Development of Flow Injection-Spectrophotometry Method for Hydroquinone Determination Based on The Formation of Blue Starch-Iodine Complex

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Abstract. Hydroquinone (1,4-dihydroxybenzene) is an aromatic organic phenolic compound used in drug and should not be used as cosmetic products. Although the use of hydroquinone has been banned in cosmetics, it is still found at low concentration for skin whitening. The effect for excess use of hydroquinone can cause irritation and changes in skin color become blackish permanently. The determination of hydroquinone can be performed by a flow injection system with spectrophotometric detection based on the redox reaction between hydroquinone and iodine. Under this method, hydroquinone can be determined based on the decreasing intensity of the blue color of the starch-iodine complex when hydroquinone was added to the solution. Hydroquinone will react with the excess iodine, thus iodine is reduced to iodide. The rest of iodine which does not react with hydroquinone, will form a blue complex with starch in an acid condition (pH = 1). The blue color of the starch-iodine complex was analyzed by visible spectrophotometry at a wavelength of 628 nm. The optimum conditions for determination of hydroquinone using the flow injection system provide at 0.05 % starch and 40 mg/L iodine by the length of mixing coil of 50 cm on the flow rate of 5.8 mL/min, and a sample volume of 100 µL. Under these optimum conditions, the flow injection method showed the linearity from 0 to 15 mg/L with a correlation (R²) of 0.9941.

1. Introduction
Hydroquinone (HQ) is an aromatic phenolic compound that has been widely used in black and white photography, chemical industries, and medicines product. Hydroquinone also has a function of melanogenesis inhibitors and widely used for the treatment of hyperpigmentation disorders. Hydroquinone has been banned to be applied in cosmetics, but it is still found at very low concentration. The effect of long-term application of hydroquinone is irritation and risk of skin cancer because this substance inhibits of the tyrosinase enzyme which reduces the melanin synthesis [1]. Terer et.al (2013) [2] reported that several retail outlets sold body lotions and cream cosmetic products content of hydroquinone with the HQ concentration below 2 % in Barton, Kenya. Hydroquinone is highly toxic even at very low concentrations, so by the United States Environment Protection Agency (US EPA) and the European Union are considered as major environmental pollutants [3]. Therefore, a
selective, accurate and fast method for determining hydroquinone is very needed to avoid the dangers of this compound.

There are many analytical methods for determining of hydroquinone, the most common analytical method is the spectrophotometric method. The study about determination of hydroquinone in cosmetic samples carried out by Elferjani (2017) [2] using a UV spectrophotometric method. The first, hydroquinone must be extracted by dissolved in 0.05 M sulfuric acid, then filtering with a filter paper and determined using a UV spectrophotometer at a wavelength of 290 nm. From this method, it shows that Beer’s law is obeyed in the linearity range of hydroquinone concentration of 10-40 µg/mL with a linear regression coefficient of 0.9994. The quantification of hydroquinone samples shows ranges from 0.008% to 0.210% [2]. Nevertheless, this method cannot be used to determine the concentration of hydroquinone less than 10 µg/ml and consume a lot of time for analysis because it must be measured one by one using quartz cuvette.

Another spectrophotometric method for determining of hydroquinone use a flow injection system based on an oxidation reaction using KMnO₄ as an oxidizing agent. The hydroquinone (HQ) will be oxidized to P-benzoquinone (BQ) in alkaline medium conditions. The green color product is formed by passing sodium hydroxide as a carrier to this system and being detected at a wavelength of 610 nm. The optimum conditions for this method use the flow rate of 2.12 mL/min by a sample loop of 227.65 µL and the length of mixing coil is 100 cm. From this optimum conditions known that limit of detection 0.0125 µg.mL⁻¹ and 0.25 µg.mL⁻¹ with recovery up to 99% [4].

The study about starch-iodine complex has been developed by Febrianti (2013) [5], iodide is oxidized by iodate (IO₃⁻) to iodine in acidic conditions. Then, the iodine reacts with starch to form a blue starch-iodine complex. The blue starch-iodine complex can be used to determined iodide in urine by the colorimetric method at the wavelength of 615 nm. In addition, Iodide (I⁻) is considered as a ligand which able to bind with mercury (II) as a metal to form tetraiodomercurate (II) [HgI₄]²⁻. The mercury reacts with excess iodide, then the rest of iodide will be oxidized by iodate in an acidic condition become iodine (I₂). The iodine will bound with starch to form a starch-iodine complex. Based on this method, the color intensity of blue starch-iodine complex will decrease if the mercury was added to the solution [6]. This research was found by Sulistyarti (2015) [6], the mercury was analyzed by Flow Injection Analysis (FIA). The optimum conditions for the operational systems were a sample loop of 250 µL by the length of mixing coil of 100 cm. For optimum chemical systems were the concentration of KIO₃ 0.01 M, the concentration of H₂SO₄ 0.01 M and starch indicator of 0.1 %.

