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VOLTAMMETRIC DETERMINATION OF REACTIVE BLACK 5 (RB5) IN WASTE WATER SAMPLES FROM THE BATIK INDUSTRY

(Penentuan Voltammetrik Bagi Reaktif Black 5 (RB5) Dalam Sampel Air Sisa Dari Industri Batik)

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Abstract

A cyclic voltammetry (CV) and differential pulse cathodic stripping voltammetry (DPCSV) studies of RB5 in Britton-Robinson buffer (BRB) at a hanging mercury dropping electrode (HMDE) are described. CV was carried out by cathodic and anodic potential scan over the range of +200 mV to -800 mV with no accumulation time and three well-defined pH-dependent cathodic peaks were observed. The effects of different scan rates, pH of BRB, repetitive cycle and increasing concentrations on the peak height and peak potential of the analyte were studied. The results showed that RB5 dye was adsorbed and irreversibly reduced at the mercury electrode, mainly under diffusion controlled in the optimum pH 4.0 of BRB. By using DPCSV, pH of BRB and various instrumental parameters such as scan rate, accumulation time, accumulation potential and pulse amplitude that may affect the peak height of RB5 were investigated and optimized for RB5 determination. Under the optimized parameters, the peak height was found to be linear with RB5 concentration in the range from 2.5×10^{-7} $molL^{-1}$ to 2.0×10^{-6} $molL^{-1}$. A detection limit (3SD/m) of 1.3×10^{-8} $molL^{-1}$ was achieved. The precision of the developed method in terms of RSD was 0.55% for intra-day measurement and 0.55%, 0.24% and 0.40%, for inter-day measurements, respectively. The developed method was successfully applied in the determination of RB5 in batik wastewaters with the mean recovery of 83.97 ± 4.78% to 97.93 ± 5.45% (0.5×10^{-6} $molL^{-1}$), 95.91 ± 2.68% to 103.49 ± 1.32% (1.0×10^{-6} $molL^{-1}$) and 99.17 ± 0.75% to 105.12 ± 0.28% (2.0×10^{-6} $molL^{-1}$). The developed method is considered sensitive, precise, accurate, rugged, robust, fast and low cost for routine analysis of RB5 dye in batik wastewaters over the UV-VIS spectrometry method.

Keywords: Reactive Black 5, differential pulse cathodic stripping, Britton-Robinson buffer, hanging mercury dropping electrode, batik wastewater samples

Abstrak

Kajian voltammetri berkitar (CV) dan voltammetri perlucutan denyut kebezaan (DPCSV) terhadap pewarna RB5 dalam larutan penimbal Britton-Robinson (BRB) menggunakan elektrod jatuhan raksa tergantung (HMDE) dihuraikan. CV dijalankan dengan mengimbas potential kathodik dan anodik dari +200 mV ke -800 mV tanpa masa pengumpulan dan tiga puncak diperhatikan selepas imbasan. Kesan perbezaan kadar imbasan, pH BRB, imbasan berkitar yang berulang dan peningkatan kepekatan RB5 ke atas ketinggian dan nilai keupayaan puncak dikaji. RB5 yang dijerap dan diturunkan secara tidak berbalik pada permukaan elektrod merkuri adalah dibawah kawalan difusi pada pH BRB yang optimum iaitu 4.0. Dengan menggunakan DPCSV, pH BRB dan parameter-parameter peralatan yang memberi kesan kepada ketinggian puncak seperti kadar imbasan, masa pengumpulan, keupayaan pengumpulan dan amplitud denyut telah dioptimumkan untuk analisis RB5. Ketinggian puncak berkadar terus dengan kepekatan RB5 dalam julat dari 2.5×10^{-7} $molL^{-1}$ hingga 2.0×10^{-6} $molL^{-1}$. Had pengesanan adalah 1.3×10^{-8} $molL^{-1}$. Ketepatan (RSD) kaedah yang dibangunkan adalah 0.55% untuk hari yang sama, manakala 0.55%, 0.24% dan 0.40% untuk tiga hari yang berbeza. Kaedah yang dibangunkan berjaya diuji bagi penentuan RB5 dalam air sisa batik dengan purata kebolehdapatan 83.97 ± 4.78% sehingga 97.93 ± 5.45% (0.5 x 10^{-6} molL^{-1}), 95.91 ± 2.68% sehingga 103.49 ± 1.32% (1.0 x 10^{-6} molL-1) dan 99.17 ± 0.75% sehingga 105.12 ± 0.28% (2.0 x 10^{-6} molL^{-1}). Kaedah yang dibangunkan adalah sensitif, tepat, lasak, teguh, cepat dan menjimatkan bagi analisis rutin kandungan RB5 dalam air sisa batik berbanding kaedah spektrofotometri UV-VIS.

Kata kunci: Reaktif Black 5, voltammetri perlucutan denyut kebezaan, penimbal Britton-Robinson, elektrod jatuhan raksa tergantung, sampel air sisa batik

Introduction

The textile industry is considered as one of the major water consumers. It utilizes a large volume of water and chemicals for the wet processing of the textiles and can contribute to the water bodies pollution [1, 2]. Homemade batik industry is a part of the textile industry and preliminary studies have shown that wastewaters discharged from this particular industry contain dyes, heavy metals, grease, wax and also suspended solids with high chemical oxygen demand (COD) [3, 4].

