Structure- Activity Relationship (Sar) Of Cyanoethylated Aromatic Amines

Odin, E.M., Onoja, P.K., Ochala, A.U.

Abstract: *Two types of aromatic amines were cyanoethylated. The cyano ethylated products were reacted with propylene oxide to give azocomponents: N-acetyl – N – β – hydroxyl propyl, N- β-propylnitrile -1, 3 – phenyl diamine and N- β hydroxypropyl, N- β- propyl nitrile aniline. These products are referred to as Azodin A and Azodin B respectively. When diazotized aromatic amines were coupled to Azodin B, eight dyes were produced, while Azodin A gave two dyes. Various elemental and spectroscopic methods were employed to elucidate the structure and properties of dyes. The UV-visible spectral data revealed that substitution(s) at the meta-position on the benzene rings of the azodyes favoured bathochromic shift more than those at ortho and para positions (dyes 5,9 and10 vs. others).The dyes were used to colour polyester materials and the exhaustion properties were measured. The result revealed that the dyes have good exhaustion and leveling properties and that the cyano ethylated products have no NH_2 group. Structure-activity relationship among the dyes were measured against some pathogens.The ability of these dyes to inhibit the growth of some micro organisms was correlated with anti-bacterial potential.*

Keywords: Aromatic amines; cyanoethylation, Azodin A and B; exhaustion; anti-bacterial; structure-activity relationship.

INTRODUCTION

N-acetyl-N- β propylnitrile 1,3 – phenyl diamine and propylnitrile aniline are products of cyano ethylation of aromatic amines. They serve as intermediates in the synthesis of disperse dyes [1]. The principal uses are the dyeing of cellulose acetate, nylon, polyester and polyacrylo-nitrile fibres. Disperse dyes are also used for dyeing wooled sheep skins and for the surface dyeing of plastics [1], [5]. Aromatic amines do not react readily with acrylonitrile in cyano ethylation process. A catalyst is employed to facilitate the reaction. The reaction can be carried out with glacial acetic acid as the catalyst or with various medium salts including zinc acetate or cuprous chloride in glacial acetic acid [10]. Sterically hindered aromatic amines must be reacted under pressure at elevated temperature [21]. Cyano ethylated products react with propylene oxide to give azocomponents, which when coupled to various diazocomponents give disperse dyes. Our previous paper [12] reported the synthesis of Disperse dyes from various diazo components and azo components using aromatic amines and propylene oxide. This present paper describes the dispersion of the dyes, the exhaustion and leveling properties and structure-activity relationship among the dyes against some pathogens.

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MATERIALS AND METHODS

Reagents and Solvents:

The N-acetyl-1,3-phenyl diamine was supplied by Prof. A. Draganov, Higher Institute of Chemical Technology, Sofia. The other reagents and solvents including the acrylonitrile were obtained from Pharmatech, Sofia and were used without further purification.

Cyanoethylation of N-acetyl-1,3-Phenyldiamine:

A mixture of N-acetyl-1,3-phenyldiamine (225 g. 15 mol), acrylonitrile (110 g, 2 mol), water (88 ml) and zinc chloride (20 g) was heated in a reaction vessel under an atmosphere of inert nitrogen for 28 hours. The temperature was gradually raised from 30 ^{0}C – 94 ^{0}C. The product, N-acetyl-N-β-propylnitrile – 1,3 phenyl diamine was recrystalised in ethanol. (Yield was 162 g, 68%).

Preparation of N-β-hydroxyl propyl N-β-propylnitrile-1,3-phenyl diamine (Azodine A):

The N-acetyl-N- β-propylnitrile-1, 3-phenyl diamine (101.5 g, 0.05 mol), propylene oxide (32 ml. 0.55 mol) were placed in an autoclave. The reaction mixture was heated in a paraffin bath at 100 ^{0}C for 3 hours. The product (Azodin A) was isolated by vacuum distillation (yield. 120 g).

