TWO APPROACHES OF DOWN SCALING OF COMPLEXOMETRIC TITRATION FOR MAGNESIUM CONTENTS DETERMINATION IN RUBBER LATEX: FIELD TEST KIT AND MICROFLUIDIC THREAD-BASED ANALYTICAL DEVICE (μTAD)

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Researcher

แนวทางที่สองเสนอการตรวจวัดแบบง่ายด้วยระบบของไหลจุลภาคที่ประดิษฐ์มาจากเส้นด้ายราคาประหยัดเพื่อใช้ในวิธีการวิเคราะห์หาปริมาณไอออนแมกนีเซียมในน้ำยาง โดยอาศัยปฏิกิริยาการไทเทรตสารประกอบเชิงซ้อนที่ใช้การตรวจวัดระยะทางของสีที่เปลี่ยนแปลงบนเส้นด้ายด้วยตาเปล่า อุปกรณ์ตรวจวัดนี้ประดิษฐ์จากการตัดเส้นด้ายชนิดคอตตอนที่ไม่มีการปรับปรุงพื้นผิวให้มีความยาว 15 เซนติเมตร ที่ตรึงอยู่บนเส้นด้ายบนอุปกรณ์รองรับ ในวิธีการทดสอบที่พัฒนาขึ้นนี้จะเกิดอันตรกิริยาระหว่างสารเคมีบนเส้นด้ายที่เคลือบไว้กับสารที่สนใจในสารตัวอย่างซึ่งจะเกิดระยะทางของสีที่เปลี่ยนแปลงไปของเส้นด้ายภายในเวลาอันสั้น ระยะทางของสีที่เกิดการเปลี่ยนแปลงจะแปรผันตรงกับความเข้มข้นของสารที่สนใจในตัวอย่าง สำหรับการไทเทรตสารประกอบเชิงซ้อนนี้จะนำเสนอการวิเคราะห์หาปริมาณไอออนแมกนีเซียมในตัวอย่างน้ำยาง โดยเริ่มแรกเส้นด้ายจะถูกปรับสภาพด้วยสารอิริโครม แบลค ที จากนั้นนำเส้นด้ายสองเส้นไปปรับสภาพด้วยสารละลายกรดเอทิลีนไดอามีนเตตราอาเซติกที่ละลายในสารละลายบัฟเฟอร์เอ็น-ไซโคลเฮกซิล-3-อะมิโนโพรพานิลซัลโฟนิค เอซิด พีเอช 10 ที่ความเข้มข้นแตกต่างกัน เมื่อมานำเส้นด้ายสองเส้นมาผูกเพื่อสร้างปมตรงกลางระหว่างเส้นด้ายทั้งสอง ก่อนที่จะนำไปตรึงบนแผ่นรองรับ จากนั้นหยดสารละลายตัวอย่าง 6 ไมโครลิตรลงบนปมตรงกลาง โดยที่ระยะของสีม่วงที่เกิดขึ้นบนเส้นด้ายจะ แปรผันตรงกับความเข้มข้นของไอออนแมกนีเซียมในสารตัวอย่างที่ให้ช่วงของการตรวจวัดตั้งแต่ 25 ถึง 1000 มิลลิกรัมต่อลิตร นอกจากนี้ ได้ศึกษาผลของไอออนที่คาดว่ารบกวนที่อยู่ในตัวอย่างน้ำยางพบว่าแนวทางที่พัฒนาขึ้นมีความจำเพาะสูงและการวิเคราะห์ในตัวอย่างจริงพบว่าแนวทางที่นำเสนอขึ้นนี้มีความสอดคล้องกับวิธีการไทเทรตดั้งเดิม

ABSTRACT

TITLE : TWO APPROACHES OF DOWN SCALING OF COMPLEXOMETRIC TITRATION FOR MAGNESIUM CONTENTS DETERMINATION IN RUBBER LATEX: FIELD TEST KIT AND MICROFLUIDIC THREAD-BASED ANALYTICAL DEVICE (μTAD)

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KEYWORDS : RUBBER LATEX (RL), MAGNESIUM IONS (Mg^{2+}), COMPLEXOMETRIC TITRATION, FIELD TEST KIT, MICROFLUIDIC THREAD-BASED ANALYTICAL DEVICE (μTAD)

First approach, a simple, low-cost and portable field test kit based on colorimetry with detection by naked eye was developed for determination of magnesium content in rubber latex (RL). The miniaturized complexometric titration between Mg^{2+} and EDTA without any masking agent was a key reaction in this development, which was designed according to the concept of green chemistry by reduction of waste generation and chemical and time consumption. The system enabled quantification of magnesium content in RL at low concentration with the detection limit being <50 mg L^{-1}, small sample volume uptake (0.18 g, sampling by a small spoon) and use of <1.5 mL reagent volume which was >70 times less than that applied in the conventional method. Moreover, with the presence of potential interference ions, greater selectivity towards magnesium was observed. Furthermore, the reagents used in our developed test kit were stable for >6 months at room temperature. The results obtained on real samples were in agreement with those obtained from the conventional complexometric titration (ISO 17403:2014(E))

method. The proposed technique provides a low-cost, rapid, simple, selective and on-site analysis of magnesium content in RL.

Second approach describes a simple complexometric titrations for analytical detection by measuring the length of color change on indicator treated thread. A novel thread-based analytical device (μTAD), fabricated from 15 cm of untreated cotton thread, provide an easy-to-use platform for the rapid measurement of analyte concentrations in aqueous solutions. Threads was fixed to a box platform to allow capillary wicking of liquid samples, free from contact with outside surfaces. In this method, interaction between deposited reagents and analytes within samples produces colored zones of differing lengths on the threads within only a few minutes. The length of the colored zones analyzed by unaided human eyes using the printed scales correlates with the concentrations of the analytes in the samples. Complexometric titration using μTAD was also proposed for Mg^{2+} monitoring in rubber latex samples. Threads were firstly pretreated with Eriochrome Black T (EBT). Two threads were then treated with two different concentrations of ethylenediaminetetraacetic acid (EDTA) in N-cyclohexyl-3-aminopropane sulfonic acid (CAPS) buffer at pH 10. They were then tied together to make a central knot before being affixed to a supporting of box platform for ready to analysis. 6 μL of sample solution was then applied to as prepared μTAD. The length of purple color product generating on thread was proportional to the concentration of Mg^{2+} in samples provided working concentration range of 25-1000 mg. L^{-1}. Greater selectivity toward Mg^{2+} compared with potential interference ions was examined. Furthermore, our developed μTAD was applied for analysis of real samples which showed results being in agreement with those obtained by classical titrations.

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CHAPTER 1
INTRODUCTION

1.1 The importance and source of this research

Para rubber tree (*Hevea brasiliensis* Muell. Arg.) (Figure 1.1) is a very important economic plant in Thailand where over three million tons a year of Rubber Latex (RL, the products of Para rubber tree) is exported [1, 2].

Figure 1.1 Para rubber tree (*Hevea brasilliensis*)

The major component in RL is cis-1, 4-polyisoprene with non-rubber components such as carbohydrates, proteins and lipid based medium for bacteria growing. pH values of normal RL solution range from 6 - 7 [3,4]. Under this condition, the surface of RL particles will become negatively charged due to the presence of carboxylate ions of protein (Alpha-globulin with pI of 4.8) and the hydrolysis of R-Lecithin phospholipid on the RL surface [4] (Figure 1.2).

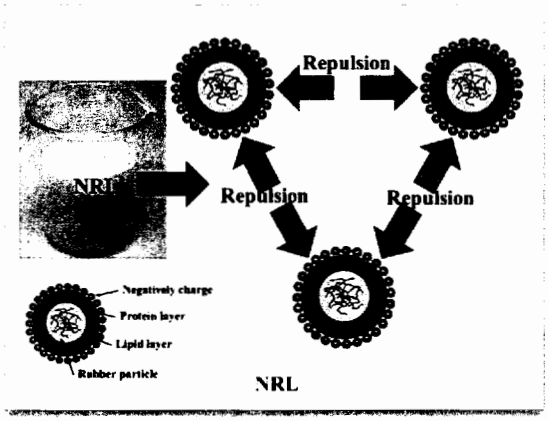


Figure 1.2 Carboxylate ions contained in the RL.

A critical step in production of RL with high quality is identification of RL components. Magnesium (Mg^{2+}) is one of the most important components suppressing RL performance and quality, *e.g.* by direct interaction of Mg^{2+} with the carboxylate ions contained in the RL. This produces insoluble, un-hydrated and un-ionized Mg^{2+} soap and insoluble Mg^{2+} hydroxide in the aqueous phase. Both of these phenomena invariably cause destabilization of RL. Furthermore, Mg^{2+} ions can form primary valence linkages between the interfaces of adjacent latex particles. This can initiate flocculation and further lead to destabilization of RL [5] Figure 1.3.

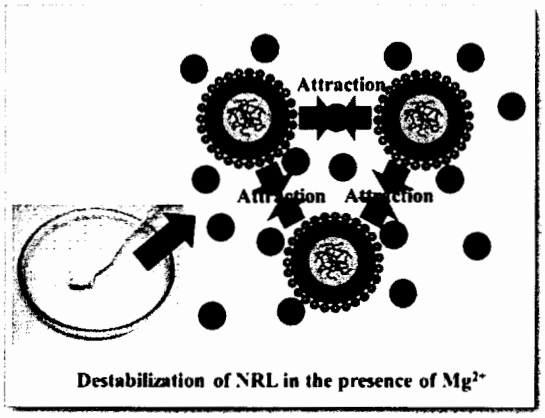


Figure 1.3 The mechanism between Mg^{2+} ions and RL.

Mg^{2+} concentration is limited to within 40 $mg.L^{-1}$ prior to distribution to manufacturers such as glove and condom companies [6]. Normally, removal of Mg^{2+} content in rubber is performed based on a precipitation reaction, *e.g.* by addition of excess amount of ammonium phosphate (>5 times of Mg^{2+} concentration) to rubber latex solution. Alternatively, diammonium hydrogen phosphate (DAHP) can be used for precipitation of Mg^{2+} with the related reaction shown in Eq. 1.1 [7-8] and Figure 1.4.

$$Mg^{2+}{}_{(aq)} + NH_{3(aq)} + HPO_4{}^{2-}{}_{(aq)} \rightarrow MgNH_4PO_{4(s)} \quad (1.1)$$

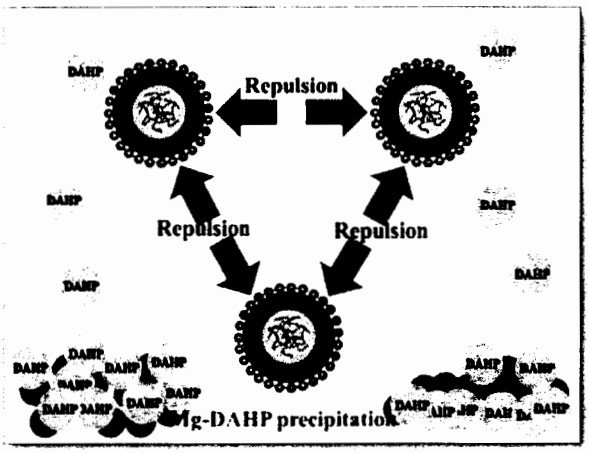


Figure 1.4 Elimination of Mg^{2+} by adding DAHP into RL.

A conventional approach involves Mg^{2+} analysis in RL based on the complexometric titration with ethylenediamine tetraacetic acid (EDTA, H_2Y^{2-}), disodium salt (soluble form) using eriochrome black T (EBT) as an indicator. Since dissociation of EDTA disodium salt and EBT depends on pH of the medium, addition of ammonium chloride (NH_4Cl)/ammonium hydroxide (NH_4OH) buffer solution is required in order to control pH of the solution to be $\geq$ 10 facilitating reaction between EBT and Mg^{2+}. At the end point of the reaction, the solution color changes from red to blue, according to the reaction shown in Eq. 1.2 [8] for Mg^{2+} analysis in RL.

$$MgIn^{-}_{(aq)} + Y^{4-}_{(aq)} + H_3O^{+}_{(aq)} \rightarrow MgY^{2-}_{(aq)} + HIn^{2-}_{(aq)} + H_2O_{(l)} \quad (1.2)$$

Apart from the well-controlled pH of the solution facilitating interaction between Mg^{2+} and EDTA, the masking agent is also added to prevent foreign ions, such as potassium (K^{+}), sodium (Na^{+}), calcium (Ca^{2+}), zinc (Zn^{2+}), iron (Fe^{3+}), copper (Cu^{2+}) and manganese (Mn^{2+}) interfering complexation between Mg^{2+} and EDTA in RL. Potassium cyanide (KCN) is a common masking agent used in the standard method [9-10]. However, it is well known that cyanide compounds are very toxic. Therefore, development of a cyanide-free method for determination Mg^{2+} in RL is still a challenge.

The first approach, a simple (no requirement of skill for analysis, not demanding sample pretreatment before analysis), low-cost (small sample and reagents volume uptake) and portable field test kit based on colorimetry using naked eye for determination of Mg^{2+} content in rubber latex (RL) was established. The novelty of this work is that miniaturized complexometric titration between Mg^{2+} and EDTA (even without using masking agent) which were designed according to the concept of "Green chemistry" reducing waste generation minimizing use of chemicals and consumption of time (at least simple two reagents (EBT indicator for reagent A; EDTA in ammonium buffer for reagent B) for test kit set up). The developed test kit will be applied for investigation of the effect of the presence of potential interference ions, preservatives used in RL and possibility for practical use in concentrated rubber latex (CRL). Stability of reagents applied in the kit and the analysis performances in real samples will be investigated and discussed.

Moreover, novel concept and application of low-cost, portable and field-based using microfluidics thread based analytical device (μTAD) will also be established using naked eye for determination of Mg^{2+} content in RL as second approach. Complexometric titrations for analytical detection by measuring the length of color change on indicator treated thread. A novel thread-based analytical device (μTAD), fabricated from 15 cm of untreated cotton thread, provide an easy-to-use platform for the rapid measurement of analyte concentrations in aqueous solutions. Threads were fixed to a box platform to allow capillary wicking of liquid samples. In this method, interaction between deposited

reagents and analytes within samples produces colored zones of differing lengths on the threads within only a few minutes. The length of the color zone analyzed by unaided human eyes using the printed scales correlates with the concentrations of the analytes in the samples. Complexometric titration using μTAD was proposed for Mg^{2+} monitoring in RL samples. Threads were firstly pretreated with EriochromeBlack T (EBT). Two threads were then treated with two different concentrations of Ethylenediaminetetraacetic Acid (EDTA) in N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer at pH 10. They were then tied together to make a central knot before being affixed to a box platform for ready to analysis. 6 μL of sample solution was then applied to as prepared μTAD. The length of purple color product generating on thread was proportional to the concentration of Mg^{2+} in samples.

1.2 Objective

1.2.1 To develop a simple test kit based on colorimetry for quantification of Mg^{2+} content in RL by miniaturized complexometric titration without using masking agent.

1.2.2 To develop a low cost microfluidic thread-based analytical device (μTAD) for determination of Mg^{2+} content in RL.

1.3 Research scope

1.3.1 First approach; the development of a simple test kit based on colorimetry for quantification of Mg^{2+} content in RL by miniaturized complexometric titration without using masking agent.

This part involves the development of a simple, low-cost and portable field test kit based on colorimetry with detection by naked eyes for determination of Mg^{2+} content in rubber latex (RL). The miniaturized complexometric titration between Mg^{2+} and EDTA without any masking agent was a key reaction in this development, which was designed according to the concept of green chemistry by reduction of waste generation and chemical and time consumption which will provide a low-cost, rapid, simple, selective and on-site analysis of Mg^{2+} content in RL. The following parameter will be exanimated;

1.3.1.2 Optimization of the developed test kit

1) Optimization of sample and reagent volume

2) Optimization of condition for reagent A and B

1.3.1.3 Validation method of the developed test kit

1.3.1.4 Study of interferences our developing test kit

1.3.1.5 Study of preservatives in RL on our developing test kit

1.3.1.6 Stability test for reagents and application of the developed test kit for practical sample analysis

1.3.1.7 Study of concentrated rubber latex (CRL) using our developed test kit

1.3.2 Second approach; The development of a low cost microfluidic thread-based analytical device (μTAD) for determination of Mg^{2+} content in RL.

A novel concept and application of low-cost, portable and field-based using microfluidics thread based analytical device (μTAD) will also be established based on colorimetry using naked eye for determination of Mg^{2+} content in RL. Complexometric titrations for analytical detection by measuring the length of color change on indicator treated thread. A novel thread-based analytical device (μTAD), fabricated from 15 cm of untreated cotton thread, provide an easy-to-use platform for the rapid measurement of analyte concentrations in aqueous solutions. Threads was fixed to a supporting polypropylene sheet to allow capillary wicking of liquid samples. In this method, interaction between deposited reagents and analytes within samples produces colored zones of differing lengths on the threads within only a few minutes. The length of the colored zones analyzed by unaided human eyes using the printed scales correlates with the concentrations of the analytes in the samples. Complexometric titration using μTAD will also proposed for Mg^{2+} monitoring in RL samples. Threads were firstly pretreated with EriochromeBlack T (EBT). Two threads were then treated with two different concentrations of ethylenediaminetetraacetic acid (EDTA) in CAPS buffer at pH 10.
They were then tied together to make a central knot before being affixed to a supporting polypropylene sheet for ready to analysis. 6 μL of sample solution was then applied to as prepared μTAD. The length of purple color product generating on thread was proportional to the concentration of Mg^{2+} in samples. The following parameters will be examined;

1.3.2.1 Validation of our developed preparation of RL sample for proposed μTAD operated by flame atomic absorption spectrophotometer (FAAS)

1.3.2.2 Study of characteristics of thread

1.3.2.3 Study of wicking property of threads

1.3.2.4 Optimization of reagent conditions and their mechanism on μTAD

1.3.2.5 Effect of reagent volume on our developed μTAD

1.3.2.6 Study of interferences on developed μTAD

1.3.2.7 Analytical characteristics and method validation of developed μTAD for Mg^{2+} detection in RL

1) Study of working range and Limit of quantification (LOQ)

2) Study of validation of develop μTAD and real sample application

1.4 Research site

Department of Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani, Thailand.

CHAPTER 2
LITERATURE REVIEWS

2.1 Conventional method for determination of magnesium ions in RL

A conventional approach involves Mg^{2+} analysis in RL based on the complexometric titration with ethylenediamine tetraacetic acid (EDTA, H_2Y^{2-}), disodium salt (soluble form) using eriochrome black T (EBT) as an indicator. Since dissociation of EDTA disodium salt and EBT depends on pH of the medium, addition of ammonium chloride (NH_4Cl)/ammonium hydroxide (NH_4OH) buffer solution is required in order to control pH of the solution to be $\geq$ 10 facilitating reaction between EBT and Mg^{2+}. At the end point of the reaction, the solution color changes from red to blue, according to the reaction shown in Eq. 1.1 and 1.2 [8]. Apart from the well-controlled pH of the solution facilitating interaction between Mg^{2+} and EDTA, the masking agent is also added to prevent foreign ions interfering complextion between Mg^{2+} and EDTA in RL.

In 1998, Karunanayake et al. [9-10] described a method used for determination Mg^{2+} contents in RL by complexometric titration and used Potassium cyanide (KCN) is a common masking agent used in the standard method. However, it is well known that cyanide compounds are very toxic. Therefore, development of a cyanide-free method for determination Mg^{2+} in RL is still a challenge.

In 2008, Satheinperakul et al. [11] methods for magnesium determination in natural rubber latex based on the potentiometric titration with a Hg-EDTA electrode. Masking agents were not applied since they disturbed the end point of the titration. Their methods showed linearity range for Mg^{2+} detection from 36 to 126 mg L^{-1}, which is in good agreement with the results obtained from atomic absorption spectrometry. Unfortunately, they found that the presence of zinc and cadmium at high levels interfered with the determination of Mg^{2+}.

In 2011, Cheewasedtham et al. [12] a method and composition for quantifying magnesium ions based on conventional complexometric titration was patented (WO2011139245) using NaHS as a selectively precipitating interfering metal ions in RL instead of using KCN as mentioned above.

In 2014, ISO 17403:2014(E) [13-15] Rubber-Determination of magnesium content of field and concentrated natural rubber lattices by titration (cyanide free method by using NaHS as a masking agent) was established.However, the reported approaches are still complicated in terms of multiple steps of analysis including need for sample pretreatment before analysis, reagent volume consumption (>105 mL), resulting in more waste and potential interference ions in RL if a masking agent was not applied (Table 2.1). Moreover, many reagents are needed for analysis which also requires skill to perform in the laboratory.

Therefore, in this approach a simple, low-cost and portable field test kit based on colorimetry with detection by naked eyes for determination of Mg^{2+} content in rubber latex (RL) was developed based on a miniaturized complexometric titration between Mg^{2+} and EDTA without using masking agent.

Table 2.1 Selectivity study towards Mg^{2+} compared with the other metal ions (Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, K^{+} and Na^{+} tested using complexometric titration reported in the tolerance concentration ($mg.L^{-1}$) (n=3).

Interference	Tolerance concentration ($mg.L^{-1}$)	With masking agent			Without masking agent		
		Mg^{2+} content ($mg.L^{-1}$)		% Relative different	Mg^{2+} content ($mg.L^{-1}$)		% Relative different
		1st collection	2nd collection		1st collection	2nd collection	
Original NRL	-	524.02 ± 11.89	256.16 ± 2.99	-	534.81 ± 17.40	289.87 ± 23.37	-
Zn^{2+}	793.62	470.60 ± 2.85	-	-9.86	611.60 ± 2.47	-	+14.36
Ca^{2+}	955.82	545.10 ± 3.72	-	+4.02	565.40 ± 7.03	-	+5.72
Fe^{3+}	157.52	-	272.60 ± 1.26	+6.42	-	315.20 ± 7.13	+8.74
Cu^{2+}	244.14	-	232.50 ± 1.53	-9.23	-	N.D.	-
Mn^{2+}	53.93	-	267.90 ± 0.77	+4.58	-	330.90 ± 3.48	+14.15
K^{+}	2171.29	-	235.20 ± 7.16	-8.18	-	228.90 ± 23.32	-21.03
Na^{+}	1356.97	-	239.50 ± 2.72	-6.50	-	225.50 ± 7.63	-22.21

U_3 was used as model sample in this study

N.D. (Not detectable; end point of the titration cannot be accomplished)

Due to the NRL can be stable around 4 hours, therefore, NRL is needed to collect several time to accomplish the experiment

$$\%\text{Relative different} = \left[\frac{Mg^{2+}\text{contents from original NRL} - Mg^{2+}\text{contents from added concentration of foreign ions})}{Mg^{2+}\text{contents from original NRL}}\right] \times 100$$

2.2 Test Kit based colorimetry for determination of Mg^{2+} contents in various samples (First approach)

Two types of test kit for Mg^{2+} detection based on colorimetry have been commercially available.

In 2013, Sigma Aldrich's company [16] proposed the type of the commercial test kit is based on an enzymatic assay performed in a 96 well flat-bottom plate coupled with spectrophotometer detection at 450 nm . The assay involves specific interaction between glycerol kinase enzyme and Mg^{2+} which results in a linear range of 1.5-7.5 $mg.L^{-1}$ without interference from foreign ions such as Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+} and Mn^{2+}.

In 2016, Marine Depot's company [17-20] have reported the field test kit in seawater based on complexometric titration without addition of masking agents. A limit of detection (LOD) is found within the range of 15-100 $mg.L^{-1}$. Moreover, interference from calcium and strontium was not observed. However, the approach as mentioned above is costly and requires expertise to perform (Table 2.2).

Table 2.2 Comparison of commercial test kit for Mg^{2+} analysis of various samples.

Products	Sample	Limit of detection ($mg.L^{-1}$)	Cost/test (USD)	Reference
A	Sea water	20	0.3699	[15]
B	Sea water	30	0.3922	[16]
C	Sea water	100	0.5198	[17]
D	Sea water	15	0.3998	[18]
E	Variety of samples	1.5	4.6050	[19]
F	RL, CRL	< 50	0.1620	Proposed method

Recently, novel microfluidic thread-based analytical devices (μTAD) have been developed due to the platform is capable if transporting aqueous and non-aqueous fluids via capillary action and possess desirable properties for building fluid transport pathway in microfluidic devices [21].Capillary channels for liquid transport provided by the fiber gaps between thread: avoid the requirement of external power sources or pumps (The capillary flow within strand of a thread is effectively confined to 1D, because the aspect ratio (length: diameter) of thread is high). Moreover, Thread enables us to fabricate 3D thread-based microfluidic device which encompass complicated microfluidic channel compared to 2D paper-based assay lateral flow system. It can be manipulated easily by globally used process such as sewing, knitting and weaving. These techniques could in principle be exploited for mass production of μTAD[22]. Threads can be swan onto various support materials to form fluid transport channels without the need for the patterned hydrophobic barriers essential for paper-based microfluidic devices[23]. Furthermore, threads offer more choices of materials than paper. Although paper can be formed using fibers other than cellulose, it would be more expensive and difficult to make with the current paper making technology [24]. These advantages allow μTAD to be portable analysis with low cost, low-volume and easy-to-use with the potential to be applied in many disciplines such as agricultural and environmental monitoring analysis. Development and application of μTAD are thus considered to be a key target.

2.3 Microfluidic thread-based analytical device, μTAD and its applications (Second approach)

2.3.1 μTAD based on electrochemical detection

In 2012, Wei et al.[25] a novel detection system with a low-cost thread-based microfluidic device for CEEC analysis was successfully demonstrated. Instead of using liquid channel for sample separation, thin polyester threads of various diameters are used as the routes for separating the samples with electrophoresis. Hot-pressed PMMA chip with protruding sleeper structures are adopted to set up the polyester threads and for

electrochemical detection of the ion samples on the thread. Plasma treatment greatly improves the wetability of thin threads and surface quality of the threads. The measured electrical currents on plasma treated threads are 10 times greater than the threads without treatment. Results indicate that nice redox signals can be obtained by measuring ferric cyanide salt on the polyester thread. The estimated detection limit for EC sensing of potassium ferricyanide ($K_3Fe(CN)_6$) is around 6.25 μM using the developed thread-based microfluidic device for determination of mixed ion samples (Cl^-, Br^- and I^-) and bio-sample in industrial or environmental applications, respectively.

