รายงานวิจัยฉบับสมบูรณ์

โครงการ

การพัฒนาชุดทดสอบภาคสนามโดยอาศัยระบบย่อส่วนและอุปกรณ์ที่ประดิษฐ์จากกระดาษ สำหรับการวิเคราะห์ปริมาณแมกนีเซียมในน้ำยางธรรมชาติ

โดย ผู้ช่วยศาสตราจารย์ ดร.ปุริม จารุจำรัส และคณะ

มิถุนายน 2560

สัญญาเลขที่ TRG5880007

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คณะผู้วิจัย	**สังกัด**
1. ผศ.ดร. ปุริม จารุจำรัส	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี
2. นางสาวณัฐพร มาลาหอม	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี
3. นายรัฐพล มีลาภสม	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี
4. ผศ.ดร. มะลิวรรณ อมตธงไชย	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี

นักวิจัยที่ปรึกษา	**สังกัด**
รศ.ดร. อทิตยา ศิริภิญญานนท์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและต้นสังกัด

Abstract

Project Code : TRG5880007

Project Title : Development of field test kits based on small scale and paper based device for identification of magnesium content in natural rubber
การพัฒนาชุดทดสอบภาคสนามโดยอาศัยระบบย่อส่วนและอุปกรณ์ที่ประดิษฐ์จากกระดาษสำหรับการวิเคราะห์ปริมาณแมกนีเซียมในน้ำยางธรรมชาติ

Investigator : Asst.Prof.Dr. Purim Jarujamrus

E-mail Address : purim.j@ubu.ac.th

Project Period : 2 Years

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 natural rubber latex (NRL) and concentrated rubber latex (CRL). 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 NRL at low concentration with the detection limit being 50 $mg.L^{-1}$ (or could be adjusted less than that), 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 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 NRL and concentrated rubber latex (CRL)

Keywords : Magnesium (Mg^{2+}), field test kit, natural rubber latex (NRL), concentrated rubber latex (CRL) complexometric titration

บทคัดย่อ

ในงานวิจัยนี้ได้พัฒนาชุดทดสอบภาคสนามอาศัยการตรวจวัดทางสีและอ่านผลด้วยตาเปล่าเพื่อใช้ในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยางธรรมชาติและน้ำยางข้น การย่อส่วนปฏิกิริยาของการไทเทรตสารประกอบเชิงซ้อนระหว่างแมกนีเซียมกับกรดเอทิลีนไดเอมีนเตตตระอะซีติกโดยไม่ใช้สารกำบังตามแนวทางของเคมีสีเขียวเป็นหัวใจหลักของพัฒนาชุดทดสอบนี้เพื่อลดการใช้สารเคมีทำให้เกิดของเสียน้อยลง มีความง่าย ราคาถูก มีความรวดเร็วในการทดสอบ จากผลการทดลองพบว่าชุดทดสอบภาคสนามที่พัฒนาขึ้นมีขีดจำกัดต่ำสุดในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยางธรรมชาติที่เท่ากับ 50 มิลลิกรัมต่อลิตร (สามารถปรับให้ต่ำกว่านี้ได้) โดยในแต่ละครั้งของการทดสอบจะใช้ตัวอย่างน้ำยางเพียง 0.18 กรัม (ใช้ช้อนขนาดเล็กในการตัก) ใช้สารเคมีน้อยกว่า 1.5 มิลลิลิตร (น้อยกว่าวิธีมาตรฐานถึง 70 เท่า) สารเคมีในชุดทดสอบที่พัฒนาขึ้นที่มีอายุการใช้งานมากกว่า 6 เดือนที่อุณหภูมิห้อง นอกจากนี้ยังพบว่าไอออนรบกวนที่เป็นองค์ประกอบภายในน้ำยางไม่มีผลต่อการวิเคราะห์ และเมื่อเปรียบเทียบผลการวิเคราะห์ที่ได้จากชุดทดสอบภาคสนามที่พัฒนาขึ้นกับเทคนิคการไทเทรตเชิงซ้อนที่ปราศจากการใช้ไซยาไนด์ตามวิธีมาตรฐานขององค์การมาตรฐานสากลภายใต้เลขมาตรฐานหมายเลขที่ 17403:2014 (E) พบว่าให้ผลสอดคล้องกัน ชุดทดสอบภาคสนามที่พัฒนาขึ้นนี้มีประสิทธิภาพที่ดี ใช้ง่าย ราคาถูก รวดเร็ว ไม่ต้องอาศัยผู้เชี่ยวชาญในการวิเคราะห์ ไม่ต้องเตรียมตัวอย่าง สามารถทำการวิเคราะห์ภาคสนามในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยางธรรมชาติและน้ำยางข้นได้เป็นอย่างดี

(คำหลัก) แมกนีเซียม ชุดทดสอบภาคสนาม น้ำยางธรรมชาติ น้ำยางข้น การไทเทรตสารประกอบเชิงซ้อน

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ

   Malahom, N.; **Jarujamrus, P*.**; Meelapsom, R.; Siripinyanond, A.; Amatatongchai, M.; Chairam, S., Simple test kit based on colorimetry for quantification of magnesium content in natural rubber latex by miniaturized complexometric titration without using masking agent *Polym. Test.* **2017,** 59, 160-167. **(Appendix A)**

2. การนำผลงานวิจัยไปใช้ประโยชน์
   - เชิงพาณิชย์ (มีการนำไปผลิต/ขาย/ก่อให้เกิดรายได้ หรือมีการนำไปประยุกต์ใช้โดยภาคธุรกิจ/บุคคลทั่วไป)
   - เชิงนโยบาย (มีการกำหนดนโยบายอิงงานวิจัย/เกิดมาตรการใหม่/เปลี่ยนแปลงระเบียบข้อบังคับหรือวิธีทำงาน)
   - เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)
     - ❖ ร่วมแสดงผลงานใน “การประชุมความร่วมมือการวิจัยพัฒนาเทคโนโลยียาง” ระหว่างวันที่ 13-14 กรกฎาคม 2560 ณ ศูนย์ประชุมอุทยานวิทยาศาสตร์ประเทศไทย จ.ปทุมธานี **(Appendix B)**
   - เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)
     - ❖ สาธิตการใช้ “ชุดทดสอบภาคสนามสำหรับวิเคราะห์ปริมาณแมกนีเซียมในน้ำยางธรรมชาติ” ให้กับนักศึกษาสาขาวิทยาศาสตร์และเทคโนโลยีการยาง ในวิชาปฏิบัติการเทคโนโลยีการยาง ภาคเรียนที่ 2/2559 คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี **(Appendix C)**

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

- **การนำเสนอผลงานในที่ประชุมวิชาการ**

  (Oral Presentation)

  \- N. Malahom, R. Meelapsom, A. Siripinyanond, M. Amatatongchai, S. Chairam and **P.Jarujamrus*** “Field test kit based on colorimetry for quantification of magnesium content in rubber latex” The 13th Asian Conference on Analytical Sciences (ASIANALYSIS XIII), 8-11 December 2016, Empress International Convention Center, Chiang Mai, Thailand **(Appendix D)**

  (Poster Presentation)

  \- S. Puchum, N. Malahom, R. Meelapsom, A. Siripinyanond, M. Amatatongchai, S. Chairam and **P. Jarujamrus***, “Simple test kit based on colorimetry for quantification of magnesium content in rubber latex“ North Eastern Science and Technology Conference (NESTC), 18 March 2017, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani, Thailand **(Appendix E)**

- **การจดสิทธิบัตร**

  \- ยื่นจดอนุสิทธิบัตรเรื่อง “ชุดทดสอบแมกนีเซียมในน้ำยางพาราภาคสนาม” เลขที่คำขอ 1603002133 เมื่อวันที่ 21 ตุลาคม 2559 **(Appendix F)**

Simple test kit based on colorimetry for quantification of magnesium content in natural rubber latex (NRL) and concentrated rubber latex (CRL) by miniaturized complexometric titration without using masking agent

Introduction:

Para rubber tree (*Hevea brasiliensis* Muell. Arg.) is a very important economic plant in Thailand where over three million tons a year of Natural Rubber Latex (NRL, the products of Para rubber tree) is exported **[1,2]**. The major component in NRL is cis-1,4-polyisoprene with non-rubber components such as carbohydrates, proteins and lipid based medium for bacteria growing. pH values of normal NRL solution range from 6 to 7 **[3,4]**. Under this condition, the surface of NRL 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 NRL surface **[4]** (**Fig. 1a**). A critical step in production of NRL with high quality is identification of NRL components. Magnesium (Mg^{2+}) is one of the most important components suppressing NRL performance and quality, *e.g.* by direct interaction of Mg^{2+} with the carboxylate ions contained in the NRL. This produces insoluble, un-hydrated and un-ionized magnesium soap and insoluble magnesium hydroxide in the aqueous phase. Both of these phenomena invariably cause destabilization of NRL. Furthermore, Mg^{2+} ions can form primary valence linkages between the interface of adjacent latex particles. This can initiate flocculation and further lead to destabilization of NRL **[5] Fig. 1b**.