Cyanoethylation of Aniline:

The process was similar to the description above. One mole of acrylonitrile was preferably employed with one mole of mono amine, while one mole of acrylonitrile was required for each gram mole of amino group in a diamine. The aniline (140 ml, 1.5 mol), water, zinc chloride were heated in a reaction vessel as described above (yield = 99.5 g. 51%).

Preparation of N- β – hydroxy Propyl-N-β-propylnitrile amine (Azodin B):

The procedure was similar to that of Azodin A. The propylnitrile aniline (73 g, 0.05 mol) and propylene oxide (29 ml, 0.05 mol) were placed in an autoclave and heated in a paraffin bath. The product was isolated using vacuum distillation (Yield = 90 g).

Synthesis of Disperse Dyes:
Disperse dyestuffs were obtained when Azodin A and B were coupled to diazonium salts of substituted amines. The following diazo components were employed: 4-chloro-2-nitro aniline, 2.6-dichloro-4-nitrol-anilne, 3-nitro-4-toluidine, 3-amino-2,2,4-triazol, 5-nitro-4-methyl-1,3-thiazole, N-acetyl p-phenyldiamine, 2,4 dinitro aniline. These components are sequentially identified with structure in Fig. 1. The process of diazotization and coupling methods have been reported [12], [14], [15].

Dyeing with Disperse Dyes:
The Methods of [5], [6], [15] were adopted and modified. In a beaker of 250 ml was dissolved 0.2 g of the dye in 100 ml water at room temperature. To the solution was added 1 g of textile material (polyester) and was stirred continuously for 15 minutes until it was completely soaked. The temperature was gradually raised to 250 ^{0}C and 0.2 g NaCl was added and stirred. To the mixture was added in two parts at interval of 15 minutes Na_2CO_3 which was dissolved in 2 ml water. The mixture was stirred intermittently while the temperature was gradually raised to 40 ^{o}C and maintained for 10 – 15 minutes. The dyed fabric was washed with ordinary water and rewashed for another 10 minutes in 50 ml, 0.5% soap solution. It was then rinsed with water and dried.

% Exhaustion:
The amount of dye put in the dye bath was weighed. After dyeing, the solution was evaporated to dryness. The amount of dye left was also weighed. The percentage exhaustion of the dyes on the fabric was calculated according to [8], [9], [15].

$$\% E = (A_d – A_b) \times 100$$

Where A_d and A_b are the quantity of the dye originally in the dye bath and of residual dye in the dye bath respectively. The results are shown in Table 1.

Fastness Properties of the Dyed Fabrics:
The finished article (dyed fabrics) were exposed to a number of conditions such as sunlight, washing with detergent and dry cleaning (heat). All the fastness evaluations were made with the aid of the Gray Scale for colour change [3], [4]. The wash fastness text was done by preparing a soap solution and placing the dyed fabrics into it. This was stirred continuously for 10 minutes at a temperature of 35 ^{0}C. At the end, the fabric was removed, rinsed with clean water and dried in free air for 24 hours. The dried fabric was compared with control sample. The light fastness assessment was carried out by exposing the dyed fabric to strong sunlight for a period of 3 hours. The exposed sample was compared with control. The heat fastness evaluation was done by heat pressing the dyed fabric with a pressing iron at a temperature of 50 – 60^{0s}C. for 3 minutes. The pressed fabric was compared with the control sample. The results are shown in Table 2

Antimicrobial Activity Test:
The antimicrobial screening method employed was the Agar-Dilution streak methods of [2], [11], [16], [19]. The antimicrobial activity was measured by determining the concentration of agent needed to inhibit the growth of test microorganisms. Clinical isolates, including *Pseudomonas species, Escherichia species, Citrobacter species, Salmonella species, Shigella species, M. Spegmatis species, Klebsiella species and S. aureus,* were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD). The Agar and Dye sample were poured into sterile dishes, allowed to set and inoculated with the organism by streaking and incubated at 37 ^{0}C for 24 hrs in duplicate. Control plates which contained no Dye sample to ensure viability and for comparison were similarly inoculated and incubated. After incubation, susceptibility and inhibition were measured by absence of growth. The result of the structure-activity relationship among the compounds are shown in table 3.

