitting (SMC, Japan) in Figure 3.5.

Figure 3.5 Gas diffusion unit (GDU)

3.3.4 Apparatus and analytical procedure

Colorimetric reagent and HCl were flowed at 20 rpm (0.4 ml /min) by a peristaltic pump into the acceptor channel and donor channel, respectively. Sample or standard solution was injected into the HCl stream by a six-port valve and generated gas SO_2. After that SO_2 is through the PTFE membrane to the acceptor stream in GDU. SO_2 can reduce iron (III) to iron (II) in the colorimetric reagent, causing the solution color to change according to the sulfite content. The analytical signal was recorded to show absorbance on the computer at wavelength 515 nm as shown manifold in Figure 3.6.

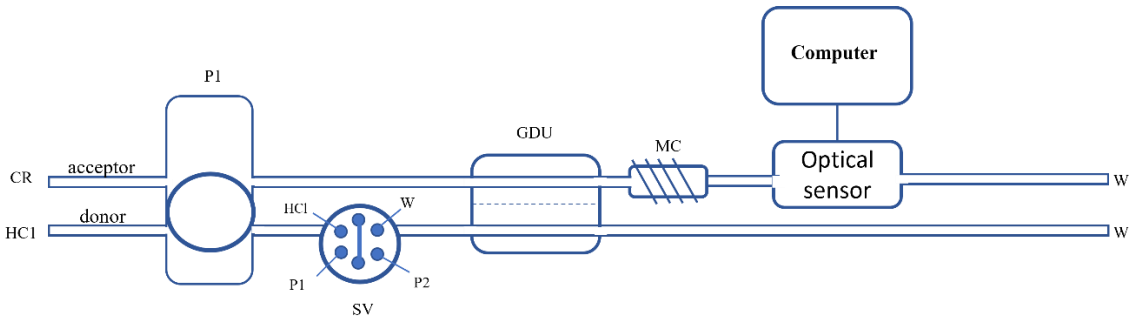

Figure 3.6 A manifold of automatic sulphite analyzer on microflow gas diffusion system via an optical sensor. CR: Colorimetric Reagent, HCl: Hydrochloric, P: Peristaltic pump, SV: Six-port Valve, GDU: Gas diffusion unit, MC: mixing coil (10 cm×1.0 mm i.d.), W: waste.

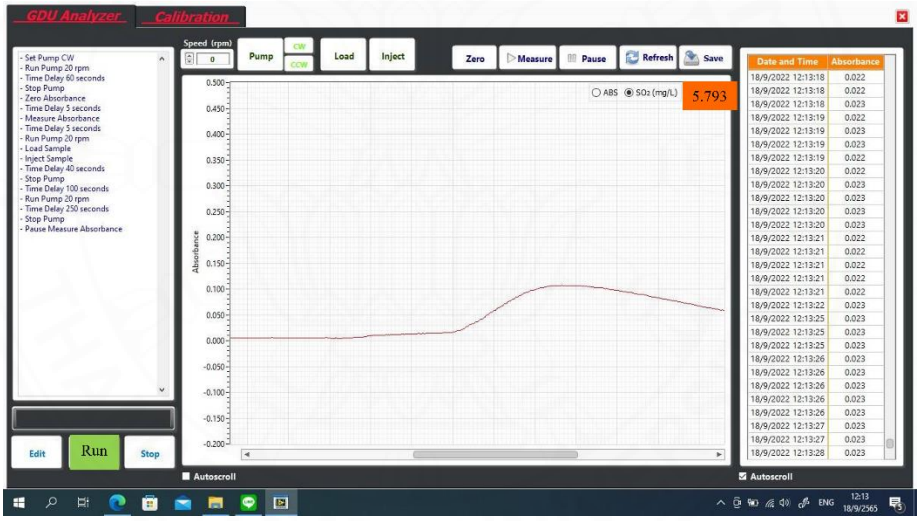

Figure 3.7 The screen shows the program running.

3.3.5 Optimization

Some parameters that need to be optimized including extraction time and concentration of reagents. The highest sensitivity of each parameter was chosen as the optimum condition. The optimization was studied using a standard sulphite solution of 10 mg L^{-1}, with initial condition consisting of 1.0 mol L^{-1} HCl, and the colorimetric reagents' (mixed between Phen and $Fe(NO_3)_3$ at concentration 6.0 and 3.0 mmol L^{-1}, respectively. The extraction time was studied at 0, 2, 4, 6, 8, 10, 12, 14, and 16 min.

The mixing coil was studied in the length 0, 10, 15, 25, 50, and 100 cm. Then, the pH of the reagent was investigated at 3, 4, 5, 5.5, 6, and 9. Next, the concentration of the reagent was optimized by varying the concentration of Fe (III) 0.1, 0.2, 0.4 0.6, 0.8, and 1.0 mmol L^{-1}. and the concentration of Phen 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26 mmol L^{-1}. Finally, the concentration of hydrochloric acid at 0.05, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, and 4.00 mol L^{-1}.

3.3.6 Sample preparation

The samples including wines, coconut water, and dried fruit were bought from the supermarket in Pathum Thani.

The wine and coconut juice samples were filtered (Whatman filter paper No.1), and after that 5.0 mL of the sample was mixed with 1 mL 0.01 mol L^{-1} of EDTA solution to prevent the catalytic action of trace amount of heavy metals. The mixture was diluted to 10 mL using DI water and was directly analyzed.

A dry fruit sample was minced. 0.2 g of a minced fruit sample was accurately weighed into a centrifuge tube, and 10 mL of distilled water was added. The mixture was centrifuged at 3000 rpm for 15 min and then filtered using (Whatman filter paper No.1). 5.0 mL of the filtrate was mixed with 1 mL 0.01 mol L^{-1} of EDTA solution. The mixture was diluted to 10 mL using DI water and finally, the sample was directly analyzed by the proposed system.

The wine and coconut juice samples were filtered after that 10 mL of the sample was pipetted into a 250mL conical flask and was titrated with iodometry.

A dry fruit sample was minced. 0.2 g of a minced fruit sample was accurately weighed into a centrifuge tube, and 10 mL of distilled water was added. The mixture was centrifuged at 3000 rpm for 15 min and then filtered using (Whatman filter

paper No.1). 10 mL of the filtrate was pipetted into a 250 mL conical flask following to 3.2.7.5.

3.3.7 Some analytical features

3.3.7.1 Standard curve

The experiments will be carried out using sulphite (mg L^{-1}) standard at concentrations from 1.0, 3.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, and 40.0 mg L^{-1} with three replicates for each concentration. The non-linearity was investigated using sulphite concentration in x-axis and absorbance in y-axis. concluded by the intercept and R_2 from the equation of the calibration curve.

