Indirect Spectrophotometry Method Based on the Formation of Chromium(VI)-Diphenylcarbazide Complex for Determination of Hydroquinone in Cosmetics

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Abstract. This research is focused on the development of a new indirect spectrophotometry for the determination of hydroquinone in cosmetics based on the capability of hydroquinone as reducing agent for chromium(VI). The principle of the determination is based on the decrease of the color intensity of the red-violet complex of chromium(VI)-Diphenylcarbazide (DPC) due to the reduction of the available chromium(VI) by hydroquinone to chromium(III). Thus, only the remaining chromium(VI) reacted with DPC to form a red-violet complex detected by spectrophotometry at 542 nm. All chemical parameters were optimized with respect to sensitivity and selectivity of measurements. Under the obtained optimum conditions (reaction time of 5 minutes, chromium(VI) of 1 mgL⁻¹, sulfuric acid of 0.05 M, and DPC of 0.0125%), the method gave linear calibration for hydroquinone concentration from 0.2-2.5 mgL⁻¹ (R² = 0.996). The proposed method also provided good sensitivity shown by low values of LoD and LoQ (0.07 and 0.1 mgL⁻¹) and satisfactory selectivity towards the common interfering ions present in cosmetics. Satisfactory results with recoveries of close to 100 % were observed when the method was applied to facial whitening and black spot removal cosmetics. This result suggests that the developed method is able to be used for monitoring of hydroquinone in skin lightening cosmetics.

1. Introduction

Whitening skin cosmetics is one of popular facial cosmetics in Asia and Africa as skin lightening agent, especially for woman with dark skin or hyperpigmentation. This type of cosmetics commonly contains active ingredients of hydroquinone, kojic acid, arbutin, glutathione, recorcinol, mercury, retinol, niacin amide, and ascorbic acid. Hydroquinone has been used for decades in creams, gels and lotions for the treatment of the hyperpigmentary disorders of the skin. Hydroquinone works by inhibiting tyrosinase for production of skin pigment (melanin); thus, skin remains fair and bright. However, research showed that hydroquinone can cause serious health complications and for prolong use it can permanently damage the skin and potentially cause cancer [1-3]. Therefore, European Union
and NADFC of Indonesia banned the utilization of hydroquinone in cosmetics. In Indonesia, hydroquinone is only allowed to be used in hair (0.3 %) and nail dyes (0.02 %), while in facial whitening cream or facial removal cream has been banned since 2008 [4].

Several analytical methods for the determination of hydroquinone in cosmetics are described, including spectrophotometry [5-9], flow injection analysis (FIA) with spectrophotometric detection [10-13], and chromatography techniques [14-16]. The present study is aimed to describe a new indirect spectrophotometry method for hydroquinone in cosmetics based on the capability of hydroquinone to reduce chromium(VI) to chromium(III), which in the presence of 1,5-diphenylcarbazide (DPC), the remaining chromium(VI) formed an intense red-violet chromium(VI)-DPC complex. The intensity of the color was reduced proportionally to the available hydroquinone. Thus, the concentration of hydroquinone can be measured spectrophotometrically based on the decrease of absorbance of the red-violet chromium(VI)-DPC complex at 542 nm. The use of chromium(VI) was considered as it showed a fast and sensitive oxidizing agent for hydroquinone [6], thus only a small concentration was used and expected to be safe for environment. In order to achieve the optimum performance of the method, the common parameters which affect redox reaction of hydroquinone and chromium(VI) as well as the stability of complex formation of chromium(VI)-DPC were optimized with respect to sensitivity. The interfering ions frequently exist with hydroquinone in cosmetics were also studied for selectivity of the method. The method was also validated by applying to determine hydroquinone in facial whitening and black spot removal creams.

2. Tools and Materials
Spectrophotometric measurements were carried out using UV-Vis 10S Genesys Spectrophotometer. Materials used in this research were hydroquinone (Sigma-Aldrich), potassium dichromate (Merck), diphenylcarbazide (C_{12}H_{14}N_{2}O, Merck), acetone (AR), sulfuric acid (Merck), ascorbic acid (C_{6}H_{8}O_{6}, Merck), lead nitrate (Merck), resorcinol (Merck), and silver nitrate (Merck). All of the chemicals applied for the research were pro-analytical grade without any purification process. De-ionized water was used for preparation of all solutions.