Azo dyes are considered as the oldest and the largest class of synthetic organic dyes. They are also considered as dyes with the largest shade range and are commonly used [5]. They account for 60% to 70% of the total organic dyes, have bright and high colour intensity, extensively used in textile, food, drug, cosmetic, automobile, leather and in the paper making industries [6, 7, 8].

Reactive Black 5 (RB5) or [2,7-naphthalenedisulfonic acid, 4-amino-5-hydroxy-3,6-bis ((4 - ((2 (sulfooxy) ethyl) sulfonyl) phenyl) azo)-tetrasodium salt], which chemical structure is shown in Figure 1, is categorized in the reactive vinyl sulphonate azo dye due to the presence of –N=N- bonds combined with vinylsulphone as the reactive groups [9]. The two azo groups which act as chromophores enable the dyes to absorb light in the visible spectrum and are also responsible for the corresponding colours. These reactive groups are covalently bonded to –OH or –NH_2 groups that are present in the materials to give excellent wash fastness property [10, 11, 12].

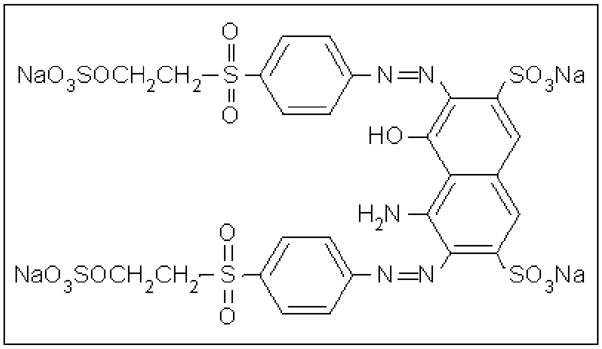

Figure 1. Chemical structure of RB5

Several analytical methods for the analysis of azo dyes and their intermediates in various samples have been developed and most of the methods are based on the electrophoresis, chromatographic and spectroscopic method [13, 14]. The voltammetric method allows for an analysis without or with minimal samples pre-treatment, has comparable selectivity and sensitivity over the chromatographic method, applicability over a wide concentration range, fast analysis, low cost on instrumentation and maintenances and is able to perform both individual and simultaneous analyses [15, 16].

This paper describes the cyclic voltammetric (CV) studies of RB5 dye using a hanging mercury dropping electrode (HMDE) as the working electrode in the Britton-Robinson buffer (BRB) to obtain qualitative information on the electrochemical reactions such as reversibility, adsorption and diffusion characteristics of a system. Differential pulse cathodic stripping voltammetric (DPCSV) studies of RB5 dye using the same HMDE and in the same BRB had then been carried out for RB5 dye determination in the wastewaters from local batik factories. With regards to the voltammetry method, no DPCSV technique for RB5 dye determination has been reported so far. It is also rarely reported for the analysis of azo dyes containing two azo groups in their chemical structures.

Materials and Methods

Materials

All chemicals used in this study were of analytical grade reagent and all solutions were prepared in deionized water. The RB5 dye standard (MW = 991.82 $gmol^{-1}$) was obtained from Sigma Aldrich, UK. For the preparation of 2.0 x 10^{-4} $molL^{-1}$ RB5 dye stock solution, 20 mg of the RB5 dye standard was dissolved in 100 mL volumetric flask. The standard working solution of 2.0 x 10^{-5} $molL^{-1}$ was prepared by the appropriate dilution of the stock solution. BRB solution as the supporting electrolyte was prepared in 1000 mL deionized water, composed of a mixture of 2.47 g boric acid (R&M Chemicals, Canada), 2.30 mL glacial acetic acid (R&M Chemicals, Canada) and 2.70 mL ortho-phosporic acid (R&M Chemicals, Canada). Sodium hydroxide (Systerm, Italy) with the concentration of 0.1 M was used to adjust the pH of the BRB solution to the desired value. High purity mercury, 99.99% (Merck, Germany) was used for HMDE.

Instrumentations

The voltammetric experiments were carried out using VA 757 Computrace Metrohm Voltammetric Analyzer with 663 VA Stand (Metrohm, Switzerland), consisted of Multi-Mode Electrode (MME) and a 40 mL capacity voltammetric cell. The MME consisted of a HMDE as the working electrode (WE), a platinum wire as the auxiliary electrode (AE) and an Ag/AgCl as the reference electrode (RE). The VA 757 was connected to a computer for data processing and for all pH measurements, the pH meter (Hanna Instruments, UK) was employed.

