

Stability of Reactive Black 5 (RB5) Standard Solution Studied In Different Conditions

Nur Syamimi Zainudin^{a,b}, Mohamad Hadzri Yaacob^{a*} and Zulkhairi Othman^a

^a*Forensic Science Programme, School of Health Sciences, Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan*

^b*Faculty of Applied Science, Universiti Teknologi Mara Cawangan (Pahang), Kampus Jengka,
26400 Bandar Jengka, Pahang*

*Corresponding author email address: hadzri@usm.my

ABSTRACT: Stability studies of Reactive Black 5 (RB5) standard solution in various conditions based on electroanalytical active azo group using UV-VIS Spectrophotometer are described. RB5 with concentrations of 20 and 200 ppm were multiple scanned at wavelength maxima of 596 nm in the range of 300 to 700 nm. The absorbances of RB5 standard solutions exposed and kept in the cupboard to room temperature for 7 hours per day for three consecutive days were measured. The average absorbances for three consecutive days for 20 ppm and 200 ppm standard solution, exposed to the ambient ranged from 0.3589 ± 0.0073 to 0.3640 ± 0.0024 and 3.5199 ± 0.0342 to 3.5643 ± 0.0481 , respectively. Within one month, the absorbances range for 20 and 200 ppm were from 0.3721 ± 0.0032 to 0.3769 ± 0.0032 and 3.4215 ± 0.0072 to 3.5361 ± 0.0180 respectively. When unexposed, the average absorbances range for respective three days of 20 and 200 ppm RB5 standard solution were from 0.3470 ± 0.0045 to 0.3640 ± 0.0024 and 3.4290 ± 0.0187 to 3.5630 ± 0.0536 , respectively. The absorbances range were from 0.3577 ± 0.0036 to 0.3668 ± 0.0050 and 3.4246 ± 0.0213 to 3.5513 ± 0.0059 for respective concentrations within one month. For stability studies that considering the effect of heat, the average range of 20 and 200 ppm for three consecutive days were 0.4013 ± 0.0022 to 0.4178 ± 0.0027 and 3.6837 ± 0.0191 to 3.6882 ± 0.0344 , respectively. The average absorbances range were from 0.3493 ± 0.0029 to 0.3586 ± 0.0025 and 3.4904 ± 0.0225 to 3.5184 ± 0.0294 for 20 and 200 ppm RB5 at pH 2.5 whereas at pH 9.0, the ranges were from 0.3515 ± 0.0043 to 0.3614 ± 0.0024 and 3.6007 ± 0.0296 to 3.6352 ± 0.0134 , respectively. It can be concluded that RB5 standard solutions were stable in all studied

conditions within a month and the azo functional group was considered was not effected by the conditions.

Keywords: Reactive Black 5, azo functional groups, UV-VIS spectrophotometry, stability

Introduction

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 structure is shown in **Figure 1** is categorised as reactive vinyl sulphonate azo dye. Its chemical formula is $C_{26}H_{25}N_5O_{19}S_6.4Na$ with molecular weight of 991.82 g/mol. It contains two $-N=N-$ bonds and two vinylsulphone as the reactive groups. The two azo groups act as chromophores to which enable the dye to absorb light in the visible spectrum region and are responsible for its colour (Méndez-Martínez *et al.*, 2012). The reactive groups are covalently bonded with $-OH$ or $-NH_2$ group that is present on fibers to give excellent wash fastness property (Esteves and Cunha, 2005; Jović *et al.*, 2013).

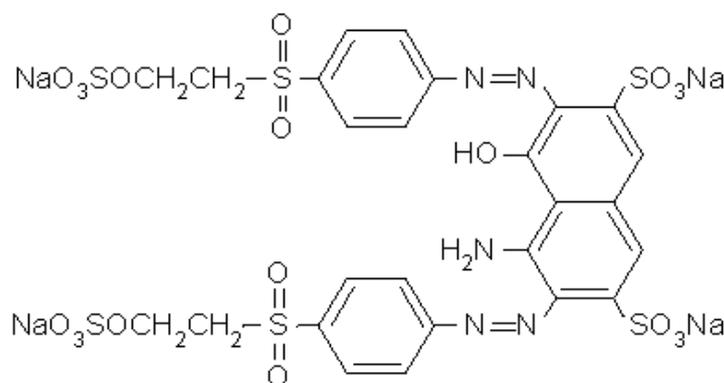


Figure 1: Chemical structure of RB5

Azo dyes are widely used in the textile, food, drug, cosmetic, automobile, leather and papermaking industries (Saratale *et al.*, 2013). When in contact with sweat, saliva and gastric juice, the azo group will reductively cleave to form carcinogenic aromatic amine like benzidine and 4-biphenylamine. After the oral ingestion of azo dyes, an aerobic intestinal microflora and mammalian azo reductase in the intestinal wall reduce them to free aromatic amines (de Lima *et al.*, 2007; Chequer *et al.*, 2011). They also tend to induce tumors in rats

and give inhibitory effects on the biosynthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins (Goyal *et al.*, 1998).