3. Method

3.1. Optimization of maximum wavelength
Optimization of maximum wavelength of chromium(VI)-DPC complex was conducted by mixing chromium(VI) in acidic condition with DPC solution to form a red-violet complex; then, the solution was scanned using UV-Vis 10S Genesys Spectrophotometer at wavelengths in the range of 400 to 800 nm.

3.2. Optimization of stability of complex.
The stability of complex chromium(VI)-DPC was optimized by monitoring the absorbance of the complex at 542 nm in the range time of 2-40 minutes. This optimization was done by mixing 0.4 mL of 10 mgL^{-1} hydroquinone solution with 1 mL of 10 mgL^{-1} chromium(VI) solution, 0.5 mL of 1 M H_{2}SO_{4}, and 0.2 mL of 0.25 % DPC, followed by dilution with de-ionized water to obtain 10 mL solution. The absorbance of chromium(VI)-DPC complex was then read at 542 nm using UV-Vis 10S Genesys Spectrophotometer from 2 to 40 minutes. The optimum time to obtain the stable complex was determined by the maximum absorbance, and this optimum condition was used for the next experiments.

3.3. Optimization of H_{2}SO_{4} concentration.
The optimum concentration of acid (H_{2}SO_{4}) was done similarly to procedure 3.2, under the optimum time; but the concentration of H_{2}SO_{4} was varied from 0.02 to 0.3 M. The optimum acid concentration was determined by the maximum absorbance, and this optimum condition was used for further experiments.
3.4. Optimization of chromium(VI) concentration.

The optimum concentration of chromium(VI) was conducted similarly to procedure 3.3, but under optimum acid concentration and the concentration of chromium(VI) was varied from 0.2 to 3 mgL⁻¹. The optimum chromium(VI) concentration was determined by the minimum absorbance which showed adequate remaining chromium(VI) for detection, and this optimum chromium(VI) concentration was used for the subsequent experiments.

3.5. Optimization of DPC concentration.

The optimum concentration of DPC was carried out using similar procedure to 3.4, but under optimum chromium(VI), and the concentration of DPC was varied from 0.005 to 0.0625 %. Chromium(VI) concentration which gave maximum absorbance was selected as optimum DPC concentration for further experiments.

3.6. Linearity of Measurement

The linear range of measurement was conducted by measuring absorbance of hydroquinone sample from 0.2-3 mgL⁻¹ under the all optimum conditions outlined in procedures 3.1 to 3.5. The absorbance of complex chromium(VI)-DPC from all hydroquinone concentrations were plotted versus the concentrations of hydroquinone and examined the coefficient determination towards linear correlation.

3.7. Selectivity of Method

In order to obtain selectivity of the method, the effect of interfering ions commonly present in cosmetics, such as ascorbic acid, resorcinol, Pb²⁺ and Ag⁺ on the absorbance of chromium (VI)-DPC were studied by adding each of those interfering substances to a certain hydroquinone concentration. The selectivity was defined by the percent error of measurement of the mixture compared to hydroquinone sample without interfering ions.

3.8. Method Validation

The proposed method was validated using standard addition procedure, followed by calculation of percent recoveries of samples after the addition of standard hydroquinone. The validity was defined with percent recovery of close to 100 %.

4. Result and Discussion

The reaction of determination of the proposed method was based on two main reactions: (1) the capability of hydroquinone to reduce chromium(VI) to chromium(III); (2) the formation of chromium(VI)-DPC complex. The redox reaction between hydroquinone and chromium(VI) under acidic condition is shown in equation (1), where hydroquinone can be oxidized to benzoquinone and chromium(VI) was reduced to chromium(III). The high potential reduction standard of chromium(VI) to chromium(III), E° = +1.33 V, is expected to give complete oxidation of hydroquinone to benzoquinone, supported by high positive potential reaction of E° = +0.631 V.

\[
3\text{C}_6\text{H}_4\text{O}_2\text{H} + \text{Cr}_2\text{O}_7^{2-} + 8\text{H}^+ \rightarrow 3\text{C}_6\text{H}_4\text{O}_2 + 2\text{Cr}^{3+} + 7\text{H}_2\text{O} \quad E^0 = +0.631 \text{ V} \quad (1)
\]

