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Synthesis characterization and antibacterial activity of Cr (III), Co (III), Fe (II), Cu (II), Ni (III) complexes of 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid based formazan dyes and their applications on leather

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Synthesis Characterization and Antibacterial Activity of Cr (III), Co (III), Fe (II), Cu (II), Ni (III) Complexes of 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid based Formazan Dyes and their Applications on Leather

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Graphical Abstract:



Keywords: Aromatic amine; hydrazine; formazan complexes; fastness properties; leather application; antibacterial activity

1	Synthesis Characterization and Antibacterial Activity of Cr (III), Co (III), Fe (II), Cu (II),
2	Ni (III) Complexes of 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene)
3	hydrazinyl) benzene sulfonic acid based Formazan Dyes and their Applications on Leather
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10	Abstract: A novel series of un-metallized and metal complexes formazan dye (12-17) have been
11	synthesized in an aqueous system, without using Buffers, with cost-effectiveness and improved
12	fastness properties. The synthetict methodology involved the synthesis of 4-(2-(((2-hydroxy-5-
13	nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid followed by
14	diazotization of 4-nitroamisnophenol which further coupled with coupler reagent i.e. 4-[(2Z)-2-
15	benzylidenehydrazinyl] benzene sulfonic acid. Multichromic metal complexes of 4-(2-(((2-
16	hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid (1:1
17	and 2:1) were synthesized with the salts of Chromium, Iron, Cobalt, Copper and Nickel. Newly
18	synthesized metal complexes were characterized by Ultraviolet-visible (UV-Vis), Fourier
19	transforms infrared (FT-IR), Proton nuclear magnetic resonance (¹ H-NMR), C ¹³ -nuclear
20	magnetic resonance (¹³ C-NMR), powder X-ray crystallography (XRD) and elemental analysis.
21	Their performance as colorant material for different fastness properties like light fastness, wash
22	fastness, perspiration fastness were evaluated on leather fabric and were shown to possess good
23	fastness properties 3-5, 4-5, 3-5, and 4-5 respectively. The synthesized formazan dyes were also

demonstrated the high percentage value of exhaustion and fixation on leather ranging from 91-24 97% and 90-98% respectively. The synthesized un-metallized and metal complex formazan dyes 25 were ranged in color from Blue, violet, Green, Brown to Red. Antibacterial activity of 26 synthesized formazan dyes was determined in solution by agar well diffusion method and on 27 leather using the dyed leather fabric with synthesized dyes as model system. Maximum 28 antibacterial activity in solution with ZOIs (19±0.05 mm, 25±0.07 mm, 23±0.09 mm, 27±0.03 29 30 mm) and significant % reduction in bacterial growth on leather (56±0.03%, 65±0.07%, 31 70±0.05%, and 72±0.08%) was demonstrated by chromium complex (15) against E. coli, S. aureus, Klebsiella and B. subtilis respectively. 32

33 Keywords: Formazan dyes; diazotization; coupling reaction; metal complex; fastness;
34 antibacterial activity

35 **1. Introduction**

Formazan dyes bear a formal resemblance to azo dyes, since they contain an azo group, but have 36 sufficient structural dissimilarities to be considered as a separate class [1]. Formazan/tetrazolium 37 and their derived metal complexes are colored compounds. They impart color due to π - π * 38 transitions of π electrons in formazan skeleton. Formazan Skeleton is (-N=N–C=N–NH-) [2]. 39 Formazans are polydentate ligands with donor atoms so that's why they have the ability to form 40 41 complexes with metal atoms. Metal complex formazan is derived from non-metal complex formazan by treating it with metal salts such as FeSO₄.7H₂O, CrCl₃.6H₂O, and CuSO₄.5H₂O, etc. 42 Formazans and their metal complex are ranging in color from red to orange as well as blue color. 43 Their color depending upon the structure either which type of chromospheres and auxochromes 44 are attached to color imparting molecule [3]. Metal complexes of tetradentate formazan were 45 first employed as dyes for the dyeing of wool by Ciba in 1947 [4]. Since then many metal 46

