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DETERMINATION OF ANILINE DEGRADED FROM SUDAN I IN CLOTHS

(Penentuan Anilin Yang Terurai Daripada Sudan I Dalam Kain)

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Abstract

A method development for the determination of an aromatic amine degraded from an azo dye in cloths was carried out. Sodium dithionite was used to cleave the azo bond of Sudan I into its aromatic amines, and the amines, mainly aniline were analyzed using high performance liquid chromatography (HPLC) with UV detection. The efficiency of the reduction process of Sudan I, based on the degree of decolorization of dye, was measured using the spectrophotometer. The optimized values of the reduction process was found effective when 1:1 ratio of 0.30% sodium dithionite to Sudan I (dit/Sud) was used at pH 8. The recovery percentage and relative standard deviation (R.S.D.) of this method was found to be 62.9 – 88.4% and 7.6 – 21.5%, respectively. The proposed method was tested on self-dyed cloth samples with Sudan I. Aniline released from the reduction of Sudan I was detected in the self-dyed cloth samples. The results of this study demonstrate the applicability of sodium dithionite for the reduction of the azo dye in the cloth samples.

Keywords: aniline, Sudan I, sodium dithionite, azo dye

Abstrak

Satu perkembangan kaedah untuk penentuan amina aromatik yang terdegradasi daripada pencelup azo dalam kain-kain telah dijalankan. Natrium ditionit telah digunakan untuk menguraikan ikatan azo Sudan I kepada amina aromatik, dan amina, terutamanya anilina telah dianalisis dengan menggunakan kromatografi cecair prestasi tinggi dengan pengesanan UV. Kecekapan proses penurunan Sudan I, berdasarkan tahap penyahwarnaan warna pencelup, telah diukur dengan menggunakan spektrofotometer. Nilai optimum dalam proses penurunan ini telah didapati berkesan apabila nisbah 0.30% natrium ditionit kepada Sudan I (dit/Sud) digunakan pada pH 8. Peratusan pemulihan dan nilai sisihan piawai relatif (SPR) bagi kaedah ini telah ditentukan sebanyak 62.9 – 88.4% dan 7.6 – 21.5%, masing-masing. Kaedah yang dicadangkan telah diuji ke atas sampel-sampel kain yang telah dicelup sendiri dengan Sudan I. Anilina yang dibebaskan daripada penurunan Sudan I telah dikesan dalam sampel-sampel kain yang dicelup sendiri. Keputusan kajian ini menunjukkan bahawa kebolehan pengunaan natrium ditionit untuk penurunan pencelup azo dalam sampel kain.

Kata kunci: anilina; Sudan I; natrium ditionit; pencelup azo

Introduction

One of the most versatile and largest class of synthetic dyes commonly used today are the azo dyes [1,2]. This is because azo dyes offer a vast spectrum of colors that have good color fastness and are cheaper to be manufactured [3]. Azo dyes are widely used in various industrial application as colorants. Products such as textiles, food, leather, paper, plastics, paints, inks, cosmetics and etc. are known to contain azo colorants [1]. The colors of azo dyes are chracterized by the chromophores associated with the azo groups (-N=N-) [3].

Azo dyes are commonly synthesized by the coupling reaction of a diazotized aromatic amine with another aromatic amine or a phenol [1]. Their structure would contain at least one azo group (-N=N-) bonded to an aromatic ring (R-NH_2) [4]. Not only is the dye itself that is hazardous, but the aromatic amine when it is cleaved from the dye poses as dangerous by-products [5]. The problem with azo dyes arises primarily from the reduction of the azo group causing them to be potentially toxic [1]. This poses a risk to consumers because some of the aromatic amines formed may be confirmed or suspected carcinogens. Azo dyes are easily reduced under anaerobic conditions to form aromatic amines and are relatively resistant to aerobic degradation [3]. The electron density of the azo bond affects the degree of degradation of the azo dye by reduction. Electron withdrawing groups can be used to reduce the electron density of the azo bond thus, causing degradation of the azo dye and releasing the colorless aromatic amines [5].