Thin Layer Chromatography:
Dyes 1,2,3,5,6,7 and 8 were run in a system of benzene: chloroform: acetone (5:2:0.5), while 4, 9 and 10 were in tetra chloromethane: methanol: water (2:2:1).The dyes were dissolved in a volatile solvent and applied unto the pretreated silica gel aluminum plate with a micropipette. The plates were developed with a solvent combination as described above. The separated compounds were located using visible light. The experiment was performed in duplicates. The rate of flow (R_f) was calculated. The results are indicated in table 1.

Spectra Data and other Analysis:
The melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The melting point was determined using the same procedure as previously reported [14]. The results are shown in Table 1. U.V. spectra were recorded on a Pye-Unican S.P 800 spectrophotometer using marched 1 cm^3 quartz cells. The absorption maxima are in nanometer. The results are also shown in Table 1. IR spectra were measured on a Pye-Unicam SP-1000 infrared spectrophotometer on a KBr disk. The ^{1}H NMR spectrum was recorded on a Varian Germini 2000 spectrophotometer operating 200 MHz Chemical shifts were recorded as δ values in ppm referenced to solvent.

RESULTS AND DISCUSSION

Azocomponent
The cyano ethylation of N-acetyl-1,3-phenyl diamine and Aniline and their subsequent reactions with propylene oxide gave two azocomponents. Azodin A and Azodin B. The structures and equations of reactions are shown in scheme 1. The two azo components were characterized by elemental analysis to confirm the cyanoethylation process. In this case some quantity of the azo salt solution with alkaline solution of β-naphtol did not form coloured substances. Indicating the absence of NH_2 groups in the cyano ethylated products. Also, the two azo components obtained gave analysis for C, O, N, and H which is in

agreement with calculated values. The infra-red (IR) spectra analysis of Azodin A revealed the presence of –NH, -CN, - CONH, - OH groups, benzene ring and absence of NH_2 group. This was supported by absence of the stretching vibration bands at 3451 and 3300 cm^{-1} (broad NH_2). IR: 1682 (CO),3421 (OH), 3265 (NH), 1593 (CN), 3016 (CH aromatic) and 2920, 2865 cm^{-1} (CH aliphatic). The IR spectra of Azodin B also revealed the presence of –CH, -OH groups, benzene ring and absence of NH_2, group. IR: 1598 (CN), 3425 (OH), 3020 (CH aromatic) and 2922-2862cm^{-1} (CH aliphatic). The ^{1}H-NMR spectral analysis was performed for the azo compounds (Azodin A and Azodin B). In order to provide further information for the spectral characteristics of the compounds. The ^{1}H-NMR spectrum of Azodin A showed a signal at δ 13.63 attributed to –OH proton. The spectrum at δ 10.69 as a multiplet is attributed to the protons of the -C(O)-CH_3. The multiplet for –NH protons appeared in the region δ 6.69 – 8.46. The –CH_3 protons in the compound showed a singlet in the region δ 2.18 – 2.27 indicating a successful reaction with propelene oxide. In the ^{1}H-NMR spectrum of Azodin B, The signal at δ 13.54 is attributed to –OH proton. The –CH_3 protons of this compound appeared as a singlet in the region δ 2.19 – 2.29. However, the ketone protons disappeared in the Azodin B spectrum.

DYE

The dyes obtained by coupling Azodin A or Azodin B to diaotized diazo components are listed in Fig. 1**.** From the results shown in Table 1, the dyes obtained from Azodin B (dye 1,2,3,4,5,7 and 8) absorbed in the range 390 nm to 431 nm. The only exception was dye No. 6 which absorbed at 560nm. This powerful bathochromic shift is probably due to the high molecular weight of the dye and the presence of two heteroatoms. The presence of such heteroatoms also may have brought about a change in intensity. This dye gave the highest absorbance recorded in the study, and the polyester material was dyed purple. Hypsochromic shift was noticed with dyes 3 and 7. They absorbed at 390nm. Dyes obtained from Azodin A (dyes 9 and 10) appeared to be more bathochromic. This was probably due to the presence of two auxochromes whose function were to intensify colour and to improve the affinity of the dye for the substrate. They gave indigo and yellowish indigo colours respectively on the polyester material. Generally, substitution(s) at the meta-position on the benzene rings of the azodyes favoured batho chromic shift more than those at ortho and para positions (dyes 5,9 and 10 vs. others).