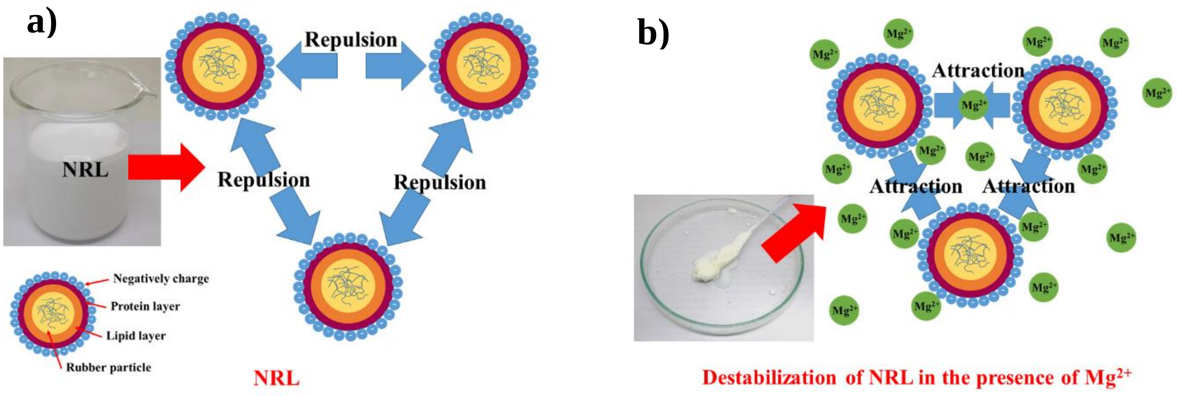


Fig. 1 Carboxylate ions contained in the NRL **a)** the proposed mechanism between Mg^{2+} ions and NRL **b**)

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 **[7-8]** and **Fig. 2**.

$$Mg^{2+}\,(aq) \;+\; NH_3\,(aq) \;+\; HPO_4^{\,2-}\,(aq) \longrightarrow MgNH_4PO_4\,(s)..............(1)$$

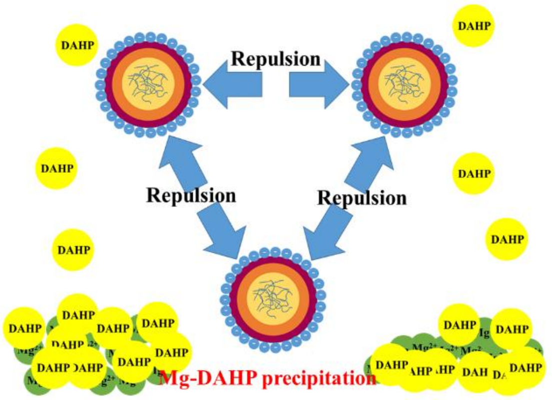


Fig. 2 Elimination of Mg^{2+} by adding DAHP into NRL

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.

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

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 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.

Satheinperakul 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 36-126 $mg.L^{-1}$ which is in good agreement with the results obtained from atomic absorption spectrometry. Unfortunately, they found that the presence of zinc and cadmium in high levels interfered the determination of Mg^{2+}. In 2011, a method and composition for quantifying magnesium ions based on conventional complexometric titration have been patented (WO2011139245) using NaHS as a selectively precipitating interfering metal ions in NRL instead of using KCN as mentioned above **[12].** Afterwards, the 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) **[12-15]** has also been established in 2014. However, the reported approaches are still complicated in terms of multiple steps of analysis including demand of sample pretreatment before analysis, reagent volume consumption (>105 mL), resulting in more waste and potential interference ions in NRL if 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 2)**. A miniaturized complexometric titration between Mg^{2+} and EDTA is thus considered to be a key reaction in this development.

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 hours, therefore, NRL is needed to collect several time to accomplish the experiment

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

Table 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	[16]
B	Sea water	30	0.3922	[17]
C	Sea water	100	0.5198	[18]
D	Sea water	15	0.3998	[19]
E	Variety of samples	1.5	4.6050	[20]
F	**NRL, CRL**	**< 50**	**0.1620**	**Proposed method**

NRL= Natural rubber latex

CRL= Concentrated rubber latex

In this work, 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 magnesium content in NRL 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 was applied for investigation of the effect of the presence of potential interference ions, preservatives used in NRL and 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.

Experimental

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 approaches 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 were used in acid digestion method of FAAS analysis . Bottle of serum container (10 mL) and eyes drop bottle (10 mL) were obtained from a pharmacy store in Ubon Ratchathani which were used as containers for reagent A and B, respectively. Plastic coffee spoons obtained from local supermarket in Ubon Ratchathani were used as sampling spoons.

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 3**.

Table 3 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

Preparation of reagents

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 for standardization. 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+} **s**tandard 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 brought up to 1 L with DI water. An indicator was prepared by dissolving EBT (0.1 g) into 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}).

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 (~0.18g). 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 reagent A and B were generated by using eye drop bottles (~0.065 mL.$drop^{-1}$).

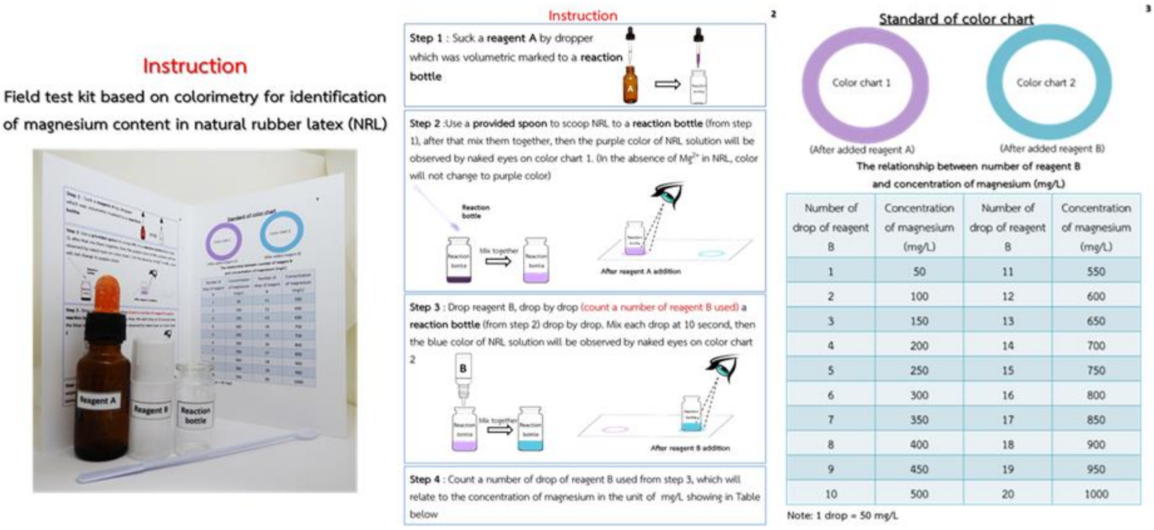
Method validation

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) into 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 ^{O}C resulting in a transparent solution which was then cooled down 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^{+}. 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 hours, fresh NRL was collected freshly several times during the experiment in order to prevent the NRL coaggulating.

For the test kit application (**Fig 3-4**), 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 together resulting in the purple color observed by naked eye, as shown by the color chart 1 in **Fig 3**. Reagent B was then added drop by drop and shaken (every 10 second) to the solution until the blue color of NRL solution was observed, see also the color chart 2 in **Fig 3**. 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 $mg.L^{-1}$.