3.3.7.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using three and ten times of the blank signal, respectively.

3.3.7.3 Selectivity

The selectivity was performed based on the addition of some ingredients in wines (100-1000 mg L^{-1}) into sulphite solution 10 mg L^{-1} as SO_2, compared to the control solution sulphite 10 mg L^{-1}(as SO_2). The studied ingredients were commonly found in beverages such as ascorbic acid, sodium nitrite, sodium sulphide, sodium bicarbonate, tartaric acid, boric acid, sucrose, fructose, and glucose.

3.3.7.4 Recovery

The recoveries were performed by spiking sulphite at the concentration of 0.3, 1.0, and 10.0 mg L^{-1} (as SO_2) in white wine and dry fruit with 3 replicate measurements, then percentage recoveries were calculated.

3.3.8 Sample analysis

The samples including wines, coconut water, and dried fruit were prepared from 3.3.6. The samples were directly analyzed by the microfluidic system in 3.3.4.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Lab on a cotton swab for sulphites determination

4.1.1 Optimization

A univariate analysis was conducted using 10 mg L^{-1} sulphite, The reaction was observed on the head of a cotton swab with red color appearance and the smartphone was used to capture the image of the color cotton head and measure the RGB value from the colorimetric app (Canal app). The value is calculated by subtraction from the intensity of a colorimetric reagent.

4.1.1.1 Influence reaction time

Three sequential reactions were performed including sulphite extraction, Fe(III) reduction, and complex formation. The extraction time is an essential variable and controls the rate of the entire process because the other two steps are very fast. The color of the head of a cotton swab was detected by smartphone after waiting for 1–24 min (in increments of 1 min). The signal increased dramatically for the first 15 min, and then more slowly (by ~8%) at 15–24 min. Therefore, the waiting time after adding acid and before image capture was fixed at 15 min. Moreover, the highest signals in the RGB results came from the green and blue values. However, the blue values had worse precision than the green ones because of the higher signal deviation in the blue value of the blank. The pale yellow color of $Fe(NO_3)_3$ of blank responded to the blue light more than the green one. Therefore, the green value was selected for the study as described below.

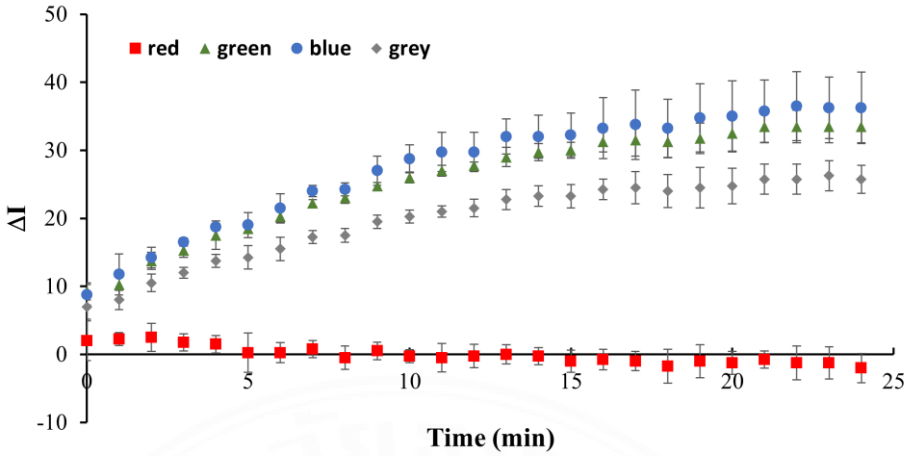

Figure 4.1 Effect of reaction time (min).

4.1.1.2 Effect concentration of hydrochloric acid

Sulphites were converted to SO_2 by acidification. The effect of acid concentration was examined by injecting 200 µL hydrochloric acid at various final concentrations of 0.05, 0.1, 0.15, 0.2, and 0.25 mol L^{-1}, with pH 1.3, 1.0, 0.8, 0.7, and 0.6. A higher acid concentration caused a more intense color on the cotton up to 1.0 mol L^{-1} and after that, there was no significant difference. Due to the molecular distribution of sulphite species, SO_3^{2-} is converted at low pH to H_2SO_3 (pH < 1), based on $K_{a2} > K_{a1}$, this result indicated the higher the value for K_a, the more the acid will dissociate into SO_2 gas above the solution in the vial. The colorimetric reaction occurs after the SO_2 is adsorbed on cotton, with correspond to distribution diagram in Figure 4.2. However, the high HCl concentration, HCl(g) can decrease the red intensity of Fe(II)-phenanthroline complex on cotton head. Therefore, 0.1 mol L^{-1} hydrochloric acid was selected for this work as shown in Figure 4.3.

$$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+ \qquad K_{a1} = 6.3\times10^{-8}$$

$$H_2SO_3 \rightleftharpoons HSO_3^- + H^+ \qquad K_{a2} = 1.4\times10^{-2}$$

$$H_2SO_3 \rightleftharpoons SO_2 + H_2O$$

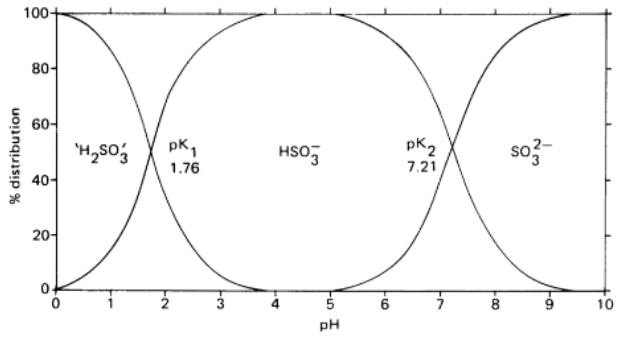

Figure 4.2 Sulfurous acid distribution diagram

Note. From "Gaseous Air Pollutants and Plant Metabolism" by (M. J. Kozioł)

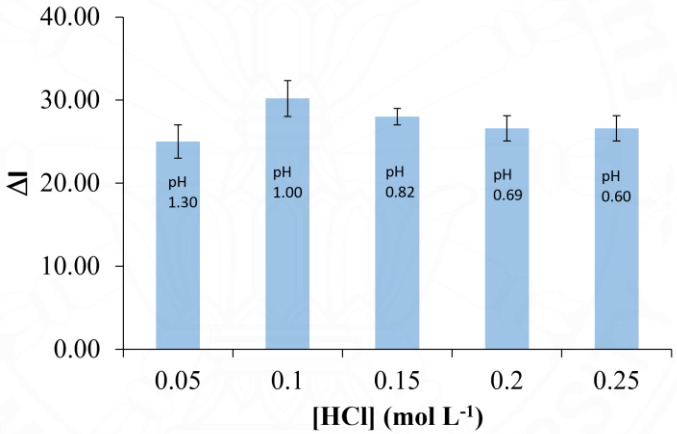

Figure 4.3 Effect of concentration of HCl (mol L^{-1}).

