

DETERMINATION OF REACTIVE BLACK 5 (RB5) BY DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRIC TECHNIQUE

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Abstract

Dyes are aromatic organic compound which have an affinity towards the substrate to which they are being applied to. The presence of dyes in wastewater samples is not safe for human even at low level. The presence of dyes in wastewater which are discharged from textile industry must be analysed. Hence, a precise, fast, accurate, simple and inexpensive analytical method with low detection limit is needed for the determination of dyes in wastewaters. The differential pulse anodic stripping voltammetric (DPASV) technique using bare glassy carbon electrode (GCE) as a working electrode and phosphate buffer at pH 4.2 as a supporting electrolyte has been proposed for Reactive Black 5 (RB5) determination. Several experimental voltammetric parameters were being optimized for obtaining a maximum response before analytical validation of the proposed technique being carried out. The optimum parameters were initial potential (E_i) = +0.3 V, end potential (E_f) = +1.0 V, scan rate (ν) = 0.04 V/s, accumulation time (t_{acc}) = 50 s, accumulation potential (E_{acc}) = 0.4 V and pulse amplitude = 0.075 V. The well-defined anodic peak appeared at 0.77972 V. The response was linear from 0.5 to 1.25 mg/L ($R^2=0.9986$) with LOD of 0.050 mg/L. The relative standard deviation (RSD) achieved were 0.08 %, 0.62 % and 0.50 %, respectively for three consecutive days. The % recovery range achieved was from 89.71 % to 111.15 %. It can be concluded that the proposed technique is precise, accurate, inexpensive, fast and has a potential to be an alternative analytical technique for RB5 analysis. The proposed method will in the future be tested for the amount of RB5 in the wastewater samples from textile industry.

Keywords: Anodic Stripping Voltammetry, Glassy Carbon Electrode, Reactive Black 5

Article History: - Received: 13 October 2020; Accepted: 16 April 2021; Published: 30 April 2021
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Introduction

Homemade batik industries in Kelantan and Terengganu are well known and famous among locals and foreign tourists. It was established in 1962, known as one of the highest demand business in Malaysia (Wan Hashim, 1996). It is found to be able to survive until now due to the high demand for batik from domestic and international markets. In Malaysia, the government has endorsed Malaysian batik as a national dress which creates opportunities among local designers to create new batik designs which normally include popular motives such as butterfly, leaves and flowers. Most batik entrepreneurs are younger family members who inherit their businesses from the older generation since it generates an established source of income (Nordin et al., 2012).

The first organic dye, Mauve, was accidentally discovered in 1856 by William Perkin, an 18-year-old student who was working on chemical synthesis of natural products. Perkin named the colour as Mauveine, after the French name of non-fast colour which was made of natural dyes (Saratale et al., 2011).

Large water volume and chemicals used for wet processing of batik could pollute the environment through its manufacturing processes. It is estimated that around 12% of the used colorants seeped into wastewaters. Discharges can also arise further downstream in the life cycle of azo colorants, such as in the landfilling and washing of dyed materials (Pinheiro et al., 2004). The colour produced by the textile wastewaters is mainly due to azo dyes and the discharge of the wastewaters into the environment is a matter of aesthetic, although at low concentrations. Improper discharge of these wastewaters into the environment will lead to a reduction in sunlight penetration, which in turn affects aquatic photosynthesis, inhibits the growth of biota, becomes toxic to organisms and depletes dissolved oxygen and water quality (Saratale et al., 2013). Therefore, it is important to have a sensitive, precise, accurate, simple, fast and inexpensive analytical method for dyes determination in textile wastewaters.

The chemical formula of RB5 is $C_{26}H_{21}N_5Na_4O_{19}S_6$ with molecular weight (MW) of $991.82 \text{ g mol}^{-1}$. As shown in Figure 1, RB5 contains two azo and two vinylsulphone functional groups as the reactive groups. The two azo groups act as the chromophores that enable the dyes to absorb light in the visible spectrum and are responsible to give colour to the textile. The reactive groups are covalently bonded with $-OH$ or $-NH_2$ groups that are present on the fibres in order to give excellent wash fastness property (Nur Syamimi et al., 2016). Therefore, the reactivity will increase due to the reaction between two reactive vinylsulphone groups and azo functional groups, but the wastewater can lead to toxicity for aquatic and other living things (Radi et al., 2012).

