บทความวิจัย

เซนเซอร์กระดาษที่มีลักษณะคล้ายบาร์โค้ดสำหรับการตรวจวัดน้ำกระด้าง

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บทคัดย่อ

การวัดระดับความกระด้างของน้ำเป็นตัวแปรที่บ่งบอกถึงคุณภาพของน้ำ ซึ่งใช้การไทเทรตสารประกอบเชิงซ้อนเป็นวิธีมาตรฐาน อย่างไรก็ตามการใช้วิธีนี้อาจมีข้อจำกัดบางประการ เช่น การวิเคราะห์มีหลายขั้นตอน ใช้สารละลายปริมาณมาก (> 105 มิลลิลิตร) และต้องอาศัยผู้ที่มีทักษะในการทดลอง เพื่อลดข้อจำกัดดังกล่าว งานวิจัยนี้จึงนำเสนอเซนเซอร์กระดาษที่มีลักษณะคล้ายบาร์โค้ดที่ประดิษฐ์ขึ้นโดยเทคนิคการพิมพ์สกรีนด้วยไข ประกอบด้วยแถบการตรวจวัดรูปสี่เหลี่ยมผืนผ้า 4 ช่อง (แต่ละแถบมีขนาด 0.3 x 5.0 เซนติเมตร) และแถบสามเหลี่ยมด้านบนคือบริเวณหยดตัวอย่าง (มีขนาด 1.9 เซนติเมตร สำหรับความสูง x 2.9 เซนติเมตรสำหรับฐาน) จากนั้นหยดสารละลายผสมระหว่าง ไดโซเดียม เอทิลีนไดเอมีนเตตระอะซีติกแอซิด และอินดิเคเตอร์ อิริโอโครม แบลค ที (EDTA-EBT) ที่มีความเข้มข้นของ EDTA ที่แตกต่างกันในแต่ละแถบใน N-cyclohexyl amino-propanesulfonic acid (CAPs) บัฟเฟอร์ pH 10.5 ปริมาตร 40.00 ไมโครลิตร เคลือบลงบนบริเวณตรวจวัด เมื่อหยดมาตรฐานแคลเซียมคาร์บอเนตหรือสารละลายตัวอย่างที่มีแคลเซียมหรือแมกนีเซียมปริมาตร 120.00 ไมโครลิตรลงบนบริเวณดังกล่าว จากนั้นไม่กี่นาทีจะปรากฏแถบสีชมพูบนพื้นหลังสีน้ำเงินบนเซนเซอร์กระดาษ ซึ่งสามารถกำหนดรูปแบบการตรวจวัดมาตรฐานสำหรับการตรวจวัดความกระด้างของน้ำอยู่ที่ช่วง 25-200 มิลลิกรัมต่อลิตร (สามารถแยกความละเอียดได้ 25 มิลลิกรัมต่อลิตร) และสามารถอ่านค่าความเข้มข้นของระดับความกระด้างของน้ำเทียบต้นแบบแถบการตรวจวัดมาตรฐานได้ด้วยตาเปล่า ดังนั้นเซนเซอร์กระดาษที่มีลักษณะคล้ายบาร์โค้ดจึงสามารถใช้งานได้จริงสำหรับการวิเคราะห์เชิงกึ่งปริมาณ นอกจากนี้ระบบยังแสดงความทนต่อไอออนรบกวนต่าง ๆ ในตัวอย่างน้ำและการประยุกต์ใช้กับตัวอย่างจริงให้ผลการวิเคราะห์ที่ไม่แตกต่างกับผลการวิเคราะห์ที่ได้จากการไทเตรตเชิงซ้อนแบบเดิม

คำสำคัญ: เซนเซอร์กระดาษที่มีลักษณะคล้ายบาร์โค้ด แคลเซียมและแมกนีเซียมไอออน การไทเทรตเชิงซ้อน ความกระด้างของน้ำ

อ้างอิงบทความนี้

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Research Article

Barcode-like paper sensor for water hardness detection

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Abstract

The measurement of water hardness has become a routine practice for water quality control where complexometric titration is used as a standard method. The use of this method, nevertheless has some disadvantages in terms of multiple steps of analysis, large reagent consumption (>105 mL) and the requirement for technical skills. Therefore, the aim of this work is to develop a barcode-like paper sensor which is a user-friendly means for water hardness detection that can overcome the aforementioned shortcomings. For this purpose, this work presents the fabrication of a barcode-like paper sensor and its practical application in the detection of total water hardness. The paper sensor was fabricated using the wax screen-printing technique to generate a barcode-like pattern design. The sensor consists of a detection zone with four bar channels (each measuring 0.3 by 5.0 cm) and a sample zone with a triangular shape of the (1.9 cm height by 2.9 cm base). The sample zone takes a few drops of reagent and the analytical technique is based on the complexometric reaction. Particularly, 40.00 µL mixed EDTA-EBT solution in N-cyclohexyl-aminopropanesulfonic acid (CAPs) buffer pH 10.5 was pre-applied to the detection zone. The sample zone, on the other hand, takes 120.00 µL of a standard solution containing $CaCO_3$ or water sample containing Ca^{2+} & Mg^{2+}. Shortly after adding the sample, a pink banding pattern would be generated on the blue background of the paper sensor. A banding pattern can uniquely identify the concentration of $CaCO_3$ in the range of 25-200 mg L^{-1} with a step size of 25 mg L^{-1}. The proposed barcode-like paper sensor can be practically used for semi-quantitative by naked eye detection. Additionally, the system exhibited high tolerance towards various interfering ions in water samples. The results of application with real samples obtained from our method agreed with those obtained from the conventional complexometric titration.

