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An iPhone-based digital image colorimeter for detecting tetracycline in milk

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ABSTRACT

An iPhone-based digital image colorimeter (DIC) was fabricated as a portable tool for monitoring tetracycline (TC) in bovine milk. An application named ColorConc was developed for the iPhone that utilizes an image matching algorithm to determine the TC concentration in a solution. The color values; red (R), green (G), blue (B), hue (H), saturation (S), brightness (V), and gray (Gr) were measured from each pictures of the TC standard solutions. TC solution extracted from milk samples using solid phase extraction (SPE) was captured and the concentration was predicted by comparing color values with those collected in a database. The amount of TC could be determined in the concentration range of 0.5–10 μg mL^{-1}. The proposed DIC-iPhone is able to provide a limit of detection (LOD) of 0.5 μg mL^{-1} and limit of quantitation (LOQ) of 1.5 μg mL^{-1}. The enrichment factor was 70 and color of the extracted milk sample was a strong yellow solution after SPE. Therefore, the SPE-DIC-iPhone could be used for the assay of TC residues in milk at the concentration lower than LOD and LOQ of the proposed technique.

1. Introduction

Digital image colorimetry (DIC) has been used for quantitative determinations in analytical chemistry (Chuan-Xiao, Xiang-Ying, Bin, & Hui-Ting, 2007; Lopez-Molinero, Linan, Sipiera, & Falcon, 2010; Suzuki, Endo, Jin, Iwase, & Iwatsuki, 2006; Zamora et al., 2011; Lima, Andrade, Barreto, Almeida, & Araujo, 2013; Lopez-Molinero, Cubero, Irigoyen, & Piazuelo, 2013). DIC could be considered as a new technique which uses digital images captured by hand-scanner (Kompany-Zareh, Mansourian, & Ravaee, 2002), digital cameras (Byrne, Barker, Pennarun-Thomas, & Diamond, 2000; Dan-Qun et al., 2010; Leon, Mery, Pedreschi, & Leon, 2006; Suzuki et al., 2006), web-cams (Gaiao et al., 2006; Bang-iam, Udnan, & Masawat, 2013; Lima et al., 2013; Torres et al., 2011) or mobile device cameras (Garcia et al., 2011; Lopez-Ruiz et al., 2012). Recently, various DIC devices have been proposed for identifying analytes, coupled with the creation of an application for mobile intelligent system; iOS (iPhone) (Choodum, Kanatharana, Wongniramaikul, & Daeid, 2013; Choodum et al., 2014; Thongprajukaew, Choodum, Sa-E, & Hayee, 2014; Yetisen, Martinez-Hurtado, Garcia-Melendrez, Vasconcellos, & Lowe, 2014) and Android (Sumsung) (Kassal, Steinberg, & Steinberg, 2013; Sumriddetchkajorn, Chaitavon, & Intaravanne, 2013, 2014) by means of photography and processing the captured images. Certain manufacturers' cameras (particularly the cameras of iPhone and Samsung) provide high resolutions that are highly capable of detecting differences in color within a small image area. The sensors of those cameras provide a larger pixel area, and hence the pictures are clear and sharp, even at high zoom. Such sensors are appropriate for the application of a technique for the analysis of substances of interest, both quantitative and qualitative. Several researchers have been developing the mobile phone based digital image colorimeter into two systems; opened system and closed system. The opened system uses only mobile phone for analysis. The external factors such as high brightness of ambient light, position of sample and focusing distance will affect the images. The closed system is better than the opened system because it uses mobile phone coupled with the fabricated photography lightbox which the external factors could be controlled. The main purpose of this research is to designed and fabricated the closed system DIC using iPhone embedded with ColorConc application for determining TC in bovine milk. Because the light source which used white light emitting diode (LED) provides not true white light affected to the captured pictures, therefore, fluorescent lamp was used instead to solve this problem in this research work.

Tetracyclines (TCs), a derivative of hydronaphthacene ring, were used as antibiotics suppressing the growth of bacteria.

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There are 7 types of TC but only 3 (tetracycline, chlortetracycline and oxytetracycline) are used in agriculture, both in plants and animals. The frequent use of TCs is high cost and continue to cause the problems of drug residues in tissues, especially in milk. Milk is a popular diary product consumed across all ages because it is a source of 5 groups of nutrients including proteins, fats, carbohydrates, minerals and vitamins. However, if the manufacturer does not check the quality of milk before selling or exporting, antibiotic residues might affect the health of consumers. TCs residues in milk can be analyzed by various methods. Sample preparation techniques especially solid phase extraction (SPE) is needed before analysis due to the advantages over traditional liquid–liquid extraction. Milk is normally processed under reversed phase or ion exchange SPE condition (Sigma–Aldrich, 2013). C18 sorbent was found to provide good recoveries (Furusawa, 2003) thus it was selected to be used in this work for extraction of TC in milk before analysis by the proposed iPhone-based digital image colorimeter comparing with the UV–Visible spectrophotometer.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade and all solutions were prepared by using deionized (DI) water (Prima Reverse Osmosis, Maxima water purification system, Elga Ltd., England). The stock solution of tetracycline (1000 μg mL^{-1}) was prepared by dissolving the appropriate amount of its hydrochloride salt (Sigma–Aldrich, Germany) in 5.0 M hydrochloric acid (HCl, Labscan, Thailand). The solutions are stable for more than 1 month when kept at 4 °C and protected from sunlight. The TC standard solutions were prepared daily from the previous solution by careful dilution with 5.0 M HCl.