According to the Sulistyarti (2015) [6] investigation, the selectivity and validity test were carried out by Permata (2017) to study the effect of CN⁻ and S²⁻ as interfering ion and also applied the method in gold mining waste. The research quantification by flow injection system-spectrophotometry with a wavelength of 618 nm and used the optimum condition. The result shows the linearity for measured the concentrations of mercury from 0 to 10 mg/L. This method showed that CN⁻ anion not interfered up to 50 mg/L, but S²⁻ has been interfering from 5 mg/L. The validation test was applied to synthetic sample and gold mining waste with recovery values from 93.8 % to 107.5 % [7].

The study of hydroquinone in this work, based on the formation of a blue starch-iodine complex. The hydroquinone will react with the excess iodine, thus iodine is reduced to iodide. The rest of iodine which does not react with hydroquinone will form a blue color complex with starch in an acidic condition (pH = 1). Under this method, hydroquinone can be determined based on the decreasing intensity of the blue color of the starch-iodine complex when hydroquinone was added to the solution. Therefore, the aim of this study is to develop an effective, simple, safe, and rapid method for determination of hydroquinone based on the formation of a blue starch-iodine complex.

2. Materials and Instrumentation

2.1. Materials
De-ionized water for the preparation of all solutions. Stock solutions of iodine dissolved in KI, hydroquinone, and sulphuric acid were prepared by dissolving the exact amount of KI (Merck), I₂
(Merck), H\textsubscript{2}SO\textsubscript{4} (Merck), salicylic acid (Merck) and hydroquinone (Merck) respectively in deionized water. A fresh starch solution of 0.05 % was prepared a few hours before work, by dissolved 0.05 g starch (Sigma) in 100 mL warm water and heated until boiling. Working solutions were prepared with taking appropriate solution by pipetting of stock solution and diluted in de-ionized water.

2.2. Instrumentation
A Shimadzu 1601 UV-Vis spectrophotometer and UV Probe 2.21 application were used for scanning and measuring absorbance. A set of flow injection manifold consists of silicone tubing, valve, sample injector, peristaltic pump, sample loop (0.75 mm I.D PTFE capillary pipe), and mixing coil (0.75 mm PTFE capillary pipe).

The flow injection system with UV-Vis spectrophotometry as a detector to determine hydroquinone can be illustrated in figure 1.

![Figure 1. A Set of Flow Injection Analysis Manifold](image)

3. Methods

3.1. Optimization of Maximum Wavelength.
Maximum wavelength of a blue starch-iodine complex was done by mixing of starch and iodine solution, then scanned by using UV-Vis spectrophotometer from 400 until 800 nm.

3.2. Optimization of Flow Injection Operational Parameters.
Optimization of flow injection analysis for operational parameters consists of the optimization of sample loop, flow rate, and length of mixing coil.

3.2.1. Optimization of Sample Loop.
Sample loop optimization was done with measuring the sample volume from the tubing by using the concentration of hydroquinone of 6 mg/L, length of mixing coil 50 cm, and flow rate of 2.8 mL/min at the wavelength of 628 nm. Volume for determination of a sample loop was carried out with the varied which were 50, 75, 100, and 125 µL. The highest starch-iodine complex absorbance was chosen as an optimum sample loop and selected as a parameter for the next experiment.

3.2.2. Optimization of Flow Rate.
Flow rate optimization was carried out to minimized dispersion of reagent and sample in PTFE hoses with the varied flow rate were 0.77, 1.5, 2.8, and 5.8 mL/min. To measure the optimum flow rate by using the concentration of hydroquinone of 6 mg/L, length of mixing coil 50 cm and sample loop 100 µL. The optimum flow rate for analysis was chosen from the highest and good shape peak of complex absorbance.