In many developing countries where the textile industry is a major industrial sector, which is a major source of discharged dye wastewater that pollutes the natural water source systems. The discharge of this large quantity of industrial wastewater containing azo dyes is a primary concern. The concerns include harming the aquatic life (both flora and fauna) as well as decreasing the aesthetic aspects of the water, since azo dyes are not biodegradable in aerobic conditions, not to mention public human health. Coloured water may reduce light penetration from the sun, causing primary aquatic plant productivity to decrease [4].

One can be exposed to azo dyes via dermal absorption, inhalation and oral ingestion [6]. Furthermore, it was discovered that the azo group can be cleaved by skin microflora, azoreductases in intestinal bacteria and liver cells [3]. Besides that, photochemical reactions through human sweat and dyes on clothings could also form aromatic amines [2]. Sudan I is one of the many types of synthetic azo dyes and it would form aniline when cleaved at the azo bond. Both Sudan I and aniline are classified under the category 3 of carcinogens by the International Agency for Research on Cancer (IARC), which means that these chemicals are harzardous, but their carcinogenicity cannot be specifically classified to humans, although some forms of bladder and liver cancer were observed in mice and rats [7,8].

Given the potential harzardous impact of aniline in daily consumer activities, it is necessary to develop a quick and inexpensive mechanism to detect and measure this compound. This study therefore aims to (i) develop an optimized method to degrade Sudan I and (ii) determine the amount of aniline which can be found in cloths dyed with Sudan I. These objectives enable researchers to measure the potential hazards of dyed textiles based on the amount of aniline that could be released from Sudan I, with the intention of raising awareness to the impacts of these dyes have towards to the consumers and environment.

Several methods have been used to degrade azo dyes to release aromatic amines from different samples such as textiles [5], leather [9], hair dyes [10] and toy products [1]. Other reagents such as hydrogen peroxide (coupled with UV radiation) [11] and Fenton's reagent [4] are oxidizing agent that could also degrade azo dyes. The methods used in these researches were mostly complex and expensive. Biological treatment on azo dyes with azoreductases [12], laccases [13], extracellular fluid protein from *Streptomyces* species [14] and bacterial strain *Shewanellaoneidensis* MR-1 [15] were also possible. However, handling of biological entities or products are demanding as incubation and pretreatment procedures are necessary. Sodium dithionite is commonly used as a reducing agent for the reduction of dye [1,5,9]. Thus, due to the effectiveness of this reagent which had been employed by other researchers in their studies; therefore it was selected to be used to degrade Sudan I azo dye in this study. In this study, the parameters such as concentration of sodium dithionite, dit/Sud ratio, temperature of reduction and pH were studied based on the degree of decolorization which was measured using the spectrophotometer. The optimized values were then applied on self-dyed cloth samples to test the applicability of the proposed method. The determination of the released aniline from the Sudan azo dye was carried out using HPLC.

Experimental

Reagents

The aromatic amine, aniline ($C_6H_5NH_2$, $\geq$ 98.5% purity) and azo dye, Sudan I ($C_{16}H_{12}N_2O$, pure) were purchased from BDH Chemicals Ltd (England) and Acros Organics (Belgium). The reducing agent, sodium dithionite ($Na_2S_2O_4$) was purchased from Systerm® (Malaysia). Ammonium dihydrogen phosphate ($(NH_4)H_2PO_4$ $\geq$99% purity) and disodium hydrogen phosphate (Na_2HPO_4, $\geq$99% purity), which were used to prepare phosphate buffer solution for chromatographic analysis were purchased from Merck (Germany) and Fisher Scientific (United Kingdom). Liquid chromatography (HPLC) grade methanol (Merck, Germany) was used as an organic solvent to dissolve aniline and Sudan I azo dye into solutions. 18 mΩ water was used to prepare HPLC mobile phase throughout the study. Stock solution of 0.1% aniline was prepared by dissolving suitable amount of aniline in 100 mL of HPLC grade methanol. A series of concentration of aniline solution from 2 to 10 ppm were prepared by diluting the stock solution with methanol followed by optimum amount of sodium dithionite solution. The pH of each prepared standard solution was adjusted to optimum pH before analyzed using HPLC. Sodium dithionite solutions were freshly prepared before used.