Keywords: Barcode-like paper sensor, Ca^{2+} and Mg^{2+}, Complexometric titration, Water hardness

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Introduction

Water is very important for food preparation, hygiene, sanitation purposes and a wide range of other uses. Therefore, controlling the water quality is of utmost importance. A common means of assessing the quality of water is to detect the level of water hardness. To do so, one may resort to detecting the level of dissolved compounds including calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, the key factors causing the temporary and permanent hardness of water. Temporary and permanent hardness together combine to result in total hardness. The latter is widely used as a standard measure for this purpose. By this basis, water can be categorized as soft, hard or very hard. Total water hardness is most commonly expressed as milligram of calcium carbonate ($CaCO_3$) equivalent per liter (mg L^{-1}). The standard quality of drinking water in sealed containers and that of groundwater for consumption are set not to exceed 100 and 300 – 500 mg L^{-1} as $CaCO_3$ of total hardness, respectively (Betz & Noll, 1950).

As for the method of determining total hardness in water samples, complexometric titration has been widely used. Nonetheless, this method still has many disadvantages including multiple steps for analysis, requirement of operational skills, need of a glassware and a high volume of reagents (>105.0 mL). To overcome these limitations, a simple method requiring inexpensive laboratory facilities, less time consumption and better user-friendliness is highly desirable (Jarujamrus et al., 2018; Jarujamrus et al., 2019; Malahom et al., 2017). For this purpose, microfluidic paper-based analytical device (μPADs) can be a powerful analytical platform due to their low cost, portability, rapid fabrication, low consumption of reagents and samples, ease-of-use, and disposability (Martinez et al., 2007). Most importantly, this platform would allow simple visualization approach where only the naked eye is required.

The fabrication of μPADs is relatively simple, since it only involves the creation of two regions on a paper hydrophilic and hydrophobic regions (Martinez et al., 2007). In general, the technique of wax-screen printing has been the standard choice for μPADs fabrication owing to the advantages including high speed, simple operation, versatility, and cost efficiency. Specifically, a large number of μPADs (>100 units) can be fabricated via the solvent free technique of wax-screen printing in a single batch (Dungchai et al., 2011; Namwong et al., 2018; Wang et al., 2012). Nonetheless, the use of microfluidic paper-based analytical devices for water hardness detection has not been fully taken advantage of. In fact, to the best of our knowledge, there has been only one report on the use of microfluidic paper-based analytical devices for the determination of Ca^{2+} and Mg^{2+} ions (Karita & Kaneta, 2016).

In order to exploit the advantages of the microfluidic paper-based system, this work employs a simple and inexpensive wax screen-printing technique for the fabrication of μPAD as barcode-like paper sensor for water hardness detection. The μPAD device developed in this work is an extension of our recent report (Jarujamrus et al., 2019). The fabricated barcode-like paper sensor in this work was optimized to feature well-defined hydrophobic barriers to allow a consistent flow of samples through the detection zone. The device was subsequently applied for the determination of total water hardness based on complexometric reaction. Particularly, the developed device performed successfully in the semi-quantitative detection of total water hardness in real samples with high tolerance towards various interfering ions. Our barcode-like paper sensor also showed satisfactory results in the method validation.

