VOLTAMMETRIC STUDIES OF REACTIVE BLACK 5 AND ITS APPLICATION IN WASTEWATER FROM BATIK INDUSTRY

by

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LIST OF ABBREVIATIONS

ACP	Alternate current polarography
AdCSV	Adsorptive cathodic stripping voltammetry
AdSV	Adsorptive stripping voltammetry
AE	Auxiliary electrode
ASV	Anodic stripping voltammetry
BDD	Boron doped diamond
BiFE	Bismuth film electrode
BRB	Britton Robinson buffer
CE	Capillary electrophoresis
CPE	Carbon paste electrode
COD	Chemical oxygen demand
CSV	Cathodic stripping voltammetry
CV	Cyclic voltammetry
DAD	Diode array detection
DB	Drimarene Blue
DCP	Direct current polarography
DME	Dropping mercury electrode
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DPCSV	Differential pulse cathodic stripping voltammetry
DPP	Differential pulse polarography
DPV	Differential pulse voltammetry
DS	Derivative spectrophotometry
GCE	Glassy carbon electrode
GDP	Gross domestic product
HMDE	Hanging mercury dropping electrode
IARC	International Agency Research on Cancer
IR	Infra red
LC-MS	Liquid chromatography-mass spectroscopy

LLE	Liquid-liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
LSV	Linear sweep voltammetry
MiFE	Mercury film electrode
MSC	Multisyringe chromatography
NMR	Nuclear magnetic resonance
NP	Normal polarography
PbFE	Lead film electrode
PGE	Pyrolytic graphite electrode
PR	Procion Red
PY	Procion Yellow
RB5	Reactive Black 5
RE	Reference electrode
RNA	Ribonucleic acid
RR120	Reactive Red 120
RR19	Reactive Green 19
SCE	Standard calomel electrode
SMDE	Static mercury dropping electrode
SPE	Solid phase extraction
SWP	Square wave polarography
SWV	Square wave voltammetry
SY	Sunset yellow
$\mathrm{TBAF_4}$	Tetrabutylammonium tetrafluoroborate
TDA	Tridodecylamine
TSS	Total suspended solid
TT	Tartrazine
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
VA	Voltammetry analyzer
WE	Working electrode

LIST OF SYMBOLS

Abs	Absorbance
Ag/AgCl	Silver-silver chloride
E_{acc}	Accumulation potential
E_i	Initial potential
E_f	Final potential
E_p	Peak potential
E_{switch}	Switch potential
g/mol	Gram per mol
HAc-NaAc	Acetic acid-sodium acetate
I_p	Peak height
M	Molar
mg/L	Miligram per liter
mL	Mililiter
mM	Milimolar
mV	Milivolt
mV/s	Milivolt per second
nA	Nano ampere
nm	Nanometer
nm/min	Nanometer per min
nM	Nano molar
rpm	Rotation per minute
s	Second
t_{acc}	Accumulation time
t_{eq}	Equilibration time
V	Volt
µg/g	Microgram per gram
µg/L	Microgram per liter
µg/mL	Microgram per mililiter
µm	Micrometer
µM	Micromolar

R^2	Correlation coefficient
λ	Wavelength
v	Scan rate

KAJIAN VOLTAMMETRI REAKTIF BLACK 5 DAN APLIKASINYA DALAM AIR SISA DARI INDUSTRI BATIK

ABSTRAK

Pewarna azo dan reaktif merupakan dua kelas pewarna organik digunakan secara meluas dalam pelbagai aplikasi terutamanya industri pembuatan batik. Pewarna Black 5 Reaktif (RB5) dikategorikan dalam pewarna azo vinylsulfonat disebabkan kewujudan ikatan –N=N- yang bergabung dengan vinylsulfonat sebagai kumpulan reaktif. Pembentukan amine aromatik yang bersifat karsinogen hasil daripada proses penurunan kumpulan azo memberi risiko pengumpulan bio dalam rantai makanan manakala pewarna reaktif mempunyai jangka hayat yang lama dalam persekitaran. Pelbagai kaedah telah digunakan untuk penentuan pewarna seperti kromatografi dan elektroforesis kapilari (CE) tetapi kaedah-kaedah tersebut kompleks dan mempunyai kos peralatan yang tinggi, keperluan dan kepelbagaian yang tinggi terhadap bahan kimia dan penyediaan sampel, penyelanggaraan serta pekerja mahir. Untuk mengatasi masalah-masalah ini, teknik voltammetri menggunakan titisan raksa tergantung (HMDE) sebagai elektrod bekerja dan larutan penimbal Britton-Robinson (BRB) sebagai elektrolit penyokong telah dicadang untuk dibangunkan dalam kajian ini. Teknik ini telah digunakan untuk mengkaji sifat elektroanalitikal dan analisis kuantitatif pewarna RB5 pada elektrod raksa. Pewarna RB5 didapati terkumpul dan melalui proses penurunan tidak berbalik pada elektrod raksa. Beberapa parameter voltammetri eksperimen telah dioptimumkan bagi memperoleh respons maksima yang dirujuk kepada puncak penurunan kedua serta pengesahan analisa untuk pembangunan kaedah bagi pewarna RB5. Parameter-parameter optimum untuk

Differential Pulse Cathodic Stripping Voltammetry (*DPCSV*) di dalam medium berasid ialah larutan BRB pada pH 2.5, keupayaan awal (E_i): +250 mV, keupayaan akhir (E_f): -600 mV, kadar imbasan (v): 5 mV/s, masa pengumpulan (t_{acc}): 80 s, keupayaan pengumpulan (E_{acc}): 0 mV and amplitud denyut: 75 mV. Untuk medium beralkali, parameter-parameter optimum ialah E_i: -100 mV, E_f: -1000 mV, v: 15 mV/s, t_{acc}: 30 s, E_{acc}: -100 mV dan amplitud denyut: 150 mV di dalam larutan BRB pada pH 9.0. Menggunakan semua parameter optimum ini, pewarna RB5 berkepekatan 2 mg/L telah menghasilkan puncak penurunan kedua pada -65.50 mV di dalam medium berasid manakala pada -570 mV bagi puncak yang sama di dalam medium beralkali. Had pengesanan (LOD) bagi pewarna RB5 ialah 12.15 µg/L dan 7.97 µg/L bagi medium berasid dan beralkali masing-masingnya. Teknik yang dibangunkan diaplikasi untuk analisa pewarna RB5 dalam sampel air sisa batik dan keputusan analisa dibanding dengan yang diperoleh menggunakan kaedah spektrofotometri UV-VIS. Kesimpulannya, teknik yang dibangunkan adalah jitu, tepat, melibatkan kos yang rendah dan berpotensi besar untuk menjadi kaedah alternatif dalam analisa kandungan pewarna RB5 di dalam air sisa batik.