In 2015, Agustini et al.[26] proposed the construction of a low cost microfluidic thread-based electroanalytical device (μTED), employing extremely cheap materials and manufacturing process free of equipments. The microfluidic channels were built with cotton threads and the estimated cost per device was only $ 0.39. The flow of solutions (1.12 μL s^{-1}) is generated spontaneously due to the capillary forces, eliminating the use of any pumping system. To demonstrate the analytical performance of the μTED, a simultaneous determination of acetaminophen (ACT) and diclofenac (DCF) was performed by multiple pulse amperometry (MPA). A linear dynamic range (LDR) of 10 to 320 μmol L^{-1} for both species, limit of detection (LOD) and limit of quantitation (LOQ) of 1.4 and 4.7 μmol L^{-1} and 2.5 and 8.3 μmol L^{-1} to ACT and DCF, respectively, as well as an analytical frequency of 45 injections per hour were reached. Therefore, the proposed device was shown potential to expand the use of μTAD, due to its simplicity, low cost and good analytical performance.

In 2015, Glavan et al.[27] described the adaptive use of conventional stainless steel pin used in unmodified form or coated with carbon paste as working, counter, and quasi-reference electrodes in electrochemical device fabricated using cotton thread or embossed omniphobic R^F paper to contain the electrolyte and sample. For some applications, these pin electrodes may be easier to modify and use than printed electrodes, and their position and orientation can be changed as needed. Electroanalytical device

capable of multiplex analysis (thread-based arrays or 96-well plates) were easily fabricated using pins as electrodes in either thread or omniphobic R^F paper (Figure 2.1).

Figure 2.1 Schematic representation of the process used for the fabrication of electrochemical cells in embossed omniphobic R^F paper.

From previous researches were demonstrated about electrochemical detection which provide high accuracy and sensitivity. However, this detection system is limited for electro active analytes and need expensive instrument for detection system. Colorimetric method has attracted interest as an alternative method due to its rapidity, simplicity and low cost and practicality in on-site analysis, for the color related to the concentration of target can be observed directly by naked eyes, without the need of expensive or sophisticated instruments.

2.3.2 μTAD based on colorimetric detection

In 2010, Li et al. [28] describes a new and simple concept for fabricating low-cost, low-volume, easy-to-use microfluidic device using threads. Thread has been used to fabricate 3D and Semi-quantitative microfluidic analytical device. An advantage of using thread as the liquid transporting channels is that it does not need patterned barriers. The developed thread-based and thread-paper-based device have potential applications in human health diagnostics, environmental monitoring, and food safety analysis, and are particularly appropriate for the developing world or remote areas, because of their relatively low fabrication costs (Figure 2.2).

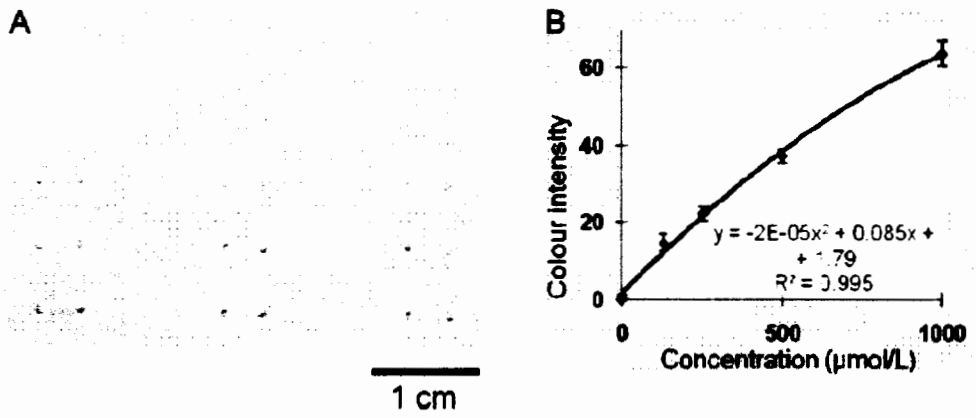


Figure 2.2 (A) Three parallel colorimetric measurements of serially diluted NO_2 solution samples. (B) the concentration calibration curve of NO_2^-.

In 2010, Reches et al. [29] also demonstrates three designs for diagnostic assays that use different characteristics of the thread. The first two designs the "woven array" and the "branching design"stake advantage of the ease with which thread can be woven on a loom to generate fluidic pathways that enable multiple assays to be performed in parallel. Moreover, they demonstrated the ability to perform colorimetric assays using the three devices by performing colorimetric assays for analytes in samples. For the detection, high intensity of thread can indicated high concentration of analytes in sample (Figure 2.3).

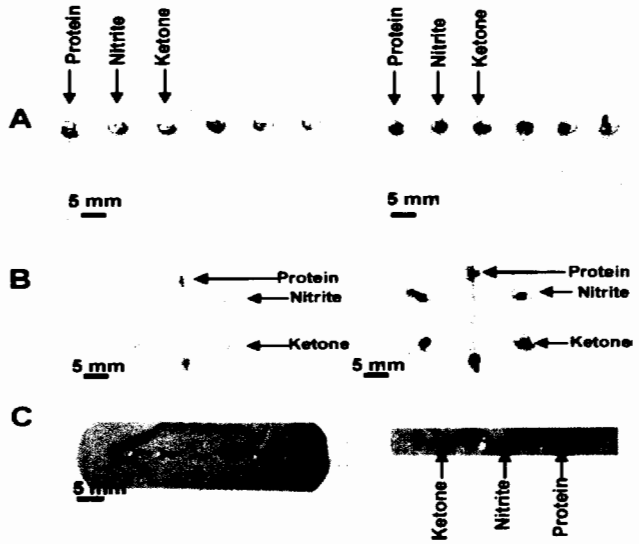


Figure 2.3 Colorimetric assays performed using the (A) woven array device, (B) branching device, (C) sewn array design.

In 2011, Ballerini et al. [30] have been studied about the use of thread as a flexible and low-cost substrate for the rapid grouping of blood. The use of a capillary substrate such as thread for blood grouping utilizes the sensitivity of the flow resistance of large particles in narrow capillary channels to separate agglutinated red blood cells (RBCs) from plasma. The principle of chromatographic separation is also exploited in this study via the use of suitable dyes to enhance the visual detection of the agglutinated RBCs and the serum phase; surprising and encouraging outcomes are obtained. Using a thread-based device, the ABO and Rh groups can be successfully determined with only 2 μL of whole blood from a pricked fingertip within 1 min and without pre-treatment of the blood sample. It is hoped that a new, inexpensive, rapid and simple method may provide an easy-to-use blood grouping platform well suited to those in developing or remote regions of the world (Figure 2.4).

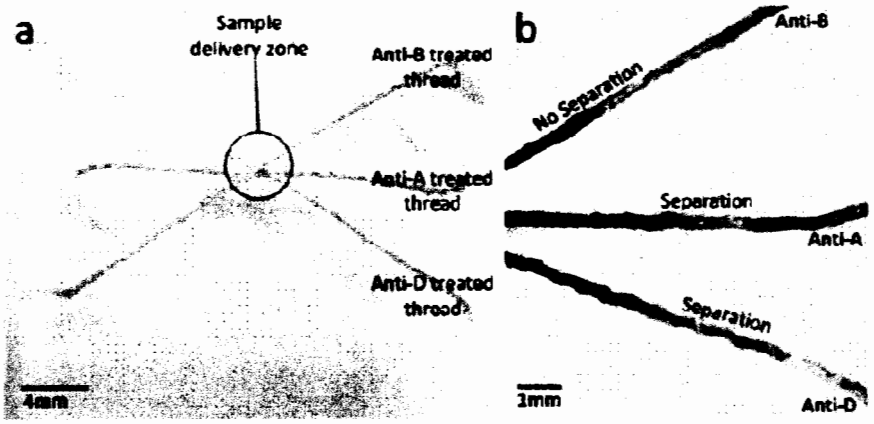


Figure 2.4 The use of thread as a flexible and low-cost substrate for the rapid grouping of blood.

In 2012, Zhou et al. [31] were demonstrated lateral-flow immunochromatographic assays are low-cost, simple-to-use, rapid tests for point-of-care screening of infectious diseases, drugs of abuse, and pregnancy. However, lateral flow assays are generally not quantitative, give a yes/no answer, and lack multiplexing. In this research were introduce the immunochromatographic assay on thread (ICAT) in a cartridge format that is suitable for multiplexing. The ICAT is a sandwich assay performed on a cotton thread knotted to a nylon fiber bundle, both of which are precoated with recognition antibodies against one target analyte. Upon sample application, the assay results become visible to the eye within a few minutes and are quantified using a flatbed scanner. Assay conditions were optimized, the binding curves for C-reactive protein (CRP) in buffer and diluted serum were established and a limit of detection of 377 μM was obtained. The possibility of multiplexing was demonstrated using three knotted threads coated with antibodies against CRP, osteopontin, and leptin proteins. The performance of the ICAT was compared with that of the paper-based and conventional assays. The results suggest that thread is a suitable support for making low-cost, sensitive, simple-to-use, and multiplexed diagnostic tests (Figure 2.5).

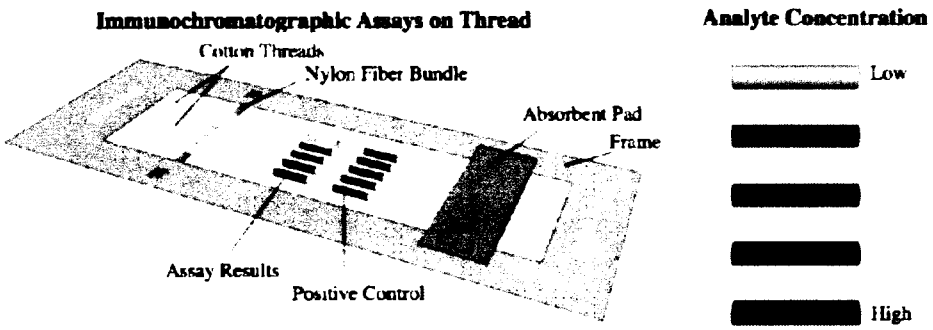


Figure 2.5 Immunochromatographic Assay on Thread.

In 2015, Nilghaz et al. [32] developed blood typing diagnostic based on a polyester thread substrate it has shown great promising for use in medical emergencies and in impoverished regions. The device is easy to use and transport, while also being inexpensive, accurate, and rapid. This research used a fluorescent confocal microscope to delve deeper into how red blood cells were behaving within the polyester thread-based diagnostic at the cellular level, and how plasma separation could be made to visibly occur on the thread, making it possible to identify blood type in a single step. Red blood cells were stained and the plasma phase dyed with fluorescent compounds to enable them to be visualized under the confocal microscope at high magnification. The mechanisms uncovered were in surprising contrast with those found for a similar, paper-based method. Red blood cell aggregates did not flow over each other within the thread substrate as expected, but suffered from a restriction to their flow which resulted in the chromatographic separation of the RBCs from the liquid phase of the blood. It is hoped that these results will lead to the optimization of the method to enable more accurate and sensitive detection, increasing the range of blood systems that can be detected.

Form above section, development and applications of μTAD as mentioned above were mainly focused on diagnostics applications based on simple quantitative and semiquantification colorimetry using naked eye for yes/no answer and quantitative information when combined with electronic detection system (digital camera, scanner or camera smart phone coupled with image processing software operated by computer,),

respectively[22-23]. Because colorimetric method is a common technique used for analysis without requirement of complex instrumental design and implementation, thus contributing to the analytical decentralization for that of applications. [33]. Moreover, thread can also be used with porous materials such as paper for improved sensitivity of semiquantitative colorimetric assay. [22]

In 2016, Gonzalez et al. [34] described a novel microfluidic thread-based analytical device (TPAD) to detect glucose through a colorimetric assay. Using commercially available nylon thread, the TPAD was trifurcated into three channels terminating at analysis sites consisting of circular zones of chromatography paper. The zones were spotted with glucose of different concentrations. A solution of GO_x, HRP, and KI wicks through the nylon thread via capillary action to the analysis sites where a yellow-brown color is observed indicating oxidation of iodide to iodine. The simplicity of the fabrication technique allows for the design and fabrication of many devices with new designs within only 2-3 hours (Figure 2.6).

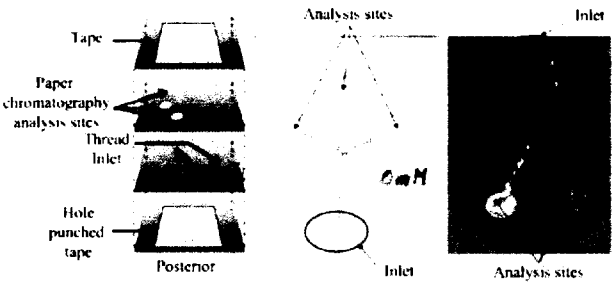


Figure 2.6 μTPAD to detect glucose through a colorimetric assay.

In 2017, Gonzalez et al. [35] described a simple and low cost μTAD to assess the activity of acetylcholinesterase (AChE). Fabrication of the device consists of two platforms, both with a nylon thread trifurcated into three channels terminating at open analysis sites at the end of the thread. 5, 5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was spotted and dried on the analysis sites. Acetylthiocholine iodide (ATC) (or cysteine, Cys),

is transported through an inlet channel of the nylon thread by capillary action due to the hydrophilic nature of nylon. AChE is transported through the other inlet channel and mixes with the ATC (or Cys) as they travel up to the analysis sites. As the solution reaches the analysis sites, an intense yellow color change occurs indicating the reaction of the thiol with DTNB to produce the yellow anion TNB^{2-}. The sites are then dried, scanned, yielding a linear range of inverse yellow mean intensity versus substrate concentration. An IC_{50} value (1.74 nM) with a known inhibitor, neostigmine bromide (NB), is obtained on the device. The multiplex design enables triplicate data collection in a device that is easy to use. μTAD have great potential to be employed in a myriad of tests including point-of-care (POC) diagnostic devices for resource-challenged settings (Figure 2.7).

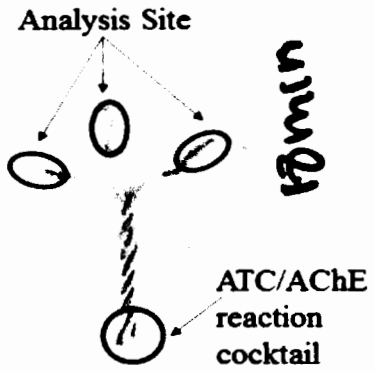


Figure 2.7 Photograph of μTAD after 18 minutes of AChE reaction time.

In 2016, S. Sateanchok et al.[36] described the thread based analytical device as an analytical platform with mobile phone has been developed as green chemical analysis for the assay of anionic surfactant. The platform involves microfluidic behavior. The thread composing a bunch of cotton fibers could serve as channel that allows anionic surfactant to move along. The part of the thread containing anionic surfactant could be seen blue when threated with methylene blue while the part without anionic surfactant

showed no blue. The platform could be then photo taken. A plot of log value of concentration of anionic surfactant versus the move distance along the thread exhibits linear: $y = 129x + 371$; $r^2 = 0.999$ where x being log (SDS concentration) and y being distance (Figure 2.8).

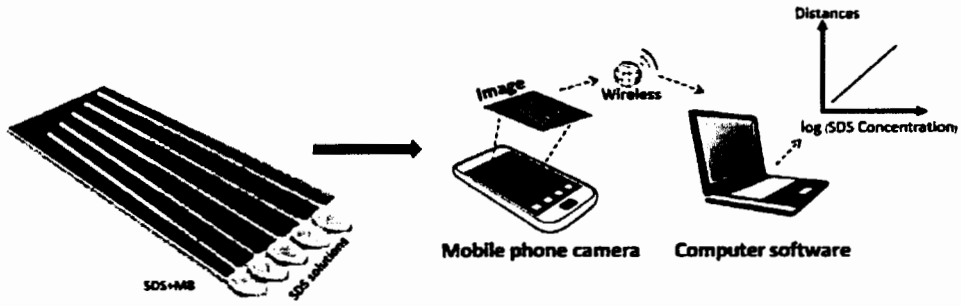


Figure 2.8 The proposed cotton thread based analytical device.

In 2017, S. Sateanchok et al. [37] described a cost-effective assay for antioxidant using simple cotton thread combining paper based device with mobile phone detection. Standard and sample solutions flow along a bunch of cotton thread treated with sodium hydroxide via microfluidic behaviors without external pumping. The total phenolic contents in the green tea samples were found to be 48–105 mg/g, with %RSD of less than 10 for that of higher 50 GAE mg/g and IC_{50} values of the samples studied were 25–50 mg/L. The results obtained by the developed methods agree with that of the standard methods.The developed assays for total phenolic content and antioxidant capacity employing Folin-Ciocalteu and DPPH reagents, respectively, have been demonstrated for real tea samples. The developed systems should be possible for at site assay, especially being useful in some remote areas and places with limited budget situations (Figure 2.9).

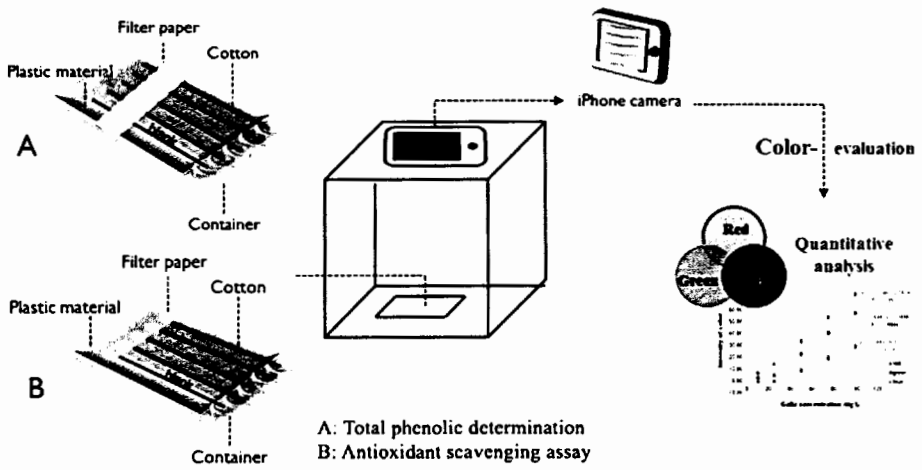


Figure 2.9 The platform of simple cotton thread combining paper based device with mobile phone detection.

However, a serious limitation of relying on electronic device is that the color hardware varies between models. This can potentially generate difference in hue, lightness and saturation, which can create errors. These errors reduces the reliability of colorimetric result interpretation (different users of the same devices may obtain different interpretation of the same color). [24]

To solve that problem, the semi quantitative analysis on μTAD by ruler was easier to detect by naked eyes without an electronic tools was established. In this method, interaction between deposited reagents and analytes within samples produces colored zones of differing lengths on the threads within only a few minutes. However, the combination of colorimetric assays with length measurement significantly reduces the reliance on electronic devices for result interpretation, and therefore reduces errors from this source.

2.3.3 Measurement of length of the colored zones

In 2014, Nilghaz et al. [38] describes a simple and semi-quantitative method for analytical detection by measuring the length of colour change on indicator treated threads using a ruler. Thread-based analytical device (μTAD), fabricated from two types of

threads (cotton and polyester), provide an easy-to-use platform for the rapid measurement of analyte concentrations in aqueous solutions. In this method, interaction between deposited reagents and analytes within samples produces coloured zones of differing lengths on the threads. The length of the coloured zones correlates with the concentrations of the analytes in the samples. Furthermore, the length measurement method can be applied to perform simultaneous assays to quantify the concentrations of different biomarkers present within the same sample. Finally, the mechanism of the length measurement method on thread-based sensors was also discussed. A comparison of this method with the currently used thread-based colorimetric method showed that the results of the length measurement method can be interpreted without the need of electronic equipment, making it well suited for use in developing regions with a lack of trained professionals (Figure 2.10 – 2.12).

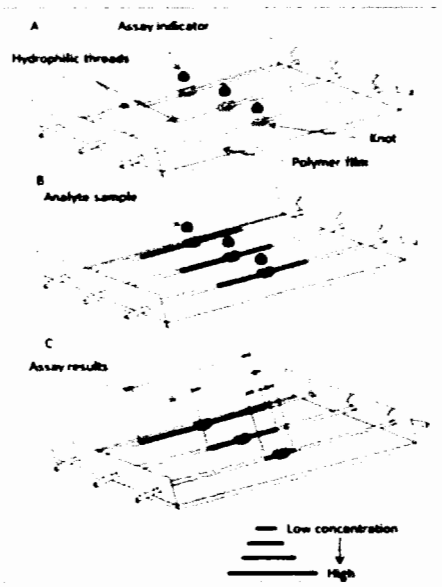


Figure 2.10 A schematic illustration of the microfluidic thread-based analytical device fabricated for the length measurement method.

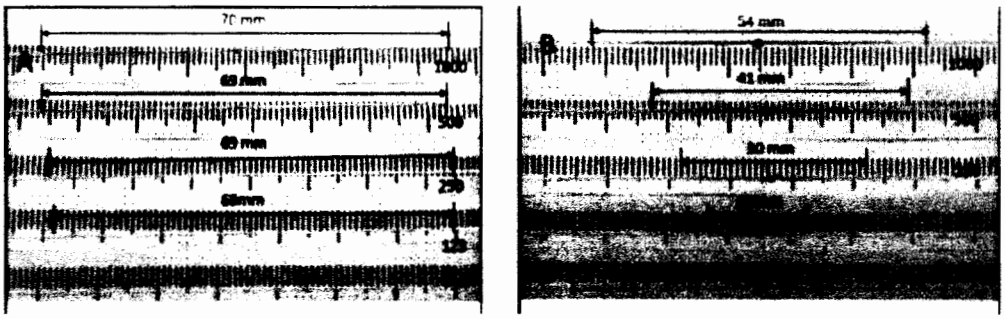


Figure 2.11 Nitrite assays performed on polyester(A) and cotton(B) threads using the length measurement method.

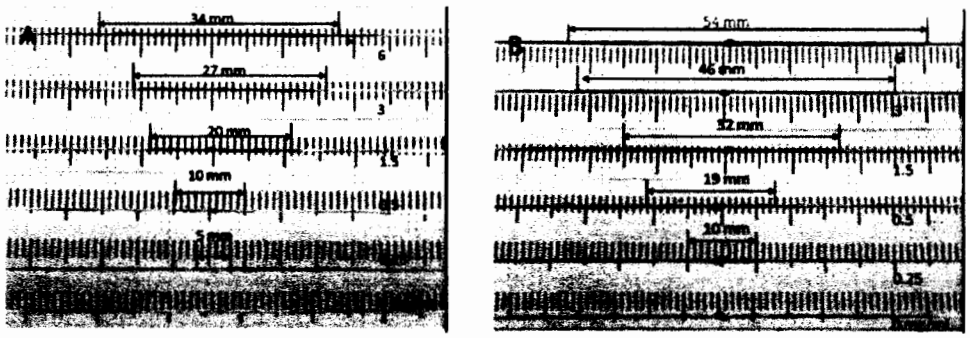


Figure 2.12 Protein assays performed using the length measurement method on polyester (A) and cotton thread (B).

Application of μTAD is particularly favored in point-of-need settings that require rapid analysis with low cost and simple operation while many of these applications are relevant to diagnostics in developing countries where resources and experts are limited, numerous applications exist in the developed world as well, for instance environmental application. Therefore, in second approach was investigated a novel concept and application of low-cost, portable and field-based using microfluidics thread based analytical device (μTAD) will also be established based on colorimetry using naked eye for determination of Mg^{2+} content in RL.

CHAPTER 3
EXPERIMENTAL

3.1 Instrumentation

Table 3.1 Instrumentation for characterizations and colorimetric measurements.

Instruments	Model	Company
Atomic absorption spectrometer	PinAAcle 900T	Perkin Elmer
Polarized light microscope	Axio Scope A1	Zeiss
Camera of iPhone	iPhone7 Plus	Apple
Chula smart lens	20x Chula Smartlens	Chula Smartlens Group
Digital camera	Olympus OMD EM10 mark II with Olympus M. Zuiko Digital ED 45 mm f1.8 lens	Olympus
Digital vernier caliper	VCD-20 (0-200 mm.)	Hummer
Stainless steel metal ruler with 15 cm/0.15 cm	-	-

3.2 Materials

Table 3.2 Materials for our developed μTAD.

Materials	Model	Company
Threads	100% cotton	Venus

3.3 Reagents and Chemicals

Table 3.3 List of reagent, grade and their suppliers.

Chemicals	Supplier
Magnesium Sulfate Heptahydrate ($MgSO_4 \cdot 7H_2O$)	Panreac
Ethylenediaminetetraacetic acid disodium salt dihydrate ($C_{10}N_2Na_2O_8 \cdot 2H_2O$, EDTA)	LABCONCO
Sodium hydrogen sulfide ($NaHS. xH_2O$)	Acros
Eriochrome Black T ($C_{20}H_{12}N_3O_7SNa$)	LABCONCO
Ethanol (C_2H_5OH)	Sigma-Aldrich
Ammonium chloride (NH_4Cl)	Fluka
Ammonium hydroxide (NH_4OH)	Fluka
Acetic acid (CH_3COOH)	Sigma-Aldrich
Diammonium hydrogen sulfide ($(NH_4)HPO_4$, DAHP)	Carlo Erba
Calcium nitrate ($Ca(NO_3)$)	Carlo Erba
Potassium sulfate (K_2SO_4)	Carlo Erba
Iron(II) sulfate ($FeSO_4$)	Carlo Erba
Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	Carlo Erba
Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Carlo Erba
Nitric acid 65% (HNO_3)	Sigma-Aldrich
Manganese sulfate ($MnSO_4$)	Carlo Erba
Sodium hydroxide (NaOH)	Carlo Erba
Paraffin oil	LABCONCO
Sodium phosphate monobasic ($NaH_2PO_4 \cdot 2H_2O$)	Carlo Erba
N-cyclohexyl-3-aminopropane sulfonic acid ($C_9H_{19}NO_3S$, CAPS)	LABCONCO

3.4 First approach; The development of a simple test kit based on colorimetry for quantification of Mg^{2+} content in RL by miniaturized complexometric titration without using masking agent was proposed.