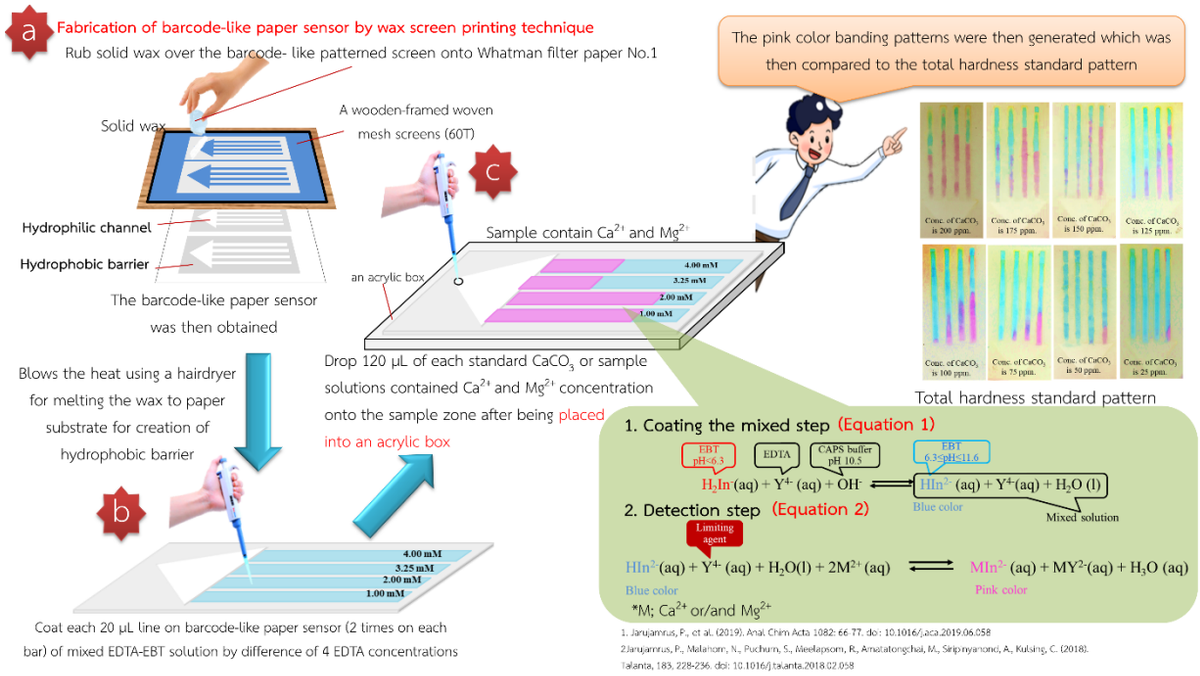

Figure 1. Scheme showing the fabrication and application of the sensor: (a) Fabrication of barcode-like paper sensor by wax screen printing technique; (b) Coating a mixed EDTA-EBT solution having four different EDTA concentrations on each line of barcode-like paper sensor; (c) Determination of total water hardness based on the standard patterns.

Experimental section

Materials and equipment

All solutions were prepared in deionized water with 18 MΩ resistance obtained from a Millipore Milli-Q purification system (Bedford, MA, USA). All of chemicals were of analytical grade. For the fabrication of the barcode-like paper sensor, Whatman No.1 (thickness 180 mm and pore size 11 mm) filter paper was purchased from Whatman International Ltd. (Maidstone, England). The solid paraffin wax (mp 53–57 °C) was purchased from a local supermarket (Ubon Ratchathani, Thailand). A hair dryer (Lesasha: tourmaline ionic shine model) that can be operated at a constant temperature was used to melt the wax. The ready-to-use wooden-framed woven mesh screens (888.32 meshes, 60T) and a squeegee were obtained from a local screen-printing shop or clothing shop in Ubon Ratchathani, Thailand. The total cost of fabricating templates for the patterned screens was 350.00 TH฿ or about 10.80 US$ per 623 cm^2 (21.0 cm × 29.7 cm). An acrylic box made from polyacrylic acid was also obtained from a local screen-printing shop or clothing shop in Ubon Ratchathani, Thailand. All images in this work were recorded using a Huawei camera phone; model nova 2i (Huawei technology company, Beijing, China). Ammonium hydroxide (NH_4OH) and calcium carbonate ($CaCO_3$) were obtained from Fluka, Switzerland. Potassium permanganate ($KMnO_4$) was obtained from Ajax Finechem, Australia. Magnesium sulfate anhydrous ($MgSO_4$) was obtained from Loba Chemie, India. Eriochrome Black T (EBT, $C_{20}H_{12}N_3O_7SNa$) was obtained from Labconco, USA. 3-(Cyclohexylamino)-1-propanesulfonic acid ($C_9H_{19}NO_3S$) or CAPS buffer and ethylenediaminetetraacetic acid disodium salt (EDTANa_2, $C_{10}H_{14}N_2Na_2O_8$) were purchased from Panreac, USA. The following chemicals were purchased from Carlo Erba, Italy and used as received for the interference study: potassium sulfate (K_2SO_4), iron(II) sulfate ($FeSO_4$), zinc sulfate ($ZnSO_4$), copper(II) sulfate pentahydrate ($CuSO_4•5H_2O$), calcium nitrate ($Ca(NO_3)_2$), manganese sulfate monohydrate ($MnSO_4•H_2O$), sodium chloride (NaCl), sodium nitrate ($NaNO_3$), methanol (CH_3OH) and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4•2H_2O$).

Fabrication of barcode-like paper sensor by screen printing using solid wax as hydrophobic material

The barcode-like paper sensor was designed and fabricated using a wax screen-printing method with a slight modification from our previous reports (Jarujamrus et al., 2019; Namwong et al., 2018). The fabrication process is shown in Figure 1a. The screen patterns were generated using Microsoft PowerPoint. To create a hydrophobic barrier, the patterned screen was placed onto a paper and rubbed over with a solid wax. The screened paper was then blown with a hair dryer (screened paper up) for ca. 15 s to melt the wax and create the hydrophobic barriers on the filter paper.

Use of barcode-like paper sensor for $CaCO_3$ detection

The barcode-like paper sensor was designed to incorporate a fork-shaped detection zone as described in our previous work (Jarujamrus et al., 2019). Specifically, the sensor contains a triangle-shaped sample zone and four detection lines as shown in Figure 1a. The design was constructed as such to allow simultaneous flow of the sample from the sample zone to the detection zone. The determination of $CaCO_3$ in standard solutions was based on complexometric reaction (Yappert & DuPre, 1997), where the concentration of $CaCO_3$ indicated by the concentration of EDTA as the limiting agent for the generation of the banding patterns. The total hardness (Ca^{2+} and Mg^{2+}) can then be determined accordingly.