In this method, chromium(VI) was added quantitatively in a certain excess to ensure reduction all hydroquinone. After the oxidation of hydroquinone has taken place, the remaining chromium(VI) was reacted with DPC to form a red-violet chromium(VI)-DPC complex as depicted in equation (2).

\[
\text{Cr}_2\text{O}_7^{2-} + 3 \text{DPC} \rightarrow \text{Cr(VI)}-\text{DPC}_3 \text{ (red-violet complex)} \quad (2)
\]

4.1. Optimization of wavelength for measurement

Wavelength for measurement was optimized in order to select a wavelength which gave maximum absorbance with maximum sensitivity for measurement. The product of reaction, chromium(VI)-DPC complex, was scanned in the range of visible wavelength from 400 to 800 nm using UV-Vis 10S
Genesys Spectrophotometer and the obtained absorption spectra is shown in Figure 1. Based on Fig. 1, chromium(VI)-DPC complex showed maximum absorbance at wavelength of 542 nm, which correlate to the complement color of red-violet [17] as obtained by the previous work [18]. Therefore, 542 nm was selected as the maximum wavelength used for the experiments.

**Figure 1.** The maximum wavelength of chromium(VI)-DPC complex.

4.2. **Optimization of Stability of Complex.**

Stability of complex was optimized in order to obtain the reaction time to achieve the equilibrium which gave maximum product of reaction of chromium (VI)-DPC complex shown by its maximum color intensity and maximum absorbance [19]. Figure 2 shows the relatively constant absorbance of chromium(VI)-DPC complex monitored from 2 to 40 minutes of reaction. Therefore, 2 minute reaction with absorbance value of 0.697 was selected for further measurements.

**Figure 2.** The optimization of stability of complex.

4.3. **Optimization of H₂SO₄ Concentration.**

Optimization of acid (H₂SO₄) concentration was conducted in order to achieve maximum oxidation of hydroquinone by chromium(VI), which thus reduces the presence chromium(VI); therefore, the optimum of acid concentration was shown by the minimum absorbance of chromium(VI)-DPC complex. As shown in Equation 1, chromium(VI) requires acidic condition for oxidizing hydroquinone. Besides, the stability of chromium(VI)-DPC complex is affected by the acidity of solution. According to APHA (American Public Health Association), chromium(VI)-DPC complex formed in interval acidity of pH 1.6 to 2.2 [20]. Furthermore, the form of chromium(VI) can be various from H₂CrO₄, HCrO₄⁻, CrO₄²⁻ to Cr₂O₇²⁻ depends on the acidity of the solution. Thus, optimization of H₂SO₄ concentration was also aimed to ensure the form of chromium(VI) in the solution as dichromate.
Figure 3 performs the effect of sulfuric acid concentration from 0.05 to 0.30 M to the absorbance of chromium(VI)-DPC complex, which shows relatively constant absorbance of chromium(VI)-DPC complex. Sulfuric acid concentration of 0.05 M, which correlates to pH suggested by APHA standard was chosen as optimal and used for further optimization.

**Figure 3.** Optimization of H$_2$SO$_4$ concentration.

4.4. *Optimization of chromium(VI) concentration.*

Optimization of the concentration of chromium(VI) was carried out to determine the concentration of chromium(VI) required for oxidizing the available hydroquinone in the sample with adequate excess to form complex with DPC for detection. The higher concentration of chromium(VI) gives the more remaining chromium(VI) and increases the color intensity shown by the high absorbance of chromium(VI)-DPC complex. The optimization of chromium(VI) concentration was monitored based on the absorbance of chromium(VI)-DPC complex, and the result is depicted in Figure 4. As expected, the higher concentration of dichromate produced higher absorbance of the complex. In this method, the adequacy of chromium(VI) for oxidizing hydroquinone is indicated by the presence of the excess of Cr(VI) observed as red-violet complex of Cr(VI)-DPC. Thus, the optimum chromium(VI) was determined by the minimum Cr(VI) which gave acceptable absorbance of Cr(VI)-DPC. In this study, 1 mgL$^{-1}$ chromium (VI) provided sufficient amounts to oxidize hydroquinone with minimum excess of chromium(VI) sufficient for detection. Therefore, 1 mgL$^{-1}$ chromium(VI) was selected for further experiments. The use of a minimum excess of chromium(VI) was also considered to fulfill the spirit of green chemistry.