complexes of tetradentate formazans have been reported and they have become an important dye 47 type, especially in the areas of reactive and acid dyes although other applications have also been 48 reported [5,6]. In addition to studies on their end-uses, investigations have been made with 49 respect to synthetic methods, as well as on the relationships between applications, colors 50 properties and chemical constitutions of the dyes [4]. Numerous literature associated to 51 formazans dyes are available. That literature shows their synthesis, structural properties, 52 photochromic transitions, tautomer formation, redox potentials [7,8] as well as the synthesis of 53 crown formazans are discussed [9,10]. Thermogravimetric analyses, dissociation and stability 54 constants, formation constants and electrochemical behaviors of Metal-complexes formazan 55 56 were evaluated [11,12]. Formazan/tetrazolium used in Brucella ring test in milk for the recognition of the presence of Brucella species. Brucellosis is a bacterial infection that spreads 57 from animals to people most often via unpasteurized milk, cheese, and other dairy products [13]. 58 59 Blue tetrazolium/formazan systems are used to exhibit enzymes activity in normal and neoplastic tissues. But it was found that it has 10 times more noxious effects in vivo (mice) than mono 60 tetrazolium/formazan system itself [14]. Formazan/tetrazolium system is relatively useful in the 61 determination of the effect of anti-cancer drugs [15,16]. However, Kebler and Furusaki states 62 that tetrazolium cannot act to do so every time, since the age of the cell and medium, both are 63 also important and take into matter. Formazan dyes have become important reactive dyes for 64 cotton. Formazan dyes are also castoff in analytical chemistry due to their high color strength of 65 numerous of their derived metal complexes [17]. As a result of their fast and intense color 66 reaction, formazans can be used for the spectroscopic determination of a variety of metal ions 67 [18]. The aims of this research work were involved too (i) synthesize formazan dyes and their 68 metal complex, (ii) Characterization of formazan and their metal complex dyes, (iii) 69

Performance evaluation of formazan and their metal complex dyes on leather with respect totheir color imparting properties.

72 **2. Experimental Work**

This research work was accompanied in the research laboratory of Department of Chemistry,
University of Management and Technology, Lahore Pakistan and in Research and Development
Lab in Shafi Reso-Chem, Lahore Pakistan. All chemicals employed in this research work were
procured from Fluka Chemische (Switzerland) as well as from BASF (Germany).

77 2.1 Physical measurement

Infrared spectra's were obtained in the range of 450-4000 cm⁻¹ by using Agilent Cary 630, 78 Agilent Technologies, USA, FTIR spectrophotometer. Electronic absorption spectra's of 79 compound (Dye 12-17) were obtained in water in the range of 200-800 nm by using UV-visible 80 spectrophotometer = Spectra Flash SF 550, Data color Inc., USA. H¹-NMR spectra's were 81 recorded in a CDCl₃ solvent by using NMR Bruker DPX 400-Operating at 300/75-¹³C MHz 82 spectrophotometer. Powder X-ray di analysis was performed by PANalytical X'Pert 83 diffractometer PRO instrument with Cu-Ka radiation (wavelength 0.154 nm) operating at 40 kV 84 and 30 mA. Measurements were scanned for di \Box raction angles (2 θ) ranging from 20° to 90° with 85 a step size of 0.02° and a time per step of 1 s. The elemental analysis of the compounds (C, H, N, 86 S) was carried out using Flash EA 1112 elemental analyzer. Melting Points were recorded using 87 Melting Point apparatus, Gallenkamp, UK. pH of the reaction and compounds was monitored 88 with Portable pH Meter Model PHB4. Application of dyes was done on leather by using Leather 89 Dying Drum Machine = Jiangsu Lianyungang Leather Machinery Factory China, Model# R-350-90 6. Light Fastness properties were evaluated by using Xenon Fad-o-meter Model# XF-15N, 91 Shimudzu Corporation Kyoto Japan. In this study, the progress of the entire reactions was 92

supervised by TLC, which was performed on 2×5 cm aluminum sheets preloaded with silica gel 60F254 to a thickness of 0.25 mm (Merck). The chromatograms were visualized under ultraviolet light (254-366 nm) or iodine vapors.

