

An Indirect Colorimetric Method for Potassium Determination in Soil Using a Paper Device and Smartphone

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ABSTRACT. This study presents a simple method for determination of potassium in microliter scale using a paper device together with a smartphone. The method begins with an ion-pair extraction of dibenzo-18-crown-6- K^+ complex into dichloromethane with an excess amount of calmagite. The aqueous phase containing the remaining calmagite is then transferred to the paper device, where a smartphone is used to capture its color and convert it to RGB value. The linear detection range was found to cover potassium concentrations from 20 $mg L^{-1}$ to 120 $mg L^{-1}$. The detection and quantification limits are 5.41 $mg L^{-1}$ and 18.03 $mg L^{-1}$, respectively. Potassium detection was carried out in a variety of actual soil samples, and the results were validated against spectrophotometric results using a paired t-test, which indicated high accuracy. The proposed method is simple, fast, and inexpensive, and it requires no complicated equipment, making it ideally suited for the detection of potassium in soil.

Keywords: Colorimetric method, paper device, potassium, smartphone, soil

INTRODUCTION

Potassium (K) is an essential soil nutrient for plants that plays a vital role in plant physiological and biochemical processes including photosynthesis, cell growth, metabolism, assimilation rate, and accumulation of sugars (Sattar et al., 2019; Wang et al., 2013). Potassium deficient plants are more susceptible to drought, frost, high salt content, stunting, spotting, leaf curl, reduced growth, and productivity shortfalls (Hafsi et al., 2014; Zhang et al., 2020). Supplying plants with sufficient quantities of nutrients is critical for increasing agricultural output. Effective fertilization of agricultural crops requires knowledge of existing nutrient concentrations in the soil prior to supplementation with fertilizers to supply what is needed while also avoiding unnecessary waste. Since potassium, along with nitrogen and phosphorus, is one of the most commonly supplemented nutrients, a convenient selective test for potassium is an important analytical tool for monitoring soil and thereby optimizing the use of fertilizer.

A variety of analytical methods have been employed to measure potassium levels. These include atomic absorption spectroscopy (AAS) (Ieggli et al., 2010), inductively coupled plasma mass spectroscopy (ICP-MS) (Paulauskas et al., 2020; Yan et al., 2017), ion chromatography (Caland et al., 2012), electrophoresis (Huang et al., 2018), and electrochemical methods

(Sedenho et al., 2013). Although these methods provide high sensitivity and resolution, they require expensive equipment, large amounts of chemicals, and a trained technician. They are also impractical for on-site detection. Another method that has been used extensively is colorimetric detection of potassium analytes. For example, Naderi et al. (2018) developed a method for colorimetric detection of potassium in urine that uses gold nanoparticles (AuNPs) and a cationic dye in an aptasensor system. The analytical linear range of the aptasensor was from 10 nM to 50 mM, and the detection limit was 4.4 nM. Qiu et al. (2019) developed a method using 4-aminobenzo-18-crown-6 modified AuNPs for potassium detection in urine samples with the linear range of 0-200 μM . Chen et al. (2013) developed a colorimetric detection method for potassium ions in urine using aptamer-modified AuNPs and an aptamer. The linear range of the colorimetric aptasensor was from 5 nM to 1 μM , and the detection limit was 5 nM. Although these methods provide sensitive detection of very low levels of potassium ions in biological samples for disease diagnosis, they would not be well suited for practical determination of higher potassium levels in soil.

In recent years, results of colorimetric analyses have been accurately and precisely digitalized by determining the RGB values of an image of the resulting color (Fu and Wang, 2018; Henares et al.,

2017; Yamada et al., 2017). Images captured with high-resolution smart phone cameras can be examined with color analysis software installed on the phone to measure color intensity, and smartphones have been widely used in this way in various on-site measurement applications (Kap et al., 2021; Shrivastava et al., 2020; Wang et al., 2020; Xing et al., 2020). In search of a simple, low cost, and portable colorimetric method for potassium detection, a paper-based device used with a smartphone is a prominent alternative method for determination of potassium in soil.