Work in this part involves the development of a simple, low-cost and portable field test kit based on colorimetry with detection by naked eyes for determination of Mg^{2+} content in rubber latex (RL). The miniaturized complexometric titration between $Mg^{2+}$and EDTA without any masking agent was a key reaction in this development, which was designed according to the concept of green chemistry by reduction of waste generation and chemical and time consumption which will provide provides a low-cost, rapid, simple, selective and on-site analysis of Mg^{2+} content in RL.

3.4.1 Preparation of reagents

3.4.1.1 Conventional method (Complexometric titration)

ISO 17403: 2014(E) [15] was applied as a conventional method with slight modification.

Calcium carbonate ($CaCO_3$) solution (0.005 mol.L^{-1})

0.50 g of $CaCO_3$ was dissolved in 1.0 L of deionized water in beaker to give a 0.500 mol.$L^{-1}$$CaCO_3$ solution.

Ethylenediaminetetraacetic acid disodium salt dihydrate, EDTA ($C_{10}N_2Na_2O_8 \cdot 2H_2O$) solution (0.005 mol.$L^{-1}$)

1.86 g of EDTA was dissolved in 1.0 L of deionized water in beaker to give a 0.500 mol.L^{-1} EDTA solution.

Ammonium buffer solution pH 10.0 (0.06 mol.L^{-1})

67.50 g of NH_4Cl in 250.0 mL of deionized water. After that, 570.0 mL of 25%, w/w NH_4OH were added to NH_4Cl solution and brought up to 1.0 L with deionized water.

Eriochrome Black T, EBT ($C_{20}H_{12}N_3O_7SNa$) as indicator solution (2.16×10^{-4} mol.L^{-1})

0.10 g of EBT was dissolved in 100.0 mL of 75%, v/v ethanol give a 2.16×10^{-4} mol.L^{-1} EBT solution.

Sodium hydrogen sulfide ($NaHS.xH_2O$) solution (0.3 $mol.L^{-1}$)

1.68 g of $NaHS.xH_2O$ was dissolved in 100.0 mL of deionized water in beaker to give a 0.3 $mol.L^{-1}$ $NaHS.xH_2O$ solution.

3.4.1.2 Test kit based on colorimetry method (our proposed)

The small-scale test kit was developed based on the conventional method. The approaches were based on complexometric titration between Mg^{2+} and EDTA using NaHS as a masking agent (or without any masking agent).

Reagent A

$2.16x10^{-4}$ $mol.L^{-1}$ of EBT indicator with the EBT: DI water volume ratio of 3:10

Reagent B

0.02 f of EDTA was dissolved in 6.67 mL of 0.06 M ammonium buffer solution pH 10.0. Followed by addition of 0.3 M $NaHS.xH_2O$ (3.33 mL) as a masking agent to give a reagent B. (Total volume was 10.0 mL)

3.4.2 Optimization of the developed test kit

3.4.2.1 Optimization of sample and reagent volume

The small spoon (Figure. 3.1) was used selected use as sample container of our developed test kit. The amount of sample using this small spoon was also investigated by weighting RL sample which was recorded and reported in terms of average with standard deviation ($x \pm S.D.$) ($n=20$).

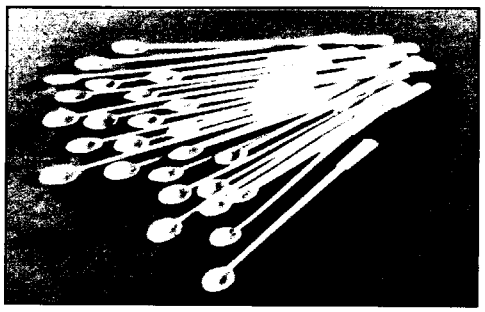


Figure 3.1 Small spoon used to scoop sample (RL)

Glass bottles with glass dropper and eye dropper (Bottle plastic; Figure. 3.2) were established to be reagent A and B volume, respectively for our developed test kit. The volume of reagent A and B were also investigated.

Figure 3.2 Glass bottles with glass dropper and eye plastic dropper

3.4.2.2 Optimization of condition for reagent A and B

In this study, reagent A (EBT, HIn^{2-}) was applied as an indicator; whilst, the EDTA, buffer solution and masking reagents were mixed into reagent B. Reagent A needs to be separated from the system prior to analysis due to the poor solubility of the indicator in the reagent B matrix, as well as the complexation between the indicator and Mg^{2+} as analysis has to be carried out before addition of EDTA as demonstrated in Eq. 1.2. (CHAPTER 1)

Reagent A was obtained by variation of a number of droplets (1-8 drops) applied in different bottles each of which had already been filled in with 50 $mg.L^{-1}$ of Mg^{2+} in DI water. In addition, Study about dilution of EBT in DI water (EBT:DI water) was shown in the ratio in the range from 1:10 to 10:10.

For reagent B was optimized by the stoichiometric amount of EDTA to react Mg^{2+} with theoretical concentrations of 25, 50 and 100 $mg.L^{-1}$ corresponded to the optimized number of droplets for reagent B being 1 drop per 50 $mg.L^{-1}$ of Mg^{2+} (with the volume of ~0.065 mL including buffer solution and masking reagents).

For the test kit application, The instruction of "Field test kit based on colorimetry for identification of Mg^{2+} content in RL was shown in Figure. 3.3. Firstly, Reagent A was transferred by a dropper up to the marked level into a reaction bottle. Then, a small spoon was used to transfer RL into the reaction bottle. The RL solution was shaken resulting in the purple color observed by naked eye, as shown by the color chart 1.1 in Figure 3.3. Reagent B was then added drop by drop and shaken (every 10 s) to the solution until the blue color of RL solution was observed, see also the color chart 2 in Figure 3.3 Count a number of drop of Reagent B used, which will relate to the concentration of Mg^{2+} in the unit of mg. L^{-1}. (Note: 1 drop ≈ 50 mg. L^{-1}).

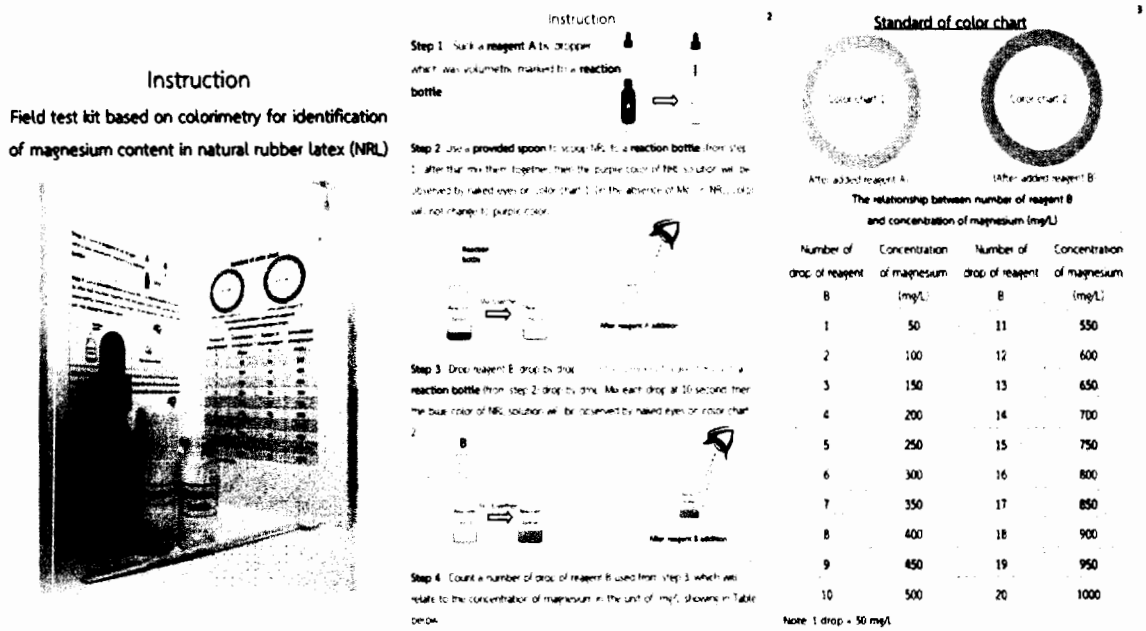


Figure 3.3 The instruction of "Field test kit based on colorimetry for identification of Mg^{2+} content in RL.

3.4.3 Method validation of the developed test kit

The RL samples consist of natural rubber latex (NRL) and concentrated rubber latex (CRL). CRL are NRL which was prepared by removal of some Mg^{2+} presented in NRL using an addition of diammonium hydrogen phosphate and/or preserved with anticoagulant reagents.

For NRL sample, Complexometric titration was performed with slight modification according to ISO 17403: 2014(E) [15]. Briefly, Natural rubber latex, NRL (2.0 g) was transferred into a conical flask followed by addition of 100 mL of DI water. 0.06 mol.L^{-1} NH_4Cl/NH_4OH buffer solution (2.0 mL) was then added to control the RL solution pH within the range of 10.0-10.5. Next, 2.16×10^{-4} mol.L^{-1} EBT indicator (1.0 mL) was dropped into the RL solution. The 0.3 mol.L^{-1} masking agent (1.0 mL) was added into the solution and also diluted in water (100 mL). The solution was then titrated with the standard $5x10^{-3}$ mol.L^{-1} EDTA solution (which was standardized with $5x10^{-3}$ mol.L^{-1} $CaCO_3$) until the red solution vanished and became pure blue. In addition, this titration approach was evaluated by comparison with FAAS which is a reliable technique with high accuracy and precision.

For CRL sample, Complexometric titration was performed with slight modification according to ISO 17403: 2014(E) [15]. Briefly, CRL sample (10.0 g) was transferred into a breaker followed by addition of 10 mL of DI water. Next, 25% acetic acid (5.0 mL) was then added to digest the CRL sample within serum. Pipette 10.0 mL of serum into a conical flask and added 0.06 mol.L^{-1} NH_4Cl/NH_4OH buffer solution (4.0 mL) was then added to control the CRL solution pH within the range of 10.0-10.5. After that, 2.16×10^{-4} mol.L^{-1} EBT indicator (1.0 mL) was dropped into the RL solution. The 0.3 mol.L^{-1} masking agent (1.0 mL) was added into the solution and also diluted in water (100 mL). The solution was then titrated with the standard $5x10^{-3}$ mol.L^{-1} EDTA solution (which was standardized with $5x10^{-3}$ mol.L^{-1} $CaCO_3$) until the red solution vanished and became pure blue. In addition, this titration approach was evaluated by comparison with FAAS which is a reliable technique with high accuracy and precision.

The sample preparation for FAAS analysis was performed with slight modification [21-22] by transferring 0.25 g of RL (Figure 3.4a) into a test tube and then 4.0 mL of 65% v/v HNO_3 were applied to the test tube as shown in Figure 3.4b. The solution was heated in an oil bath at 165 °C (Figure 3.4c) resulting in a transparent solution which was then cooled down and diluted with HNO_3 (2%, v/v) prior to the FAAS analysis as shown in Figure 3.4d.

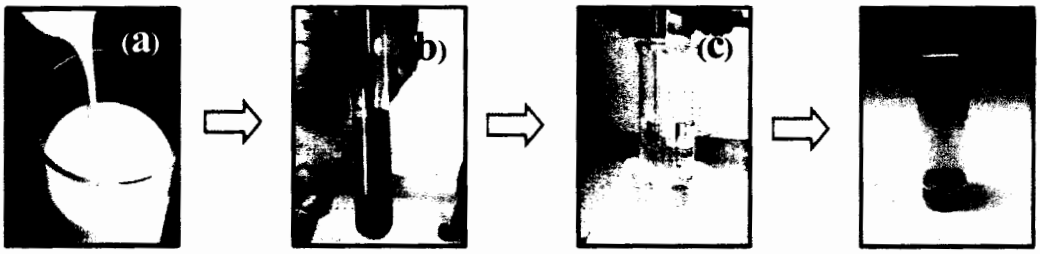


Figure 3.4 The preparation of RL sample for determination of Mg^{2+} by FAAS method.

3.4.4 Study of interferences in RL on our developing test kit

In this study, the investigated ions were Fe^{3+}, Cu^{2+}, Mn^{2+}, K^{+}, Zn^{2+}, Ca^{2+} and Na^{+}. The tested concentrations of the foreign ions were 50, 100, 150, 250, 500, 750, 1000 and 1500 $mg.L^{-1}$, respectively. The results obtained from the complexometric titration with the masking agent were then compared with those obtained without the masking agent. NRL number of U_3 was used as model sample in this study. As the NRL can be stable for approximately 4 h, fresh NRL was collected freshly several times during the experiment in order to prevent the NRL coagulating.

3.4.5 Study of preservatives in RL on our developing test kit

The preservative solutions of 0.2%, w/v NH_4OH and mixture of 0.2%, w/v NH_4OH, 0.025%, w/w ZnO and 0.025%, w/w TMTD, respectively, were added to the RL sample. Mg^{2+} content in the treated RL was then quantified and the result was compared with that obtained by the conventional method (complexometric titration).

3.4.6 Study of stability test for reagents and application of the developed test kit for practical sample analysis

Reagents A and B were left at room temperature for 6 months prior to the application with the test kit for Mg^{2+} determination. The results were compared with that obtained by the conventional complexometric titration method.

Samples were collected from several regions in Ubon Ratchathani province (U_2-U_3) and Kalasin province (K_1-K_3), Thailand. In each analysis, identification of Mg^{2+} was performed by applying the developed test kit, which were operated by three different users. The results were averaged and compared with those obtained by the conventional complexometric titration method.

3.4.7 Study of concentrated rubber latex (CRL) using our developing test kit

In this thesis, two types (CRL-Type A and CRL-Type B) of CRL sample were studied. CRL-Type A are Mg^{2+} in NRL were removed by addition of diammonium hydrogen phosphate (DAHP) [7-8] and CRL-Type B are clear serum with acidic medium (pH 3) was obtained from CRL by diluting 10 g of CRL with 10.0 mL of water and coagulate with 5.0 mL of 25% acetic acid water [15].

3.5 Second approach; the development of a low cost microfluidic thread-based analytical device (μTAD) for determination of Mg^{2+} content in RL

3.5.1 Preparation of reagents

3.5.1.1 Conventional method (Complexometric titration [15])

All reagents used in conventional method were same as reagent mentioned in section 3.4.1.1

3.5.1.2 μTAD based on colorimetry method (our proposed)

The μTAD was developed based on miniaturized complexometric titration without using masking agent

Eriochrome Black T, EBT ($C_{20}H_{12}N_3O_7SNa$) as indicator solution (2.16×10^{-4} mol.L^{-1})

0.1 g of EBT was dissolved in 100 mL of 75%, v/v ethanol.

N-cyclohexyl-3-aminopropanesulfonic acid, CAPS ($C_9H_{19}NO_3S$) as buffer solution pH 10.0 ($9.99x10^{-3}$ mol.L^{-1})

2.21 g of CAPS was dissolved in 1000 mL of deionized water.

Mixture solution of various concentrations (mg. L^{-1}) of EDTA in CAPS ($C_9H_{19}NO_3S$) pH 10.0 solution

The concentrations of EDTA in CAPS solution were 6.17, 8.23, 10.29, 20.57, 30.86, 41.14 mM and demand of weight at various concentrations were shown in Table.3.4. Next, EDTA was dissolved in 25 mL of CAPs buffer solution. The mixture need to be tuned to basic condition at pH 10.0 by adding of 1 M NaOH until the pH was around 10.0 before then the volume was adjusted to final of 25 mL.

Table 3.4 Weight of solid EDTA and dissolved in 25 mL of CAPS buffer at pH 10 reporting in concentration (mM).

Concentration of EDTA (mM)	Weight of EDTA (g) in 25 mL of CAPS buffer at pH 10
6.17	0.0573
8.23	0.0764
10.29	0.0955
20.57	0.1909
30.86	0.2092
41.14	0.2790

3.5.2 Preparation of RL samples

3.5.2.1 Digestion of RL samples for conventional method (Complexometric titration)

For complexometric analysis, the digestion of RL samples was reported method of concentrated rubber latex (CRL) from ISO17403:2014 [15]. 10.0 g of RL was transferred into a breaker followed by addition 1.0 mL of 25% v/v acetic acid. The

separation between serum and sludge was occurred. Around 2.4 mL of Clear serum of RL was obtained at pH 4-5.

3.5.2.2 Digestion of RL samples for developed μTAD method

For developed μTAD, RL samples were collected in Ubon Ratchathani province. Before analysis on developed μTAD, a simple sample preparation of RL was developed by modification from ISO 17403:2014, method of concentrated rubber latex (CRL) [15]. RL samples were transferred into micro centrifuge tubes and digested by 25% v/v acetic acid. RL serum was obtained in acidic condition at pH 4-5. After that, pH of RL serum was adjusted the pH to be 7 before analysis by transferring RL serum 100 μL into a micro centrifuge tube. CAPS buffer solution (500 μL) and 1 $mol.L^{-1}$ NaOH (50 μL) were then added, respectively as demonstrated in Figure 3.5

Moreover, our developed μTAD was also applied determine Mg^{2+} contents in water sample (such as tap, canal and mineral waters). The water samples without pretreatment including tap and canal waters were collected and mineral water was obtained from local super market at Warin Chamrap, Ubon Ratchathani, Thailand. (See also the result in Appendix B).

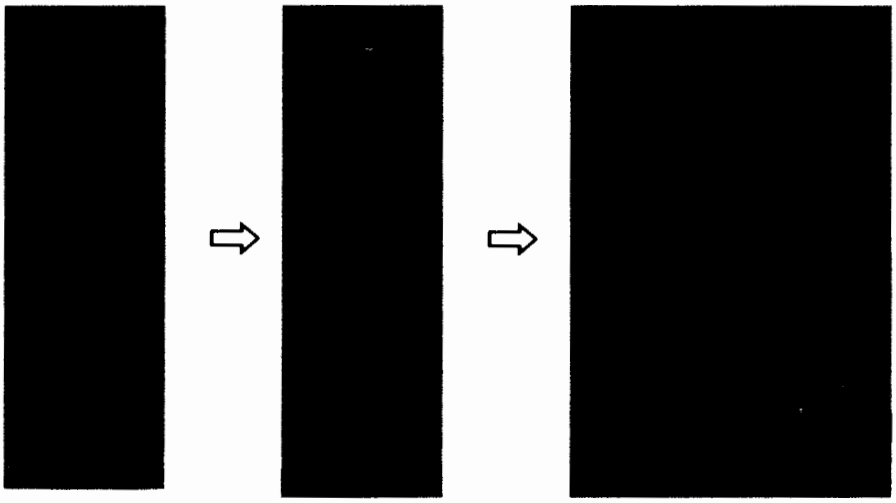


Figure 3.5 Our developed preparation of RL sample based on digestion method which was modified from ISO 17403: 2014(E). [15]

3.5.3 Method validation of our developed preparation of RL sample for proposed μTAD operated by flame atomic absorption spectrophotometry (FAAS)

From section 3.4.2.2, the separation between serum and sludge was occurred. The sample preparation for FAAS analysis was performed with slight modification [21-22] by transferring 0.25 g of original RL, serum and sludge into an each test tube and then 4.0 ml of 65% v/v HNO_3 were applied to the test. The solution was heated in an oil bath at 165 °C resulting in a transparent solution which was then cooled down and diluted with HNO_3 (2%, v/v) prior to the FAAS analysis.

3.5.4 Study of physical properties of threads

3.5.4.1 Study of percentage of cotton on threads

In this study, four types (A,B,C and D) of commercial threads will be investigated for fabricating μTAD. 15 cm of thread samples were weighted and reported as W_0. Next, threads sample was then immersed in 10 mL of 70% of sulfuric acid for 20 min. Insoluble fraction of threads were washed with deionized water and dried at room temperature. Dried fraction of threads were weight and reported as W. Last, percentage of cotton was calculated by $((W_0\text{-}W) \times 100)/W_0$ whereas, W_0 and W are a dried weight of thread before and after immersing in sulfuric acid, respectively. (Figure 3.6)

Figure 3.6 Producer our method for investigated of percentage of cotton on threads.

3.5.4.2 Study of diameter measurement of threads

For diameter of threads (80%, 90%, 100% cotton and polyester thread) investigation, the digital vemier caliper was used to measure a diameter of various threads (n=10) and which was reported in terms of average with standard deviation ($\bar{x} \pm$ S.D.)

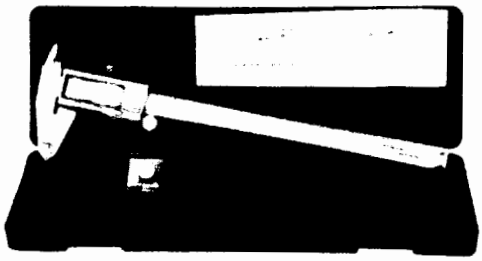


Figure 3.7 Digital vernier caliper.

3.5.5 Study of wicking property of threads used for fabrication of μTAD

In this study, the wicking properties of the thread both as untreated and treated with chemical treatment were investigated. The method for removal of wax covered on thread using by Na_2CO_3 as a chemical treatment as demonstrated in Figure 3.8.

After that, 3 μL of the red color solutions of food dye was applied to these threads (untreated and treated of threads), length 10 cm for 1 minute thereafter we measured the average rate of wicking of the threads before untreated and after treated with a ruler scale.

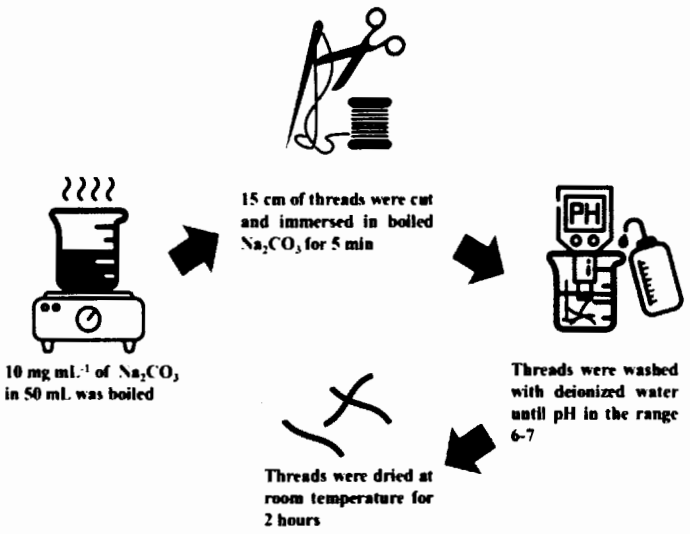


Figure 3.8 The removal of wax covered on thread using by Na_2CO_3 as a chemical treatment.

3.5.6 Optimization of reagent conditions on μTAD

Study of condition of EDTA in two medium were in DI (as Condition A) and CAPS buffer solution (as Condition B) Mg^{2+} standard solution in range of 0, 25, 50, 250, 750 and 1000 mg. L^{-1} were tasted and measured the length of color change. Concentration of EDTA in CAPS buffer solution was optimized. The concentrations of EDTA in CAPS solution were 6.17, 8.23, 10.29, 20.57, 30.86, 41.14 mM in CAPS buffer solution. The mixture need to be tuned to basidic condition at pH 10.0 by adding 1 M of NaOH until adjust to pH around 10.0. In experiment, the test solution was tested with the standard solution of Mg^{2+}; concentration were 0, 25, 50, 100, 500, 750 and 1000 mg. L^{-1} and interpreted by measuring the length of color change on indicator treated thread(soaked threads with EBT for 10 minute and leave it dry).

3.5.7 Fabrication and operating procedures of complexometric titration on μTAD

Our developed μTAD were prepared by using untreated 100% cotton threads with the diameter of 300 ± 0.2 μm as a substrate material which can be wicked by the sample solution according to capillary action without use of external pumping devices. The threads were cut by a scissor to a length of 15 cm as shown in Figure 3.9.

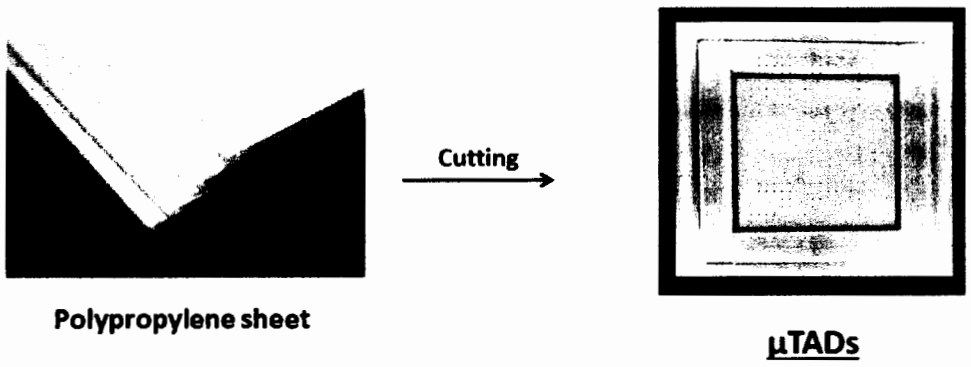


Figure 3.9 Fabrication of box platform was used in develop μTAD.

For operating procedures, μTADs in all analyses were prepared by using untreated 100% cotton threads with the diameter of 300 ± 0.2 μm as substrate materials which can be wicked by the sample solution according to capillary action without use of external pumping devices. The threads were cut by a scissor to a length of 15 cm. Complexometric analysis employed a foam sheet holder as the support which was fabricated by cutting a foam sheet with the dimension of 15 cm × 15 cm. A millimeter scale ruler was also made on the foam sheet. Step 1: threads (15 cm) were initially soaked into EBT (2.16×10^{-4} M) for 10 min and then dried at room temperature. Step 2: two threads were further modified by addition of 3 μL of EDTA with different concentrations (8.23 and 30.86 mM) in CAPS solution at pH 10, which changed the thread color from red wine to blue. Step 3: both threads were tied together with a central knot before being attached to a box prior to analysis (threads treated with 8.23 and 30.86 mmol L^{-1} of EDTA were vertically aligned for detection of low range of Mg^{2+} concentration and the other thread treated with 30.86 mmol L^{-1} of EDTA was horizontally aligned for detection of high range of Mg^{2+} concentration, respectively). Step 4: the sample (6 μL) can be added onto the crossing threads and the length of the color change can be measured with a ruler (summation of length from the central knot: Up and down for detection of low range of Mg^{2+} concentration, left and right for detection of high range of Mg^{2+} concentration). The analyte in samples then reacted with indicator reagents deposited on μTADs. This instantly produced the length of red-violet color zone on μTAD being proportional to the concentration of Mg^{2+} in samples (Step 5) as demonstrated in Figure 3.10.