In this study, stabilities of RB5 standard solution in various conditions such as exposed and unexposed to room temperature, prepared in different pH and also after heating and cooling at room temperature were examined using UV-VIS Spectrophotometer as a part of method development using voltammetric technique. This study was to ensure that all tested conditions did not affect the azo functional group of RB5 dye which may further effect its voltammetry analysis.

Methodology

Instrumentations and Materials

Jasco single beam UV-Visible Spectrophotometer with 1 cm matched plastic cell connected to a computer loaded with Jasco PC Software was used for all spectral measurements. For pH measurements, Hanna Instruments Microprocessor pH Meter was employed. RB5 standard was obtained from Sigma-Aldrich with a quantity of 100 g per bottle. All chemicals used were of analytical grade reagent and all the solutions were prepared in deionised water obtained from Sastec Laboratory Water Purification System.

Preparation of RB5 Standard Stock Solution

Approximately 100 mg of RB5 powder was weighed and dissolved in deionized water in 50 mL volumetric flask which gave final concentration of 2000 ppm of stock solution. From the stock solution, 200 and 20 ppm working concentrations were prepared by further dilution with deionised water in 250 mL volumetric flask.

Stability Analysis of RB5 Standard Solution

The freshly prepared 20 and 200 ppm working solutions were scanned using Jasco UV-VIS Spectrophotometer in the wavelength range of 300-700 nm. The deionised water was used as the blank. Both solutions were divided into four portions for the following experiments:

Stability of RB5 Dye Exposed to Ambient

The solutions were exposed to room temperature by exposing them to the lamp light. A 3 mL of these working solutions were measured every hour for seven hours in three days and within 1 month.

Stability of RB5 Dye Unexposed to Ambient

The RB5 solutions were kept in cupboard. A same volume of these stock solutions were scanned starting from wavelength (λ) of 700 nm to 300 nm, followed the same procedures and period of time as previously mentioned.

Stability of RB5 Dye in Different pH

The pH of RB5 solutions were adjusted to 2.5 and 9.0 using 0.1 M NaOH solution and 0.1 M HCl solution for acidic and basic conditions, respectively and have been exposed to the room temperature. The stability studies of this dye in two conditions were monitored from consecutive seven hours per day for three days.

Stability of RB5 Dye After Heating and Cooling at Room Temperature

The RB5 solutions were heated to the boiling point (98 °C) and cooled to the room temperature before being scanned using the same procedures as the previously mentioned.

Results and Discussion

The RB5 shows maximum absorption at wavelength (λ) of 596 nm, as shown in **Figure 2**. UV-VIS spectra of the RB5 dye in visible region is characterised by a band at 596 nm. The band is assigned to HOMO-LUMO transition, which is a π - π^* transition between π system of the azo groups, naphthalene ring and the lone pair of the NH_2 group for the HOMO. For the LUMO, there is π^* of the C-O substituent (Sahel *et al.*, 2007). The band is characteristic to a long conjugated π system, linked by two azo groups of RB5 dye (Rivera *et al.*, 2011; Radi *et al.*, 2012).

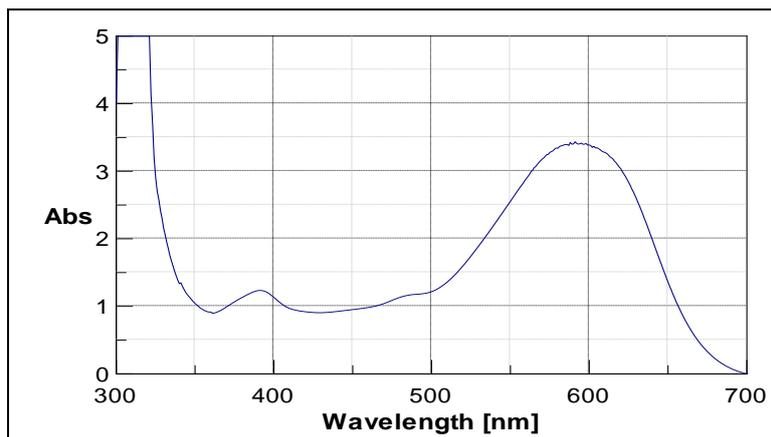


Figure 2: Spectrum of the prepared 200 ppm RB5 standard solution (pH 5.1)

In the stability studies of the 20 ppm RB5 solution under exposed ambient, the average ranges of absorbances obtained were from 0.3548 ± 0.044 to 0.3796 ± 0.0027 , 0.3609 ± 0.0013 to 0.3682 ± 0.0035 and 0.3568 ± 0.0012 to 0.3672 ± 0.0022 for the first, second and third days respectively. The average ranges of absorbances for 200 ppm RB5 were from 3.4740 ± 0.0119 to 3.5647 ± 0.0077 , 3.4551 ± 0.0435 to 3.6034 ± 0.0081 and 3.5193 ± 0.0074 to 3.5844 ± 0.0106 , as shown in **Figure 3**. The average ranges of absorbance obtained for three consecutive days for the 20 ppm RB5 were from 0.3589 ± 0.0073 to 0.3640 ± 0.0024 whereas for the 200 ppm were from 3.5199 ± 0.0342 to 3.5643 ± 0.0481 .

