ภาคผนวกที่ 1

ภาคผนวกที่ 2

ภาคผนวกที่ 3

ภาพกิจกรรมการนำผลงานวิจัยไปใช้ประโยชน์

ผลงานวิจัยเรื่อง การพัฒนาเซนเซอร์แบบตรวจวัดสีบนโทรศัพท์เคลื่อนที่สำหรับตรวจวัดเมทแอมเฟตามีนภาคสนาม

กิจกรรม: การแลกเปลี่ยนเรียนรู้ภายใต้โครงการวิเคราะห์และออกแบบนวัตกรรมเพื่อการบริหารสถาบันพัฒนาการสอบสวนคดีพิเศษ

วันเวลา วันที่ 1 เมษายน 2558

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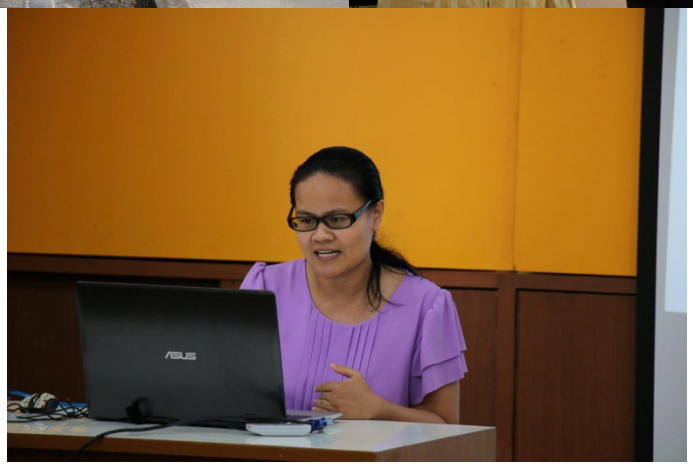

ภาคผนวกที่ 4

ต้นแบบสำหรับการจำหน่ายเชิงพาณิชย์

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A sol–gel colorimetric sensor for methamphetamine detection

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ABSTRACT

A sol–gel colorimetric sensor was successfully developed for the detection of methamphetamine (MA). Simon's reagents were entrapped within the polymeric network of the sol–gel matrix. The sol–gel MA sensor was fabricated within a micro-PCR tube to which the sample solution could be directly added for in-tube detection. This resulted in a small and easy to carry sensor. The sensor was used to demonstrate the rapid quantitative analysis of MA in illicit methamphetamine tablets (Yaba) in conjunction with digital image colorimetry. Real-time Red-Green-Blue (RGB) basic color data of the colorimetric product from the sensor was obtained using an application installed on a mobile phone. The concentrations of MA detected in the illicit tablets by the sol–gel sensor were comparable to values obtained from gas chromatograph-flame ionization detector (GC-FID) analysis. Method validation indicated good precision both intra- and inter-day (0.85–2.41% and 1.76–4.51%, respectively). The sensor was also applied to spiked urine samples and low relative errors in the range of +4 to −9% were obtained. The sol–gel sensor was capable of being stored for almost 3 months (84 days) in a freezer (−18 °C) with only a +4.89% change in the results compared to analysis carried out on the day of preparation. These results demonstrate that the sol–gel MA sensor has the potential to be used as a colorimetric sensor for MA detection in a variety of media. When the sensor was used in combination with a color analysis application installed on a mobile phone, it provided an ideal novel platform for the rapid quantitative analysis of MA.

1. Introduction

Methamphetamine (MA) has been increasingly abused, even though it has been strictly controlled, causing widespread social problems. It is a synthetic stimulant which affects the central nervous system [1] causing serious health problems. MA is the main component in illicit preparations such as. "Ice" (MA in a clear crystal form), "Yaba" (MA in tablet form), and "crank" (MA as a powdered hydrochloride salt). It has been classified as a Category I narcotic under the Narcotic Act B.E. 2522 in Thailand, a Class A Schedule 2 drug under The Misuse of Drugs Act 1971 in the UK, and a Schedule II substance under the UN Convention on Psychotropic Substances in the USA. The ability to quickly detect MA in a range of formulations as well as in urine would provide a very useful and deployable tool in providing intelligence information in counteracting criminal activity relating to this drug.

The typical analytical technique used for the quantitative analysis of MA is gas chromatography using a flame ionization detector (GC-FID) and/or mass spectrometry (GC-MS) [2–8]. High performance liquid chromatography/mass spectrometry [9,10] and electrochemiluminescence detection [11,12] have also been reported. These techniques are usually laboratory based requiring sample extraction and preparation and the appropriate expertise for effective operation.

Recently, digital image colorimetry has been reported as being effective for the rapid quantitative analysis of MA suggesting its use as part of a deployable field test available for first responders [13,14]. This method is based on the measurement of the intensity

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of basic red green blue (RGB) color from a digital image of the colorimetric product produced as a result of a simple chemical presumptive test. Although rapid and accurate quantitative analysis of MA is achieved using such methods, the necessity to use liquid reagents in field tests is not always desirable. The development of a colorimetric sensor in which the chemical reagents are already encapsulated avoids the need to carry reagents and negates any associated risks of spillage, thus increasing the convenience of the method.

The optical sensor for MA detection has been developed by entrapping a bisazo dye in plasticized poly vinyl chloride (PVC) [15,16]. The dye, (4-[4-(4-trifluoroacetylphenylazo)-1-naphthylazo]-*N*,*N*-dioctylaniline), was prepared by using a diazotization procedure lasting more than 5 h. It was then dissolved with plasticizer and PVC in tetrahydrofuran before being spin coated on rotating polyester foil. This sensor was originally reported as a detector for amphetamine, which causes its color to change from blue to red, with a detection limit of 0.1 mmol L^{-1} [16]. However, it was suggested that because of MA's higher lipophilicity and the sensor response would be improved by a factor of 1.2. It was also suggested that the sensor responded to simple aliphatic amines with between 10 and 100 times less sensitivity than the amphetamine response.

Fluorescence chemosensors have also previously been developed for MA [17,18]. These fluorine-based sensors were synthesized using a complicated Suzuki-Miyaura cross-coupling reaction lasting 72 h. The resultant polymers needed a further 2 days of purification revealing a yield of 33–85% [17,18]. Poly[(9,9-dioctylfluorenyl-2,7-diyl)-alt(2,1,3-benzothiadiazole-4,7-diyl)] revealed the best selectivity and sensitivity to MA vapor with a detection limit of 180 ppb [18].

In this work, a novel colorimetric sensor was developed using a simple and rapid procedure involving a sol–gel process. The commonly used Simon's reagent [1] was entrapped within a sol–gel matrix and reacted directly with MA eliminating the necessity for liquid reagents. Digital image analysis was used to detect the color intensity of the product on the sensor achieving a rapid, portable, and accurate quantitative method for analysis of MA.

2. Materials and methods

2.1. Materials

Methamphetamine crystal (purity ~98.5%) and illicit tablets (Yaba) were obtained from the Drug Control Division, Food and Drug Administration Thailand (license number: 1003.2/790). Acetaldehyde was purchased from Aldrich Chemical Co. Ltd. (Dorset, England), sodium nitroprusside dihydrate (>98.0%) was purchased from Fluka (Sigma-Aldrich Chemie, Steinheim, Germany). Anhydrous sodium carbonate and ethanol were obtained from Fisher Scientific. Tetraethoxysilane (TEOS) was obtained from Merck (Darmstadt, Germany) and concentrated hydrochloric acid (HCl) from Carlo Erba (Spain).

MA stock solution (10 mg mL^{-1}) was prepared by dissolving the appropriate amount of methamphetamine in ultrapure water (Barnstead EasyPure II, Thermo fisher scientific, OH). Working standard solutions were prepared daily by diluting the MA stock solution with ultrapure water to the desired concentrations.

Simon's reagent was prepared by mixing the two reagents (reagent 1 and 2) in a ratio of 1:2 (*v*/*v*). These reagents consisted of 10%*v*/*v* acetaldehyde in aqueous sodium nitroprusside solution (1%*w*/*v*) (reagent 1) and 2%*w*/*v* sodium carbonate in water (reagent 2) [1].

The sol–gel solution consisted of TEOS, 0.04 mol L^{-1} HCl, and ethanol in the ratio of 2:1:2 (*v*/*v*) [19]. All the ingredients were mixed and magnetically stirred for 15 min at room temperature.

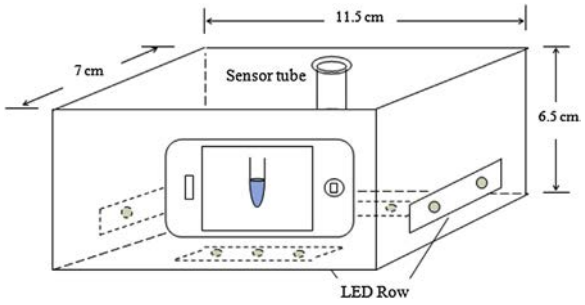

Fig. 1. Real time on-mobile color analysis system for methamphetamine detection.

2.2. Preparation of the sol–gel MA sensor

The sol–gel MA sensor was prepared by mixing the sol–gel solution with Simon's reagent in a ratio of 1:2 (*v*/*v*). The resultant mixture (75 μL) was then transferred into a flat cap micro-PCR tube (200 μL) and the lid of the tube was quickly closed in order to avoid the evaporation of any components. The tube was left at room temperature for 30 min to obtain a sol–gel MA sensor inside the tube before being stored in a freezer (−18 °C) prior to further use.

The sol–gel MA sensor morphology was investigated using a scanning electron microscope (SEM) (Quanta400, FEI, Czech Republic). It was also characterized by Fourier transform infrared spectroscopy (FTIR) (Equinox55, Bruker, Germany). Spectral analysis was performed over a range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using KBr pellet preparation.

2.3. Colorimetric test of MA

The sol–gel MA sensors were pierced using a small tip immediately before used to allow the entrapped Simon's reagents and MA to rapidly react. Each MA standard solution (50 microliters) was transferred to the flat cap micro-PCR tubes containing the pierced sol–gel MA sensor and mixed for 2 min. The intensity of the color product inside the tube was detected using the ColorAssist app (FTLapps, Inc.) on an iPhone 4.0 in the flash-off mode.