96 2.3 General procedure for the synthesis of 4-[(2Z)-2-benzylidenehydrazinyl] benzene
97 sulfonic acid (5)

98 Synthesis of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid which act as coupler 99 reagent, comprises of the conversion of 4-aminobenzenesulphonic acid (1) to diazonium salt (2) 100 which on reduction yielded 4-hydrazinylbenzenesulfonic acid (3) which further react with 101 benzaldehyde to form required product [19].



102

103 **Scheme 1:** Synthesis of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5)

104 2.3.1 Diazotization of 4-[(2Z)-2-benzylidenehydrazinyl]

Technical grade 4-aminobenzenesulphonic acid (1) corresponding to 173g (1mole) of 100 per 105 cent purity added in 1500 ml water in 3 L beaker and also added 65 g of Na₂CO₃ then given a 106 mixing for complete dissolution. Then the solution is filtered off to remove undissolved particles 107 and impurities. Then the made ice jacketed around the beaker to attain temperature 0-5°C. Then 108 during continuous agitation, the addition of 400 ml of HCl was made gradually. Then the ice 109 110 flakes about 100 g added to the solution to maintain temperature 0-5°C, and the addition of Sodium nitrite solution i.e. 73 g of NaNO₂ in 150 ml water was done dropwise in 15 minutes at 111 temperature 0-5°C. Then the congo-iodo paper was checked which was positive by giving blue 112

coloration. Then the reaction mixture allowed for 1:30 hr for reaction completion. Afterward,
diazonium salt filtered off with filtration unit associated with vacuum pump [20]. Scheme 1
showed the synthesis of diazonium salt (2) of 4-aminobenzenesulphonic acid (1).

116 **2.3.2** Reduction of diazonium salt (2) of 4-aminobenzenesulphonic acid (1)

To a beaker of 3 L, 565 g of crystalline Na₂SO₃.7H₂O had been added in 830 ml water and made 117 a clear solution of it. Solution of Sodium sulfite cooled in an ice bath to attain 0-5°C 118 temperature. At that temperature moist paste of diazonium salt (2) added in one hour. Then the 119 120 orange coloration appears in solution without turbidity. Then the stirring made continuously for 1 hour. Afterwards, the reaction solution was heated to boil-age with continuous stirring and 664 121 122 concentrated HCl was added in half an hour. The solution color lightens and finally very light yellow color appeared at the end of reaction. Reaction mixture cooled down over a period of 123 overnight. Precipitate formation takes place and these are filtered off with sanction unit, washing 124 125 of precipitates was done with cold water. Precipitates of 4-hydrazinylbenzenesulfonic acid (3) dried in an electric oven at 100°C. Purification was done by employing the method as described 126 by [19]. % age yield= 96% of the theoretical amount. Scheme 1 was represented the synthesis of 127 4-hydrazinylbenzenesulfonic acid (3). 128

129 **2.3.3** Synthesis of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5)

To a beaker of 3 L, quantity 198.75 g (1mole) of 4-hydrazinylbenzenesulfonic acid (**3**) weighed on analytical balance by considering it 95% pure and dissolved it by the addition of 2508 ml of water as well as 99.99 ml of NaOH added to attain pH~10, heated with continuous stirring for 30 minute at 45°C. Afterwards, cool down and filter it to remove impure and undissolved materials. Solution was shifted in 3 liter reactor and heated to 55-60°C. Condensation reaction was done by the drop-wise addition of benzaldehyde (**4**) 106.2 g (1mole) in 30 minute at 55°C with

136 continuous stirring. Stirring plus heating continue till complete reaction. Reaction completed in 3 hour. Then the precipitation achieved by acidification as well as salting out process at pH~2.5 at 137 room temperature which is done by employing the 25 ml HCl and 20% of NaCl of the total 138 volume of the solution. After, filtration was done through filtration unit at ambient temperature. 139 Filtered cake was dried in electric oven at 60°C. % age yield was 97% of the theoretical amount. 140 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) was that coupler reagent which was 141 142 employed for the synthesis of formazan dyes by the azo coupling reaction. This product (5) was purified by following the method as described by [20,21]. Scheme 1 exhibited the synthesis of 4-143 [(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5). 144