The aim of this study is the development of a microliter operation for the determination of potassium in soil. This indirect determination of potassium involves two stages where potassium is first extracted into dichloromethane with dibenzo-18-crown-6 and an excess amount of calmagite as counter ion. Then the remaining calmagite is colorimetrically determined using a paper device and smartphone, with no need for any expensive equipment. The net decrease in color intensity of the calmagite on the paper device is proportional to the amount of potassium present in the original soil sample, because the missing calmagite was lost by binding with the original potassium that is now in the crown-potassium-calmagite complex. It is noteworthy that the extracted potassium is not measured directly on the paper device because crown-potassium-calmagite complex does not show a color change response in accordance with potassium concentration lower than 120 mg L^{-1} . The proposed method requires only small amounts of chemicals, and it is simple and quick making it suitable for on-site quantification of potassium in soil.

EXPERIMENTAL SECTION

Reagents and Chemicals

All chemicals used in this study were analytical grade. Deionized water (DI water) was used in the preparation of all solutions in the experiments. Potassium chloride was purchased from Ajax Finechem (Australia). Dibenzo-18-crown-6 was purchased from Sigma-Aldrich (USA). Calmagite was purchased from Carlo Erba (Italy). Lithium hydroxide and ethylenediaminetetraacetic acid (EDTA) were purchased from Loba Chemie (India). Whatman No.1 and No.5 qualitative filter paper were purchased from Whatman International, Ltd. (UK).

Apparatus

The stencil screen, made with polyester fabric on a wooden frame (35 cm x 45 cm) was custom ordered from a local screen-printing shop in Phitsanulok, Thailand. An Apple iPhone 7 plus with RGB color value application was used for colorimetric analysis. A photo light box was made with wood and LED lights (15x15x15 cm; width x length x height). A synergy H1 hybrid multi-mode microplate reader (BioTex, USA) was used for the spectrophotometric method.

Preparation of the Paper Device

The pattern for applying the epoxy resin was initially designed using Microsoft Word software, and a stencil screen was then created from this pattern by a local screen-printing shop. To apply the epoxy resin, the filter paper is placed under the stencil screen, and then 20 mL of epoxy resin solution (2:1 v/v of epoxy resin in curing agent) is poured onto the screen. A squeegee is used to distribute the solution across the screen and gently push the solution through. The epoxy resin needs to penetrate to the bottom of the filter paper. The papers thus printed are then left to dry at room temperature. After drying, the paper device is ready to use. The appearance is shown in **Figure 1**.

Procedure for Indirect Colorimetric Determination of Potassium

For the ion-pair extraction of potassium, 300 μL of potassium solution, 300 μL of pH 3.5 buffer, and 300 μL of 0.8% w/v dibenzo-18-crown-6 in dichloromethane are placed in a microcentrifuge tube and shaken for 10 seconds. After that, 100 μL of 0.04% w/v calmagite is added. The solutions were shaken for 10 seconds, and then the aqueous phase is allowed to separate from the organic layer for 5 minutes. For spectrophotometric detection, the upper (aqueous) phase was carefully drawn off using a micropipette and transferred to a well plate to be read in a microplate spectrophotometer at 530 nm. This spectrophotometry procedure is based on Madrakian et al (2016). For colorimetric detection using paper device and smartphone, 5 μL of the upper phase is carefully drawn off using a micropipette and dropped onto the circular hydrophilic area of the paper device. The color is then photographed in a photo light box using a smartphone. The images are then imported into RGB color value software to measure color intensity. The G parameter was used as an analytical quantifier because it was linearly correlated with the potassium concentration. A decrease in the pink color intensity of the calmagite is proportional to the amount of potassium that was originally present. The procedure of the indirect colorimetric determination of potassium is summarized in **Figure 2**.

Application in Soil Samples

Five soil samples were collected from rice fields in five provinces of Thailand: Phitsanulok, Chiang Mai, Lampang, Kamphaeng Phet, and Khon Kaen. The steps for the soil extraction followed the methods of Jones (2001). All soil samples were dried and then sieved to a particle size of 2 mm. A 5.0 g sample of the soil was placed in a centrifuge tube. Deionized water was added for a final volume of 25 mL, and the tube was shaken for 15 min. The mixture was filtered through Whatman No.5 filter paper and the filtrate was used for the potassium analysis. The results using the paper device and smartphone were compared with the results obtained from spectrophotometry.