2.4. RGB capturing system and procedure

A custom-built RGB detection box was modified from previous work [14] (Fig. 1). The box (7.0 cm × 11.5 cm × 6.5 cm) was made of opaque black corrugated plastic board with an internal white background. The flat cap micro-PCR containing sol–gel MA sensor was hung at the top of the box. A small cover was used to eliminate any effects from environmental light. Ten white high intensity light emitting diodes (LEDs) were placed at the bottom of the box as the light sources. A hole was made in one side of the box to enable the built-in digital camera of an iPhone 4.0 to detect the RGB intensity of the colorimetric product.

2.5. Analytical performance and method validation

Each colorimetric test was repeated three times using three different sol–gel sensors across all concentrations of MA tested. The average intensities of the red, green and blue colors from the three replications were used to establish a single point in a calibration graph for each color. The linear range was investigated from 0 to 5 mg mL^{-1}. The limit of detection was calculated using standard methods (y at limit of detection = $y_B + 3S_B$ where y_B is the intercept of the calibration curve and S_B is the standard deviation of blank) [20]. Precision was expressed as a percentage of the relative standard deviation (%RSD) of the RGB values for each color from the three replicate analyzes. Accuracies were evaluated

as a percentage relative error ($(x_e - x_{control})/x_{control} \times 100$) generated by analyzing a known concentration of MA standard solution ($x_{control}$ = 0.75 mg mL^{-1}) and quantified using an external calibration equation (x_e).

2.6. Analysis of case work samples

Five seized methamphetamine tablets (Yaba samples) obtained from the Drug Control Division of the Food and Drug Administration of Thailand were analyzed. Ten milligrams from each Yaba sample were extracted with 1 mL of water and sonicated for 5 min in line with previously reported preparative method [14]. The supernatant was analyzed using the sol–gel MA sensor and quantified using the calibration equation for the green intensity (I_G) of the digital image. The results were compared with those obtained from gas chromatographic analysis [14].

Five MA spiked urine samples were also investigated. Five blank urine samples obtained from five volunteers were spiked with MA at a concentration of 1 mg mL^{-1}. They were analyzed using the sol–gel MA sensor and quantified as described previously.

3. Results and discussion

3.1. Preparation and characterization of the sol–gel MA sensor

In a sol–gel process, a precursor alkoxide undergoes hydrolysis and condensation to form a transparent gel. Silica sol was prepared by mixing an alkoxide (TEOS) with water and a catalyst (HCl) in alcohol solution (ethanol). During gelation of the sol, a series of hydrolysis and condensation reactions occur at room temperature in which reagents can be entrapped within a polymeric network of the porous gel [19,21,22].

In this study, the composition of both Simon's reagents and the sol–gel solution were optimized to achieve an optimal sol–gel MA sensor. Optimization was conducted by varying one parameter while keeping the other parameters constant. Different ratios of TEOS, HCl (0.02, 0.04, 0.06 mol L^{-1}), and ethanol were evaluated and it was found that the ratio of 2:1:2 (*v*/*v*) of TEOS: 0.04 mol L^{-1} HCl: ethanol provided a suitable formula for the transparent sol–gel as reported previously [19]. This mixture was then used to conduct further optimization. For the Simon's reagents, ratios of 3:1, 2:1, 1:1, 1:2, and 1:3 (*v*/*v*) of 10%*v*/*v* acetaldehyde in aqueous sodium nitroprusside solution (1%*w*/*v*) (reagent 1) and 2%*w*/*v* sodium carbonate in water (reagent 2) were systematically evaluated by mixing with the sol–gel solution at a ratio of 1:1. sol–gel MA sensors were obtained from all the formulas, and after testing it was found that a ratio of 1:2 (*v*/*v*) reagent 1: reagent 2 provided the darkest blue product within 15 s of mixing, it was thus selected as the optimum mixture of Simon's reagents.

Because the porosity of the sol–gel material depends on the ratio of reagent and precursor [19], the ratio of sol–gel solution to Simon's reagents was investigated to obtain the best sol–gel MA sensor. The sol–gel solution was mixed with Simon's reagents (reagent 1 and reagent 2 at a ratio of 1:2 *v*/*v*) at ratios of 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, and 1:4 (*v*/*v*). When the volume of sol–gel solution was decreased (at ratios of 4:1, 3:1, 2:1 and 1:1), a darker blue product was obtained from the reaction of MA and the sensor. However, when the volume of Simon's reagent was increased (1:1, 1:2, 1:3, and 1:4) the gelation time increased. At ratios of 1:3 and 1:4, the sensors remained soft even though the mixture was left for 16 h. This may be due to an excess of reagents being entrapped within the polymeric network of the gel. As a consequence, a ratio of sol–gel solution and Simon's reagents of 1:2 was selected for the preparation of the optimized sol–gel MA sensor.

The image from scanning electron microscopy (SEM) of the optimized sol–gel MA sensor prepared under optimum conditions is shown in Fig. 2a and b. Large pores were observed in the network-like sensor which facilitated the penetration of MA into the sol–gel matrix allowing the reaction with the entrapped Simon's reagents to occur. Very similar SEM images from three sensors with approximately 5 μm average pore size were observed indicating the excellent reproducibility of the sensor.

FTIR spectra of the optimized sol–gel MA sensor exhibited a broad peak at 3458 cm^{-1} (Fig. 2c) which corresponded to hydroxyl (–OH) vibration [23–26]. An absorption peak at 1641 cm^{-1} was probably due to the OH vibration of ethanol [25] or water [24,26]. A number of peaks observed at 450–1100 cm^{-1} were attributed silica. The largest peak observed at 1077 cm^{-1} was attributed to asymmetric stretching of the silicon atom in the siloxane bonds (Si–O or Si–O–Si) [23,25–27]. This can be attributed to C–O stretching of the TEOS and ethanol [24]. The absorption peak at 465 cm^{-1} was assigned to the rocking motion of the oxygen atoms bridging the silicon atoms in the siloxane bonds (Si–O–Si) [23,24], while the symmetric vibration of the silicon atoms in the siloxane bond occurred at 797 cm^{-1} [23,27]. The peaks at 570 and 958 cm^{-1} were probably due to a stretching vibration of the Si–O–Si and Si–OH, respectively [24]. The absorption peaks at 1943–2144 cm^{-1} were attributed to vibration peaks from the Simon's reagents as sodium nitroprusside has been reported to show peaks at 1950 and 2150 cm^{-1} [28]. Although the characteristic peaks in the FTIR spectra of the optimized sol–gel exhibited more absorption peaks than those of the Simon's reagents, the appearance of a light brown color in the sensor due to sodium nitroprusside confirmed the presence of Simon's reagents in the sensor (Fig. 2d).

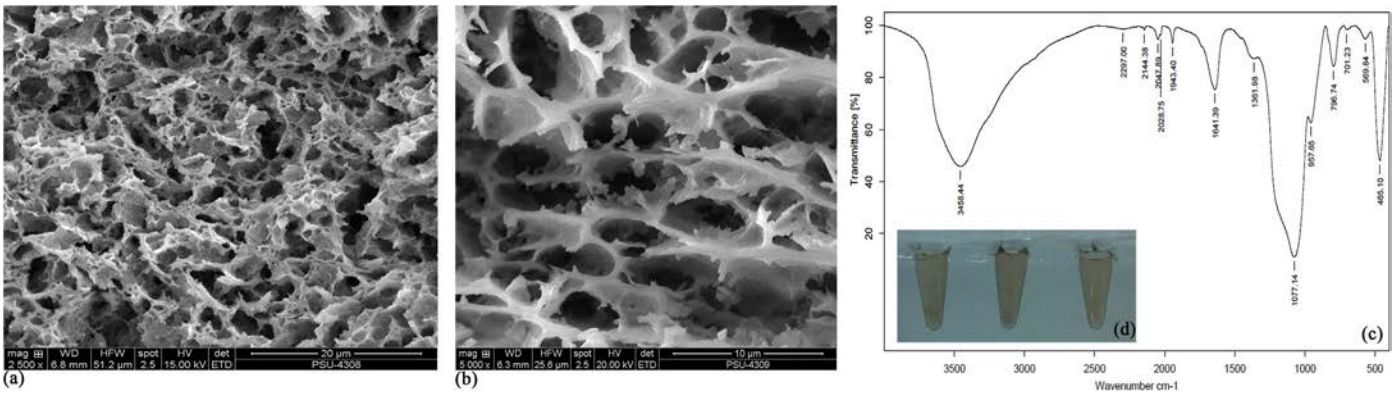

Fig. 2. (a–b) SEM image of the sol–gel MA sensor, (c) FTIR spectra of the sol–gel MA sensor, (d) the sol–gel MA sensors with 50 microliters of ultrapure water.

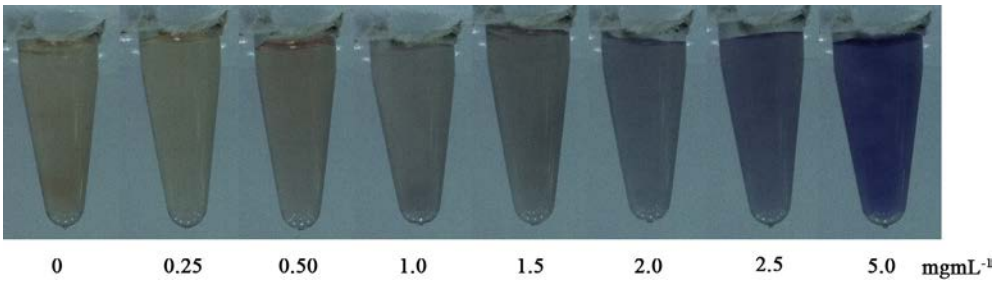

Fig. 3. Colorimetric products obtained from the use of the sol–gel MA sensor with various concentrations of methamphetamine (0–5.0 mg mL^{-1}).

Besides being biocompatible, the optimized sol–gel sensor is chemically, photo chemically and thermally stable compared to organic polymers. It has the advantages of being easy to prepare, economically viable and environmental friendly. The shape and size of the gel could be easily controlled because the sol is initially liquid prior to gel formation. In this study, the transparent gel was formed at the bottom of a flat cap micro-PCR tube resulting in a small and easy to carry sensor to which the sample solution could be directly added for in-tube detection of MA.