To fabricate the sensor for the detection zone, a mixed solution of EDTA and EBT was prepared at the desired concentration and pH 10.5 with the use of CAPS buffer. Specifically, the 0.02 mol L^{-1} CAPS buffer solution was prepared by dissolving CAPS (0.442 g) in a mixed solution between 50.00 mL of deionized water and 10.00 mL of methanol. Then, the solution was adjusted to pH 10.5 using 1.00 mol L^{-1} NaOH and the total volume was adjusted to 100.00 mL with deionized water. An indicator (2.16×10^{-4} mol L^{-1} of EBT) was prepared by dissolving EBT (10.00 mg) in 10.00 mL of 75% v/v ethanol. EDTA solutions with concentrations in the range of 0.25 – 4.00 mmol L^{-1} were prepared by dissolving solid EDTA in 25.00 mL of 0.02 mol L^{-1} CAPS buffer at pH 10.5. Additionally, 1.00 mL of 2.16×10^{-1} mol L^{-1} EBT was added where the pH was adjusted by 1.00 mol L^{-1} NaOH and the final volume was adjusted to 25.00 mL).

Finally, 20.00 µL of the mixed solution was coated onto the detection lines twice. The detection zone then turns into blue color before letting dry at room temperature (Figure 1b). For the detection step, 120.00 µL of either standard $CaCO_3$ or a sample containing $CaCO_3$ was dropped onto the sample zone. Pink banding patterns would subsequently be appeared on the blue background of the detection zone (Figure 1c). For the standard $CaCO_3$, 17 different concentrations were used, ranging from 0 to 400 mg L^{-1} with a 25 mg L^{-1} increment.

Selectivity of the developed sensor

The selectivity towards total hardness determination (Ca^{2+} and Mg^{2+}) was also investigated. The potential interference ions under study include iron (Fe^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), chloride (Cl^-), sulfate (SO_4^{2-}) and nitrate (NO_3^-) with concentrations ranging from 25-1000 mg L^{-1}. Each type of foreign ion was separately added to the 125 mg L^{-1} $CaCO_3$ solution. The corresponding tolerance concentration (mg L^{-1}) was identified as the highest concentration that could be added while maintaining all banding patterns within 10% standard deviation (S.D.) of those generated from the 125 mg L^{-1} standard $CaCO_3$ solution.

Real samples application and method validation

For the application of real samples, water samples including mineral drinking water and tap water were spiked with 100 mg L^{-1} of $CaCO_3$. Then, 120.00 µL of un-spiked and spiked water samples were tested on the barcode-like paper sensor (Figure 1b, c). As for method validation, complexometric titration was used. Complexometric titration was performed with slight modification according to a previously reported standard method (Yappert et al., 1997). Briefly, 0.01 mg of each $CaCO_3$ containing sample was transferred into an

Erlenmeyer flask. In order to control the pH of the solution within the range of 10.0 -10.5, 10.00 mL deionized water and 2.00 mL CAPS buffer at 0.01 mol L^{-1} were also added. Next, 5 drops of 2.16×10^{-4} mol L^{-1} EBT indicator were added. The resulting solution was then titrated with 0.02 mol L^{-1} EDTA standard solution until the color of the solution changed from red to pure blue. The standard solution was standardized with 0.02 mol L^{-1} $CaCO_3$ where 0.01 g of which was dissolved in deionized water.

Results and discussion

Optimization and mechanism on paper strip sensor for total hardness determination

For the optimization and mechanism study of the barcode-like paper sensor for total hardness determination, standard $CaCO_3$ solutions were used to represent the total hardness determination (Ca^{2+} and Mg^{2+}). First, Whatman No.1 filter paper was cut into a strip shape (0.2×5.0 cm). Then, each strip was soaked in a mixed EDTA-EBT solution containing different EDTA concentrations ranging from 0.25 - 4.00 mmol L^{-1} with an increment of 0.25 mmol L^{-1} in CAPS buffer pH 10.5. The presence of different EBT species at different pH values would turn color on the strip into blue color within 5 min as described in Equation 1 (Figure 1b). Subsequently, 15 µL of each standard $CaCO_3$ solution (25-400 mg L^{-1} with a step size of 25 mg L^{-1}) was dropped onto one end of the strip. In this step, the complexation between Ca^{2+} and EDTA (limiting agent) was firstly formed. The formation was induced by the differences in the effective formation constants (K_f) of Ca-EDTA and Mg-EDTA complexes (5.0×10^{10} and 4.9×10^{8}, respectively) as compared to those of Ca-EBT and Mg-EBT complexes (4.4×10^{3} and 4.9×10^{5}, respectively) (Lindstrom & Diehl, 1960). The excess amount of Ca^{2+} and Mg^{2+} can then bind with EBT. Consequently, pink color would appear on the blue background in the detection zone based on Equation 2 (Figure 1b). It is important to note that the distance of pink color on blue color depends on the concentration of standard $CaCO_3$ or total hardness samples.