VOLTAMMETRIC STUDIES OF REACTIVE BLACK 5 AND ITS APPLICATION IN WASTEWATER FROM BATIK INDUSTRY

ABSTRACT

Azo and reactive dyes are two classes of organic dye mostly used in various applications especially in batik industry. Reactive Black 5 (RB5) dye is categorized in vinylsulphonate azo dye group due to the presence of –N=N- bonds combined with vinylsulphone as the reactive group. The production of carcinogenic aromatic amines, resulted from azo reduction has a significant risk of bioaccumulation in the food chains whereas reactive dyes have a long half-life in environment. Various methods have been used for dye measurements such as a chromatography and capillary electrophoresis (CE) but they are well-known for their complex and high cost instrumentation, high and multiple requirements on chemicals and sample preparation, maintenance and skilled labour. To overcome these problems, the voltammetric technique using Hanging Mercury Dropping Electrode (HMDE) as a working electrode and Britton-Robinson Buffer (BRB) as a supporting electrode was proposed to be developed. This technique was used for investigating the electroanalytical properties and quantitative analysis of RB5 dye at the mercury electrode. The RB5 dye was found to be accumulated and undergone irreversible reduction process at the mercury electrode. The experimental voltammetric parameters were optimized in order to obtain a maximum response which referred to the second reduction peak with analytical validation of the technique for RB5 dye. The optimum parameters for the differential pulse cathodic stripping voltammetry (DPCSV) technique in acidic medium were the BRB at pH 2.5, initial potential (E_i):

+250 mV, final potential (E_f): -600 mV, scan rate (v): 5 mV/s, accumulation time (t_{acc}): 80 s, accumulation potential (E_{acc}): 0 mV and pulse amplitude: 75 mV. In basic medium, the optimum parameters were E_i: -100 mV, E_f: -1000 mV, v: 15 mV/s, t_{acc}: 30 s, E_{acc}: -100 mV and pulse amplitude: 150 mV in BRB at pH 9.0. Using both optimized parameters, RB5 dye at a concentration of 2 mg/L has produced second reduction peak at -65.50 mV in acidic medium whereas the same peak at -570 mV in basic medium. The limit of detection (LOD) of RB5 dye was approximately 12.15 µg/L and 7.97 µg/L for acidic and basic medium, respectively. The developed technique was applied for the analysis of RB5 dye in batik wastewater samples and the results were compared with those obtained by the UV-VIS Spectrophotometry method. It can be concluded that the developed technique is precise, accurate, rugged, robust, low cost, fast and has potential to be an alternative method for routine analysis of RB5 dye in batik wastewater samples.

CHAPTER I

LITERATURE REVIEW

1.1 Overview

East Coast of Peninsular Malaysia, especially in Kelantan and Terengganu is very well known for its homemade textile industry or more precisely the batik industry among local people. This industry is one of the oldest industries in Malaysia, which believed to have existed around the year of 1921 (Wan Hashim, 1996). It is traditionally inherited from generation to generation and becomes a source of income for many local families since there is a high demand from both local and foreign markets. Furthermore, it has also becomes one of the main attractions for tourists to visit these places (Ahmad *et al.*, 2002; Hapsah, 2006; Rashidi *et al.*, 2012).

Batik is defined as a fabric which traditionally uses a manual wax-resist dyeing technique. Modern advances in the textile industry have led to extended of this term to include fabrics which add in traditional patterns and designs even if they are not produced using the wax–resist dyeing techniques. Generally, the making of batik can be classified into three processes which are fabric preparation (waxing), dyeing and de-waxing (Diana, 2010).

In the making of batik, the first step includes washing of cotton fabric in order to remove starch. The fabric is then dried, ironed and stretched on a wooden frame. After that, a rough charcoal sketch is made as guidance for the painting. Wax, a melted mixture of resin, paraffin and yellow bee wax is poured into a brass shaped pen or canting tool called Tjanting and then applied to the sketch. The Tjanting is like a small cup with single or double spout. The wax becomes a protective agent in the dyeing process since it protects dye from spreading.

In the second process, dyeing is applied to colour the sketched area. Chemical substance like ludigol is added into dilute dye to obtain a brighter and more vibrant colour. Alum is used in pre-treatment process of fabric before marbling to ensure the sketched design can be applied well on the fabric, whereas sodium alginate is used to thicken the dye for screening, printing, painting and control spreading purposes. Dyeing needs skills in order to produce a good combination of colours.

In the third process, the decorated and painted fabric is boiled to remove all the wax that protects the dye from spreading during the painting and dyeing process. This process can be repeated until the entire wax has been removed from the fabric. The fabric is then rinsed in cold water and dried under mild sun to protect the colours. The rinsed water after the boiling process is normally discharged without any treatment and can be considered as wastewater from the batik industry (Ahmad *et al.*, 2002).

1.2 Batik Wastewaters, Characteristics and Environment Impacts

Textile industry is considered as one of the major water consumers as it utilizes large volume of water and chemicals for wet processing of the textiles and thus contributes to the river pollution (Norasikin *et al.*, 2010; Saratale *et al.*, 2011; Ellouze *et al.*, 2012; Radi *et al.*, 2013a). Homemade batik industry is a part of this textile industry. Preliminary studies show that discharged wastewaters from the homemade batik industry contain dyes, heavy metals, grease, wax, suspended solid (TSS) and have high chemical oxygen demand (COD) (Nora'aini and Suhaimi, 2009; Rashidi *et al.*, 2012).