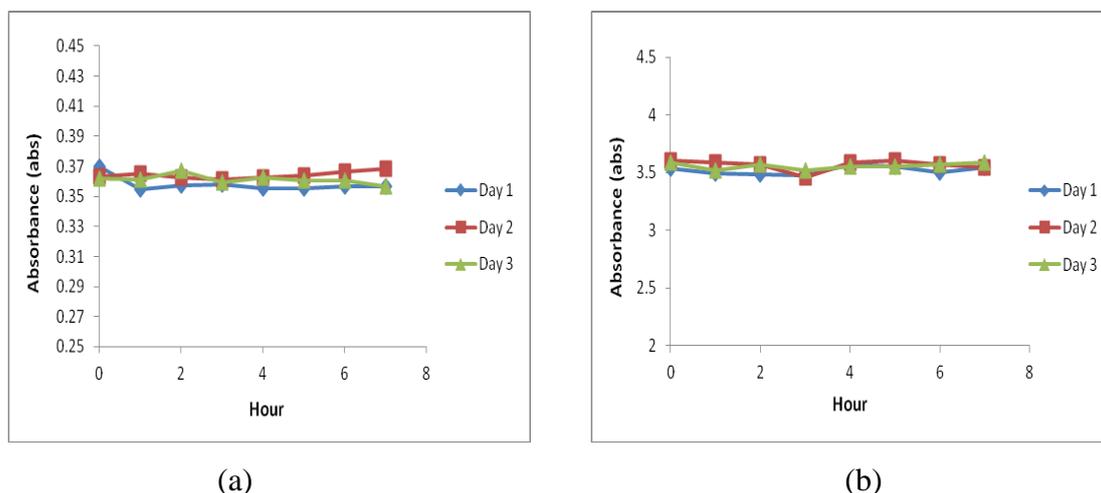


Figure 3: Absorbances of the RB5 standard solutions of (a) 20 and (b) 200 that were exposed to the room temperature up to seven hours for three consecutive days

Within one month, the absorbance ranges for the 20 and 200 ppm were from 0.3721 ± 0.0032 to 0.3769 ± 0.0032 and 3.4215 ± 0.0074 to 3.5361 ± 0.0180 , respectively. The results show that

RB5 dye standard solution were stable with % RSD less than 2 %. **Figure 4** illustrates the absorbances of RB5 after being exposed to the ambient within one month, whereas **Figure 5** shows the spectra of the RB5 standard solution.

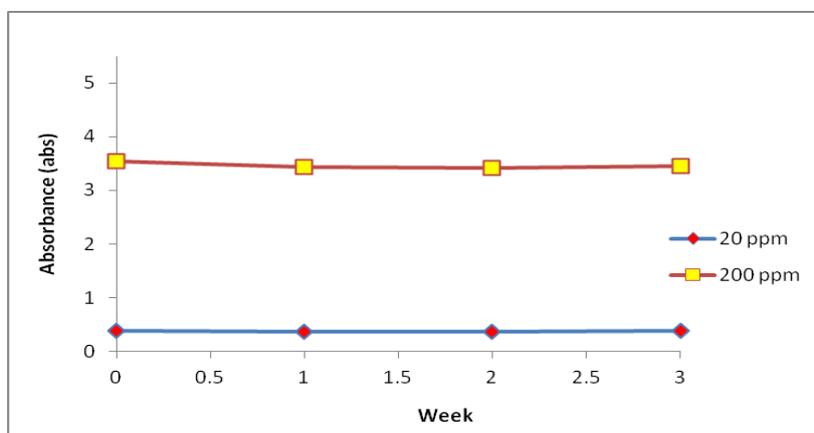


Figure 4: Absorbances of 20 and 200 ppm of the RB5 standard solutions that were exposed to the room temperature within one month