Instruction

Field test kit based on colorimetry for identification of magnesium content in natural rubber latex (NRL)

Instruction

Step 1 : Suck a **reagent A** by dropper which was volumetric marked to a **reaction bottle**

Step 2 :Use a **provided spoon** to scoop NRL to a **reaction bottle** (from step 1), after that mix them together, then the purple color of NRL solution will be observed by naked eyes on color chart 1. (In the absence of Mg^{2+} in NRL, color will not change to purple color)

Step 3 : Drop reagent B, drop by drop (count a number of reagent B used) a **reaction bottle** (from step 2) drop by drop. Mix each drop at 10 second, then the blue color of NRL solution will be observed by naked eyes on color chart 2

Step 4 : Count a number of drop of reagent B used from step 3, which will relate to the concentration of magnesium in the unit of mg/L showing in Table below

Standard of color chart

Color chart 1 (After added reagent A)

Color chart 2 (After added reagent B)

The relationship between number of reagent B and concentration of magnesium (mg/L)

Number of drop of reagent B	Concentration of magnesium (mg/L)	Number of drop of reagent B	Concentration of magnesium (mg/L)
1	50	11	550
2	100	12	600
3	150	13	650
4	200	14	700
5	250	15	750
6	300	16	800
7	350	17	850
8	400	18	900
9	450	19	950
10	500	20	1000

Note: 1 drop = 50 mg/L

Fig 3. The instruction of “Field test kit based on colorimetry for determination of magnesium content in natural rubber latex (NRL)”

1. Addition of optimized reagent A (which was marked the level on the dropper of reagent A bottle) in the reaction bottle

2. Addition of NRL sample (comparing with standard color chart 1 shown in **Fig 3**)

3. Addition of reagent B (16 drops) 1 drop = 50 $mg.L^{-1}$ Mg^{2+} (comparing with standard color chart 2 shown in **Fig 3**)

Fig. 4 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)

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).

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 were operated by three different users. The results were averaged and compared with those obtained by the conventional complexometric titration method.

Results and Discussion

Optimization of the developed test kit

The optimized sample amount scooped by using a small spoon was 0.1731 ± 0.02 g (**Table 4**) and the optimized reagent volume dropped by using an eye drop bottle was 0.065 ± 0.004 mL **(Table 5).** 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 2.

Table 4 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

Average weight of NRL sample/sampling is 0.1731 ± 0.0200 g

Table 5 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

Average weight/drop is 0.065 ± 0.004 g

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 5a**) 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 5b**). 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.

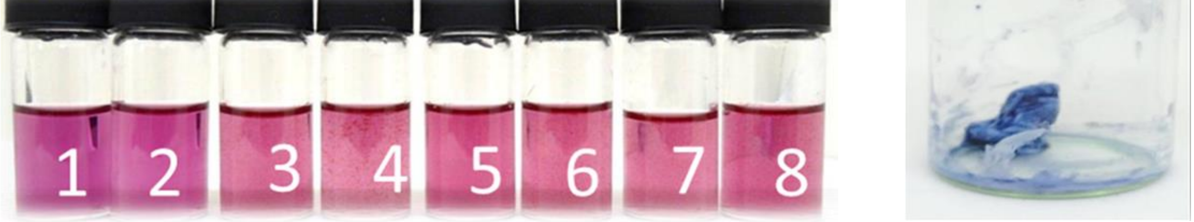


Fig 5 Variation of number of EBT droplets (1-8 drops) containing 50 mg.L^{-1} Mg^{2+} in DI water **a)** and precipitation of NRL sample when 3 drops of EBT were applied **b)**.

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 6** and **7**. 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 8**.

Table 6 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 2.74×10^{-3} mol·L^{-1} EDTA, ** containing 5.48×10^{-3} mol·L^{-1} EDTA, *** containing 10.98×10^{-3} mol·L^{-1} EDTA

Table 7 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 NRL	**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

$$\%\textbf{Relative difference} \quad = [\frac{\mathbf{Mg^{2+}contents\ from\ complexometric\ titration\ -\ Mg^{2+}contents\ from\ our\ developing\ method)}}{\mathbf{Mg^{2+}contents\ from\ complexometric\ titration}}] \times \mathbf{100}$$

Table 8 The monitoring of pH in our developing test kit system

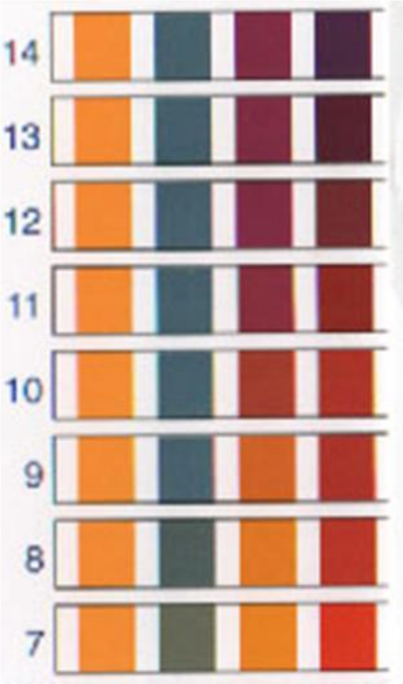
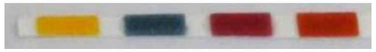
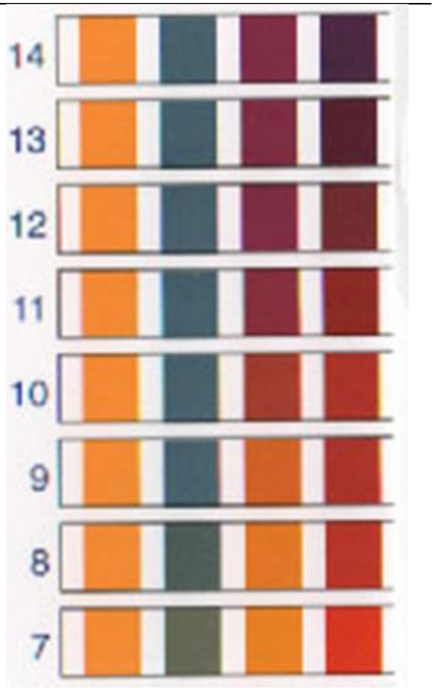
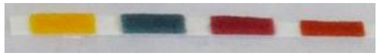
Number of droplets of reagent B	Total concentration of buffer presented in reagent B (M)	Color change of pH paper		Standard color chart of pH paper
		Before addition of reagent B (NRL sample and reagent A were applied in the reaction bottle)	After addition of reagent B	
10	0.02			
20	0.03			
30	0.04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

Table 8 (Continue)

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 (NRL sample and reagent A were applied in the reaction bottle)	**After addition of reagent B**	
70	0.05	 **(pH 7-8)**		
80	0.05			
90	0.05			
100	0.06		 **(pH 10-11)**	

[29] Buffer capacity was calculated by this equation;

$$\beta = 2.3 \frac{C_{BOH} \times C_B}{C_{BOH} + C_B}$$

$\beta = Buffer\ capacity$

$C_{BOH} = Concentration\ of\ base\ (M)$

$C_B = Concentration\ of\ salt\ (M)$

In this experiment, we used 3.67 M NH_4OH and 1.274 M NH_4Cl as basic and salt solutions for buffer preparation.

$$\beta = 2.3 \frac{3.67 \times 1.274}{3.67 + 1.274}$$

$$= 2.3\ (\frac{4.676}{4.944})$$

$$= 2.3 \times 0.946$$

$$= 2.17\ M/pH$$

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 9)**.

Table 9 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

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 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 10**). 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 11**).

Table 10 Our developed test kit (without masking agent) performed with the presence of potential interference ions in NRL 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 NRL**								
(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

** NRL from U_3 sample

Table 11 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		

Table 11 (Continue)

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
U_2 added 80 $mg.L^{-1}$ of Mg^{2+}	560.30 ± 6.05	12	600	583.3	100.6
		11	550		
		12	600		

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 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+}, **Fig 6**) **[27]**.

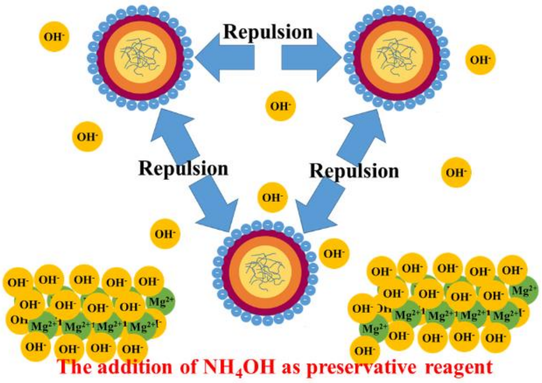


Fig 6 The proposed mechanism when NH_4OH as preservative were performed

ZnO and TMTD were added in 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**

12. 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.

Table 12 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 differrent**
Original NRL	775.3 ± 9.25	15	750.0	-3.3
		15		
		15		
NRL preserved with 0.2% NH_4OH	500.7 ± 4.39	10	500.0	-0.1
		10		
		10		
NRL preserved with 0.2% NH_4OH, 0.025% ZnO and 0.025%TMTD	611.7 ± 14.01	11	550.0	-10.1
		11		
		11		

U_2 was used as model sample in this study

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

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%

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 13**. 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 14**.

