

## Optimization of Flow Injection (FI) – Spectrophotometry for Hydroquinone Analysis

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### ABSTRACT

Hydroquinone is one of the phenolic compounds used in various cosmetic products for skin lightening as it can inhibit tyrosinase enzyme in producing melanin. However, hydroquinone is classified as a toxic compound, therefore, several countries such as Africa, Canada, and Indonesia prohibits hydroquinone usage in cosmetics. This research was focused on the development of a method for hydroquinone analysis using flow injection (FI) combined with spectrophotometry based on the reaction of hydroquinone with phloroglucinol in alkaline condition producing orange complex detected at 493 nm. The FI method was optimized based on operational factors and chemical factors in order to achieve sensitivity. The maximum sensitivity of FI method was achieved under operational condition of 100  $\mu$ L sample volume, 100 cm mixing coil 1, 50 cm mixing coil 2 and 2.8 mL/min with the chemical condition of 0.005 mol/L NaOH and 0.001 mol/L phloroglucinol. Under these optimum conditions, the proposed method showed linearity in the range concentration of 2 – 80 mg/L and the method was applied to cosmetic sample with acceptable recovery.

Keywords: hydroquinone, flow injection, phloroglucinol, optimization

### INTRODUCTION

Hydroquinone (1,4-dihydroxybenzene) is an aromatic organic compound classified as a phenolic compound used in cosmetic for skin whitener. This compound plays a role in inhibiting tyrosinase (producing melanin in the skin) enzyme thus the skin color becomes white [1]. However, the usage is limited or even prohibited in some countries because of its toxicity effect. The European Union has banned hydroquinone usage in cosmetics [2]. Indonesia through BPOM Number: HK.00.05.42.1018 only allows hydroquinone in nail polish and hair dye by 0.02 and 0.3%. Meanwhile, in America, the FDA allows the hydroquinone in various cosmetic products with levels of 1.5 – 2%. Although hydroquinone has been banned, hydroquinone is still widely found in various cosmetics. The cosmetics usage containing hydroquinone in West African countries is known as 26 – 67% [3]. Meanwhile, in Indonesia some cosmetics contain hydroquinone with a concentration of 3 – 9% were also found in Sidoarjo [4].

Hydroquinone (HQ) can be determined using several methods including test kits using phloroglucinol (PG) reagents [5], spectrophotometry using redox reactions with KMnO<sub>4</sub> as the oxidizing agents [6], chromatography using high-performance liquid chromatography

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(HPLC) [7], and amperometry using SPCE electrode which is modified with nanomaterial. However, some of these methods use a batch method that has many disadvantages, such as consuming large amounts of reagents and samples, time-consuming and costly [8]. Therefore, it needs to be combined with a method that takes a relatively short time and has a good level of accuracy such as flow injection (FI) method [9].

In a routine analysis spectrophotometric method was carried out frequently and give good results. However, the method requires the development or improvement so that its performance is better and more efficient. In this work, a spectrophotometric method is combined with FI to determine hydroquinone in cosmetic. The hydroquinone determination is based on the complex formation of hydroquinone and phloroglucinol in alkaline condition. The complex will be detected at 493 nm using the FI-spectrophotometry which can save samples and less time-consuming. Flow injection (FI) performance is strongly influenced by operational factors including sample volume (loop sample), mixing coil and flow rate while chemical factors which include the concentration of NaOH and phloroglucinol reagent. Therefore, the proposed method is expected to produce a method of determining hydroquinone which is effective, fast, efficient, sensitive, easy and accurate [5].

## **EXPERIMENT**

### **Chemicals and instrumentation**

Hydroquinone was purchased from Sigma Aldrich (China), phloroglucinol and sodium hydroxide (99%) were purchased from Merck (Germany) and 95% ethanol were purchased from Sigma Aldrich (China).

Flow injection analyzer (including ismatec sa peristaltic pump, injector sample, silicon tubing, sample loop and mixing coil (PTFE i.d 0.75 mm)), UV-Vis 1601/ Shimadzu spectrophotometer, and UV Probe 2.21 application.