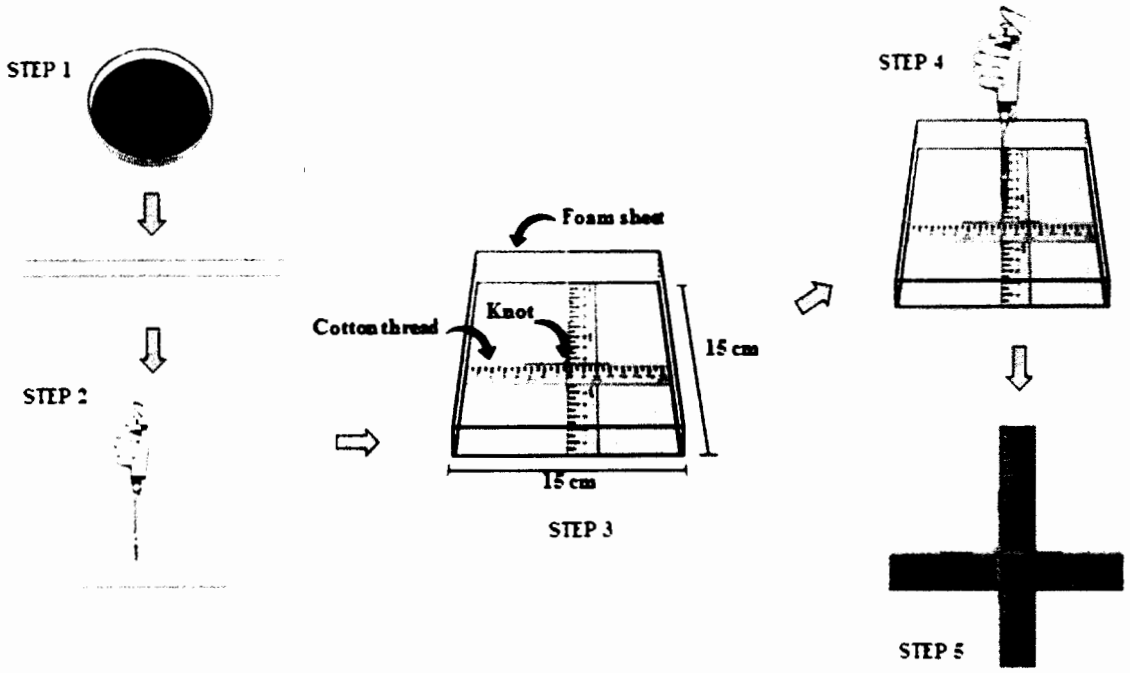


Figure 3.10 Operating procedures of complexometric titration on developed μTAD.

3.5.8 Optimization of regent volume on developed μTAD

Reagent volume on develop μTAD was investigated in the range of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 μL were investigated by micropipette. Using 41.14 mM of EDTA in CAPS buffer solution (pH=10.0) at various volumes (μL) were dropped onto as prepared threads. (EBT was soaked for 10 min) and observed the length of generating color (blue color).

3.5.9 Study of interferences on μTAD

Study of selectivity towards Mg^{2+} and chloride ion compared with potential interference ions was investigated. In complexometric analysis of Mg^{2+} ,the investigated cations were Fe^{3+}, Cu^{2+}, Mn^{2+}, K^{+}, Zn^{2+}, Ca^{2+}, $Na^{+}$and the anions were OH^{-}, SO_4^{2-}, NO_3^{-}, PO_4^{3-} and Cl^{-}. The tested concentrations of each ion were 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 750 and 1000 $mg.L^{-1}$ containing 50 and 250 $mg.L^{-1}$ for low and high range of Mg^{2+} detection, respectively.

3.5.10 Analytical characteristics of developed μTAD

3.5.10.1 Study of working range

Standard solution of Mg^{2+} were prepared by diluting amount of Mg^{2+} with deionized water to give working solution in the range of 25 to 1000 mg mL^{-1}, with the three triplicate measurements. The tasted of this present work were carried out under the optimized conditions (reagent volume: 3 μL; sample volume: 6 μL; 8.23 mM and 30.86 mM of EDTA in CAPS buffer solution was vertically and horizontally aligned for low and high concentration of Mg^{2+}). In interpretations of this reaction was observed by measure of change color from purple to blue on μTAD.

3.5.10.2 Study of limit quantification (LOQ)

The LOQ was evaluated from lowest concentration of Mg^{2+}at which can detected by naked eyes using developed μTAD considering from the length of color change on μTAD.

3.5.10.3 Study of recovery on μTAD

For recovery test, RL sample was spiked with 50 and 100 $mg.L^{-1}$ of standard Mg^{2+}. Moreover, for water samples are consist of tap, canal and mineral waters were spiked with 100 $mg.L^{-1}$ of standard Mg^{2+}.(see also the result in Appendix B)

3.5.11 Method validation of the μTAD and real sample application

For validation method, complexometric titration was performed with slight modification according to ISO 17403: 2014(E) [15]. Briefly, serum of RL (2.4 g) (method for preparation was shown in section 4.3.2) was transferred into conical flask followed by addition of 0.06 $mol.L^{-1}$ NH_4Cl/NH_4OH buffer solution (2.0 mL) and $2.16x10^{-4}$ $mol.L^{-1}$ EBT indicator (1.0 mL) was dropped into the sample solution. The solution was then titrated with the standard $5x10^{-3}$ $mol.L^{-1}$ EDTA solution (which was standardized with $5x10^{-3}$ $mol.L^{-1}$ $CaCO_3$) until the red solution vanished and became pure blue.

For real RL samples application, the proposed method for Mg^{2+} determination in RL was evaluated by analyzing real sample from Ubon Ratchathani province.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 First approach; The development of a simple test kit based on colorimetry for quantification of Mg^{2+} content in RL by miniaturized complexometric titration without using masking agent

4.1.1 Optimization of the developed test kit

4.1.1.1 Optimization of sample and reagents volume

The optimized sample amount scooped by using a small spoon was 0.1731 ± 0.02 g (Table 4.1.) and the optimized reagent volume dropped by using an eye drop bottle was 0.065 ± 0.004 mL (Table 4.2).

Table 4.1 Optimization of sample weight per sampling by using a spoon (n = 20).

Replication	Weight (g)	Replication	Weight (g)
1	0.1741	11	0.1967
2	0.1495	12	0.1760
3	0.1759	13	0.1657
4	0.1528	14	0.1789
5	0.1431	15	0.1683
6	0.1742	16	0.1793
7	0.1981	17	0.1953
8	0.1795	18	0.1841
9	0.1863	19	0.1698
10	0.1645	20	0.1507

Table 4.2 Optimization of volume of reagent B (weight/drop).

Replication	Weight (g)	Replication	Weight (g)	Replication	Weight (g)
1	0.070	6	0.063	11	0.062
2	0.067	7	0.067	12	0.066
3	0.066	8	0.065	13	0.064
4	0.062	9	0.065	14	0.064
5	0.065	10	0.065	15	0.067

4.1.2 The condition of reagents on developed test kit

In this study, reagent A (EBT, HIn^{2-}) was applied as an indicator; whilst, the EDTA, buffer solution and masking reagents were mixed into reagent B. Reagent A needs to be separated from the system prior to analysis due to the poor solubility of the indicator in the reagent B matrix, as well as the complexation between the indicator and Mg^{2+} as analysis has to be carried out before addition of EDTA as demonstrated in equation 1.2.

The optimized condition for reagent A was obtained by variation of a number of droplets (1-8 drops) applied in different bottles each of which had already been filled in with 50 mg. L^{-1} of Mg^{2+} in DI water. The optimized number of droplets was three drops (Figure 4.1a) since the use of >3 drops resulted in precipitation of RL whilst using of 1-2 drops led to low color intensity of the end point of the titration reaction which is difficult to see by naked eyes. However, the addition of three drops into practical RL samples resulted in precipitation of RL since EBT was dissolved in ethanol which precipitated RL (Figure 4.1b). EBT solution was thus diluted with DI water with the selected EBT:DI water ratio of 3:10, which was marked at the level shown on the dropper for sampling of reagent A. Due to the fact that, EDTA is a limiting reagent in the complexometric titration reaction for Mg^{2+} determination. The stoichiometric amount of EDTA to react Mg^{2+} with theoretical concentrations of 25, 50 and 100 mg.L^{-1} corresponded to the optimized number of droplets for reagent B being 1 drop per 50 mg.L^{-1} of Mg^{2+} (with the volume of ~0.065 mL including buffer

solution and masking reagents), detail of information and explanation shown in Table 4.3 and 4.4.

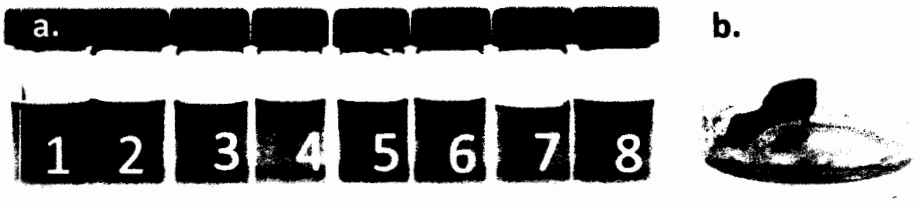


Figure 4.1 Variation of number of EBT droplets (1-8 drops) containing 50 mg.L^{-1} Mg^{2+} in DI water a) and precipitation of RL sample when 3 drops of EBT were applied b).

Table 4.3 The stoichiometric amount of EDTA in the reagent B to react Mg^{2+} with theoretical concentration of 25, 50 and 100 mg.L^{-1}.

Amount of Mg^{2+} (mg·L^{-1})	Number of drops of reagent B		
	a drop of reagent B* = 25 mg·L^{-1} of Mg^{2+}	a drop of reagent B** = 50 mg·L^{-1} of Mg^{2+}	a drop of reagent B*** = 100 mg·L^{-1} of Mg^{2+}
50	2	1	1
100	4	2	1
150	5	3	2
200	8	4	2
250	10	5	3

* containing 2.74 x 10^{-3} M EDTA

** containing 5.48 x 10^{-3} M EDTA

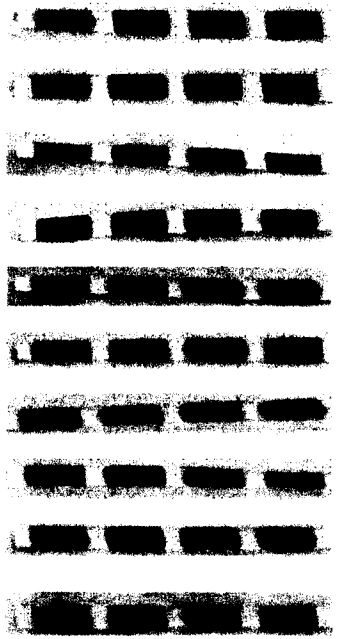
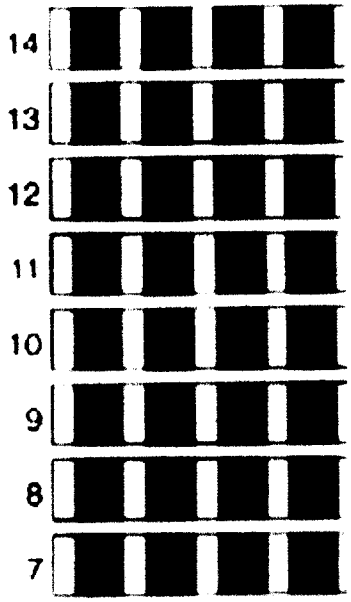
*** containing 10.98 x 10^{-3} M EDTA

Table 4.4 Comparison of percentage error between 1 drop of reagent B (1 drop of reagent B ≈ 50 mgL^{-1} of Mg^{2+}) and (1 drop of reagent B ≈100 mgL^{-1} of Mg^{2+}) on our developed test kit.

Area of RL	Mg^{2+} content by complexometric titration ($mg \cdot L^{-1}$) (n=3)	%Relative difference (n = 3) from our developing test kit	
		1 drop of reagent B ≈50 $mg \cdot L^{-1}$ of Mg^{2+}	1 drop of reagent B ≈100 $mg \cdot L^{-1}$ of Mg^{2+}
U2	748.5 ± 7.1	0.3	6.9
U2	770.9 ± 8.2	3.8	3.8
U2	812.2 ± 3.6	4.7	10.8
U3	306.2 ± 5.3	14.3	30.6
U3	322.5 ± 4.4	8.5	24.0
U3	375.2 ± 26.1	6.6	6.6

This condition was obtained by taking in account solution miscibility and buffer capacity (β) where a drop of reagent B was found with β = 2.17 M per 0.1 pH unit. This is sufficient for the resistance to pH change in the developed test kit as well as being applicable even when excess amount of reagent B was applied, see also in (Table 4.5).

Table 4.5 The monitoring of pH in our developing test kit system.

Number of droplets of reagent B	Total concentration of buffer presented in reagent B (M)	Color change of pH paper		Standard color chart of pH paper
		Before addition of reagent B (RL sample and reagent A were applied in the reaction bottle)	After addition of reagent B (at pH 10-11)	
10	0.02	 (pH 7-8)		
20	0.03			
30	0.04			
40	0.04			
50	0.05			
60	0.05			
70	0.05			
80	0.05			
90	0.05			
100	0.06			

4.1.3 Validation of the developed test kit

Comparison of the analysis results between complexometric titration (standard method) and FAAS techniques (reference method) was made for Mg^{2+} content in RL samples obtained from U_1 and U_3. The results obtain from t-test showed $t_{stat} = 1.02$ whereas $t_{critical} = 2.78$ revealed that there is no statistical difference between these two methods with a confidence level of 95%. (Table 4.6)

Table 4.6. Comparison of Mg^{2+} content ($mg.L^{-1}$) at different area of RL between Flame Atomic Absorption Spectroscopy, FAAS and complexometric titration (n=3).

Area of RL	Mg^{2+} content ($mg.L^{-1}$)	
	FAAS method	Complexometric titration method
U_1	746.90 ± 0.002	748.48 ± 7.07
U_1	800.02 ± 0.008	770.90 ± 8.22
U_1	873.81 ± 0.001	812.17 ± 3.62
U_3	283.12 ± 0.001	306.18 ± 5.30
U_3	342.55 ± 0.001	322.52 ± 4.39
U_3	363.89 ± 0.001	375.13 ± 26.08

U_1 and U_3 were collected 3 times

4.1.4 Effect of interferences on our developed μTAD

For interferences study, a masking agent was added in order to reduce interference in Mg^{2+} analysis by undergoing complexation with the foreign ions in the RL solution. Due to the simplicity and lower analysis cost, the titration method was selected as the benchmark method for determination of Mg^{2+} in RL samples.

In addition, our test kit was developed based on scaling down the process for the complexometric titration between Mg^{2+} and EDTA with [13-15] without NaHS as a masking agent. In order to study the ability of NaHS to be used as a masking agent, interferences in Mg^{2+} analysis caused by complexation with the potential

foreign ions in the RL solution (Fe^{3+}, Cu^{2+}, Mn^{2+}, K^{+}, Zn^{2+}, Ca^{2+}, and Na^{+}) were assessed.

The resulting tolerance concentration ($mg.L^{-1}$, which was defined as the added concentrations of foreign ions that reveal significant changes of % error to be within ± 10%) data are shown in Table 4.7. The % error values obtained from complexometric titration using the masking agent were lower than those obtained without masking agent, especially with the presence of Cu^{2+} where the end point of the titration could not be identified without the masking agent. This indicates the requirement for addition of masking agent (here being NaHS) for improved analysis reliability. According to the observed high tolerance concentrations, it can be concluded that the investigated ions could not interfere the analysis of practical RL samples. Note that the result revealed relatively low tolerance concentrations for Fe^{3+}, Cu^{2+}, and Mn^{2+}. However, the concentrations of these ions in the real RL are very much lower than the studied concentrations [8, 23-24] and see further explanation in Table 4.8.

The function of NaHS as a masking agent for Mg^{2+} analysis in RL can be explained by formation of complexes with foreign metal ions according to the Hard-Soft acid-base reaction. Na^{+} can be considered as hard acid and HS^{-} is soft base which does not prefer to react with Mg^{2+} (hard-acid). However, interference ions in RL are mostly soft acids favorably interacting with HS^{-}. The resulting complexes are stable and not likely to react with EDTA. [25]. These investigations corresponded to the results obtained in reported works [13-15]. Therefore, the evaluated complexometric titration was further used as the reference method by comparison with our developed test kit for quantification of Mg^{2+} from U_1 and U_2 as well as these samples spiked with 40 and 80 $mg.L^{-1}$ Mg^{2+}, respectively. The complexometric titration results were insignificantly different from the results obtained from the test-kit approach (t_{stat} = 6.42 and $t_{critical}$ = 2.78). Furthermore, the analytical recoveries were also in an acceptable range of 100.6- 102.4 for RL sample (Table 4.9).

Table 4.7 Selectivity study towards Mg^{2+} compared with the other metal ions (Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, K^{+} and Na^{+} tested using complexometric titration reported in the tolerance concentration ($mg.L^{-1}$) (n=3).

Interference	Tolerance concentration ($mg.L^{-1}$)	With masking agent			Without masking agent		
		Mg^{2+} content ($mg.L^{-1}$)		% Relative different	Mg^{2+} content ($mg.L^{-1}$)		% Relative different
		1st collection	2nd collection		1st collection	2nd collection	
Original RL	-	524.02 ± 11.89	256.16 ± 2.99	-	534.81 ± 17.40	289.87 ± 23.37	-
Zn^{2+}	793.62	470.60 ± 2.85	-	-9.86	611.60 ± 2.47	-	+14.36
Ca^{2+}	955.82	545.10 ± 3.72	-	+4.02	565.40 ± 7.03	-	+5.72
Fe^{3+}	157.52	-	272.60 ± 1.26	+6.42	-	315.20 ± 7.13	+8.74
Cu^{2+}	244.14	-	232.50 ± 1.53	-9.23	-	N.D.	-
Mn^{2+}	53.93	-	267.90 ± 0.77	+4.58	-	330.90 ± 3.48	+14.15
K^{+}	2171.29	-	235.20 ± 7.16	-8.18	-	228.90 ± 23.32	-21.03
Na^{+}	1356.97	-	239.50 ± 2.72	-6.50	-	225.50 ± 7.63	-22.21

U_3 was used as model sample in this study

N.D. (Not detectable; end point of the titration cannot be accomplished)

Due to the RL can be stable around 4 hours, therefore, RL is needed to collect several time to accomplish the experiment

$$\%\text{Relative different} = \left[\frac{\text{Mg}^{2+}\text{contents from original RL} - \text{Mg}^{2+}\text{contents from added concentration of foreign ions)}}{\text{Mg}^{2+}\text{contents from original RL}}\right] \times 100$$

Table 4.8 Our developed test kit (without masking agent) performed with the presence of potential interference ions in RL sample.

Elements	Reported* ($mg.L^{-1}$)	Found by FAAS ($mg.L^{-1}$)	Spiked elements ($mg.L^{-1}$)	Total ($mg.L^{-1}$)	Complexometric titration ($mg.L^{-1}$) n=3	Developed Test Kit without masking agent n=3		% Relative different
						Drop	$mg.L^{-1}$	
Original RL** (Mg)	-	-	-	0	571.572 ± 2.38	12	600	4.97
Fe	0.27	57.6	60	117.6	576.85 ± 11.00	12	650	13.72
Cu	0.27	1.5	2	3.5	584.07 ± 6.02	12	600	4.97
Mn	7.45	4.9	5	9.9	578.51 ± 2.40	12	600	4.97
K	816	1215.8	1300	2515.8	573.31 ± 13.79	13	650	13.72
Zn	16.02	42.3	50	92.3	573.41 21.27	13	650	13.72
Ca	8.9	905.4	150	1055.4	593.67 ± 3.35	13	650	13.72
Na	966	870.7	1000	1870.7	555.06 ± 23.80	13	650	13.72

* [23-24] operated by inductively coupled plasma (ICP) atomic emission spectroscopy technique and FAAS

** RL from U_3 sample

Table 4.9 Recovery study by comparison between complexometric titration and our developed test kit (n=3)

Area of RL	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	Our developed test kit			
		Number of drops (1 drop≈50 $mg.L^{-1}$)	Mg^{2+} content ($mg.L^{-1}$)	Average of Mg^{2+} content ($mg.L^{-1}$)	% Recovery
Original U_1	748.48 ± 7.07	15	750	750.0	-
		15	750		
		15	750		
U_1 added 40 $mg.L^{-1}$ of Mg^{2+}	770.90 ± 8.22	16	800	800.0	101.3
		16	800		
		16	800		
U_1 added 80 $mg.L^{-1}$ of Mg^{2+}	812.17 ± 3.62	17	850	850.0	102.4
		17	850		
		17	850		
Original U_2	431.96 ± 0.84	10	500	500.0	-
		10	500		
		10	500		
U_2 added 40 $mg.L^{-1}$ of Mg^{2+}	488.49 ± 14.55	11	550	550.0	101.9
		11	550		
		11	550		
U_2 added 80 $mg.L^{-1}$ of Mg^{2+}	560.30 ± 6.05	12	600	583.3	100.6
		11	550		
		12	600		

4.1.5 Effect of preservatives in RL on our developing test kit

In general, preservation of RL can be long-term with the aim to maintain RL quality during storage and transportation by addition of preservatives to the samples. Short-term preservation involves a few days storage of liquid samples prior

to further processing. The related additives are anticoagulant in RL. A mixture of 0.2%, w/v NH_4OH, 0.025%, w/w ZnO and 0.025%, w/w TMTD are normally used in formulation processes in Thailand as alternative to the sole use of NH_4OH which has a pungent smell as well as causing environmental pollution and respiratory system irritation when released into the atmosphere [26]. Ammonia solution (NH_4OH) is conventionally added into the samples as a primary preservative in the concentration ranging from 0.2 to 0.5%, w/v which can inhibit reaction with bacteria under high pH condition and precipitate $Mg(OH)_2$ (reduction of free Mg^{2+}, Figure 4.2) [27].

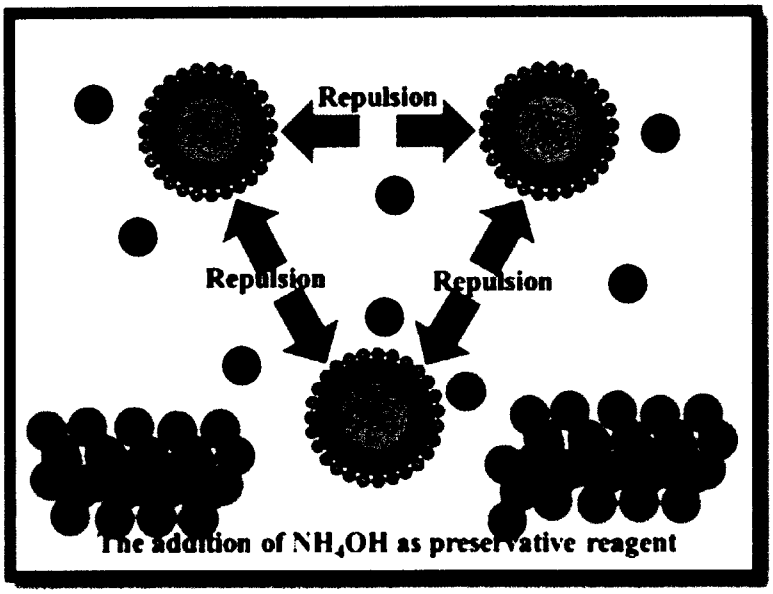


Fig. 4.2 The proposed mechanism when NH_4OH as preservative were performed.

ZnO and TMTD were added in RL as secondary preservatives which stabilize RL dispersed system. ZnO and TMTD can preserve natural rubber latex (by inhibiting bacteria growth) in the presence of small amounts of ammonia [4, 28]. The results are shown in Table 4.10. When the preservative solutions of 0.2%, w/v NH_4OH, 0.025%, w/w ZnO and 0.025%, w/w TMTD were added to the RL samples, Mg^{2+} content quantified by complexometric titration was slightly decreased in the system containing 0.2%, w/v NH_4OH. Moreover, Mg^{2+} content in the treated RL analyzed by our developed test kit was not statistically different from that obtained by

the complexometric titration. This suggests that the preservatives used in RL did not affect the developed test kit system.

Table 4.10 Effect of preservatives used in RL on our developed test kit (n=3).

Type of samples	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	Our developed test kit		
		Number of drops (1 drop ≈ 50 $mg.L^{-1}$)	Average of Mg^{2+} content ($mg.L^{-1}$)	% Relative differrent
Original RL	775.3 ± 9.25	15 15 15	750.0	+3.3
RL*	500.7 ± 4.39	10 10 10	500.0	+0.1
RL**	611.7 ± 14.01	11 11 11	550.0	+10.1

U_2 was used as model sample in this study.

RL* : Original RL preserved with 0.2% NH_4OH.

RL** : Original preserved with 0.2% NH_4OH, 0.025% ZnO and 0.025%TMTD.

%Relative different

$$= \left[\frac{Mg^{2+}\text{contents from complexometric titration} - Mg^{2+}\text{contents from our developing test kit)}}{Mg^{2+}\text{contents from complexometric titration}}\right] \times 100$$

The results from these two methods showed $t_{stat} = 1.02$ and $t_{critical} = 12.71$ which are not statistically different with 95% confidence level.

4.1.6 Effect of stability of reagents and real sample application of the developed test kit

Stability of reagents applied in the developed test kit method was studied. Reagent A and B were left at room temperature for 6 months. The resulting Mg^{2+} concentrations determined in both cases were compared with the analysis obtained by using freshly prepared reagents. The results showed good stability of both reagents, as shown in Table 4.11.

Samples were collected from several regions in Ubon Ratchathani province (U_2-U_3) and Kalasin province (K_1-K_3), Thailand. In each analysis, identification of Mg^{2+} was performed by applying the developed test kit, which were operated by three different users. The results were averaged and compared with those obtained by the conventional complexometric titration method.

Table 4.11 Stability of our developed test kit (reagent A and B) (n=3).