3.2. Colorimetric test of sol–gel MA sensor

A colorimetric test of MA with Simon's reagents produced a blue color product associated with the Simon-Awe complex. MA firstly reacts with acetaldehyde to produce an enamine intermediate. This intermediate then reacts with sodium nitroprusside to produce an immonium salt which can be hydrolyzed to a Simon-Awe complex [13,29] and purple to blue products have been reported [14]. Using the sol–gel MA sensor, a purple to blue product was obtained after the addition of MA standard solution corresponding to previous literature [14]. Darker blue products were obtained with increasing MA concentrations (Fig. 3). The homogeneous blue products observed from the color test using the MA sensor suggested a homogenous entrapping of Simon's reagent within the sol–gel matrix.

3.3. Digital image colorimetry for MA detection

Quantitative analysis of MA using optical sensors reported in the literature usually involves the use of a spectrophotometer [15,16] requiring a transparent and homogeneous sensor. Although a sol–gel colorimetric sensor is suitable for spectrophotometric detection due to its optical transparency [19,30,31], the expense and bulky nature of such an instrument limits its usefulness. In this work, digital image colorimetry was used for the quantitative analysis of MA for the first time using a sol–gel matrix. The method was not only more convenient than conventional colorimetry involving solution chemistry, but also employed a cheaper detection device, making use of the digital camera of an iPhone 4, compared to a spectrophotometer.

Digital image colorimetry is based on the analysis of the intensity from digital images of colorimetric products. This analysis provides analytical data in the form of basic red green blue (RGB) color which can be used for the quantitative analysis of analytes of interest. Our previous work [14] showed that the ColorAssist iPhone application can be used to capture the RGB values, resulting in a much shorter analysis time than other commonly used programs *e.g.* Matlab [32,33], Kylix [34,35], Visual basic [36,37], and Adobe Photoshop [13,38–41].

The intensity of the RGB values (I_R, I_G, and I_B) obtained from the sol–gel MA sensor images were correlated with the MA concentrations as illustrated in Fig. 4a. The intensities of the red and green channels decreased with increasing MA concentrations from 0 to 5 mg mL^{-1} as has been previously noted [14]. The blue intensity remained constant in the analyzed concentration range and provided the highest intensity which was expected given the blue color of the product. The green channel showed higher intensities than the red channel deviating from the previous reported results where both channels provided very similar intensities over the concentration range studied [14]. This difference may have contributed to the darker color of the Simon's reagents when entrapped in the sol–gel matrix. However, it should be noted that the RGB capturing systems used in the both studies were slightly different.

The molecular absorption of the blue products was estimated using an equation previously reported [13,38–40,42]. As expected, the blue channel provided the lowest estimated absorption compared to the red and green channels (Fig. 4b) as it is the most reflected light. The red channel, which aggregates light in a spectral range from 580 to 700 nm [43] was found to have the highest absorption. This agrees with reported spectrophotometric analysis which demonstrates the maximum absorption peak of this Simon-Awe complex to be at 580 nm [14,44].

The relationships derived from the combination of the total RGB data both in terms of intensity ($I_{TOTAL} = I_R + I_G + I_B$) and absorbance ($A_{TOTAL} = A_R + A_G + A_B$) are illustrated in Fig. 4c and d. The sensitivity of the total absorption obtained in this study was lower than that obtained in previous work (0.203 ± 0.005 a.u.mL mg^{-1} compared to 0.56 ± 0.03 a.u.mL mg^{-1} [14]), but covered a wider linear range (0–5.0 mg mL^{-1} compared to 0.1–2.5 mg mL^{-1}[14]).

Because the color of the sol–gel MA sensor and/or samples may affect the RGB values of the purple-blue product, the RGB values of the blank were subtracted them from the RGB values of the product. The relationships between the subtracted RGB values and MA concentrations both as individual and total values are presented in Fig. 5. All subtracted RGB values increased with increasing MA concentrations indicating a greater difference between the color of the blank and the color of the product when MA concentrations were increased. The intensity of the green channel demonstrated higher sensitivity than the red channel, but the latter provided higher sensitivity for absorption.

3.4. System performance and method validation

The calibration equations and analytical performance including linear range, linearity, accuracy, and limit of detection (LOD) of the proposed method are presented in Table 1. The sensitivities (slope of the calibration equations) of the intensity of the red channel both blank subtracted and non-subtracted were lower than those of the green channel. Both blank subtracted and non-subtracted total absorption provided wider linear ranges than total intensity. The precisions expressed by %RSD from three repetitions of the analysis of MA (2.5 mg mL^{-1}) using the sol–gel MA sensors within the same day (intra-day) were in the range of 0.85–2.41%, and were in the range of 1.76–4.51% for analysis performed on three

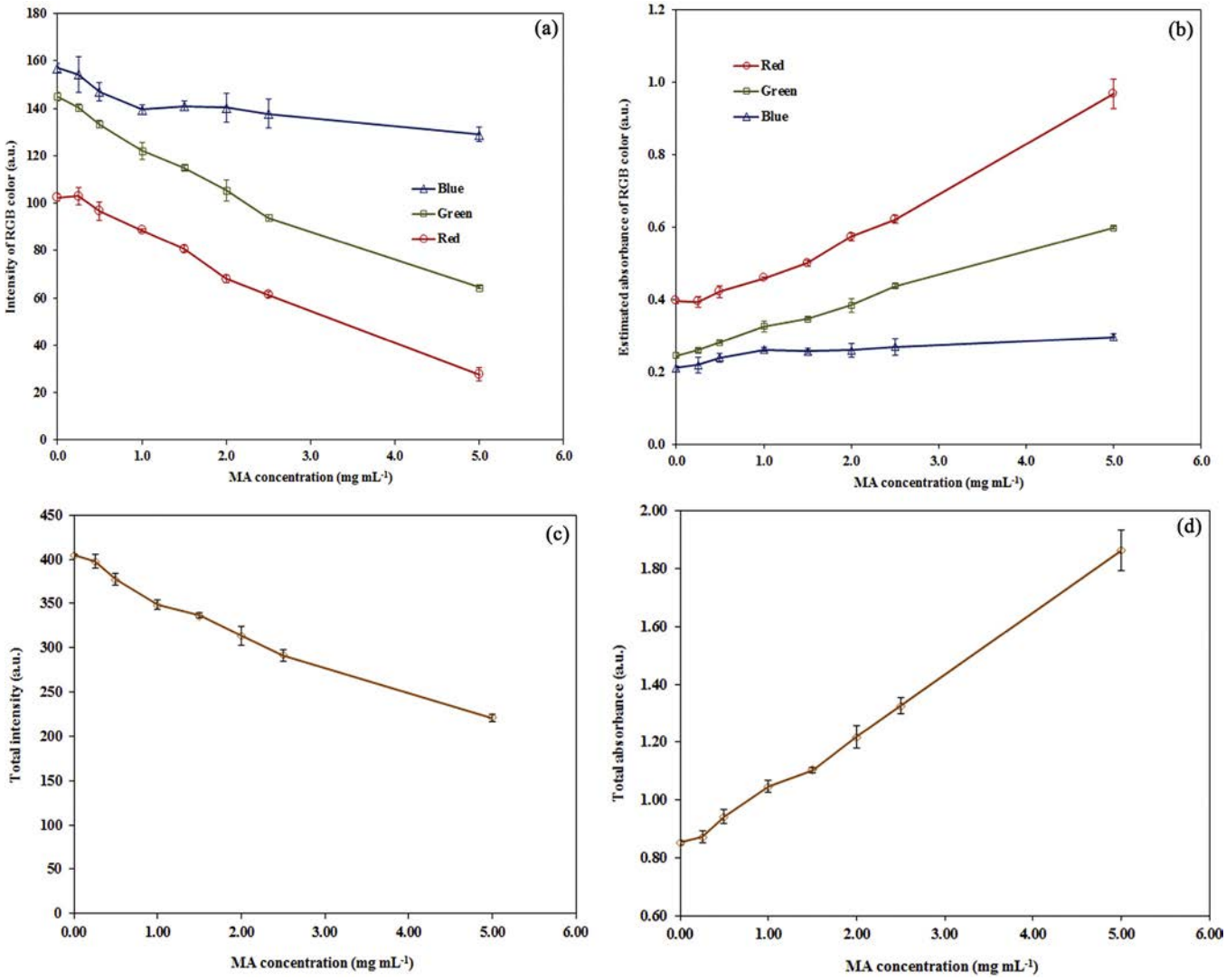

Fig. 4. Relationships between MA concentrations and (a) individual RGB values, (b) absorbance, (c) total intensity, (d) total absorbance of RGB values.

Table 1
Calibration equations and analytical performance of the proposed method.

Relationship	Calibration equation (y = a.u., x = mg mL^{-1})	Linear range (mg mL^{-1})	Linearity (R^2)	Accuracy (%RE[a])	LOD[b] (mg mL^{-1})
I_R	$y = -(18.6 \pm 0.7)x + (107 \pm 1)$	0.25–2.5	0.9955	+8.0	0.223 ± 0.009
I_G	$y = -(20.1 \pm 0.6)x + (144.3 \pm 0.9)$	0–2.5	0.9940	+5.3	0.207 ± 0.006
A_R	$y = (0.124 \pm 0.006)x + (0.33 \pm 0.02)$	0.50–5.0	0.9908	+9.3	0.52 ± 0.02
A_G	$y = (0.071 \pm 0.002)x + (0.246 \pm 0.004)$	0–5.0	0.9964	+0.1	0.318 ± 0.008
$\lvert I_R - I_{Rblank} \rvert$	$y = (18.2 \pm 0.7)x - (4 \pm 1)$	0.25–2.5	0.9943	+2.2	0.212 ± 0.008
$\lvert I_G - I_{Gblank} \rvert$	$y = (19.8 \pm 0.8)x + (1 \pm 1)$	0.25–2.5	0.9941	+9.3	0.225 ± 0.009
$\lvert A_R - A_{Rblank} \rvert$	$y = (0.124 \pm 0.006)x - (0.06 \pm 0.02)$	0.50–5.0	0.9908	+9.3	0.53 ± 0.02
$\lvert A_G - A_{Gblank} \rvert$	$y = (0.071 \pm 0.002)x + (0.002 \pm 0.005)$	0.25–5.0	0.9957	+6.7	0.35 ± 0.01
I_{Total} ($I_R + I_G + I_B$)	$y = -(41 \pm 2)x + (395 \pm 4)$	0.50–2.5	0.9893	+0.9	0.29 ± 0.02
A_{Total} ($A_R + A_G + A_B$)	$y = (0.203 \pm 0.005)x + (0.83 \pm 0.01)$	0–5.0	0.9965	+0.8	0.310 ± 0.008
$\lvert I_{Total} - I_{Totalblank} \rvert$	$y = (32 \pm 2)x + (25 \pm 5)$	1.0–5.0	0.9906	+9.3	0.53 ± 0.03
$\lvert (A_{Total} - A_{totalblank}) \rvert$	$y = (0.206 \pm 0.005)x + (0.03 \pm 0.01)$	0.25–5.0	0.9971	+6.7	0.59 ± 0.01