Apparatus and operating conditions

The equipment consists of a vacuum degasser, autosampler, UV/Vis Detector and pump (Series 200, Perkin Elmer, United States) was used to determine aniline in the reduced Sudan I azo dye solution. The system is computer-controlled (Windows XP Professional operating system), using the the Total Chrom Navigator software package. HPLC separations were carried out using a reversed phase column of ZORBAX SB-Phenyl column (4.6 mm I.D x 250 mm length, and 5 µm particle size, Agilent Technology, United States). The guard-column was of the same type of separation column (4.6 mm I.D. x 12.5 mm length and 5 µm particle size). The chromatographic eluent consisted of 70% methanol and 30% of ammonium dihydrogen phosphate/disodium hydrogen phosphate buffer solution at pH 6.9. For analysis, 15 µL of the sample solution were injected at a flow-rate of 1 mL/min and at room temperature. The analysis run time was 10 min. The UV-spectra were recorded at 231 nm. MERCK UV-Spectroquant Pharo 100 spectrophotometer was used to measure the color absorption of Sudan I azo dye solution at 476 nm using 10 mL cell tube.

Optimization of dit/Sud ratio, reduction temperature, pH and recovery

Various concentrations of sodium dithionite aqueous solutions were mixed with various amount of 10 ppm Sudan I azo dye solution in methanol to study the effect of the sodium dithionite concentration toward the decolorization of the Sudan I azo dye solution. Each mixture was shaken for 1 minute and left at room temperature for 15 minutes before measured using spectrophotometer at 476 nm. The reduction temperature of Sudan I azo dye to aniline was studied from room temperature to 70 ^{o}C. The effect of the pH of the sodium dithionite solution toward the reduction of the Sudan I azo dye to aniline was studied from pH 2 to 10. 10 - 200 ppm of Sudan I azo dye solutions were treated under the optimum conditions before analyzed using HPLC. The detected aniline amounts were calculated and compared to the stoichiometric amount of aniline released from the reduction of Sudan I. Each concentration was repeated three times.

Sample preparation and extraction

The application of the proposed method was carried out on a self-dyed cloth. Three pieces of 20 x 20 cm of plain white cotton cloth weighted approximately 9 gram were washed in hot distilled water followed by 100 mL of 0.1 M nitric acid to eliminate any possible colorant that adsorbed on the cloths before dried in oven at 100 ^{o}C. Each cloth was soaked in 100 mL of 0.5% Sudan I azo dye solution in methanol for up to 12 hours. The dyed cloths were then air dried at room temperature. Sudan I was extracted from the cloths using 100 mL of methanol at room temperature for 1 hour. 5 mL of the extracted solution containing Sudan I was subjected to the optimum conditions treatment before analyzed using the HPLC.

Results and Discussion

Optimization of sodium dithionite concentration

Sodium dithionite is an effective reducing agent to cleave Sudan I azo dye to aromatic amine compounds. However, the optimum amount needs to be determined. Three different concentrations of sodium dithionite solution were used. Each concentration of sodium dithionite was mixed with different volume of Sudan I azo dye solution. Each mixture absorbance was measured using spectrophotometer to determine the decrement of the absorbance. Measured absorbances were compared with the blank without sodium ditionite. The results are shown in Figure 1. It indicates that higher sodium dithionite concentrations solution decolourized Sudan I azo dye solution at a lower dit/Sud ratio. In general, all three different sodium dithionite concentrations decreased the Sudan I azo dye solution absorbance when the higher ratio of dit/Sud was used. The dit/Sud ratios that completely decolourized the Sudan azo dye solution occurs at 7:3, 5:5 and 3:7 for 0.21%, 0.25% and 0.30% sodium dithionite solution, respectively. This is because higher concentration of reducing agent provides more reducing power to reduce the dye even at lower ratio.