Voltammetric measurement:

Cyclic voltammetry

BRB solution with the volume of 9 mL was placed into a voltammetric cell and purged by a stream of purified nitrogen for at least 15 minutes. RB5 dye standard solution (2.0 x 10^{-3} $molL^{-1}$) with the volume of 1 mL was then spiked to give a final concentration of 2.0 x 10^{-4} $molL^{-1}$ and the solution was repurged for 2 minutes. The RB5 dye was scanned from +200 mV to -800 mV (cathodic direction) and switched to +200 mV (anodic direction) with a scan rate (v) of 25 mV/s in various pH of the BRB solution (pH 2.0 to 12.0). The effect of various v (25 to 200 mV/s), the effect of repetitive cycle (1 to 5 cycles) and the effect of dye concentrations on the peak height (I_p) and peak potential (E_p) of RB5 dye were investigated.

Differential pulse stripping voltammetry

BRB solution with the volume of 9 mL was placed into the voltammetric cell and purged by a stream of purified nitrogen for at least 15 minutes. RB5 dye standard solution (2.0 x 10^{-5} $molL^{-1}$) with the volume of 1 mL was then spiked to give the final concentration of 2.0 x 10^{-6} $molL^{-1}$ and the solution was repurged for 2 minutes. The initial operational parameters applied for the voltammetric measurements were the initial potential (E_i) = +150 mV, final potential (E_f) = -700 mV, v = 15 mV/s, accumulation time (t_{acc}) = 0 s, accumulation potential (E_{acc}) = 0 mV and pulse amplitude = 50 mV. Parameters such as v, t_{acc}, E_{acc} and pulse amplitude were optimized to increase the response of the developed method.

The developed method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, repeatability, accuracy, ruggedness and robustness to verify the suitablity for the extended purposes according to the approach proposed by Miranda et al. [17]. The LOD and LOQ were calculated by the equations; LOD = 3 SD/m and LOQ = 10 SD/m, where SD is the standard deviation of the three replicate determinations of the RB5 dye at the lowest concentration on the calibration curve and m is the slope of the regression equation [18]. The RB5 dye standard solution at the concentration of 2.0 x 10^{-6} $molL^{-1}$ was applied for intra-day and inter-day precision, ruggedness and robustness with five replicate (n=5) measurements. The ruggedness of the developed method was investigated using the same voltammetry analyzer (VA 757), but had been conducted by two different analysts [19] and was also analyzed by using two different voltammetry analyzers (VA 757 and VA 797) but only conducted by one analyst [20] under the same optimum parameters. The robustness was carried out by examining the effect of a small variation of v, t_{acc}, time interval analysis after RB5 dye standard preparation and pH of BRB solution on the response.

Collection, preservation and analysis of wastewaters from the batik industry

Wastewaters from the batik industry were obtained from nine batik factories, located at Kota Bharu and Pantai Cahaya Bulan, Kelantan, Malaysia. The wastewaters were collected from two different dyebaths at each factory. The wastewaters were analyzed using the developed DPCSV technique without the prior samples pre-treatment and extraction by spiking 1 mL of the wastewater samples into the 9 mL BRB solution.

Recovery studies of the RB5 dye in wastewaters from the batik industry

To study the accuracy and reproducibility of the developed DPCSV technique, recovery experiments were carried out using the standard addition method.

UV-VIS Spectrophotometry analysis of the RB5 dye

The results obtained by the developed DPCSV technique were compared with the UV-VIS Spectrophotometry method using Spectrophotometer Cary 100, USA. The double beam mode was employed for the overall analysis from the start wavelength of 700 nm to the stop wavelenghth of 300 nm with the rate of 600 nm/min.

Results and Discussion

Electrochemical behaviour of the RB5 dye

Cyclic voltammetric studies of the RB5 dye were carried out in 0.04 M BRB solution from pH 2.0 to 12.0 with v of 25 mV/s. From the cyclic voltammogram obtained, BRB solution at pH 4.0 was observed to be the optimum condition with I_p of 298 nA for RB5 dye reduction at the HMDE, as shown in Figure 2. Three well-defined reduction peaks at -79.80 mV, -258 mV and -568 mV were obtained for the first, second and third peak, respectively. No oxidation peaks were observed which confirmed that the RB5 dye was irreversibly reduced at the mercury electrode. The second reduction peak was referred to as the analytical signal due to its higher sensitivity compared to the first and third reduction peak of the RB5 dye [21].

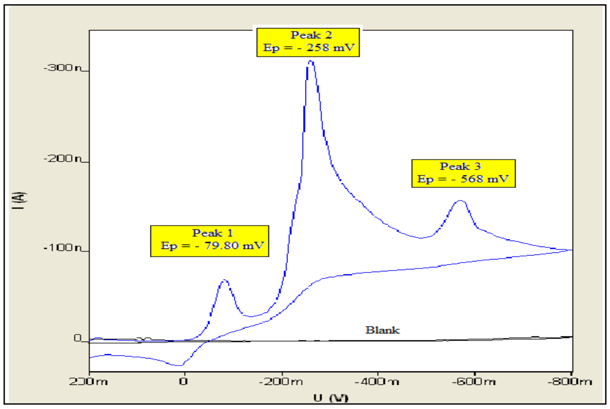

Figure 2. Cyclic voltammogram of 2 x 10^{-4} $molL^{-1}$ RB5 dye in BRB solution at pH 4.0.Experimental condition: E_i = +200 mV, E_{switch} = -800 mV, E_f = +200 mV and v = 25 mV/s

The first two obtained reduction peaks were suggested due to the subsequence reduction process of the two azo groups to amines. Both hydroxyl and amino groups were electron donating substituents in the RB5 dye compound. The first reduction peak was suggested from the reduction process of the azo with the hydroxyl group and followed by the reduction process of the other azo with amino group, which gave the second reduction peak on the voltammograms. The reduction process of the azo with amino group was more difficult due to the electron distribution in this functional group [22].