Dyeing and Exhaustion

From the dyeing results in Table 1, exhaustion varied between 98.5% and 70%. The highest exhaustion properties were from dyes 6, 9 and 10. All the dyes gave very good exhaustion and fibre penetration. The dyeing obtained also showed very good levelness. With all the dyes examined, the absorption wavelength (λmax) and % exhaustion have directly proportional relationship but inverse to melting point (Table 1).

Thin Layer Chromatography:

Thin layer chromatography was also used to characterize the dyes. The result as presented in table 1 supports the fact that relatively minor differences in molecular structure between one absorbing substance and another may bring about a shift in absorption wavelength, λmax. As one or more unsaturated linkages occur in the molecule, so λmax is displaced towards longer wavelength. This shows that structural factors determine whether or not a molecule will absorb in the visible. They also decide where such absorption will occur [1], [16]. The high melting point of the synthesized compounds (9, 10, 4,6 and others) is an indication of stability of the compounds.

Antimicrobial Activity:

The structure activity relationship among the synthesized azo dyes were measured against eight pathogens [5], [16]. The activity of the compounds against bacterial strains are provided in table 3. Their activity varies depending on structure. From table 3, all the compounds were active on the eight micro organisms. Compound 10 showed the greatest activity of 0.82 µg/ml against *klebsiella species, 0,91 µg/ml to 0.96 µg/ml* against *shigella species* and *Escherichia coli* respectively. The compound also exhibited an inhibition of 1.54 µg/ml against *S. aureus* and 2.17 µg/ml against *Salmonella species.* Table 3 also revealed compound 10 to be a more active drug on *Klebsiella species, shigella species, Escharichia coli, S. aureus and salmonella species* than the standard antibiotic, Streptomycin SO_4. Generally, all the compounds showed activity between 0.82 µg/ml against *Klebsiella species* and 5.4 µg/ml against *M. smegmatis.* Compound 9,6 and 4 also showed remarkable activity on all the micro organisms. Examination of all the compounds revealed some important structural requirements for the antibacterial activity. The presence of acetyl, hydroxyl and nitro groups probably account for the higher activity of compounds 9 and 10,while the activity of compounds 4 and 6 may be attributed to the presence of hetero atoms in the dye molecules. The similarity on the activity of the ten compounds on all the micro organisms could be attributed to the presence of OH group on the azo component of the dyes, while the presence of acetyl and hydroxyl groups on compounds 9 and 10 might be responsible for their higher activity. The presence of acetyl group in compounds 9 and 10 enhanced their activity above others. This tends to suggest that the mode of antibacterial actions may be aided by the presence of acetyl and hydroxyl groups on these dyes, and to some extent the presence of hetero atoms on the diazo components (compounds 4 & 6). The compounds possessing these groups displayed appreciable antibacterial activity, while those which completely lack these groups exhibited decrease activity. This suggests that the substitution pattern is a strong contributory factor to the degree of activity of the compounds. The OH group increased the lipophilic character of the compounds (9 & 10). This group increased the ability of the compounds to permeate the cell-wall of the micro organisms. The acetyl group enhanced this permeation. Instead of the compounds being trapped in the bacterial that have high cell-wall lipid content, they are able to permeate the organism [18]. The exact role of the hetero atoms is not yet understood but its importance to antimicrobial activity of some pyridazines has been reported [16].