Table 13 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 differrent**	**Average of Mg^{2+} content (mg.L^{-1})**	**% Relative differrent**
U_2	Original NRL	719.1±3.3	683.3	-5.0	700.0	-2.7
	NRL mixed with preservatives*	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*	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 NRL

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

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

Table 14 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

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

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%

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 2**). Our developed test kit (even without masking agent) can be applicable even with the presence of potential interference ions (see also in **Table 10**) 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 6** and **7** providing the detection limit of magnesium being 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 can be 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 15**) 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.

Table 15 Study 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 ($mg \cdot L^{-1}$)**	**Our developed test kit**	
		Number of drops (1 drop = 50 $mg.L^{-1}$)	**Mg^{2+} content ($mg.L^{-1}$)**
CRL*	55.54 ± 3.34	1 ± 1	50.00 ± 50.00
CRL**	54.25 ± 1.27	1 ± 1	50.00 ± 50.00

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

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

CRL**: 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]**

The results in **Table 15** indicated that the established test kit were not only applicable for NRL analysis, but it is also for practical use in CRL even in acidic medium of CRL sample which is sufficient in the developed test kit (pH 7 in the system was obtained after reagent A was applied, then pH 10 was accomplished in the system when just one drop of reagent B was applied)

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Appendix

Appendix A

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Analysis Method

Simple test kit based on colorimetry for quantification of magnesium content in natural rubber latex by miniaturized complexometric titration without using masking agent

Nutthaporn Malahom [a], Purim Jarujamrus [a, *], Rattapol Meelapsom [a], Atitaya Siripinyanond [b], Maliwan Amatatongchai [a], Sanoe Chairam [a]

[a] Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

[b] Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

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ABSTRACT

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 natural rubber latex (NRL). 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 NRL 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 NRL.

1. Introduction

Para rubber tree (*Hevea brasiliensis* Muell. Arg.) is a very important economic plant in Thailand where over three million tons a year of Natural Rubber Latex (NRL, the product of Para rubber tree) is exported [1,2]. The major component in NRL is *cis*-1,4-polyisoprene with non-rubber components such as carbohydrates, proteins and lipid based medium for bacteria growing. pH values of normal NRL solution range from 6 to 7 [3,4]. Under this condition, the surface of NRL 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 NRL surface [4] (Fig. 1a). A critical step in production of NRL with high quality is identification of NRL components. Magnesium (Mg^{2+}) is one of the most important components suppressing NRL performance and quality, *e.g.* by direct interaction of Mg^{2+} with the carboxylate ions contained in the NRL. This produces insoluble, un-hydrated and un-ionized magnesium soap and insoluble magnesium hydroxide in the aqueous phase. Both of these phenomena invariably cause destabilization of NRL. Furthermore, Mg^{2+} ions can form primary valence linkages between the interface of adjacent latex particles. This can initiate flocculation and further lead to destabilization of NRL [5] Fig. 1b.

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+} 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) [7,8] and Fig. 1c.

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* Corresponding author.
E-mail address: purim.j@ubu.ac.th (P. Jarujamrus).

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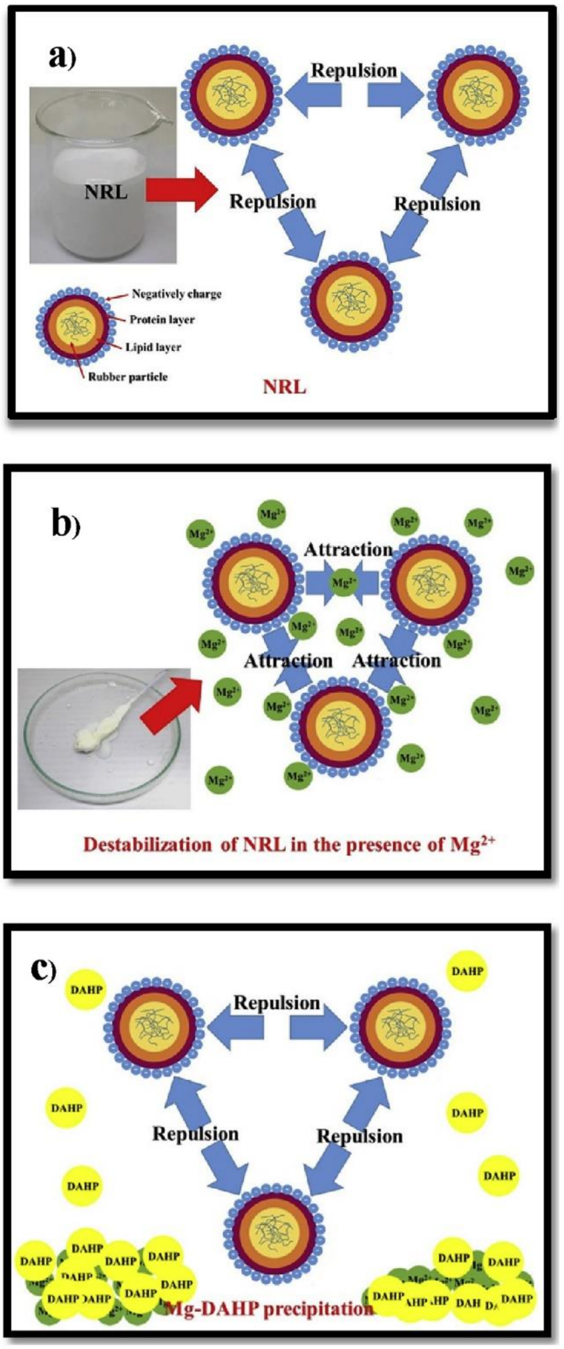

Fig. 1. Carboxylate ions contained in the NRL **a)** the proposed mechanism between Mg^{2+} ions and NRL **b)** Elimination of Mg^{2+} by adding DAHP into NRL **c)**.

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

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 ≥ 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.

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

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.

Satheinperakul 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.

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

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+} **s**tandard 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 $\beta = 2.17$ 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

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}).

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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 $\beta = 2.17$ 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.

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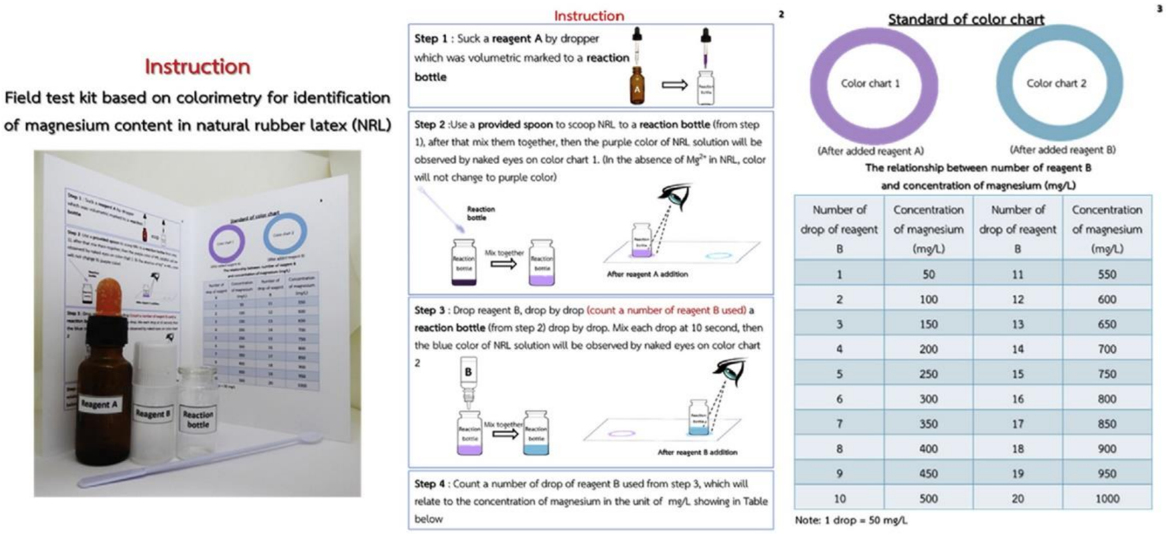


Instruction

Field test kit based on colorimetry for identification of magnesium content in natural rubber latex (NRL)

Instruction

Step 1 : Suck a **reagent A** by dropper which was volumetric marked to a **reaction bottle**