The effect of pH of BRB solution on the E_p was studied from pH 2.0 to 12.0. The results showed that the E_p of RB5 dye was shifted to the more negative potentials with the increased pH indicating that the electron transfer was accompanied by proton transfer in the reduction process [23, 24]. The reduction process was easier to take place in a medium rich with H^+ ions and more difficult in a medium with low H^+ ions [25] and the reduction process of RB5 dye was pH dependent [26, 27].

The effects of scan rate (v) from 25 to 200 mV/s on the E_p and I_p of the RB5 dye were observed under the same experimental conditions. The results showed that by increasing the v, the E_p shifted to more negative potentials and accompanied with the increase of the I_p. The increase of I_p indicated an adsorption phenomenon of the RB5 dye at the surface of the mercury electrode [28, 29]. A linear relationship was observed between log I_p against log v with a slope of 0.5282 (R^2 = 0.9982, n = 6), as shown in Figure 3. The slope of 0.5282 was close to the theoretical value of 0.5, which indicated that the reduction process of RB5 dye was mainly controlled by diffusion [30, 31].

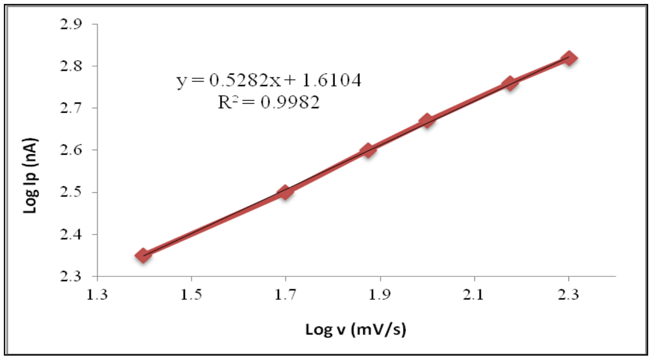

Figure 3. Plot of I_p to the log v for 2 x 10^{-4} molL^{-1} RB5 dye in BRB solution at pH 4.0

The effect of the RB5 dye concentrations was investigated and the results showed that the E_p of RB5 dye at -258 mV showed no significant change in the peak position and the I_p of the RB5 dye increased with the increasing concentration of RB5 dye. This phenomenon confirmed the adsorption of the RB5 dye at the mercury electrode surface [32]. No new peak was observed with the increasing RB5 dye concentration at both the cathodic and anodic directions, which indicated that the obtained reduction peaks were due to respective RB5 dye and not from other electroactive species that may be present in the BRB solution [33].

The effect of repetitive cycles on the I_p of the RB5 dye was studied with five cycles. The I_p decreased in the second cycle and was almost constant for the third, fourth and fifth cycle, as shown in Figure 4. This meant that the variation of I_p was controlled by diffusion in the experimental condition [34] and RB5 dye had the adsorption behaviour at the surface of the mercury electrode [35].

Differential pulse stripping voltammery of the RB5 dye: The effects of pH of BRB solution

Differential pulse cathodic stripping voltammetry of 2 x 10^{-6} molL^{-1} RB5 dye was studied in 0.04 M BRB solution from pH 2.0 to 12.0. BRB solution at pH 2.5 was considered as the optimum pH since the highest I_p and well-defined shape of the first reduction peak were obtained. The voltammogram of RB5 dye in BRB solution at pH 2.5 is shown in Figure 5. The E_p of RB5 dye peak shifted to more negative potentials as the pH of BRB solution increased, indicating the involvement of H^+ in the reduction process of RB5 dye [36].

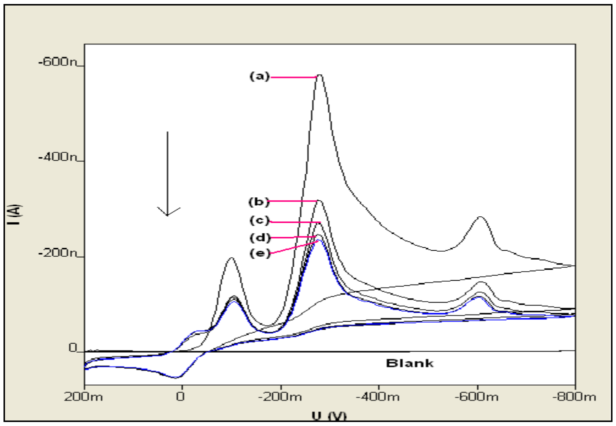

Figure 4. Cyclic voltammograms of repetitive cycles of 2 x 10^{-4} $molL^{-1}$ RB5 dye in BRB solution at pH 4.0. (a) First (b) second (c) third (d) fourth and (e) fifth cycle