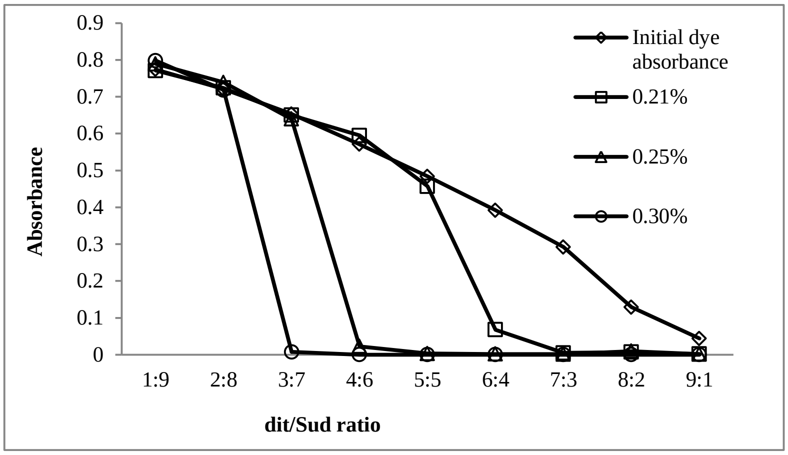


Figure 1. Effect of the sodium dithionite concentration on the reduction of Sudan I azo dye at different ratios of dit/Sud.

Optimization of reduction temperature

The mixtures of various concentrations of sodium dithionite solution with the Sudan I azo dye solution at various ratios were heated at four different temperatures for 15 minutes before spectrophotometry measurement was carried out to study the effect of the temperature on the reduction of Sudan I azo dye to aniline. The obtained results are shown in Figure 2a to Figure 2c. The observed general trend is that Sudan I azo dye absorbances decrease when dit/Sud ratios increase at these four different reduction temperatures. As shown in Figure 2a, fully decolorization of Sudan I azo dye occurred at ratio 7:3 for reduction temperature at room temperature, 40, 50 and 60 ^{o}C. Reduction temperature at 70°C accelerated the Sudan I azo dye fully decolorization from ratio 7:3 to 6:4. Ratios below 6:4 does not show an orderly decrement of absorbance when the reduction temperature increases from room temperature to 70 ^{o}C. The effects of reduction temperature were more obvious (Figure 2b) when 0.25% sodium dithionite was used. The increment of the reduction temperature from room temperature to 70 ^{o}C accelerated the decolorization of the dye from ratio 6:4 to 4:6. However, as shown in Figure 2c, the increasing reduction temperatures does not show any significant effect on the decolorization of dye when 0.30% sodium dithionite solution was used. In this concentration, effect of the concentration is more significant than the temperature toward the decolorization of the Sudan I azo dye. Since there were no obvious temperature effect on the decolorization of the Sudan I azo dye in 0.30% sodium dithionite solution. Therefore, heating is unnecessary to be incorporated into the developed method if 0.30% sodium dithionite was chosen to be used to decolorize the dye.

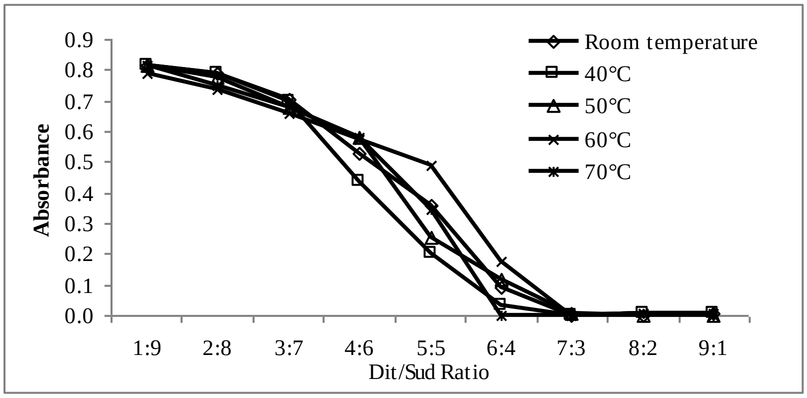


Figure 2a. Effect of temperature on the reduction of dye in 0.21% sodium dithionite.