Area	Type of sample	Mg^{2+} content by complexo-metric titration ($mg.L^{-1}$)	6 months of reagent A		6 months of reagent B	
			Average of Mg^{2+} content ($mg.L^{-1}$)	% Relative differrent	Average of Mg^{2+} content ($mg.L^{-1}$)	% Relative differrent
U_2	Original RL	719.1±3.3	683.3	-5.0	700.0	-2.7
	RL mixed	611.7±14.0	566.5	-7.4	566.5	-7.4
U_3	with	667.1±7.5	633.3	-5.1	615.5	-7.6
	preservatives*	582.4±1.4	600.0	+3.0	600.0	+3.0

*Preservatives is a mixed solution of 0.2%NH_4OH, 0.025% ZnO and 0.025%TMTD in RL For stability test of Reagent A, the reagent B was freshly prepared. On the other hand, for stability test of Reagent B, the reagent A was freshly prepared.

Furthermore, the proposed method for Mg^{2+} determination in RL was evaluated by analyzing real samples from U_1, U_2 and K_1-K_3 with concentrations of

Mg^{2+} within in the ranges of 450-600 $mg.L^{-1}$ and 500-550 $mg.L^{-1}$, respectively. The results for all the samples were in good agreement with the values obtained from complexometric titration, see also Table 4.12.

Table 4.12 Real sample application of RL using our developed test kit.

Area	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	Subject	Our developed test kit		% Relative different
			Number of drops	Mg^{2+} content ($mg.L^{-1}$)	
U_1	523.1 ± 13.0	1	11	550	+5.14
		2	12	600	+14.70
		3	11	550	+5.14
U_2	465.1 ± 6.9	1	10	500	+7.50
		2	9	450	-3.25
		3	10	500	+7.50
K_1	518.0 ± 11.4	1	11	550	+6.18
		2	11	550	+6.18
		3	11	550	+6.18
K_2	491.1 ± 2.8	1	10	500	+1.81
		2	10	500	+1.81
		3	10	500	+1.81
K_3	558.4 ± 6.4	1	10	500	-10.46
		2	10	500	-10.46
		3	10	500	-10.46

$$\%\text{Relative different} = \left[\frac{(Mg^{2+}\text{contents from complexometric titration} - Mg^{2+}\text{contents from our developing method})}{Mg^{2+}\text{contents from complexometric titration}}\right] \times 100$$

The results from these two methods showed $t_{stat} = 0.009$ and $t_{critical} = 3.18$ which are not statistically different with the confidence level of 95%.

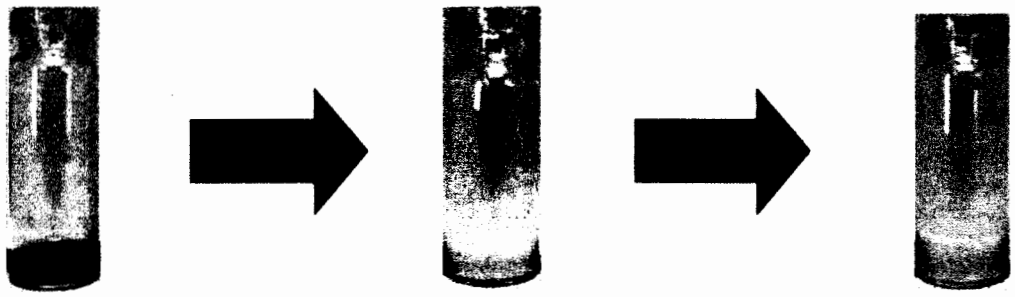


Figure 4.3 The operation of developed test kit based on colorimetry using naked eyes for identification of Mg^{2+} content in rubber latex (RL) operated in RL sample containing 775 mg.L^{-1} Mg^{2+}.

Table 4.13 Effect of concentrated rubber latex (CRL) using our developing test kit by comparison with complexometric titration (n = 3).

Type of CRL sample	Mg^{2+} content by complexometric titration (mg·L^{-1})	Our developed test kit	
		Number of drops (1 drop ≈ 50 mg.L^{-1})	Mg^{2+} content (mg.L^{-1})
CRL-Type A	55.54 ± 3.34	1	50.00
CRL- Type B	54.25 ± 1.27	1	50.00

(U_2 was used as model RL sample in this study which was contained 811.11 ± 28.40 mg·L^{-1} of original Mg^{2+} content conducting by complexometric titration).

CRL-Type A: Mg^{2+} in RL (U_2) were removed by addition of diammonium hydrogen phosphate (DAHP) [7-8].

CRL-Type B: Clear serum with acidic medium (pH 3) was obtained from CRL* by diluting 10 g of CRL* with 10.0 mL of water and coagulate with 5.0 mL of 25% acetic acid water [15].

4.2 Second approach; the development of a low cost microfluidic thread-based analytical device (μTAD) for determination of Mg^{2+} content in RL

4.2.1 Validation of our developed preparation of RL sample for proposed μTAD operated by flame atomic absorption spectrophotometer (FAAS)

In table 4.14, the result were demonstrated successful of our developed preparation of RL sample that suggested this method can digested Mg^{2+} into serum. The concentration of Mg^{2+} of original and serum of RL were 582.9 ± 0.0002 and 534.4 ± 0.0011 $mg.L^{-1}$ revealed that there is no difference. Furthermore, for Mg^{2+} in sludge of RL was less occurred.

Table 4.14 Method validation of our developed preparation of RL sample for proposed μTAD operated by FAAS

Sample	Concentration of Mg^{2+}, $mg.L^{-1}$
	Flame Atomic Absorption spectrophotometer (FAAS) ($mg.L^{-1}$)
Original RL	582.9 ± 0.0002
Serum of RL	534.4 ± 0.0011
Sludge of RL	26.5 ± 0.0003

*FAAS (PinAAcle 900T; Perkin Elmer, US) equipped with hollow cathode lamps (HCL) of Mg at 285.2 nm with lamp current at 84 mA with operating conditions as followed; slit width 0.7 nm, air flow rate and acetylene flow rate at 8.0 and 2.5 $m^3.s^{-1}$, respectively

4.2.2 Characterization of threads on our developed μTAD

4.2.2.1 Effect of percentage of cotton on thread

Four types of threads are A, B, C and D were investigated percentage of cotton on threads. From Figure 4.4, solubility of various threads was occurred. For sample A-C, threads were soluble, small fraction of thread is insoluble and dark precipitation, respectively. In contrast, thread of sample D was insoluble. Moreover, in

table 4.15, percentage of cotton on thread of different type show that sample A,B and C are 100%, 90% and 80% cotton, respectively, whereas sample D is non-cotton.

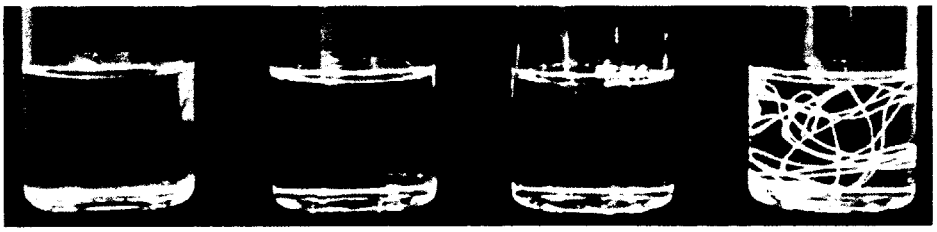


Figure 4.4 Produce for testing percentage of cotton in thread.

Table 4.15 The percentage of cotton on various threads.

Sample	W_0 (g)	W (g)	Percentage of cotton in thread $((W_0-W)\times100)/W_0$	Type of thread
A	0.0247±0.0082	-	-	100% cotton
B	0.0301±0.0151	0.0028±0.0012	90.70	90% cotton
C	0.0238±0.0079	0.0051±0.0017	78.57	80% cotton
D	0.0175±0.0058	0.0173±0.0065	1.14	Non cotton

4.2.2.2 Effect of diameter measurements of threads

The average±S.D. diameter of threads (cotton 80%, cotton 90%, cotton 100%, polyester 100%) measured by using digital vernier caliper were 300 ± 0.5, 290 ± 0.1, 300 ± 0.2 and 180 ± 0.1 μm, respectively (Table 4.16).

Table 4.16 The measurement of diameter of various types of thread using Vernier caliper (n=10).

Sample	Replication of diameter measurements of thread (μm)										Average (μm)	S.D. (μm)	Average ± S.D. (μm)
	1	2	3	4	5	6	7	8	9	10			
Cotton 80%													
Sample 1	340	360	300	270	330	340	310	280	330	330	320	0.284	300 ± 0.5
Sample 2	310	330	400	270	290	180	210	380	340	340	300	0.815	
Sample 3	310	240	310	320	260	280	300	340	270	360	300	0.369	
Cotton 90%													
Sample 1	320	290	320	290	270	280	300	290	290	290	290	0.157	290 ± 0.1
Sample 2	280	290	290	300	300	290	280	300	290	280	290	0.081	
Sample 3	300	300	270	290	290	310	300	310	300	290	300	0.117	
Cotton 100%													300 ± 0.2
Sample 1	340	310	270	300	310	270	290	300	300	290	300	0.204	
Sample 2	280	290	290	330	300	300	270	320	300	290	300	0.176	
Sample 3	290	300	310	310	290	290	330	340	320	320	310	0.176	
Polyester													
Sample 1	210	210	190	210	160	180	190	190	180	200	190	0.162	180 ± 0.1
Sample 2	150	180	160	160	170	160	180	190	190	170	170	0.137	
Sample 3	160	180	180	170	180	180	180	190	180	190	180	0.088	

4.2.3 Effect of wicking property of the threads

For wicking property study, we also examined four different types of thread (cotton 80%, cotton 90%, cotton 100%, polyester 100%) were utilized for fabricating μTAD (for wicking fluid in thread for fabricating μTAD). To perform, The firstly were cotton 80%, cotton 90%, cotton 100% and polyester 100% threads; It's have average diameter of 300±0.5, 290±0.1, 310±0.2 and 180±0.1 μm, subsequently. The fibers structures of thread are therefore different; the rate of transport of fluids and liquid penetration are different. The wicking properties of the threads with untreated and treatment with 10 $mg.L^{-1}$ of Na_2CO_3 for covered wax removal were examined in order to ensure the reproducibility of our results on μTAD. This is because fluid wicking rate along thread relies on the surface properties of theirs fibbers. Any surface heterogeneity will result in uneven rates of wicking. Moreover, natural cotton thread (un-mercerized) is hydrophobic [39]. To allow the wicking of aqueous solution on cotton threads, a chemical treatment is required to remove surface contaminants. Conversely, the synthetic, polyester thread is inherently hydrophilic, allowing aqueous liquids to wick by capillary action between fibers. The wicking rate (distance, cm/min) of threads before and after treated measured according to a ruler scale were shown in Figure 4.5 and Table 4.17. 80% cotton did not wick before treatment because of the thread hydrophobicity as mentioned above. After treatment, 80% cotton increased the wicking rate. However, the wicking rates for 90% cotton, 100% cotton and 100% polyester decreased after treatment. This can be explained by the degeneration of the fiber structures after treatment. The result show that the most suitable thread of wicking rate was 100 % cotton thread due to the good wicking and dyeing properties, which was selected for μTAD fabrication in this study. Note that 100% polyester showed the best wicking property but it is hard to dye which is not suitable for the measurement of length of color zone on μTAD.

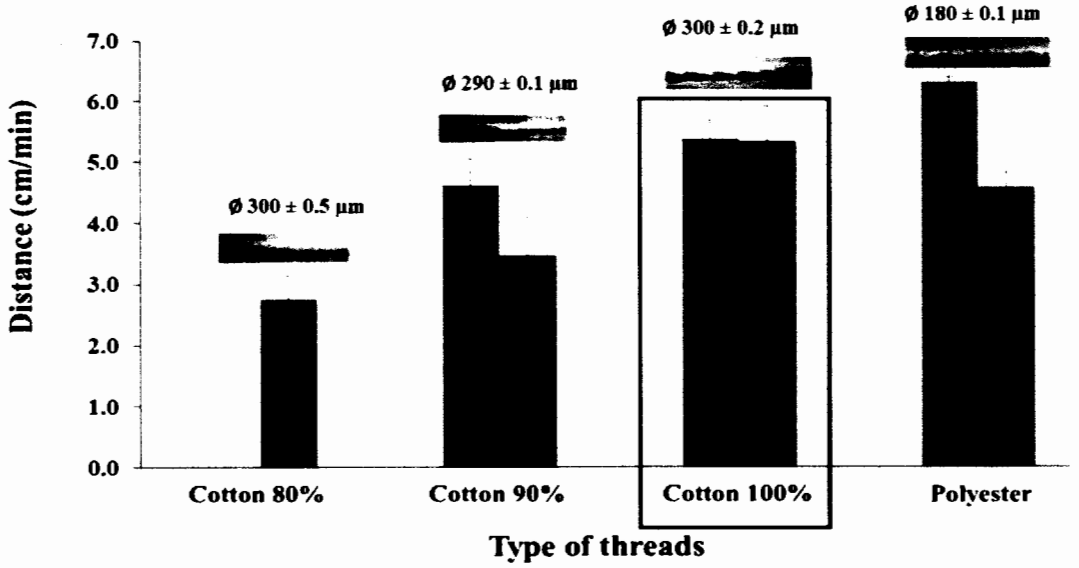


Figure 4.5 Wicking properties of untreated thread (■) and treated thread (■), (n=3).

Table 4.17 Wicking property of the threads.

Type of threads	Untreated (cm/min)	Treated (cm/min)
cotton 80%	0.00 ± 0.00	2.76 ± 0.38
cotton 90%	4.62 ± 0.42	3.47 ± 0.64
cotton 100%	5.38 ± 0.30	5.33 ± 0.59
polyester 100%	6.31 ± 0.09	4.58 ± 0.24

Note: Red color solution of food dye (3 µL) was added into central each thread (each with the length of 10 cm) using a micropipette for 1 minute and measurement by stainless steel metal ruler with 15 cm/0.15 cm.

4.2.4 Optimization of reagent conditions and their mechanism on μTAD

For complexometric method, the optimized condition used in our developed μTAD for complexometric analysis of Mg^{2+} was based on conventional benchmark, ISO 17403: 2014(E) [15] and our previous study [7] using EBT, EDTA and NH_4Cl/NH_4OH buffer solution. A well known reaction mechanism for the Mg^{2+} analysis in aqueous (water and RL sample) solution is determined by EDTA titration after the sample has been buffered to pH 10 with EBT indicator, can serve as indicator in this titrations. The purple complex between Mg^{2+} and EBT will be formed before titration. At end point, after complex between Mg^{2+} and EDTA is completed. EBT will return to blue color as an original form Eq.1.1 in CHEAPTER 1. To develop the simple complexometric titrations for analytical detection by measuring the length of color change on indicator treated thread. From preliminary study on μTAD (data not shown), the evaporation of ammonium buffer on μTAD is a main problem for the complexometric titration on μTAD. Thus, CAPS buffer was replaced and used as buffer for titration on μTAD. The mechanism for the complexometric titration on μTAD is described in Eq.4.1-4.3. Threads were firstly pretreated with EBT (H_2In^-), which the purple color was observed (Figure 4.6 and Eq.4.1).

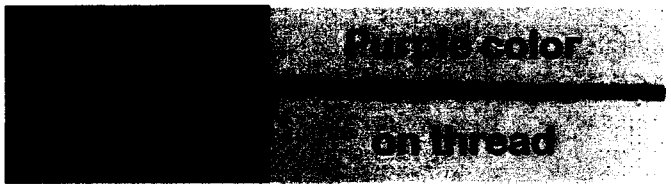


Figure 4.6 Purple color on thread after dipped thread with EBT.

$$\underset{\text{pH} < 6.3}{\underset{\text{(red)}}{H_2In^-(aq)}} \overset{\text{purple}}{\leftrightarrow} \underset{6.3 < \text{pH} < 11.6}{\underset{\text{(blue)}}{HIn^{2-}(aq)}} \qquad (4.1)$$

μTAD was then treated with EDTA in CAPS buffer at pH 10 where the purple color on μTAD as previous step was changed to blue color due to the

deprotonation of EBT and fully-protonated form of EDTA (Y^{4-}) is ready to react with Mg^{2+} (Figure 4.7 and Eq.4.2).

Figure 4.7 Blue color on thread after dropped EDTA (Y^{4-}) in CAPS to thread.

$$H_2In^-(aq) + Y^{4-}(aq) + OH^-(aq) \leftrightarrow HIn^{2-}(aq) + Y^{4-}(aq) + H_2O(l) \quad (4.2)$$

Next, sample solution containing Mg^{2+} was then applied to as prepared μTAD. The length of purple color zone was again generated on μTAD (Figure 4.6 and Eq.4.3) due to the fact that the formation constant between Mg^{2+} and EDTA is higher (stable) than Mg^{2+} and EBT (K_f of $[MgY^{2-}]$ (7.1×10^8) and K_f of $[MgIn^-]$ (1.0×10^7) [40]. At end point, after complexation between Mg^{2+} and EDTA is completed. As EDTA is limiting agent, the remained Mg^{2+}will form with EBT, result in the purple color again (Figure 4.8 and Eq.4.3).

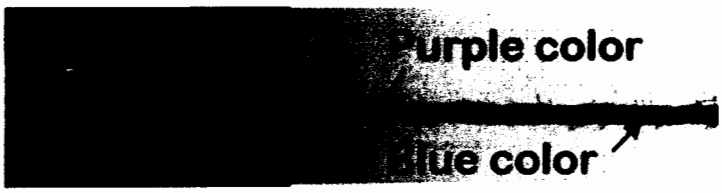


Figure 4.8 Purple color zones in blue color after dropped sample (Mg^{2+}) to the thread.

$$2Mg^{2+}_{(aq)} + HIn^{2-}_{(aq)} + Y^{4-}_{(aq)} + OH^-_{(aq)} \leftrightarrow MgY^{2-}_{(aq)} + MgIn^-_{(aq)} + H_3O^+_{(aq)} \quad (4.3)$$

From previous section, it's corresponded to the zoom up pictures obtained from microscope. Therefore, the concentration of EDTA and suitable pH are key element on this developed reaction on μTAD. (Figure 4.9)

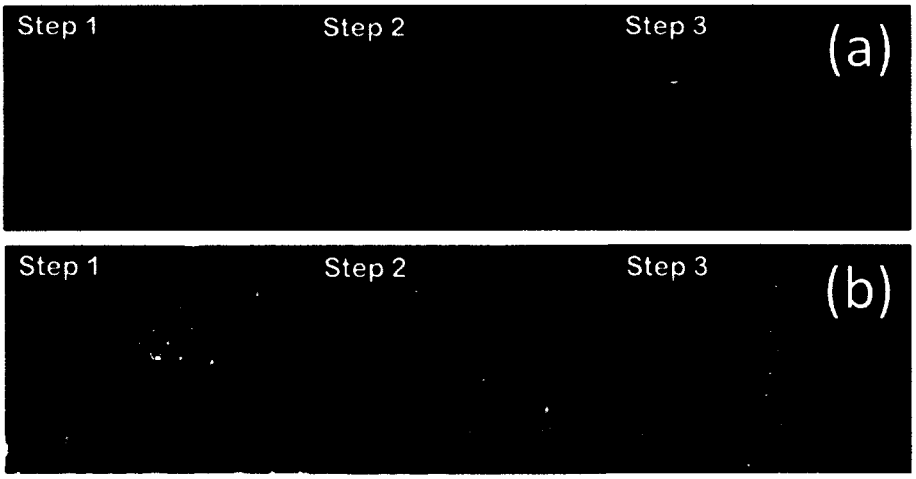


Figure 4.9 The pictures obtained from microscope at along thread type (a) and cross section type (b), respectively.

To prove the mechanism and function of EDTA in suitable pH (threads were then tied together for clear observation), thread were pretreated with EBT. After that, μTAD was then treated with EDTA in CAPS buffer at pH 10 and EDTA in DI water at pH 6. It is clearly seen that only condition with EDTA in suitable pH (condition A) can be observed the length of purple color zone in the presence of Mg^{2+} (Figure 4.10). Furthermore, the condition B (EDTA in CAPS buffer) was further investigated the effect of EDTA in CAPS buffer at pH 10.0 by comparison between in the presence of EDTA and in the absence of EDTA in CAPS buffer at pH 10 for performing complexometric titration on μTAD as demonstrated in Table 4.18 It is clearly seen that the length of purple color product generating on μTAD was proportional to the various concentration of Mg^{2+} for both presence and absence of EDTA in CAPS buffer at pH 10. The length of purple color product at every concentration of Mg^{2+} occurred in the absence of EDTA in CAPS buffer at pH 10 condition was higher than that of the presence of EDTA in CAPS at pH 10 and limited at 750 $mg.L^{-1}$ Mg^{2+} since no complexation between Mg^{2+} and EDTA corresponding to our proposed mechanism as mentioned above. This observation can confirm that the concentration of EDTA in suitable pH are key element on this developed complexometric titration on μTAD.

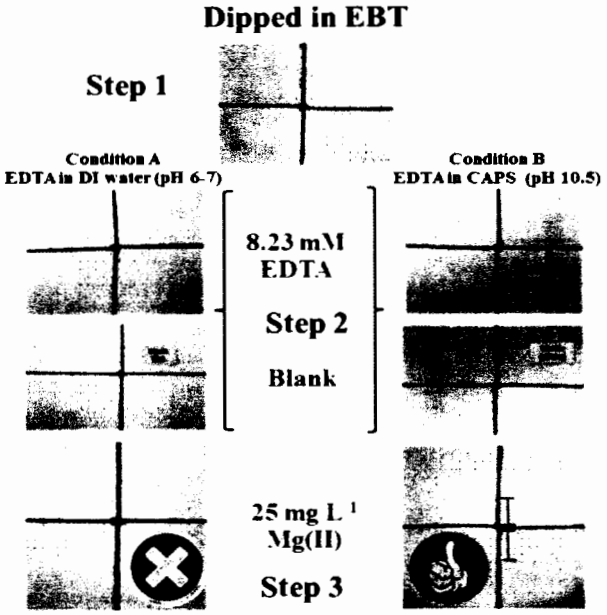


Figure 4.10 Effect of EDTA in different diluents for performing complexometric titration on μTAD.

Table 4.18 Effect of the presence and absence of EDTA in CAPS buffer (pH 10.0) for performing complexometric titration on μTAD.

Concentration of Mg^{2+} ($mg.\ L^{-1}$)	Length (cm) of purple color change	
	The presence of EDTA in CAPS buffer (pH 10.0)	The absence of EDTA in CAPS buffer (pH 10.0)
Blank*	0	0
50*	0.6	0.8
250**	2.1	2.5
500**	2.5	4
750**	3	4.7
1000**	3.4	4.7

Note: Measurement by stainless steel metal ruler with 15 cm/0.15 cm.

*Using EDTA 8.23 mM and **Using EDTA 30.86 mM

The optimization of EDTA concentration in CAPS buffer at pH 10 for fabrication of complexometric titration on μTAD was examined as demonstrated in Table 4.19. The studied EDTA concentrations were in the range of 6.17- 41.14 mM. The suitable concentration of EDTA were 8.23 mM and 30.86 mM of EDTA in CAPS solution at pH 10 for low (25-100 $mg.L^{-1}$) and high range (250-1000 $mg.L^{-1}$) of Mg^{2+} detection, respectively, considering from proportional length of purple color product generated on μTAD provided wide working range of Mg^{2+} detection.

Table 4.19 Optimization of EDTA concentration (mM) for complexometric titration on μTAD.

Concentration of Mg^{2+} ($mg.L^{-1}$)	**Length (cm) of purple color change**					
	Concentration of EDTA (mM)					
	6.17	**8.23**	**10.29**	**20.57**	**30.86**	**41.14**
0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
25	0.7	0.6	N.D.	N.D.	N.D.	N.D.
50	1	0.8	0.8	N.D.	N.D.	N.D.
100	1.3	1.3	1.2	1.1	N.D.	N.D.
250	-	-	-	2	1.5	1.1
300	-	-	-	2.3	1.8	1.8
500	-	-	-	3.1	2.2	2.2
750	-	-	-	-	2.7	2.8
1000	-	-	-	-	3.7	4

Note: Measurement by stainless steel metal ruler with 15 cm/0.15 cm.

* N.D. defined as "Not detectable" ;the length of purple color zone was not generated on μTAD

- defined as the length of purple color zone was fully generated along μTAD, indicating that the concentration of EDTA is limited

Therefore, box platform of μTAD was designed and fabricated as mentioned in Figure 4.11. These devices consisted of two threads which were pretreated with EBT and then treated with different concentration of CAPS buffer at pH 10. Both threads were then tied together with a central knot before being attached to a box platform prior to analysis. The small sample (6 μL) can be added onto the crossing threads and the length of the color change can be measured with a ruler (summation of length from the central knot: Up and down for detection of low range of Mg^{2+} concentration, left and right for detection of high range of Mg^{2+} concentration). The analyte in samples then reacted with indicator reagents deposited on μTADs. This instantly produced the length of purple color zone on μTAD being proportional to the concentration of Mg^{2+} in samples.

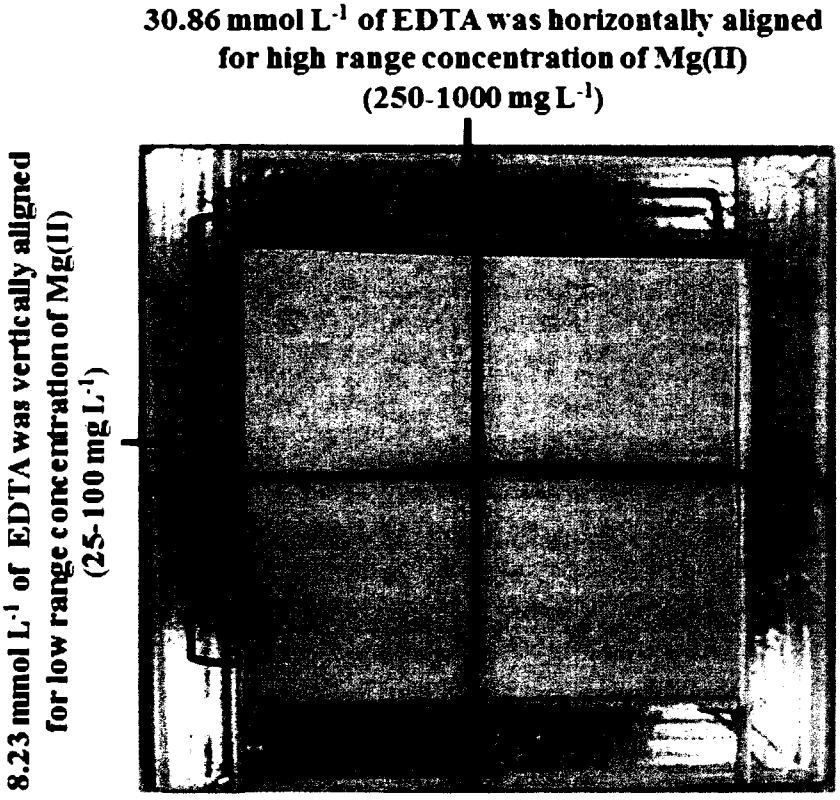


Figure 4.11 The images illustrate that different concentrations of Mg^{2+} produced a color zone of differing length on developed μTAD for complexometric analysis.