3.2.3. Optimization the Length of Mixing Coil.
The length of mixing coil has an effect on the starch-iodine complex formation to determination of hydroquinone. How long the time for the reaction between reagent and sample in PTFE hoses
influenced the complex color formation and dispersion of the sample. Variation of mixing coil for optimization by the PTFE hose with different length of 25, 50, 75, and 100 cm. Optimization the length of the mixing coil was done by 6 mg/L hydroquinone, sample loop of 100 µL and flow rate of 5.8 mL/min. The highest and good shape peak blue complex absorbance was chosen as an optimum mixing coil and used for the optimization of chemical parameters.

3.3. Optimization of Flow Injection Chemical Parameters
Optimization of flow injection analysis for operational parameters consists of a concentration of starch and iodine.

3.3.1. Optimization Concentration of Iodine.
The concentration of iodine as an oxidizing agent was optimized to ensure the forming of blue color starch-iodine complex without added hydroquinone. The optimization also carried out to get long linearity range. The iodine concentration was done with variation from 30 mg/L until 100 mg/L by the optimum operational parameters was sample loop of 100 µL, length of mixing coil of 50 cm, and the flow rate of 5.8 mL/min. The optimum iodine concentration was chosen based on the highest and good shape peak absorbance of the blue starch-iodine complex.

3.3.2. Optimization Concentration of Starch.
Starch as an indicator for the starch-iodine complex was optimized to examine the influence of increasing concentration starch for the blue color absorbance. Optimization of starch concentration was carried out with variation of 0.01, 0.02, 0.05, 0.07, and 0.1 %. The flow injection analysis operated by an operational system of sample loop of 100 µL, length of mixing coil of 50 cm, and the flow rate of 5.8 mL/min. The concentration of starch was chosen by the highest absorbance and good shape peak to measure the linearity of hydroquinone concentration.

3.3.3. Linearity Measurement of Hydroquinone Concentration.
In order to determine the linearity measurement of hydroquinone concentration was carried out based on the optimum result of flow injection operational and chemical parameters. The concentrations of hydroquinone from 0 to 15 mg/L were measured by Shimadzu 1601 UV-Vis spectrophotometer as a detector. The data obtained made a curve of the relationship between sample concentration (x-axis) and absorbance (y-axis).

4. Results and Discussion
The redox reaction between hydroquinone and iodine is shown below, where hydroquinone is oxidized to quinone by iodine and the excess iodine is reduced to iodide by hydroquinone, the reaction below [8]:

\[
C_6H_4O_2H_2 + I_2 \text{ (excess)} \rightarrow C_6H_4O_2 + 2I^- + 2H^+
\]

Then, the rest of iodine which does not react with hydroquinone, bound by starch to form a starch-iodine complex [9].

\[
I_2 \text{ (rest)} + \text{Amilum} \rightarrow I_2\text{-Amilum} \text{ (blue color complex, } \lambda \text{ 628 nm)}
\]

The starch indicator consists of the linear and helical amylose and branched amylopectin compounds, which the amylose is a part that can be dissolved in water (hot temperature). Meanwhile, the amylopectin compounds is a branched polysaccharide consisting of glucose molecules that bounds one to others through 1,6-glycosidic bonds every 20-25 unit of glucose molecules and cannot be dissolved in water [10]. The blue color of starch-iodine is formed because the iodine molecules are bound to the surface of amylose, because of the affinity of iodine towards amylose about 20%, meanwhile towards amylopectin only less than 1% [11]. Iodine will be trapped into the helical amylose structure and give a blue/purple color solution depend on the length of the amylose molecule [12].
4.1. Optimization of Maximum Wavelength
The starch-iodine complex was scanned in the visible absorption spectrum at the wavelength of 400 to 800 nm that shown in figure 3. From figure 3, the spectrum shows the maximum absorption band of the starch-iodine complex at 628 nm. The maximum wavelength was chosen as the basis of flow injection measurement with spectrophotometric detection.

![Figure 2. The Maximum Wavelength of Starch-Iodine Complex](image)

4.2. Optimization of Operational Parameters

4.2.1. Optimization of Sample Loop.
Sample loop optimization has a function to observe the influence of increasing injected sample volume to the signal formed. Noteworthy, determination of the optimum sample loop parameter not only based on the highest absorbance formed, however should be viewed from the peak shape and the height of intensity resulted [7]. The sample loop with various volumes (50, 75, 100, 125 µL) were optimized using a different length of the sample loop. Under the resulted from figure 4, showed that 100 µL was chosen as the optimum sample loop based on the highest absorbance peak with good shape peak.