Overall, the aforementioned phenomenon indicates that the optimum concentration of EDTA and the pH in the system are the key factors affecting the performance of the developed barcode-like paper sensor. Especially, the large magnitude of the K_f values of Ca-EDTA and Mg-EDTA indicates that the above-mentioned reactions can go to completion if EDTA is present in its completely deprotonated form (Y^{4-}) (Yappert et al., 1997). Correspondingly, to ensure that EDTA is in the Y^{4-} form, pH values should be greater than 12. Nonetheless, the precipitation of Ca^{2+} as $CaCO_3$ and of Mg^{2+} as $Mg(OH)_2$ prevented the reaction from proceeding at such high pH values. To overcome this obstacle, the complexation was carried out at pH 10.5 at which EDTA is still present in its Y^{4-} form. In light of this context, the optimum 4 concentrations of EDTA (including 1.00, 2.00, 3.25 and 4.00 mmol L^{-1}) in the mixed EDTA-EBT solution were chosen since they can generate distinguishable banding patterns over the range of $CaCO_3$ concentration of 25-200 mg L^{-1} with a step size of 25 mg L^{-1} (Figure 2).

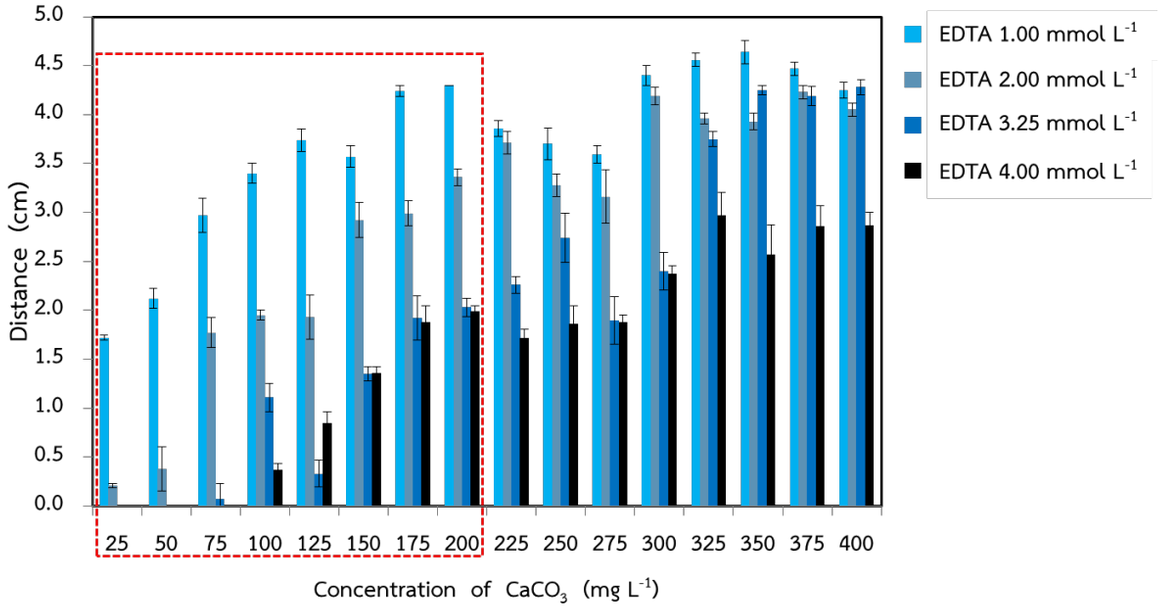

Figure 2. Banding patterns on detection zone of the sensor representing different concentrations of $CaCO_3$.

Total hardness determination (Ca^{2+} and Mg^{2+}) on barcode-like paper sensor

In order to develop a practical sensor for total hardness determination, the fork-shaped barcode-like paper sensor as discussed in our previous work (Jarujamrus et al., 2019). The key components of the paper sensor consist of the sample zone and the detection zone. On the triangle-shaped sample zone, a liquid sample can be dropped through a specified hole on an acrylic box (Figure 1b, c). The fork-shaped detection zone includes four detection lines (Figure 1a). This design facilitates the sample flow since a design with smaller base-to-height ratio could result in inferior flow characteristics of the sample. The paper sensor employs the use of Whatman No.1 paper and the four different EDTA concentrations (1.00, 2.00, 3.25 and 4.00 mmol L^{-1}) in the mixed EDTA-EBT solution. Distinguishable banding patterns over a range of concentrations of $CaCO_3$ of 25-200 mg L^{-1} can be generated. The level of total hardness (based on Ca^{2+} and Mg^{2+} concentrations) can be detected with the step size of 25 mg L^{-1} which corresponds to one of eight different banding patterns (Figure 3a, b). It is to be noted that the use of a smaller step size may improve the precision but will deteriorate the distinguishability of the banding patterns and ultimately compromise the practicality of the analysis. Therefore, the step of 25 mg L^{-1} was chosen for the purpose of semi-quantitative analysis in this work where the limit of quantification (LOQ) of 25 mg L^{-1}.