### **Procedure reaction**

#### **Optimization of Operational Factors**

##### **Sample Volume**

Sample loop optimization was carried out using 6 mg/L hydroquinone, length of mixing coil 90 cm and a constant flow rate of 5 ml/min. The sample volume was varied which were 50, 75, 100, 125 and 150  $\mu$ L. The sample volume that provides the optimum analysis was shown by the highest absorbance value of the HQ-FG complex.

##### **Mixing Coil**

The length of the mixing coil was optimized by varying the length of PTFE hose 25, 50, 75, 100 and 125 cm. Optimization of mixing coil 1 and 2 was carried out using an optimum sample loop, hydroquinone concentration of 6 mg/L, and a constant flow rate of 5 mL/min. The length of the Mixing Coil optimum is shown by the highest absorbance value of the HQ-FG complex and the best peak.

##### **Flow Rate**

The flow rate of reagent solution was varied respectively 0.8, 1.5, 2.8 and 5 ml/min. The optimization of the flow rate was carried out using an optimum parameter of hydroquinone concentration of 6 mg/L. The flow rate optimum is shown by the highest absorbance value of the HQ-FG complex and the best peak.

## Optimization of Chemical Factors

### The concentration of Sodium Hydroxide

The optimization of sodium hydroxide concentration was carried out using hydroquinone concentration of 6 mg/L and phloroglucinol 200 mg/L. Sodium hydroxide concentrations were varied respectively 0.5, 0.05, 0.005, and 0.0005 mol/L. The sodium hydroxide concentration optimum is shown by the highest absorbance value of the HQ-FG complex.

### Concentration of Phloroglucinol

Optimization of phloroglucinol concentration was carried out in optimum variable before by using hydroquinone concentration of 6 mg/L and flow rate of 5 mL/min. Phloroglucinol concentrations were varied respectively 50, 100, 150, 200 and 250 mg/L. Phloroglucinol concentration optimum is shown by the highest absorbance value of the HQ-PG complex.

The FI-Spectrophotometry system to determine the hydroquinone can be illustrated in figure 1 below.

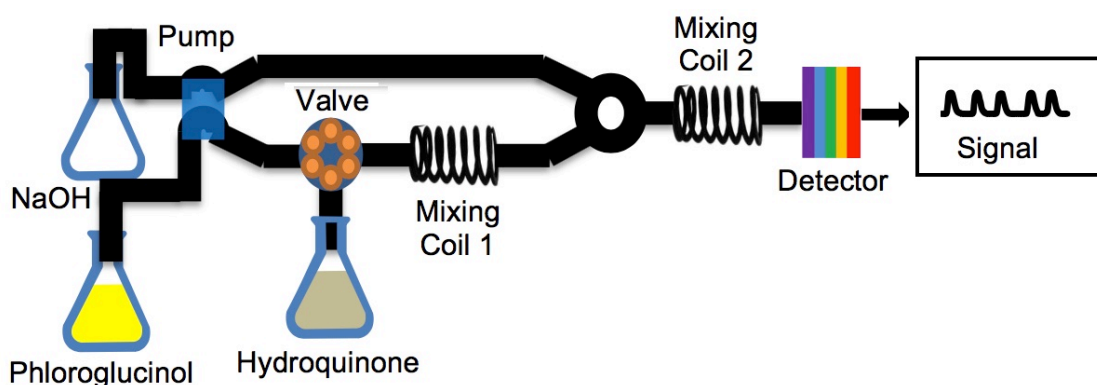


Figure 1. The manifold of flow injection analysis

## RESULT AND DISCUSSION

Hydroquinone could be detected by reaction with phloroglucinol in alkaline solution to produce an orange complex detected at 493 nm. The presence of sodium hydroxide will produce enolate from phloroglucinol as shown in Figure 2, and react with hydroquinone to produce complex compound by hydrogen bonding [10].