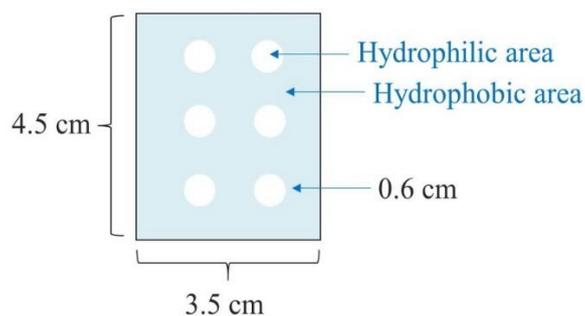


Figure 1. The finished paper device

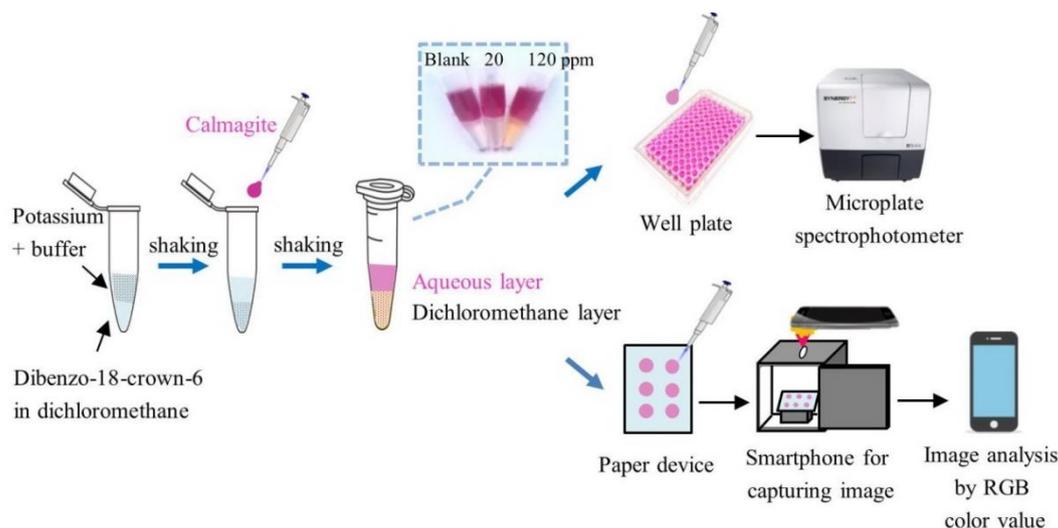


Figure 2. Procedure for indirect colorimetric method of potassium determination

RESULTS AND DISCUSSION

Optimization of Ion-pair Extraction Conditions

The ion-pair extraction of potassium ions uses dibenzo-18-crown-6 as the complexing agent and excess calmagite as the counter ion. It is well known that K^+ , which has a radius of 133 pm, fits very well into the cavity of 18-crown-6 (Frański et al., 2009; Gul et al., 2019). As a result, K^+ can be chelated well with dibenzo-18-crown-6. When an excess of calmagite, an anionic chromophoric reagent, is added, ion-pairs form between the positively charged dibenzo-18-crown-6- K^+ complex and calmagite anion through the electrostatic attraction and anion- π interactions (Frański et al., 2009). These ion pairs are then extracted into dichloromethane. The mechanism of extraction is shown in **Figure 3**.

The effects of three parameters on the ion-pair extraction process used in the indirect determination of potassium were investigated using spectrophotometric detection. The parameters were: pH, concentration of dibenzo-18-crown-6, and reaction time. Since the goal is to adjust the extraction process for most effective extraction of potassium in the form of crown-potassium-calmagite complex, all three parameters were optimized to obtain the lowest possible calmagite absorbance values (least amount of remaining calmagite) in the aqueous layer. The effect of pH was

studied in the range of 3-6. A graph plotting potassium concentration against absorbance of remaining calmagite was constructed to find the linearity and thereby determine the optimal pH. The obtained linear equations and correlation coefficients (R^2) are shown in **Table 1**. The highest linearity was observed at pH 3.5, so this was used as the optimal pH for the potassium extraction.

The effect of dibenzo-18-crown-6 concentration was studied from 0.025 to 1% w/v. **Figure 4a** shows how increasing dibenzo-18-crown-6 concentration caused decreasing absorbance, indicating less calmagite remaining in the aqueous phase, until the concentration was 0.8% w/v, at which point the absorbance bottomed out. The high crown ether concentration required here for efficient potassium extraction might be due to the relatively weak complex formed between dibenzo-18-crown-6 and potassium (Dadfarnia and Shamsipur, 1992). Based on these findings, 0.8% w/v dibenzo 18-crown-6 was selected as the optimal concentration. Furthermore, the reaction time was studied in the range of 5-60 minutes. The effects of varying reaction time are shown in **Figure 4b**. Absorbance from the calmagite remaining in the aqueous phase initially fell and then stabilized at about 5 minutes. The optimal reaction time was thus set at 5 min.