4.5. *Optimization of DPC concentration.*

Optimization of DPC is required to determine the suitable concentration of DPC to react with the remaining chromium(VI) forming maximum chromium(VI)-DPC complex. It was reported that the formation of chromium (VI)-DPC complex can be achieved under mole ratio of 1:8 for Cr(VI):DPC [13]. From the experimental results (Figure 5) showed that the absorbance of complex increased by increasing DPC concentration from 0.005 to 0.0125 %, but it slightly decreased for higher concentration of 0.025% and relatively constant up to 0.0625 %. The concentration of 0.0125 % DPC which have mole ratio of more than 1 to 8 and gave the highest absorbance of chromium (VI)-DPC complex was chosen as the optimum concentration of DPC and used for the subsequent experiment.
4.6. Linearity of Measurement
The linearity of measurement was conducted by measuring various concentration of standard hydroquinone from 0.1 to 3 mgL\textsuperscript{-1} under the obtained optimum parameters, i.e. 1 mgL\textsuperscript{-1} dichromate as chromium(VI), 0.05 M H\textsubscript{2}SO\textsubscript{4}, 0.0125\% DPC, and spectrophotometric detection at 542 nm. Then, the delta (\(\Delta\)) absorbance (absorbance difference) between blank solution and sample (hydroquinone) solution was plotted versus hydroquinone concentration. Figure 6 shows that the method performed linear correlation (\(R^2\), of 0.9961) between the concentration, \(x\), and the delta absorbance, \(y\), with linear equation of \(y=0.2293x+0.0362\) for concentrations of hydroquinone from 0.2 to 2.5 mgL\textsuperscript{-1}.

4.7. Selectivity of Method
The selectivity of method was tested by monitoring the effect of the interfering substances to the absorbance of the standard hydroquinone, and the difference of absorbance was performed as percent error. This procedure was conducted by adding substances which commonly present in whitening cosmetics, such as silver and lead ions, resorcinol, and vitamin C (ascorbic acid) to standard hydroquinone solutions. Under the addition of 0-5 mgL\textsuperscript{-1} of each substance, the method was not interfered by silver and lead ions, as well as resorcinol, supported by percent error of less than 8\%. Based on the t-test with a confidence level of 95\% and \(\alpha = 0.05\), the recoveries of hydroquinone in the presence of the three substances did not show significant different shown by the smaller value of \(t_{\text{count}}\) compared to \(t_{\text{table}}\). The presence of ascorbic acid in hydroquinone sample up to 1 mgL\textsuperscript{-1} can be tolerated by the proposed method; however, higher concentrations of ascorbic acid (2.5-5 mgL\textsuperscript{-1}) resulted in high percent error of much more than 10\% Table 1). The interfering effect of ascorbic acid to the measurement of hydroquinone in consequence of its nature as strong reducing agent which
capable to behave as hydroquinone in reducing chromium(VI) to chromium(III); thus gave results to higher recoveries of hydroquinone (> 0.5 mgL\(^{-1}\)) and high percent errors.

![Graph showing linear relationship](image)

**Figure 6.** Linearity of the method

**Table 1.** The selectivity of method

| [HQ] (mgL\(^{-1}\)) | Interfering ions | [Interfering ions] (mgL\(^{-1}\)) | Measurable concentration (mgL\(^{-1}\)) | % Error |
|----------------------|------------------|-------------------------------|----------------------------------------|---------|
| 0.00                 |                  | 0.50 ± 0.03                   | 0.00                                   |
| 0.50                 |                  | 0.51 ± 0.05                   | 2.00                                   |
| 1.00                 |                  | 0.48 ± 0.13                   | 4.00                                   |
| 2.50                 |                  | 0.47 ± 0.04                   | 6.00                                   |
| 5.00                 |                  | 0.47 ± 0.07                   | 6.00                                   |
| 0.00                 | Ag\(^+\)         | 0.50 ± 0.04                   | 0.00                                   |
| 0.50                 |                  | 0.51 ± 0.04                   | 2.00                                   |
| 1.00                 |                  | 0.48 ± 0.13                   | 4.00                                   |
| 2.50                 |                  | 0.47 ± 0.04                   | 6.00                                   |
| 5.00                 |                  | 0.47 ± 0.07                   | 6.00                                   |
| 0.00                 | Pb\(^{2+}\)      | 0.49 ± 0.04                   | 2.00                                   |
| 0.50                 |                  | 0.49 ± 0.01                   | 2.00                                   |
| 1.00                 |                  | 0.49 ± 0.03                   | 2.00                                   |
| 2.50                 |                  | 0.48 ± 0.06                   | 4.00                                   |
| 5.00                 |                  | 0.50 ± 0.03                   | 0.00                                   |
| 0.00                 | Resorcinol       | 0.49 ± 0.04                   | 4.00                                   |
| 0.50                 |                  | 0.51 ± 0.02                   | 2.00                                   |
| 1.00                 |                  | 0.48 ± 0.04                   | 4.00                                   |
| 2.50                 |                  | 0.47 ± 0.03                   | 6.00                                   |
| 5.00                 |                  | 0.46 ± 0.03                   | 8.00                                   |
| 0.00                 | Ascorbic acid    | 0.50 ± 0.04                   | 0.00                                   |
| 0.50                 |                  | 0.51 ± 0.05                   | 2.00                                   |
| 1.00                 |                  | 0.54 ± 0.01                   | 8.00                                   |
| 2.50                 |                  | 0.78 ± 0.03                   | 56.00                                  |
| 5.00                 |                  | 1.05 ± 0.01                   | 110.00                                 |