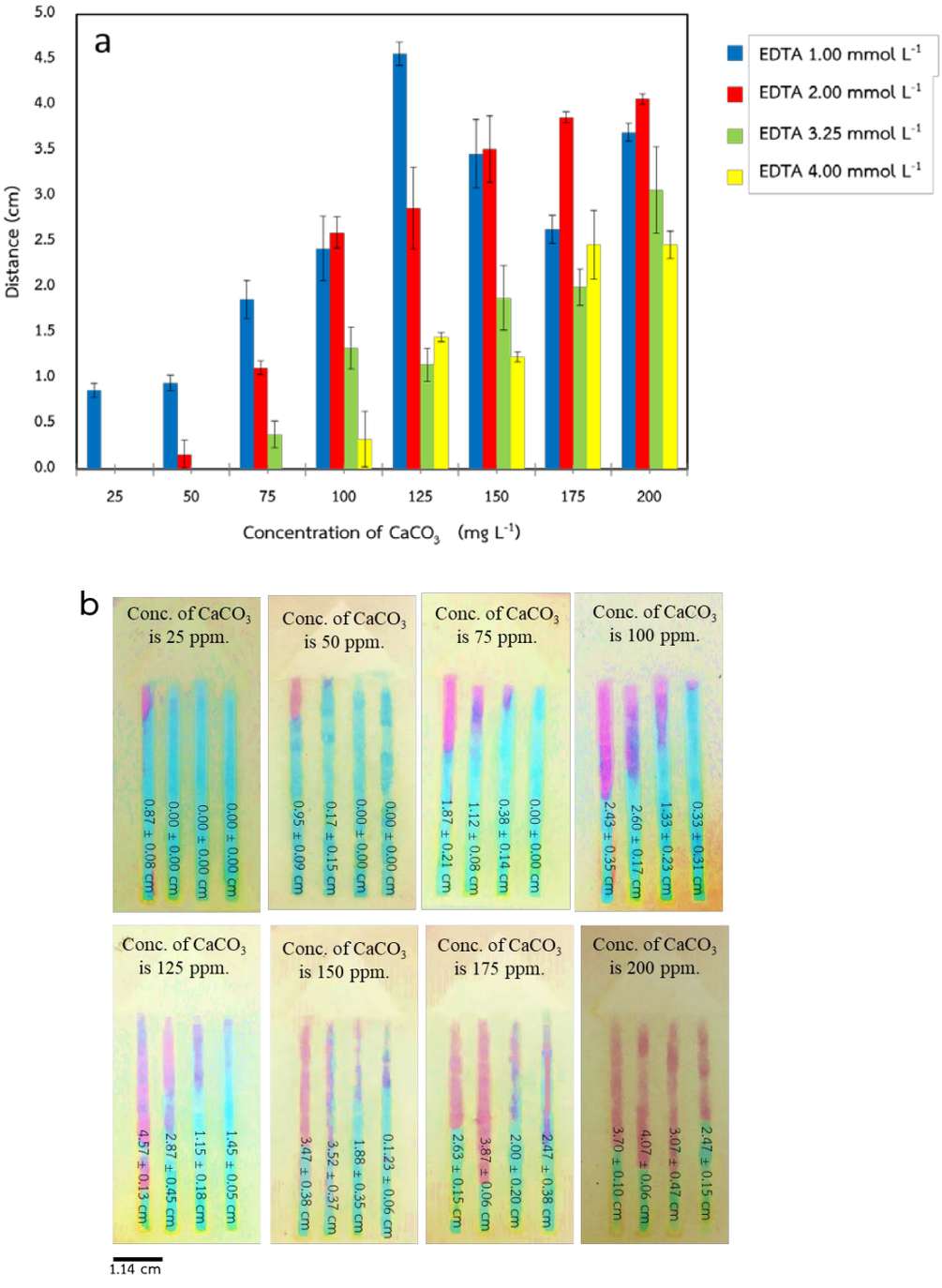

Figure 3. Standard banding patterns representing different concentrations of $CaCO_3$ on the barcode-like paper sensor (a) and the corresponding actual appearances on the detection zone (b).

Interference of foreign ions

The effect of seven different foreign ions on the banding patterns of the barcode-like paper sensor was investigated. Each ion was separately added to 125 mg L^{-1} $CaCO_3$ solution. The corresponding tolerance concentration (mg L^{-1}) was identified as the highest concentration that could be added while all banding patterns were maintained within one standard deviation of the pattern of the 125 mg L^{-1} standard $CaCO_3$ solution without interference. The results are reported in Table1. It was found that Cl^- and Na^+ were the two species with the highest tolerance concentrations of 1000 and 500 mg L^{-1}, respectively. On the other hand, Fe^{2+}, Mn^{2+}, Zn^{2+} and SO_4^{2-} and NO_3^{2-} species had the tolerance concentrations of 50, 25, 50, 100 and 75 mg L^{-1}, respectively. The lower tolerance concentrations of the latter ions may be attributed to the behavior of

these ions being relatively more similar to Ca^{2+} and Mg^{2+} with respect to the tendency to form a complex with EDTA. In any case, the concentrations of several foreign ions in real water samples are likely to be much lower than their corresponding tolerance concentrations such as the case of Mn^{2+} (Jarujamrus et al., 2018). This result suggests the high selectivity of the developed barcode-like paper sensor towards $CaCO_3$ solution.