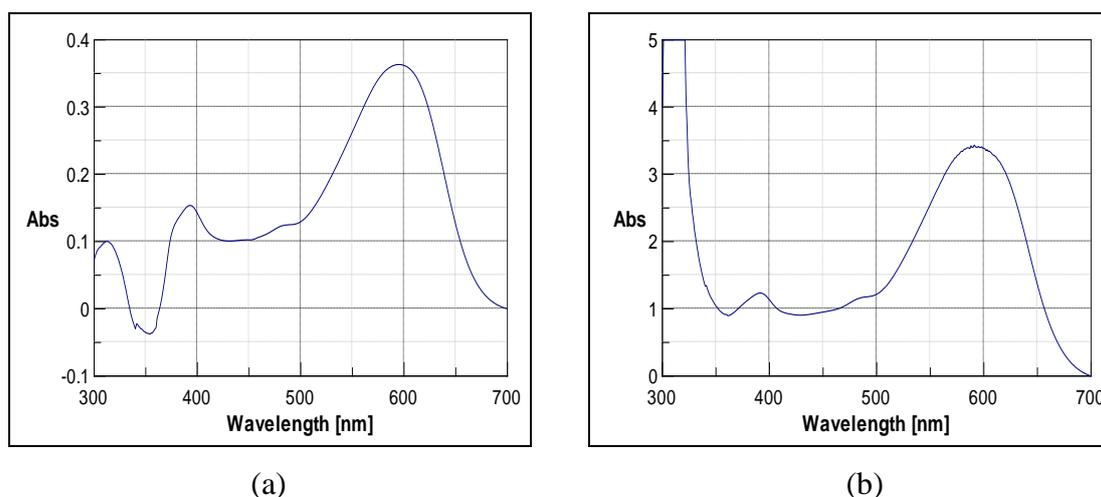


Figure 5: Spectra of the (a) 20 and (b) 200 ppm RB5 standard solutions that were exposed to the room temperature within one month

Under unexposed ambient, the average absorbance ranges for the 20 ppm RB5 were from 0.3396 ± 0.021 to 0.3535 ± 0.0041 , 0.3612 ± 0.0034 to 0.3684 ± 0.0053 and 0.3530 ± 0.0031 to 0.3613 ± 0.0024 for the first, second and third days respectively. For the RB5 dye standard solution with concentrations of 200 ppm, the ranges were from 3.4270 ± 0.0073 to 3.4507 ± 0.0040 , 3.5262 ± 0.0037 to 3.5641 ± 0.0048 and 3.5287 ± 0.0041 to 3.6583 ± 0.0059 , as shown in **Figure 6**.

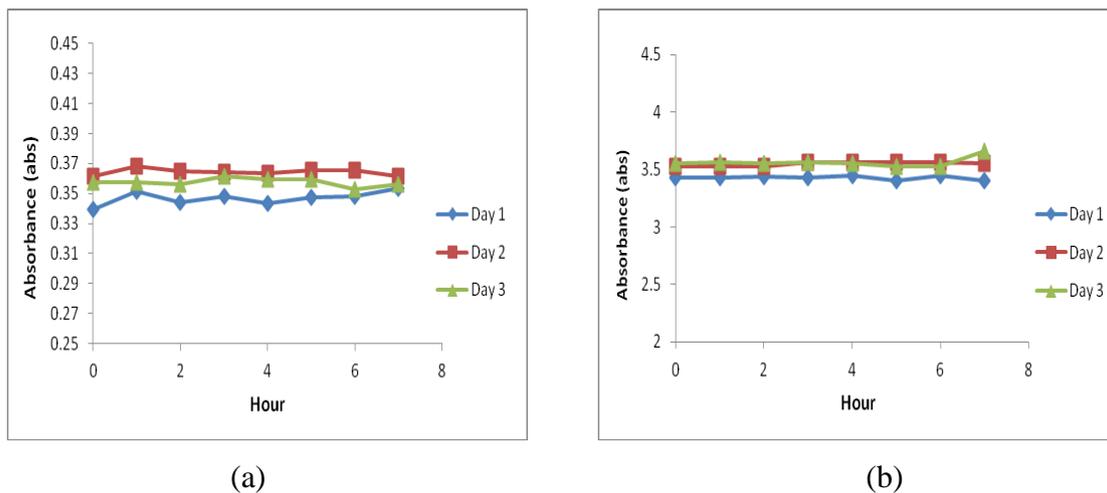


Figure 6: Absorbances of the RB5 standard solutions of (a) 20 and (b) 200 that were unexposed to the room temperature up to seven hours for three consecutive days

The average ranges for the 20 and 200 ppm RB5 standard solutions were from 0.3470 ± 0.0045 to 0.3640 ± 0.0024 and 3.4290 ± 0.0187 to 3.5630 ± 0.0536 for three days measurement, respectively. The absorbance ranges were from 0.3577 ± 0.0036 to 0.3668 ± 0.0050 and 3.4246 ± 0.0213 to 3.5513 ± 0.0059 for the respective concentrations within one month. **Figure 7** and **Figure 8** show the results of these stability studies. The absorbances show that the RB5 standard solutions were stable within one month with % RSD less 2%. There is no significant difference of absorbance obtained for RB5 solutions that exposed and unexposed to the ambient within a month.