Step 2 :Use a **provided spoon** to scoop NRL to a **reaction bottle** (from step 1), after that mix them together, then the purple color of NRL solution will be observed by naked eyes on color chart 1. (In the absence of Mg^{2+} in NRL, color will not change to purple color)

Step 3 : Drop reagent B, drop by drop (count a number of reagent B used) a **reaction bottle** (from step 2) drop by drop. Mix each drop at 10 second, then the blue color of NRL solution will be observed by naked eyes on color chart 2

Step 4 : Count a number of drop of reagent B used from step 3, which will relate to the concentration of magnesium in the unit of mg/L showing in Table below

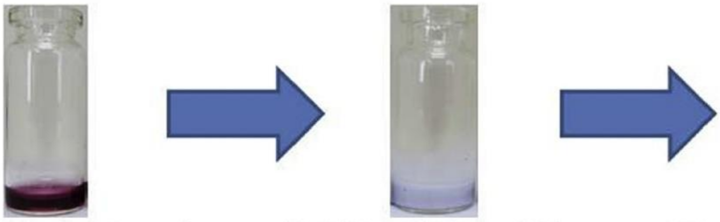
Standard of color chart

The relationship between number of reagent B and concentration of magnesium (mg/L)

Number of drop of reagent B	Concentration of magnesium (mg/L)	Number of drop of reagent B	Concentration of magnesium (mg/L)
1	50	11	550
2	100	12	600
3	150	13	650
4	200	14	700
5	250	15	750
6	300	16	800
7	350	17	850
8	400	18	900
9	450	19	950
10	500	20	1000

Note: 1 drop = 50 mg/L

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.

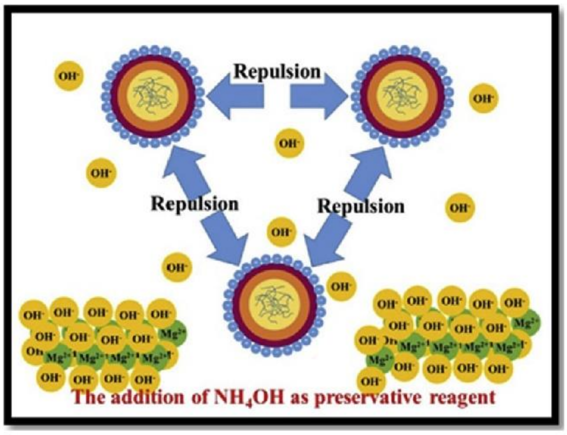


Fig. 4. The proposed mechanism when NH_4OH as preservative were performed.

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 differrent
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.

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

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.

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

[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

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

The results from these two methods showed *tstat* = 0.009 and *tcritical* = 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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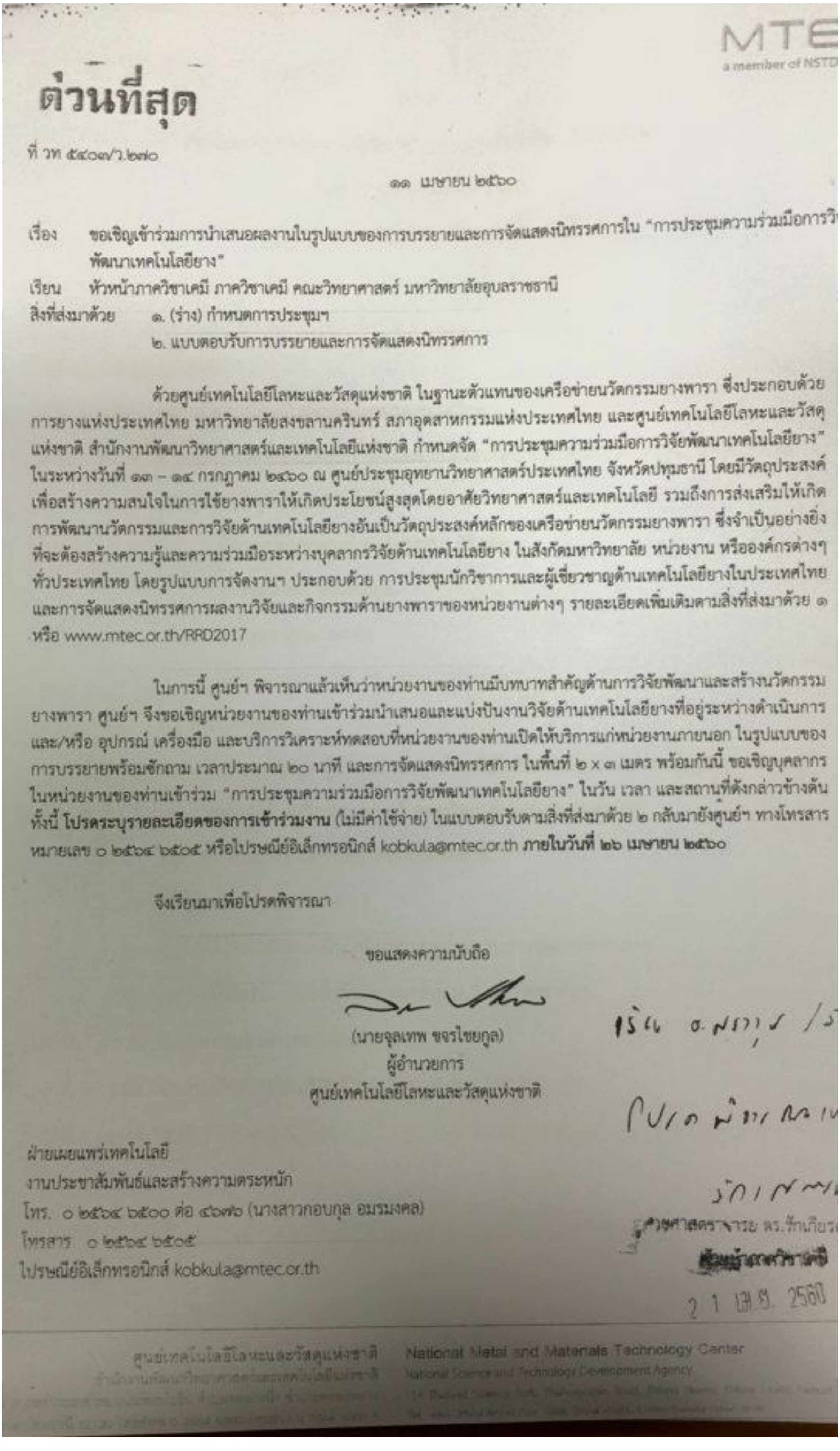
Appendix B



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ด่วนที่สุด

ที่ วท ๕๔๐๗/ว.๒๗๐

๑๑ เมษายน ๒๕๖๐

เรื่อง ขอเชิญเข้าร่วมการนำเสนอผลงานในรูปแบบของการบรรยายและการจัดแสดงนิทรรศการใน "การประชุมความร่วมมือการวิ
พัฒนาเทคโนโลยียาง"

เรียน หัวหน้าภาควิชาเคมี ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี

สิ่งที่ส่งมาด้วย ๑. (ร่าง) กำหนดการประชุมฯ
๒. แบบตอบรับการบรรยายและการจัดแสดงนิทรรศการ

ด้วยศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ ในฐานะตัวแทนของเครือข่ายนวัตกรรมยางพารา ซึ่งประกอบด้วย การยางแห่งประเทศไทย มหาวิทยาลัยสงขลานครินทร์ สภาอุตสาหกรรมแห่งประเทศไทย และศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ กำหนดจัด "การประชุมความร่วมมือการวิจัยพัฒนาเทคโนโลยียาง" ในระหว่างวันที่ ๑๓ – ๑๔ กรกฎาคม ๒๕๖๐ ณ ศูนย์ประชุมอุทยานวิทยาศาสตร์ประเทศไทย จังหวัดปทุมธานี โดยมีวัตถุประสงค์เพื่อสร้างความสนใจในการใช้ยางพาราให้เกิดประโยชน์สูงสุดโดยอาศัยวิทยาศาสตร์และเทคโนโลยี รวมถึงการส่งเสริมให้เกิดการพัฒนานวัตกรรมและการวิจัยด้านเทคโนโลยียางอันเป็นวัตถุประสงค์หลักของเครือข่ายนวัตกรรมยางพารา ซึ่งจำเป็นอย่างยิ่งที่จะต้องสร้างความรู้และความร่วมมือระหว่างบุคลากรวิจัยด้านเทคโนโลยียาง ในสังกัดมหาวิทยาลัย หน่วยงาน หรือองค์กรต่างๆ ทั่วประเทศไทย โดยรูปแบบการจัดงานฯ ประกอบด้วย การประชุมนักวิชาการและผู้เชี่ยวชาญด้านเทคโนโลยียางในประเทศไทย และการจัดแสดงนิทรรศการผลงานวิจัยและกิจกรรมด้านยางพาราของหน่วยงานต่างๆ รายละเอียดเพิ่มเติมตามสิ่งที่ส่งมาด้วย ๑ หรือ www.mtec.or.th/RRD2017

