Abstract

Digital-image colorimetry has been used for the determination of formaldehyde using purpald reaction on a simply fabricated paper-based analytical device (PAD). The PAD can be simply fabricated by cutting filter paper to small piece (6 mm diameter) using a puncher. The reaction between formaldehyde and purpald reagent was performed and generated the purple product on the PAD. A photograph of the resulting PAD, placed in a home-made mini-studio box, was taken by a smart phone’s camera with the fixed camera settings: ISO value, white balance and shutter speed. The photograph then was analyzed the color intensity by ImageJ computer software. Using 5.0 µL of purpald reagent and the reaction time of 720 s, the standard calibration graph was in linear-log model at the range of 1.0-20 mg L⁻¹ formaldehyde with $R^2$ of 0.9919. This method will be an alternative method which is simple and low-cost for the formaldehyde determination.

Keywords: Digital-imaging colorimetry, Formaldehyde detection, Paper-based analytical device

Introduction

Detection of formaldehyde is very important in the field of healthcare because formaldehyde is a well-known toxic compound that is widely used as preservative in household products, pharmaceutical products, paints and even cosmetics. Formaldehyde is a colorless gas emitted into the indoor environment by furniture and many other sources. Although it is not allowed to be used in foods, agricultural industries, in some area of the third-world country still add formaldehyde in fertilizer that can be contaminated in soil and the agricultural products [1]. Formaldehyde is health hazard because it can also cause irritation to eyes and nose, central nervous system, damage immune system and respiratory disease. Moreover, in 2006, formaldehyde is classified by the International Agency for Research on Cancer (IARC) that it is carcinogen to human even at low concentrations [2]. The guideline value of formaldehyde concentration determined by the World Health Organization (WHO) is 82 µg L⁻¹.
Although, there have been several methods for determination of formaldehyde concentration in recent years, for example, fluorescence spectroscopy [3, 4], gas chromatography-mass spectrometry [5] and colorimetry [6, 7], some severe problems such as toxic reagents or expensive cost of instruments are still unsolved. A simple, low-cost and sensitive method for determination of formaldehyde, therefore, is required in chemical point-of-care. Recently, colorimmetrical quantification method have been developed using a paper-based device (PAD), which is a low-cost material, coupled with an inexpensive technique, digital-imaging colorimetry.

Digital-imaging colorimetry is a new analytical method based on analysis of colorimetrical reaction that can be taken a photograph by a digital camera, a web-cam or a hand scanner. The photograph was recorded in digital mode by camera sensors, such as CMOS (complementary metal-oxide semiconductors) and CCD (charge coupled detectors). And then the photograph can be analyzed the color intensity of the chemical reaction by an image manipulation software [8].

In this research, hence, the digital-imaging colorimetry has been developed for formaldehyde detection by purpald reaction on a PAD. The proposed method can be an alternative approach with inexpensive, simple and portable technique for any chemical quantification by digital-imaging colorimetry.

Materials and Methods

Chemicals and reagents

Formaldehyde solution (HCHO, 37%) and sodium hydroxide solution (NaOH, 97%) were purchased from Ajax Finechem (Australia). Stock standard solutions of 1,000, 100 and 10 mg L⁻¹ formaldehyde were prepared by dilution with deionized water. Purpald reagent (>99%) obtained from Sigma-Aldrich (Singapore) was accurately weighed 20.0 mg and then diluted with 5% (w/v) of NaOH to be 0.273 M purpald reagent. All chemicals and reagents were used without further purification. The working standard solutions were prepared from these stock solutions to the desired concentrations.

Fabrication of the paper-based analytical devices (PADs)

A piece of filter paper, Whatman No. 1 (Cole-Parmer, USA) was simply cut by a common puncher into small pieces with 6 mm diameter. The small pieces were used as a PAD for the determination of formaldehyde. It can be kept in a desiccator at the room temperature.

Experimental procedure and camera settings

A PAD was placed on a watch glass. A 5.0 µL of 0.273 M purpald reagent was dropped on the PAD, and then allowed to dry at the room temperature for a few minute. Next, a 5.0 µL of standard or sample solution was dropped onto the PAD that the reaction between purpald reagent and formaldehyde would then occur. The change of the PAD’s color from colorless to purple was observed by taking photograph using a mobile
phone (Asus Zenfone 2, Asus, Thailand) and a home-made mini-studio box.