[a] %RE = $(x_e - x_{control})/x_{control} \times 100$, $x_{control}$ = a known-concentration of MA standard solution (0.75 mg mL^{-1}), x_e = concentration of MA quantified using external calibration.
[b] LOD = limit of detection calculated by y at LOD = $y_B + 3S_B$ where y_B is intercept of calibration curve and S_B is standard deviation of blank) [20].

separate days (inter-day). Accuracies were evaluated as %relative error (%RE = $(x_e - x_{control})/x_{control} \times 100$) generated by analyzing a known concentration of MA standard ($x_{control}$ = 0.75 mg mL^{-1}) and quantified using an external calibration equation (x_e). A relative error of +0.1% to 9.3% was obtained indicating good accuracy of analysis. The limit of detection calculated using the standard method (LOD = $y_B + 3S_B$ where y_B is the intercept of the calibration curve and S_B is the standard deviation of blank) [20] were in a range of 0.207 ± 0.006 to 0.59 ± 0.01 mg mL^{-1}.

3.5. Stability of the sol–gel MA sensor

The stability of the sol–gel MA sensor was evaluated by preparing forty sensors using the same ingredients at the same time.

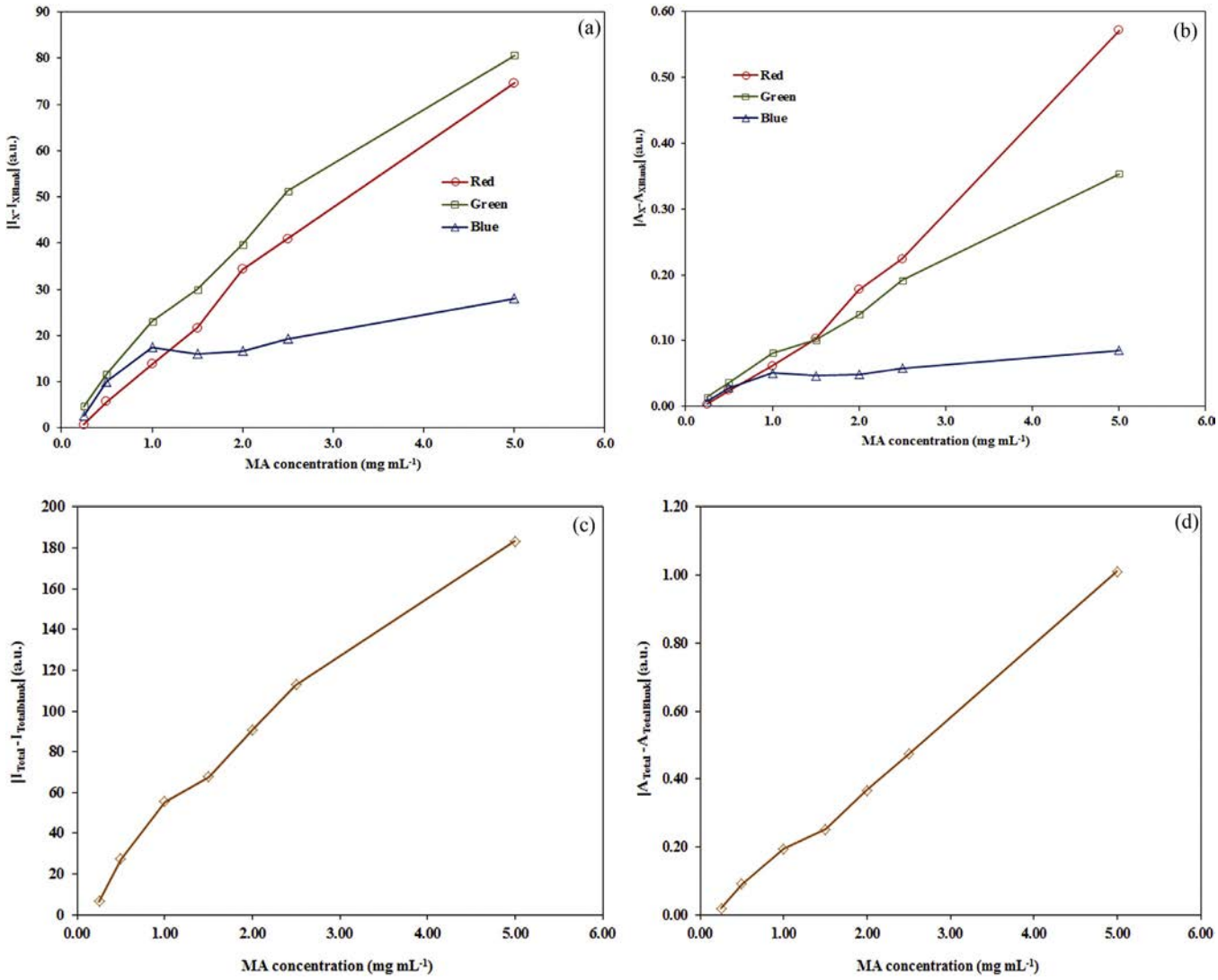

Fig. 5. Relationship between blank subtracted RGB values and MA concentrations (a) individual intensity, (b) individual absorbance, (c) total intensity, (d) total absorbance.

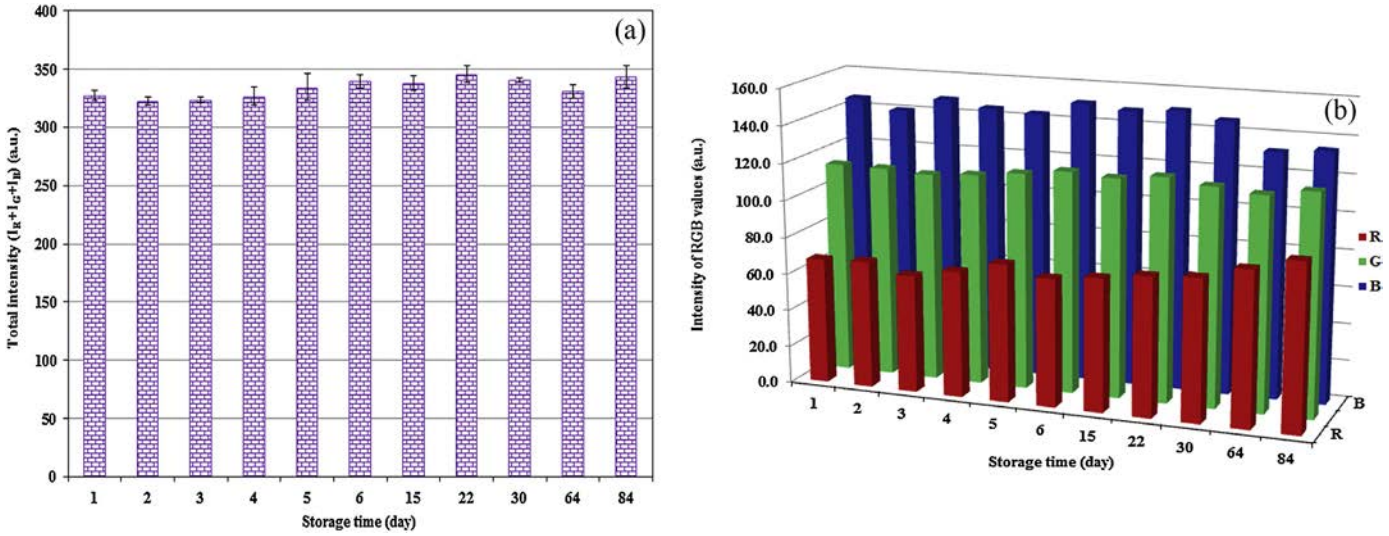

Fig. 6. Stability of the sol–gel MA sensor (a) total intensity, (b) individual intensity.

Three of the prepared sensors were used for MA detection (at 2.5 mg mL^{-1}) on the day of preparation, and the remaining stored in a freezer (−18 °C). Three sensors were subsequently removed from the freezer and used for MA detection every day over a one week period and then on a weekly basis for a further 3 weeks and finally after 2 and 3 months and the results are presented in Fig. 6. The total intensity of the color product obtained from the use of the sol–gel MA sensors stored for almost 3 months (84 days) varied by only +4.89% when compared with the results obtained from analysis of gels on the day of preparation (Fig. 6a). This indicated an excellent stability for the sol–gel MA sensor. When individual RGB values were considered, the intensities of the green and blue channels at 84 days had changed by +4.36% and −7.78%, respectively (Fig. 6b) while the intensities of the red channel had changed by 5.91%, 13.30%, and 33.00% at 15, 30, and 84 days after preparation, respectively. From the obtained data, the green channel was viewed as the most effective for the rapid quantitative analysis of MA using the sol–gel MA sensor delivering stable and repeatable result over a 3 month period when stored in the freezer prior to use.

In order to further ascertain any temperature and light affects during filed deployment, 6 sol–gel sensors were taken out from the freezer and kept at room temperature (27 °C) inside and outside the sampling box for 3 h, while a further 3 sensors were kept in the freezer (−18 °C) prior to use. The sensors outside of the sampling box had darkened slightly, most likely as a result of exposure to light, in comparison to those kept inside of the sampling box and those kept in the freezer. This color change affected the performance for the analysis of MA only very slightly. However, the sensor kept within the opaque box at room temperature provided no difference with those kept in the freezer indicating no influence of temperature performance and refining the parameters required for potential field deployment.

3.6. Determination of MA in case work samples

The sol–gel MA sensor combined with digital colorimetry was applied for the rapid quantitative analysis of MA in Yaba samples. Five seized Yaba samples were extracted by the proposed method and the results compared with those obtained from GC-FID analysis [14]. The results (Table 2) clearly demonstrated good quantitative agreement across both methods.

