



Synthesis, Antimicrobial Assessment and Application of 2, 4-Dinitrophenylhydrazine Based Azodye on Chrome and Vegetable Tanned Leather

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ABSTRACT

An Azodye was synthesized using 4-aminophenol acid as the primary aromatic amine and 2, 4-dinitrophenylhydrazine as the coupling agent and labelled AXD1. The antimicrobial activity of the dye was tested on *Staph aureus* and *Staph wanei*. The result showed that the AXD1 dye has the potential to inhibit and limit the spread of the said microorganisms. The melting point of the synthesized dye was found to be 192 °C, the wavelength of maximum absorption (max) was 372.00nm. From the FTIR analysis, the following functional group were discovered: C-H, C-H 1, 4- disubstitution, C-H, N-H, C-N, C=C-C, -N-N-, carboxylate. The dye was applied on chrome and vegetable tanned leather which gave well to very good rub and light fastness on both the chrome and vegetable tanned leather. From the results obtained, it can be concluded that the Synthesized Azo dye can be used in the dyeing of both chrome and vegetable tanned leather.

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INTRODUCTION

Azo dyes represent the largest production volume of dye chemistry, today and their relative importance may even increase in the future. They play a crucial role in the governance of the dye and printing market. These dyes are synthesized from a simple method of a diazotization and coupling. Different routes and modification are made to obtain the desired colour properties, yield and particle. Size of the dye for improved dispersibility (Benkhaya 2018). Azo dyes are the most used dyes and account for more than 60% total dyes (2, 3). Approximately 70% of all the dyes used in industry are azo dyes (4, 5). These compounds are characterized by the functional group (-N=N-) uniting two symmetrical and/or asymmetrical, identical or non azo allelyl or aryl radicals. Azo dyes are the most important synthetic colorant which have been widely used in textile, printing, paper manufacturing etc. as well as their harmful effects of azo dyes on humans and aquatic life, have aroused urgent calls for the treatment of effluents containing azo dyes to eliminate them or convert them into useful and safe products. In addition, the distribution of dyes

in water increased with the increase in the molecular weight of the azo dyes, probably caused by the increase in the molecular weight of the azo dyes in the form of increase azo dyes bonds, resulting in decrease in the rate degradation of azo dyes (Heligo 2020). Most azo dyes are synthesized by characterization of an aromatic primary amine following by coupling with one or more electron rich nucleophiles such as amino and hydroxyl (Dickey 2019).

METHODOLOGY

Diazotization reaction

About 0.5g (2.8mmol) of 4-Aminophenol was placed and 5ml of water was added to a clear bath to obtain a clear solution. 0.2g of sodium nitrate solution was prepared in 1ml of distilled water and added to 4-Aminophenol solution. The resulting solution was then added to a test tube containing 0.5ml of concentrated hydrochloric acid (HCl) and placed on an ice bath until a significant amount of solid was precipitated.

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Coupling reaction

In a 25ml round bottom flask, 0.4g (2.6mmol) of was added and 2ml of 2.5mol of sodium-hydroxide was added and stirred. The mixture containing diazonium salt was added to the round bottom flask and was heated until boiling point is reached. 1g of sodium chloride was added and heated continuously until mixture dissolve. The whole mixture was stirred continuous and was cooled to room temperature. After which the round bottom flask was placed on an ice bath for 15 minutes. The solid precipitate form was filtered and washed with sodium-chloride solution. The solid was kept to air dry and then was weighed. The solid azo dye was labelled ADX1

Preparation of Mcphana Solution

0.2g of the ADX1 dye was weighed and was delivered into 2ml of DMSO for 100ml of solution, after which a serial dilution was made to get 100ml, 50ml, 25ml, and 12.5ml, respectively.

Determination of Zone of Inhibition

Muller Hinton agar was prepared and two petri (one for each microorganism) dish was half filled with the agar and was left to solidify. 0.1ml of microorganisms (*staph waneri* and *staph aureus*) was inoculated into the agar plate, four strain was then made and was labeled according to concentration made (100ml, 50ml, 25ml, 12.5ml) on both agar plate. Each hole made from the strain was fill with the concentration starting from the lowest to highest concentration (12.5ml, 25ml, 50ml, and 100ml respectively). After which it was inoculated for 18-24 hours at 36°C. After which it was inoculated for 18-24 hours at 36°C. After incubating, result was checked if there is any zone of inhibition.