The home-made mini-studio box, shown in Figure 1, was made of acrylic glass (PMMA) by ourselves. There is a series of light emitting diodes (LEDs) in the box. The box has 2 layers of 3 mm thick acrylic glass; inside is white for scattering the light in the box, and outside is black for preventing the interloping light. A mobile phone (Asus Zenfone 2, Asus, Thailand) was placed on the box that it can taking a photograph through a hole on the box. To fix factors of camera, all photographs were taken by the same mobile phone using the camera application in the manual mode with specific parameters as shown in Table 1.

![Figure 1. Mini-studio box made of acrylic glass](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO value</td>
<td>100</td>
</tr>
<tr>
<td>White balance</td>
<td>6500K</td>
</tr>
<tr>
<td>Speed shutter</td>
<td>1/250 s</td>
</tr>
</tbody>
</table>

**Digital-imaging colorimetry**

A photograph of the resulting PAD was analyzed by ImageJ computer software [9] by following orders. First, the photograph was converted to be 8-bit or gray-scale type, and then changed to be negative by inverse-scale function. A specific area of the resulting PAD was selected and measured the average of the color intensity. Furthermore, to normalize the obtained color intensity, a piece of paper with black and white was placed in the same photograph’s frame of the PAD. The black and white area of the paper were also measured the color intensity to get darkness limit and brightness limit, respectively, of the camera’s sensor. The color intensity of the PAD can be normalized by the following equation:

\[
\text{normalized color intensity (\%)} = \frac{\text{measured color intensity} - \text{brightness limit}}{\text{darkness limit}} \times 100
\]

**Results and Discussion**

**Effect of reagent volume with fixed concentration of formaldehyde**

Three different volumes of 1.0, 3.0 and 5.0 µL of 0.273 M purpald reagent were dropped on each PAD. A working solution of 50 mg L\(^{-1}\) formaldehyde was consequently dropped 5.0 µL
volume onto the PAD. The reaction between formaldehyde and purpald reagent occurred that the PAD’s color changed to purple. After the PAD was put in the mini-studio box for exactly 720 s (or 12 minutes), the photograph of the PAD was taken by the mobile phone with chosen parameters as shown in Table 1. The resulting photographs of each PAD were analyzed the color intensity by ImageJ. The results in Figure 2 showed that the color intensity increased when the volume of the reagent increased, and the small reagent volume of 1.0 µL could not spread on a whole area of the PAD that resulted in the high standard deviation of the obtained color intensity.

According to the results, it was clear that the volume of purpald reagent had to be used more than 1.0 µL, however, the optimum reagent volume could not be chosen by this experiment with the fixed formaldehyde concentration because it was not only the sensitivity refers to the analytical performance, but also the linearity was needed to be concerned. The further experiment was, therefore, set to investigate the effect of reagent volume with various concentration of formaldehyde.

**Figure 2.** Effect of reagent volume on the obtained color intensity of the solution containing 50 mg L⁻¹ formaldehyde with the reaction time of 720 s; the inset is the typical photographs of each PAD.

**Effect of reagent volume with various concentrations of formaldehyde**

Volume of purpald reagent was varied in the range of 2.0-5.0 µL to investigate the effect on the digital-imaging colorimetry for the determination of formaldehyde at various concentrations between 0.10 and 100 mg L⁻¹ formaldehyde. The formaldehyde standard solution was dropped 5.0 µL volume onto the reagent-dropped PAD. The PAD was taken a photograph using the mini-studio box at the reaction time of 720 s, and then the photograph was analyzed the color intensity by ImageJ (Figure 3). It was found that the obtained color intensity dramatically increased when the concentration of
formaldehyde was from 1.0 and 25 mg L\(^{-1}\) for all series of the used reagent volume; whilst, the intensity slightly changed in the ranges of lower concentration (0.10-1.0 mg L\(^{-1}\)) and higher concentration (25-100 mg L\(^{-1}\)). Therefore, the dynamic range of the relationship between the intensity and the formaldehyde concentration would be in the range of 1.0-25 mg L\(^{-1}\).