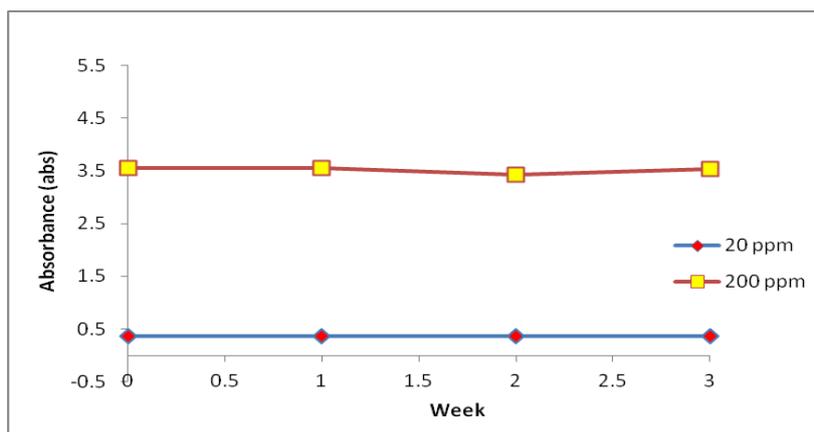


Figure 7: Absorbances of 20 and 200 ppm of the RB5 standard solutions that were unexposed to the room temperature within one month

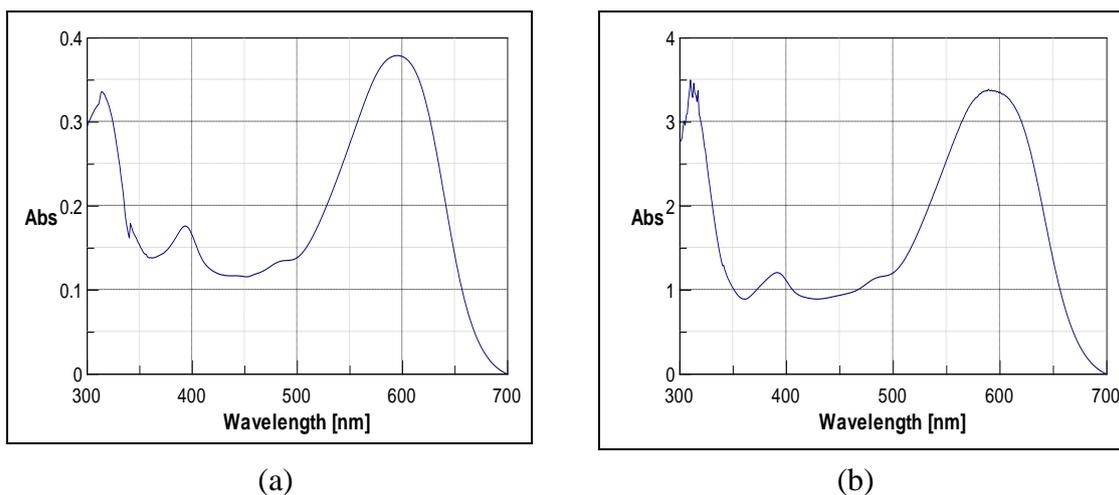


Figure 8: Spectra of the (a) 20 and (b) 200 ppm RB5 standard solutions that were unexposed to the room temperature within one month

For the RB5 standard solution with concentration of 20 ppm in the acidic medium, the average ranges of absorbances obtained were from 0.3456 ± 0.027 to 0.3525 ± 0.0034 , 0.3521 ± 0.0047 to 0.3612 ± 0.0023 and 0.3551 ± 0.0046 to 0.3639 ± 0.0079 for the first, second and third days respectively. Meanwhile, the average ranges were from 3.4668 ± 0.0055 to 3.5296 ± 0.0113 , 3.4766 ± 0.0177 to 3.5519 ± 0.0149 and 3.4927 ± 0.0111 to 3.5253 ± 0.0080 for RB5 standard solution with concentration of 200 ppm. For the 20 and 200 ppm RB5 standard solutions which in pH 2.5, the average ranges were from 0.3493 ± 0.0029 to 0.3586 ± 0.0025 and 3.4904 ± 0.0225 to 3.5184 ± 0.0294 within three days, **Figure 9**.