4.2.5 Effect of reagent volume

For reagent volumes, liquid wicking tests were carried out by applying aqueous solutions of color solution to the threads. There are six different volumes of color solution a wide range of volume from 1.0 to 6.0 μL were introduced onto the central of the threads and observing by length of color change on μTAD with ruler scale. Ultimately, the solution of 3 μL wicked along the threads and filled the device within one minute. A 3 μL solution was introduced onto the threads with a micropipette; the test solution rapidly wicked along the threads.

4.2.6 Effect of foreign ions on the distance change of added ions at various concentrations containing of Mg^{2+} in RL sample

Effect of foreign ions on the length of color change of added ions at various concentrations containing of Mg^{2+} at low range and high range of Mg^{2+} detection were investigated considering from deviation of length of purple color measurement of Mg^{2+} standard at 50 $mg.L^{-1}$ and 250 $mg.L^{-1}$ without foreign ions were 0.71 cm ± 0.10 cm and 2.08 cm ± 0.20 cm (n = 10), respectively (deviation of length of color change of added ions was less than SD of Mg^{2+} standard). The results shown in Table 4.20 that no interference from foreign ions only interference by hydroxide ions (OH^-) at low range of Mg^{2+} detection which can be sorted out by pH adjustment before sample applications. Effect of foreign ions on the length of color change of added OH^- at 10 $mg.L^{-1}$ containing of Mg^{2+} at low range of Mg^{2+} detection was investigated considering from deviation of length of purple color measurement compared to bare Mg^{2+} standard at 50 $mg.L^{-1}$ without foreign ions. The negative deviation of length of color change of added ions was higher than SD of bare Mg^{2+} standard. The mechanism can be explained by Eq. 4.4.

$$3Mg^{2+}_{(aq)} + HIn^{2-}_{(aq)} + Y^{4-}_{(aq)} + 3OH^-_{(aq)} \rightleftharpoons Mg(OH)_{2(s)} + MgY^{2-}_{(aq)} + MgIn^-_{(aq)} + H_2O_{(l)} \quad (4.4)$$

However, the concentrations of these ions in the real water samples and rubber latex are very much lower than the studied concentrations. [15]

Table 4.20 Effect of foreign ions on the length of color change of added ions containing 50 mg L^{-1} and 250 mg L^{-1} of Mg^{2+}

Foreign ions	Compounds	Low range of Mg^{2+} detection at 50 mg.L^{-1}		High range of Mg^{2+} detection at 250 mg.L^{-1}	
		Tolerance concentration (mg.L^{-1})	Distance error (cm)	Tolerance concentration (mg.L^{-1})	Distance error (cm)
K^+	K_2SO_4	700.0	0.02	750.0	0.11
Na^+	Na_2SO4	90.0	0.05	450.0	0.10
Fe^{2+}	$FeSO_4.7H_2O$	50.0	0.06	250.0	0.12
Mn^{2+}	$MnSO_4$	150.0	0.02	200.0	0.05
Ca^{2+}	$Ca(NO_3)_2$	20.0	0.01	500.0	0.09
Cu^{2+}	$CuSO_4.5H_2O$	50.0	0.06	150.0	0.05
Zn^{2+}	$ZnSO_4$	80.0	0.01	200.0	0.03
OH^-	NaOH	10.0	-0.38	20.0	0.11
SO_4^{2-}	$MgSO_4.7H_2O$	140.0	0.02	150.0	0.11
NO_3^-	$Ca(NO_3)_2$	72.2	0.01	140.0	0.05
PO_4^{3-}	$NaH_2PO_4.2H_2O$	50	0.01	300.0	0.14
Cl^-	NaCl	250	0.01	1000.0	-0.20

Note: Measurement by stainless steel metal ruler with 15 cm/0.15 cm.

*Length measurement of Std. Mg^{2+} 50 mg.L^{-1} was 0.71 cm ± 0.10 cm (n = 10)

Length measurement of Std. Mg^{2+} 250 mg.L^{-1} was 2.08 cm ± 0.20 cm (n = 10)

4.2.7 Analytical characteristics and method validation of developed μTAD for Mg^{2+} detection in RL

4.2.7.1 Working range and Limit of quantification (LOQ)

Working range performed on developed μTAD for complexometric analysis of Mg^{2+} determination was demonstrated in Appendix A. The linearity ranges

of the calibration curves (length of purple color (cm) and concentration of Mg^{2+} ($mg.L^{-1}$)) for complexometric analysis of Mg^{2+} were found to be 25-225 $mg.L^{-1}$ (Figure 4.12(a), Table. 21 and A.1) and 300-1000 mg L-1(Figure 4.12(b), Table 2.1 and A.2) with a good precision of intraday and interday (%RSD <6) (Table 4.22). Moreover, the LOQ was evaluated from lowest concentration of Mg^{2+} (25 $mg.L^{-1}$) which can be observed the length of color change on μTAD by naked eyes.

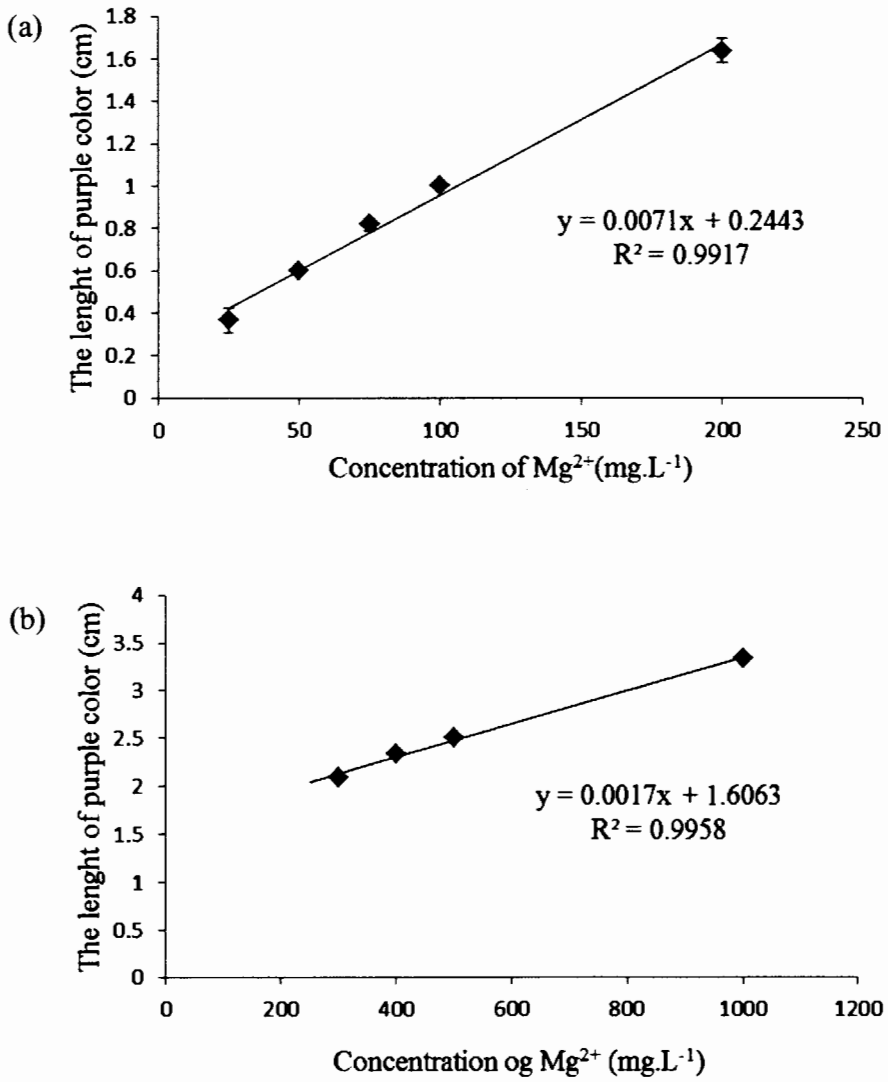


Figure 4.12 The images illustrate that working range performed on developed μTAD of Mg^{2+} determination for low range concentration of Mg^{2+}(a) and high range concentration of Mg^{2+}(b).

Table 4.21. The illustration of working range of Mg^{2+} detection (threads treated with 8.23 and 30.86 mmol L^{-1} of EDTA were vertically aligned for detection of low range of Mg^{2+} concentration and the other thread treated with 30.86 mmol L^{-1} of EDTA was horizontally aligned for detection of high range of Mg^{2+} concentration, respectively).

Range	The length of purple color (cm), n=3		
	Conc. of Mg^{2+} (mg L^{-1})	Up & Down	Left & Right
Low	0	N.D.	N.D.
	25	0.4 ± 0.38	N.D.
	50	0.6 ± 0.00	N.D.
	75	0.8 ± 0.03	N.D.
	100	1.0 ± 0.00	N.D.
	150	1.4 ± 0.06	N.D.
	200	1.6 ± 0.06	N.D.
	225	-	N.D.
High	250	-	1.8 ± 0.06
	300	-	2.1 ± 0.08
	400	-	2.3 ± 0.06
	500	-	2.5 ± 0.00
	750	-	3.0 ± 0.06
	1000	-	3.3 ± 0.06
	1250	-	-
	1500	-	-

Note: Measurement by stainless steel metal ruler with 15 cm/0.15 cm and the length of the purple color change can be measured with a ruler (summation of length from the central knot: Up and down for detection of low range of Mg^{2+} concentration, left and right for detection of high range of Mg^{2+} concentration)

* N.D. defined as "Not detectable" ;the length of purple color zone was not generated on μTAD and - defined as the length of purple color zone was fully generated along μTAD, indicating that the concentration of EDTA is limited

Table 4.22 The %RSD of intraday and interday at low and high range.

Range	Concentration of Mg^{2+} ($mg.L^{-1}$)	%RSD	
		Intraday precision	Interday Precision
Low	50	0.0	0.0
	100	0.0	4.8
High	250	3.0	2.4
	750	1.9	1.7

4.2.7.2 Validation of develop μTAD method and real sample application

The developed complexometric titration on μTAD was applied for analysis of Mg^{2+}in real RL samples shown in table 4.23. Complexometric titration was selected as the conventional technique for Mg^{2+}analysis which showed results being in agreement with our μTAD approach. Moreover, the recovery of the developed μTAD for spiked RL and waters sample are good and acceptable.

Table 4.23 Determination of Mg^{2+} in real samples using proposed μTAD compared with classical complexometric titration method (n=3)

Type of sample	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	Our developed μTAD			
		Average of distance of purple color (cm)	Average of Mg^{2+} content ($mg.L^{-1}$)	%Recovery	% Relative error
RL no.1	351.24± 2.65	2.18 ± 0.02	351.18 ± 16.03	-	-0.01
RLno.1*	402.09 ± 2.86	2.28 ± 0.02	406.74 ± 16.03	101.38	1.15
RL no.1**	459.67 ± 2.65	2.52 ± 0.02	453.03 ± 16.03	100.41	-1.45

* RL no.1 : RL added 50 $mg.L^{-1}$ of Mg^{2+}

** RL no.1 : RL added 100 $mg.L^{-1}$ of Mg^{2+}

CHAPTER 5
CONCLUSIONS

First approach, the development of a simple test kit based on colorimetry for quantification of Mg^{2+} content in RL by miniaturized complexometric titration without using masking agent was investigated. A simple and portable field test kit for colorimetric determination of magnesium content in rubber latex (RL) was successfully developed according to the concept of green chemistry by reducing waste generation, minimizing the use of chemicals and consumption of time (at least simple two reagents (EBT indicator for reagent A; EDTA in ammonium buffer for reagent B) for test kit set up). These were found to be effective and non-instrumental approaches with low cost, simple (no requirement of skill for analysis), not demanding sample pretreatment before analysis, small sample volume uptake (0.18 g, sampling by a small spoon) and use of <1.5 mL reagent volume which was >70 times less than when compared with conventional methods and the other commercial test kits. Our developed test kit (even without masking agent) can be applicable even with the presence of potential interference ions (see also in Table 4.8) and preservatives in RL due to the effect of minimizing scale of reagents and sample. Moreover, the EDTA concentration in reagent B could be adjusted for matching with theoretically expected magnesium concentration. The performance of the approach meets the requirement for analysis of magnesium content in practical RL samples which can be performed within a minute and observed by naked eye based on comparison with a color chart. Moreover, our developed test kit is stable at room temperature for more than 6 months. The established approaches were not only applicable for RL analysis, but it is also for practical use in concentrated rubber latex since our developed test kit in terms of reagent B can provide enough buffer capacity system (even one drop was applied into the sample) which is suitable for acidic samples such as CRL.

Second approach, described the development of a low cost microfluidic thread analytical device (μTAD) for determination of Mg^{2+} content in RL. A developed μTAD, fabricated from untreated cotton thread based on complexometric titrations provide an easy-to-use platform for rapid measurement of magnesium concentrations in rubber latex by measuring the length of color change on indicator treated thread. In this method, interaction between deposited reagents and analytes within samples produces colored zones of differing lengths on the threads within only a few minutes analyzed by unaided human eyes using the printed scales correlates with the concentrations of the analytes in the samples. Furthermore, the analysis of real samples using the developed μTAD were agreed well with those obtained by classical titrations. Our developed system can facilitate a rapid, simple, sensitive (providing a low detection limit and wide dynamic range of detection: a significant reduction in the Mg^{2+} LOD is achieved by in situ pre-concentration (multiple applications of the testing zone on μTAD). Moreover, our developed μTADs to be used as length measurement could lead toward the development of other interesting analytes with broad applications, offering a convenient and cost-effective alternative to the conventional laboratory-based equipment.

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APPENDICES

APPENDIX A

The images illustrate that low and high range concentration of Mg^{2+}detection on developed µTAD for complexometric analysis

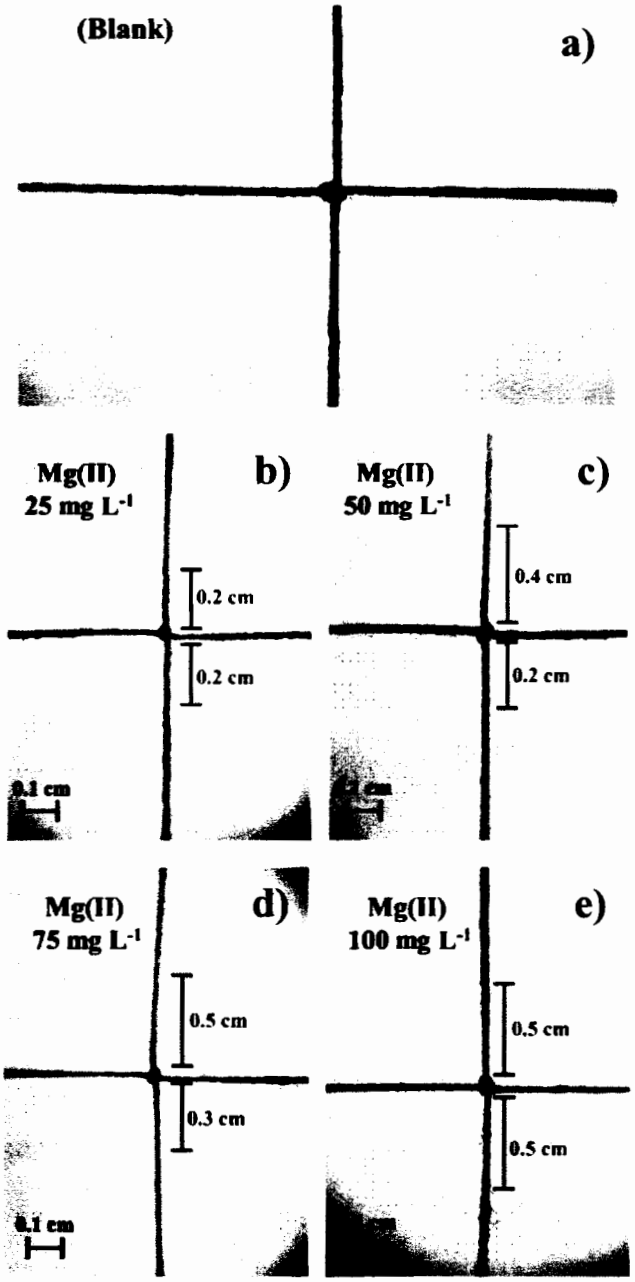

Figure A.1 The images of illustrate blank a) and low range concentration of Mg^{2+}detection 25b), 50c), 75d) and 100e) mg L^{-1}, respectively, on developed μTAD for complexometric analysis. (Photo taken by Iphone7 combine with Chula smart lens)

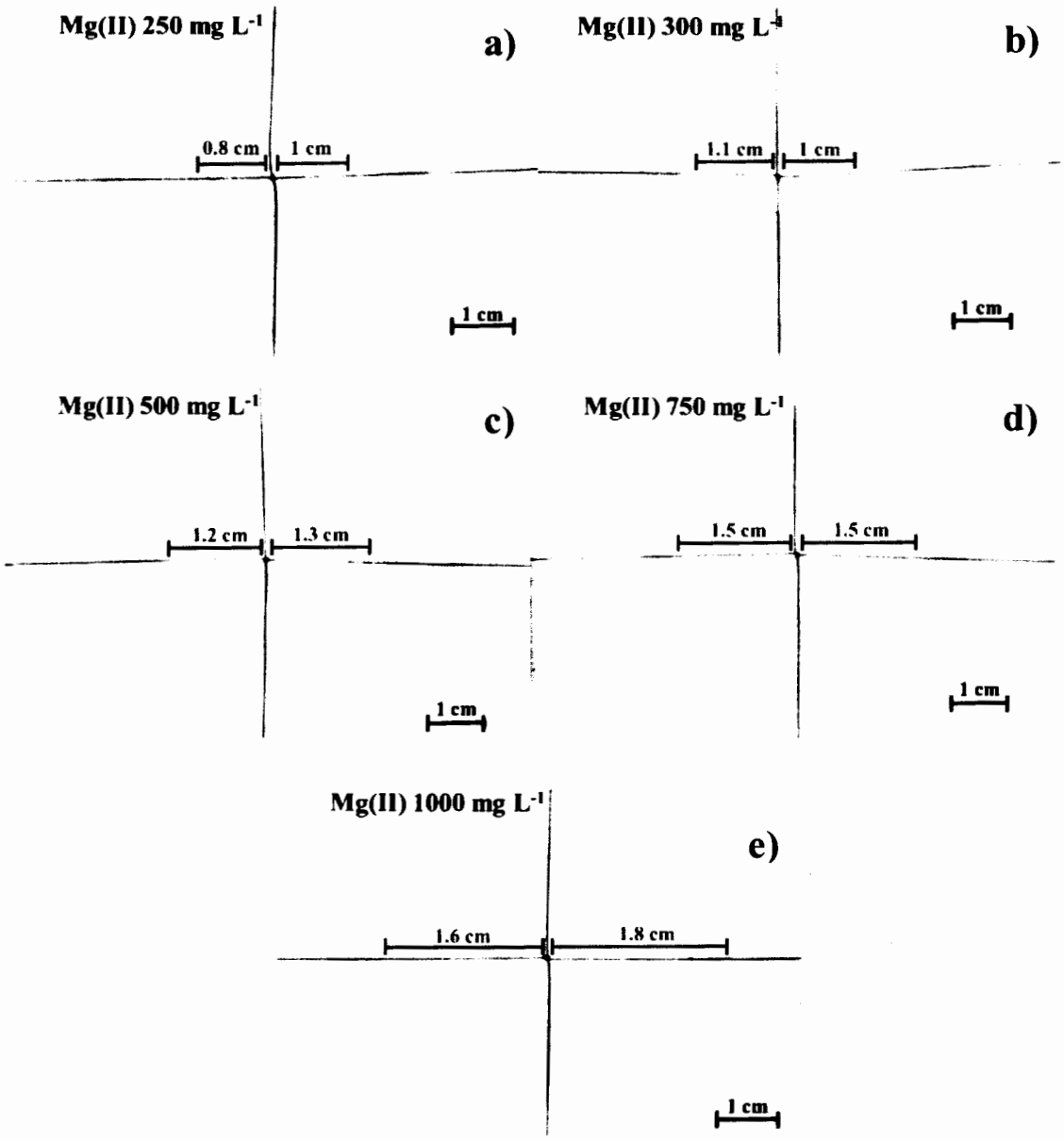

Figure A.2 The images illustrate that high range concentration of Mg^{2+} detection 250a), 300b), 500c), 750d) and 1000e) mg L^{-1}, respectively, on developed μTAD for complexometric analysis (Photo taken by digital camera, Olympus).

APPENDIX B

The result of Mg^{2+} content in water samples by using developed μTAD

Table B.1 Determination of Mg^{2+} in real water samples using proposed μTAD compared with conventional complexometric titration method.

Sample	Complexometric titration		Proposed method			
	Concentration of Mg^{2+} ($mg.L^{-1}$)	% Recovery	Length of red-violet color (cm)	Concentration of Mg^{2+} ($mg.L^{-1}$)	% Recovery	% Relative different
Tap water	1.32 ± 0.70	-	0.0	-	-	-
Spiked 100 mg L^{-1} of Mg^{2+}	95.23 ± 0.60	94.0	1.0	98.04 ± 0.03	-	-3.0
Canal water	10.12 ± 1.50	-	0.0	-	-	-
Spiked 100 mg L^{-1} of Mg^{2+}	109.20 ± 0.90	99.2	1.05	103.91 ± 3.80	-	-4.8
Mineral water	54.96 ± 0.69	-	0.70	51.78 ± 11.17	-	5.8
Spiked 100 mg L^{-1} of Mg^{2+}	151.12 ± 1.35	97.5	1.20	162.89	107.3	7.8

$$\%\text{Relative difference} = \left[\frac{Mg^{2+}\text{contents from our developed method} - Mg^{2+}\text{contents from complexometric titration}}{Mg^{2+}\text{contents from complexometric titration}}\right] \times 100$$

APPENDIX C

Effect of interference of complexometric reaction on μTAD

Effect of foreign ions on the length of color change of added metal ions (M^{2+}) at various containing of Mg^{2+} at low and high range of Mg^{2+} detection on developed μTAD was described in Eq. C.1.

$$M^{2+}_{(aq)} + 2Mg^{2+}_{(aq)} + HIn^{2-}_{(aq)} + 2Y^{4-}_{(aq)} + 2OH^{-}_{(aq)} \leftrightarrow MY^{2-}_{(aq)} + MgY^{2-}_{(aq)} + MgIn^{-}_{(aq)} + H_2O_{(l)} \quad (C.1)$$

*$MgIn^{-}_{(aq)}$ is excess

M^{2+} means other metal in RL such as Ca^{2+} and Mn^{2+}

That no interference from foreign ions because the concentrations of these ions in the rubber latex are very much lower than the studied concentrations. [15]

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1. N. Malahom, P. Jarujamrus*, R. Meelapsom, A. Siripinyanond, M. Amatatongchai and S. Chairam. "Simple test kit based on colorimetry for quantification of magnesium content in natural rubber latex by miniaturized complexometric titration without using masking agent" **Polymer Testing**. 59, 160-167.
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N.Malahom[1], R. Meelapsom[1], A. Siripinyanond[2], M. Amatatongchai[1], S. Chairam[1] and P. Jarujamrus[1*], "Development of field test kit based on colorimetry for quantification of magnesium content in natural rubber latex" **The 13th Asian Conference on Analytical Sciences (ASIANALYSIS XIII)** 8-11 December, 2016. The Empress International Convention Center, Chiang Mai, Thailand.

POSTER PRESENTATION

S. Puchum1, N. Malahom[1], R. Meelapsom[1], A. Siripinyanond[2], M. Amatathongchai[1], S. Chairam[1] and P. Jarujamrus[1*] "Simple test kit based on colorimetry for quantification of magnesium content in rubber latex" **North Eastern Science and Technology Conference 2017: NESTC2017**, 18 March 2017, Ubon Rachathani University, Thailand.

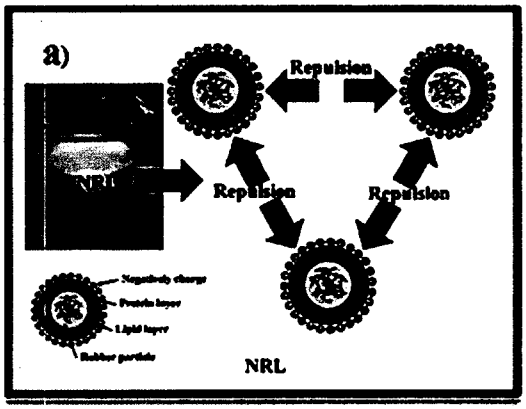


Fig. 1. Carboxylate ions contained in the NRL a) the proposed mechanism between Mg^{2+} ions and NRL b) Elimination of Mg^{2+} by adding DAHP into NRL c).

$$Mg^{2}(aq) + NH_3(aq) + HPO_4{}^{2-}(aq) \rightarrow MgNH_4PO_4(s) \quad (1)$$

A conventional approach involves Mg^{2+} analysis in NRL based on the complexometric titration with ethylenediamine tetraacetic acid (EDTA, H_2Y^{2-}), disodium salt (soluble form) using eriochrome black T (EBT) as an indicator. Since dissociation of EDTA disodium salt and EBT depends on pH of the medium, addition of ammonium chloride (NH_4Cl)/ammonium hydroxide (NH_4OH) buffer solution is required in order to control pH of the solution to be $\geq$ 10, facilitating reaction between EBT and Mg^{2+}. At the end point of the reaction, the solution color changes from red to blue, according to the reaction shown in Eq (2) [8]. for Mg^{2+} analysis in NRL.

$$\underset{(red)}{MgIn^-}(aq) + Y^{4-}(aq) + H_3O^+(aq) \rightarrow MgY^{2-}(aq) + \underset{(blue)}{HIn^{2-}}(aq) + H_2O(l) \quad (2)$$

Apart from the well controlled pH of the solution facilitating interaction between Mg^{2+} and EDTA, a masking agent is also added to prevent foreign ions, such as potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), zinc (Zn^{2+}), iron (Fe^{3+}), copper (Cu^{2+}) and manganese (Mn^{2+}) interfering complexation between Mg^{2+} and EDTA in NRL. Potassium cyanide (KCN) is a common masking agent used in the standard method [9,10]. However, it is well known that cyanide compounds are very toxic. Therefore, development of a cyanide-free method for determination Mg^{2+} in NRL is still a challenge.