*An average of 3 repetitions

4.8. **Validation Method**

The proposed method was validated by applying to determine hydroquinone concentration in four types of cosmetics (A, B, C, and D) consisted of facial whitening creams and black spot removal creams, followed by standard addition procedure. In this standard addition procedure, three different concentrations of hydroquinone were added separately to each of the four extract creams and the hydroquinone concentrations were determined using the proposed method. The recoveries of the hydroquinone are summarized in Table 2 showing that the proposed method provided good accuracy.
shown by the high % recoveries of close to 100 % with good precisions (typical SD ≤ 0.1 and RSD < 10 %). The limits of detection and quantification (LoD and LoQ) were determined by measuring blank solutions, and the LoD and LoQ values were derived from the addition of the signal of blank with 3 times of the standard deviation for LoD and with 10 times of the standard deviation for LoQ, resulting respective values of 0.07 mgL⁻¹ and 0.10 mgL⁻¹. These low values of LoD and LoQ showing that the proposed method provided adequate sensitivity required for the determination of hydroquinone in whitening cosmetics. Table 2 also showed that the hydroquinone in all cosmetics used in this study were measurable under the developed method. Therefore, the proposed method can be considered as an alternative method for hydroquinone determination in cosmetics.

| Table 2. The validity of method |
|---------------------------------|
| Cosmetic samples               | HQ addition concentration (mgL⁻¹) | Measurable concentration±SD (mgL⁻¹) | % Recovery |
| A                               | 0.00                             | 0.53 ± 0.05                         | -          |
| A                               | 0.30                             | 0.85 ± 0.04                         | 103.77     |
| A                               | 0.50                             | 1.06 ± 0.03                         | 105.66     |
| A                               | 0.00                             | 0.50 ± 0.06                         | -          |
| B                               | 0.30                             | 0.80 ± 0.03                         | 100.00     |
| B                               | 0.50                             | 1.00 ± 0.02                         | 100.00     |
| B                               | 0.00                             | 0.40 ± 0.10                         | -          |
| C                               | 0.20                             | 0.60 ± 0.04                         | 100.00     |
| C                               | 0.40                             | 0.79 ± 0.08                         | 97.50      |
| C                               | 0.00                             | 0.23 ± 0.05                         | -          |
| D                               | 0.20                             | 0.44 ± 0.04                         | 104.30     |
| D                               | 0.40                             | 0.63 ± 0.06                         | 100.00     |

*An average of 3 repetitions

5. Conclusion
Indirect spectrophotometry method for the determination of hydroquinone in whitening cosmetics based on the decrease absorbance of the chromium(VI)–DPC complex has been successfully developed. This method has high selectivity towards Ag⁺, Pb²⁺, and resorcinol, except of ascorbic acid and has been applied to cosmetics sample with satisfactory results (recoveries of close to 100 %). This result suggests that the method mentioned above is able to be used for monitoring of hydroquinone in skin lightening cosmetics.

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Acknowledgments
The authors are grateful to University of Brawijaya Malang Indonesia for financial support through “Hibah Guru Besar dan Doktor 2019” research grants and research facilities.