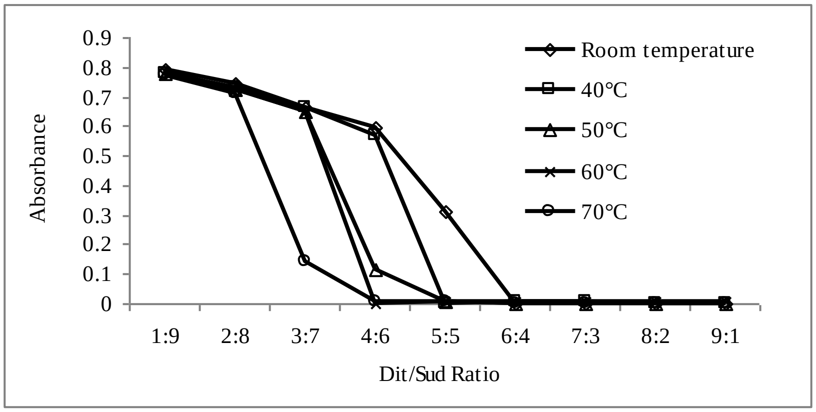


Figure 2b. Effect of temperature on the reduction of dye in 0.25% sodium dithionite.

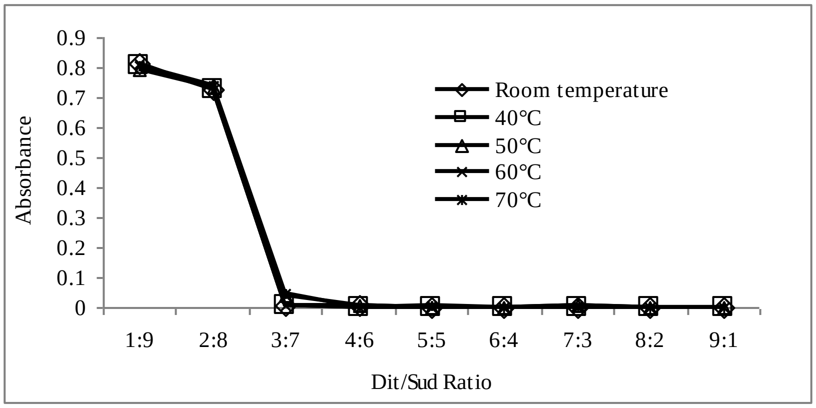


Figure 2c. Effect of temperature on the reduction of dye in 0.30% sodium dithionite.

Optimization of reduction pH

The effects of the reducing agent pH on the Sudan I azo dye decolorization was studied by altering the sodium dithionite solution pH using either HCl or NaOH. The pH ranges from 2 to 10 was studied. The effects of the sodium dithionite pH on the dye decolorization in 0.21% and 0.30% of sodium dithionite solution are presented in Figure 3a, 3b and 3c. All figures show a significant and obvious trend that alkaline sodium dithionite solution decolorized the Sudan I azo dye faster than the acidic sodium dithionite solution. The alkaline curves are slightly below the neutral curve. This shows that alkaline sodium dithionite did not enhance much the reduction of the dye compared to the neutral sodium dithionite solution.. However, curve that produced by pH 2 sodium dithionite shows a significant decrement of the decolorization of the dye in all three concentrations. This is because sodium dithionite is unstable in acidic condition and decomposes to sodium thiosulfate and sodium bisulfate [16]. pH 8 was chosen as optimum value instead of pH 10. This is because it is enough to maintain the stability of the sodium dithionite at pH 8. Moreover, the degrees of decolorization of Sudan I azo dye solution by sodium dithionite at pH 8 and 10 are almost similar in 0.30% sodium dithionite.