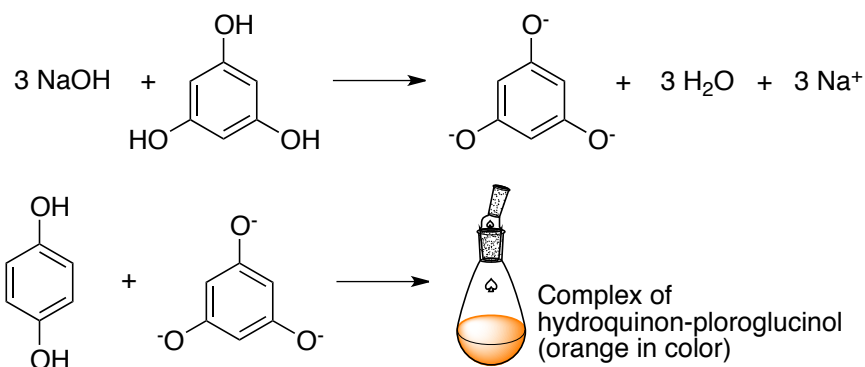
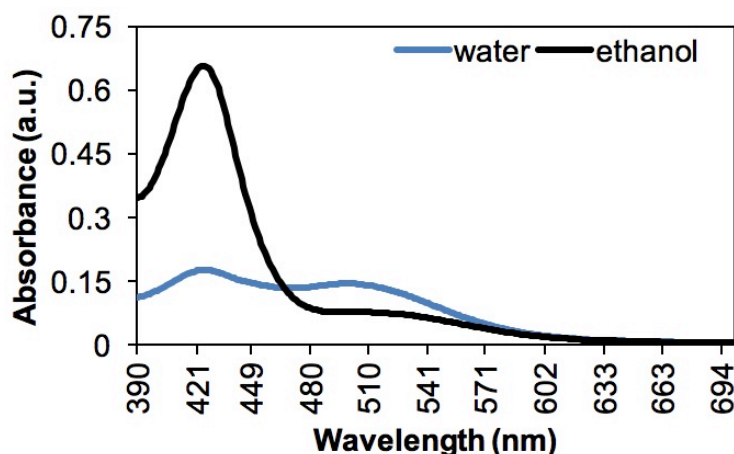


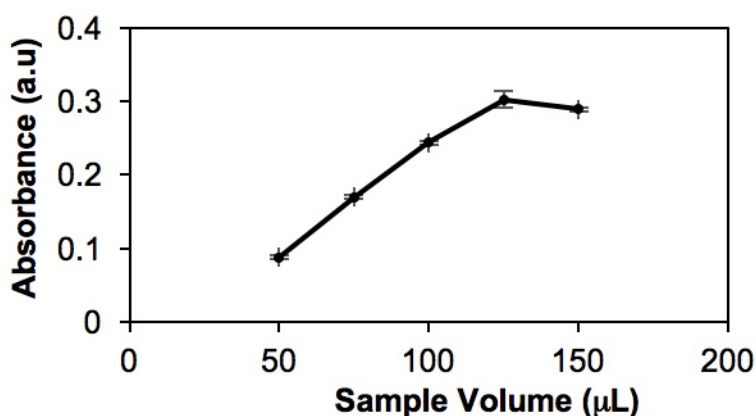
Figure 2. Formation of hydroquinone and phloroglucinol complex's compound in basic condition (adopted from reference [5])

The selection of hydroquinone solvent affects the complex HQ-PG formation. The difference of hydroquinone solvent using ethanol and distilled water are shown by the absorption of UV-Vis spectrophotometer in Figure 3. The absorbance intensity is increased by the different substituent in solvent (auxochrome) such as hydroxyl, amine, alkoxy, and halide group called hyperchromic [11].



**Figure 3.** The maximum wavelength of hydroquinone with distilled water and ethanol solvent

The distilled water was chosen as the hydroquinone solvent based on Figure 3 because distilled water gives the minimum interaction with the sample than ethanol. It is showed by the absorbance of ethanol solvent is higher than the distilled water solvent because the hydroxyl group of ethanol can interact with the hydroquinone by the hydrogen bonding. The interaction between ethanol and hydroquinone formed the complex by the hydrogen bonding donor [12].