To confirm that, the experiment was re-investigated by using formaldehyde concentration in the range of 1.0-20 mg L\(^{-1}\). The color intensity, obtained from that used the various volume of purpald reagent, was not significantly different with the other at the same concentration of formaldehyde (Figure 4). Hence, the linearity from each different volume of purpald reagent were examined that the equations of linear regression and the determination coefficient (R\(^2\)) were reported in Table 2. The relationship of those calibration graph were not linear that the R\(^2\) were in the range of 0.7066 to 0.7919. However, the calibration graphs were illustrated in a linear-log model, a relationship between the color intensity and the logarithmic function of formaldehyde concentration. It was found that the linear-log models of all series of reagent volume provided linear relationships with good R\(^2\) as shown in Table 2. According to the results, the linearity from using 4.0 and 5.0 µL of purpald reagent showed higher sensitivity than the others with good R\(^2\) of 0.9926 and 0.9919, respectively. In the further experiment, 5.0 µL of purpald reagent was chosen.
Figure 4. Effect of reagent volume on the digital-imaging colorimetry for the determination of formaldehyde at various concentrations between 1.0 and 20 mg L\(^{-1}\) with the reaction time of 720 s.

Table 2. Summary of linear regression with \(R^2\) in linear-linear model and linear-log model for the determination of formaldehyde in the range of 1.0-20 mg L\(^{-1}\) concentration by the digital-imaging colorimetry using various volume of purpald reagent.

<table>
<thead>
<tr>
<th>Volume of purpald reagent (µL)</th>
<th>Linear-linear model</th>
<th>Linear-log model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equation</td>
<td>(R^2)</td>
</tr>
<tr>
<td>2.0</td>
<td>(y = 2.06x + 34.81)</td>
<td>0.7066</td>
</tr>
<tr>
<td>3.0</td>
<td>(y = 2.69x + 30.77)</td>
<td>0.7638</td>
</tr>
<tr>
<td>4.0</td>
<td>(y = 3.11x + 27.32)</td>
<td>0.7919</td>
</tr>
<tr>
<td>5.0</td>
<td>(y = 3.04x + 26.94)</td>
<td>0.7867</td>
</tr>
</tbody>
</table>

\(x\) is concentration of formaldehyde and \(y\) is normalized color intensity.

Effect of reaction time

The kinetic of the colorimetric reaction between purpald reagent and formaldehyde on the PAD was also studied. Two different concentrations of formaldehyde of 0.1 and 50 mg L\(^{-1}\) was determined by the proposed method using 5.0 µL of 0.273 M purpald reagent. Each resulting PAD was placed in the mini studio-box, and then taken photographs every 10 s for 15 minutes. The photographs were analyzed by ImageJ. The changes of the color intensity related with the reaction time are shown in Figure 5. For the higher concentration of 50 mg L\(^{-1}\) formaldehyde, the obtained intensity rapidly changed and then was stable within 150 s. On the other hand, the purple color of the PAD determining the lower concentration of 0.1 mg L\(^{-1}\) formaldehyde leisurely appeared; its analyzed intensity of purple color was stable after 700 s reaction time. Therefore, the reaction time of at least 150 s were recommended to be used for the determination of formaldehyde by digital-imaging colorimetry using purpald reagent, however, the result obtained at the reaction time of 700 s would provide good precision for trace analysis.
Figure 5. Relationship between the color intensity and the time of the reaction between purpald reagent and formaldehyde in various concentration of 0.1 mg L$^{-1}$ (dotted line) and 50 mg L$^{-1}$ (solid line).

Conclusions

The simple paper-based analytical device (PAD) has been developed for formaldehyde detection by digital-imaging colorimetry. It clearly presented that the mobile phone’s camera can be used as a colorimetrical transducer providing good results under the fixed camera settings: ISO value of 600, white balance of 6500K and shutter speed of 1/250 s. The calibration graph for the determination of formaldehyde appropriated with the linear-log model in the range of 1.0-20 mg L$^{-1}$ formaldehyde from the experiment using 5.0 µL of 0.273 M purpald reagent and 720 s reaction time (Figure 6). In addition, this work can be a promising prototype of an alternative method by digital-imaging colorimetry for chemical quantification with many advantages including a low-cost device, a low consumption of sample and reagent, a rapid analytical method and also an anywhere-laboratory.

Figure 6. Calibration graph of the linear-log model for the determination of formaldehyde in the range of 1.0-20 mg L$^{-1}$ concentration by the digital-imaging colorimetry using 5.0 µL of 0.273 M purpald reagent.
Acknowledgements

The authors are grateful for financial support from the Thailand Research Fund through Research Team Promotion Grant (RTA5780005). E.Punrat also thanks the Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University for the Postdoctoral Fellowship.

References