145 2.4 General Procedure for the Synthesis of formazan dyes (12-17)

In this research work, first 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) were synthesized (Scheme 1) which act as coupler reagent. In second step, 4-(2-(((2-hydroxy-5nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid (12) were synthesized which was un-metallized formazan dye. Further un-metallized formazan dye (12) was converted to metal complexes with the salts of Fe, Cu, Cr, Ni and Co and their characterization was done by using different spectroscopic techniques. The syntheses of each formazan dye of this series were given in the scheme 2.



Scheme 2: Synthesis of un-metallized formazan dyes (12), metal complexes of Fe (13), Cu (14),
Cr (15), Ni (16) and Co (17)

153

156 2.4.1 Synthesis of 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 157 hydrazinyl) benzene sulfonic acid (12)

158 To a 500 ml beaker, 150 ml water added along with 15.41g (0.1mole) on 100% pure basis of 4nitroaminophenol (10) on continuous stirring with multiple agitators. Then 40 ml of HCl was 159 added allowed for 15 minutes for complete dissolution. After that solution is filtered off by 160 employing suction unit to remove impurity. Then the solution beaker jacketed with ice and 30 g 161 of ice flakes was also added to attain the temperature 10°C. Afterward, a solution of NaNO₂ 162 /water (7.4/15ml) added in 4 portions. Positivity of Congo red paper for HCl and Iodo paper for 163 NaNO₂ checked. Then the reaction solution was stirred for 1hr at 15-17 °C. Congo-iodo= +ve 164 was to be checked during stirring for 1 hour after every 15 mins. Iodo paper made negative by 165

166 the addition of 5g of Sulfamic acid (H₃NSO₃). Again purification of diazo (11) was done by the filtration process. Diazonium salt (11) is ready for the further process. This diazonium salt of 4-167 nitroaminophenol (11) was coupled with 28.48g (0.1mole) on 97% percent pure basis of 276.31g 168 of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) that was achieved by dissolving 169 (5) in 400 ml H₂O at highly alkaline pH~10-11 at temperature 15-17 °C. Alkaline pH was 170 achieved by the addition of 35g of Na_2CO_3 . The addition of diazonium salt (11) to coupler 171 172 reagent (5) was done in 1 hour. The reaction took 2 hours for its completion. The dye (12) was brought to room temperature; pH was reduced up to 4.5 by HCl and 15% NaCl of the total 173 volume of dye was also added for salting out purposes. The dye (12) was filtered and dried at 60-174 175 70° C till constant weight. The obtained yield was 85% over theoretical calculation. The reaction for the synthesis of formazan dye 12 is presented in scheme 2. 176

177 2.4.2 Synthesis of [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 178 hydrazinyl) benzene sulfonic acid] iron II complex (1:1) (13)

For the synthesis of dye (13), un-metallized formazan dye [4-(2-(((2-hydroxy-5-nitrophenyl) 179 diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] (12) of 0.1 mole was prepared 180 according to the method as described in scheme 2. Dye (12) was acting as tridentate ligand. Its 181 metallization with iron was achieved by treating it with equimolar (0.1mole) concentration i.e. 182 27.80g of FeSO₄.7H₂O at slightly acidic pH~6.50 at elevated temperature i.e. 65-70°C. Slightly 183 acidic pH achieved with the addition of HCl in dye (12) then heated it for 65-70°C. At required 184 temperature, addition of FeSO₄.7H₂O was done in 30 minute. Afterwards, reaction mixture was 185 heated for 3 hour for the completion of metallization and final product was dye 13 i.e. [4-(2-(((2-186 hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] iron II 187 complex (1:1). The dye (13) was brought to room temperature; pH was reduced up to 4.5 by HCl 188

and 15% NaCl of total volume of dye was also added for salting out purposes. The dye (13) was
filtered and dried at 60-70°C till constant weight. The obtained yield was 90% over theoretical
calculation. The reaction for the synthesis of formazan dye (13) is presented in scheme 2.