All of the Yaba samples analyzed were red tablets. The tablet color did not interfere with the ability of the sol gel to deliver a quantitative result for the methamphetamine within the tablet indicating that the sol–gel MA sensors were not influenced by any color ingredient within the sample being analyzed. This is a critical finding for operational viability of the sensor.

The sol–gel MA sensors were also used to analyze urine samples. Urine, spiked with MA, was added to the sol–gel sensor as a direct liquid sample and the results are presented in Table 3. It was found that the quantified MA concentrations obtained from the I_G equation provided a very good correlation with the spiked concentrations (%relative error of +3 to −9%). These results again confirmed that the sol–gel MA sensors were not influenced by any color component within the sample.

Table 2
Analysis of Yaba samples.

Sample no.	% of MA in Yaba samples		%Relative error
	Sol–gel MA sensor[a]	GC-FID[b]	
1.	18 ± 1%	19.9 ± 0.4%	−9.5%
2.	18 ± 1%	17.4 ± 0.1%	+3.4%
3.	18 ± 2%	18.7 ± 0.2%	−3.7%
4.	19 ± 2%	19.3 ± 0.3%	−1.6%
5.	19.9 ± 0.3%	18.1 ± 0.5%	+9.9%

[a] Quantified by using I_G equation: $y = -(20.1 \pm 0.6)x + (144.3 \pm 0.9)$.
[b] Using the same sample set as previous report [14].

Table 3
Analysis of spiked urine samples.

Sample no.	Spiked	Quantified[a]	%Relative error
1.	1.0	0.94	−6%
2.	1.0	1.04	+4%
3.	1.0	1.03	+3%
4.	1.0	1.04	+4%
5.	1.0	0.91	−9%

[a] Quantified by using I_G equation: $y = -(20.1 \pm 0.6)x + (144.3 \pm 0.9)$.

4. Conclusion

A sol–gel colorimetric sensor for methamphetamine detection was successfully developed by entrapping Simon's reagents within the polymeric network of a sol–gel matrix. This made the preparation of the sensor much easier than those described in previous reports. The developed sensor was fabricated within a small flat cap micro-PCR tube making it easy to transport and use. A small volume of the sample solution could be directly added into the tube during testing and the sensor was stable for almost 3 months when kept in a freezer (−18 °C). When the sensor was used in combination with digital image colorimetry, rapid quantitative accurate analysis of methamphetamine was achieved without interferences from other colored additives or products.

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References

[1] United Nations International Drug Control Programme, Rapid Testing Methods of Drugs of Abuse: Manual for Use by National Law Enforcement and Narcotics Laboratory Personnel, United Nation, Vienna, 1994.
[2] F.M. Dayrit, M.C. Dumlao, Impurity profiling of methamphetamine hydrochloride drugs seized in the Philippines, Forensic Sci. Int. 144 (2004) 29–36.
[3] K. Kuwayama, H. Inoue, J. Phorachata, K. Kongpatnitiroj, V. Puthaviriyakorn, K. Tsujikawa, H. Miyaguchi, T. Kanamori, Y.T. Iwata, N. Kamo, T. Kishi, Comparison and classification of methamphetamine seized in Japan and Thailand using gas chromatography with liquid–liquid extraction and solid-phase microextraction, Forensic Sci. Int. 175 (2008) 85–92.
[4] K. Tsujikawa, K. Kuwayama, H. Miyaguchi, T. Kanamori, Y.T. Iwata, H. Inoue, Chemical profiling of seized methamphetamine putatively synthesized from phenylacetic acid derivatives, Forensic Sci. Int. 227 (2013) 42–44.
[5] S. Choe, S. Heo, H. Choi, E. Kim, H. Chung, J. Lee, Analysis of pharmaceutical impurities in the methamphetamine crystals seized for drug trafficking in Korea, Forensic Sci. Int. 227 (2013) 48–51.
[6] J.S. Lee, H.S. Chung, K. Kuwayama, H. Inoue, M.Y. Lee, J.H. Park, Determination of impurities in illicit methamphetamine seized in Korea and Japan, Anal. Chim. Acta 619 (2008) 20–25.
[7] J.X. Zhang, D.M. Zhang, X.G. Han, Identification of impurities and statistical classification of methamphetamine hydrochloride drugs seized in China, Forensic Sci. Int. 182 (2008) 13–19.
[8] A.R. Khajeamiri, M. Faizi, F. Sohani, T. Baheri, F. Kobarfard, Determination of impurities in illicit methamphetamine samples seized in Iran, Forensic Sci. Int. 217 (2012) 204–206.
[9] M. del Mar Ramírez Fernández, S.M.R. Wille, V. di Fazio, M. Gosselin, N. Samyn, Analysis of amphetamines and metabolites in urine with ultra performance liquid chromatography tandem mass spectrometry, J. Chromatogr. B 878 (2010) 1616–1622.
[10] M. Nieddu, G. Boatto, M.A. Pirisi, E. Baralla, Multi-residue analysis of eight thioamphetamine designer drugs in human urine by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Sp. 23 (2009) 3051–3056.
[11] Z. Cai, Z. Lin, X. Chen, T. Jia, P. Yu, X. Chen, Electrochemiluminescence detection of methamphetamine based on a Ru(bpy)32 + -doped silica nanoparticles/Nafion composite film modified electrode, Luminescence 25 (2010) 367–372.

[12] H. Dai, Y. Wang, X. Wu, L. Zhang, G. Chen, An electrochemiluminescent sensor for methamphetamine hydrochloride based on multiwall carbon nanotube/ionic liquid composite electrode, Biosens. Bioelectron. 24 (2009) 1230–1234.
[13] A. Choodum, N. Nic Daeid, Digital image-based colorimetric tests for amphetamine and methamphetamine, Drug Test. Anal. 3 (2011) 277–282.
[14] A. Choodum, K. Parabun, N. Klawach, N. Nic Daeid, P. Kanatharana, W. Wongniramaikul, Real time quantitative colorimetric test for methamphetamine detection using digital and mobile phone technology, Forensic Sci. Int. 235 (2014) 8–13.
[15] G.J. Mohr, New chromoreactands for the detection of aldehydes, amines and alcohols, Sensor Actuat. B-Chem. 90 (2003) 31–36.
[16] G.J. Mohr, M. Wenzel, F. Lehmann, P. Czerney, A chromoreactand for optical sensing of amphetamine, Anal. Bioanal. Chem. 374 (2002) 399–402.
[17] Y. Fu, L. Shi, D. Zhu, C. He, D. Wen, Q. He, H. Cao, J. Cheng, Fluorene–thiophene-based thin-film fluorescent chemosensor for methamphetamine vapor by thiophene–amine interaction, Sensor Actuat. B-Chem. 180 (2013) 2–7.
[18] D. Wen, Y.Y. Fu, L.Q. Shi, C. He, L. Dong, D.F. Zhu, Q.G. He, H.M. Cao, J.G. Cheng, Fine structural tuning of fluorescent copolymer sensors for methamphetamine vapor detection, Sensor Actuat. B-Chem. 168 (2012) 283–288.
[19] O. Bunkoed, F. Davis, P. Kanatharana, P. Thavarungkul, S.P.J. Higson, Sol–gel based sensor for selective formaldehyde determination, Anal. Chim. Acta 659 (2010) 251–257.
[20] J.N. Miller, J.C. Miller, Statistic and Chemometric for Analytical Chemistry, 5th ed., Pearson Education Limited, Essex, 2005.
[21] A. Persad, K.-F. Chow, W. Wang, E. Wang, A. Okafor, N. Jespersen, J. Mann, A. Bocarsly, Investigation of dye-doped sol–gels for ammonia gas sensing, Sensor Actuat. B-Chem. 129 (2008) 359–363.
[22] S.-A. Wallington, T. Labayen, A. Poppe, N.A.J.M. Sommerdijk, J.D. Wright, Sol-gel entrapped materials for optical sensing of solvents and metal ions, Sensor Actuat. B-Chem. 38 (1997) 48–52.
[23] S. Ibrahem, H. Ibrahem, Synthesis and study the effect of H_2O/TEOS ratio of the silica xerogel by sol-gel method, Inter. Arch. App. Sci. Technol. 5 (2014) 1–5.
[24] R.K. Nariyal, FTIR measurements of SiO_2 Glass prepared by sol-gel technique, Chem. Sci. Trans. 3 (2014) 1–3.
[25] A. Gungor, H. Demirtas, I. Atilgan, M. Yasar, Synthesis and Characterization of SiO_2 Films Coated on Stainless Steel by Sol-gel Method, International Iron and Steel Symposium, Karabuk, Turky, 2012.
[26] I. Garcia-Lodeiro, A. Fernandez-Jimenez, M. Teresa Blanco, A. Paomo, FTIR study of the sol-gel systhesis of cerementitious gels: C-S-H and N-A-S-H, J. Sol-Gel Sci. Technol. 45 (2008) 63–72.
[27] J.C. Echeverría, P. de Vicente, J. Estella, J.J. Garrido, A fiber-optic sensor to detect volatile organic compounds based on a porous silica xerogel film, Talanta 99 (2012) 433–440.
[28] Sigma-Aldrich, Sodium Nitroprusside Dihydrate: FTIR Spectrum.
[29] K.-A. Kovar, M. Laudszun, Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursors Chemicals, Pharmazeutisches Institut der Universitat Tubingen, Germany, 1989.
[30] S. Dhanya, J. Joy, T.P. Rao, Fabrication and characterization of rhodamine 6G entrapped sol–gel film test strip for virtually specific and sensitive sensing of nitrite, Sensor Actuat. B-Chem. 173 (2012) 510–516.
[31] E. Wang, K.-F. Chow, V. Kwan, T. Chin, C. Wong, A. Bocarsly, Fast and long term optical sensors for pH based on sol–gels, Anal. Chim. Acta 495 (2003) 45–50.
[32] L.M. Goddijn, M. White, Using a digital camera for water quality measurements in Galway Bay, Estuar. Coast. Shelf S. 66 (2006) 429–436.
[33] A. Lopez-Molinero, D. Liňan, D. Sipiera, R. Falcon, Chemometric interpretation of digital image colorimetry. Application for titanium determination in plastics, Microchem. J. 96 (2010) 380–385.
[34] E.d.N. Gaiao, V.L. Martins, W.d.S. Lyra, L.F.d. Almeida, E.C.d. Silva, M.C.U. Araújo, Digital image-based titrations, Anal. Chim. Acta 570 (2006) 283–290.
[35] W. Silva Lyra, V.B. dos Santos, A.G.G. Dionízio, V.L. Martins, L.F. Almeida, E. Nóbrega Gaião, P.H.G.D. Diniz, E.C. Silva, M.C.U. Araújo, Digital image-based flame emission spectrometry, Talanta 77 (2009) 1584–1589.
[36] N. Maleki, A. Safavi, F. Sedaghatpour, Single-step calibration, prediction and real samples data acquisition for artificial neural network using a CCD camera, Talanta 64 (2004) 830–835.
[37] Y. Suzuki, M. Endo, J. Jin, K. Iwase, M. Iwatsuki, Tristimulus colorimetry using a digital still camera and its application to determination of iron and residual chlorine in water samples, Anal. Sci. 22 (2006) 411–414.
[38] A. Choodum, P. Kanatharana, W. Wongniramaikul, N. Nic Daeid, Using the iPhone as a device for a rapid quantitative analysis of trinitrotoluene in soil, Talanta 115 (2013) 143–149.
[39] A. Choodum, P. Kanatharana, W. Wongniramaikul, N. Nic Daeid, Rapid quantitative colorimetric tests for trinitrotoluene (TNT) in soil, Forensic Sci. Int. 222 (2012) 340–345.
[40] A. Choodum, N. Nic Daeid, Rapid and semi-quantitative presumptive tests for opiate drugs, Talanta 86 (2011) 284–292.
[41] K. Thongprajukaew, A. Choodum, B.E. Sa, U. Hayee, Smart phone: a popular device supports amylase activity assay in fisheries research, Food Chem. 163 (2014) 87–91.
[42] M. Kompany-Zareh, M. Mansourian, F. Ravaee, Simple method for colorimetric spot-test quantitative analysis of Fe(III) using a computer controlled hand-scanner, Anal. Chim. Acta 471 (2002) 97–104.
[43] K. Cantrell, M.M. Erenas, I. de Orbe-Payaí, L.F. Capitaín-Vallvey, Use of the Hue parameter of the Hue, saturation, value color space as a quantitative analytical parameter for bitonal optical sensors, Anal. Chem. 82 (2009) 531–542.
[44] B. Bruijns, Illicit Drugs Analysis on Chip: The Use of Lab-on-a-chip Technology for Forensic Science Applications, University of Amsterdam, 2011.