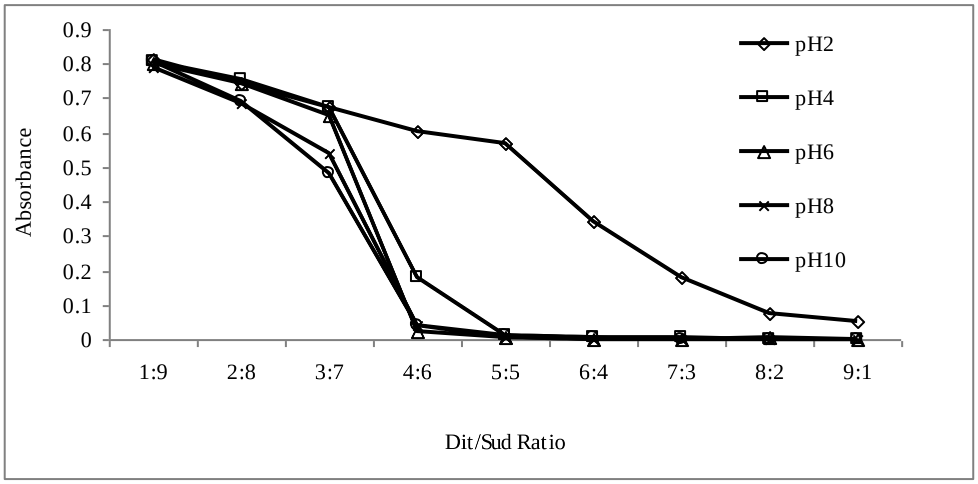


Figure 3a. Effect of the pH on the reduction of dye in 0.21%sodium dithionite.

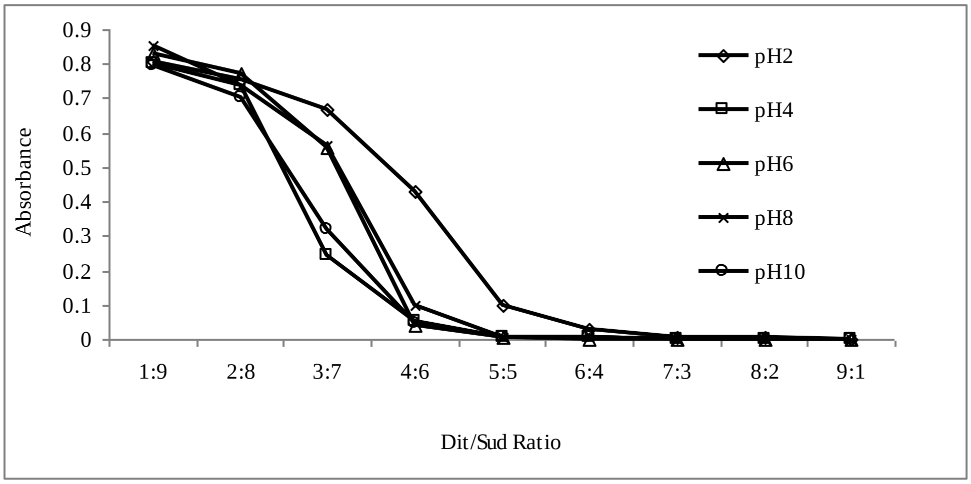


Figure 3b. Effect of the pH on the reduction of dye in 0.25% sodium dithionite.

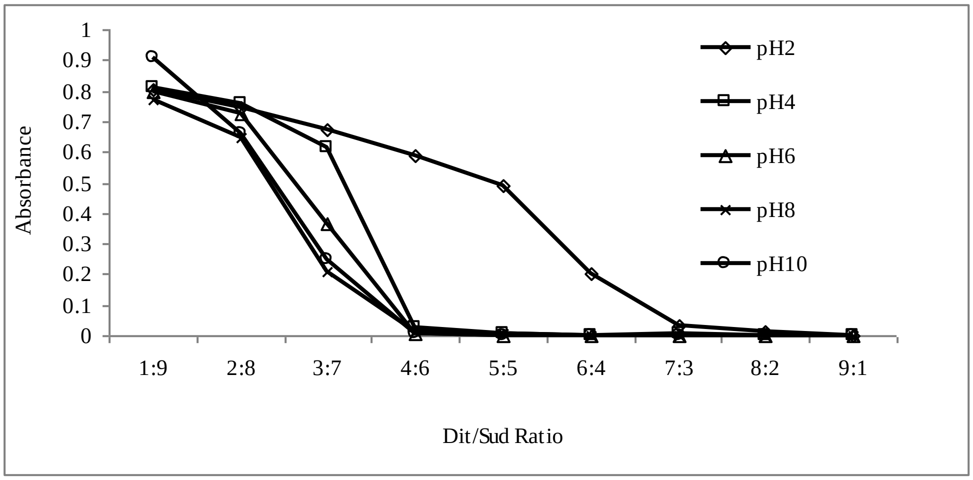


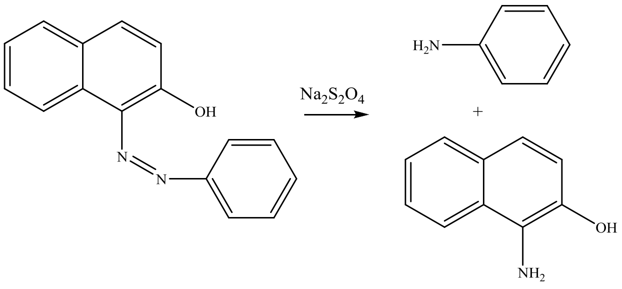
Figure 3c. Effect of the pH on the reduction of dye in 0.30% sodium dithionite.