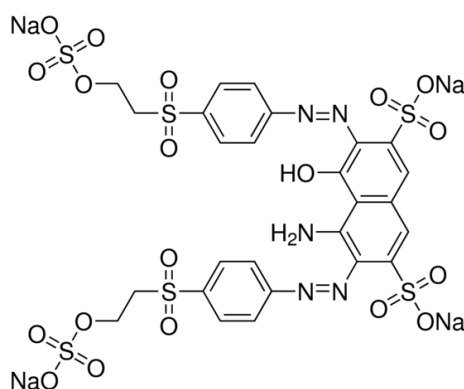


Figure 1. Chemical Structure of Reactive Black 5 (RB5)

Differential pulse cathodic stripping voltammetric (DPCSV) has been proposed for RB5 determination using the hanging mercury dropping electrode (HMDE) and Britton-Robinson buffer (BRB) as supporting electrolyte. The DPCSV technique was based on the reduction of azo group on the surface of HMDE (Nur Syamimi et al., 2016). The usage of HMDE is not environmentally friendly as it is toxic. With regards to the voltammetry method, no DPASV technique which uses solid-state glassy carbon electrode for RB5 determination has been reported so far.

Chromatography, spectrophotometry and voltammetry are commonly used analytical methods for textile azo dyes. Instead of offering very low detection limit, the chromatography method is well known for its complex and high-cost instrumentations, demanding requirements on maintenance, skilled labours, chemicals and samples preparation as well as low speed of analysis. On the other hand, the spectrophotometry method is reported to be limited by the fact that absorption of the certain compounds may appear in the same range of the electromagnetic spectrum which leads to spectral interference operational steps even though it offers very low running and instrumental costs (Santos et al., 2013). Pinheiro et al. (2004) have proposed spectrophotometric method for textile azo dyes analysis. The textile wastewater samples were measured at wavelength, λ range of 200 to 350 nm to avoid interference by visible colour which would limit the applicability of other proposed spectrophotometric methods for amine analysis.

The voltammetric method seems to have high potential as a promising alternative electroanalytical method for determination of dyes in batik wastewater. Differential pulse stripping voltammetric (DPSV) technique has a good discrimination against capacitive current which resulting in improved resolution, higher sensitivity, low detection limit and an effectiveness to be applied in the analysis of various electrochemically active compounds at their trace amounts at a reasonable cost and through a simple and fast analysis. The purpose of this study is to propose DPASV technique for RB5 determination at the bare glassy carbon electrode using phosphate buffer as the supporting electrolyte.

Methods

Materials

Analytical grade reagents were used throughout the experiment. All solutions were prepared using deionized water. A 50 mg RB5 powder was then dissolved in 100 mL volumetric flask for the preparation of 500 mg L⁻¹ RB5 stock solution. Appropriate dilution of the prepared stock solution was carried out in order to obtain a standard working solution series. Phosphate buffer solution (PBS) was prepared by dissolving 27.218 g of potassium dihydrogen phosphate and 71.630 g of disodium hydrogen phosphate in a 1L volumetric flask with deionized water and this was followed by adding 2.70 mL of ortho phosphoric acid. The deionized water was added up to the calibration mark of the volumetric flask. Sodium hydroxide with concentration of 0.1 M was prepared and then used to adjust the pH of the PBS solution to 4.2.

Instrumentation

Autolab Potentiostat (Metrohm, Switzerland) was used in the overall voltammetric experiment. The Autolab consists of a three-electrodes system. A glassy carbon electrode (GCE) acts as the working electrode (WE), a platinum wire as the counter electrode (AE) and an Ag/AgCl (3M KCl) as the reference electrode (RE). The GCE was polished with alumina powder and then rinsed with deionized water. The Autolab was connected to NOVA 1.1 software for data processing.

Voltammetric Measurement

Differential Pulse Anodic Stripping Voltammetry (DPASV)

The measuring solution was prepared by pipetting desired volume of prepared RB5 standard solution and 40 mL of PBS into the measuring vessel to give a final concentration of 0.5 mg L⁻¹. A 0.5 mg L⁻¹ RB5 standard solution was used throughout the optimization procedure. A small size of stirrer bar was put into the measuring vessel and the solution was then stirred well. After a period of time, the stirrer was switched off before voltammetric measurements proceeded.

The initial potential (E_i) = 0.3 V, final potential (E_f) = 1.0 V, accumulation time (t_{acc}) = 10 s, scan rate (v) = 0.04 V/s, accumulation potential (E_{acc}) = 0.4 V and pulse amplitude = 0.075 V were applied as unoptimized parameters. These instrumental parameters were then optimized to increase the response (current) of the proposed DPASV technique in RB5 analysing.