Table 1. Effect of foreign ion interference

Foreign ions	Compounds containing foreign ions	Tolerance concentrations (mg L^{-1})	Deviation of distance (cm) of pink bars on the detection lines with respect to those representing standard 125 mg L^{-1} $CaCO_3$*			
			1.00 mmol L^{-1} EDTA	2.00 mmol L^{-1} EDTA	3.25 mmol L^{-1} EDTA	4.00 mmol L^{-1} EDTA
Na^{+}	Na_2SO_4	500	+ 0.21	+ 0.37	+ 0.49	- 0.07
Fe^{2+}	$FeSO_4.7H_2O$	50	+ 0.05	- 0.57	- 0.54	- 0.23
Mn^{2+}	$MnSO_4$	25	- 0.23	- 0.28	- 0.26	- 0.15
Zn^{2+}	$ZnSO_4$	50	+ 0.07	- 0.08	- 0.13	- 0.18
Cl^{-}	NaCl	1000	- 0.18	- 0.13	+ 0.18	- 0.25
SO_4^{2-}	K_2SO_4	100	+ 0.32	+ 0.15	+ 0.23	+ 0.17
NO_3^{2-}	$Ca(NO_3)_2$	75	+ 0.22	- 0.22	+ 0.33	+ 0.11

*Distances of pink bars representing standard 125 mg L^{-1} $CaCO_3$ ion on the 1.00, 2.00, 3.25, and 4.00 mmol L^{-1} EDTA detection lines were 4.57 ± 0.10, 2.87 ± 0.45, 1.15 ± 0.18 and 1.45 ± 0.05 cm, respectively (n = 3).

Real sample application and method validation

The use of barcode-like paper sensor for water hardness detection was validated by complexometric titration technique, a conventional technique widely used for similar purposes. The validation applied spiked and un-spiked samples of mineral water and tap water, and the results are reported in Table 2.

Table 2. Application of the sensor to the real samples and method validation (n=3).

Sample	Concentration of standard $CaCO_3$ (mg L^{-1})	Complexometric titration		Proposed method	
		Found ± SD (mg L^{-1})	%Recovery	Found (mg L^{-1})	%Recovery
Mineral drinking water	0	171.57 ± 0.04	-	150	-
	100	263.71 ± 0.03	92.14	250	100
Tap water	0	19.06 ± 0.04	-	25	-
	100	114.38 ± 0.05	95.32	125	100

Overall, water hardness detection by our barcode-like paper sensor largely agreed with the standard method. Moreover, the barcode-like paper sensor exhibited superior performance as compared to the complexometric titration in several ways, including smaller volume requirement of reagents and samples, user-friendly for unskilled operators, and low-cost of fabrication (Table 3).

Table 3. Analytical performances comparison between the classical method and the proposed method

Comparison parameters	**Traditional complexometric titration (Classical method)[1]**	**Barcode-like paper sensor based on complexometric reaction (Proposed method)**
Operator	Expertise required	Expertise not required
Principle of detection	The concentration of a water hardness is directly calculated from the volume of EDTA required to reach the end point as judged by the naked eye using an indicator	The distance of color change observed by the naked eye on the barcode-like paper sensor based on the complexometric reaction is compared to established water hardness standard pattern
Volume of sample and reagent solutions	In the orders of magnitude of mL to L	**Preparation device step**: the mixed solution between EDTA and EBT in CAPS buffer pH 10.5 (40 µL) onto 4 detection channels (total volume 160 µL) **Detection step:** 120 µL of sample solution
On site analysis	Yes; but more complicated in terms of equipment set up	Yes; device is disposable
Analysis time	Relatively long depending on the technical skill of operators	~2 mins
Accuracy & precision	Good accuracy and precision but require the technical skill of operator (error of quantification by the estimation of the volume of titrant for end point evaluation can be minimized)	Analysis in real samples (recovery in the range of 92.14 - 100% for accuracy)
Working range	0.001M- 1M	25-200 mg L^{-1} (with step of 25 mg L^{-1})
Shortcomings	Requires a substantial amount of glassware during the titration, relatively large volumes of solutions, considerable technical skills, and comparably long analysis time.	Only suitable for semi-quantitative analysis. Additionally, the stability of the device is needed to be further investigate

Conclusion

A low-cost, simple, and rapid barcode-like paper sensor was fabricated using a wax screen-printing method and successfully applied for the determination of total water hardness. The simple fabrication procedures involved coating different detection lines with different concentrations of EDTA solution. The barcode-like paper sensor can generate visibly different banding patterns after being exposed to different concentrations of $CaCO_3$ solution (25 -200 mg L^{-1} with a step size of 25 mg L^{-1}). Each pattern could be uniquely assigned to each $CaCO_3$ concentration within the range. By requiring only a small sample amount (280 µL) and not requiring skilled operators, the paper sensor can be practically used for semi-quantitative analysis of the level of total hardness of water (Ca^{2+} and Mg^{2+}, which was reported as mg L^{-1} $CaCO_3$). The detection using the paper sensor was validated by complexometric titration and exhibited high tolerance towards various interference ions. Ultimately, the developed sensor serves as a pioneering model for future developments of other analytes that offer a broad range of practical applications.