192 2.4.3 Synthesis of [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 193 hydrazinyl) benzene sulfonic acid] copper II complex (1:1) (14)

For the synthesis of dye (14), un-metallized formazan dye [4-(2-(((2-hydroxy-5-nitrophenyl) 194 195 diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] (12) of 0.1 mole was prepared 196 according to the method as described in scheme 2. Dye (12) was acting as tridentate ligand. Its metallization with copper was achieved by treating it with equimolar (0.1mole) concentration i.e. 197 198 24.9685g of CuSO₄.5H₂O at slightly acidic pH~6.50 at elevated temperature i.e. 65-70°C. Slightly acidic pH achieved with the addition of HCl in dye (12) then heated it for 65-70°C. At 199 required temperature, addition of CuSO₄.5H₂O was done in 30 minute. Afterwards, reaction 200 201 mixture was heated for 3 hour for the completion of metallization and final product was dye 14 i.e. [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene 202 sulfonic acid] copper II complex (1:1). The dye (14) was brought to room temperature; pH was 203 reduced up to 4.5 by HCl and 15% NaCl of total volume of dye was also added for salting out 204 purposes. The dye (14) was filtered and dried at 60-70° C till constant weight. The obtained yield 205 was 89% over theoretical calculation. The reaction for the synthesis of formazan dye 14 is 206 presented in scheme 2. 207

208 2.4.4 Synthesis of Bis-[4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 209 hydrazinyl) benzene sulfonic acid] chromium III complex (2:1) (15)

For the synthesis of dye (15), un-metallized formazan dye [4-(2-(((2-hydroxy-5-nitrophenyl)
diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] (12) of 0.1 mole was prepared

212 according to the method as described in scheme 2. Dye (12) was acting as tridentate ligand. Its metallization with chromium as 2:1 complex was achieved by treating it with 0.05 mole 213 concentration i.e. 13.32 g of 26.64 g of CrCl₃.6H₂O at slightly acidic pH~6.70 at elevated 214 temperature i.e. 90-100°C. Slightly acidic pH achieved with the addition of HCl in dye (12) then 215 heated it for 90-100°C. At required temperature, addition of CrCl₃.6H₂O was done in 30 minute. 216 Afterwards, reaction mixture was heated for 5 hour for the completion of metallization and final 217 product was dye **15** i.e. Bis-[4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 218 219 hydrazinyl) benzene sulfonic acid] chromium III complex (2:1). The dye (15) was brought to room temperature; pH was reduced up to 1.5 by HCl and 20% NaCl of total volume of dye was 220 221 also added for salting out purposes. The dye (15) was filtered and dried at 60-70 °C till constant weight. The obtained yield was 91% over theoretical calculation. The reaction for the synthesis 222 of formazan dye (15) is presented in scheme 2. 223

224 2.4.5 Synthesis of [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 225 hydrazinyl) benzene sulfonic acid] nickel II complex (1:1) (16)

For the synthesis of dye (16), un-metallized formazan dye [4-(2-(((2-hydroxy-5-nitrophenyl) 226 diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] (12) of 0.1 mole was prepared 227 according to the method as described in scheme 2. Dye (12) was acting as tridentate ligand. Its 228 metallization with copper was achieved by treating it with equimolar (0.1mole) concentration i.e. 229 28.086g of NiSO₄.7H₂O at slightly acidic pH~6.50 at elevated temperature (65-70°C). Slightly 230 acidic pH achieved with the addition of HCl in dye (12) then heated it for 65-70°C. At required 231 temperature, addition of NiSO₄.7H₂O was done in 30 minute. Afterwards, reaction mixture was 232 heated for 3 hour for the completion of metallization and final product was dye (16) i.e. [4-(2-233 (((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] 234

nickel II complex (1:1). The dye (16) was brought to room temperature; pH was reduced up to
3.5 by HCl and 15% NaCl of total volume of dye was also added for salting out purposes. The
dye (16) was filtered and dried at 60-70 °C till constant weight. The obtained yield was 87% over
theoretical calculation. The reaction for the synthesis of formazan dye (16) is presented in
scheme 2.