Validation of the Proposed DPASV Technique

Validation of the proposed DPASV technique which included an appropriate linear range with acceptable correlation coefficient (R^2), limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and recovery of RB5 standard in the simulation of batik wastewater were investigated under optimum conditions. The purpose of validation was to verify the compatibility and ability of the proposed DPASV technique for RB5 analysis as proposed by Miranda et al. (2012). The LOD was estimated by measuring the additional lower concentration of RB5 standard solution until a significantly different response from the response of PBS solution was achieved. The LOQ was calculated as 3.333 times the value of the obtained LOD.

The RB5 standard solution at the concentration of 0.5 mg L⁻¹ was used for both intra-day and inter-day precision, as well as ruggedness study with five replicates ($n=5$) measurement. The ruggedness of the

proposed DPASV technique was investigated using the same instrument (Autolab, Metrohm) but had been conducted by two different operators under the same optimum parameters. Statistical *F*-test was carried out for the ruggedness. The accuracy of the proposed DPASV technique was examined by spiking three known different concentrations of RB5 standard solution into the voltammetric cell.

Simulation Sample of Batik Wastewater

About 0.01 gram of congo red and 0.01 g RB5 standard were measured in a weighing plate using the Shimadzu ATX 224 analytical balance. Then, each dye was transferred and dissolved into 500 mL volumetric flask with tap water until it reached the calibration mark. About 0.5 mL simulation sample of batik wastewater was spiked into the voltammetric cell which consisted the PBS solution.

Recovery of RB5 Standard Solution in the Simulation Sample

Recovery of the RB5 standard solution in simulation sample was determined by spiking the three known amounts standard solution to achieve the concentrations of 0.5, 0.7 and 1.0 mg L⁻¹ in the voltammetric cell. The recovery study was performed with three replicates (n=3) measurement per spiked concentration of the simulation sample into the voltammetric cell. Regression equation was applied in order to determine the recovered concentrations of the spiked RB5 standard solution into the simulation batik wastewaters.

Results and Discussion

Optimization of the Proposed DPASV Technique

Effect of Accumulation Time

The accumulation time (t_{acc}) strongly affected the response (peak current) in electroanalytical measurements (Sezgin et al., 2016). The t_{acc} is a time given for RB5 to be accumulated onto the surface of GCE before being analyzed. The effect of t_{acc} on peak current (I_p) and peak potential (E_p) was determined at the range of 10 s to 100 s. The I_p was increased with t_{acc} until 50 s and then it remained unchanged after 50 s. The t_{acc} of 50 s was considered as the optimum condition since the highest I_p and well-defined shape were obtained which was 360 nA at E_p of 0.77972 V.

The increasing of I_p with t_{acc} was due to the accumulation of RB5 at the GCE surface in a higher amount since a longer time was given for the accumulation to take place. The almost constant value of I_p that obtained at longer 50 s of t_{acc} is suggested because of RB5 saturation at the GCE surface.

Effect of Scan Rate

The relationships of I_p and E_p of RB5 to various ν in the range of 0.01 V/s to 0.07 V/s were studied. The optimum response obtained was 360 nA at E_p of 0.77972 V with the optimum ν of 0.04 V/s in PBS solution (pH 4.2).

Effect of Accumulation Potential

An accumulation time (E_{acc}) is defined as a potential where the RB5 is accumulated on the surface of GCE and optimum E_{acc} is the most appropriate potential for this accumulation process to occur. The E_{acc} values used were varied from +0.2 V to +0.7 V. The maximum I_p was observed at E_{acc} of +0.4 V. Hence, it indicated that an accumulation of RB5 at the GCE surface effectively occurred at E_{acc} +0.4 V in which the obtained I_p and E_p were 360 nA and 0.77972 V respectively.

Effect of Pulse Amplitude

Pulse amplitude also plays a major factor to increase the sensitivity of voltammetric measurement (Kounaves, 1997). The examined values of pulse amplitude were 0.025 V to 0.115 V in PBS solution (pH 4.2). The I_p had increased sharply from 0.025 V to 0.075 V and then it slowly decreased. The pulse amplitude of 0.075 V was chosen as the optimum condition for the analysis due to the highest I_p obtained which was 360 nA with E_p of 0.77972 V.

Validation of the Proposed DPASV Technique

Linear Curve, Detection Limit and Quantification Limit

The I_p of RB5 as the concentrations were increased from 0.5 to 1.25 mg L⁻¹. As shown in Figure 2, the linear equation obtained was I_p (nA) = 266.07x + 14.322 with a correlation coefficient (R^2) value of 0.9986 at five different concentrations of RB5 standard solution. The R^2 value (0.9986) was considered fit for the obtained I_p against concentrations to the regression line as the value was more than 0.9980 (Gazy, 2004). The LOD was 0.050 mg L⁻¹ and the LOQ was 0.167 mg L⁻¹.