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Real time quantitative colourimetric test for methamphetamine detection using digital and mobile phone technology

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ABSTRACT

The Simon presumptive color test was used in combination with the built-in digital camera on a mobile phone to detect methamphetamine. The real-time Red-Green-Blue (RGB) basic color data was obtained using an application installed on the mobile phone and the relationship profile between RGB intensity, including other calculated values, and the colourimetric product was investigated. A wide linear range (0.1–2.5 mg mL^{-1}) and a low detection limit (0.0110 ± 0.0001–0.044 ± 0.002 mg mL^{-1}) were achieved. The method also required a small sample size (20 μL). The results obtained from the analysis of illicit methamphetamine tablets were comparable to values obtained from gas chromatograph-flame ionization detector (GC-FID) analysis. Method validation indicated good intra- and inter-day precision (2.27–4.49%RSD and 2.65–5.62%RSD, respectively). The results suggest that this is a powerful real-time mobile method with the potential to be applied in field tests.

1. Introduction

Methamphetamine is a synthetic stimulant which affects the central nervous system [1]. It is increasingly abused and has caused serious health and social problems. It is usually called "crank" if in the form of the powder hydrochloride salt, "Yaba" in Thai when in the form of tablets, and "Ice" in its clear crystal form. This illegal drug has been classified as a Category I narcotic under the Narcotic Act B.E. 2522 of Thailand, while it has been reclassified from a Class B to a Class A Schedule 2 drug under The Misuse of Drugs Act 1971 (UK) in 2007 [2].

Colourimetric presumptive tests are common methods used for field test. The Marquis and Simon presumptive chemical tests are recommended for methamphetamine by the United Nations International Drug Control Programme [1]. The Marquis test produces an orange-brown reaction, while a blue reaction is obtained from the Simon test. The latter test can be used to differentiate this drug from amphetamine *via* the selective reaction of the Simon's reagents with the secondary amine within methamphetamine. These tests are currently widely used as the presumptive tests for methamphetamine in most forensic laboratories because they are rapid, simple, and reliable. However they are currently only used to produce qualitative results. Quantitative analysis of methamphetamine is generally achieved within a forensic science laboratory using gas chromatography which may coupled with flame ionization detector (GC-FID) and/or mass spectrometry (GC–MS) [3–9].

Our previous work has shown a great potential for digital image-based analysis to be used to develop fast and direct quantitative determinations [10–12] which extend the potential value of colourimetric presumptive test methods. The method has been used for rapid quantitative analysis by color test for amphetamine and methamphetamine [10], opiates [12], and the explosive trinitrotoluene [11]. It has also been reported in other analytical applications [13–19]. These applications have been based on the analysis of basic red green blue (RGB) color data obtained from digital images [12,14] generated by a digital camera. During image processing, the reflected light from the colored products of a colourimetric test pass through three different filters, red, green, and blue, and is detected and recorded by image sensors

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such as charge-coupled devices (CCD) [14,19] or complementary metal oxide semiconductors (CMOS). The final color of the digital image is composed from the additive data of the three RGB filters after scaling and adjustment to compensate for variations in the conditions of capture. The RGB values of digital images can be measured for example using the image processing tool box in Matlab [15,20], Kylix [17,21], Visual basic [16,18], or Adobe Photoshop [10–12] and can be used to calibrate the images, concentrating on the analytes of interest. This method, thus, provides a basis on which quantitative colourimetric analysis of samples containing an unknown quantity of analyte can be undertaken.

In previous studies, standard digital cameras *e.g.* digital single-lens reflex (DSLR) cameras have been used to produce the color images. However, images can also be conveniently produced using built-in digital cameras in commonly available smart phones.

This study used the built-in digital camera of an iPhone and an iPhone application (app) for color analysis instead of any other image processing programs. This resulted in a more convenient analysis. The RGB values from the color products were also immediately available without the necessity to connect to an external computer as reported in previous studies. The rapid, portable, and accurate quantitative analysis of methamphetamine was achieved by using a simple colourimetric test only.

2. Materials and methods

2.1. Materials

Crystal methamphetamine standard (Ice; purity ~98.5%) and Yaba (as samples seized by law enforcement agencies) were obtained from the Drug Control Division, Food and Drug Administration Thailand (license number: 1003.2/790). Methamphetamine standard solutions were prepared with ultrapure water (Barnstead EasyPure II, Thermo fisher scientific, OH). Acetaldehyde was purchased from Aldrich Chemical Co. Ltd. (Dorset, England), and anhydrous sodium carbonate was purchased from Fisher Chemicals, Fisher Scientific UK Limited (Loughborough, UK). Sodium nitroprusside dihydrate (>98.0%) was purchased from Fluka (Sigma–Aldrich Chemie, Steinheim, Germany).

2.2. Colourimetric presumptive test (Simon test).

Two reagents were required for the Simon test, 10% (v/v) acetaldehyde in aqueous sodium nitroprusside solution (1%, w/v) (reagent 1) and 2% (w/v) sodium carbonate in water (reagent 2) [1]. The reagents were prepared under optimized conditions as follows: 10 μL of reagent 1 was added to 20 μL of the methamphetamine solution in a micro-tube. This was followed by 80 μL of reagent 2. The solution was then mixed by shaking and left to stand for 2 min prior to detecting its color intensities.

Each experiment was repeated 3 times. The linear range was investigated from 0.05 to 10 mg mL^{-1}. The average intensities of the red, green and blue colors from 3 replications were used to establish a calibration graph for each color. The limit of detection was calculated using standard methods (limit of detection = $y_B + 3S_B$ where y_B is the intercept of the calibration curve and S_B is the standard deviation of the blank) [22]. Precision was expressed as the percentage relative standard deviation for each color from the 3 replicate analysis.

2.3. RGB capturing system and procedure

A custom-built color detection box (Fig. 1) was used to eliminate any effects from environmental light. The box (8.0 cm × 11.0 cm × 5.5 cm) was made of opaque black corrugated plastic board with an internal white background. A flat cap PCR micro-tube was hung at the top of the box as a reaction container. A 5 W cool daylight Philips light bulb was put at the top of the box as the upper light source, 2 cm from the micro-tube (this was found to be the optimal distance). The intensity of the color product inside the micro-tube was detected using the ColorAssist app (FTLapps, Inc.) for an iPhone 4.0 in the flash-off mode.

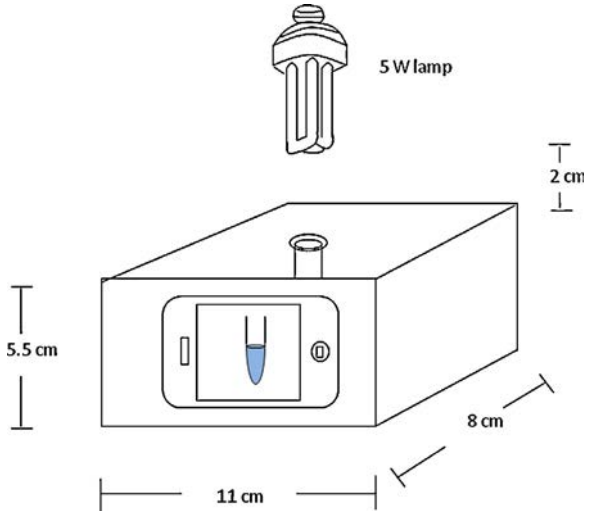

Fig. 1. Real time on-mobile color analysis system for methamphetamine detection.

2.4. Gas chromatography-flame ionization detector analysis

An Agilent 7980A gas chromatograph equipped with a flame ionization detector (Agilent Technologies, China) was used for sample quantification. Samples of 1 μL were injected and separated using a HP-5 capillary column (30 m length × 0.32 mm mm id × 0.25 μm film thickness). The column was kept at 100 °C for 1 min, increasing to 260 °C at a rate of 20 °C min^{-1} and held for 3 min [10]. A split ratio of 25 to 1 with a carrier gas (high-purity-grade helium) flow rate of 1.0 mL min^{-1} was used. The flow rate of fuel (hydrogen), oxidant (air zero), and make-up gas were 30, 300, and 25 mL min^{-1}, respectively. The inlet and detector were kept at 260 °C and 275 °C, respectively. The instrument was calibrated for methamphetamine in the range of 0.0025–2 mg mL^{-1} where $n = 6$ for each calibration standard injected.