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References

Betz, J., and Noll, C. (1950). Total-hardness determination by direct colorimetric titration. **Journal-American Water Works Association**, 42(1), 49-56.

Dungchai, W., Chailapakul, O., and Henry, C. S. (2011). A low-cost, simple, and rapid fabrication method for paper-based microfluidics using wax screen-printing. **Analyst**, 136(1), 77-82.

Jarujamrus, P., Malahom, N., Puchum, S., Meelapsom, R., Amatatongchai, M., Siripinyanond, A., Chairam, S., and Kulsing, C. (2018). Complexometric and argentometric titrations using thread-based analytical devices. **Talanta**, 183, 228-236.

Jarujamrus, P., Meelapsom, R., Naksen, P., Ditcharoen, N., Anutrasakda, W., Siripinyanond, A., Amatatongchai, M., and Supasorn, S. (2019). Screen-printed microfluidic paper-based analytical device (muPAD) as a barcode sensor for magnesium detection using rubber latex waste as a novel hydrophobic reagent. **Analytica Chimica Acta**, 1082, 66-77.

Karita, S., and Kaneta, T. (2016). Chelate titrations of Ca(2+) and Mg(2+) using microfluidic paper-based analytical devices. **Analytica Chimica Acta**, 924, 60-67.

Lindstrom, F., and Diehl, H. (1960). Indicator for the titration of calcium plus magnesium with (ethylenedinitrilo) tetraacetate. **Analytical Chemistry**, 32(9), 1123-1127.

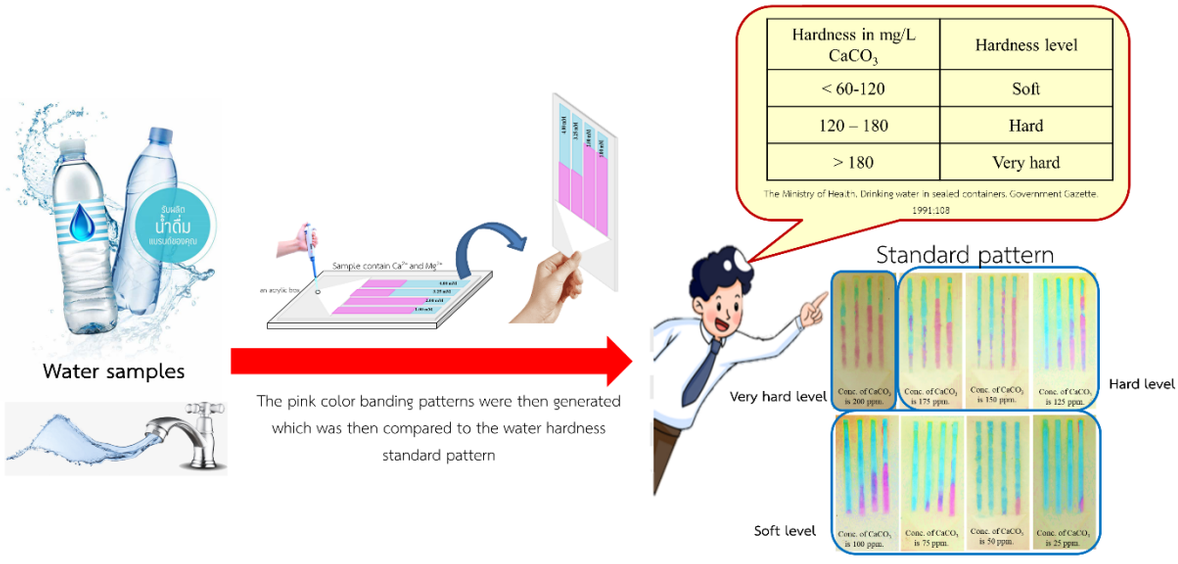
Malahom, N., Jarujamrus, P., Meelapsom, R., Siripinyanond, A., Amatatongchai, M., and Chairam, S. (2017). Simple test kit based on colorimetry for quantification of magnesium content in natural rubber latex by miniaturized complexometric titration without using masking agent. **Polymer Testing**, 59,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

Martinez, A. W., Phillips, S. T., Butte, M. J., and Whitesides, G. M. (2007). Patterned paper as a platform for inexpensive, low-volume, portable bioassays. **Angewandte Chemie International Edition**, 46(8), 1318-1320.

Namwong, P., Jarujamrus, P., Amatatongchai, M., and Chairam, S. (2018). Fabricating Simple Wax Screen-Printing Paper-Based Analytical Devices To Demonstrate the Concept of Limiting Reagent in Acid–Base Reactions. **Journal of Chemical Education**, 95(2), 305-309.

Wang, S., Ge, L., Song, X., Yu, J., Ge, S., Huang, J., and Zeng, F. (2012). Paper-based chemiluminescence ELISA: lab-on-paper based on chitosan modified paper device and wax-screen-printing. **Biosens Bioelectron**, 31(1), 212-218.

Yappert, M. C., & DuPre, D. B. (1997). Complexometric titrations: competition of complexing agents in the determination of water hardness with EDTA. **Journal of Chemical Education**, 74(12), 1422.



"Barcode-like paper sensor for water hardness detection"

- Graphical abstract –