ในการนี้ ศูนย์ฯ พิจารณาแล้วเห็นว่าหน่วยงานของท่านมีบทบาทสำคัญด้านการวิจัยพัฒนาและสร้างนวัตกรรมยางพารา ศูนย์ฯ จึงขอเชิญหน่วยงานของท่านเข้าร่วมนำเสนอและแบ่งปันงานวิจัยด้านเทคโนโลยียางที่อยู่ระหว่างดำเนินการ และ/หรือ อุปกรณ์ เครื่องมือ และบริการวิเคราะห์ทดสอบที่หน่วยงานของท่านเปิดให้บริการแก่หน่วยงานภายนอก ในรูปแบบของการบรรยายพร้อมซักถาม เวลาประมาณ ๒๐ นาที และการจัดแสดงนิทรรศการ ในพื้นที่ ๒ x ๓ เมตร พร้อมกันนี้ ขอเชิญบุคลากรในหน่วยงานของท่านเข้าร่วม "การประชุมความร่วมมือการวิจัยพัฒนาเทคโนโลยียาง" ในวัน เวลา และสถานที่ดังกล่าวข้างต้น ทั้งนี้ **โปรดระบุรายละเอียดของการเข้าร่วมงาน** (ไม่มีค่าใช้จ่าย) ในแบบตอบรับตามสิ่งที่ส่งมาด้วย ๒ กลับมายังศูนย์ฯ ทางโทรสารหมายเลข ๐ ๒๕๖๔ ๖๕๐๕ หรือไปรษณีย์อิเล็กทรอนิกส์ kobkula@mtec.or.th **ภายในวันที่ ๒๖ เมษายน ๒๕๖๐**

จึงเรียนมาเพื่อโปรดพิจารณา

ขอแสดงความนับถือ

(นายจุลเทพ ขจรไชยกูล)
ผู้อำนวยการ
ศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ

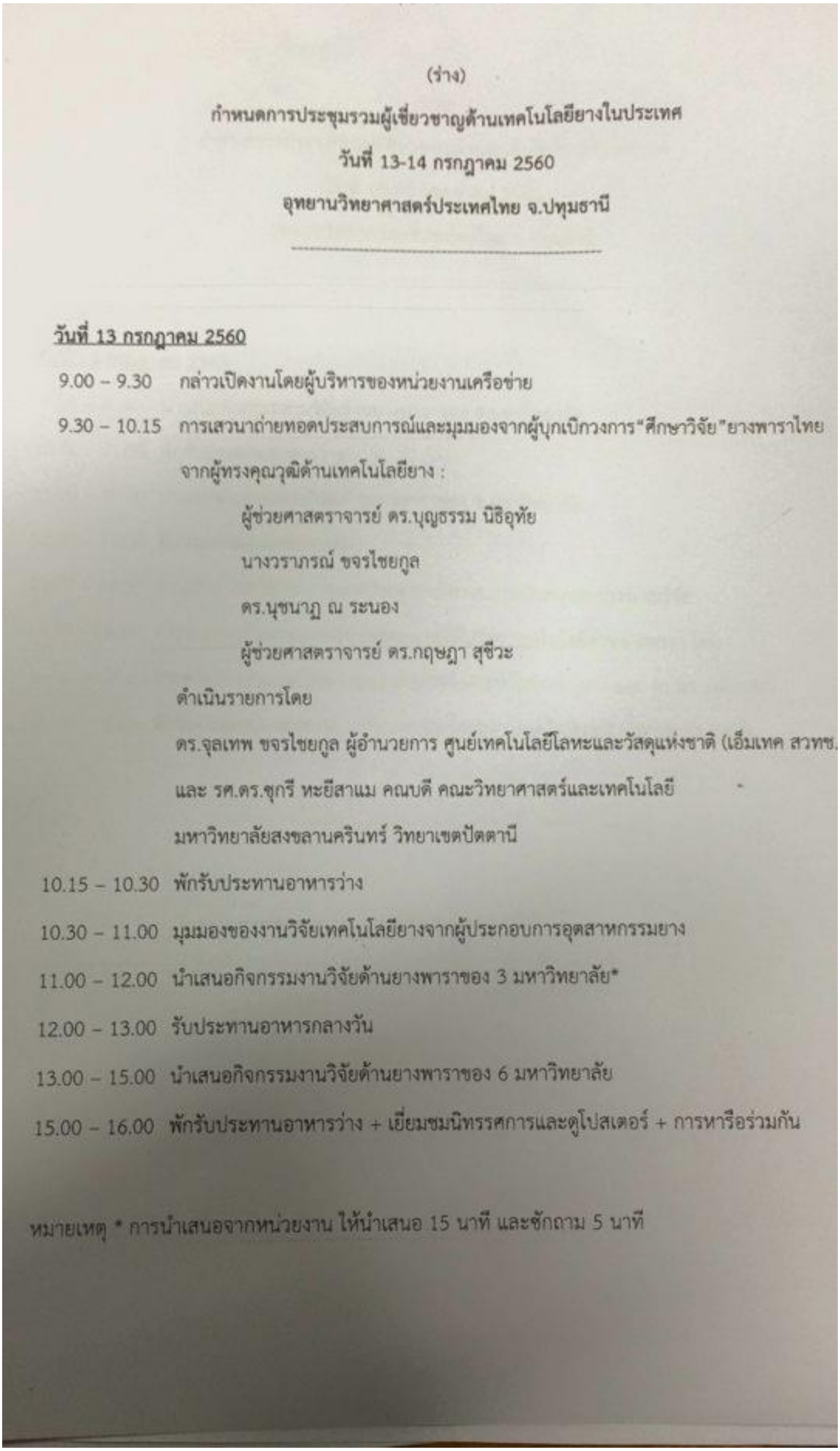
เรียน อ.[illegible]

[illegible]

รัก[illegible]

ศาสตราจารย์ ดร.รักเกียร
หัวหน้าภาควิชาเคมี
21 เม.ย. 2560

ฝ่ายเผยแพร่เทคโนโลยี
งานประชาสัมพันธ์และสร้างความตระหนัก
โทร. ๐ ๒๕๖๔ ๖๕๐๐ ต่อ ๔๖๗๖ (นางสาวกอบกุล อมรมงคล)
โทรสาร ๐ ๒๕๖๔ ๖๕๐๕
ไปรษณีย์อิเล็กทรอนิกส์ kobkula@mtec.or.th



(ร่าง)

กำหนดการประชุมรวมผู้เชี่ยวชาญด้านเทคโนโลยียางในประเทศ

วันที่ 13-14 กรกฎาคม 2560

อุทยานวิทยาศาสตร์ประเทศไทย จ.ปทุมธานี

วันที่ 13 กรกฎาคม 2560

9.00 – 9.30 กล่าวเปิดงานโดยผู้บริหารของหน่วยงานเครือข่าย

9.30 – 10.15 การเสวนาถ่ายทอดประสบการณ์และมุมมองจากผู้บุกเบิกวงการ"ศึกษาวิจัย"ยางพาราไทย
จากผู้ทรงคุณวุฒิด้านเทคโนโลยียาง :
ผู้ช่วยศาสตราจารย์ ดร.บุญธรรม นิธิอุทัย
นางวราภรณ์ ขจรไชยกูล
ดร.นุชนาฏ ณ ระนอง
ผู้ช่วยศาสตราจารย์ ดร.กฤษฎา สุชีวะ
ดำเนินรายการโดย
ดร.จุลเทพ ขจรไชยกูล ผู้อำนวยการ ศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ (เอ็มเทค สวทช.
และ รศ.ดร.ซุกรี หะยีสาแม คณบดี คณะวิทยาศาสตร์และเทคโนโลยี
มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี

10.15 – 10.30 พักรับประทานอาหารว่าง

10.30 – 11.00 มุมมองของงานวิจัยเทคโนโลยียางจากผู้ประกอบการอุตสาหกรรมยาง

11.00 – 12.00 นำเสนอกิจกรรมงานวิจัยด้านยางพาราของ 3 มหาวิทยาลัย*

12.00 – 13.00 รับประทานอาหารกลางวัน

13.00 – 15.00 นำเสนอกิจกรรมงานวิจัยด้านยางพาราของ 6 มหาวิทยาลัย

15.00 – 16.00 พักรับประทานอาหารว่าง + เยี่ยมชมนิทรรศการและดูโปสเตอร์ + การหารือร่วมกัน