2.5. Analysis of Yaba samples

Five seized Yaba samples obtained from the Drug Control Division of the Food and Drug Administration of Thailand. A sample of ten milligrams from each Yaba sample was extracted with 1 mL of water and sonicated for 5 min. The supernatant was analyzed using the Marquis and Simon test. Samples were also quantified using the iPhone method under test and using GC-FID. For gas chromatographic analysis, methanol was used to extract the methamphetamine from the Yaba.

3. Results and discussion

3.1. Simon test for methamphetamine

Methamphetamine reacts with acetaldehyde to produce an enamine intermediate which then reacts with sodium nitroprusside to produce an immonium salt. This salt can then be hydrolyzed to a Simon-Awe complex to give a blue colored product [10,23]. At high concentrations (>1 mg mL^{-1}) the blue product remains for 3–4 min before gradually changing to purple which becomes deeper

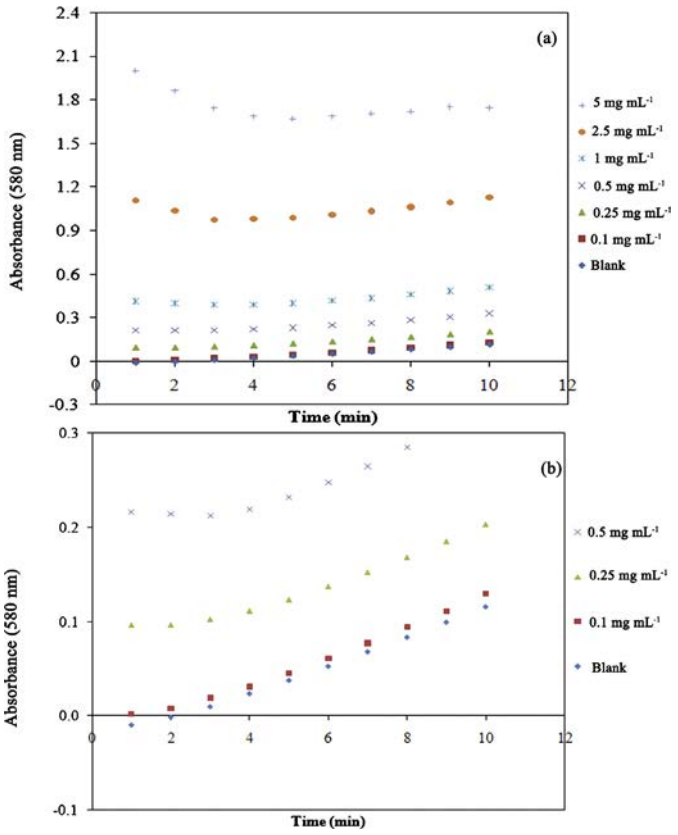

Fig. 2. Spectrophotometric detection (580 nm) of Simon-Awe product from methamphetamine.

over time. In this study, the molecular absorption (580 nm [24]) of these blue products was found to decrease within 3–4 min before increasing (Fig. 2a). The decrease in absorption seems to relate to the color change indicating the decomposition of the Simon-Awe product. At low concentrations of methamphetamine (<1 mg mL^{-1}), 1), the blue product was observed for only a few seconds and then rapidly changed to purple and the absorption remained constant for 2–3 min before increasing (Fig. 2b). Two minutes post reagent addition was thus selected as the optimum time frame for the digital image capture however some changes in absorption of blue product was observed at higher concentrations of methamphetamine (2.5 and 5.0 mg mL^{-1}). The molecular absorption of the blue products increased with methamphetamine concentration and a calibration equation of $y = (0.38 \pm 0.01)x + (0.01 \pm 0.03)$ was obtained for the detection of methamphetamine ranging from 0.1 to 5.0 mg mL^{-1}.

3.2. Digital image analysis

Objects selectively absorb and reflect certain wavelengths of light [10,14]. The reflected light passes through the camera filters (RGB) when the object is being photographed and is read by the image sensors (CCD or CMOS) within the camera. The additive data of the three RGB filters then presents the final color in the digital image [10,15].

The analysis of a digital image provides analytical RGB data ranging from 0 to 255 for each channel. The user obtains the R, G, B values of 0, 0, 0 when the digital color is black, and similarly 255, 255, 255 for a white image. In this study, the ColorAssist iPhone application was used to capture the RGB values in real-time resulting in a much shorter analysis time than other commonly used to determine RGB values *e.g.* Matlab [15,20], Kylix [17,21], Visual basic [16,18], and Adobe Photoshop [10–12].

The simple detection system previously described was used to capture the RGB values of the product from the Simon test without any interfering light. A flat capped PCR micro-tube was used as the reaction tube to eliminate any effect from reflected light which can commonly occur in glass tubes [16] and the clear lid of the micro-tube allowed the white light to pass through the sample which was then selectively absorbed (or reflected).

3.2.1. Individual RGB values

The RGB intensity values (I_R, I_G, and I_B) obtained from the image captured by the iPhone app were related to concentrations of methamphetamine as illustrated in Fig. 3. The intensities of red, green, and blue decreased with increasing methamphetamine concentrations from 0.1 to 2.5 mg mL^{-1} indicated by the appearance of the purple color at these concentration ranges. The blue intensity was highest and remained constant for longer time frames at higher concentrations (2.5–10 mg mL^{-1}). These findings corresponded to the results obtained from a spectrophotometer as described in Section 3.1.

The calculated molecular absorption of the products [10–12,25] increased with methamphetamine concentrations of up to 2.5 mg mL^{-1} and above that, became constant (Fig. 4). Although

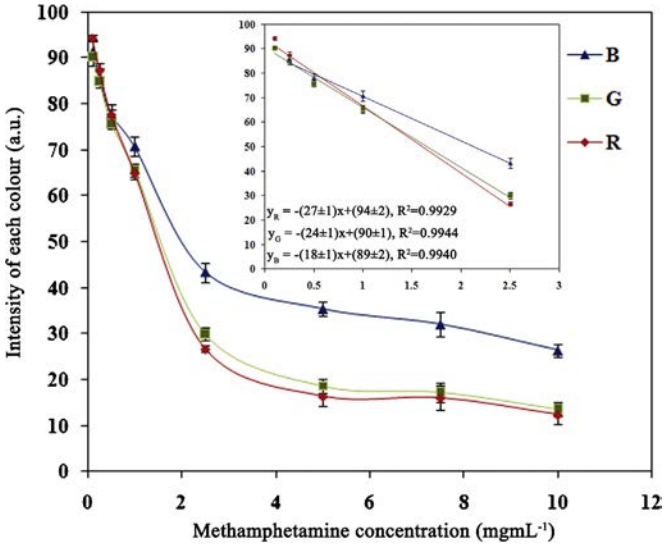

Fig. 3. Relationship between intensity of each color and methamphetamine concentration (all were point-to-point lines, except linear portions which were regression lines).

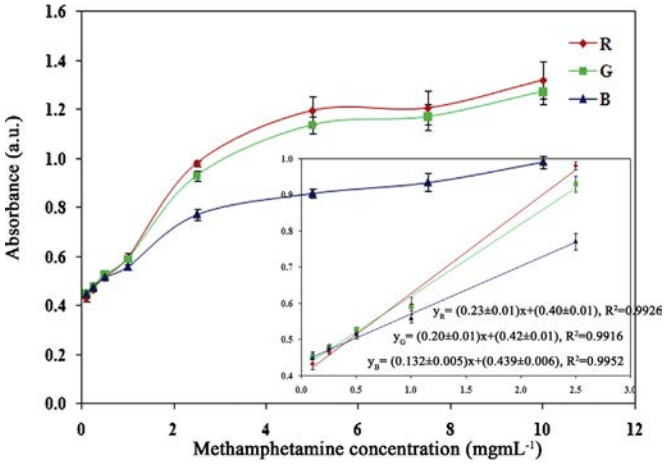

Fig. 4. Relationship between absorbance of each color and methamphetamine concentration.

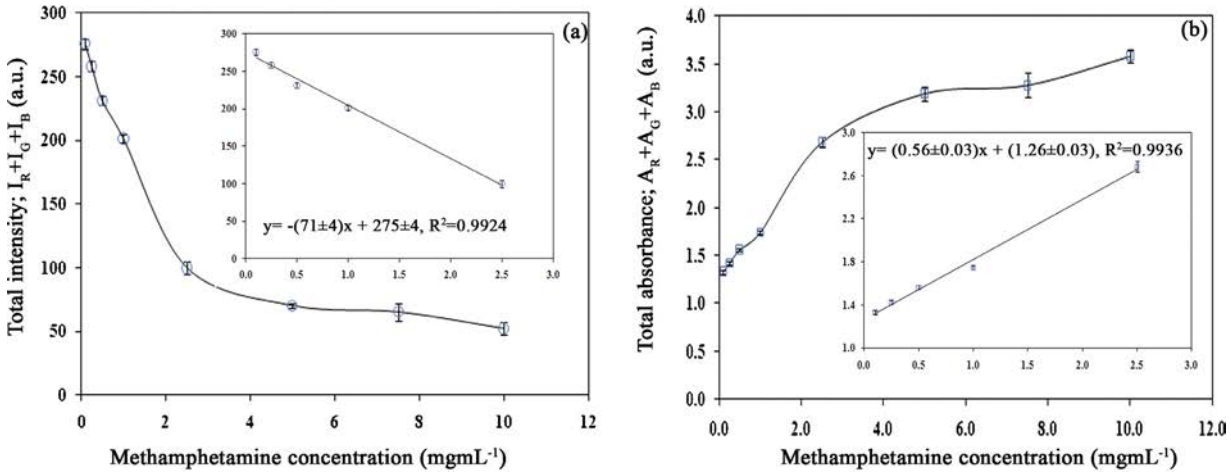

Fig. 5. Relationship between (a) total intensity (b) absorbance and methamphetamine concentration.

these relationships were similar to the results from spectrophotometric methods, the sensitivity (slope of the calibration graph) of the digital image–RGB method for the red channel was lower (0.23 ± 0.01 a.u. mL mg^{-1} against 0.38 ± 0.01 a.u. mL mg^{-1}). The linear range of this method was also shorter (0.1–2.5 mg mL^{-1} compared with 0.1–5.0 mg mL^{-1}). The lowest absorption was obtained from the blue channel due to its higher reflectivity resulting in the appearance of this color as the test result.

