SMARTPHONE – BASED COLORIMETRIC ANALYSIS FOR DETERMINATION OF PHENOL IN INDUSTRIAL EFFLUENT

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TÓM TẮT

XÁC ĐỊNH PHENOL TRONG NƯỚC THẢI CÔNG NGHIỆP BẰNG PHƯƠNG PHÁP ĐO MÀU SỬ DỤNG ĐIỆN THOẠI THÔNG MINH

Một phương pháp mới, đơn giản, rẻ tiền, đáng tin cậy và có độ nhạy cao để phân tích phenol có trong nước thải công nghiệp đã được phát triển và xác nhận giá trị sử dụng. Phương pháp này dựa trên phản ứng oxy hóa khử của phenol với hỗn hợp thuốc thử $K_3[Fe(CN)]_6$ và $FeCl_3$ để tạo thành phức Prussian Blue màu xanh. Phenol được xác định sau khi sử dụng điện thoại thông minh chụp hình phức Prussian Blue và số hóa hình ảnh này bằng phần mềm ImageJ với màu xanh lam của không gian màu RGB. Các điều kiện phản ứng ảnh hưởng đến sự hình thành phức Prussian Blue đã được khảo sát và tối ưu hóa. Phương pháp này đã được xác nhận giá trị sử dụng hoàn toàn theo hướng dẫn của ICH và đã áp dụng thành công để xác định phenol trong nước thải của nhà máy giấy. Đồ thị hồi quy tuyến tính trong phạm vi nồng độ của phenol từ 0.01ppm đến 1.5ppm với hiệu suất thu hồi trung bình là 98.6 ± 0.29 (%), giới hạn phát hiện là 3.0ppb và giới hạn định lượng là 11ppb. Sử dụng một lượng rất nhỏ mẫu và thuốc thử là một trong ưu điểm đáng chú ý khác của phương pháp này (20 µL/một lần phân tích) do đó nó đáp ứng được với những nguyên tắc của phân tích xanh. Phân tích dựa trên điện thoại thông minh cho kết quả tốt tương tự như UV/Vis, do đó, có thể sử dụng điện thoại thông minh làm công cụ thay thế cho các phân tích đo màu xác định phenol. Phương pháp này đơn giản, chi phí thấp, thân thiện với môi trường, có thể mang xách, nhạy, và đặc biệt, không yêu cầu nhân viên được đào tạo nên có thể sử dụng ở vùng sâu vùng xa với nguồn lực hạn chế hoặc trong công việc hàng ngày của các phòng thí nghiệm trong các ngành công nghiệp.

Từ khóa. *phenol, nước thải công nghiệp, phức Prussian blue, đo màu dựa trên smartphone, hình ảnh được số hóa.*

1. INTRODUCTION

Phenol is a toxic substance that is widely used in the manufacture of synthetic phenolic resins, dyes, plastics, paints, pesticides, insecticides, herbicides and synthetic intermediates. It is mostly released to the environment through industrial wates from coke oven plants, petroleum refineries, drug manufactures, wood factories, the pulping and paper industries. Owing to its toxicity, both the Environmental Protection Agency (EPA) of the United States and the European Union (EU) have included

phenol in their lists of priority pollutants. The Occupational Safety and Health Administration (OSHA) has set a limit of 5 ppm in air, for workers during 8-hour work shifts [1,2]. Due to the wide applicability and toxicity, phenol is subject to many studies on methods for their detection; for example, universal methods of GC/FID, GC/MSD, HPLC, electrochemical, X-ray diffraction (XRD), and spectrophotometric methods using the reagents of 4-aminoantipyrine (TCVN 6216:1996), N-diphenylbenzamidine, p-nitroanilin (64TCN 102:1997), a mixture of phosphotungstic acid $H_3PW_{12}O_{40}$ and phosphomolybdic acid $H_3PMo_{12}O_{40}$ (Folin-Ciocalteu reagent) [1,3]. Due to their simplicity, short time, adaptability at various experimental conditions, accuracy, inexpensive equipment, etc., the spectrophotometric methods are more suitable for industrial laboratories. To estimate the concentration of total phenolic compounds in juices and teas, the Prussian Blue method firstly suggested by Price and Butler (1977) and modified by Graham (1992) with adaptation to microplates was used with minor modifications [3,4]. As the formation of the Prussian Blue complex offered a sensitive and rapid method for the colorimetric determination of total phenols, most researchers apply the way to determine or monitor the total phenolic content in food extracts, herbs, nutritional supplements, and beverages. In the previous study, we reported the effective spectrophotometric method for the determination of phenol in the industrial effluent using a Prussian Blue (PB) reagent mixture [5]. In the digital age, smartphones is a modernized personal digital assistant with a mobile operation system, wireless connectivity, large internal memory, and most of them have integrated cameras with autofocus, digital zooms and high-resolutions. Therefore, smartphone cameras can recognize tiny differences in color tone. Several applications of smartphones in analytical chemistry, e.g., immunosensors or blood analysis sensors, have been introduced thus far [6]. The most convenient color space for smartphone image processing used commonly in colorimetric sensor researches is RGB color space [6,7]. RGB is an additive color model wherein color is a combination between the three primary colors of red (R), green (G), and blue (B). A color's RGB value indicates its red, green, and blue color intensity. Each intensity value is on a scale of 0 to 255. However, RGB values are highly susceptible to ambient light noise[6]. In this sequential study, the application of smartphone as a simple, portable colorimetric analytical device for detection of phenol in effluent using the Prussian Blue reagent was examined. This new method was also validated according to the ICH guidelines (International Conference on the Harmonization) [8,9].