4.1.1.3 Volume of reagent on a cotton swab

The colorimetric reagent was pipetted on the center head of the cotton swab with 20, 30, 40, and 50 µL. It was found that the signal ΔI was 31.3±1.5, 44.3±2.5, 40.0±1.0, and 41.7±0.6, respectively. Due to volume of colorimetric reagent on the cotton head increases while the mole of sulfur dioxide be the same, so the red complex can dilute on cotton head. The optimized reagent volume was 30 µL because of the highest signal presented as shown in Figure 4.4.

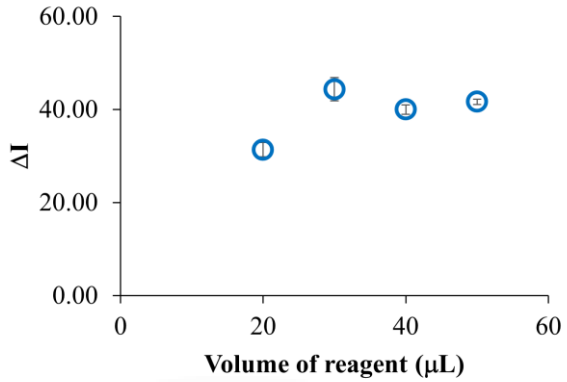

Figure 4.4 Effect volume of colorimetric reagent on a signal (ΔI) of a cotton swab method.

4.1.1.4 Concentration of Fe(III) nitrate

The concentration of $Fe(NO_3)_3$ in colorimetric reagent at 0.2, 0.5, 0.7, 0.8, and 1 mmol L^{-1} were studied. It was found that the intensity increases up to 0.8 mmol L^{-1} and after that decreases due to an increase in the value of the blank while the value of the standard has no difference therefore the optimal signal detection was 0.8 mmol L^{-1} $Fe(NO_3)_3$ as shown in Figure 4.5.

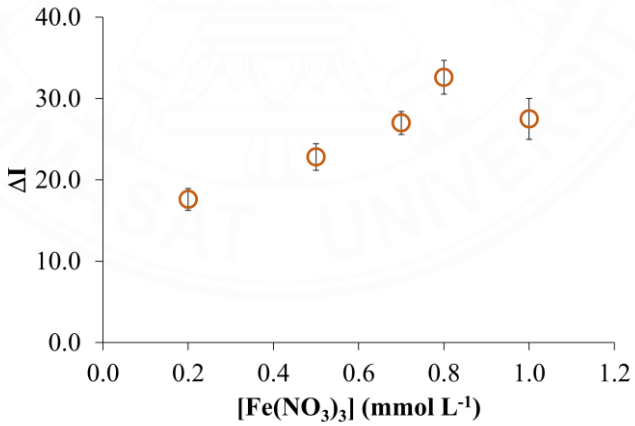

Figure 4.5 Concentration of $Fe(NO_3)_3$.

4.1.1.5 Concentration of 1,10-phenanthroline

The concentration of 1,10-phenanthroline in colorimetric reagent at 3, 5, 10, 15, and 20 mmol L^{-1} were studied and the result was shown in Figure

4.6. The optimal concentration was 15 mmol L^{-1} because of its highest signal after that it was stable as shown in Figure 4.6.

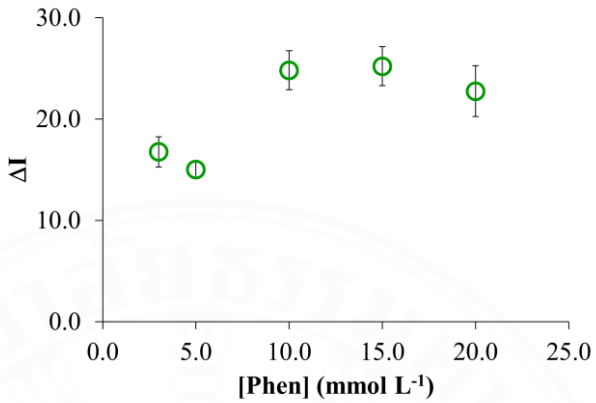

Figure 4.6 Concentration of 1,10-phenanthroline.

4.1.1.6 pH of colorimetric reagent

The colorimetric reagent at pH buffer 3.0, 4.0, 5.2, 5.5, 5.8, 6.0, and 6.8 were studied and the result is presented in Figure 4.7. The graph showed that the intensity increased up to pH 5.5 after that it decreased. The stability of complex should be in acidic medium because at higher pH of alkaline solution, $Fe(OH)_3$ will be precipitated. Therefore, the pH 5.5 was selected as optimal pH for colorimetric reagent.

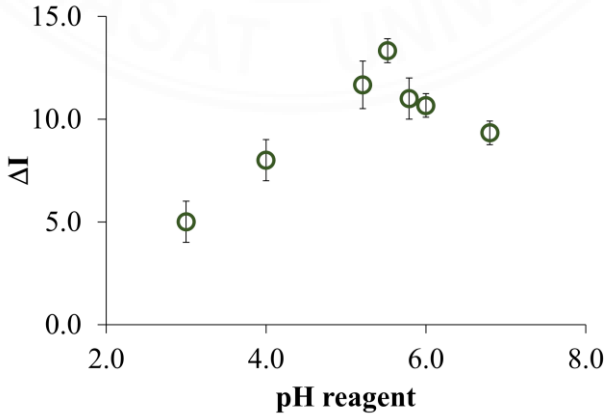

Figure 4.7 pH of reagent

4.1.2 Analytical performance

4.1.2.1 Linearity, LOD and LOQ

Under the optimized experiment, the linearity of the method was studied. The method showed two linear ranges of 0.32-2.40 and 2.40-24.00 mg L^{-1} with R^2 = 0.9997 and 0.9936, respectively. The results were presented in Figure 4.8. The two linear ranges were obtained because the shape of cotton is three dimensions, and the higher concentration of SO_2 has adsorbed not only the surface of the cotton head but also diffused inside of the cotton. Whereas the image was captured only the surface of one side.

The LOD and LOQ defined as 3 and 10 times the standard deviation of the blank, were calculated to be 300 and 600 μg L^{-1}as SO_2, respectively.