Optimization of dithionite/Sudan I ratio

The optimization of dit/Sud ratio was studied throughout the optimization process for concentration, temperature and pH parameters. As shown in the results, the dit/Sud ratio where complete decolorization of Sudan I azo dye mostly occurs from ratio 5:5 and above. Therefore, the suitable optimum ratio to be used is 1:1 of dit/Sud (v/v).

Recovery percentage

Five different concentrations of Sudan I azo dye solutions were subjected to sodium dithionite treatment at optimum conditions. Each concentration was repeated three times to obtain each concentration relative standard deviation. The recovery percentage of aniline released from Sudan I azo dye reduction is calculated based on the stoichiometric amount of aniline, which theoretically released from the Sudan I azo dye in accordance to the following chemical equation (1):



(1)

Analyzed results were calibrated using aniline spiked standard in sodium dithionite solution. Aniline peak was detected at around 3.84 min throughout the 10 min run time. The linear correlation value (R^2) obtained from the standard curve is 0.9975 and the minimum detection limit calculated based on the calibration line equation is approximately 0.6 ppm by substituting zero to the y value.

The recovery percentage results are shown in Table 1. The recovery percentage at lower concentrations are slightly below 70%; whereas, higher concentrations are more than 70%. The overall recoveries in the range of concentration from 10 to 200 ppm are within 62.9 - 88.4% with R.S.D. values at 7.6 - 21.5%.

Table 1. The recovery percentages and R.S.D. of different Sudan I concentrations.

Concentration. Of Sudan I (ppm)	Recovery %				R.S.D.
	Duplicate 1	Duplicate 2	Duplicate 3	Average	(%)
10	58.3	67.8	62.7	62.9	7.6
30	72.9	70.7	61.1	68.2	9.2
80	99.1	83.8	64.8	82.6	20.8
150	100.0	82.3	83.1	88.4	11.3
200	82.1	100.2	64.9	82.4	21.5

Note: RSD represents Relative Standard Deviation

Application of the optimized method on self-dyed cloth sample

Sudan I azo dye was extracted from a self-dyed cloth using methanol. The extracted dye solution was treated with sodium dithionite under optimum conditions to reduce the dye into its aromatic amine. Amount of aniline which was released into the solution, was determined using HPLC. The obtained average result is 28.3 mg/kg with 1.2% of the relative standard deviation.

Conclusion

Sodium dithionite is a very effective reducing agent to reduce Sudan I azo dye in alkaline condition. It is able to cleave Sudan I azo dye to 1-aminonaphthalen-2-ol and aniline. In this study, it was found that 0.3% of sodium dithionite needs to be used to decolorize Sudan I azo dye at dit/sud ratio of 1:1 at pH 8. When 0.3% of sodium dithionite was used, heating of the mixture of the sodium dithionite and the Sudan I azo dye is unnecessary because the decolorization of the dye is mostly contributed by the concentration of the sodium dithionite rather than the temperature. Moreover, high heating temperature could decompose sodium dithionite. The minimum amount of the aniline that could be detected by the HPLC is approximately 0.6 ppm equivalent to 6.7 mg/kg if 9 gram of sample was used. Overall recoveries were 62.9 – 88.4% with R.S.D. values at 7.6 – 21.5%. Three pieces of cloths were dyed with Sudan I azo dye before extracted using methanol. Extracted Sudan I azo dye was subjected to optimum conditions treatment before analyzed using HPLC to determine the amount of aniline released from the adsorbed Sudan I azo dye onto the cloths. The obtained average result for the self-dyed cloth is 28.3 mg/kg with R.S.D. value at 1.2%.

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