หมายเหตุ * การนำเสนอจากหน่วยงาน ให้นำเสนอ 15 นาที และซักถาม 5 นาที

(ร่าง)

กำหนดการประชุมรวมผู้เชี่ยวชาญด้านเทคโนโลยียางในประเทศ

วันที่ 13-14 กรกฎาคม 2560

อุทยานวิทยาศาสตร์ประเทศไทย จ.ปทุมธานี

<u>วันที่ 14 กรกฎาคม 2560</u>

9.00 – 10.40	นำเสนอกิจกรรมงานวิจัยด้านยางพาราของ 4 มหาวิทยาลัย
10.40 – 11.00	พักรับประทานอาหารว่าง
11.00 – 12.00	นำเสนอกิจกรรมงานวิจัยด้านยางพาราของ 3 มหาวิทยาลัย
12.00 – 13.00	รับประทานอาหารกลางวัน
13.00 – 14.30	การนำเสนอหรือเสวนาจากหน่วยงานบริหารและสนับสนุนงบประมาณวิจัย
14.30 - 15.30	การนำเสนอกิจกรรมการวิจัยพัฒนาและให้บริการเทคโนโลยีด้านยางพารา โดย การยางแห่งประเทศไทย (กยท.) กรมวิทยาศาสตร์บริการ (วศ.) และ ศว.พว. (เอ็มเทค)
15.30 – 16.30	พักรับประทานอาหารว่าง + เยี่ยมชมนิทรรศการและดูโปสเตอร์ + การหารือร่วมกัน

ประมวลภาพกิจกรรม

“การประชุมความร่วมมือการวิจัยพัฒนาเทคโนโลยียาง” ระหว่างวันที่ 13-14 กรกฎาคม 2560

ณ ศูนย์ประชุมอุทยานวิทยาศาสตร์ประเทศไทย จ.ปทุมธานี

Appendix C

ประมวลภาพกิจกรรม

สาธิตการใช้ "ชุดทดสอบภาคสนามสำหรับวิเคราะห์ปริมาณแมกนีเซียมในน้ำยางธรรมชาติ" ให้กับนักศึกษา สาขาวิทยาศาสตร์และเทคโนโลยีการยาง ภาคการศึกษาที่ 2/2559 คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี








Appendix D

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

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

Nutthaporn Malahom[1], Rattapol Meelapsom[1], Atitaya Siripinyanond[2], Maliwan Amatatongchai[1],
Sanoe Chairam[1] and Purim Jarujamrus[1,*]

[1] *Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science,*
Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
[2] *Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
**E-mail: purim.j@ubu.ac.th*

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

Appendix E

ชุดทดสอบภาคสนามที่อาศัยการตรวจวัดทางสีเพื่อวิเคราะห์หาปริมาณของแมกนีเซียมในน้ำยาง

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

สดใส ภูชุม[1], ณัฐพร มาลาหอม[1], รัฐพล มีลาภสม[1], อทิตยา ศิริภิญญานนท์[2],
มะลิวรรณ อมตธงไชย[1], เสนอ ชัยรัมย์[1] และ ปุริม จารุจำรัส[1*]
[1]ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี, อำเภอวารินชำราบ , จังหวัดอุบลราชธานี, 34190
[2]ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล, ถ.พระรามที่ 6 แขวงทุ่งพญาไท เขตราชเทวี, กรุงเทพมหานคร, 10400

S. Puchum[1], N. Malahom[1], R. Meelapsom[1], A. Siripinyanond[2],
M. Amatathongchai[1], S. Chairam[1] and P. Jarujamrus[1*]

[1]Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
[2]Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400,Thailand

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บทคัดย่อ

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

ภาคสนามที่พัฒนาขึ้นเทียบกับเทคนิคการไทเทรตเชิงซ้อนที่ปราศจากการใช้ไซยาไนด์ตามวิธีมาตรฐานขององค์การมาตรฐานสากลภายใต้เลขมาตรฐานหมายเลขที่ 17403:2014 (E) ให้ผลสอดคล้องกัน ชุดทดสอบภาคสนามที่พัฒนาขึ้นนี้มีประสิทธิภาพที่ดี ง่าย ราคาถูก รวดเร็ว ไม่ต้องอาศัยผู้เชี่ยวชาญในการวิเคราะห์ ไม่ต้องเตรียมตัวอย่าง สามารถทำการวิเคราะห์ภาคสนามในการวิเคราะห์หาปริมาณแมกนีเซียมในน้ำยางได้อย่างดี

คำสำคัญ: แมกนีเซียม ชุดทดสอบภาคสนาม น้ำยาง เคมีสีเขียว การไทเทรตสารประกอบเชิงซ้อน

Abstract

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, 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.

Keywords: Magnesium, Test kit, Rubber latex (RL), Green chemistry, Complexometric titration

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*Corresponding author. E-mail address: purim.j@ubu.ac.th

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

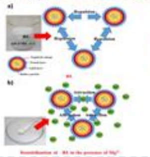
S. Puchum[1], N. Malahom[1], R. Meelapsom[1], A. Siripinyanond[2], M. Amatathongchai[1], S. Chairam[1] and P. Jarujamrus[1*]

[1] *Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand*
[2] *Department of Chemistry and Center of Excellent for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
**E-mail: purim.j@ubu.ac.th*

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 platforms 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 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.

A major cause of destabilization of RL :

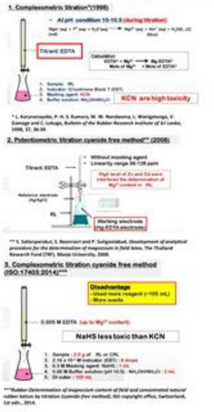


- Negatively charge due to the presence of carboxylate ions of protein (Alpha-globulin with pI of 4.8) and the hydrolysis of R-Lecithin phospholipid on the RL surface
- The direct interaction of Mg^{2+} with carboxylate ions contained in the RL producing insoluble, unhydrated and unionized magnesium soap
- The insoluble magnesium hydroxide may also precipitate in the aqueous phase

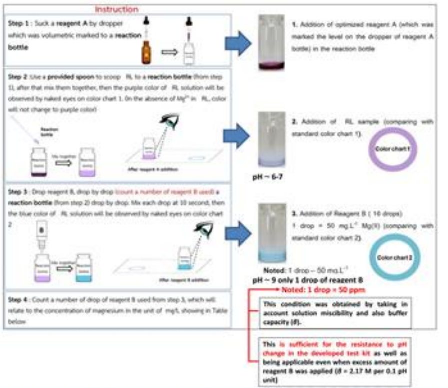
Routine monitoring is needed…….

Pollution Control Department : $Mg^{2+} \leq 50$ ppm
AFET : $Mg^{2+} \leq 40$ ppm

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



Determination of Mg^{2+} in RL with miniaturized complexometric titration:



Step 1 : Suck a **reagent A** by dropper which was volumetric marked to a **reaction bottle**

Step 2 : Use a **provided spoon** to scoop RL to a **reaction bottle** (from step 1), after that mix them together, then the purple color of RL solution will be observed by naked eyes on color chart 1. (In the absence of Mg^{2+} in RL, color will not change to purple color)

Step 3 : Drop reagent B, drop by drop (count a number of reagent B used) a **reaction bottle** (from step 2) drop by drop. Mix each drop at 10 second, then the blue color of RL solution will be observed by naked eyes on color chart 2

Step 4 : Count a number of drop of reagent B used from step 3, which will relate to the concentration of magnesium in the unit of mg/L showing in Table below

1. Addition of optimized reagent A (which was marked the level on the dropper of reagent A bottle) in the reaction bottle
2. Addition of RL sample (comparing with standard color chart 1).

pH ~ 6-7

3. Addition of Reagent B (10 drops) 1 drop = 50 $mg.L^{-1}$ Mg(II) (comparing with standard color chart 2).

Noted: 1 drop ~ 50 $mg.L^{-1}$
pH ~ 9 only 1 drop of reagent B
Noted: 1 drop = 50 ppm

This condition was obtained by taking in account solution miscibility and also buffer capacity (β).