240 2.4.6 Synthesis of [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) 241 hydrazinyl) benzene sulfonic acid] cobalt III complex (1:1) (17)

242 For the synthesis of dye (17), un-metallized formazan dye [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid] (12) of 0.1 mole was prepared 243 244 according to the method as described in scheme 2. Dye (12) was acting as tridentate ligand. Its metallization with copper was achieved by treating it with equimolar (0.1mole) concentration i.e. 245 24.91g of (CH₃COO.4H₂O) at slightly acidic pH~6.50 at elevated temperature i.e. 65-70°C. 246 Slightly acidic pH achieved with the addition of HCl in dye (12) then heated it for 65-70°C. At 247 required temperature, addition of NiSO₄.7H₂O was done in 30 minute. Afterwards, reaction 248 mixture was heated for 3 hour for the completion of metallization and final product was dye (17) 249 i.e. [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene 250 sulfonic acid] cobalt III complex (1:1). The dye (17) was brought to room temperature; pH was 251 reduced up to 2.5 by HCl and 15% NaCl of total volume of dye was also added for salting out 252 purposes. The dye (17) was filtered and dried at 60-70 °C till constant weight. The obtained yield 253 was 88% over theoretical calculation. The reaction for the synthesis of formazan dye (17) is 254 presented in scheme 2. 255

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258 **2.5 Dyeing method for assessment of color shade development on leather**

Assessment of color shade development of un-metallized formazan dye (12) and metal complex 259 formazan dyes (13-17) was done by the application of dyes at 2% dyeing on goat leather fabric 260 by using the standard method as described by [21,22]. Dye solution (250 ml, 1.0 g dye equivalent 261 to 2% of goat crust weight) was taken in a dyeing drum. The pH of the dye-bath was adjusted to 262 5.5 by adding acetic acid solution (5.0 ml. 10% w/v) solution. The total volume of the dye-bath 263 was adjusted to 500 ml by adding the required amount of water. The leather pieces of 50 g of 264 goat crust were introduced into the dye-bath with continuous stirring at 24 rpm. The contents of 265 the dye-bath were stirred for 1 hour at room temperature (30°C) and then the temperature was 266 gradually raised to 50-55°C over a period of 35 minutes and maintained for 1 hour. The dye-bath 267 was kept rotating during the process of dyeing; formic acid 5.0 ml was added and pH adjusted to 268 3.6 and run the drum for half hour more till dye fixed on leather. After this, the dye liquor was 269 taken in 500 ml volumetric flask. The fabric was washed with cold water and the combined 270 solution of dye liquor and washings was then further diluted to 500 ml with water. From this 271 diluted solution, 2.0 ml was further diluted to 100 ml with water and the absorbance of this 272 solution was measured to find out the exhaustion of dye on leather sheet. The dyed leather piece 273 was piled up overnight to dry; provided the chukrum and toggle and finally, mounted on shade 274 card. A weighed amount of leather piece was stirred in boiling acidified pyridine which dissolves 275 the unfixed dye from fabric and from the absorbance of this solution percentage fixation was 276 determined. 277

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- 279
- 280

281 **2.6** Valuation of fastness properties on leather

Valuation of fastness properties like color change, staining on adjacent fiber, light, wash and
perspiration for the synthesized un-metallized formazan dyes and metal complex formazan dyes
series (12-17) was checked by the application of 2% dye on Goat leather as per standard methods
described by [23].