Heavy metals such as copper (Cu), zinc (Zn) and chromium (Cr) are harmful wastes that give a risk of contaminating groundwater and other water resources (Normala and Goh, 2010). They are also not biodegrade in the environment and this condition makes them tend to accumulate in living organisms (Chuah *et al.*, 2005). The contaminated groundwater and aquatic food then may lead to the diseases like cancer, tumor and may disrupt the endocrine system of human as well (Imtiazuddin *et al.*, 2012).

Lokhande *et al.* (2011) have mentioned that the discharged of untreated industrial effluents, which contain various amounts of metals have the potential to contaminate crops, enter the food chain and finally will affect human health. The discharged wastewaters with high COD may cause the depletion of dissolved oxygen (DO), thus leads to anaerobic condition (Diana, 2010).

1.3 Dyes in General

Dyes are generally be defined as coloured substances which have the affinity to the applied substrates to produce significant degree of coloration. The first organic dye, Mauve was accidentally discovered by English chemist, William Henry Perkin in 1856 while he was trying to synthesize quinine in aniline (Saratale *et al.*, 2011). Before that time, colouring materials were extracted from natural sources like barks, roots, seeds, leaves and shellfish (www.dyes-pigments.com). The discovery of organic dyes has overwhelmed the role of natural dyes since they have low cost, brighter colours, better resistance towards environmental factors and easy to apply to fabrics (Ngah *et al.*, 2011).

Nowadays, as the world population increases, the demand for clothing has also increases with improving sense of fashion and lifestyle. As a result, textiles are extensively produced to fulfill the demand, which subsequently intensify the development of the organic dye industries. In some countries, like India and Sri Lanka, textile production becomes the main source of income that highly contributes to their Gross Domestic Product (GDP) (Ahmad *et al.*, 2006; Normala and Goh, 2010).

More than 700,000 tons and 100,000 types of dyes are annually produced worldwide in large quantities and commercially used (Cristóvão *et al.*, 2009). In 2008, the worldwide market for organic dyes increases up to $11 billion (Salari *et al.*, 2009). They are mostly used in textile, food, printing, pharmaceutical, cosmetic, nutritional and tannery industries (Joe *et al.*, 2008; Karatas *et al.*, 2009; Tanyldizi, 2011).

1.4 Organic Dyes and the Subclasses

The organic dyes can be namely according to the chemical structure of their particular chromophoric groups (www.dyes-pigments.com). They can be classified into several subclasses such as azo dyes, vat dyes, reactive dyes, basic dyes, acid dyes and sulphur dyes (www.iiem.com). Azo, reactive and vat dyes are as follows;

1.4.1 Azo Dyes

The azo dyes are considered as the oldest and the largest class of synthesized organic dyes with 3000 different varieties and are also usually considered as the class with the fullest shade range and mostly used (Xu *et al.*, 1995). They account for 60 % to 70 % of the total organic dyes, have bright and high colour intensity. They are most widely used dyes in the textile, food, drug, cosmetic, automobile, leather and papermaking industries (Gupta *et al.*, 2007; Muthukumar *et al.*, 2007; Karipcin *et al.*, 2009; Bogdanowicz *et al.*, 2013; Saratale *et al.*, 2013).

The azo dyes are characterized by the presence of one or more azo groups (-N=N-), produced by diazotization of aromatic amines and coupling of the diazonium salts with a phenol or aromatic amine at free ortho or para position (Jayadevappa *et al.*, 2006). The azo group, which is chromophore, enables the dyes to absorb light in the visible spectrum and responsible for the corresponding colour of the dyes (Colindres *et al.*, 2010; Méndez-Martínez *et al.*, 2012). The benzene or naphthalene group which is attached to the azo group may consists of many substituents like chloro (-Cl), methyl ($-CH_3$), nitro ($-NO_2$), amino ($-NH_2$), hydroxyl (-OH) and carboxyl (-

COOH) (Saratale *et al.*, 2011). According to Aguedach *et al.* (2005), half-lives of some azo dyes in sunlight are usually greater than 2000 hours.

The colour produced by the textile wastewaters is mainly due to azo dyes and the discharged of the wastewaters into the environment is a matter of aesthetic, although at low concentrations, which are from 10 mg/L to 50 mg/L. Improper discharge of these wastewaters into environment will lead to reduction in sunlight penetration, which in turn affects aquatic photosynthesis and inhibits the growth of biota, depletion of DO, water quality and toxic to organisms (Koupaie *et al.*, 2011; Hanafiah *et al.*, 2012; Sarala and Venkatesha, 2012; Solís *et al.*, 2012; Saratale *et al.*, 2013).

The azo group that has reductively cleaved when in contact with sweat, saliva or gastric juices is responsible for the aromatic amines formation. Examples of such aromatic amines are benzidine and 4-biphenylamine. After the oral ingestion of azo dyes, an aerobic intestinal microflora and mammalian azo reductase in the intestinal wall can reduce them to free aromatic amines. Hence, the intestines would be one of the first target organs to suffer by the ingestion of the azo dyes (Xu *et al.*, 1995; Umbuzeiro *et al.*, 2005; de Lima *et al.*, 2007; Chequer *et al.*, 2011). Goyal *et al.* (1998) have mentioned that various azo dyes tend to induce tumors in rats and mice. The azo dyes give inhibitory effects on the biosynthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins.

1.4.2 Reactive Dyes

The reactive dyes become one of the most significant innovation technologies for dyes field in the 20th century. They are covalently bonded with -OH or –NH_2 groups that present on the fibers, expectedly to give excellent wash fastness property compared to an ordinary dye-substrate system (Karcher *et al.*, 2002; Esteves and Cunha, 2005; Jović *et al.*, 2013). This excellent property has made them not easy to be biodegraded. Fixation efficiency of 60 % to 90 % causes large amounts of the unfixed dyes to be discharged into the environment (Riera-Torres and Gutiérrez, 2010). The mechanism of vinylsulphone reactive dye fixation to the cellulose polymer fabric is shown in Figure 1.1 (www.chm.bris.ac.uk).