Determination of Minimum Inhibitory Concentration

For each microorganism, four (4) test tubes was collected with an extra one as blank, sterilize in the autoclave. After sterilization, each test tube was labeled according to concentration (100ml, 50ml, 25ml, and 12.5ml respectively) for both microorganism. To each test tube, 3ml of Muller Hintonbroth was put into each test tube. 0.1ml of microorganism (*staphylococcus waneri*)

into first four test, 0.1ml of the microorganism (*staphylococcus aureus*) into the remaining test tubes. 0.2ml of dye was put into each test tube from the lowest to highest concentration (12.5ml, 25ml, 50ml, 100ml respectively) for the two microorganism. After each is done it was put into the incubator and incubate for 18-24 hours at room temperature. After the incubation period is reach, the result was checked (i.e. if it is turbid or clear).

Determination of Minimum Bacteriocidal Concentration

The same steps of determination of minimum inhibitory concentration, after the result is gotten; culture all clear tubes in minimum inhibitory concentration and the dilution was started from the first at which no growth was observed, the preparation was allowed to solidify. After being well solidify the plate was further divided into two, after which it is well solidified, the agar plate will then be divided into two with the help of a marker or maxing tape, one part was labeled *staphylococcus aurus* and the other part *staphylococcus waneri*. Each section was labeled according to concentration (200ml, 100ml, 50ml and 25ml) respectively. From minimum inhibitory concentration, the agar plate was streaked with the use a wire loop of according to concentration made from the determination of minimum inhibitory concentration. Starting from the lowest to highest (12.5ml, 25ml, 50ml and 100ml) respectively. For both microorganisms. It was then inoculated for about 18-24hrs at 36°C.

Preparation of Dye Bath Liquor

About 1% solution of the dye was prepared with a liquor ratio of 50:1. Also, 0.5g of the fiber was used. The volume required from each solution was calculated using the formula below;

$$V = \frac{P \times W}{C}$$

Where: P = percent shade

W = weight of fibre

C = percentage concentration of stock

Dyeing of Substrates

The substrates were netted and thoroughly squeezed to remove excess water. It

was immersed into a dye bath at 40°C and was carried out for about an hour at a temperature of about 100°C with constant agitation. At the end of the dyeing process, the dyed substrate was removed and squeezed and rinsed thoroughly under running tap water and allowed to dry at room temperature.

Fastness Properties

The dyed sample was subjected to wash fastness test by the following procedures. The specimens was prepared by cutting the dye substrate 7cm by 2cm into dimensions. It was made into composites by stitching the test specimen made of the dyed sample place between white cotton or dimension of 8cm by 4cm. the composite was agitated in the solution made up of the following;

- Soap solution 5g/l
- Sodium carbonate 2g/l

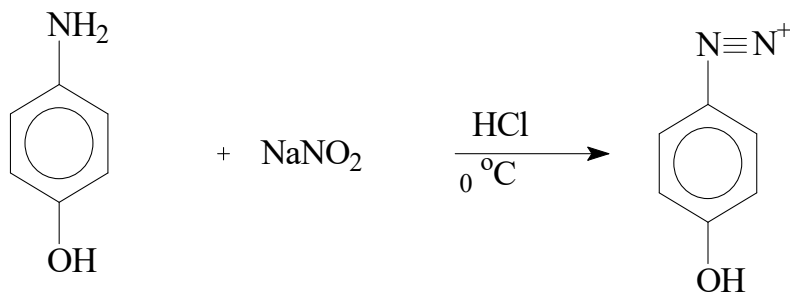
Liquor ration 50:1

The washing was maintained at a temperature of 50°C for 45minutes with continuous agitation. At the end of the washing test, the composite specimen was removed, rinsed in a running tap water and the adjacent fabric was accessed using the appropriate grey scale.

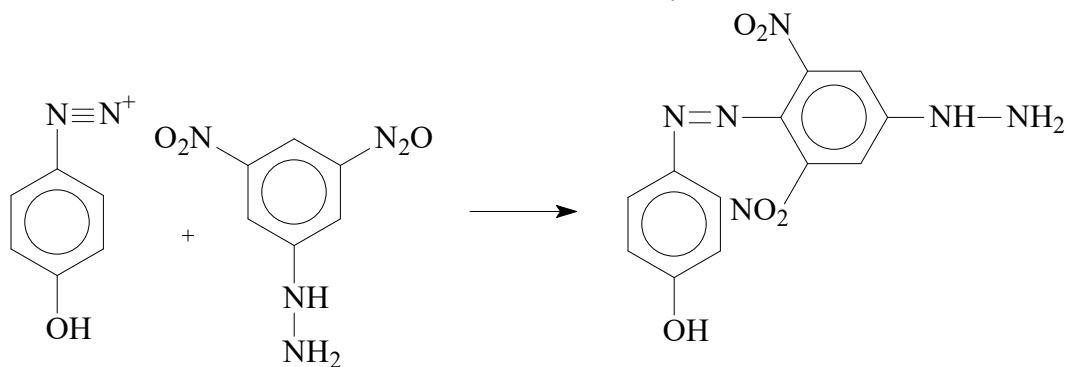
Light Fastness Test

The dyed sample was exposed facing due south and inclined at an angle of the horizontal approximately equal to latitude of the place where the exposure is being made. Adequate ventilation of the sample during exposure to UV radiation for 7 days. The exposed sample was examined at interval and the change in the colour of the sample was compared visually with change that occurs in the standard.