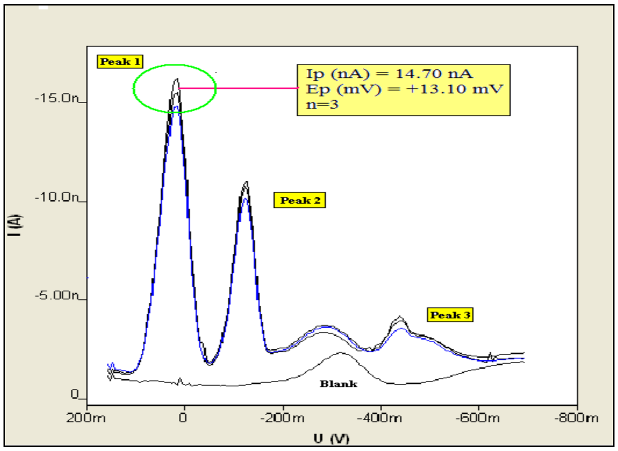

Figure 5. Voltammogram of 2 x 10^{-6} $molL^{-1}$ RB5 dye in BRB solution at pH 2.5. Parameters conditions: E_i = +150 mV, E_f = -700 mV, v = 15 mV/s, t_{acc} = 0 s, E_{acc} = 0 mV and pulse amplitude = 50 mV

Optimization of the operational parameters

It is very important to optimize all the parameters that may influence the measurements in order to obtain the optimum response [37]. The effects of scan rate (v), accumulation time (t_{acc}), accumulation potential (E_{acc}) and pulse amplitude on the I_p of 2 x 10^{-6} $molL^{-1}$ RB5 dye were studied in the BRB solution at pH 2.5.

The effect of scan rate (v)

The relationships of I_p of the RB5 dye to various v such as 5, 10, 15, 30 and 40 mV/s were observed using the unoptimum parameters. The v of 5 mV/s was chosen as the optimum condition since it gave the highest I_p of the first reduction peak of the RB5 dye which was 16.10 nA.

The effect of accumulation time (t_{acc})

Accumulation of electroactive species at the electrode contributed to a high response in the electroanalytical measurements [38]. Accumulation time (t_{acc}) is the period of time given for the RB5 dye to accumulate onto the mercury electrode surface before being analyzed. The dependence of I_p on the t_{acc} was studied at concentration of 2 x 10^{-6} $molL^{-1}$ RB5 dye over the range from 0 to 110 s.

At t_{acc} of 0 s, the first reduction peak of RB5 dye gave a higher I_p compared to the second reduction peak. The I_p of the second reduction peak had become dominant, starting from t_{acc} of 10 s and onwards. The second reduction peak was referred as the analytical signal for further optimization since it showed higher I_p as the t_{acc} increased. From Figure 6, the I_p increased with the increasing t_{acc} from 0 to 80 s, then became constant with the longer times. The t_{acc} of 80 s was chosen as the optimum condition with I_p of 121 nA at E_p of -88 mV.

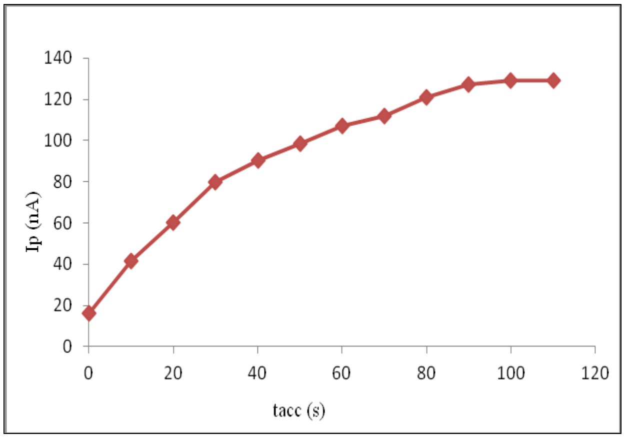

Figure 6. Effect of various t_{acc} on the I_p of second reduction peak of 2 mg/L RB5 dye in BRB solution at pH 2.5. Initial instrumental parameters were E_i = +150 mV, E_f = -650 mV, v = 5 mV/s, E_{acc} = 0 mV and pulse amplitude = 50 mV

As suggested by Tavares et al. [39], the increase of I_p with t_{acc} was due to the accumulation of RB5 dye at the mercury electrode surface in a larger amount, since longer time was given for the accumulation process to occur. The constant I_p that was obtained at longer 90 s had been suggested due to the saturation of the accumulated RB5 dye at the electrode surface [40].

The effect of accumulation potential (E_{acc})

Accumulation potential (E_{acc}) was the potential for the RB5 dye to be accumulated at the surface of mercury electrode, whereas an optimum E_{acc} was the most appropriate potential for the accumulation process to take place. The effect of E_{acc} on the I_p of the second reduction peak was evaluated over the range of 0 to -100 mV for 2 x 10^{-6} molL^{-1} RB5 dye. The maximum response was observed at E_{acc} of 0 mV with I_p of 120 nA at E_p of -82.10 mV. It indicated that the accumulation of RB5 dye at the electrode surface effectively occured at 0 mV.