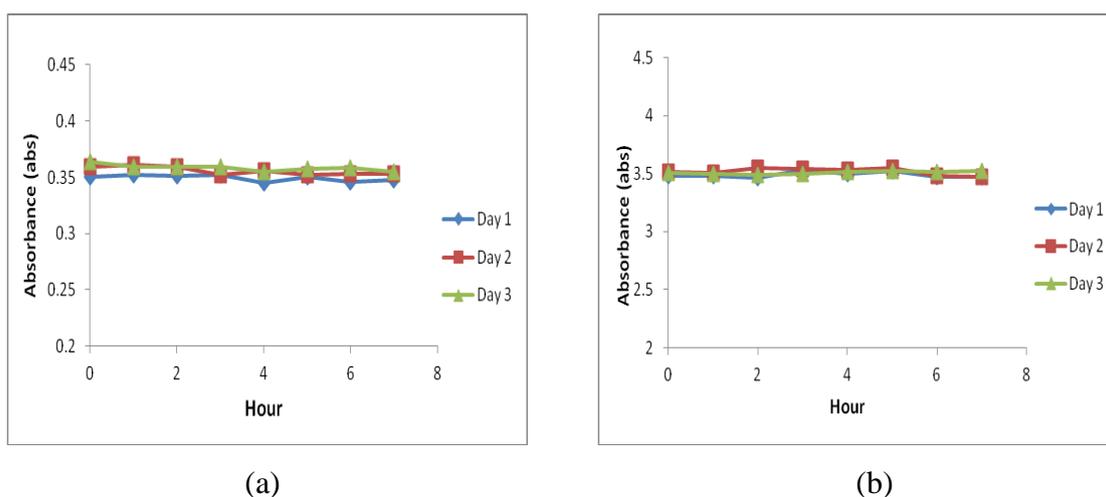


Figure 9: Absorbances of the RB5 standard solutions of (a) 20 and (b) 200 ppm at pH 2.5 up to seven hours for three consecutive days

The absorbance values show that RB5 standard solution is stable within three days, also with % RSD lower than 2%. The spectra are shown in **Figure 10**.

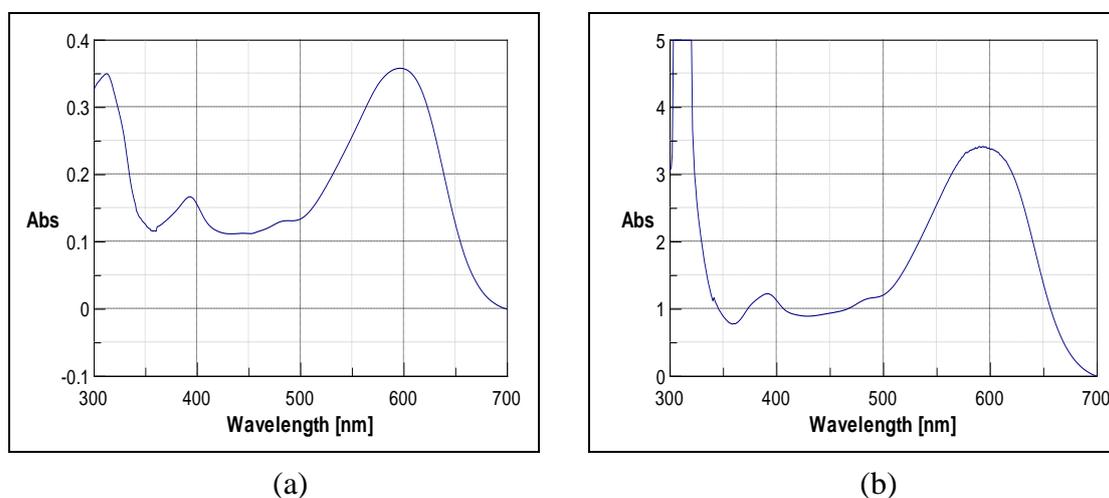


Figure 10: Spectra of (a) 20 and (b) 200 ppm RB5 standard solutions at pH 2.5 within three days

In basic medium, the average ranges of absorbances for the 20 ppm RB5 standard solution were from 0.3434 ± 0.085 to 0.3580 ± 0.0020 , 0.3501 ± 0.0020 to 0.3639 ± 0.0056 and 0.3576 ± 0.0045 to 0.3649 ± 0.0062 for the first, second and third days, respectively. While, the average ranges of absorbances for the 200 ppm RB5 standard solution were from 3.5592 ± 0.0067 to 3.6522 ± 0.0056 , 3.6088 ± 0.0656 to 3.6523 ± 0.0097 and 3.5637 ± 0.0036 to 3.6461 ± 0.0086 . The average absorbance ranges for the 20 and 200 ppm RB5 standard solutions at pH 9.0 were from 0.3515 ± 0.0043 to 0.3614 ± 0.0024 and 3.6007 ± 0.0296 to 3.6352 ± 0.0134 within three days. These stability results are as shown in **Figure 11**. The % RSD values for the absorbances obtained were less than 2%. Hence, the RB5 standard solutions were stable in the basic medium. The spectra are shown in **Figure 12**. The results suggest that acidic and basic medium did not give any significant effect to the azo functional group of RB5 dye.