Sotheinperakul et al. (2008) [11] have reported methods for magnesium determination in natural rubber latex based on the potentiometric titration with a Hg-EDTA electrode. Masking agents were not applied since they disturbed the end point of the titration. Their methods showed linearity range for Mg^{2+} detection from 36 to 126 mg L^{-1}, which is in good agreement with the results obtained from atomic absorption spectrometry. Unfortunately, they found that the presence of zinc and cadmium at high levels interfered wih the determination of Mg^{2+}. In 2011, a method and composition for quantifying magnesium ions based on conventional complexometric titration was patented (WO2011139245) using NaHS as a selectively precipitating interfering metal ions in NRL instead of using KCN as mentioned above [12]. Afterwards, ISO 17403: 2014(E), Rubber-Determination of magnesium content of field and concentrated natural rubber latices by titration (cyanide-free method by using NaHS as a masking agent) [13–15] was established in 2014. However, the reported approaches are still complicated in terms of multiple steps of analysis including need for sample pretreatment before analysis, reagent volume consumption (>105 mL), resulting in more waste and potential interference ions in NRL if a masking agent was not applied (Table 1.). Moreover, many reagents are needed for analysis which also requires skill to perform in the laboratory.

Nowadays, two types of test kit for magnesium detection based on colorimetry have been commercially available. One is the field test kit in seawater based on complexometric titration without addition of masking agents. A limit of detection (LOD) is found within the range of 15–100 mg L^{-1} [16–19]. Moreover, interference from calcium and strontium was not observed. Another type of the commercial test kit is based on an enzymatic assay performed in a 96 well flat-bottom plate coupled with spectrophotometer detection at 450 nm [20]. The assay involves specific interaction between glycerol kinase enzyme and Mg^{2+} which results in a linear range of 1.5–7.5 mg L^{-1} without interference from foreign ions such as Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+} and Mn^{2+}. However, the approach as mentioned above is costly and requires expertise to perform (Table A). A miniaturized complexometric titration between Mg^{2+} and EDTA is thus considered to be a key reaction in this development.

In this work, a simple (no requirement of skill for analysis, not

Table 1
Selectivity study towards Mg^{2+} compared with the other metal ions (Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, K^+ and Na^+ tested using complexometric titration reported in the tolerance concentration ($mg.L^{-1}$) (n = 3).

Interference	Tolerance concentration ($mg.L^{-1}$)	With masking agent			Without masking agent		
		Mg^{2+} content ($mg.L^{-1}$)		% Relative different	Mg^{2+} content ($mg.L^{-1}$)		% Relative different
		1st collection	2nd collection		1st collection	2nd collection	
Original NRL	–	524.02 ± 11.89	256.16 ± 2.99	–	534.81 ± 17.40	289.87 ± 23.37	–
Zn^{2+}	793.62	470.60 ± 2.85	–	−9.86	611.60 ± 2.47	–	+14.36
Ca^{2+}	955.82	545.10 ± 3.72	–	+4.02	565.40 ± 7.03	–	+5.72
Fe^{3+}	157.52	–	272.60 ± 1.26	+6.42	–	315.20 ± 7.13	+8.74
Cu^{2+}	244.14	–	232.50 ± 1.53	−9.23	–	N.D.	–
Mn^{2+}	53.93	–	267.90 ± 0.77	+4.58	–	330.90 ± 3.48	+14.15
K^+	2171.29	–	235.20 ± 7.16	−8.18	–	228.90 ± 23.32	−21.03
Na^+	1356.97	–	239.50 ± 2.72	−6.50	–	225.50 ± 7.63	−22.21

U_3 was used as model sample in this study.
N.D. (Not detectable: end point of the titration cannot be accomplished).
Due to the NRL can be stable around 4 h, therefore, NRL is needed to collect several time to accomplish the experiment.

$$\%\text{Relative different} = \left[\frac{(\text{Mg}^{2+}\text{ contents from original NRL} - \text{Mg}^{2+}\text{ contents from added concentration of foreign ions})}{\text{Mg}^{2+}\text{ contents from original NRL}}\right] \times 100.$$

demanding sample pretreatment before analysis), low-cost (small sample and reagents volume uptake) and portable field test kit based on colorimetry using naked eye for determination of magnesium content in NRL was established. The novelty of this work is the use of a miniaturized complexometric titration between Mg^{2+} and EDTA (even without using masking agent) which were designed according to the concept of 'Green chemistry' reducing waste generation, and minimizing use of chemicals and consumption of time (at least simple two reagents (EBT indicator for reagent A; EDTA in ammonium buffer for reagent B) for test kit set up). The developed test kit was applied for investigation of the effect of the presence of potential interference ions, preservatives used in NRL and the possibility for practical use in concentrated rubber latex (CRL). Stability of reagents applied in the kit and the analysis performances in real samples were investigated and discussed.

2. Experimental section

2.1. Chemicals and materials

All chemicals were analytical grade. All solutions were prepared in deionized water with 18 MΩ resistance (obtained from a Millipore Milli-Q purification system, Bedford, MA, USA). The NRL in this research was collected from 6 areas (U_1-U_3 and K_1-K_3) in Ubon Ratchathani province (U) and Kalasin province (K). Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, Panreac), calcium carbonate ($CaCO_3$, Fluka), ethylenediamine tetraacetic acid ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$; EDTA, Fisher Chemical) and eriochrome black T ($C_{20}H_{12}N_3O_7SNa$; EBT, LABCONCO), ethanol 99%, v/v (C_2H_5OH, Sigma-Aldrich), ammonium chloride (NH_4Cl, Fluka) and ammonium hydroxide (NH_4OH, Fluka) and sodium hydrogen sulfide ($NaHS \cdot xH_2O$, ACROS ORGANICS) were used as reagents in the conventional complexometric titration and our developed small-scale test kit for Mg^{2+} identification in NRL. NH_4OH, zinc oxide (ZnO, Carlo Erba) and tetramethyl thiuram disulfide ($C_6H_{12}N_2S_4$, TMTD, Sigma-Aldrich) were used as preservatives in NRL. The following chemicals were used as received: calcium nitrate ($Ca(NO_3)_2$), potassium sulfate (K_2SO_4), sodium chloride (NaCl), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), and manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$), which were obtained from Carlo Erba. Iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, Unilab), zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$, Fluka), concentrated nitric acid 65%, v/v (HNO_3, Sigma-Aldrich) were used in interference study. Paraffin oil purchased from LABCONCO was used in the acid digestion method of FAAS analysis. Serum container (10 mL) and eyes drop bottles (10 mL) were obtained from a pharmacy store in Ubon Ratchathani and used as containers for reagents A and B, respectively. Plastic coffee spoons obtained from a local supermarket in Ubon Ratchathani were used as sampling spoons.

2.2. Instruments

A flame atomic absorption spectrophotometer (PinAAcle 900T; Perkin Elmer, US) equipped with hollow cathode lamps (HCL) was exploited to determine concentrations of Mg^{2+} and interfering ions (Cu^{2+}, Mn^{2+}, K^+, Fe^{3+}, Zn^{2+}, Ca^{2+} and Na^+) in NRL samples for method validation with the operated conditions shown in Table 2.

2.3. Preparation of reagents

2.3.1. Conventional method

ISO 17403: 2014(E) [15] was applied as a conventional method with slight modification. Briefly, $CaCO_3$ (0.500 g) was dissolved in 1 L of DI water (5×10^{-3} mol L^{-1}) as a primary standard. EDTA (1.86 g) was dissolved in 1 L of DI water (5×10^{-3} mol L^{-1}). The equivalent mole ratio between Mg^{2+} standard and the EDTA solution is 1:1. pH of the system was adjusted to be 10.5 by using 0.06 mol L^{-1} NH_4Cl/NH_4OH buffer solution (which was prepared by dissolving 67.5 g of NH_4Cl in 250 mL of DI water). After that, 570 mL of 25%, w/w NH_4OH were added to NH_4Cl solution and brought up to 1 L with DI water. An indicator was prepared by dissolving EBT (0.1 g) in 100 mL of 75%, v/v ethanol (2.16×10^{-4} mol L^{-1}). A masking agent, $NaHS.xH_2O$ (1.68 g) was dissolved in 100 mL of DI water (0.3 mol L^{-1}).

Table 2
The operating conditions of FAAS.

FAAS parameter	Wavelength (nm)/Lamp current (mA)
Mg	285.2/84
Ca	422.7/86
Zn	213.9/67
Cu	324.8/89
Fe	248.3/62
Mn	279.5/66
K	766.5/116
Na	589.0/77
Slit width (nm)	0.7
Air flow rate ($m^3.s^{-1}$)	8.0
Acetylene flow rate ($m^3.s^{-1}$)	2.5

2.3.2. Test kit based colorimetry

The small-scale test kit was developed based on the conventional method. The approaches were based on complexometric titration between Mg^{2+} and EDTA using NaHS as a masking agent (or without any masking agent). The optimized procedure was as follows; Samples were transferred by plastic spoons (~0.18 g). Reagent A was 2.16×10^{-4} mol L^{-1} EBT indicator with the EBT:DI volume ratio of 3:10. Reagent B was 5.48×10^{-3} mol L^{-1} EDTA which was prepared by dissolving EDTA (0.02 g) ito a 0.06 mol L^{-1} NH_4Cl/NH_4OH buffer (6.67 mL), followed by addition of 0.3 mol L^{-1} $NaHS.xH_2O$ (3.33 mL) as a masking agent. The droplets of reagents A and B were generated by using eye drop bottles (~0.065 mL.drop^{-1}).

2.4. Method validation

Complexometric titration was performed with slight modification according to ISO 17403: 2014(E) [15]. Briefly, NRL (2.0 g) was transferred into a conical flask followed by addition of 100 mL of DI water. 0.06 mol L^{-1} NH_4Cl/NH_4OH buffer solution (2.0 mL) was then added to control the NRL solution pH within the range of 10.0–10.5. Next, 2.16×10^{-4} mol L^{-1} EBT indicator (1.0 mL) was dropped into the NRL solution. The 0.3 mol L^{-1} masking agent (1.0 mL) was added ito the solution and also diluted in water (100 mL). The solution was then titrated with the standard 5×10^{-3} mol L^{-1} EDTA solution (which was standardized with 5×10^{-3} mol L^{-1} $CaCO_3$) until the red solution vanished and became pure blue. This titration approach was evaluated by comparison with FAAS which is a reliable technique with high accuracy and precision. The sample preparation for FAAS analysis was performed with slight modification [21,22] by transferring NRL (0.25 g) to a test tube followed by addition of concentrated HNO_3 (65% v/v) (4.0 mL). The solution was heated in an oil bath at 165 °C resulting in a transparent solution which was then cooled and diluted with HNO_3 (2%, v/v) prior to the FAAS analysis.

For interferences study, a masking agent was added in order to reduce interference in Mg^{2+} analysis by undergoing complexation with the foreign ions in the NRL solution. In this study, the investigated ions were Fe^{3+}, Cu^{2+}, Mn^{2+}, K^+, Zn^{2+}, Ca^{2+} and Na^+ at concentrations of 50, 100, 150, 250, 500, 750, 1000 and 1500 mg L^{-1}, respectively. The results obtained from the complexometric titration with the masking agent were then compared with those obtained without the masking agent. NRL number of U_3 was used as model sample in this study. As the NRL can be stable for approximately 4 h, fresh NRL was collected freshly several times during the experiment in order to prevent the NRL coaggulating.

For the test kit application (Figs. 2–3), reagent A was transferred by a dropper up to the marked level into a reaction bottle. Then, a small spoon was used to transfer NRL into the reaction bottle. The NRL solution was shaken resulting in the purple color observed by naked eye, as shown by the color chart 1 in Fig. 2. Reagent B was then added drop by drop and shaken (every 10 s) to the solution until the blue color of NRL solution was observed, see also the color chart 2 in Fig. 2. The number of drops was recorded. For accuracy study, the recovery test was performed by spiking Mg^{2+} to NRL solution at concentrations of 40 and 80 mg L^{-1}.

2.5. Study of preservatives in NRL on our developing test kit

The preservative solutions of 0.2%, w/v NH_4OH and mixture of 0.2%, w/v NH_4OH, 0.025%, w/w ZnO and 0.025%, w/w TMTD, respectively, were added to the NRL sample. Mg^{2+} content in the treated NRL was then quantified and the result was compared with that obtained by the conventional method (complexometric titration).

2.6. Stability test for reagents (A and B) and application of the developed test kit for practical sample analysis

Reagents A and B were left at room temperature for 6 months prior to the application with the test kit for Mg^{2+} determination. The results were compared with that obtained by the conventional complexometric titration method.

Samples were collected from several regions in Ubon Ratchathani province (U_2-U_3) and Kalasin province (K_1-K_3), Thailand. In each analysis, identification of Mg^{2+} was performed by applying the developed test kit, which was operated by three different users. The results were averaged and compared with those obtained by the conventional complexometric titration method.

3. Results and discussion

3.1. Optimization of the developed test kit

The optimized sample amount scooped by using a small spoon was 0.1731 ± 0.02 g (Table B1) and the optimized reagent volume dropped by using an eye drop bottle was 0.065 ± 0.004 mL (Table B2). In this study, reagent A (EBT, HIn^{2-}) was applied as an indicator; while the EDTA, buffer solution and masking reagents were mixed in reagent B. Reagent A needs to be separated from the system prior to analysis due to the poor solubility of the indicator in the reagent B matrix, as well as the complexation between the indicator and Mg^{2+}, as analysis has to be carried out before addition of EDTA, as demonstrated in equation (2).

The optimized condition for reagent A was obtained by variation of a number of droplets (1–8 drops) applied in different bottles each of which had already been filled in with 50 mg L^{-1} of Mg^{2+} in DI water. The optimized number of droplets was three drops (Fig B1a) since the use of >3 drops resulted in precipitation of NRL whilst using of 1–2 drops led to low color intensity of the end point of the titration reaction which is difficult to see by naked eye. However, the addition of three drops into practical NRL samples resulted in precipitation of NRL since EBT was dissolved in ethanol which precipitated NRL (Fig B1b). EBT solution was thus diluted with DI water with the selected EBT:DI water ratio of 3:10, which was marked at the level shown on the dropper for sampling of reagent A.

EDTA is a limiting reagent in the complexometric titration reaction for Mg^{2+} determination. The stoichiometric amount of EDTA to react Mg^{2+} with theoretical concentrations of 25, 50 and 100 mg L^{-1} corresponded to the optimized number of droplets for reagent B being 1 drop per 50 mg L^{-1} of Mg^{2+} (with the volume of ~0.065 mL including buffer solution and masking reagents), details and explanation are shown in Tables B3 and B4. This condition was obtained by taking in account solution miscibility and buffer capacity (β) where a drop of reagent B was found with β = 2.17 M per 0.1 pH unit. This is sufficient for the resistance to pH change in the developed test kit as well as being applicable even when excess amount of reagent B was applied, see also Table B5.

3.2. Validation of the developed test kit

Comparison of the analysis results between complexometric titration (standard method) and FAAS techniques (reference method) was made for Mg^{2+} content in NRL samples obtained from U_1 and U_3. The results obtain from t-test showed t_{stat} = 1.02 whereas $t_{critical}$ = 2.78 revealed that there is no statistical difference between these two methods with a confidence level of 95% (Table 3). Due to the simplicity and lower analysis cost, the titration method was selected as the benchmark method for determination of Mg^{2+} in NRL samples. In addition, our test kit was developed

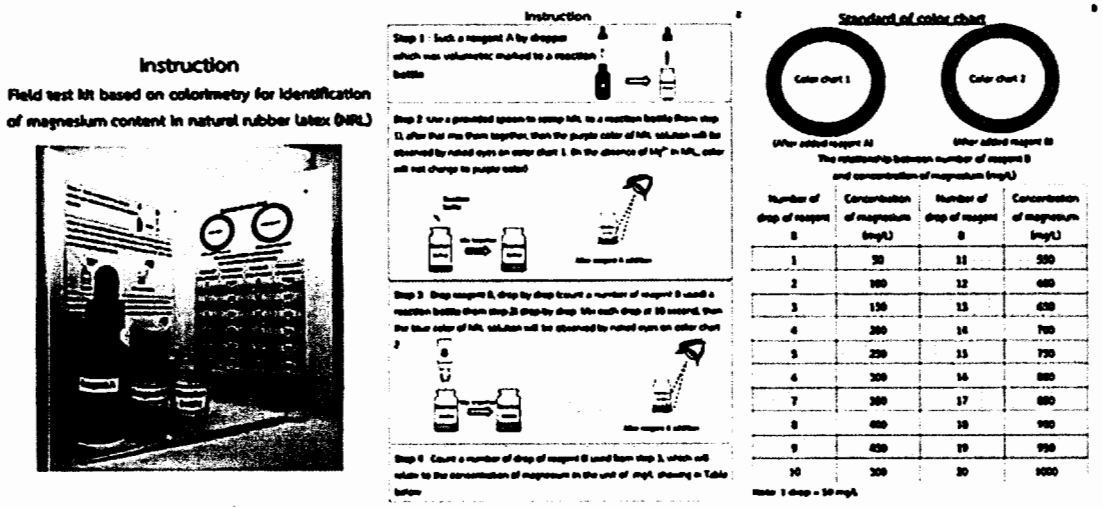
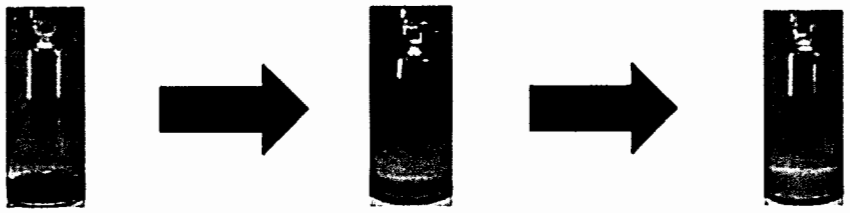


Fig. 2. The instruction of "Field test kit based on colorimetry for determination of magnesium content in natural rubber latex (NRL)".



1. The optimized reagent A was taken by a dropper up to the marked level into a reaction bottle.

2. Addition of NRL sample by a small spoon was used to transfer NRL into the reaction bottle The NRL solution was shaken together resulting in the purple color observed by naked eyes, as shown by the color chart 1 in Fig 2.

3. Reagent B was then added drop by drop (every 10 second) into the solution until the blue color of NRL solution was observed, see also the color chart 2 in Fig 2.

Fig. 3. The operation of developed test kit based on colorimetry using naked eyes for determination of magnesium content in natural rubber latex (NRL) operated in NRL sample containing 775 mg L^{-1} Mg^{2+} (16 drops were performed).

Table 3
Comparison of Mg^{2+} content ($mg.L^{-1}$) at different area of NRL between Flame Atomic Absorption Spectroscopy, FAAS and complexometric titration (n = 3).

Area of NRL	Mg^{2+} content ($mg.L^{-1}$)	
	FAAS method	Complexometric titration method
U_1	746.90 ± 0.002	748.48 ± 7.07
U_1	800.02 ± 0.008	770.90 ± 8.22
U_1	873.81 ± 0.001	812.17 ± 3.62
U_3	283.12 ± 0.001	306.18 ± 5.30
U_3	342.55 ± 0.001	322.52 ± 4.39
U_3	363.89 ± 0.001	375.13 ± 26.08

U_1 and U_3 were collected 3 times.

based on scaling down the process for the complexometric titration between Mg^{2+} and EDTA with [13–15]without NaHS as a masking agent. In order to study the ability of NaHS to be used as a masking agent, interferences in Mg^{2+} analysis caused by complexation with the potential foreign ions in the NRL solution (Fe^{3+}, Cu^{2+}, Mn^{2+}, K^+, Zn^{2+}, Ca^{2+}, and Na^+) were assessed. The resulting tolerance concentration ($mg.L^{-1}$, which was defined as the added concentrations of foreign ions that reveal significant changes of % error to be within ±10%) data are shown in Table 1. The % error values obtained from complexometric titration using the masking agent were lower than those obtained without masking agent, especially with the presence of Cu^{2+} where the end point of the titration could not be identified without the masking agent. This indicates the requirement for addition of masking agent (here being NaHS) for improved analysis reliability. According to the observed high tolerance concentrations, it can be concluded that the investigated ions could not interfere the analysis of practical NRL samples. Note that the result revealed relatively low tolerance concentrations for Fe^{3+}, Cu^{2+}, and Mn^{2+}. However, the concentrations of these ions in the real NRL are very much lower than the studied concentrations [8,23,24] (see further explanation in Table C). The function of NaHS as a masking

agent for Mg^{2+} analysis in NRL can be explained by formation of complexes with foreign metal ions according to the Hard-Soft acid-base reaction. Na^+ can be considered as hard acid and HS^- is soft base which does not prefer to react with Mg^{2+} (hard-acid). However, interference ions in NRL are mostly soft acids favorably interacting with HS^-. The resulting complexes are stable and not likely to react with EDTA [25]. These investigations corresponded to the results obtained in reported works [13–15].

Therefore, the evaluated complexometric titration was further used as the reference method by comparison with our developed test kit for quantification of Mg^{2+} from U_1 and U_2 as well as these samples spiked with 40 and 80 mg L^{-1} Mg^{2+}, respectively. The complexometric titration results were insignificantly different from the results obtained from the test-kit approach (t_{stat} = 6.42 and $t_{critical}$ = 2.78). Furthermore, the analytical recoveries were also in an acceptable range of 100.6–102.4 for NRL sample. (Table 4).

3.3. Effect of preservatives in NRL on our developed test kit

In general, preservation of NRL can be long-term with the aim to maintain NRL quality during storage and transportation by addition of preservatives to the samples. Short-term preservation involves a few days storage of liquid samples prior to further processing. The related additives are anticoagulant in NRL. A mixture of 0.2%, w/v NH_4OH, 0.025%, w/w ZnO and 0.025%, w/w TMTD are normally used in formulation processes in Thailand as alternative to the sole use of NH_4OH, which has a pungent smell as well as causing environmental pollution and respiratory system irritation when released into the atmosphere [26]. Ammonia solution (NH_4OH) is conventionally added to the samples as a primary preservative in the concentration ranging from 0.2 to 0.5%, w/v which can inhibit reaction with bacteria under high pH condition and precipitate $Mg(OH)_2$ (reduction of free Mg^{2+}, Fig. 4) [27]. ZnO and TMTD were added to NRL as secondary preservatives which stabilize NRL dispersed system. ZnO and TMTD can preserve natural rubber latex (by inhibiting bacteria growth) in the presence of small amounts of ammonia [4,28]. The results are shown in Table 5. When the preservative solutions of 0.2%, w/v NH_4OH, 0.025%, w/w ZnO and 0.025%, w/w TMTD were added to the NRL samples, Mg^{2+} content quantified by complexometric titration was slightly decreased in the system containing 0.2%, w/v NH_4OH. Moreover, Mg^{2+} content in the treated NRL analyzed by our developed test kit was not statistically different from that obtained by the complexometric titration. This suggests that the preservatives used in NRL did not affect the developed test kit system.

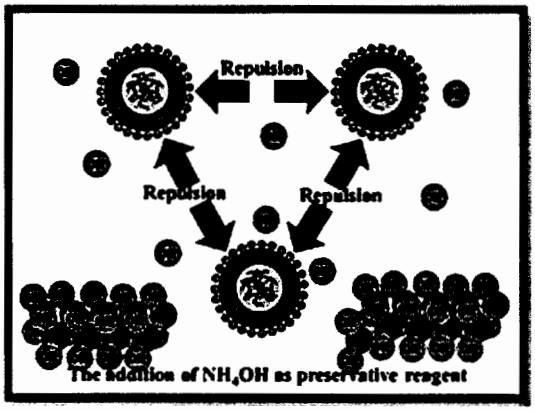


Fig. 4. The proposed mechanism when NH_4OH as preservative were performed.

3.4. Stability of reagents (A and B) and real samples application

Stability of reagents applied in the developed test kit method was studied. Reagent A and B were left at room temperature for 6 months. The resulting Mg^{2+} concentrations determined in both cases were compared with the analysis obtained by using freshly prepared reagents. The results showed good stability of both reagents, as shown in Table 6. Furthermore, the proposed method for Mg^{2+} determination in NRL was evaluated by analyzing real samples from U_1, U_2 and K_1-K_3 with concentrations of Mg^{2+} within in the ranges of 450–600 mg L^{-1} and 500–550 mg L^{-1}, respectively. The results for all the samples were in good agreement with the values obtained from complexometric titration, see also Table 7.

Table 4
Recovery study by comparison between complexometric titration and our developed test kit (n = 3).

Area of NRL	Mg^{2+} content by complexometric titration (mg.L^{-1})	Our developed test kit			
		Number of drops (1 drop = 50 mg L^{-1})	Mg^{2+} content (mg.L^{-1})	Average of Mg^{2+} content (mg.L^{-1})	% Recovery
Original U_1	748.48 ± 7.07	15	750	750.0	–
		15	750		
		15	750		
U_1 added 40 mg L^{-1} of Mg^{2+}	770.90 ± 8.22	16	800	800.0	101.3
		16	800		
		16	800		
U_1 added 80 mg L^{-1} of Mg^{2+}	812.17 ± 3.62	17	850	850.0	102.4
		17	850		
		17	850		
Original U_2	431.96 ± 0.84	10	500	500.0	–
		10	500		
		10	500		
U_2 added 40 mg L^{-1} of Mg^{2+}	488.49 ± 14.55	11	550	550.0	101.9
		11	550		
		11	550		
U_2 added 80 mg L^{-1} of Mg^{2+}	560.30 ± 6.05	12	600	583.3	100.6
		11	550		
		12	600		

Table 5
Effect of preservatives used in NRL on our developed test kit (n = 3).