The effect of pulse amplitude

According to De Lima et al. [41], pulse amplitude is one of the factors that affect the sensitivity of voltammetric measurements. The effect of pulse amplitude on the I_p was studied by measuring 2 x 10^{-6} molL^{-1} RB5 dye within the range of 25 to 125 mV of pulse amplitude, under the previous optimum parameters. Figure 7 shows that the I_p has sharply increased at the range of pulse amplitude from 25 to 75 mV and slowly decreased at higher values. The E_p has shifted towards less negative directions as the values of pulse amplitude increased.

The pulse amplitude of 75 mV was noted as the optimum condition due to the highest I_p observed which was 223 nA with E_p at -65.50 mV. By changing the pulse amplitude, the E_i was changed from +150 mV to +250 mV and the E_f was changed from -650 mV to -600 mV in order to observe the first reduction peak on the voltammograms.

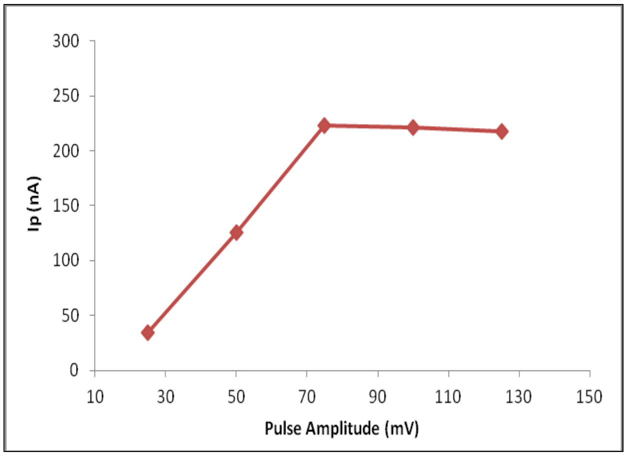

Figure 7. Effects of various pulse amplitudes on the I_p of 2 mg/L RB5 dye in BRB solution at pH 2.5. Initial instrumental parameters were E_i = +150 mV, E_f = -650 mV, v = 5 mV/s, t_{acc} = 80 s and E_{acc} = 0 mV

By applying these optimized parameters, the I_p was enhanced about 24 times, compared to the obtained I_p by using the unoptimized parameters which was 9 nA at E_p of -121 mV. Table 1 shows the optimum supporting electrolyte and instrumental parameters of the developed DPCSV technique of 2 x 10^{-6} $molL^{-1}$ RB5. These optimum parameters were then used to validate the developed method.

Table 1. Optimum supporting electrolyte and instrumental parameters of the developed DPSCV technique

Supporting Electrolyte and Instrumental Parameters	Optimum Value
Britton-Robinson Buffer (BRB)	pH 2.5
Initial Potential, E_i (mV)	+250
End Potential, E_f (mV)	-600
Scan Rate, v (mV/s)	5
Accumulation Time, t_{acc} (s)	80
Accumulation Potential, E_{acc} (mV)	0
Pulse Amplitude (mV)	+75

Validation of the developed method: Linearity, limit of detection, limit of quantification

The applicability of the developed DPCSV technique as an analytical method for the determination of RB5 dye standard solution was examined by measuring the obtained I_p as the function of concentration under the optimum operational and instrumental parameters as shown in Table 1.

Two linear regions were observed and the I_p of RB5 dye tended to maintain, starting at the concentration of 1.6 x 10^{-5} $molL^{-1}$. As suggested by Geremedhin et al. [42] and Radi et al. [43], this phenomenon may be due to the limited adsorption of RB5 dye at the mercury electrode, which resulted from the saturation of the electrode surface. As shown in Figure 8, the first linear region demonstrated its linearity from 2.5 x 10^{-7} $molL^{-1}$ to 2.0 x 10^{-6} $molL^{-1}$ at five different concentrations of RB5 dye standard solution. The constructed calibration graph was represented by the linear equation of I_p (nA) = 115.61 x – 10.31 with a correlation coefficient (R^2) of 0.9998. The second region was linear from 3.0 x 10^{-6} $molL^{-1}$ to 1.4 x 10^{-5} $molL^{-1}$ with the regression equation of I_p (nA) = 11.76 x + 211.56 and R^2 of 0.9987. The first linear region demonstrated a bigger slope than the second region, thus it was applied for RB5 dye determination since a higher sensitivity had been offered for analysis [44]. The calculated LOD for the standard solution was 1.3 x 10^{-8} $molL^{-1}$ and the LOQ was found to be 4.3 x 10^{-8} mol L^{-1}.