**Figure 4.** Effect of Sample Volume on HQ-FG Absorbance

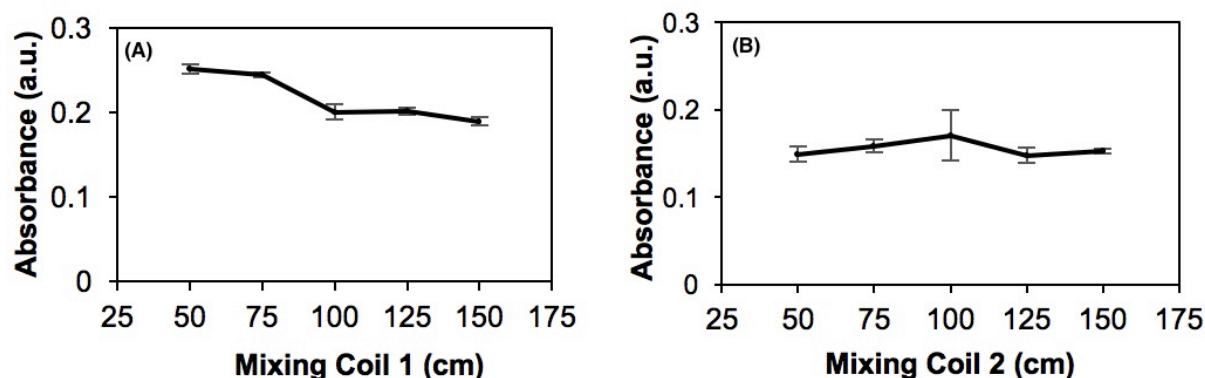
#### Optimization of Sample Volume

The peak or FI gram were affected by sample volume which injected on the FI system. The increased sample volume will be impacted by the dispersion of the solution. However, increasing the sample volume must produce a maximum peak value with minimum

dispersion. The effect of sample volume is given in Figure 4. The best absorbance of sample volume is 125  $\mu\text{L}$  with the value 0.3022. However, the sample volume of 125  $\mu\text{L}$  gives the shoulder peak because the reaction between hydroquinone and phloroglucinol were not maximum. Besides, the sample volume of 100  $\mu\text{L}$  and 125  $\mu\text{L}$  did not have a significant difference. Therefore, 100  $\mu\text{L}$  was chosen as the best peak and absorbance. Theoretically, the higher sample volume will produce higher peaks as shown in Figure 4 [13]. The higher sample volume affects the physical dispersion and produces a widened peak. Therefore the sample volume is not only chosen by the highest signal but also best peak results [14].

### Optimization of Mixing Coil

Mixing coil is the PTFE hose where the reaction occurred by mixing reagent and sample. There are two mixing coils for a maximum reaction that are mixing coil 1 (when the phloroglucinol mixes with hydroquinone) and mixing coil 2 (the result of mixing coil 1 mixes with sodium hydroxide). The length and diameter of the mixing coil is very influential on the maximum reaction based on Figure 5.



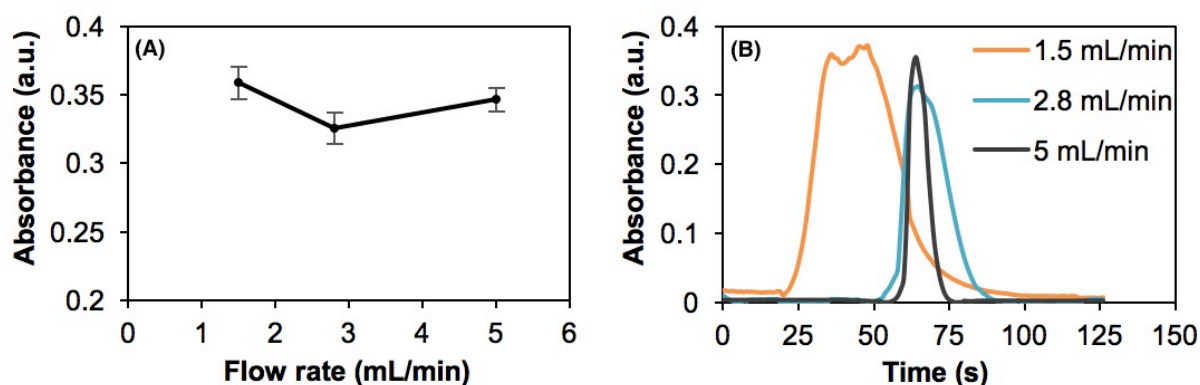
**Figure 5.** Effect of Mixing Coil 1 (a) and Mixing Coil 2 (b)

The length of coil length would obviously change the observed signal because increased coil length increases the reaction time the and decreased coil length decreases the reaction time since as chemistry equilibrium has not reached [9]. Figure 5 (a) shows the signal slightly decreased with the longer coil. The longer coil will maximize the reaction between phloroglucinol and hydroquinone by the hydrogen bond from the hydroxyl group of phloroglucinol and hydroquinone [12]. When the mixed solution in coil 1 was reacted with the sodium hydroxide, the complex formation will not be maximum and decrease the signal. Therefore, 50 cm was chosen as the best mixing coil 1 because give the best signal.