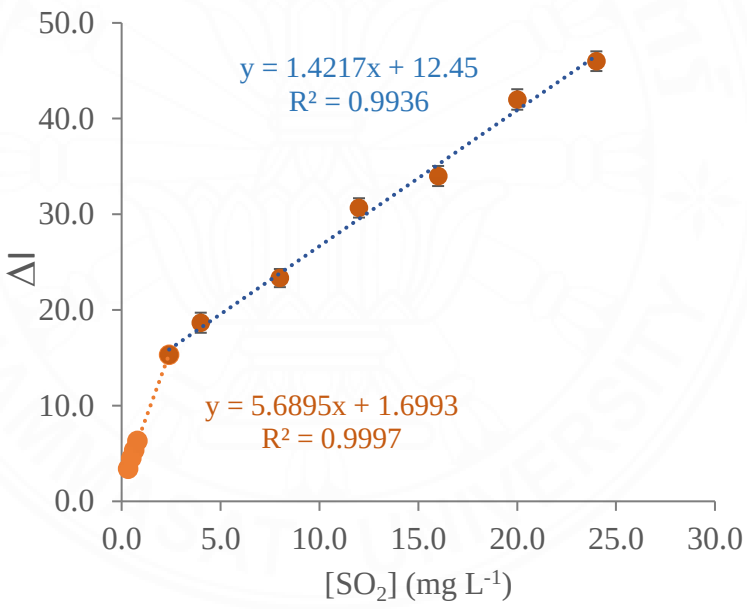

Figure 4.8 Two linearity ranges; 0.32-2.40 and 2.40-24.00 mg L^{-1} (as SO_2)

The color of cotton head of each concentration can be detected and distinguished with naked-eye, that is presented in Figure 4.9. At higher concentration of sulphite, more intense of red color was observed.

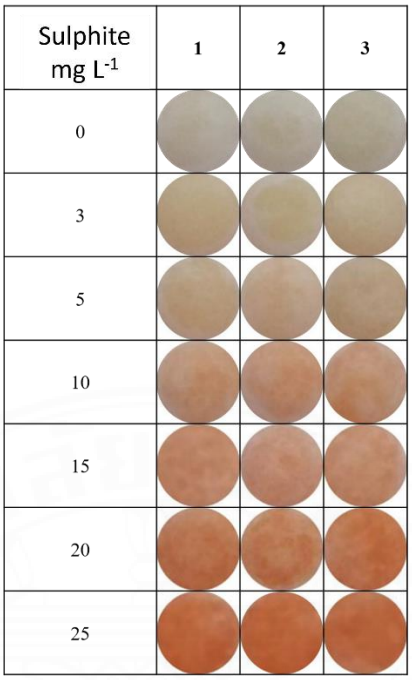

Figure 4.9 The images of the reaction of some standard concentrations on cotton.

4.1.2.2 Recovery

Different wine samples were chosen to evaluate the accuracy of this proposed method for the analysis of sulphites. The recoveries were performed by spiking standard sulphites 3.75, 7.5, and 15 mg L^{-1}. The results are shown in Table 4.1, the percentage recoveries in the range of 90.5-107.8% of SO_2 (N=3). For comparison, the samples were analyzed by the Ripper method. The results are tabulated in the table. No significant difference between headspace microextraction via lab a cotton swab and ripper methods at P=0.05, $t_{stat} < t_{crit}$ (0.2 < 2.18) (n=3).

Table 4.1

Recovery of the method for sulphite analysis.

Sample	Added (mg L^{-1} as SO_2)	Found (mg L^{-1} as SO_2)	Recovery (%)
Sparkling1	3.75	3.87 ± 0.58	103.2
	7.50	7.62 ± 0.53	101.6
	15.00	14.39 ± 0.58	95.9
White wine1	3.75	3.45 ± 0.31	92.1
	7.50	6.79 ± 0.53	90.5
	15.00	15.53 ± 0.73	103.5
Red wine 1	3.75	3.64 ± 0.53	97.0
	7.50	7.21 ± 0.54	96.1
	15.00	14.55 ± 0.53	97.0
Rose wine 1	3.75	3.50 ± 0.52	93.4
	7.50	7.05 ± 0.31	94.0
	15.00	14.32 ± 0.41	95.5
Rose wine 2	3.75	4.04 ± 0.53	107.8
	7.50	7.95 ± 0.58	106.0
	15.00	13.67 ± 0.71	91.2

4.1.2.3 Interference study

The study was carried out by adding a known concentration of the possible interfering compound to a standard solution containing sulphites 10 mg L^{-1} (as SO_2). The percentages of error were evaluated, and the results showed in Table 4.2. It was found that the signal of solution after adding each interference at the studied concentration, the error of each was less than 5%, excluding nitrite and sulphide. The concentration of nitrite and sulphide ion with 10 mg L^{-1} affected to signal of sulphite with more than 5 % error. Nitrite is commonly used as an antimicrobial and color enhancer and sulfides can be generated by bacteria in food (Zaruba et al., 2016) which nitrite can oxidize sulfite to sulphate. The reason is that SO_2 decreases its negative error.

In other hand, the sulphide ion presented a positive error due to a double displacement reaction between sodium sulphide and acid to produce H_2S gas that could reduce Fe(III) to Fe(II) as same as SO_2. Fortunately, conventional wines presented nitrite and sulphide of less than 10 mg L^{-1}.

Table 4.2

The interference studies.

Interference	Concentration (mg L^{-1})	% error
Glucose	1000	-4.1
Fructose	1000	1.0
Sucrose	1000	1.0
Oxalic acid	100	-3.1
Ascorbic acid	100	-1.1
Tartaric acid	100	-1.0
Boric acid	100	2.2
Carbonate	100	-4.1
Bicarbonate	100	-3.3
Nitrite	10	-9.1
Sulphide	10	6.3
Ethanol	20*	3.8

* % by volume

4.1.3 Sample analysis

The wine samples were studied including 7 bottles of white wine; 3 bottles of red wine and rose wine; and 3 bottles of sparkling wine. The amount of sulphite in these samples was analyzed in form of SO_2 by the proposed method compared to the ripper method. It was found that there was no significant difference between the results analyzed by the proposed method and ripper methods at $P=0.05$, and the t_{stat} value was 0.20, whereas t_{crit} is 2.18. The results were shown in Table 4.3. Almost samples contained sulphites of more than 10 mg L^{-1}(as SO_2), and those samples

specified the label "contain sulphites". The regression for comparing the results from both methods showed excellent correlation with the equation, y =1.0258x – 0.4435 and R^2 0.9923, presenting the good agreement with the results of both methods.

Table 4.3

Comparison of method for determination of sulphites in wine samples (n=3).