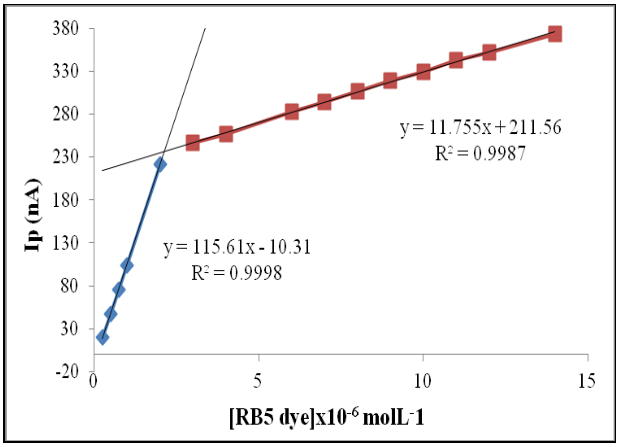

Figure 8. Two linear curves of RB5 dye in BRB solution at pH 2.5. (a) 2.5 x 10^{-7} to 2.0 x 10^{-6} $molL^{-1}$ and (b) 3.0 x 10^{-6} to 1.4 x 10^{-5} $molL^{-1}$

Precision

The precision of the developed technique for RB5 dye determination was determined in five replicate measurements (n=5) at a concentration of 2.0 x 10^{-6} $molL^{-1}$ [45]. The relative standard deviation (RSD) of 0.55% had been achieved for the measurements which indicated that the developed method was precise and confidence as the value was less than 2% [46]. The intra-day and inter-day repeatability studies were carried out by measuring 2.0 x 10^{-6} $molL^{-1}$ RB5 dye for five replicates in three consecutive days using the developed technique. The RSD values for day 1, 2 and 3 were 0.55%, 0.24% and 0.40%, respectively which also indicated that the developed method was precise and confidence.

Accuracy

The accuracy of the developed technique was tested by spiking three known different concentrations of RB5 dye which were 5 x 10^{-7} $molL^{-1}$, 1.0 x 10^{-6} $molL^{-1}$ and 2.0 x 10^{-6} $molL^{-1}$ into a voltammetric cell containing BRB solution [47]. These spiked RB5 dye standard solutions were measured in three replicates (n=3) and the discovered amounts of the spiked solutions in the cell were calculated using the equation of I_p (nA) = 115.61 x – 10.31, where x is the found concentrations of RB5 dye in the voltammetric cell. The satisfactory recoveries of 99.94 ± 3.15%, 99.16 ± 1.80% and 100.61 ± 1.09% were respectively achieved for the spiked concentrations of 5 x 10^{-7} $molL^{-1}$, 1.0 x 10^{-6} $molL^{-1}$ and 2.0 x 10^{-6} $molL^{-1}$.

Ruggedness

The RSD values were 0.49% and 0.38% for the first and second analyst, respectively for the measurements using the same voltammetry analyzer (VA 757). The RSD values were 0.49% and 0.20% for the analysis using the respective VA 757 and VA 797 (different voltammetry analyzers) by the first analyst. From the two-tailed *F* test, there were no significant differences between the obtained variances for RB5 dye when the measurements were performed by two different analysts with the same voltammetry analyzer and by the same analyst with two different voltammetry analyzers at the 5% significance level. Hence, the results indicated that the developed technique was considered rugged.

Robustness

By the two -tailed *F* test at 95% confidence level, none of small variations of the v (3 and 7 mV/s), t_{acc} (78 and 82 s), time interval analysis after the standard preparation (0, 30 and 60 minutes) and pH of BRB solution (pH 2.3 and 2.7) had significantly influenced the obtained I_p of RB5 dye at the concentration of 2.0 x 10^{-6} $molL^{-1}$ with five replicate (n=5) measurements [48, 49].

Determination of RB5 dye in wastewaters collected from the batik industry

To evaluate the application to a real samples analysis, the developed DPCSV technique was used to analyze RB5 dye in wastewater samples which were collected from the local batik factories. The wastewater samples were analyzed by the developed technique under optimum parameters without any sample pre-treatment. Table 2 shows the content of RB5 in the collected batik wastewater samples.

Table 2. The concentrations of RB5 dye in the batik wastewater samples from the first and second dyebath

Samples (First Dyebath)	Concentration of RB5 (x 10^{-6} mol/L)	Samples (Second Dyebath)	Concentration of RB5 (x 10^{-6} mol/L)
S1-A1	0.493	S1-B1	N.D
S2-A1	N.D	S2-B1	N.D
S3-A1	0.324	S3-B1	N.D
S4-A1	0.236	S4-B1	N.D
S5-A1	N.D	S5-B1	N.D
S6-A1	0.421	S6-B1	0.235
S7-A1	0.183	S7-B1	N.D
S8-A1	N.D	S8-B1	N.D
S9-A1	N.D	S9-B1	N.D