The complex formation was occurred in the mixing coil 2 by the reaction of mixing solution from mixing coil 1 with sodium hydroxide. Mixing coil 2 of 100 cm shows the best complex formation HQ-PG based on the highest signal in Figure 5 (b) as the explanation above that the longer mixing coil will increase the reaction time.

### Optimization of Flow Rate

In order to increase the sensitivity and reducing the variability, the flow rate should be optimization. Moreover, the aim of flow rate optimization is getting the best reaction, material efficiency and more precise determination which is shown in Figure 6.

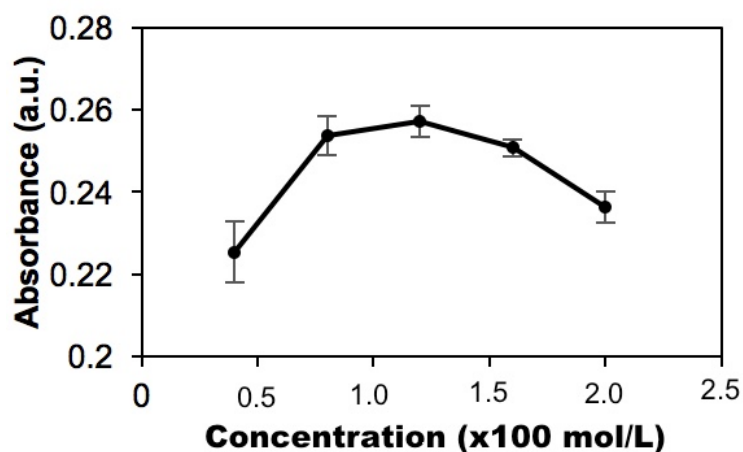


**Figure 6.** Effect flow rate: absorbance (a) and FIA-gram (b)

One of the factors to control the dispersion is flow rate since as increasing flow rate leads turbulent flow in tubular reactors to cause a decrease in the dispersion [16]. The flow rate of 1.5 ml/min in Figure 6 (a) gives the highest absorbance than all. However, the signal in Figure 6 (b) gives splitting peak which shows the reaction between phloroglucinol and hydroquinone are not maximum. The flow rate of 2.8 and 5 ml/min did not have the difference significantly. Therefore, the flow rate of 2.5 was chosen for the best sensitivity with acceptable peak shape and minimum of material consumption.

### Optimization of phloroglucinol

Phloroglucinol formed the complex by medium hydrogen bonding of the hydroxyl group [15]. The stability of the OH group is caused by the position and value of Bond Dissociation Enthalpies (BDE). The value of phloroglucinol BDE is higher than Hydroquinone respectively 354.0 and 329.2 kJ/mol. The Higher BDE, the compound is more stable to form complex association [10]. The correlation of phloroglucinol to form complex association shows in Figure 7.



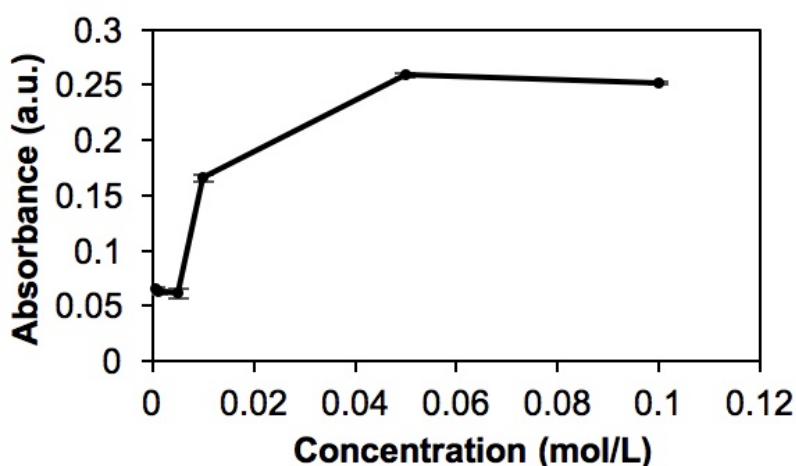
**Figure 7.** Effect of phloroglucinol reagent to form complex HQ-PG