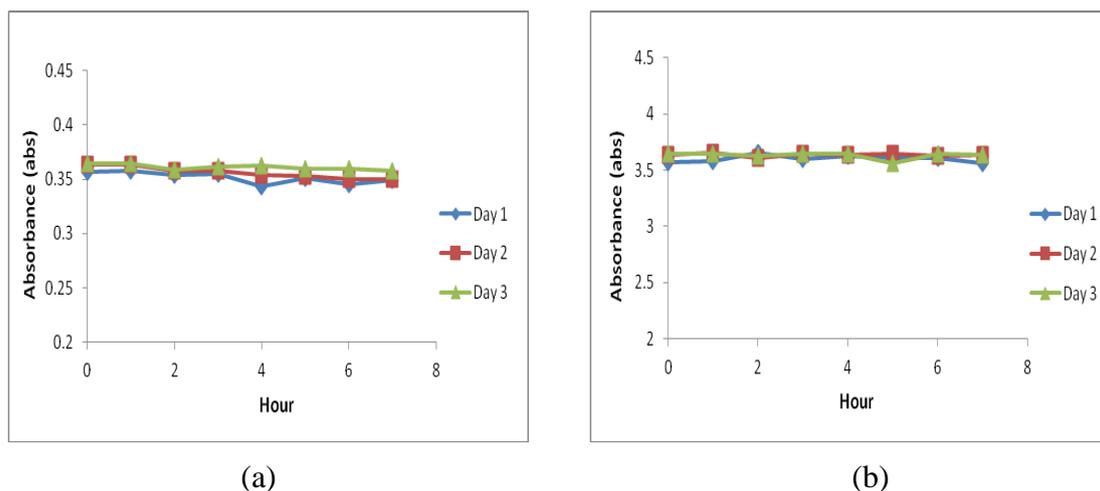


Figure 11: Absorbances of the RB5 standard solutions of (a) 20 and (b) 200 ppm at pH 9.0 up to seven hours for three consecutive days

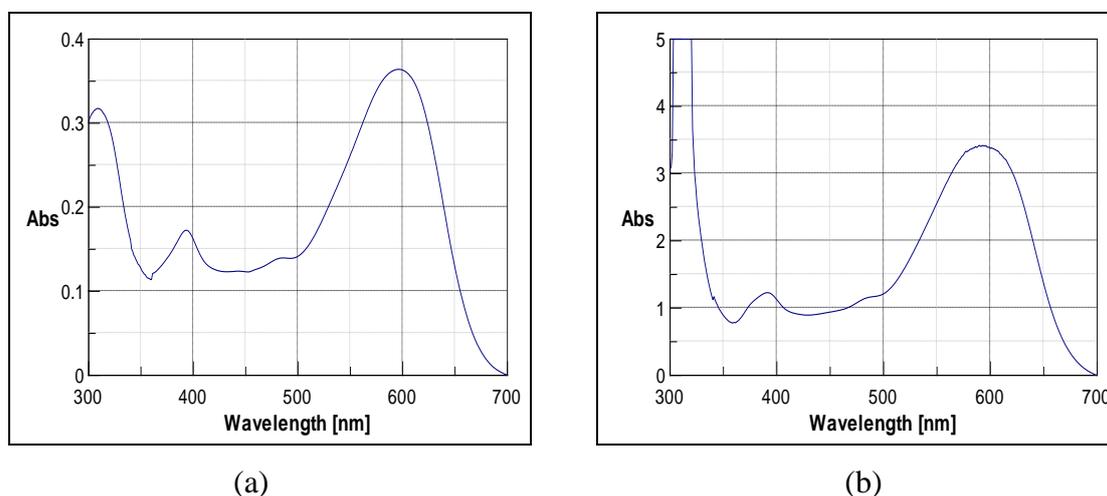


Figure 12: Spectra of (a) 20 and (b) 200 ppm RB5 standard solutions at pH 9.0 within three days

Another stability studies that had been performed was the effect of heat. For the first, second and third days, the average absorbance ranges for the 20 ppm RB5 standard solution were from 0.4129 ± 0.0038 to 0.4210 ± 0.0030 , 0.3994 ± 0.0038 to 0.4125 ± 0.0038 and 0.3978 ± 0.0030 to 0.4041 ± 0.0078 , respectively. Meanwhile, for the 200 ppm RB5 standard solution, the average absorbance ranges were from 3.7109 ± 0.0036 to 4.1675 ± 0.0274 , 3.6575 ± 0.0130 to 3.7144 ± 0.0115 and 3.6573 ± 0.0052 to 3.7145 ± 0.0099 . The average absorbance ranges of the 20 and 200 ppm standard solution for three consecutive days measurement were 0.4013 ± 0.0022 to 0.4178 ± 0.0027 and 3.6837 ± 0.0191 to 3.6882 ± 0.0344 , respectively. The results are as shown in **Figure 13**. The % RSD values with less 2 % were obtained for the

absorbance measurements of the stability study of the heated RB5 standard solutions. Hence, the heat did not affect the azo functional group of RB5. **Figure 14** shows the spectra.