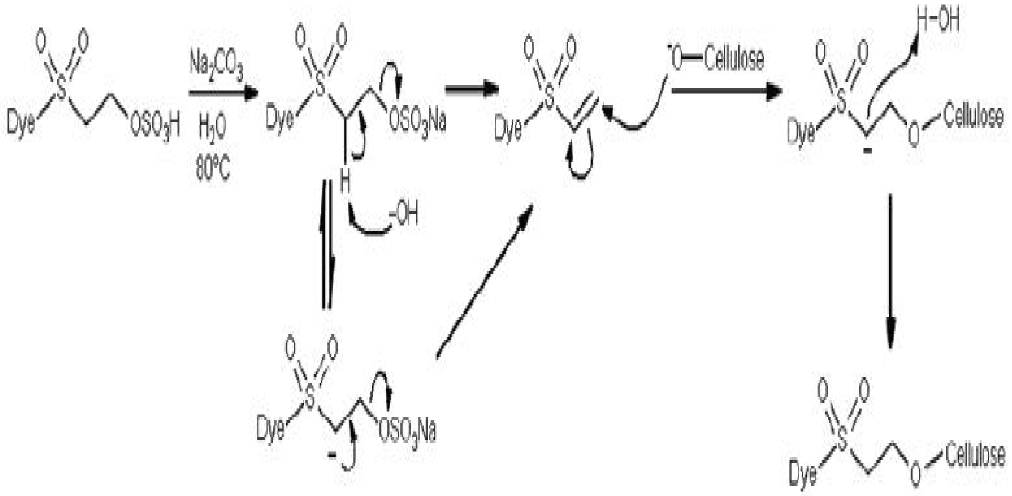

Figure 1.1 Mechanism of vinylsulphone reactive dye fixation to the cellulose polymer fabric

The fibre-reactive group may react with the OH^- ions in the alkali solution and become hydrolysed. The alkali used for maintaining pH depends on brand of dye and dyeing temperature. Generally, caustic soda, soda ash or sodium carbonate is used as alkali, depending on reactivity of the dyes. They create proper pH in dye bath and act as the dye-fixing agent (textilelearner.blogspot.com). According to Zanoni *et al.* (1997), typically about 12 % of reactive dyes are lost to waste during manufacturing processes and about 20 % of these losses are discharged into the environment through textile effluents. The reactive dyes have shown potential to have a long half-life in the neutral medium of environment.

For instance, in voltammetric analysis of reactive dye, Turquoise Blue 15 based on copper-phthalocyanine as chromophore and a monochlorotriazine as a reactive group has been investigated for its electrochemical behaviour and quantitative determination using cyclic voltammetry (CV) and cathodic stripping voltammetry (CSV) technique by Osugi *et al*. (2003).

1.4.3 Vat Dyes

The vat dyes made up 15 % of total consumption of textile dyes, which have high requirement on fastness to produce coloured fibers with excellent colour fastness as well a particularly to light, washing and chlorine bleaching. The dyes fall into two groups which are indigoid and anthraquinonoid. Besides, the vat dyes are insoluble in water, but with the presence of an alkali or reducing agent, they can be reduced to form a soluble dye, called leuco dyes (Carneiro and Fogg, 2005; Kariyajjanavar *et al*., 2013). As an example, Komboonchoo *et al*. (2009) have studied indirect cathodic reduction of the vat dye indigo by cathodically reduced Lawsone, the major natural colourant in the *henna* leaves in the alkaline aqueous solution.

1.5 Analytical Methods for Determination of Textile Azo Dyes

Several analytical methods have been employed to determine textile azo dyes and the intermediates in various samples such as electrophoresis, chromatography, infra red (IR) spectroscopy, nuclear magnetic resonance (NMR) and spectrophotometry method (Bersier and Bersier, 1986). Zanoni *et al.* (1999) have mentioned that chromatography and spectrophotometry method are extensively used for determination of the textile azo dyes. Meanwhile, Santos *et al.* (2013) have mentioned that the most common used methods to determine these dyes are spectrophotometry and voltammetry methods.

1.5.1 Spectrophotometric Determination of Textile Azo Dyes

The UV-VIS spectrophotometric method for textile azo dye analysis for comparison purpose with voltammetric technique was studied by Radi *et al.* (2013a). In this study, they have measured Reactive Red 231 dye in phosphate buffer at pH 3.77 using wavelength, λ range of 200 to 600 nm. The absorbance was linear to dye concentration at maximum λ of 536 nm from 20 to 200 µM. A limit of detection (LOD) was 10 µM, 50 times higher than the LOD obtained in voltammetric study. They have reported that this spectrophotometric method was not able to analyze the dye in real samples at a lower concentration than 10 µM due to its low sensitivity.

In addition, Radi *et al.* (2012) have also studied the UV-VIS spectrophotometric method for Reactive Black 5 dye determination. A series of dye concentration from 5.0×10^{-6} to 1.0×10^{-4} M was measured in the range 200 to 800 nm and the highest absorbance was obtained at λ of 600 nm. The obtained regression equation was y = 0.029 x + 0.015, while the LOD and quantification limit (LOQ) were 4.7 x 10^{-6} M and 15.6 x 10^{-6} M, respectively. However, an application of this developed method in real samples analysis was not carried out.

This 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). Moreover, Pinheiro *et al.* (2004) have mentioned that this method gives only the moderate LOD.

Derivative spectrophotometry (DS) was used to analyze the binary mixture of five textile dyes which were yellow, scarlet, red, blue and navy blue by Nateri and Ekrami (2009). The absorption spectrum of the binary mixture that prepared in different ratios was recorded between λ of 400 to 700 nm. The obtained spectrum was divided by standard spectrum of individual component of the binary mixture to calculate the derivative spectra. Other applications of DS method for textile dyes determination were reported by Turabik (2008), Almeida *et al.* (2009), Gözmen *et al.* (2009), Gao *et al.* (2010), Nateri (2010) and Özer and Turabik (2010).

Although the DS method is developed to give higher sensitivity and minimize spectral interference in terms of overlapping spectrum compared to the normal spectrophotometry, it is mentioned to have main disadvantage for very dependence on instrument parameters such as scan rate and slit width. The instrument parameters for recording parent zero-order spectrum should have given strict attention since it strongly influences the shape and intensity of the derivative (Ojeda and Rojas, 2013).