The Mode of Action

The mechanisms of action [7] of the new Disperse azo-dyes consist of the following categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function and inhibition of protein synthesis. In gram-positive bacteria, the cell wall consist largely of a thick layer of peptidoglycan, which gives the cell rigidity and maintain a high internal osmotic pressure. In gram-negative bacteria, this layer is thinner and the internal osmotic pressure is correspondingly lower. The action of the compounds is to block peptidoglycan synthesis, which severely weakens the cell wall. The compounds also interfere with protein synthesis by inhibiting the binding of aminoacyl tRNA to the recognition site and prevent peptide bonds from forming.

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[22].

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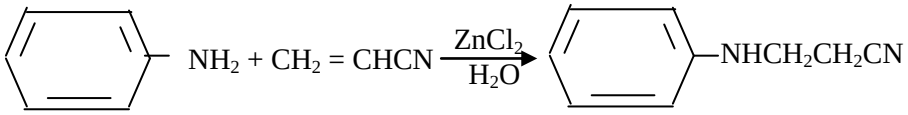

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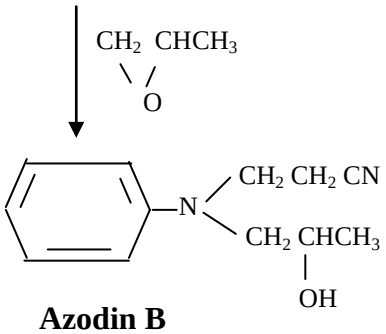

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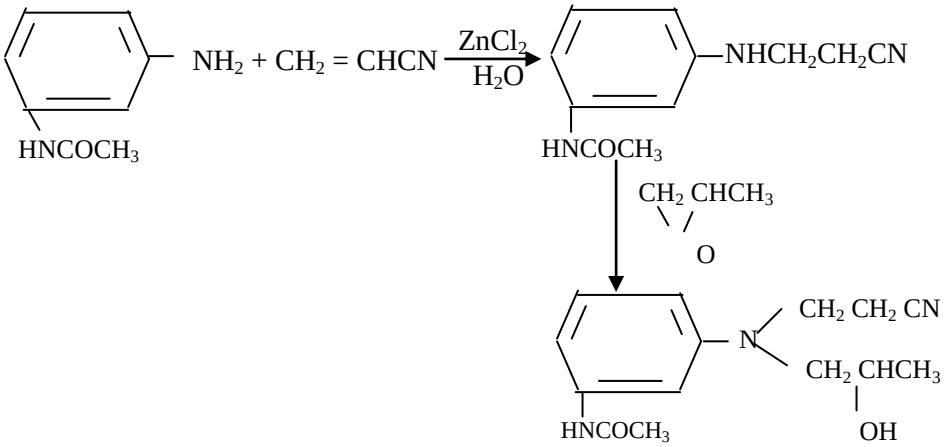
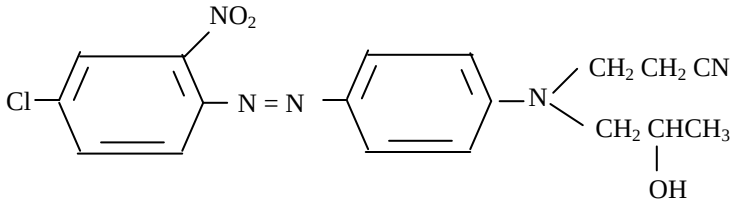


Azodin A

SCHEME 1: Preparation and Structure of Azocomponents

1.



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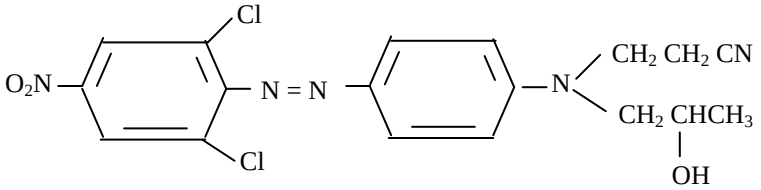