2.2. Sample preparation procedures

TC residues were determined in three commercial types of milk (no flavor): (a) ultra-high temperature processing (UHT), (b) pasteurized and (c) sterilized milk obtained from the local market of Phitsanulok area of Thailand in 2014. An aliquot of 5 mL of milk sample was placed into 15 mL a plastic centrifuge tube and 5 mL of 10% (v/v) trichloroacetic acid (TCA, Fisher Scientific, UK) was added. The solution was shaken using vortex mixture (Vortex-genie G-560E Scientific Industries, U.S.A.) at room temperature in the middle level for 5 min then centrifuged (Hettich zentrifugen D-7200 Tuttlingen, Germany) at 2150×*g* for 10 min. The supernatant was then passed through a 0.45 μm pore size nylon filter followed by applied to a SPE cartridge (VertiPak C18-LP SPE Tube 6 mL, 500 mg, Vertical Chromatography Co., Ltd.) preconditioned with 4 mL methanol and 4 mL water. The cartridge was washed with 4 mL water and allowed to dry for 2 min before eluted with 3 mL methanol. Nitrogen gas was used for solvent purging until the extracted solution was 2.5 mL then photographed in the fabricated DIC-iPhone and measured with UV–Vis Spectrophotometer.

2.3. Apparatus

Double beam UV–Vis Spectrophotometer (Lambda 20 Perkin Elmer, USA) was used for the results comparison with the DIC-iPhone measurement.

2.4. DIC system

A schematic of the iPhone-based digital image colorimeter is depicted in Fig. 1. In order to protect the system from outside light, a photography lightbox was made from plywood (10 × 16.5 × 25 cm) with the internal walls sprayed with black paint. An iPhone model 5S (Apple Inc., USA) which has the developed ColorConc application was utilized for capturing digital image from outside the lightbox via the drill hole. The TC solution filled in the sample cell (quartz cuvette, 10 mm Hellma, U.S.A.), which was located in a sample cell holder made by a white plastic, was photographed under fluorescent light (5 W, Philips, USA). The focusing distance was fixed at 10.5 cm.

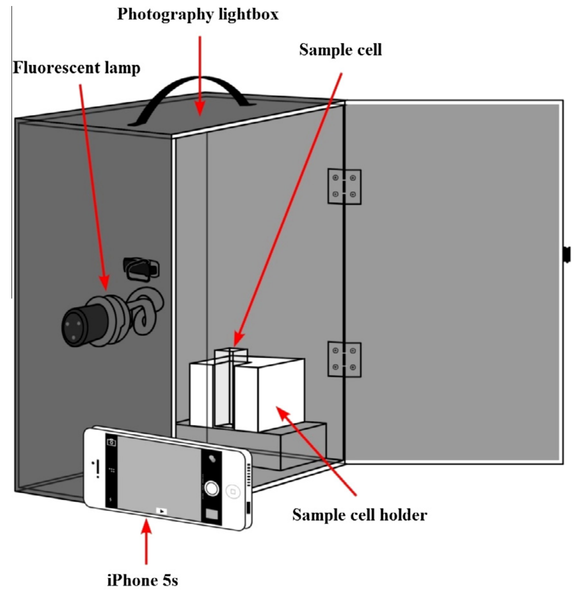

Fig. 1. Schematic diagram of the iPhone-based digital image colorimeter.

2.5. iOS application

A mobile application named as ColorConc was developed for iPhone devices on the iOS operating system. The screenshots which show how the app operates are shown in Fig. 2(A–E). The application consists of a calibration or 'learning' module and a 'testing' module. The application stores data in a local database so that multiple projects can be maintained independently.

2.5.1. Calibration/learning

In order to identify samples correctly in the 'testing' module, first the 'learning' module calibrates the software with a set of images obtained from reference concentrations. Each image will be associated with a concentration level. On creation of a new project, the set of images will be empty (Fig. 2A and B). First, the user will prepare the chemical solution and place it within the photography lightbox. Then the user will slide the iPhone device into position tightly positioning the iPhone's camera at the center of the opening on the lightbox (thus no external light can interfere). The user will manually press a button in the app to take the photo. The image data is processed by cropping to a small central 300 × 300 pixel square area of the image (Fig. 2C). Next, an average color value is calculated from the individual red, green and blue components of the pixels in the image:

$$R_{avg} = \frac{\sum_{i=1}^{n} R_i}{n}$$

$$G_{avg} = \frac{\sum_{i=1}^{n} G_i}{n}$$

$$B_{avg} = \frac{\sum_{i=1}^{n} B_i}{n}$$