1.5.2 Chromatographic Determination of Textile Azo Dyes

Fernández *et al.* (2010) have developed a method based on multisyringe chromatography (MSC) with monolithic column using UV detection to determine sulphonated azo dyes Acid Yellow 23, Yellow 9 and Red 97. Ion pair reagent used was dodecyltrimethylammonium bromide, whereas mobile phase used was mixture of acetonitrile and water. The first dye to elute to column is Acid Yellow 9, followed by Yellow 23 and Red 97. Tap water, well water and lagoon biological wastewater were spiked with different amounts of each dye for validation purpose.

García-Lavandeira *et al.* (2010) have optimized procedure based on ultrasound assisted-extraction of textile samples with 20 mL methanol under controlled conditions and followed by separation and analysis using liquid chromatography-mass spectroscopy (LC-MS) for the control of dyes in textile products. In this study, the LOD found to be lower than 0.4 µg/g. Ràfols and Barceló (1997), Sarzanini *et al.* (1997), Smyth *et al.* (1999), De Lima *et al.* (2007) and Vaněková *et al.* (2007) have also studied chromatography method for textile azo dyes determination.

Instead of offering very low detection limit, the chromatography method is well-known for its complex and high cost instrumentations, high and multiple requirements on maintenance, skilled labours, chemicals and samples preparation, and low speed of analysis as well. A part from that, the samples need to be pretreated before being analyzed to minimize interferences during analysis (Pinheiro *et al.*, 2004; Al-Degs *et al.*, 2008).

It is necessary for the solid phase extraction (SPE) to be carried out as the pre-treatment step in azo dyes analysis using chromatography. However, the extraction materials of polymeric SPE sorbents are non-selective and interferences are extracted together from the complex matrices such as textile effluents. In addition, co-elution of the most polar analytes with humic substances for surface water samples exhibits peak at the beginning of the chromatogram. Although additional clean up using silica can be performed to overcome the problem, it is always laborious and time intensive (Bouzige *et al.*, 1999).

1.5.3 Capillary Electrophoresis Determination of Textile Azo Dyes

Peláez-Cid *et al.* (2008) have studied non-aqueous capillary electrophoresis (CE) with electrochemical detection for Basic Blue 41, Blue 9, Green 4, Violet 16 and Violet 3 dye determinations. The electrophoresis buffer for the dyes separation was a solvent mixture of 75:25 volume of acetonitrile/methanol which containing 1 M acetic acid and 10 mM sodium acetate. The LOD found to be from 0.1 to 0.7 μg/mL. The SPE was successfully carried out for distilled, river and water samples pre-treatment.

Pérez-Urquiza *et al.* (2000) have developed capillary zone electrophoresis coupled with photodiode-array detection (DAD) to determine several sulfonated azo dyes such as Acid Orange 7, Orange 12, Orange 52, Red 26, Red 27 and Red 88. They used electrophoresis buffer that contains a 1:5 dilution of 10 mM phosphoric acid and tetrabutylammonium hydroxide buffer and 25 nM of triethylamine. The obtained LOD was range from 0.1 to 4.53 μg/mL. The spiked river waters were analyzed after acidification at pH 3.0 and SPE pre-concentration step.

There are many limitations in applying capillary electrophoresis (CE) method for textile dyes determination. There is requirement of matrix to change from the aqueous to non-aqueous medium for determination of basic dyes in real aqueous samples. Noisy signal and distorted peaks will be obtained if the aqueous samples are directly injected to the non-aqueous CE with electrochemical detection (Peláez-Cid *et al.*, 2008).

Besides, precipitation of organic salts results in complicated dyes determination which leads to time consuming sample pre-treatments like liquid-liquid extraction (LLE) and SPE. The LLE is only appropriate for spiked samples such as distilled water but not for more complexes aqueous matrices such as lake and river water (Peláez-Cid *et al.*, 2008). Furthermore, the usage of organic solvent system in non-aqueous CE method instead of aqueous buffer to overcome hydrophobic interaction of dyes with the capillary wall in aqueous system causes difficulty in selecting the appropriate solvents and ratios. The choice of solvents and ratios is very important in order to achieve good resolution and selectivity of the method (Peláez-Cid *et al.*, 2008).

1.5.4 Voltammetric Determination of Textile Azo Dyes

Modern polarography and voltammetric methods are particularly appropriate for azo dyes analysis due to their high sensitivity, applicability over an unusually wide concentration range, high satisfactory results, relatively short time and direct analysis without any sample extraction or pre-treatment procedure and also low running cost (Barek *et al*., 1996; Fogg *et al*., 1998; Radi *et al*., 2013a).

The electrochemical behaviour and quantitative analysis of textile azo dye, Reactive Red 231 has been investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) technique, respectively. Glassy carbon electrode (GCE) was used as the working electrode (WE) and phosphate buffer at pH 3.77 as the supporting electrolyte. The oxidation of hydroxyl-substituted azo dye exhibited two well-defined anodic peaks on the voltammograms when scanned from 0.0 V to +1.0 V. A linear plot between peak height (I_p) of the first anodic peak and the concentrations of the dye were in the range from 2 to 200 µM with the LOD of 0.20 µM. The method was evaluated by determining the dye in river water, synthetic effluent and tap water samples with recoveries from 89 % to 97 % (Radi *et al*., 2013a).

Santos *et al.* (2013) have studied adsorptive cathodic stripping voltammetry (AdCSV) technique using hanging mercury dropping electrode (HMDE) for quantification of two textile azo dyes, Procion Red (PR) and Procion Yellow (PY). In Britton-Robinson buffer (BRB) at pH 8.0, the PR has exhibited two cathodic peaks and PY has only exhibited one cathodic peak, which corresponding to the azo group reduction. The linearity ranges obtained by plotting I_p at -0.422 V and -0.538 V for the PY and PR as a function of concentration were from 30.15 to 633.15 nM and from 62.56 to 982.291 nM, respectively. The method was then applied in synthetic textile dyes samples.