Sample	SO_2 (mg L^{-1})	
	The proposed method	Ripper method
White wine 1	6.45 ± 0.12	6.03 ± 0.18
White wine 2	12.28 ± 0.55	11.94 ± 1.19
White wine 3	38.00 ± 0.65	39.40 ± 1.19
White wine 4	39.42 ± 0.71	41.57 ± 1.53
White wine 5	35.62 ± 0.71	34.42 ± 0.92
White wine 6	40.53 ± 0.58	40.96 ± 0.73
White wine 7	15.65 ± 1.53	12.79 ± 0.71
Red wine 1	1.69 ± 1.53	1.37 ± 1.10
Rose wine 1	23.87 ± 1.41	25.1 ± 0.98
Rose wine 2	20.66 ± 1.15	19.44 ± 0.92
Sparkling 1	3.06 ± 0.38	3.92 ± 0.76
Sparkling 2	9.43 ± 0.36	10.11 ± 0.43
Sparkling 3	13.76 ± 0.53	14.32 ± 0.82

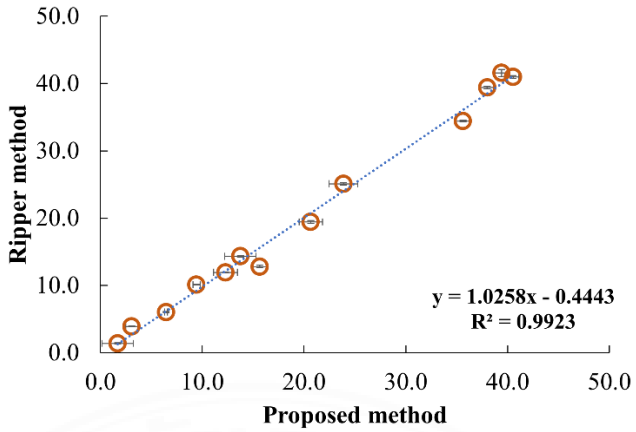

Figure 4.10 Amounts of sulphites determined by the proposed method compared to the standard ripper method.

4.1.4 Analysis of wine sample using various brands and lots of smartphone

Eleven Android phones of different models and brands were used to test the universal clamp sample holder and the proposed analytical method. Sulphites in white wine samples were measured in seven replicates. Although the phone camera was located at various places (e.g., toward a corner or at the middle of the top edge), The clamp can be used to fix at camera of these smartphones. We were able to clip the clamp holder onto all the smartphones. The sulphite analysis results are shown in Figure 4.11. From the one-way analysis of variance, there was no significant difference between the amount of sulphites determined by various smartphones, with an F-value of 1.58 (F-critical = 1.98, $\alpha = 0.05$).

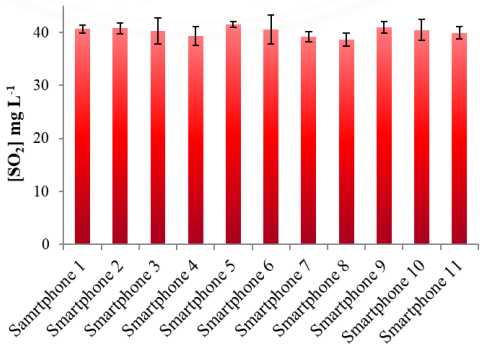

Figure 4.11 Amounts of sulphites in the same wine sample were determined using different smartphones.

4.2 Microfluidic system for sulphites analysis

The automatic flow-based analysis via gas diffusion unit was used throughout this part. based on three reactions as same as the system in section 4.1.

4.2.1 Optimization

4.2.1.1 Extraction time

After the 10 mg L^{-1} sulphite standard solution reacts with hydrochloric acid in a donor stream, sulfur dioxide gas was diffused through membrane and dissolved in an acceptor stream, then Fe(III) was reduced to Fe(II). The time of gas diffusion was studied by varying the time of stopping (at 0, 2, 4, 6, 8, 10, 12, 14, and 16 min), after propelling standard solution into the donor stream. The longer time stopping , the higher signal was obtained until 8 min, after that the signal was relatively stable (Figure 4.12). However, the stopping time was set at 5 min to compromise between sensitivity and speed of analysis.

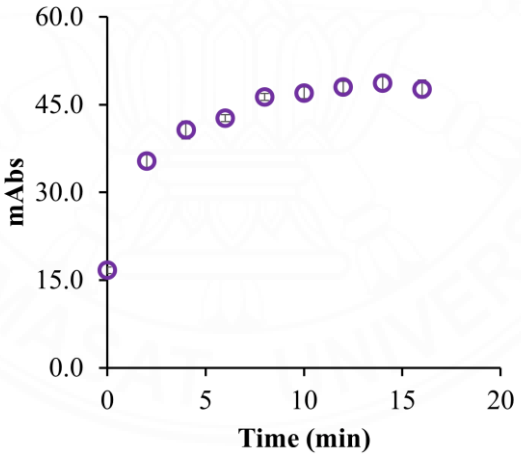

Figure 4.12 Effect of gas diffusion

4.2.1.2 Mixing coil

The effects of various mixing coil lengths were studied at 0, 10, 15, 25, 50, and 100 cm for mixing between the diffused SO_2 and colorimetric reagent. Although increasing the length of the coil gets the better mixing, more dispersion will

be obtained. This leads to larger peak but lower peak height.In addition, the analysis time has also been increased. So, this work selected the highest absorbance of red complex was obtained with 10 cm of coil length (Figure 4.13).

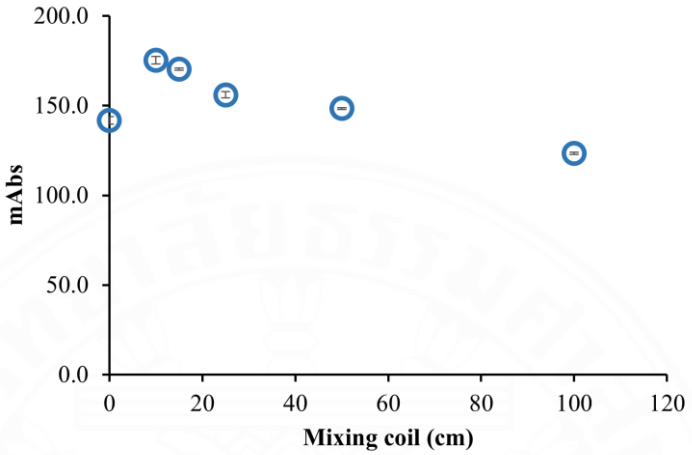

Figure 4.13 Effect of mixing coil length

4.2.1.3 Concentration of HCl

Hydrochloric acid solution influences the conversion of sulphites to sulfur dioxide. According to the literature review, sulfur dioxide species thrive at pH < 1 (Natalie Chiaverini and Tom Mortier, 2015). Therefore, the acid concentration at 0.05, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, and 4.00 mol L^{-1} was studied by reacting with sulphites. At higher concentrations of HCl, a higher absorbance of red complex was presented, which related to the suitable pH of sulphites conversion as shown in Figure 4.14. Therefore, the acid concentration of 1.5 mol L^{-1} was chosen for this research.