Type of samples	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	Our developed test kit		
		Number of drops (1 drop = 50 $mg\ L^{-1}$)	Average of Mg^{2+} content ($mg.L^{-1}$)	% Relative different
Original NRL	775.3 ± 9.25	15 15 15	750.0	+3.3
NRL preserved with 0.2% NH_4OH	500.7 ± 4.39	10 10 10	500.0	+0.1
NRL preserved with 0.2% NH_4OH, 0.025% ZnO and 0.025%TMTD	611.7 ± 14.01	11 11 11	550.0	+10.1

U_2 was used as model sample in this study.

$$\%\text{Relative different} = \left[\frac{\text{Mg}^{2+}\text{contents from complexometric titration} - \text{Mg}^{2+}\text{contents from our developing test kit}}{\text{Mg}^{2+}\text{contents from complexometric titration}}\right] \times 100.$$

The results from these two methods showed t_{stat} = 1.02 and $t_{critical}$ = 12.71 which are not statistically different with the confidence level of 95%.

Table 6
Stability of our developed test kit (reagent A and B) (n = 3).

Area	Type of sample	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	6 months of reagent A		6 months of reagent B	
			Average of Mg^{2+} content ($mg.L^{-1}$)	% Relative different	Average of Mg^{2+} content ($mg.L^{-1}$)	% Relative different
U_2	Original NRL	719.1 ± 3.3	683.3	−5.0	700.0	−2.7
	NRL mixed with preservatives[a]	611.7 ± 14.0	566.5	−7.4	566.5	−7.4
U_3	Original NRL	667.1 ± 7.5	633.3	−5.1	615.5	−7.6
	NRL mixed with preservatives[a]	582.4 ± 1.4	600.0	+3.0	600.0	+3.0

For stability test of Reagent A, the reagent B was freshly prepared. On the other hand, for stability test of Reagent B, the reagent A was freshly prepared.

$$\%\text{Relative different} = \left[\frac{\text{Mg}^{2+}\text{contents from complexometric titration} - \text{Mg}^{2+}\text{contents from our developing method}}{\text{Mg}^{2+}\text{contents from complexometric titration}}\right] \times 100.$$

[a] Preservatives is a mixed solution of 0.2%NH_4OH, 0.025% ZnO and 0.025%TMTD in NRL.

Table 7
Real sample application of NRL using our developed test kit.

Area	Mg^{2+} content by complexometric titration ($mg.L^{-1}$)	Subject	Our developed test kit		% Relative different
			Number of drops	Mg^{2+} content ($mg.L^{-1}$)	
U_1	523.1 ± 13.0	1	11	550	+5.14
		2	12	600	+14.70
		3	11	550	+5.14
U_2	465.1 ± 6.9	1	10	500	+7.50
		2	9	450	−3.25
		3	10	500	+7.50
K_1	518.0 ± 11.4	1	11	550	+6.18
		2	11	550	+6.18
		3	11	550	+6.18
K_2	491.1 ± 2.8	1	10	500	+1.81
		2	10	500	+1.81
		3	10	500	+1.81
K_3	558.4 ± 6.4	1	10	500	−10.46
		2	10	500	−10.46
		3	10	500	−10.46

$$\%\text{Relative different} = \left[\frac{\text{Mg}^{2+}\text{contents from complexometric titration} - \text{Mg}^{2+}\text{contents from our developing methods}}{\text{Mg}^{2+}\text{contents from complexometric titration}}\right] \times 100.$$

The results from these two methods showed t_{stat} = 0.009 and $t_{critical}$ = 3.18 which are not statistically different with the confidence level of 95%.

4. Conclusions

A simple and portable field test kit for colorimetric determination of magnesium content in natural rubber latex (NRL) was successfully developed according to the concept of green chemistry by reducing waste generation, minimizing use of chemicals and consumption of time (at least simple two reagents (EBT indicator for reagent A; EDTA in ammonium buffer for reagent B) for test kit set up). These were found to be effective and non-instrumental approaches with low cost, simple (no requirement of skill for analysis), not demanding sample pretreatment before analysis, small sample volume uptake (0.18 g, sampling by a small spoon) and use of <1.5 mL reagent volume which was >70 times less than when compared with conventional methods [12–15] and the other

commercial test kits (see in Table A). Our developed test kit (even without masking agent) can be applicable even with the presence of potential interference ions (see also in Table C) and preservatives in NRL due to the effect of minimizing scale of reagents and sample. Moreover, the EDTA concentration in reagent B could be adjusted for matching with theoretically expected magnesium concentration; see further explanation in Table B3 and B4 providing the detection limit of magnesium of 50 mg L^{-1} (or could be adjusted less than that). The performance of the approach meets the requirement for analysis of magnesium content in practical NRL samples which can be performed within a minute and observed by naked eye based on comparison with a color chart. Moreover, our developed test kit is stable at room temperature for more than 6 months. The established approaches were not only applicable for NRL analysis, but it is also for practical use in concentrated rubber latex (CRL; see further explanation in Table D) since our developed test kit in terms of reagent B can provide enough buffer capacity system (even one drop was applied into the sample) which is suitable for acidic samples such as CRL.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymertesting.2017.01.023.

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Further reading

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ภาควิชาเคมี
เลขรับที่ 114
วันที่ 9 ธ.ค. 2559
เวลา 14.15 น.

คณะวิทยาศาสตร์
เลขรับ 4462
วันที่ 8 ธ.ค. 2559
เวลา 14.53 น.

SCAN

บันทึกข้อความ

ส่วนราชการ โครงการอุทยานวิทยาศาสตร์ สำนักงานอธิการบดี โทร.๑๑๘๕

ที่ ศธ ๐๕๒๙.๑.๔.๗/ ๕๖/๖๓ **วันที่** ๗ ธันวาคม ๒๕๕๙

เรื่อง แจ้งดำเนินการด้านทรัพย์สินทางปัญญา

เรียน คณบดีคณะวิทยาศาสตร์

ตามที่คณะวิทยาศาสตร์ มีความประสงค์ยื่นคำขอรับอนุสิทธิบัตร "ชุดทดสอบแมกนีเซียมภาคสนามในน้ำยางพารา" จำนวน ๑ คำขอ โดยผู้ประดิษฐ์คือ นายบุริม จารุจำรัสและคณะ นั้น

บัดนี้ โครงการอุทยานวิทยาศาสตร์ ซึ่งเป็นหน่วยประสานงาน ได้ดำเนินการยื่นคำขอรับอนุสิทธิบัตรดังกล่าวต่อกรมทรัพย์สินทางปัญญา ผ่านสำนักงานพาณิชย์จังหวัดอุบลราชธานีเรียบร้อยแล้ว รายละเอียดปรากฏตามเอกสารแนบ

จึงเรียนมาเพื่อโปรดทราบ และแจ้งผู้เกี่ยวข้องทราบ

(ผู้ช่วยศาสตราจารย์ชวลิต ถิ่นวงศ์พิทักษ์)
ผู้อำนวยการโครงการอุทยานวิทยาศาสตร์

รองคณบดีฝ่ายวิจัย

เรียน ผศ. บุริม จารุจำรัส
(✓) เพื่อโปรดทราบ
( ) เพื่อโปรดแจ้งเวียนให้ทราบทั่วกัน
( ) เพื่อโปรดพิจารณา

แบบ สป/สผ/อสป/001-ก

หน้า 1 ของจำนวน 3 หน้า

คำขอรับสิทธิบัตร/อนุสิทธิบัตร

☐ การประดิษฐ์
☐ การออกแบบผลิตภัณฑ์
☑ อนุสิทธิบัตร

ข้าพเจ้าผู้ลงลายมือชื่อในคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ขอรับสิทธิบัตร/อนุสิทธิบัตร ตามพระราชบัญญัติสิทธิบัตร พ.ศ. 2522 แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่2) พ.ศ. 2535 และพระราชบัญญัติสิทธิบัตร (ฉบับที่3) พ.ศ. 2542

สำหรับเจ้าหน้าที่	
วันรับคำขอ 21 ต.ค. 2559	เลขที่คำขอ
วันยื่นคำขอ 21 ต.ค. 2559	1603002133
สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	
ใช้กับแบบผลิตภัณฑ์ ประเภทผลิตภัณฑ์	
วันประกาศโฆษณา	เลขที่ประกาศโฆษณา
วันออกสิทธิบัตร/อนุสิทธิบัตร	เลขที่สิทธิบัตร/อนุสิทธิบัตร
ลายมือชื่อเจ้าหน้าที่	

1.ชื่อที่แสดงถึงการประดิษฐ์/การออกแบบผลิตภัณฑ์
ชุดทดสอบแมกนีเซียมภาคสนามในน้ำยางพารา

2.คำขอรับสิทธิบัตรการออกแบบผลิตภัณฑ์นี้เป็นคำขอสำหรับแบบผลิตภัณฑ์อย่างเดียวกันและเป็นคำขอลำดับที่
ในจำนวน คำขอ ที่ยื่นในคราวเดียวกัน

3.ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร และที่อยู่ (เลขที่ ถนน ประเทศ)	
ดู แนบ สป/สผ/อสป 001-ก หน้า 3	3.1 สัญชาติ
	3.2 โทรศัพท์
	3.3 โทรสาร
	3.4 อีเมล์

ยกเว้นค่าธรรมเนียม
...พ.ศ. ๒๕๔๘ ...

4.สิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร
☐ ผู้ประดิษฐ์/ผู้ออกแบบ ☑ ผู้รับโอน ☐ ผู้ขอรับสิทธิโดยเหตุอื่น

5.ตัวแทน (ถ้ามี)/ที่อยู่ (เลขที่ ถนน จังหวัด รหัสไปรษณีย์)	
นางสาวอาภรณ์ สมรักษ์	5.1 ตัวแทนเลขที่ 2337
ที่อยู่ โครงการอุทยานวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี	5.2 โทรศัพท์ 0 4543 3456, 08 6087 5348
85 ถนนสถลมาร์ค ตำบลเมืองศรีไค อำเภอวารินชำราบ จังหวัดอุบลราชธานี	5.3 โทรสาร 0 4543 3456
34190	5.4 อีเมล์ arporn.somrak@gmail.com

6.ผู้ประดิษฐ์/ผู้ออกแบบผลิตภัณฑ์ และที่อยู่ (เลขที่ ถนน ประเทศ)
ดู แนบ สป/สผ/อสป 001-ก หน้า 3

7.คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม
ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ในวันเดียวกับคำขอรับสิทธิบัตร
เลขที่ วันยื่น เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ
☐ คำขอเดิมมีการประดิษฐ์หลายอย่าง ☐ ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ ☐ ขอเปลี่ยนแปลงประเภทของสิทธิ

หมายเหตุ ในกรณีที่ไม่อาจระบุรายละเอียดได้ครบถ้วน ให้จัดทำเป็นเอกสารแนบท้ายแบบพิมพ์นี้ โดยระบุหมายเลขกำกับข้อและหัวข้อที่แสดงรายละเอียดเพิ่มเติมดังกล่าวด้วย

สำนักงานจัดการสิทธิเทคโนโลยี โครงการอุทยานวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี
ชั้น 1 อาคารศูนย์เครื่องมือกลางและปฏิบัติการเทคโนโลยีชีวภาพ มหาวิทยาลัยอุบลราชธานี
85 ถนนสถลมาร์ค ตำบลเมืองศรีไค อำเภอวารินชำราบ จังหวัดอุบลราชธานี 34190
โทรศัพท์ 0 4535 3000 ต่อ 3185, 0 4543 3456, 08 6087 5348 โทรสาร 0 4543 3456
Email: aroom.somrak@gmail.com

หนังสือนำส่งเอกสาร คำขอรับอนุสิทธิบัตร
ในนาม มหาวิทยาลัยอุบลราชธานี
ผ่านสำนักงานพาณิชย์จังหวัดอุบลราชธานี

หนังสือนำส่งเอกสาร คำขอรับอนุสิทธิบัตรในนามมหาวิทยาลัยอุบลราชธานีฉบับนี้ ขอยืนยันว่า มหาวิทยาลัยอุบลราชธานี ได้นำส่งเอกสาร คำขอรับอนุสิทธิบัตร เรื่อง "ชุดทดสอบแมกนีเซียมภาคสนามในน้ำยางพารา" แก่กรมทรัพย์สินทางปัญญา ผ่านสำนักงานพาณิชย์จังหวัดอุบลราชธานี

เมื่อวันที่.....21..... เดือน ...ตุลาคม... พ.ศ. ...2559..... และเจ้าหน้าที่ประจำสำนักงานพาณิชย์จังหวัด ผู้รับคำขอ ได้รับเอกสารดังกล่าวเรียบร้อยแล้ว

ลงชื่อ .....
(นางสาวชามรณ์ สมรักษ์)
ตำแหน่ง เจ้าหน้าที่วิจัย
สำนักงานจัดการสิทธิเทคโนโลยี โครงการอุทยานวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี

วันที่ 21 / ต.ค. / 59

ลงชื่อ .....
(.....)
ตำแหน่ง .....
เจ้าหน้าที่ประจำสำนักงานพาณิชย์จังหวัดอุบลราชธานี
ผู้รับคำขอ

วันที่ 21 ต.ค. 2559 / .....

The 13th Asian Conference on Analytical Sciences (ASIANALYSIS XIII)
c/o Center of Excellence for Innovation in Analytical Science and Technology (I-ANALY-S-T) Office
Science Complex Building II (SCB 2), Room 2323, Faculty of Science, Chiang Mai University,
Chiang Mai 50200, THAILAND
Tel. /Fax. +66-53-941-917, E-mail asianalysis.thailand@gmail.com,
Website: http://asianalysis13.cmu.ac.th, Facebook: Asianalysis Cnx

15 October 2016

Dear Nutthaporn Malahom; Rattapol Meelapsom; Atitaya Siripinyanond; Maliwan Amatatongchai; Sanoe Chairam; Purim Jarujamrus,

The 13th Asian Conference on Analytical Sciences (ASIANALYSIS XIII) will take place at The Empress International Convention Center, Chiang Mai, the Kingdom of Thailand from 8–11 December 2016. The ASIANALYSIS XIII aims to provide a timely forum for the analytical scientists to disseminate their recent research works, to exchange the ideas and experiences on a broad range of analytical sciences. Moreover, it passionately pursues the spirit of international collaboration and friendship.

On behalf of the organizing committee, it is my great pleasure to inform you that your paper entitled "Field test kit based on colorimetry for quantification of magnesium content in rubber latex" has been accepted for presentation at ASIANALYSIS XIII.

Please visit our website at http://asianalysis13.cmu.ac.th for the latest information.

I thank you very much for your contribution to the ASIANALYSIS XIII. We look forward to welcoming you in Chiang Mai, Thailand.

Yours sincerely,

Kate Grudpan

Kate Grudpan
Chairperson, ASIANALYSIS XIII Organizing Committee
Center of Excellence for Innovation in Analytical Science and Technology (I-ANALY-S-T),
Faculty of Science, Chiang Mai University,
Chiang Mai 50200 THAILAND

ชุดทดสอบภาคสนามที่อาศัยการตรวจวัดทางสีเพื่อวิเคราะห์หาปริมาณของแมกนีเซียมในน้ำยาง

Simple test kit based on colorimetry for quantification of magnesium content in rubber latex

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บทคัดย่อ

การพัฒนาชุดทดสอบภาคสนามโดยอาศัยการตรวจวัดทางสีและอ่านผลด้วยตาเปล่าเพื่อใช้ในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยาง พัฒนาขึ้นจากปฏิกริยาของการไทเทรตเชิงซ้อนที่เกิดขึ้นระหว่างแมกนีเซียมกับกรดเอทธิลลีนไดเอมีนเตตตร้าอะซีติกโดยไม่ใช้สารมาร์สกิ้งเอเจนต์ตามแนวทางของเคมีสีเขียว ในชุดทดสอบที่พัฒนาขึ้นนี้จะอาศัยระบบย่อส่วนเพื่อลดการใช้สารเคมีเพื่อให้มีความง่าย ราคาถูก ลดเวลาที่ใช้ในการทดสอบ จากผลการทดลองพบว่าระบบชุดทดสอบภาคสนามที่พัฒนาขึ้นมีขีดจำกัดต่ำสุดในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยางธรรมชาติที่น้อยกว่า 50 มิลลิกรัมต่อลิตร โดยในแต่ละครั้งของการทดสอบจะใช้ตัวอย่างน้ำยางเพียง 0.18 กรัม (ใช้ข้อนขนาดเล็กในการตัก) ใช้สารเคมีน้อยกว่า 1.5 มิลลิลิตร(น้อยกว่าวิธีมาตรฐานถึง 70 เท่า)และสามารถทำซ้ำได้ สารเคมีในชุดทดสอบที่พัฒนาขึ้นที่มีอายุการใช้งานมากกว่า 6 เดือนที่อุณหภูมิห้อง นอกจากนี้ยังพบว่าไอออนรบกวนอื่นๆที่เป็นองค์ประกอบภายในน้ำยางไม่มีผลต่อการวิเคราะห์ เมื่อเปรียบเทียบผลการวิเคราะห์ที่ได้จากชุดทดสอบ

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Field test kit based on colorimetry for quantification of magnesium content in rubber latex

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Rubber latex (RL) is very important economic plant in Thailand which is driven by quality products, leading to a fundamental need for chemical analysis. A critical step in development of RL with high quality is identification of RL components. Magnesium (Mg^{2+}) is one of the most important components suppressing RL performance and quality. In this work, simple, low-cost and portable platforms of field test kit based on colorimetry detected using naked eyes was developed for determination of Mg^{2+} content in RL. The miniaturized complexometric titration between Mg^{2+} and EDTA with NaHS as a masking agent was a key reaction in this development which was designed according to the concept of green chemistry by reduction of waste generation and chemical and time consumption. The developed system enabled quantification of magnesium content in RL at low concentration with the detection limit being <50 $mg.L^{-1}$, small sample volume uptake (0.18 g, sampling by a small spoon) and use of <1.5 mL reagent volume which was >70 times less than that applied in the conventional method. Moreover, with the presence of potential interference ions, greater selectivity towards magnesium was observed. Furthermore, the reagents used in our developed test kit were stable for >6 months at the room temperature. The results obtained in real samples were in agreement with those obtained from the conventional complexometric titration (ISO 17403: 2014(E)) method. The proposed technique provides a low-cost, rapid, simple, selective and on-site analysis of magnesium content in RL.

Keywords : Magnesium (Mg^{2+}), field test kit, rubber latex (RL), green chemistry, complexometric titration

ชุดทดสอบภาคสนามที่อาศัยการตรวจวัดทางสีเพื่อวิเคราะห์หาปริมาณของแมกนีเซียมในน้ำยาง

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บทคัดย่อ

การพัฒนาชุดทดสอบภาคสนามโดยอาศัยการตรวจวัดทางสีและอ่านผลด้วยตาเปล่าเพื่อใช้ในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยาง พัฒนาขึ้นจากปฏิกริยาของการไทเทรตเชิงซ้อนที่เกิดขึ้นระหว่างแมกนีเซียมกับกรดเอทธิลีนไดเอมีนเตตร้าอะซีติกโดยไม่ใช้สารมาร์สกิ้งเอเจนต์ตามแนวทางของเคมีสีเขียว ในชุดทดสอบที่พัฒนาขึ้นนี้จะอาศัยระบบย่อส่วนเพื่อลดการใช้สารเคมีเพื่อให้มีความง่าย ราคาถูก ลดเวลาที่ใช้ในการทดสอบ จากผลการทดลองพบว่าระบบชุดทดสอบภาคสนามที่พัฒนาขึ้นมีขีดจำกัดต่ำสุดในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยางธรรมชาติที่น้อยกว่า 50 มิลลิกรัมต่อลิตร โดยในแต่ละครั้งของการทดสอบจะใช้ตัวอย่างน้ำยางเพียง 0.18 กรัม (ใช้ช้อนขนาดเล็กในการตัก) ใช้สารเคมีน้อยกว่า 1.5 มิลลิลิตร(น้อยกว่าวิธีมาตรฐานถึง 70 เท่า)และสามารถทำซ้ำได้ สารเคมีในชุดทดสอบที่พัฒนาขึ้นที่มีอายุการใช้งานมากกว่า 6 เดือนที่อุณหภูมิห้อง นอกจากนี้ยังพบว่าไอออนรบกวนอื่นๆที่เป็นองค์ประกอบภายในน้ำยางไม่มีผลต่อการวิเคราะห์ เมื่อเปรียบเทียบผลการวิเคราะห์ที่ได้จากชุดทดสอบ

Simple test kit based on colorimetry for quantification of magnesium content in rubber latex

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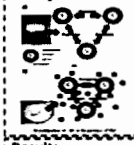
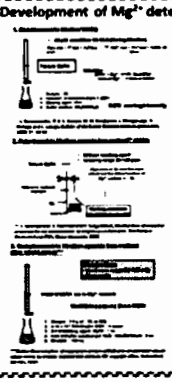
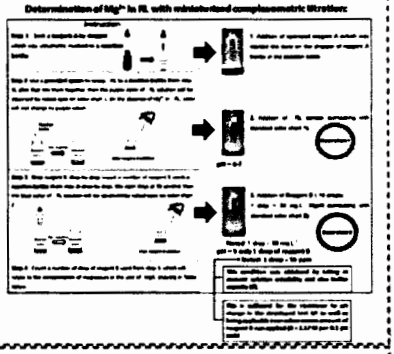
Abstract : Export industries, especially rubber, are driven by quality of products, leading to a fundamental need for chemical analysis. In this work, simple, low-cost and portable platform-of field test kit based on colorimetry detected by using naked eyes was developed for determination of magnesium content in rubber latex (RL). The miniaturized complexometric titration between Mg^{2+} and EDTA without any masking agent was a key reaction in this development which was designed according to the concept of green chemistry by reduction of waste generation and chemical and time consumption. The developed system enabled quantification of magnesium content in RL at low concentration with the detection limit being < 50 ppm small sample volume optimize (0.18 g, sampling by a small spoon) and used of < 1.5 mL reagent volume which was > 70 times less than that applied in the conventional method. Moreover, with the presence of potential interferences ions, greater selectivity towards magnesium was observed. Furthermore, the reagents used in our developed test kit were stable for > 6 months at the room temperature. The results obtained in real samples were in agreement with those obtained from the conventional complexometric titration (ISO 17483: 2014(E)) method. The proposed technique provides a low-cost, rapid, simple, selective and on-site analysis of magnesium content in RL.

A major cause of destabilization of RL :



Development of Mg^{2+} determination in RL :





Results :

Table 1.

Area of RL	Mg^{2+} content (ppm)	
	PAAS method	Complexometric titration based on cyanide free method
U1	746.90 ± 0.002	748.48 ± 7.87
U1	808.07 ± 0.008	778.90 ± 8.22
U1	873.81 ± 0.001	812.17 ± 3.62
U2	363.13 ± 0.001	306.18 ± 5.30
U2	342.56 ± 0.001	322.52 ± 4.39
U2	363.89 ± 0.001	375.13 ± 26.08

Table 2.

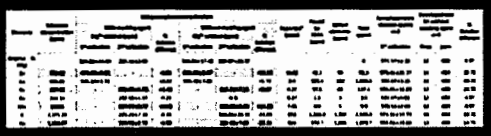


Table 3.

Type of samples	Mg^{2+} content by complexometric titration (ppm)	Our developed test kit		
		Number of drops (1 drop = 50 ppm)	Average of Mg^{2+} content (ppm)	% Relative difference
Original RL	775.3 ± 9.25	15 15 15	750.0	+3.3
RL preserved with 0.2% NH_4OH	809.7 ± 4.39	16 16 16	800.0	+0.1
RL preserved with 0.2% NH_4OH, 0.025% EtOH and 0.025% TritonX100	811.7 ± 14.88	16 16 16	800.0	+30.1

Table 4.

Area	Type of sample	Mg^{2+} content by complexometric titration (ppm)	6 months of reagent A		6 months of reagent B	
			Average of Mg^{2+} content (ppm)	% Relative difference	Average of Mg^{2+} content (ppm)	% Relative difference
U2	Original RL	758.1 ± 3.3	693.3	-8.5	703.0	-7.2
	RL mixed with preservatives*	611.7 ± 14.0	566.5	-4.7	566.5	-4.7
U3	Original RL	697.1 ± 7.5	693.3	-1.5	693.5	-0.7
	RL mixed with preservatives*	582.4 ± 1.4	600.0	+0.3	600.0	+3.0

Table 5.

Type of CRL sample	Mg^{2+} content by complexometric titration (ppm)	Our developed test kit	
		Number of drops (1 drop = 50 ppm)	Mg^{2+} content (ppm)
CRL*	55.34 ± 1.94	1 ± 1	50.00 ± 50.00
CRL**	54.25 ± 1.27	1 ± 1	50.00 ± 50.00

Table 6.

Area	Mg^{2+} content by complexometric titration (ppm)	Subject	Our developed test kit		
			Number of drops	Mg^{2+} content (ppm)	% Relative different
U1	523.1 ± 13.0	1	11	550	+5.14
		2	12	600	+14.79
		3	11	550	+5.14
U2	485.1 ± 6.9	1	10	500	+7.50
		2	9	450	-3.25
		3	10	500	+7.50
U3	518.0 ± 11.4	1	11	550	+6.18
		2	11	550	+6.18
		3	11	550	+6.18
U2	495.1 ± 2.8	1	10	500	+1.01
		2	10	500	+1.01
		3	10	500	+1.01
U3	588.4 ± 6.4	1	10	500	-18.66
		2	10	500	-18.66
		3	10	500	-18.66

Conclusion :

- Simple and portable platforms of field test kit for colorimetric determination of Mg^{2+} content in RL were successfully developed according to the concept of green chemistry by reducing waste generation, minimizing use of chemicals and consumption time (at least simple two reagents (EBT indicator for reagent A, EDTA in ammonium buffer for reagent B) for test kit set up), No masking is possible
- The detection limit of Mg^{2+} is 50 ppm (can be adjusted)
- Small sample volume consume (0.18 g, sampling by a small spoon)
- Use of < 1.5 mL reagent, volume which was > 70 times less than that applied in the conventional method
- Good stability of reagent A and B (more than 6 months)

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2. [illegible]

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