RESULTS AND DISCUSSION



Scheme 1: Diazotization of 4-Aminophenol



Scheme 2: synthesis of ADX1 dye by coupling reaction with 2,4-dinitrophenylhydrazine



Table 1: Determination of Zone of Inhibition (mm)

ADX1 Conc.	200mg	100mg	50mg	25mg
<i>Staph aureus</i>	25.00	23.00	22.00	20.00
<i>Staph waneri</i>	26.00	19.00	15.00	12.00

Table 2: Determination of Minimum Inhibitory Concentration

ADX1 Conc.	200mg	100mg	50mg	25mg
<i>Staph aureus</i>	++	++	+	+
<i>Staph waneri</i>	++	++	+	+

Table 3: Determination of Minimum Bacteriocidal Concentration

ADX1 Conc.	200mg	100mg	50mg	25mg
<i>Staph aureus</i>	-	-	-	-
<i>Staph waneri</i>	-	-	-	-

Table 4: Physical properties of the synthesized dye.

Sample	Melting point (°C)	Percentage yield %	Colour
ADX1 dye	175	70	Light brown

Table 5: Fourier transforms infrared spectroscopy (FT-IR) of synthesized dye

Sample	Functional group	Frequency Range (cm ⁻¹)	Experimental frequency (cm ⁻¹)
ADX1 dye	C-H disubstitution (para)	860 – 800	820.4
	Aromatic C-H stretch	1225 – 950	976.6
	C = C-Aromatic stretch	1510 – 1450	1509.6
	Secondary amine CN stretch	1190 – 1130	1133.1
	Open chain 970 (- N = N -)	1630 – 1575	1587.8
	Phenol OH bend	1410 – 1310	1371.1

Table 6: Fastness properties of the dye

Dyed sample	Light fastness	Rub fastness	Colour on substrate
Chrome tanned leather	3	3/4	Light brown
Vegetable tanned leather	4	3	Light brown

Gray scale for fastness properties

5 = Excellent, 4 = Very Good, 3 = Good, 2 = Fair and 1 = Poor

The result from tables' 1-3 show that ADX1 dye has high antimicrobial activity against the test organisms. The zones of inhibition showed that the dye is able to inhibit the growth and spread of *Staph. aureus* and *Staph. waneri* the highest activity being at a dye concentration of 200 mg/l. The melting point of the dye was determined by using electro thermal melting point apparatus in which the dye has the melting point of 192 °C which indicates the purity of the synthesized compound. UV – visible spectra indicated its highest wavelength of maximum

absorbance as 353.00nm. An orange colour dye was observed.

Fourier transform infrared spectroscopy (FTIR) table of the synthesized dye, the FTIR result was observed as follows: C – H, C – H monosubstitution, C – H disubstitution, C = C, C = C – C stretch, C – H1, 2 – disubstitution, NH bend, NH stretch, NH₂, CN stretch, SO₂, - N = N -. The fastness properties of the dyed leather ranges from good to very good, from 4 – 5 the increase in the fastness properties of the dyed leather can be attributed in the chromophore group. It can also be attributed to the hydrophilic nature of the collagen



which enhances the penetration of the dye. Table above indicate good to very good light fastness in attempting to trace the relationship between chemical structure and fastness. It was observed by Finar, (2015), that there is no absolute value for the light fastness of a dye. The rating obtained for a given colorant for any fading test are; conc. and degree of aggregation of dye within the fabric nature in which the dye is dispersed; molecular structure of the dye substantivity, nature of the fabric in which the dye is dispersed. However, the light fastness of dyes on chrome and vegetable tanned leather ranges from 3-4 which could be ascribed to coupling component used. The dye solubility was tested and was observed that the dye is soluble in water and ethanol.

CONCLUSION

Azo dye was synthesized using 4-aminophenol and 2, 4-dinitrophenylthiazine as the coupling component. The antimicrobial activity of the dye showed that it is a viable antimicrobial agent most especially at a relatively high concentration. FTIR spectroscopy of the dye showed the presence of functional groups that aligned with the structural properties of the dye. The dye gave a good shade on both leather (vegetable and chrome tanned leather) hence both dyes exhibited good fastness property on the substrate. From the properties exhibited by the dye on chrome and vegetable tanned leather, it showed that the dye is excellent in the dyeing of leather.

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