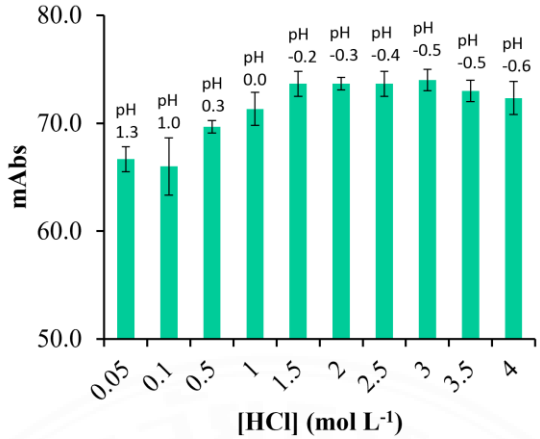

Figure 4.14 Effect of concentration of HCl

4.2.1.4 Concentration of Fe(III) nitrate

The concentration of $Fe(NO_3)_3$ in colorimetric reagent at 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol L-1 were studied. It was found that the absorbance increased until the concentration was 0.4 mmol L^{-1}, after which it is relatively stable (Figure 4.15). For selecting the optimal condition, not only sensitivity but also robustness need to be considered. Therefore, the optimal signal detection was 0.6 mmol L^{-1} of $Fe(NO_3)_3$.

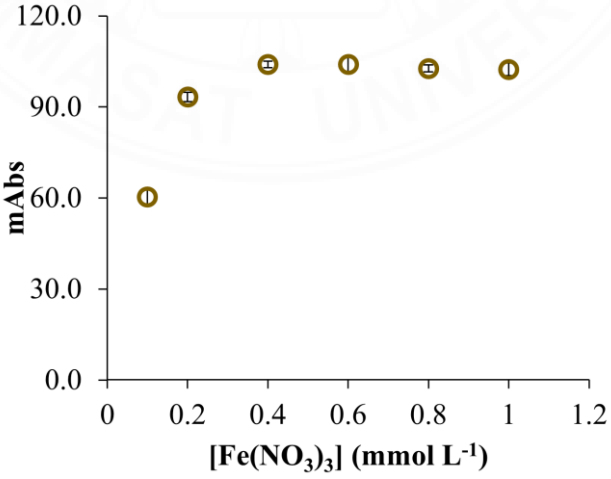

Figure 4.15 Effect of concentration of $Fe(NO_3)_3$ on acceptor line

4.2.1.5 Concentration of 1,10-phenanthroline

The concentration of 1,10-phenanthroline in colorimetric reagent at 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 22.0, 24.0, and 26 mmol L^{-1} were studied and the result is shown in Figure 4.16. At higher concentrations of this reagent involved, a higher signal was obtained until at 22 mmol L^{-1}. Therefore, this concentration was selected.

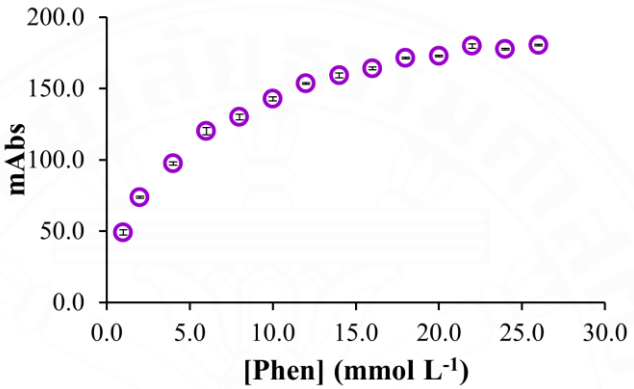

Figure 4.16 Effect of concentration of 1,10-phenanthroline

4.2.1.6 pH of colorimetric reagent

The colorimetric reagent at pH buffer 3.0, 4.0, 5.0, 5.5, 6.0, and 9.0 were studied and the result is presented in Figure 4.17. The graph showed that the intensity slightly increased up to pH 5.5 after that it decreased. At higher pH, the solution would contain higher amount of hydroxide ion that may be caused precipitation of $Fe(OH)_3$, especially in alkaline solution. It is corresponding to the stability of complex. In addition, the reduction of Fe^{3+} is suitable in acidic solution.

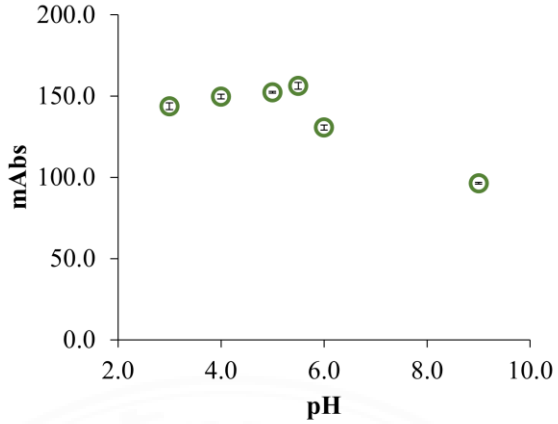

Figure 4.17 Effect of pH colorimetric reagent

4.2.2 Analytical performance

4.2.2.1 Standard curve, LOD and LOQ

Under the optimized experiment, the standard curve of the method was studied. At concentration range 1.0-10.0 mg L^{-1}, the calibration obeyed Beer-Lambert's Law, with linear equation y = 10.064x + 44.164 and R^2 = 0.9843 However, the method showed non-linear calibration graph in polynomial the range of 1.0-35.0 mg L^{-1} with equation y= -0.1354x^2+14.766x+21.755 R^2 = 0.9991. The results are presented in Figure 4.18. The system involved not only complex formation and reduction of Fe^{3+}, but also the microextraction with gas diffusion unit that may be led to the non-linear calibration at higher concentrations. In addition, this phenomenon may be caused by dispersion in FIA that presented the wider peak but the height is slightly increase at higher concentration range.

The LOD and LOQ defined as 3 and 10 times the standard deviation of the blank, were calculated to be 0.32 and 1.08 mg L^{-1} as SO_2 respectively.