2. MATERIALS AND METHODS

2.1. Materials

All chemicals used in this experiments were of analytical reagent grade and were used without supplementary purifications. HCl solution was used for dilution of 5%$FeCl_3.6H_2O$ [5,6] and the double distilled water was used for dilution solution of 5%$K_3[Fe(CN)_6]$. A reagent mixture was prepared by mixing solutions including 5%$FeCl_3.6H_2O$ in 0.1M HCl and 5%$K_3[Fe(CN)_6]$. Phenol stock solution was 0.5mg/mL prepared by the double distilled water from the phenol standard. Working standards were prepared by the appropriate dilution of the stock. Industrial effluent samples including untreated and treated wastewater were collected from A.F.C Paper Company., LTD.

2.2. Methods

2.2.1. Measuring Process

Some modifications based on previous studies were performed for this measuring process [6,7]. A system built for the capturing image of sample solutions consisted of a wooden box with approximate dimensions of 20×30×50 (cm) and was minimized the entry of external light to ensure the reliability and reproducibility of the obtained RGB data. The inside of chamber was covered a uniform black color to avoid the effects of reflections that could affect the quality of the captured images. Inside the box, two white LED lights (5W/light) was fixed

on the box top to shine a light toward a 24-well plate contained sample solutions. The camera integrated Xiaomi Redmi Note 5 smartphone was located exactly parallel under 24-well plate and opposite to the LEDs. The image capturing of sample solutions was controlled by the free Teamview software. The captured image was transmitted directly to a computer located outside the box for following processing (Fig. 1). The free ImageJ software was used to digitalized the image colors using RGB color space. In additon, the color measurement can be performed by various quantitative applications as Color Analysis, Color Piker, … that be always offered for free download on App Stores by smartphone manufacturers. The color change of digitalized images was observed in red, green and blue channels. However, the blue channel was selected for this work due to its color similarity with the typical color of Prussian Blue complex and its higher sensibility in comparison with other channels. Both the experiments and the color measurements were carried out in triplicate. Phenol also was determinted by spectrophotometric method using Genesys 10S UV-Vis as the comparative method.

Figure 1. System built for capturing images of sample solutions

2.2.2. Effect of reaction conditions on the formation of Prussian Blue complex

The different parameters of reaction affecting the formation and the stability of PB complex were studied and optimized to give the maximum color intensity value and hence the highest sensitivity of the proposed method. Variables were optimized by changing each in turn, while, keeping all others constant. 10mL flask was added consistently by the aliquot volume of reagent mixture and a volume of phenol stock solution, then were placed in ultrasonic bath until the completed reactions and the fully developed-color. Solutions were diluted up to the mark with distilled water and then quantified colors, against a blank of reagent mixture prepared in the same way, but without phenol (a blank). The color intensity value of samples were expressed as the absolute value of the subtracting the color intensity value of a sample from the color intensity value of a blank. The reaction conditions as the volume ratio of reagent mixture ($5\%FeCl_3/5\%K_3[Fe(CN)_6]$), the concentration of reagent mixture, the time and temperature of reaction were investigated. Moreover, the sufficient concentration of HCl solution to dilute $FeCl_3$ solution was examined by the preparation of $5\%FeCl_3$ solutions using the concentration range of HCl solution from 0.01M to 0.4M. To improve reliability of results, all above examinations were carried out with three concentrations of the phenol stock solution, low (1ppm), medium (5ppm), and high (20ppm), using the same procedure.