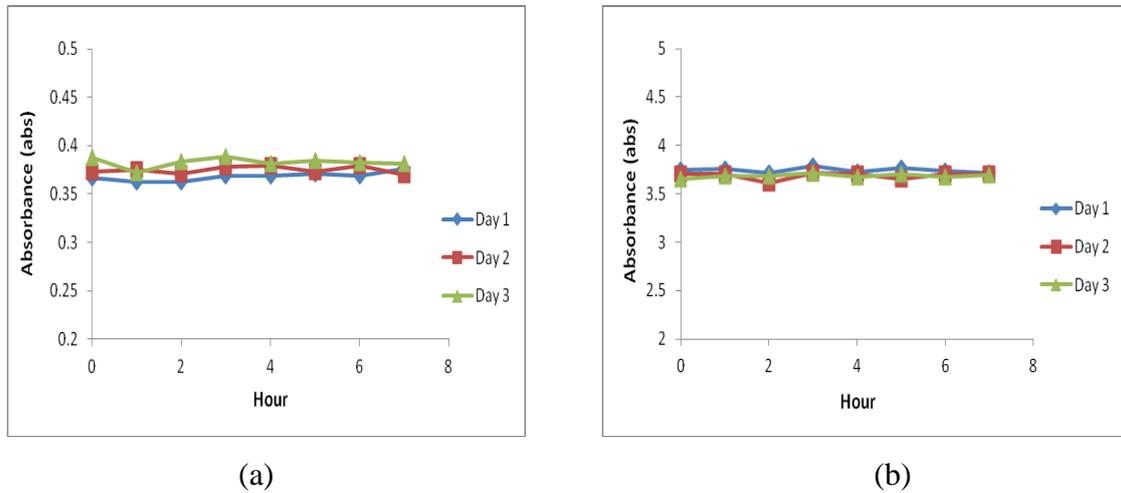


Figure 13: Absorbances of the RB5 standard solutions of (a) 20 and (b) 200 ppm after heating and cooling to room temperature up to seven hours for three consecutive days

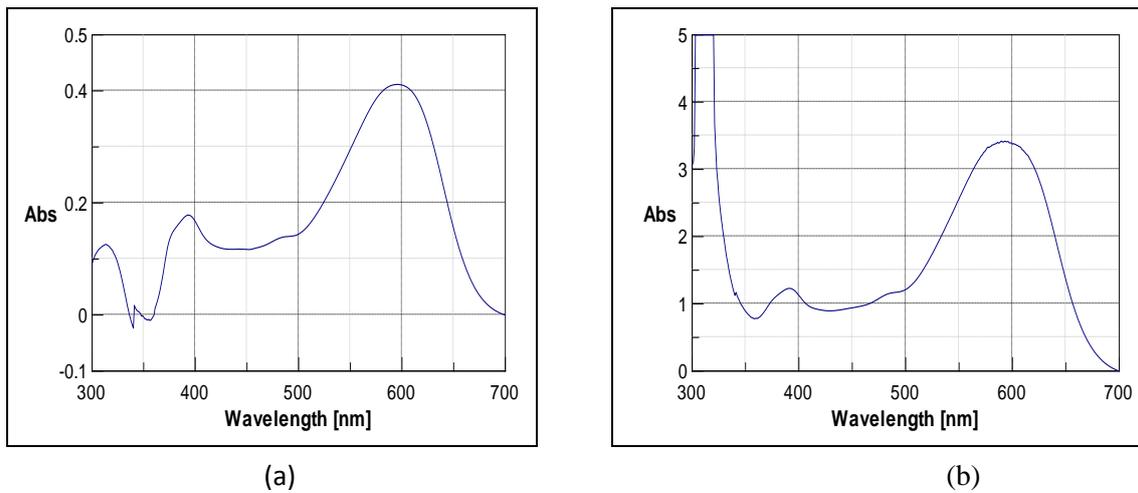


Figure 14: Spectra of (a) 20 and (b) 200 ppm RB5 standard solution after heating and cooling to the room temperature within three days

The results of the entire study is summarised in **Table 1**.

Table 1: Summary of absorbance range for RB5 standard solutions in the studied conditions

Condition	Absorbances of RB5 Dye	
	20 ppm	200 ppm
Exposed to the ambient	Three consecutive days:	
	0.3589±0.0073 to 0.3640±0.0024	3.5199±0.0342 to 3.5643±0.0481
	Within 1 month:	
	0.3721±0.0032 to 0.3769±0.0032	3.4215±0.0072 to 3.5361±0.0180
Unexposed to the ambient	Three consecutive days:	
	0.3470±0.0045 to 0.3640±0.0024	3.4290±0.0187 to 3.5630±0.0536
	Within 1 month:	
	0.3577±0.0036 to 0.3668±0.0050	3.4246±0.0213 to 3.5513±0.0059
Acidic medium	Three consecutive days:	
	0.3493±0.0029 to 0.3586±0.0025	3.4904±0.0225 to 3.5184±0.0294
Basic medium	Three consecutive days:	
	0.3515±0.0043 to 0.3614±0.0024	3.6007±0.0296 to 3.6352±0.0134
Heating	Three consecutive days:	
	0.4013±0.0022 to 0.4178±0.0027	3.6837±0.0191 to 3.6882±0.0344

Conclusion

RB5 standard solutions were stable in the different studied conditions within one month period of time as azo functional group of the RB5 dye which is electroanalytically active was not being affected.

Acknowledgements

The authors gratefully acknowledges the Universiti Sains Malaysia (USM) for awarding the Short Term Grant No 304/PPSK/61312057 and also would like to thank all the Forensic Sciences laboratory staff for their co-operation and assistance.

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