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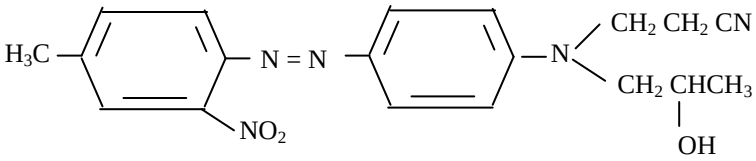

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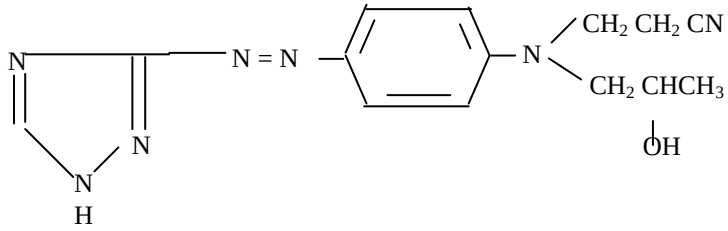


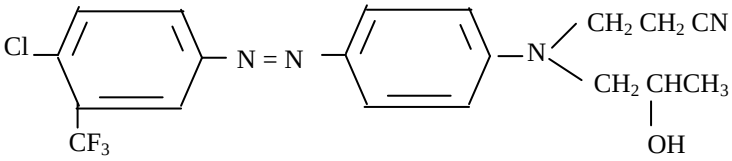
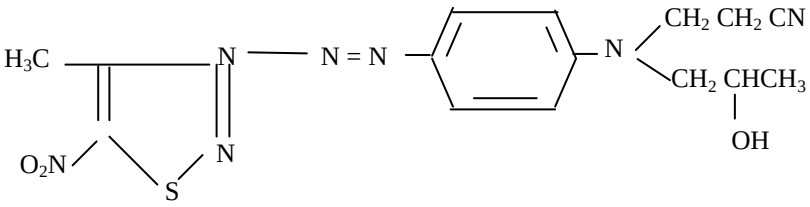


Fig. 1 Structures of the Dyes

6.



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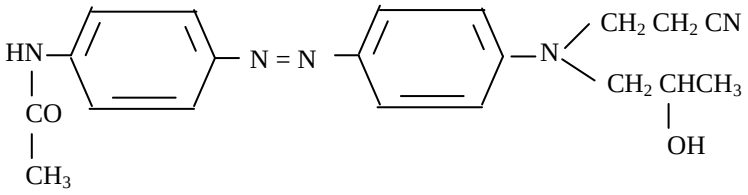

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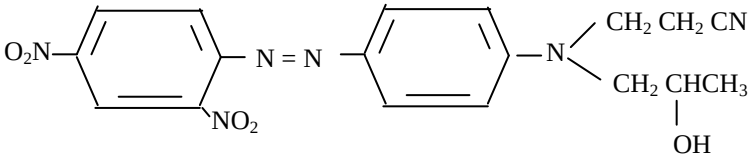

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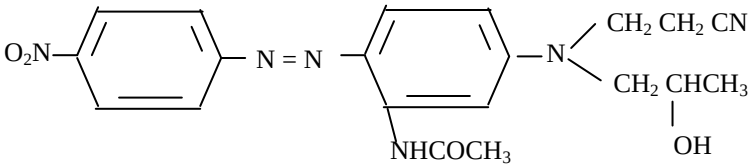


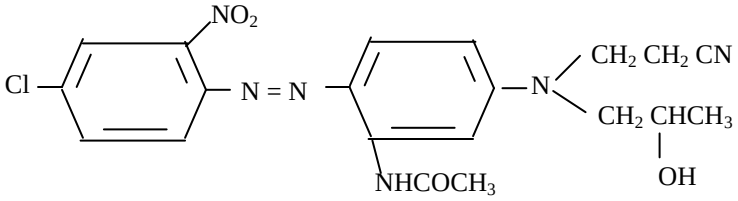


Fig. 1 cont.