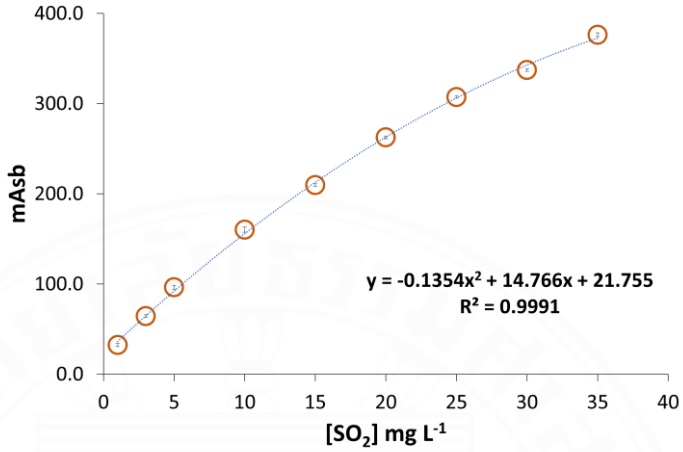

Figure 4.18 Non-linearity calibration curve in the range of 1.0-35.0 mg L^{-1} (as SO_2)

4.2.2.2 Recovery

Wine and dried fruit samples were chosen to evaluate the accuracy of this proposed method for the analysis of sulphite. The recoveries were performed by spiking standard sulphite 0.3, 1.0 and 10.0 mg L^{-1}. The results are shown in the Table 4.4, the percentage recoveries in the range 94.4-107.7 of SO_2 (N=3).

Table 4.4

Recovery of the method for sulphites analysis

Sample	Added (mg L^{-1} as SO_2)	Found (mg L^{-1} as SO_2)	Recovery (%)
White wine	0.30	0.30 ± 0.001	99.0
	1.00	1.03 ± 0.004	103.0
	10.00	9.87 ± 0.002	98.7
Dried fruit	0.30	0.32 ± 0.001	107.7
	1.00	0.97 ± 0.001	97.3
	10.00	9.44 ± 0.004	94.4

4.2.2.3 Interference study

The study was carried out by adding a known concentration of the possible interfering compound to a standard solution containing sulphite 10 mg L^{-1} (as SO_2). The percentages of error were evaluated, and the results showed in table 4.5. It was found that the signal of solution after adding each interference at the studied concentration, the error of each was less than 5%, excluding nitrite and sulphide. The concentration of nitrite and sulphide ion with 10 mg L^{-1} affected to signal of sulphite with more than 5 % error. Nitrite is commonly used as an antimicrobial and color enhancer and sulfides can be generated by bacteria in food (Zaruba et al., 2016) which nitrite can oxidize sulfite to sulphate. This led to decreasing of SO_2 concentration and showing the negative error.

In the other hand, the sulphide ion presented a positive error due to a double displacement reaction between sodium sulphide and acid to produce H_2S gas that could reduce Fe(III) to Fe(II) as same as SO_2. Fortunately, conventional wines presented nitrite and sulphide of less than 10 mg L^{-1}

Table 4.5

The Interference studies

Interference	Concentration (mg L^{-1})	% Error
Glucose	1000	-2.9
Fructose	1000	-1.1
Sucrose	1000	-3.2
Oxalic acid	100	-3.2
Ascorbic acid	100	-3.2
Tartaric acid	100	-0.7
Boric acid	100	-2.1
Carbonate	100	3.0
Bicarbonate	100	-2.6
Nitrite	100	-70.3
Sulphide	10	30.5
Ethanol	10*	-4.6

* % by volume

4.2.3 Sample analysis

The developed flow-injection system was introduced. to find the sulfite content in wine, coconut water, and dried fruit sample that are sold in general department stores in Thailand. All samples were also analyzed by the Ripper method Therefore, the results of both methods of analysis were used statistically assessed by paired t-test, it was found that the values obtained from the developed method gave no significant difference to the Ripper method at 95 % confidence level (t_{stat} 0.05 < t_{crit} 2.57), as summarized in Table 4.6

Table 4.6

Comparison of method for determination of sulphites in samples (n=3)

Sample	SO_2 (mg L^{-1})	
	The proposed method	The Ripper method
White wine 1	8.20±0.003	8.68±0.01
White wine 2	6.62±0.004	6.52±0.03
White wine 3	10.32±0.003	9.87±0.04
Sparkling wine	7.20±0.003	7.23±0.02
Coconut water	5.51±0.003	5.25±0.04
Dried fruit	5.26±0.002	5.52±0.04

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

This research has focused on the design and invention of two sulphite analysis systems as followed: (1) lab on a cotton swab with smartphone detection and (2) automate microfluidic system with gas diffusion for sulphites analysis.

First, a headspace microextraction (HS-ME) was studied for sample pre-treatment and determination of sulphite. The method is based on 3 reactions; 1) conversion of sulphite to volatile sulphur dioxide (SO_2) by acidification in the 1.5-ml vial, 2) reduction of Fe(III) to Fe(II) by SO_2 and 3) complex formation of Fe(II)-phenanthroline. The last two reactions were demonstrated on the cotton swab and the red color was observed and detected by a universal clamp sample holder and the colorimetric Sensor mobile application was presented as a versatile tool for colorimetric analysis with smartphones. The app colorimetric application was used to evaluate the intensity of red product color related to the amount of sulphite in the samples. The developed system provided a linear calibration plot for sulphite 0.32-2.40 mg L^{-1} with R^2 0.9997 and 2.40-24.00 mg L^{-1}(as SO_2) with R^2 0.9936. The LOD and LOQ of the proposed method were 300 and 600 μg L^{-1}, respectively, and recoveries were 90.5-107.8 %. This method was applied to the determination of sulphites in wines from a supermarket in Thailand. The result obtained by the proposed method was compared to the Ripper method, there was no significant difference at the 95% confidence level. Moreover, the microfluidic cotton swab-based analytical device (μCSAD) device and head space microextraction have the major advantages of eco-friendliness, simplicity, and portability.

Second, a flow-based analysis via a microflow gas diffusion system with an optical sensor. A gas diffusion unit (GDU) was printed using 3D printing which used to prevent interference from sample matrices. The sulphite was mixed with HCl stream in the donor line of GDU, then generated sulfur dioxide gas (SO_2) and diffused through the PTFE hydrophobic membrane into a colorimetric reagent in the acceptor line. The colorimetric reagent consisted of Fe(III) and 1,10- phenanthroline. The Sulfur dioxide can reduce Fe(III) to Fe(II), then Fe(II)-phenanthroline complex was formed and

detected with optical sensor (LED 515 nm and photodiode). The calibration standard of sulphite was non-linear in the range of 1.00-35.00 mg L^{-1} with equation $y= -0.1354x^2+14.766x+21.755$ $R^2 = 0.9991$. The LOD and LOQ of the proposed method were 0.32 and 1.08 mg L^{-1} as SO_2, respectively. and recoveries were 94.4-107.7%. This method was applied to the determination of sulphites in wines and dried fruit from a supermarket in Thailand. The result obtained by the proposed method was compared to the Ripper method, there was no significant difference at the 95% confidence level. In the proposed method, no complicated instrument and highly skilled operator were needed, that led the method be simple and miniaturized. In addition, the method can avoid interference in samples by using microflow gas diffusion.