3.2.2. Total RGB values

The relationships derived from the combination of the total RGB data, may provide additional valuable information not available

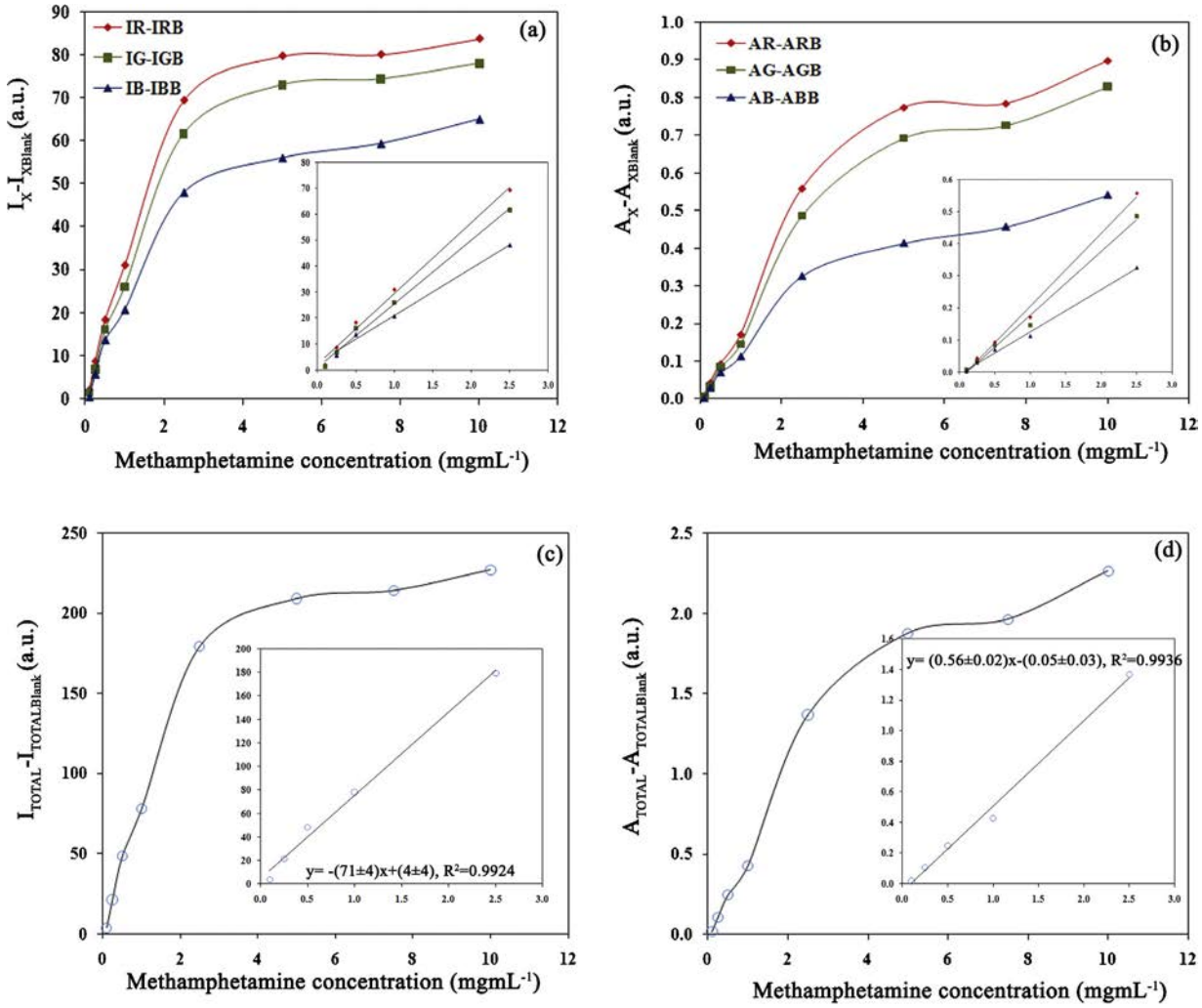

Fig. 6. Relationship between blank subtracted RGB values and methamphetamine concentration (a) each individual intensity (b) absorbance (c) total intensity (d) total absorbance.

Table 1
Analytical performance of the proposed method.

Relationships[a]	Sensitivity (a.u. mL mg^{-1})	Linear range (mg mL^{-1})	LOD[b] (mg mL^{-1})
I_R	27 ± 1	0.1–2.5	0.044 ± 0.002
I_G	24 ± 1	0.1–2.5	0.0110 ± 0.0001
I_B	18 ± 1	0.25–2.5	0.0243 ± 0.0007
A_R	0.23 ± 0.01	0.1–2.5	0.035 ± 0.001
A_G	0.20 ± 0.01	0.1–2.5	0.0161 ± 0.0003
A_B	0.132 ± 0.005	0.1–2.5	0.0216 ± 0.0005
I_{TOTAL}	71 ± 4	0.1–2.5	0.0200 ± 0.0005
A_{TOTAL}	0.56 ± 0.03	0.1–2.5	0.01378 ± 0.00009
$I_R - I_{Rblank}$	27 ± 1	0.1–2.5	0.123 ± 0.005
$I_G - I_{Gblank}$	24 ± 1	0.1–2.5	0.142 ± 0.002
$I_B - I_{Bblank}$	18 ± 1	0.25–2.5	0.196 ± 0.006
$A_R - A_{Rblank}$	0.23 ± 0.01	0.1–2.5	0.137 ± 0.006
$A_G - A_{Gblank}$	0.20 ± 0.01	0.1–2.5	0.150 ± 0.003
$A_B - A_{Bblank}$	0.132 ± 0.005	0.1–2.5	0.191 ± 0.005
$(I_{TOTAL} - I_{TOTALblank})$	71 ± 4	0.1–2.5	0.147 ± 0.003
$(A_{TOTAL} - A_{TOTALblank})$	0.56 ± 0.02	0.1–2.5	0.150 ± 0.002

[a] I, intensity; A, absorbance.
[b] LOD = $y_B + 3S_B$ [22].

from the individual values [15]. These were calculated both in terms of intensity ($I_{TOTAL} = I_R + I_G + I_B$) and absorbance ($A_{TOTAL} = A_R + A_G + A_B$) and related to methamphetamine concentrations and are illustrated in Fig. 5. It was found that the sensitivity of the total values was higher than those of the individual values (0.56 ± 0.03 a.u. mL mg^{-1} compared to 0.23 ± 0.1 a.u. mL mg^{-1} for the red channel), but covered the same linear range (0.1–2.5 mg mL^{-1}).

3.2.3. Blank subtracted RGB values

Methamphetamine pills available in Thailand (sold as Yaba) are commonly orange in color, but are occasionally white, green, and blue. These various colors may affect the RGB values of the blue-purple product. The RGB values of the blank were therefore taken into account by subtracting them from the RGB values of the product. Simon's reagents and the ultrapure water without methamphetamine standard was used as the blank in this section, but Yaba extract in ultrapure water without Simon' reagents was recommended for illicit sample analysis. The relationships between the subtracted RGB values and the methamphetamine concentrations both as individual and total intensities, and their absorbances are presented in Fig. 6. Both the intensity and absorbance of the subtracted RGB values increased with increasing methamphetamine concentration indicating a greater difference between the color of the blank and the color of the product with increasing drug concentrations. Red demonstrated the highest sensitivity echoing the results when the individual RGB values were considered.

3.3. Analytical performance and validation

The analytical performance including the sensitivity, linear range, and limit of detection of the proposed method is presented in Table 1. The precisions expressed by %RSD from three repetitions of the analyses (1 mg mL^{-1}) within the same day (intra-day) were in the range of 2.27–4.49%, and were in the range of 2.65–5.62% for analyses performed on three separate days (inter-day). Accuracies were evaluated as a percentage relative error ($(x_e - x_{control})/x_{control}$*100) generated by analyzing a known concentration of standard methamphetamine solution ($x_{control}$ = 0.75 mg mL^{-1}) and quantified using an external calibration of the I_G equation (x_e). The relative error of +1.48% was obtained indicating good accuracy.

3.4. Analysis of Yaba samples

Five seized Yaba samples were extracted and analyzed using GC-FID (Fig. 7). The GC-FID was calibrated and demonstrated good linearity in the range of 0.0025–1.0 mg mL^{-1} of methamphetamine in methanol ($y = 408.85x - 2.78$; $R^2 = 0.9996$; %RSD < 4.4). The results displayed in Table 2, clearly demonstrated a very good correlation between the accurately determined concentration of methamphetamine in the samples using GC-FID and the real-time mobile quantitative method. This also indicated that the color tests

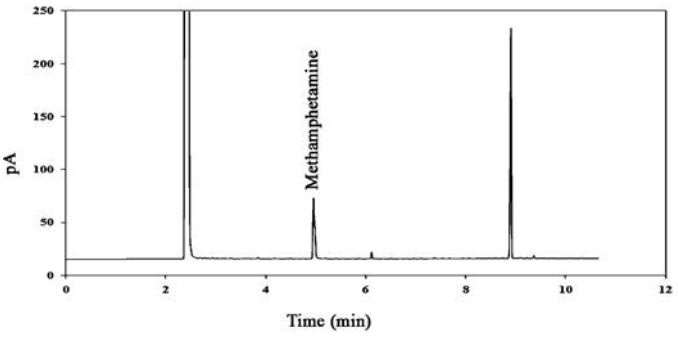

Fig. 7. Chromatogram of Yaba tablet (sample 3).

Table 2
Analysis of Yaba samples.

Sample no.	% of methamphetamine in Yaba samples		% relative error	$P = 0.05$
	Digital image method	GC-FID		
1.	20 ± 1%	19.9 ± 0.4%	+0.2%	ns[a]
2.	16 ± 1%	17.4 ± 0.1%	−7.3%	ns
3.	20.1 ± 0.9%	18.7 ± 0.2%	+7.1%	ns
4.	19 ± 2%	19.3 ± 0.3%	−1.2%	ns
5.	19 ± 1%	18.1 ± 0.5%	+4.3%	ns

[a] ns, no significant difference.