2.2.3. Analytical method validation

For validation of the analytical method, the guidelines established by the ICH (TCVN ISO/IEC 17025:2017) were employed[8,9]. Under reaction conditions optimized above, the typical analytical characteristics of method validation including linearity range, sensitive, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy, and bias were investigated. To establish the linearity range of the method, various concentration of phenol standard solution from 0.005ppm to 20ppm were prepared in a series of 10mL flasks, followed by adding a volume of reagent mixture. The reaction was performed at above optimized conditions. Data were analyzed by linear regression using the least squares method. The form of the linear regression equation was $y = ax + b$, where y and x were the blue color difference (ΔB) and concentration of phenol standard stock solution, respectively. The correlation coefficients R^2 was used to characterize linearity of the method. LOD and

LOQ were calculated according to Eq. (1 and 2) by the color measure of 21 blank samples, where SD is the standard deviation of the intercept and a is the slope of the calibration curve.

$$LOD = 3\frac{SD}{a} \quad (1)$$

and $$LOQ = 10\frac{SD}{a} \quad (2)$$

The accuracy of the method was assessed as the percentage recovery which was determined by adding phenol standard solution of 0.5ppm and 1.0ppm (C_t) to the effluent sample determined the phenol concentration (C_m). Recovery was calculated by Eq. 3, where C_{m+t} is the concentration of solution after addition of phenol standard solution.

$$H\% = \frac{C_{m+t} - C_m}{C_t}.100 \quad (3)$$

The repeatability of the proposed method was checked by the analysis of two effluent samples from the same wastewater source. Precision of the method was expressed as the percentage RSD according to Eq. 4, where $\bar{\chi}$ is the mean of all measurements.

$$\%RSD = \frac{SD}{\bar{\chi}}.100\% \quad (4)$$

In addition, analytical results using the validated method were referred simultaneously to the results of spectrophotometric method that we developed previously over the same effluent samples and the same reagent mixture.

2.2.4. Statistical analysis

Statistical analyses were performed using descriptive statistics, ANOVA, F-test, and t-test (considering $p < 0.05$ as significant), with Data Analysis of Excel software. The results were expressed as mean values.

2.2.5. Determination of phenol in the effluent sample

Effluent samples were collected from A.F.C Paper Company according to TCVN 5999:1995 and were treated according to 64 TCN 102:1997 standard of industries. Phenol must be determined within 24 hours after effluent samples collected. Aliquot 150mL of the initial effluent sample eliminated absolutely sulphur compounds and non-volatile impurities was recorded V(mL). Aliquot 20µL of the treated sample was added to 10mL volumetric flask, then added the reagent mixture of $FeCl_3/K_3[Fe(CN)_6]$. The reaction was processed to complete under above optimized conditions. After that, the flask was filled up to mark by distilled water. Finally, 1mL sample solution was transferred to 24 well-plate via pipette for capturing an image and followed by the digital image-based colorimetric analysis for phenol determination. C_{phenol} in effluent samples was calculated according to Eq. 5, where C_A is the phenol concentration calculated from the linear equation determined above.

$$C_{Phenol}(ppm) = C_A(ppm)\frac{1000.V\,(mL)}{150} \quad (5)$$

3. RESULTS AND DISCUSSION

3.1. Effect of reaction conditions

The reagent mixture of $FeCl_3/K_3[Fe(CN)_6]$ plays a significant role in the formation of PB complex and the selectivity of the reaction that were recognized by the color intensity difference. In this study, the effect of the reagent mixture on complexation including the concentration of reagent mixture and the volume ratio of $5\%FeCl_3$ and $5\%K_3[Fe(CN)_6$ in reagent mixture were evaluated. Firtsly, the concentrations of reagent mixture were varied from 1.10^{-3}(%) to 10.10^{-3}(%). Thus, as shown in Fig. 3, the maximum difference of color intensity achieved at 5.10^{-3}(%) concentration of reagent mixture. The further increasing reagent mixture did not show a positive color development. Then, the volume ratios of $5\%FeCl_3$ and $5\%K_3[Fe(CN)_6$ in the reagent mixture were varied from 1/9 to 9/1. As the results from Fig. 4, the 5/5 volume ratio of $5\%FeCl_3$ and $5\%K_3[Fe(CN)_6$ was found to be optimum. Increasing the volume ratio made the difference of color intensity decrease significantly. It also was found that the investigated concentrations of phenol resulted similarly.