![Figure 3. The Effect of Variation Sample Loop on Hydroquinone Absorbance](image)
4.2.2. Optimization of Flow Rate.
The optimization of flow rate to minimize dispersion between reagent and sample, because the optimum flow rate can be increasing the sensitivity of this method. Flow rate variations were carried out of 0.77, 1.5, 2.8, and 5.8 mL/min.

![Figure 4. The Effect of Flow Rate on Hydroquinone Absorbance](image)

Based on figure 5, the flow rate of 1.5 and 5.8 mL/min did not give significant difference signal peak. The flow rate of 1.5 mL/min produced a widened peak with a shoulder peak, it was consumed a lot of time for analysis. Shoulder peak indicated that the reaction between reagent starch and hydroquinone-I₂ did not optimum which was may be influenced by a slow flow rate, so that produced widened peak and dispersion on solutions [13]. The dispersion as the main factor which gave a consequence of losing insensitivity and limit of detection [14]. Therefore, the flow rate of 5.8 mL/min was chosen as an optimum flow rate because had the best sensitivity with an acceptable peak.

![Figure 5. The Effect of Flow Rate FIA-gram](image)

4.2.3. Optimization of Mixing Coil.
Mixing coil by using a PTFE hose, as one a part of reaction zone where very influential on the reaction between reagents and sample because the length of mixing coil would affect to the chemical equilibrium [15]. The sensitivity also can be improved by decreasing or increasing the diameter and length in the mixing coil as one part of reaction zones [13]. As described in figure 7, more along the
length of the mixing coil shows that the signal was decreased. Decreasing by the signal intensity caused of reaction between starch and hydroquinone-I₂ were too long in mixing coil so that the chemical reaction has not reached equilibrium and gives the effect of dispersion in the solution [14]. Under these result, the length of 50 cm of mixing coil provided the highest absorbance and indicated the best length as a reaction zone for this method.

![Figure 6](image)

**Figure 6.** The Effect of Length Mixing Coil on Hydroquinone Absorbance

4.3. **Optimization of Chemical Parameters**

4.3.1. **Optimization Concentration of Iodine.**
Optimization of iodine as an oxidizing agent to ensure get a long linearity range from the forming of blue color starch-iodine complex. It can be seen from figure 8 gives information about the higher concentration of iodine, also increase the absorbance of the starch-iodine complex because of the color formed of these complex more toughly. The absorbance from the concentration of iodine 40 mg/L with 50 mg/L very slightly different, but increase for the next concentration.

![Figure 7](image)

**Figure 7.** The Effect of Concentration of Iodine Reagent to Form Starch-I₂ Complex

Higher complex absorbance does not mean the peak of FIA gram shape will be accepted. As describe figure 9 below, the influence of higher iodine concentration can be obtained should peak and shoulder peak clearly appear in the highest concentration of iodine. It was caused by dispersion and the chemical reaction has not reached equilibrium so that gave shoulder peaks [14]. The resulted show that 40 mg/L had a good peak shape without shoulder peak and acceptable as an optimum peak.
4.3.2. Optimization Concentration of Starch.
Starch as an indicator solution also optimized to find out the maximum absorbance of the starch-iodine complex. From figure 10, at the concentration of 0.05% to 0.1% absorbance values ranges respectively from 0.66 until 0.69 which indicates there is no significant difference. It was known that optimum concentration starch of 0.05%.

4.4. Linearity Measurement of Hydroquinone Concentration
Under the optimum of operational and chemical conditions outline above (i.e. operational conditions such as sample loop of 100 µL, flow rate of 5.8 mL/min, and length of mixing coil of 50 cm, also chemical conditions for concentration iodine of 40 mg/L and concentration of starch of 0.05%) this method gives results to be linear (y = -0.0551x + 0.8854 with R² = 0.9941). Based on the reduction of iodine to iodide by hydroquinone, so that the intensity will be decreasing when hydroquinone was added to the solution. Calibration curve as describe figure 11 below, can measure the linearity range from 0 to 15 mg/L with an analysis time of 4 minutes.
5. Conclusion
The Flow Injection-spectrophotometry method to determine of hydroquinone based on the blue starch-iodine complex was successfully developed with the maximum product by optimum resulted of operational and chemical parameters. This method employed safe, rapid, availability of raw materials and low-cost reagents with linearity 0.9941.

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