The analytical features of two proposed method were compared to another methods as presented in Table 5.1. The proposed methods were not presented the lowest detection limits, however, the LOQs are low enough for analysis sulphites in food samples. In addition,the proposed methods present wider ranges of linearity as compared to capillary electrophoretic (CE), electrochemical sensor and optical probe. Although the methods are narrower range than LC-MS and ion-exchange chromatography with conductivity detection, it is better cost consumption for analytical laboratory, especially in small and medium company

Both proposed systems are designed to use in different purposes, the μCSAD via smartphone detection is for field analysis and suitable for local entrepreneurs, however, the automatic sulphite analyzer is set in laboratory and suitable for industry or company.

Table 5.1

Various method for determination of sulphites

Author (Year)	Sample	Detection method	Range	LOD
(Bener et al., 2020)	Wine and Vinegar	UV-vis spectrophotometry	0.32-19.08 mg L^{-1} SO_2	0.084 mg L^{-1}
(Kraikaew et al., 2019)	White wine	On-line cone reservoirs membrane less gas-liquid separation flow system with conductivity detector (C4D).	8-160 mg L^{-1} SO_2	–
(Norouzi & Parsa, 2018)	Weak liquor	Electrochemical Sensor Based on Ni/Poly (4-Aminobenzoic Acid)/Sodium Dodecyl sulfate/Carbon Paste Electrode	0.08–0.8 and 0.8-8 mg L^{-1} SO_3^{2-}	0.04 mg L^{-1}
(Zaruba et al., 2016)	Wine, Jam, and Juice.	Optical Probe as the Microdrop Holder in Headspace Single Drop Microextraction	0.032−0.320 mg L^{-1} SO_2	0.008 mg L^{-1}
(Katherine S. Robbins et al., 2015)	dried fruits, vegetables, frozen seafood, sweeteners, and juices.	Liquid Chromatography−Tandem Mass Spectrometry	0.25−114 mg L^{-1} SO_2	–
(Centonze et al., 2009)	Fresh meats and shrimps	Ion-exchange chromatography with conductivity detection	8.2–160 mg kg^{-1} SO_2	2.7 mg kg^{-1}

Table 5.1

(Continued)

Author (Year)	Sample	Detection method	Range	LOD
(Jankovskiene et al., 2001)	Wine	Capillary electrophoretic (CE) method with UV-Vis spectrophotometry	0.8-6.4 mg L^{-1} SO_3^{2-}	0.16 mg L^{-1}
(Lowinsohn & Bertotti, 2001)	Wine	Coulometric titration	25.6-76.8 mg L^{-1} SO_2	0.64 mg L^{-1}
(Yamada et al., 1983)	–	Flow injection system with chemiluminescent detection	0.9-35 ng SO_3^{2-}	0.9 ng
Part 4.1	Wines	Lab on cotton swab via sample clamp holder	0.32-2.40 mg L^{-1} and 2.40-24.00 mg L^{-1} SO_2	300 μg L^{-1}
Part 4.2	Wines and dried fruit	A flow-based analysis via a microflow gas diffusion system	1.0-35.0 mg L^{-1}	0.32 mg L^{-1}

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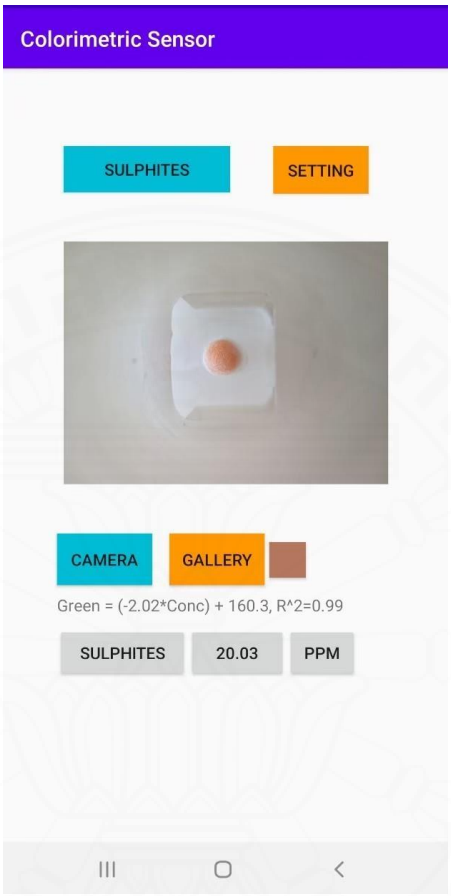
APPENDICES

APPENDIX A

The screen of colorimetric sensor application



APPENDIX B

t-test of lab on cotton swab and Ripper methods

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	20.03226	20.10538
Variance	201.40078	213.57404
Observations	13	13
Pearson Correlation	0.996164	
Hypothesized Mean Difference	0.00	
df	12	
t Stat	-0.198188	
P(T<=t) one-tail	0.423108	
t Critical one-tail	1.78229	
P(T<=t) two-tail	0.846216	
t Critical two-tail	2.17881	

Therefore, t_{cal} (0.20) < t_{crit} (2.18), no significant difference at the 95% confidence level between lab cotton swab and the Ripper method, analyzed by t-test.

APPENDIX C

t-test of microfluidic system and Ripper methods

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	7.178749	7.185167
Variance	3.282437	3.538236
Observations	6	6
Pearson Correlation	0.983679	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.04615	
P(T<=t) one-tail	0.48249	
t Critical one-tail	2.01505	
P(T<=t) two-tail	0.96498	
t Critical two-tail	2.57058	

Therefore, t_{cal} (0.05) < t_{crit} (2.57), no significant difference at the 95% confidence level between microfluidic system and the Ripper method, analyzed by t-test.

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Publications

Khamkhajorn, C.; Pencharee, S.; Jakmunee, J.; Youngvises, N., Smartphone-based colorimetric method for determining sulfites in wine using a universal clamp sample holder and microfluidic cotton swab-based analytical device. *Microchemical Journal* 2022**,** *174*, 107055. Doi: https://doi.org/10.1016/j.microc.2021.107055

Presentation

Khamkhajorn C., Pencharee S., Jakmunee J., and Youngvises N. Analysis of Sulphite Based on Head Space Microextraction and Smartphone Detection. Poster presentation at Pure and Applied Chemistry International Conference (PACCON 2020), Bangkok, Thailand.

Khamkhajorn C., Suwanboriboon J., Meesiri W., and Youngvises N. An automatic sulphite analyzer based on microflow gas diffusion via optical. Poster presentation at Pure and Applied Chemistry International Conference (PACCON 2022), Bangkok, Thailand.