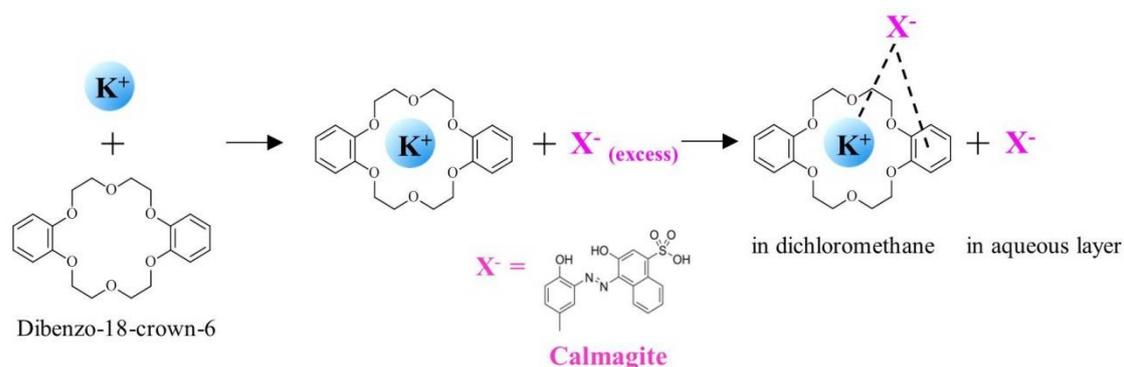


Figure 3. Schematic diagram to show the mechanism for ion-pair extraction of potassium

Table 1. The effect of pH on linearity of potassium determination

pH	Linear equation	Correlation coefficient (R^2)
3	$y = -0.0010x + 0.5889$	0.6926
3.5	$y = -0.0013x + 0.6186$	0.9935
4	$y = -0.0009x + 0.5994$	0.8161
4.5	$y = -0.0010x + 0.6278$	0.5357
5	$y = -0.0011x + 0.6506$	0.7567
5.5	$y = -0.0010x + 0.6718$	0.6863
6	$y = -0.0017x + 0.7059$	0.9771

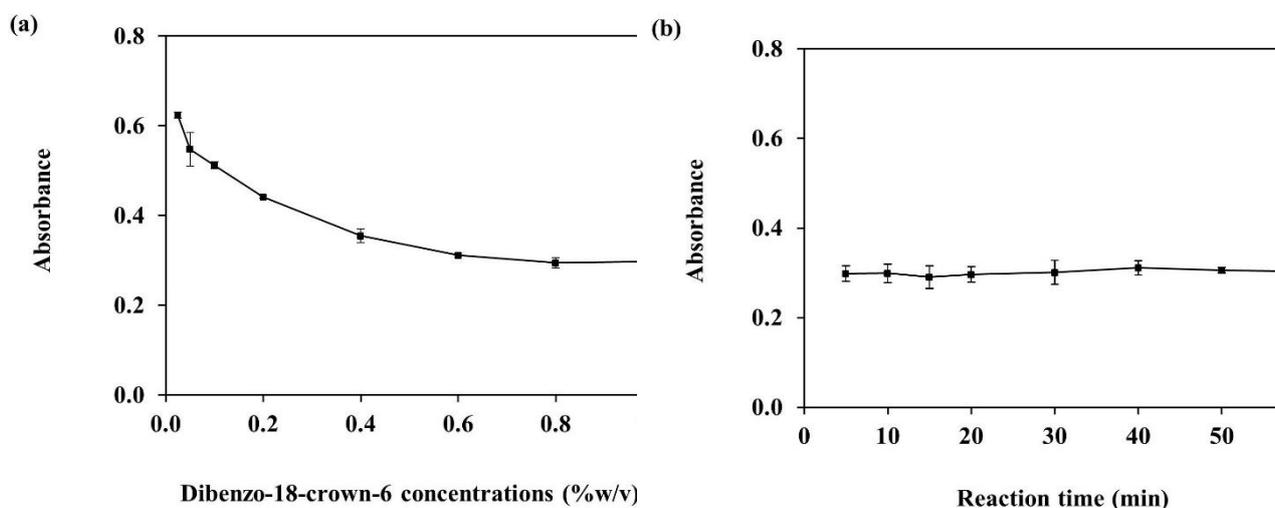


Figure 4. The effect of (a) dibenzo-18-crown-6 concentration and (b) reaction time on the ion-pair extraction