The principle of complex formation is the reaction between ionic phloroglucinol and hydroquinone. The ionic phloroglucinol is formed by the alkaline solution from NaOH when in the highest pH based on all of pKa of phloroglucinol respectively 8, 9.2 and 14 [17]. The

maximum reaction of hydroquinone and phloroglucinol is shown by the highest absorbance in Figure 7. The phloroglucinol concentration of 0.001 mol/L is the best concentration to form the complex HQ-PG for the best sensitivity.

### Optimization of Sodium Hydroxide

The reaction of Hydroquinone-phloroglucinol is affected by the alkalinity solution to convert phloroglucinol become ionic in complex formation. For the best ionic transformation, the phloroglucinol has to the pH 14 in solution to change all of the hydroxyl group of phloroglucinol. Therefore, the concentration of NaOH is varied by 0.0005 – 0.1 mol/L in Figure 8 to get the best concentration in the formation of ionic phloroglucinol and maximize the complex formation.



**Figure 8.** Effect of NaOH concentration to form ionic phloroglucinol and complex formation

Maximum ionic formation of phloroglucinol is formed in the pH 14 based on the pKa. Therefore the NaOH concentration was varied in the range of pH 14 that are 0.0005 – 0.1 mol/L. the best peak is showed by the highest peak of 0.05 mol/L means the optimum ionic formation of phloroglucinol and then complex formation are in 0.05 mol/L concentration of NaOH, due to all hydroxyl groups of phloroglucinol transform to ionic forms optimally in pH 14 [15]. The ionic part of phloroglucinol then react with hydroquinone and form HQ-PG complex.

### Linearity

The linearity of the FIA method was determined by the calibration curve of hydroquinone. By the calibration curve can obtain the linear concentration range, the limit of detection and sensitivity by hydroquinone measurement 2 – 100 mg/L. However, the concentration of 100 mg/L deviated from the linear range. Therefore, the calibration curve uses the range concentration of 2 – 80 mg/L. Figure 9 shows the calibration curve of hydroquinone measurement and shows the correlation between the concentration of hydroquinone with the absorbance of HQ-PG complex. The sensitivity was obtained from the slope of regression equation  $y = 0.039[\text{hydroquinone}]$  by the result of 0.039 mg/L.A. The linearity was obtained from the  $R^2$  is 0.9981 and limit detection (S/N=3) of hydroquinone measurement from FIA is 0.04 mg/L.

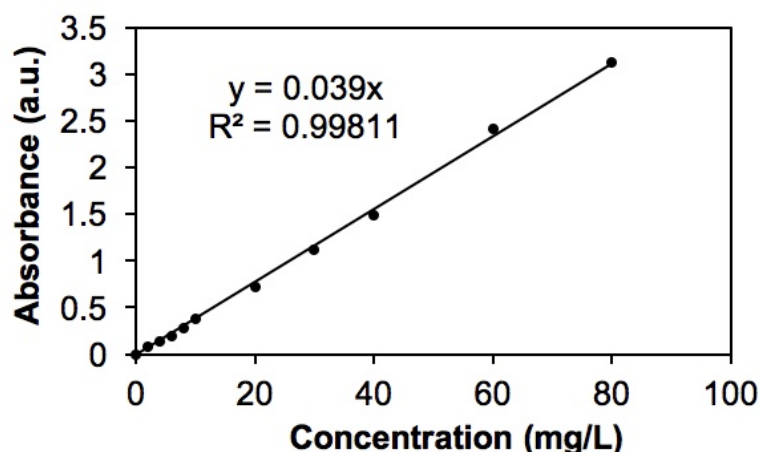


Figure 9. Calibration curve of hydroquinone

## CONCLUSION

The FI-Spectrophotometry method was successful to determine hydroquinone with an optimum result of the operational and chemical factor with the maximum product of the reaction. The current method offers advantages of selective, simple and rapid analysis to detect hydroquinone with the linearity 0.9981 and limit of detection 0.04 mg/L.

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