On the other hand, Nur Syamimi et al. (2016) have reported the linear equation of I_p (nA) = 115.61 x – 10.31 with R^2 of 0.9998, LOD of 0.0122 mg L⁻¹ and the LOQ of 0.041 mg L⁻¹ for RB5 analysis at HMDE in BRB solution (pH 2.5) based on reduction of azo functional group by applying DPASV technique.

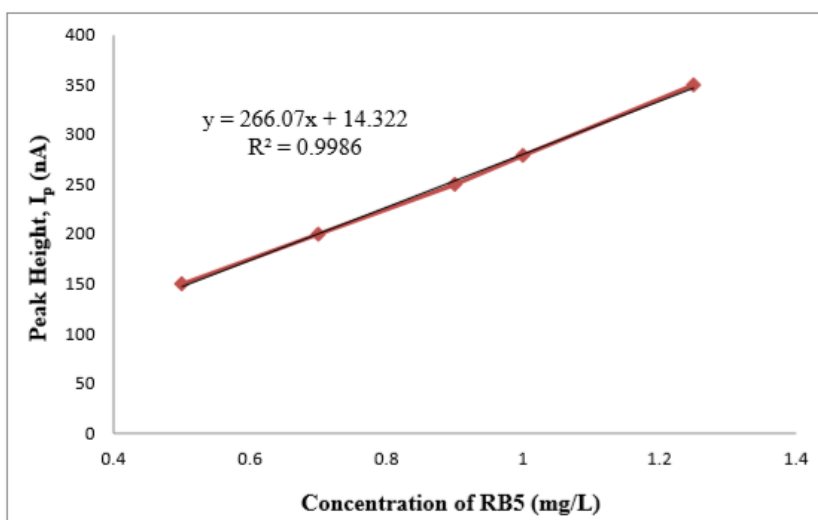


Figure 2 Linear curve of I_p against concentration of RB5 in phosphate buffer solution pH 4.2

Precision

The precision of the proposed DPASV technique for RB5 analysis was investigated in three replicates ($n=3$) at the concentrations of 0.5 mg L⁻¹ for three consecutive days. The RSD values obtained were 0.08 %, 0.62 % and 0.50 % respectively. The measurements using the proposed DPASV technique were considered precise and confidence since all the RSD values found to be less 5%.

Accuracy

The accuracy of the proposed DPASV technique was investigated by spiking three known amounts of RB5 which gave the final concentration of 0.7 mg L⁻¹, 0.9 mg L⁻¹ and 1.25 mg L⁻¹ in a voltammetric cell containing PBS solution at pH 4.2. The amounts of the spiked solutions in the cell were calculated using the equation of I_p (nA) = 266.07x + 14.322, where x is the found concentrations of spiked RB5 in the voltammetric cell. The satisfactory recoveries of 92.80%, 102.20% and 103.20% were-achieved for the proposed technique to be considered accurate.

Ruggedness

The RSD values of 1.03% to 1.36% were achieved as measurements were carried out by the first and second operator using the same instrument (Autolab, Metrohm). From the statistical two-tailed F test, there were no significant differences between the obtained variances when the measurements of RB5 were conducted by two different operators using the same instrument at the 5% significance level. Hence, the results indicated that the proposed DPASV technique was rugged.

Recovery Studies of RB5 in the Simulation Wastewater Samples

No oxidation peak of RB5 was observed for the analysis of simulation wastewater samples. The recovered concentration of spiked RB5 standard solution into simulation samples of dye was calculated using the regression equation, $y = 266.07x + 14.32$, which was obtained from the previous data. The recovery achieved for 0.5, 0.7 and 1.0 mg L⁻¹ of RB5 standard solution was 104.40 %, 89.71 %, and 111.15 %, respectively. According to Kergaravat et al. (2012), all of the variance coefficients (RSD) values obtained in the recovery study was acceptable since they were lower than 15%. According to the *t*-test, there were no significant differences between recovery and added value at the 95% confidence level with degree of freedom ($n-1 = 2$) since all the calculated *t* values in the experiment are lower than theoretical *t* value, which is 4.303 (Bergamini et al., 2010).

Conclusion

The proposed DPASV technique was successfully tested to detect and determine RB5 in the simulation wastewater samples. The technique was advantageous because it was practically rapid, convenient, sensitive, accurate, precise, rugged and inexpensive. The proposed method will in the future be tested for RB5 determination in real wastewater samples from batik industry.

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