Selectivity of Detecting K^+

In order to evaluate the selectivity of the detection method, K^+ and other ions including Na^+ , Cu^{2+} , Pb^{2+} , Fe^{2+} , and Mn^{2+} as the potential interference in the ion-pair extraction (Ahmed et al, 2020) were tested under the optimal extraction conditions using a spectrometer. For each of these tests, 100 mg L^{-1} solution of the tested ion, which is higher than the level in soil (Khan et al, 2011), was added. The effects of these ions on remaining calmagite absorbance are shown in Figure 5. All ions, except for Na^+ , decreased the absorbance, when compared with the blank sample. This indicates that there is interference on the extraction from Cu^{2+} , Pb^{2+} , Fe^{2+} , and Mn^{2+} . Ethylenediaminetetraacetic acid

(EDTA), one of the most widely used chelating agents, forms sufficiently stable complexes with most metal ions except the alkali metals (Joanna and Alan, 2019). For this reason, EDTA was added as a complexing reagent to prevent the extraction of other ions during testing. All the ions were tested with added EDTA to gauge its effect. When EDTA was added, only K^+ decreased the absorbance and no significant change in the absorbance was observed for any of the other ions (Figure 5) due to the strong complexation of the other ions with EDTA, preventing interference from these ions. In addition, a one-way analysis of variance (ANOVA) was performed for comparison of all interference and the blank samples. The results

showed that the absorbance obtained from all other ions and blank were not significantly different ($p = 0.84$). These results indicated that other ions cannot interfere with potassium detection in the presence of EDTA.

Method Validation of Colorimetric Detection on Paper using a Smartphone

An indirect colorimetric determination of potassium using the paper device and smartphone as detection method was developed. Validity of this method employed for analysis of potassium was evaluated by determining linearity, sensitivity, precision, and

accuracy. The calibration curve results are shown in **Figure 6**. The linear equation for the range of 20-120 mg L^{-1} was found to be: $y = 0.692x + 172.96$ with correlation of determination (R^2) of 0.9906. Limit of detection (LOD) and limit of quantification (LOQ) were determined using calibration standards. LOD and LOQ were calculated as $3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the intensity of a blank sample and S is the slope of the calibration curve (Jayawardane et al., 2015 and Apilux et al., 2012). LOD was determined to be 5.41 mg L^{-1} , and LOQ was found to be 18.03 mg L^{-1} .

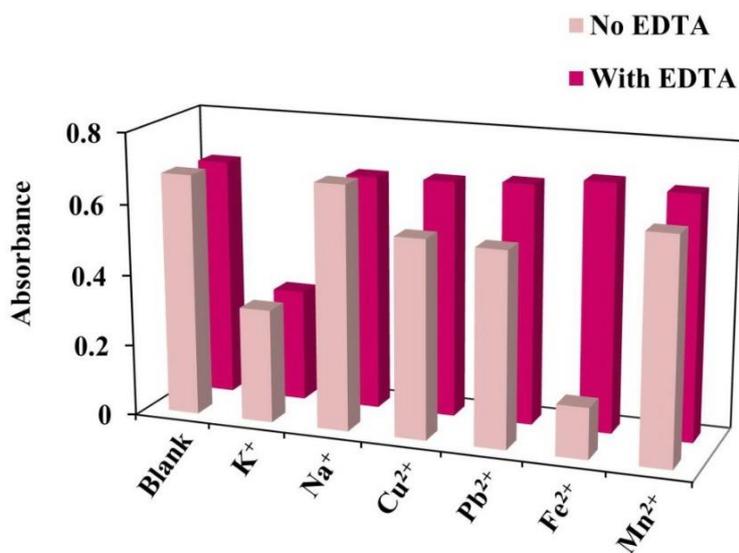


Figure 5. The effect of K^+ and other interference ions, compared with the blank, during extraction, with and without EDTA