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 (β = 2.17 M per 0.1 pH unit)

Results :

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

Area of RL	Mg^{2+} content (ppm)	
	FAAS method	Complexometric titration based on cyanide free method
U1	746.90 ± 0.002	748.48 ± 7.07
U1	800.02 ± 0.008	770.90 ± 8.22
U1	873.81 ± 0.001	812.17 ± 3.62
U3	283.12 ± 0.001	306.18 ± 5.30
U3	342.55 ± 0.001	322.52 ± 4.39
U3	363.89 ± 0.001	375.13 ± 26.08

The results from these two methods showed t_{stat} = 1.02 and $t_{critical}$ = 2.78 which are not statistically different with the confidence level of 95%

Table 2. Our developed test kit (without masking agent) performed with the presence of potential interference ions in RL sample (n=3)

Elements	Tolerance concentration (ppm)	Using complexometric titration						Reported* (ppm)	Found by FAAS (ppm)	Spiked elements (ppm)	Total (ppm)	Complexometric titration (ppm) n=3	Developed test kit without masking agent n=3		% Relative different
		With masking agent			Without masking agent										
		Mg^{2+} content (ppm)		% Relative different	Mg^{2+} content (ppm)		% Relative different								
		1st collection	2nd collection		1st collection	2nd collection						3rd collection	Drop	ppm	
Original RL (Mg)	-	524.02±11.89	256.16±2.99	-	534.81±17.40	289.87±23.37	-	-	-	-	0	571.57±2.38	12	600	4.97
Zn	793.62	470.60±2.85	-	-9.86	611.60±2.47	-	+14.36	16.02	42.3	50	92.3	573.41±21.27	13	650	13.72
Ca	955.82	545.10±3.72	-	+4.02	565.40±7.03	-	+5.72	8.9	905.4	150	1,055.4	593.67±3.35	13	650	13.72
Fe	157.52	-	272.60±1.26	+6.42	-	315.20±7.13	+8.47	0.27	57.6	60	117.6	576.85±11.00	12	600	13.72
Cu	244.14	-	232.50±1.53	-9.23	-	N.D.	-	0.27	1.5	2	3.5	584.07±6.02	12	600	4.97
Mn	53.93	-	267.90±0.77	+4.58	-	330.90±3.48	+14.15	7.45	4.9	5	9.9	578.51±2.40	12	600	4.97
K	2,171.29	-	235.20±7.16	-8.18	-	228.90±23.32	-21.03	816	1,215.8	1,300	2,515.8	573.31±13.79	13	650	13.72
Na	1,356.97	-	239.50±2.72	-6.50	-	225.50±7.63	-22.21	966	870.7	1,000	1,870.7	555.06±23.80	13	650	13.72

*Operated by inductively coupled plasma (ICP) atomic emission spectroscopy technique and FAAS

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

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

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

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

Area	Type of sample	Mg^{2+} content by complexometric titra*ion (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	719.1 ± 3.3	683.3	-0.5	700.0	-7.2
	RL mixed with preservatives*	611.7 ± 14.0	566.5	-4.7	566.5	-4.7
U3	Original RL	667.1 ± 7.5	633.3	-1.5	615.5	-6.7
	RL mixed with preservatives*	582.4 ± 1.4	600.0	+0.3	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.

Table 5. Study 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 (ppm)	Our developed test kit	
		Number of drops (1 drop = 50 ppm)	Mg^{2+} content (ppm)
CRL*	55.54 ± 3.34	1 ± 1	50.00 ± 50.00
CRL**	54.25 ± 1.27	1 ± 1	50.00 ± 50.00

*Mg^{2+} in RL (U_3) were removed by addition of diammonium hydrogen phosphate (DAHP)
** 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

Table 6. Real sample application of RL using our developed test kit

Area	Mg^{2+} content by complexometric titration (ppm)	Subject	Our developed test kit		% Relative different
			Number of drops	Mg^{2+} content (ppm)	
U1	523.1±13.0	1 2 3	11 12 11	550 600 550	+5.14 +14.70 +5.14
U2	465.1±6.9	1 2 3	10 9 10	500 450 500	+7.50 -3.25 +7.50
K1	518.0±11.4	1 2 3	11 11 11	550 550 550	+6.18 +6.18 +6.18
K2	491.1±2.8	1 2 3	10 10 10	500 500 500	+1.81 +1.81 +1.81
K3	558.4±6.4	1 2 3	10 10 10	500 500 500	-10.46 -10.46 -10.46

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%

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 uptake (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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Appendix F

ภาควิชาเคมี
เลขรับที่ 1114
วันที่ 9 ธ.ค. 2559
เวลา 16.13 น.

คณะวิทยาศาสตร์
เลขรับ 4462
วันที่ 8 ธ.ค. 2559
เวลา 10.53 น.

SCAN

บันทึกข้อความ

ส่วนราชการ โครงการอุทยานวิทยาศาสตร์ สำนักงานอธิการบดี โทร.๓๑๘๕

ที่ ศธ ๐๕๒๙.๑.๔.๓/ ๕๗๑๒ **วันที่** ๗ ธันวาคม ๒๕๕๙

เรื่อง แจ้งดำเนินการด้านทรัพย์สินทางปัญญา

เรียน คณบดีคณะวิทยาศาสตร์

ตามที่คณะวิทยาศาสตร์ มีความประสงค์ยื่นคำขอรับอนุสิทธิบัตร “ชุดทดสอบแมกนีเซียมภาคสนามในน้ำยางพารา” จำนวน ๑ คำขอ โดยผู้ประดิษฐ์คือ นายปุริม จารุจำรัสและคณะ นั้น

บัดนี้ โครงการอุทยานวิทยาศาสตร์ ซึ่งเป็นหน่วยประสานงาน ได้ดำเนินการยื่นคำขอรับอนุสิทธิบัตรดังกล่าวต่อกรมทรัพย์สินทางปัญญา ผ่านสำนักงานพาณิชย์จังหวัดอุบลราชธานีเรียบร้อยแล้ว รายละเอียดปรากฏตามเอกสารแนบ

จึงเรียนมาเพื่อโปรดทราบ และแจ้งผู้เกี่ยวข้องทราบ

(ผู้ช่วยศาสตราจารย์ชวลิต ถิ่นวงศ์พิทักษ์)
ผู้อำนวยการโครงการอุทยานวิทยาศาสตร์

[illegible]
แจ้งผู้เกี่ยวข้องทราบด้วยครับ

(ผศ.ดร. ประสงค์สุม บุญยอุปพัทธ์)
รองคณบดีฝ่ายวิจัย

เรียน ดร. ปุริม จารุจำรัส
(✓) เพื่อโปรดทราบ
( ) เพื่อโปรดแจ้งเวียนให้ทราบทั่วกัน
( ) เพื่อโปรดพิจารณา

๙ ธ.ค. 59

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: arporn.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 ....อาภรณ์ สมรักษ์....	ลงชื่อ ....
(นางสาวอาภรณ์ สมรักษ์)	(....นางสาวภัทราพร ตัณฑ์พูนเกียรติ....)
ตำแหน่ง เจ้าหน้าที่วิจัย	ตำแหน่ง ....นักวิชาการพาณิชย์ชำนาญการ....
สำนักงานจัดการสิทธิเทคโนโลยี โครงการอุทยานวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี	เจ้าหน้าที่ประจำสำนักงานพาณิชย์จังหวัดอุบลราชธานี ผู้รับคำขอ
วันที่ ...21... / ....10.... / ...59...	วันที่ ....21 ต.ค. 2559.... / ....

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 1603002133
วันยื่นคำขอ 21 ต.ค. 2559	
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)
ต่อ แบบ สป/สผ/อสป 001-ก หน้า 3
3.1 สัญชาติ
3.2 โทรศัพท์
3.3 โทรสาร
3.4 อีเมล์

ยกเว้นค่าธรรมเนียม
ตามกฎกระทรวง พ.ศ. ๒๕๔๗ ว่าด้วยอัตราค่าธรรมเนียมและการยกเว้นค่าธรรมเนียม
เกี่ยวกับสิทธิบัตร/อนุสิทธิบัตร และประกาศคณะกรรมการสิทธิบัตร

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

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

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

7.คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม

ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ในวันเดียวกับคำขอรับสิทธิบัตร เลขที่ วันยื่น เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ
☐ คำขอเดิมมีการประดิษฐ์หลายอย่าง ☐ ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ ☐ ขอเปลี่ยนแปลงประเภทของสิทธิ

หมายเหตุ ในกรณีที่ไม่อาจระบุรายละเอียดได้ครบถ้วน ให้จัดทำเป็นเอกสารแนบท้ายแบบพิมพ์นี้โดยระบุหมายเลขกำกับข้อและหัวข้อที่แสดงรายละเอียดเพิ่มเติมดังกล่าวด้วย