Radi *et al.* (2012) have also studied the electrochemical behaviour of Reactive Black 5 (RB5) dye and its quantitative determination using the GCE in phosphate buffer at pH 4.20. The CV and DPV technique were employed for this study. The dye exhibited one well-defined anodic peak due to amino group oxidation at +0.560 V against silver-silver chloride (Ag/AgCl) by using the DPV technique. The linear plot of I_p and dye concentrations enabled the DPV to determine the dye over a wide concentration range, which was from 6.0 x 10^{-7} to 1.5 x 10^{-6} M. The LOD, LOQ and accuracy of 4.0 x 10^{-7} M, 1.1 x 10^{-6} M and 98.2 % were respectively obtained.

Radi *et al.* (2011) have investigated the electrochemical behaviour and determination of Drimarene Blue X-BLN (DB), also by employing the CV and DPV technique. In CV, oxidation of DB at the GCE in phosphate buffer found to exhibit two pairs of quasi-reversible anodic and cathodic peaks on the voltammograms. Using DPV technique and phosphate buffer at pH 2.85, linear relationship between I_p and concentrations of dye was observed in the range of 2.0 x 10^{-6} to 3.0 x 10^{-5} M and 6.0 x 10^{-6} to 3.0 x 10^{-5} M for the first and second anodic peak, respectively. The LOD for the first and second peak were 8.7 x 10^{-7} M and 5.7 x 10^{-7} M, while the LOQ were 2.9 x 10^{-6} M and 1.9 x 10^{-6} M. The method was successfully tested to the DB in tap water samples.

The textile azo dye, Reactive Red 238 has been studied using the CV and DPV technique for its electrochemical behaviour and quantitative analysis at the GCE by Radi *et al.* (2010). The voltammetric behaviour showed that the dye exhibited one irreversible anodic peak at +0.692 V against Ag/AgCl in phosphate buffer at pH 3.34. A linear I_p with dye concentration from 2.0 x 10^{-6} to 1.0 x 10^{-5} M was obtained. The respective LOD and LOQ were 4.49 x 10^{-7} M and 1.49 x 10^{-6} M with satisfactory recovery when applied in the synthetic wastewater samples.

The CV has been performed in sulphuric acid medium using GCE as the WE for electrochemical behaviour study of Cibacron Navy WB. The potential range used for the dye scanning was from +700 to -450 mV. The electrochemical reaction exhibited three cathodic peaks at +50 mV, -155 mV and -317 mV. Two anodic peaks were shown at +382 mV and +547 mV. These results have indicated quasi-reversible electrochemical nature of the dye (Kariyajjanavar *et al.*, 2010).

Gupta *et al.* (2007) have studied CV of Reactofix Red 3 BFN at the platinum electrode using potassium chloride (KCl) as the supporting electrolyte. The dye exhibited one reduction peak in the forward scan and one oxidation peak in the reverse scan. At different scan rates, a reduction peak was exhibited at +0.75 V and a corresponding oxidation peak at +0.65 V. In this study, the electrode reaction was found to be mainly diffusion-controlled with some adsorption contribution.

Li *et al.* (2007) have studied CV and linear sweep voltammetry (LSV) of Basic Brown G in ammonia-ammonia chloride (NH_3-NH_4Cl) at pH 9.1. One cathodic peak was exhibited at -0.67 V against the Standard Calomel Electrode (SCE). From the

CV results, the electrochemical irreversible reaction was controlled by diffusion. The I_p was linear to the dye concentration over the range of 3.0 x 10^{-8} to 1.0 x 10^{-4} M at the HMDE by using the LSV. The LOD was found to be 9.0 x 10^{-9} M and the recoveries were varied from 95.3 % to 102 %.

Guo *et al.* (2004) have examined Orange II in acetic acid-sodium acetate (HAc-NaAc) buffer at pH 3.62, on the mercury electrode using CV and LSV technique. Using CV, they have found that the dye exhibited one cathodic peak at -0.364 V and no anodic peak, which indicate irreversible electrode reaction. A linear relationship between I_p and concentrations of Orange II was observed in the range of 1.0 x 10^{-8} to 1.0 x 10^{-3} M with LOD of 2.0 x 10^{-9} M by using LSV. Recovery test was performed in artificial samples and it varied from 95 % to 104 % with mean recovery of 99.1 %.

Guaratini *et al.* (2001) have studied Reactive Red 120 (RR120) and Reactive Green 19 (RG19) using CV and cathodic stripping voltammetry (CSV) technique with HMDE as the WE. Britton-Robinson buffer (BRB) was used as the supporting electrolyte. BRB solution at pH 4.0 and 3.0 were the optimum conditions for RR120 and RG19 analysis, respectively. Linear calibration graphs were obtained, which were from 1.5 x 10^{-8} to 1.4 x 10^{-7} M and from 1.2 x 10^{-8} to 2.0 x 10^{-7} M with accumulation time (t_{acc}) of 180 s and 240 s for respective RR120 and RG19. The LOD of 7 x 10^{-10} M was obtained for RR120 and 5 x 10^{-10} M for RG19.

The vinylsulphone based reactive group dyes are generally used to dye silk fabric. Zanoni *et al.* (1999) have determined vinylsulphone azo dye, remazol orange 3R in BRB solution by the CSV technique at HMDE. One cathodic peak was exhibited against Ag/AgCl over the whole pH range (pH 2.0 to 12.0) attributed to reduction of azo group. Under optimum parameter conditions, the I_p was linear to dye concentration from 30 to 300 μg/L in BRB at pH 4.0 and from 6.2 to 62 μg/L at pH 10.0 with t_{acc} of 60 s and 40 s, respectively. The LOD was found to be 1.5 μg/L in BRB at pH 10.0 with t_{acc} of 120 s.

Goyal *et al*. (1998) have investigated electrochemical reduction of Direct Orange 31 at a pyrolytic graphite electrode (PGE) using LSV and CV in the phosphate buffer with pH range from pH 2.0 to 10.3. Two reduction peaks corresponding to the reduction of azo groups and two anodic peaks corresponding to oxidation of the generated products were exhibited on the voltammograms. They have found that the reduction process does not stop at the hydrazo stage but subsequently reduced to give off amino compounds as the final products in acidic medium.