286 2.7 Antibacterial activity

287 The antibacterial activity of each type of synthesized formazan dyes (12-17) was examined in solution [24] as well as on substrate against different bacterial strains i.e. Staphylococcus aureus 288 (ATCC 25923), Bacillus subtilis (ATCC 9637), Klebsiella (ATCC 10031) and Escherichia coli 289 290 (ATCC 25922). The antibacterial effect of synthesized formazan dyes (12-17) on agar plates was studied by known agar well diffusion method as described by [25,26]. These bacterial strains 291 were obtained from Pakistan Chemical Scientific Industrial Research (PCSIR) laboratories 292 293 complex, Lahore, Pakistan. Cefradine was used as a standard antibiotic drug (positive control) for each type of bacterial species. 294

295 2.7.1 Antibacterial activity of synthesized formazan dyes (12-17) in solution

In the first step, sample solutions with 1M strength of each type of synthesized formazan dyes 296 (12-17) and standard drug were prepared with distilled water separately in 1L volumetric flask 297 [27]. In the second step, solution of nutrient agar was prepared in 1L volumetric flask by 298 dissolving 28 g nutrient agar in 1000 mL distilled water with continuous stirring to dissolve well 299 and to make the solution uniform; then mouth of flask was covered with yarn to preserve the 300 culture media. Then sterilized it in an autoclave at 120-121 °C and maintains pressure at 15 LD/ 301 sq inch for 20-25 min and then cooled that solution. This medium used for the growth of the 302 bacterial strains for the preparation of inoculum [28]. 303

In the third step, slants of each frozen bacteria that were already present in the stock slants of bacterial culture were taken and a loop full of culture or frozen bacteria was added to the sterilized nutrient agar in the test tube and preserved for future use. After that, bacterial strains (*S. aureus, B. subtilis, Klebsiella and E. coli*) from stock slants were taken and added into four conical flasks in which nutrient agar (25 ml) was already added. These four cultures of four different bacteria were incubated at 37 °C for 24 hours in a shaker. These cultures used as inoculum [29].

In the fourth step, freshly prepared molten nutrient agar (200 mL) was poured in 28 sterilized 311 petri dishes as a basal layer. After that 1 mL inoculum of each bacterial strain (S. aureus, B. 312 subtilis, Klebsiella and E. coli) was added in 28 petri dishes. Then put lids on the dishes and 313 allowed them to cool and solidify. The solidify agar core 2 mm in diameter was bored at three 314 peripheral positions by sterilized hollow iron rod. After that holes were filled with the already 315 316 prepared samples and standard drug. Then the petri dishes were allowed to place for 1 hour and after that incubated the dishes in incubator at 37 °C for 24 hours in order to complete the reaction 317 for bioactivity. After the completion of incubation period the diameters of the clear zones were 318 measured and recorded [30]. 319

320 2.7.2 Antibacterial activity of synthesized formazan dyes (12-17) on substrate (Leather 321 fiber)

In the next set of experiments, the antibacterial activity of leather specimens dyed with synthesized formazan dyes (**12-17**) and standard drug (Cefradine) was determined and leather fabric was dyed as per following the methods described in the dyeing method section. The 1 inch² each fabric specimens (dyed and standard) were introduced separately in the 200 mL nutrient agar inoculated with the different bacterial strains i.e. *S. aureus, B. subtilis, Klebsiella* *and E. coli* and incubated at 37 °C overnight (16 h). The same set of experiment was also repeated for the undyed leather fabric as done with dyed leather fabric [31]. The % reduction of bacterial growth by synthesized formazan dyes (**12-17**) and standard drug (Cefradine) was expressed as follows:

331

$$R = B - A/A \times 100$$

where R= % reduction in bacterial population; B= absorbance (660 nm) of the media inoculated with bacterial strains and undyed fabric; A= absorbance (660 nm) of the media inoculated with bacterial strains and dyed fabric.

335 **3. Results and Discussion**

In current research work, un-metallized formazan dyes (12) and metal complex formazan dyes (13-17) were synthesized by employing different metal salts of Fe, Cu, Co, Ni, and Cr. Synthesized formazan dyes had been characterized by UV-visible spectroscopy, IR spectroscopy, ¹H-NMR, ¹³C-NMR, powder XRD as well as by elemental analysis. Performance evaluation of these dyes was checked on leather by employing leather processing drums.