Table 1: Some Physical Characteristics of Synthesized Dyes:

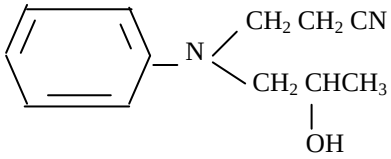
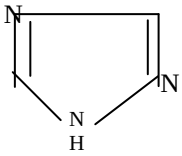
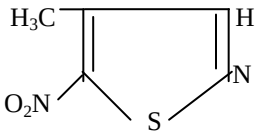
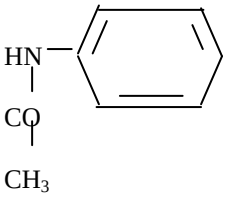
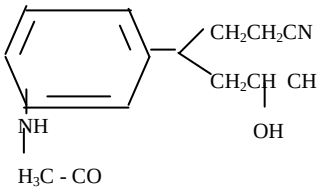
Dye No.	λmax (nm)	%Exhaustion	Melting point (^{0}C)	R_f (cm)
1.	430	86	125 - 128	0.6, 0.8
2.	420	85	165 - 168	0.18, 0.58
3.	390	70	132 – 134	0.18, 0.57
4.	410	82	160 – 162	
5.	431	88	163 – 165	0.19, 0.63
6.	560	98.5	160 – 162	0.02, 0.65
7.	390	78	158 – 160	0.29, 0.55
8.	410	83	155 – 156	0.47, 0.81
9.	520	97	170	0.83, 0.86
10.	510	96	185	0.74, 0.78

Table 2. Gray Scale Assessment

Dye	**Wash Fastness**	**Light fastness**	**Heat fastness**
Dye 1	4	4	5
Dye 2	4	5	5
Dye 3	3	4	5
Dye 4	5	5	5
Dye 5	4	4	4
Dye 6	5	5	5
Dye 7	3	4	5
Dye 8	4	4	4
Dye 9	5	5	5
Dye 10	5	5	5

The Scale was rated 1-5
Scale 1 = poor result; Scale 5 = very good result [17], [20].

Table 3: Structure and Antimicrobial Activity (MIC) of Disperse dyes against some pathogens.

Disperse dyes	Substituent Diazo Compound	Azo Compound	Pathogens (MIC, mg/ml) K.s	P.s	Sh.s	Ec	S.s	C.s	S. a	M.s
1.	**NO_2, Cl**		**2.58**	**4.16**	**5.34**	**4. 38**	**4.06**	**3.58**	**5.40**	**5.24**
2.	**NO_2, di-Cl**	"	**2.47**	**4.09**	**5.09**	**4.29**	**3.98**	**3.53**	**5.28**	**5.19**
3.	**NO_2, CH_3**	"	**2.69**	**4.34**	**5.20**	**4.68**	**4.20**	**3.62**	**5.15**	**5.26**
4.		"	**1.90**	**3.87**	**2.69**	**3.16**	**3.52**	**3.37**	**4.13**	**4.97**
5.	**CF_3, Cl**	"	**2.52**	**4.11**	**4.98**	**4.51**	**3.49**	**3.61**	**5.38**	**5.38**
6.		"	**1.86**	**3.89**	**2.58**	**2.94**	**3.47**	**2.99**	**3.40**	**4.55**
7.		"	**2.63**	**4.38**	**5.29**	**5.11**	**4.42**	**3.69**	**5.13**	**5.42**
8.	**NO_2, NO_2**	"	**2.48**	**4.24**	**4.90**	**4.20**	**3.94**	**3.44**	**3.60**	**5.16**
9.	NO_2		1.74	3.28	1.18	1.84	3.36	2.79	3.23	4.22
10.	**Cl, NO_2** "		**0.82**	**3.17**	**0.91**	**0.96**	**2.17**	**1.54**	**3.19**	**3.94**
Streptomycin	**SO_4**	"	**3.56**	**3.52**	**4.11**	**5.91**	**4.49**	**5.38**	**2.72**	

3.44K.s:
Klebsiella species; P.s: Pseudomonas species; Sh.s: Shigella species; Ec: coli; S.s Salmonela species; C.s: Citrobacter species; S.a: S. aureus: sM.S: M.smegmatis