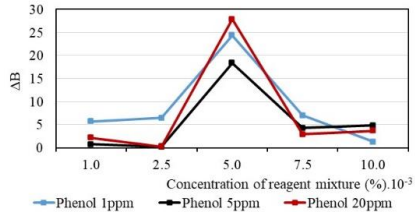

Figure 2. Effect of concentration of reagent mixture

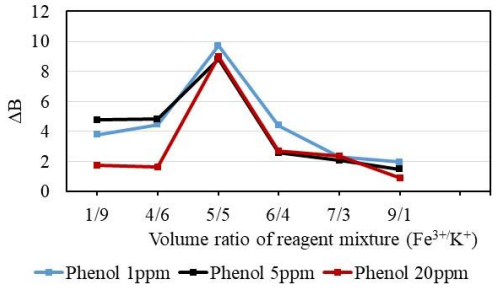

Figure 3. Effect of volume ratio of reagent mixture

In order to select the most suitable concentration of HCl for the dilution of $FeCl_3$, the effect of HCl concentration was also studied over the HCl concentration range from 0.0M to 0.4M. As shown in Fig. 4, it was found that, the increasing HCl concentration made the color intensity difference markedly increase. The maximum color intensity difference was achieved at 0.1M of HCl solution. However, at the higher HCl concentration, there was a decline in the color analytical signal. The results obtained from the investigated concentrations of phenol performed a similar tendency.

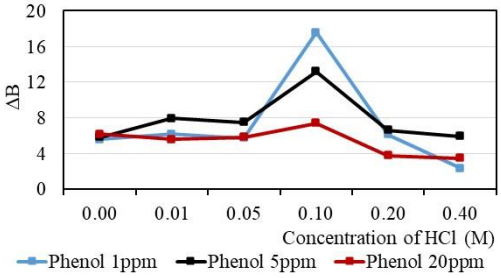

Figure 4. Effect of HCl concentration

The optimal reaction time and reaction temperature for formation of the PB complex are necessary to complete the reaction and achieve a strong analytical signal, corresponding to the highest difference of color intensity. Different times and temperatures of reaction were investigated to select the best condition for the developed procedure. As shown in Fig. (5 and 6), the optimum time and temperature of reaction was obtained to be 20 minutes and 50°C; respectively. The increasing of reaction temperature or the lasting of reaction time affected unfavorably in the color analytical signal. Generally, this tendency was similar to the investigated concentrations of phenol.

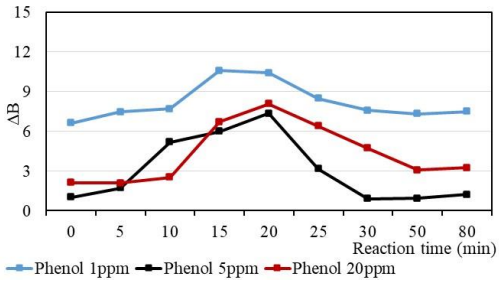

Figure 5. Effect of the reaction time

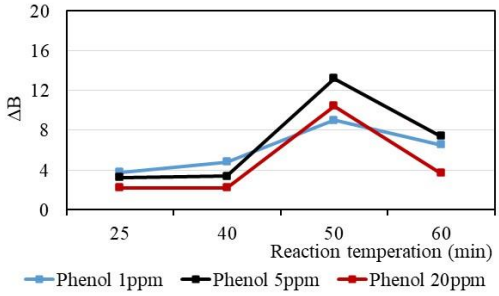

Figure 6. Effect of reaction temperature

Based on the above results, it is easy to find that the reaction of phenol and inexpensive reagent mixture of $FeCl_3/K_3[Fe(CN)_6]$ performs quite simply at mild conditions, and produces the stable colored PB complex that can be detected easily by smartphones. Due to highlight advantages, the proposed method can be applied widely at industrial laboratories to determine phenol in effluent in routine analysis.

3.2. Validation of analytical method

According to the results obtained from Fig. (7 and 8), a linear relationship between the color intensity diferrence and phenol concentration was in the range of 0.01-1.5ppm and was described by the linear regression equation y=13.016x + 5.4959 with correlation coefficient of 0.9989. It reveals that 99.89% of the total variation arounds the mean and residual (error) is only 0.11%. Regression characteristics like slope, intercept, and relative standard deviations were presented in Tab. 1.