Fogg *et al*. (1998) have used polarography and voltammetry method for determination of triazine-based reactive azo dyes which containing two different leaving groups, 4-carboxypyridyl and 1,4-diazabicyclo[2,2,2]octanyl. One cathodic peak and other peaks at more negative potentials were observed, respectively attributed to the reduction of azo group and reactive groups. Based on the azo group reduction, the calibration curve was found to be linear from 1.0×10^{-7} to 1.0×10^{-5} M with LOD of 0.5×10^{-7} M and 1.0×10^{-8} to 5.0×10^{-7} M with LOD of 5.0×10^{-8} M for differential pulse polarography (DPP) and differential pulse cathodic stripping voltammetry (DPCSV) technique, respectively. Other voltammetric determinations of azo dyes and their hydrolysis products were also studied by Fogg *et al*. (1997), Zanoni *et al*. (1997) and Barek *et al*. (1996).

1.6 Voltammetric Determination of Azo Dyes in Various Applications

A determination of azo dyes in various samples by voltammetric techniques with low LOD has been developed and reported in the literature. For example, the voltammetric determination of azo dyes such as Sudan I in food samples. According to Wu (2010), Sudan I is an organic azo dye, commonly used as a colorant in chemical industries and an additive for those foods containing chili powders due to its strong red-orange colour. The presence of this dye at any level is not safe for human and the International Agency for Research on Cancer (IARC) has classified it as a category 3 carcinogen (Yin *et al*., 2011).

Mo *et al.* (2010) have studied a gemini surfactant – ionic liquid – multiwalled carbon nanotube composite film modified glassy carbon electrode for voltammetric determination of Sudan I in chili powder and ketchup samples. The obtained LOD for this study was 0.03 µM. The determination of Sudan I has also been carried out at the single-walled carbon nanotubes and iron (III) - porphyrin modified glassy carbon by square wave voltammetry (SWV). The obtained LOD was 0.01 µM. Chili powder, chili juice and ketchup samples were used to find out the potential of this method for Sudan I detection (Wu, 2010).

Yang *et al.* (2009) have determined Sudan I at an ordered mesoporous carbon modified GCE. The linear range was from 0.403 to 66 µM and the LOD was down to around 0.00244 µM by using DPV technique. In this study, ketchup was used as the sample. Other examples of azo food dyes were tartrazine (TT) and sunset yellow (SY) which have been studied by DPV technique using pretreated boron-doped diamond (BDD) as the WE. The obtained LOD were 62.7 nM and 13.1 nM for TT and SY, respectively. This method was applied in powdered juice drinks, gelatins and nutrient-enhanced sport drink beverages samples (Medeiros *et al*., 2012). Other applications of voltammetric techniques for azo dyes detection in food samples were reported by Song (2010), Zhang *et al*. (2010) and Ma *et al*. (2013).

In an automobile application, dye is commonly added to fuels in order to act as a visible marker for quality control purpose in order to distinguish liquids with chemical and physical similar properties (Trindade and Zanoni, 2009). In Brazil, the gasoline is a mixture of standard gasoline and concentration of 20 to 26 % of dehydrated ethanol, which produced from sugar cane. However, the gasoline is being adulterated by using ethanol with higher amount of water or by adding a higher amount of ethanol. Since the adulterated fuels may give problems to the car engine, health hazards and cause loss of tax revenues to the government, a dye with orange coloration is prescribed by law to be added to dehydrated ethanol for quality control purpose (Romanini *et al*., 2009). Voltammetric determination of azo dye in fuel samples has been studied by Romanini *et al*. (2009). They have developed SWV technique to determine dye solvent Orange 7 in fuel ethanol using GCE and the obtained LOD was 0.9×10^{-7} M.

Moreover, an application of azo dye in drugs analysis has been reported by Pereira *et al*. (2002). In this study, they have used Reactive Red 120 (RR120) which has two azo groups as chromophore and two monochlorotriazine groups as reactive site for derivatization of a clotrimazole. Clotrimazole, an effectively used antimycotic agent in against dermal infections and vulvovaginal candidiasis was mentioned not suitable for direct reduction due to its ill-defined peak. The DPP analysis of a mixture of RR120 dye and clotrimazole with various concentrations exhibited a new peak that was well separated from all the RR120 dye peaks. Thus, the indirect determination of the clotrimazole can be carried out in the presence of RR120 dye excess.

1.7 Voltammetric Techniques

Voltammetry technique is reported to offer several important advantages such as has possibility to analyze real matrices without preliminary complicated and time consuming separations for individually or simultaneously determination of analytes, has similar selectivity as chromatography, offers faster analysis, low expenses on the instrument and also can be carried out in turbid solutions (Amorello and Orecchio, 2013; Švorc *et al.*, 2013). It offers high sensitivity and low waste emissions (Jian and Li-Ping, 2013).

The application of voltammetric techniques has been reported in literature for various samples such as food samples (Meiju *et al.*, 2007; Gambarra-Neto *et al.*, 2009; Hajian and Ensafi, 2009; Santos *et al.*, 2009; Makhotkina and Kilmartin, 2010; Sia *et al.*, 2010; Tyszczuk *et al.*, 2011), pharmaceutical samples (Ghoneim and Tawfik, 2004; Torriero *et al.*, 2004; Álvarez-Lueje *et al.*, 2008; Starek, 2009; Tyszczuk and Korolczuk, 2010; Gupta *et al.*, 2011a; Jain and Sharma, 2012; Jain and Yadaz, 2012), toxicology and environment samples (Arancibia and Muñoz, 2007; Gibbon-Walsh *et al.*, 2010; Mahesar *et al.*, 2010; Kamat *et al.*, 2011; Li *et al.*, 2011; Koper and Grabarczyk, 2012) and forensic samples (Woolever *et al.*, 1999;

Pournaghi-Azar and Dastangoo, 2000; Woolever and Dewald, 2001; Bowman *et al.*, 2007; Balbino *et al.*, 2012; Salles *et al.*, 2012).