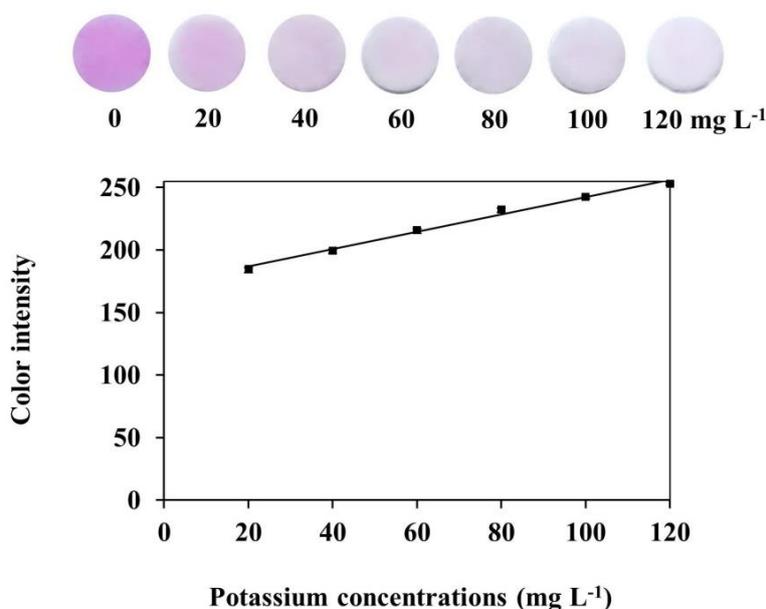


Figure 6. Images of color intensity of remaining calmagite on paper device for various concentrations of potassium and calibration curve of potassium obtained from indirect colorimetric determination using a paper device and smartphone

Table 2. Comparison of potassium detection results from soil samples using the paper device with a smartphone and using the spectrophotometric method

Sample	Added/mg kg ⁻¹	Paper device with smartphone		Spectrophotometric method	
		Found/mg kg ⁻¹	Recovery/%	Found/mg kg ⁻¹	Recovery/%
Soil 1	0.0	41.3 ± 4.2	–	37.7 ± 2.1	–
	250.0	315.9 ± 11.0	109.8 ± 2.9	301.6 ± 4.3	105.6 ± 1.7
Soil 2	0.0	50.9 ± 7.2	–	46.7 ± 3.2	–
	250.0	320.7 ± 18.2	107.9 ± 4.4	304.4 ± 6.2	103.1 ± 2.4
Soil 3	0.0	34.0 ± 4.2	–	32.8 ± 10.5	–
	250.0	296.6 ± 7.2	105.0 ± 1.7	282.2 ± 10.5	99.7 ± 4.2
Soil 4	0.0	48.5 ± 8.3	–	44.0 ± 5.5	–
	250.0	270.1 ± 11.0	88.6 ± 6.0	290.1 ± 2.2	98.5 ± 2.8
Soil 5	0.0	60.5 ± 8.3	–	66.2 ± 1.2	–
	250.0	318.3 ± 14.5	103.1 ± 3.3	321.7 ± 4.3	102.2 ± 1.3

To evaluate the precision of the proposed method, intra-repeatability ($n = 6$) and inter-reproducibility (5 days) were measured, both using potassium concentrations of 20, 60, and 100 mg L⁻¹, and expressed as %RSD. The percentage values of RSD for repeatability and reproducibility are 2.02-3.51% and 2.42-3.86%, respectively. The low %RSD values (< 5%) obtained indicate that the proposed method is precise enough for the analysis of potassium. To evaluate the accuracy of the developed method, five soil samples with and without 250 mg kg⁻¹ of standard potassium, were used to test the accuracy of potassium detection. Results from the soil samples using the paper device and smartphone detection are compared with results using spectrophotometry in **Table 2**. The paired t -test at 95% confidence level showed that there was no significant difference between the two methods ($t_{stat} = 0.85$, $t_{critical} = 2.26$). Percentage recoveries of 88.6–109.8% were obtained, indicating good accuracy. Therefore, it can be concluded that this proposed method for potassium determination in soil samples is both effective and reliable.

CONCLUSIONS

Measuring remaining calmagite after ion-pair extraction of potassium by using a paper colorimetric device together with a smartphone has been shown to be a reliable new method for the determination of potassium in soil. This developed method performed well in all precision and accuracy evaluations after analysis of soil samples. Soil samples containing other ions had no significant effect on the potassium determination, because EDTA is used to prevent any potential interference. The prominent features of the new method are that it is simple, fast, selective, inexpensive, and that it consumes a very small amount of reagents and sample. Thus, this proposed method is well suited for essential agricultural purposes as well as a wide range of other applications.

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