N.D: not detected

To verify these results, recovery experiments were carried out by spiking three different concentrations of RB5 standard solution with the concentrations of 0.5 x 10^{-6}, 1.0 x 10^{-6} and 2.0 x 10^{-6} $molL^{-1}$ into the wastewaters collected from the batik factories that did not contain RB5 dye. The experiments were carried out using four wastewater samples (S1-B1, S2-B1, S5-B1 and S8-B1) with three replicate (n=3) measurements per each spiked concentration. The recoveries achieved for 0.5 x 10^{-6}, 1.0 x 10^{-6} and 2.0 x 10^{-6} $molL^{-1}$ RB5 dye were from 83.97 ± 4.78 to 97.93 ± 5.45%, 95.91 ± 2.68 to 103.49 ± 1.32% and 99.17 ± 0.75 to 105.12 ± 0.28%, respectively. According to the *t*-test (two-tailed), there were no significant differences between the recovery and spiked value at the 95% confidence level with degree of freedom (n-1=2), since all the calculated *t* values in the experiment were lower than the theoretical *t* value, which was 4.303 [50, 51]. These good recovery values indicated that the matrixes did not affect the determination of RB5 dye in batik wastewater samples and it was suggested that the developed method can be applied for RB5 dye determination in the batik wastewater samples without any sample pre-treatment. The recovery results are listed in Table 3.

Comparison of the results by the developed DPCSV technique and spectrophotometry method

The performance of the developed DPCSV technique in determining the RB5 dye was then compared against UV-VIS spectrophotometry [52]. Spectrophotometry measurements of RB5 dye in the range of 300 to 700 nm had shown that the highest absorbance was obtained at 596 nm which was attributed to the diazo groups and responsible for the blue colour of the dye solution [53]. The calibration curve was constructed and a linear range was found from 1.0 x 10^{-6} to 1.5 x 10^{-4} $molL^{-1}$ with the regression equation of $y = 0.0225 x + 0.0287$ ($R^2 = 0.9991$), as shown in Figure 9. The LOD and LOQ were 0.4 x 10^{-7} $molL^{-1}$ and 1.3 x 10^{-7} $molL^{-1}$, respectively.

Table 3. Analytical results of RB5 dye in batik wastewater samples using the developed technique

Samples	**Spiked[a] amount ($\times10^{-6}$ molL^{-1})**	**Found[a] amount ($\times10^{-6}$ molL^{-1})**	**Recovery ± SD (%)**	**RSD (%)**
S1-B1	0.5	0.44	88.30 ± 7.69	8.71
	1.0	0.97	97.37 ± 1.42	1.46
	2.0	1.99	99.75 ± 0.66	0.66
S2-B1	0.5	0.44	88.07 ± 7.99	9.07
	1.0	0.98	98.30 ± 3.60	3.66
	2.0	2.10	105.12 ± 0.28	0.27
S5-B1	0.5	0.42	83.97 ± 4.78	5.69
	1.0	0.96	95.91 ± 2.68	2.79
	2.0	1.99	99.61 ± 0.43	0.43
S8-B1	0.5	0.49	97.93 ± 5.45	5.57
	1.0	1.03	103.49 ± 1.32	1.28
	2.0	1.98	99.17 ± 0.75	0.76

[a] Average of three replicate measurements for each spiked concentration

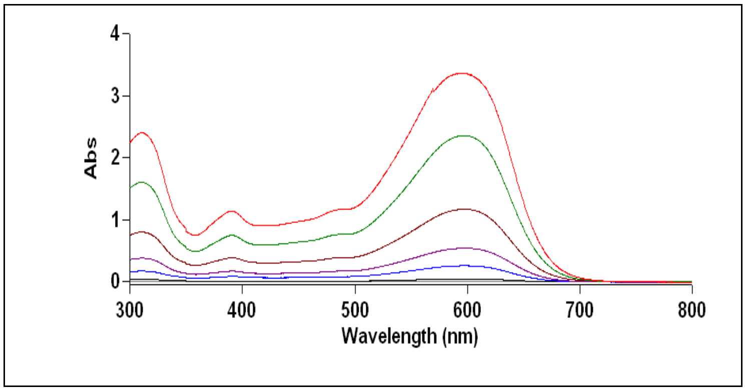

Figure 9. Spectra of RB5 dye with increasing concentration from 1.0 x 10^{-6} to 1.5 x 10^{-4} molL^{-1}

The results obtained by both developed DPCSV and spectrophotometry method in analyzing RB5 dye in the several wastewaters samples collected from the batik factories were then compared, as shown in Table 4. The developed DPCSV technique can be considered more sensitive as it had an ability to detect and determine the dye at a lower level compared to the UV-VIS Spectrophotometry method.

Table 4. The concentrations of RB5 dye in the batik wastewater samples using two different methods

Samples	Concentration of RB5 (x 10^{-6} mol/L)	
	DPSV	UV-VIS
S1-A1	0.493	0.471
S2-A1	N.D	N.D
S3-A1	0.324	N.D
S4-A1	0.236	N.D
S5-A1	N.D	N.D
S6-A1	0.421	N.D
S7-A1	0.183	N.D
S8-A1	N.D	N.D
S9-A1	N.D	N.D

N.D: not detected

Conclusion

The developed DPCSV technique had been succesfully applied to determine the RB5 dye in wastewaters from the batik industry. The present method had an advantage that no prior extractions or samples pre-treatment were required. It was also found to be practically rapid, convenient, sensitive, accurate, precise, rugged, robust and low in cost compared to the UV-VIS Spectrophotometry method. Therefore, it could be an excellent alternative method for the routine determination of RB5 dye in batik wastewaters samples.

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