1.7.1 Instrumentation in Voltammetric Measurement

The voltammetric techniques consist of three electrodes system in a voltammetric cell, which are working electrode (WE), auxiliary electrode (AE) and reference electrode (RE). There are also included magnetic stirrer and gas line for purging the supporting electrolyte. The RE is placed close to the WE, while the WE is placed between the RE and AE. A potentiostat monitors voltage over the WE and AE which is automatically adjusted to give correct applied potential (Fifield and Haines, 2000; Christian, 2004). Figure 1.2 shows the typical arrangement for electrochemical cell of voltammetry (Fifield and Haines, 2000).

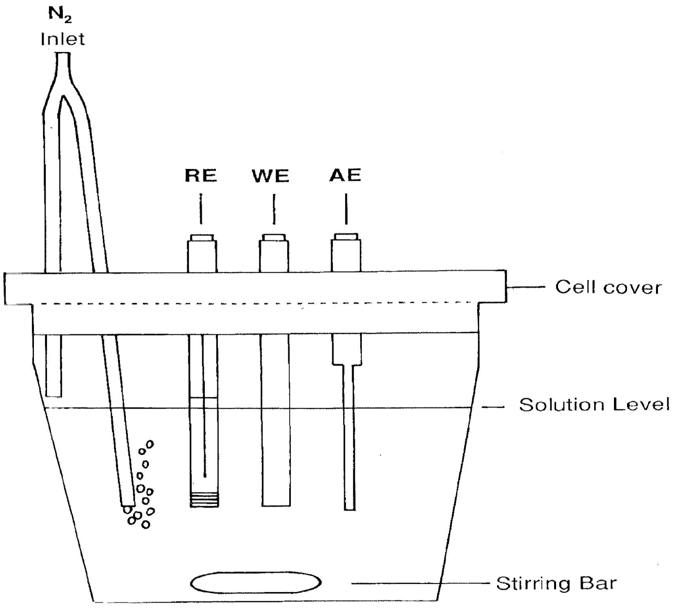

Figure 1.2 Typical arrangements for electrochemical cell of voltammetry (AE: Auxiliary Electrode, WE: Working Electrode, RE: Reference Electrode)

1.7.1.1 Working Electrode (WE)

Working electrode (WE) provides a site for the redox reaction of electroactive species and also for the charge transfers from and to electroactive species to take place (www.cem.msu.edu). It strongly influences the performance of the voltammetric technique. The ideal characteristics of this electrode are has wide potential range, low resistance and be able to provide a high signal-to-noise response. The WE must also has a characteristic of chemical inertness, which means it should be made from the materials that do not react with solvent and any compounds of the solutions over as wide a potential range as possible.

There are two classes of electrode for the WE, which are liquid electrode like mercury and solid electrode such as carbon, gold and platinum. Mercury electrode offers several advantages to act as the WE. Its large activation overpotential for reduction of hydronium ion to form hydrogen enables the reduction of many species in acidic solution (Fifield and Kealey, 2000). In addition, mercury has ability to form amalgam with the most metals and provide uncontaminated surface from previous analysis due to readily formed new drops or new thin mercury films.

The only disadvantage of mercury is itself oxidizing at potential of +0.3 V and cannot be used at positive applied potentials more than +0.4 V against the SCE (Fifield and Haines, 2000; Fifield and Kealey, 2000). Figure 1.3 shows the different types of WE and their potential windows against SCE in various supporting electrolyte (Skoog *et al.*, 2004).

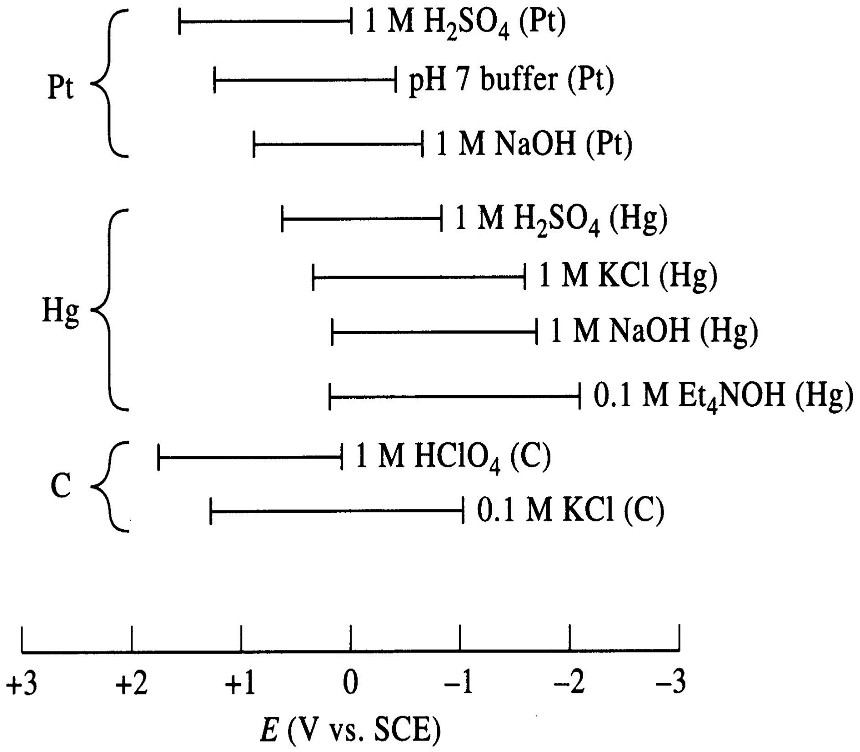

Figure 1.3 Different types of the WE and their potential windows

Dropping mercury electrode (DME), hanging mercury drop electrode (HMDE) and static mercury drop electrode (SMDE) are the three main types of mercury electrode in voltammetry. The DME is used in polarography, an earlier subclass of voltammetric technique. In the DME, each self-renewing mercury drop at the end of a narrow capillary after continues growing due to gravity gives an advantage of uncontaminated surface from the previous analysis by any adsorbed electroactive species. In addition, this type of mercury electrode does not need to be polished or cleaned like solid electrodes such as carbon electrode.

The HMDE is reported as the only electrode type that sufficiently sensitive for metal speciation and drug residues in complex matrices such as natural waters and