Blank 0.01 ppm 0.02 ppm 0.05 ppm 0.1 ppm 0.5 ppm 0.8 ppm 1 ppm 1.5 ppm

Figure 7. Color change of PB complex in the concentration range of 0.01-1.5 (ppm) phenol

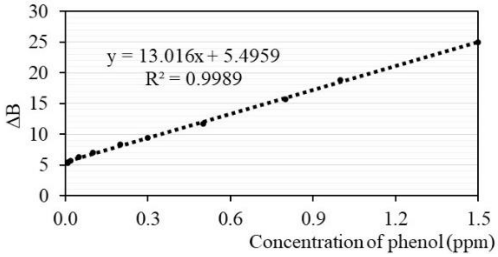

Figure 8. Calibration curve of phenol

Table 1. Statistical data of the regression equation for determination of phenol

	Coefficients	Standard Error	t_{stat}
Intercept (b)	5.4959	0.1021	53.8199
Slope (a)	13.0164	0.1560	83.4201
$SD_{(y)}$		0.7444	
$SD_{(b)}$		0.3229	
$SD_{(a)}$		0.4934	
ε_b		0.2355	
ε_a		0.3598	
t_{tab}		2.3060	
F_{stat}		6958.9073	
F_{tab}		5.3177	

Table 2. Statistical data of the regression equation for determination of phenol

	Coefficients	Standard Error	t_{cal}
Intercept (b)	0.01398	0.00375	3.72657
Slope (a)	0.80535	0.00616	130.70716
$SD_{(y)}$		0.033	
$SD_{(b)}$		0.01300	
$SD_{(a)}$		0.02134	
ε_b		0.00836	
ε_a		0,01373	
t_{tab}		2.22814	
F_{cal}		17084	
F_{tab}		4.96460	

Data was validated by ANOVA, where the statistic values of F_{stat} and t_{stat} were bigger than their tabulated values (F_{tab} and t_{tab}; respectively). Consequently, the regression equation was described fully as follows: $y = (13.0164 \pm 0.3598)x + (5.4959 \pm 0.2355)$, where x is concentration of phenol (ppm). The calculated values of LOD and LOQ were 3.0ppb and 11ppb; respectively, which indicated the high sensitivity of the proposed method.

The results of the recovery studies were shown in Tab. 2. The mean recovery values were about 98 % with low RSD (about 0.28%) confirmed the accuracy and precision of the proposed method.

Table 3. Results of recovery studies

Initial conc. (ppm)	Added (ppm)	Found (ppm)	Recovery (%)	RSD (%) (n=6)
0.077	0.5	0.570	98.72	0.2772
0.077	1.0	1.157	98.50	0.2982

Precision was evaluated according to the repeatability which its results was shown in Tab. 3. For repeatability examinations, the percent relative standard deviation (RSD) from three replicate analyses was 0.29 %, which was smaller the requirements of AOAC standards (11%). Therefore, the results proved the precision of the proposed method.

The proposed method was successfully applied for determination of phenol in untreated effluent from A.F.C paper company (Tab.3). The results of the digital image-based colorimetry method were statistically compared with those of the referenced spectrophotometric method using Student's t-test and F-test with respect to accuracy and precision. It was noted that $|t_{stat}| = 1.3407 < t_{tab} = 2.7764$ and $F_{stat} = 1.0158 < F_{tab} = 19$. It revealed that the variances of the two results were equal and the means were the same. As you can see, it was no significant difference between results of the proposed and spectrophotometric methods as the calculated values did not exceed the theoretical values at 95% confidence level. Hence, the accuracy and precision of the proposed method were satisfactory.

Table 4. Results of the proposed method in comparison with the referenced method

	Spectrophotometer	Proposed method
Sample 1 (ppm)	44.2426	44.5244
Sample 2 (ppm)	44.3723	44.6367
Sample 3 (ppm)	44.5020	44.3800
Mean (ppm)	44.3723	44.3800
SD	0.1297	0.1287
RSD %	0.2923	0.2900
ε	0.3222	0.3197
$\|t_{stat}\|$	1.3407	
F_{stat}	1.0158	
$t_{tab} = t_{(\alpha, n1+n2-2)}$	2.7764	
$F_{tab} = F_{(\alpha, n1-1, n2-1)}$	19	

4. CONCLUSION

Following the first publications on the determination of phenol in industrial effluent using the spectrophotometric method with $FeCl_3/K_3[Fe(CN)_6$ reagent mixture, there is the new method using digital image-based colorimetric method via a smartphone as a detector. The validation of the proposed method was performed according to the ICH guidelines and complied with the requirements for analytical application and to ensure the reliability of the results. A different considerable advantage of the method is to use a small amount of sample and reagents (20µL sample/analysis), therefore, displaying agreement with the precepts of green analysis. The method is a simple, low-cost, benign, sensitive, portable, particularly, does not require trained personnel and can be used in a remote area with limited resources or in routine work of industrial laboratories.

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