- 341 **3.1 Spectral Studies**
- 342 3.1.1. 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene
 343 sulfonic acid (12).
- Reddish Brown (Solid). Yield (85%). Melting Point (390°C). λ_{max} in nm (log ε): λ_{max1} 470 (0.470), λ_{max2} 340 (0.687). FTIR (KBr, Cm⁻¹) ***max:** 1500 (C=N, str), 1358 (N=N, str), 1600 (aromatic C=C), 750 (CN=NC, str), 3410 (N-H, str), 1130 (Ar-OH, str), 1420 (SO₃H, str), 1335 (Ar-NO₂, str). ¹H-NMR (CDCl₃, 400 MHz) δ: 5.35, (s 1H, Aromatic-OH), 1.14, (s 1H, N-H), 11.28 (s 1H, SO₃H), 6.50-7.60 (m 12H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ: 155 (C=N), 102, 109.8, 117.6, 125.4, 126.3, 127.3, 128.8, 131, 134.7, 139.2, 140.5, 146.8, 164.1 (Aromatic

- 350 carbons). Anal. Calc. for C₁₉H₁₅N₅O₆S (MW: 441.417 g/mol): C, 51.7; H, 3.43; O, 21.75; N,
- 351 15.87; S, 7.26% and Found: C, 51.3; H, 3.3; O, 21.55; N, 15.65; S, 7.21%.
- 352 3.1.2. [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene
 353 sulfonic acid] iron II complex (1:1) (13).
- Reddish Brown (Solid). Yield (90%). Melting Point (450°C). λ_{max} in nm (log ε): λ_{max1} 380 (0.950), λ_{max2} 280 (1.05). FTIR (KBr, Cm⁻¹) ***max:** 1540 (C=N, str), 1440 (N=N, str), 1580 (aromatic C=C), 830 (CN=NC, str), 1385 (SO₃H, str), 1360 (Ar-NO₂, str), 680 (Metal-O, str), 3410 (O-H, str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.10 (s 1H, SO₃H), 6.70-8.20 (m 12H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ: 155.2 (C=N), 111.5, 116.5, 117.6, 125.4, 127.3, 127.7, 128.8, 131.0, 134.7, 137.0, 139.2, 149.3, 150.5, 159.5, (Aromatic carbons). Anal. Calc. for C₁₉H₁₉FeN₅O₉S (MW: 549.292 g/mol): C, 41.54; H, 3.49, O, 26.21; N, 12.75; S, 5.84; Fe,
- 361 10.17%. Found: C, 41.30; H, 3.35; O, 26.15; N, 12.6; S, 5.56; Fe, 10.05%.
- 362 3.1.3. [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene
 363 sulfonic acid] copper II complex (1:1) (14).
- Greyish Black (Solid), Yield (89%), Melting Point (420°C). λ_{max} in nm (log ε): λ_{max1} 340 (0.758), 364 λ_{max2} 218 (0.90). FTIR (KBr, Cm⁻¹) "max: 1499 (C=N, str), 1305 (N=N, str), 1595 (aromatic 365 C=C), 840 (CN=NC, str), 1290 (SO₃H, str), 1395 (Ar-NO₂, str), 610 (Metal-O, str), 3490 (O-H, 366 str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.20 (s 1H, SO₃H), 6.85-8.55 (m 12H, Ar-H). ¹³C-NMR 367 (CDCl₃, 75 MHz) δ: 155.2 (C=N), 116.8, 117.6, 125.2, 125.4, 126.3, 127.3, 128.8, 131.0, 131.8, 368 134.7, 139.2, 140.5, 150.5, 155.2, 164.7 (Aromatic carbons). Anal. Calc. for C₁₉H₁₉CuN₅O₉S 369 (MW: 556.932 g/mol): C, 40.97; H, 3.44; O, 25.85; N, 12.57; S, 5.76; Cu, 11.41% and Found: C, 370 40.81; H, 3.39; O, 25.71, N, 12.41; S, 5.61; Cu, 11.29%. 371

- 372 3.1.4. Bis-[4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl)
 373 benzene sulfonic acid] chromium III complex (2:1) (15).
- Greyish Brown (Solid), Yield (91%), Melting Point (460°C). λ_{max} in nm (log ε): λ_{max1} 360 (0.85),
- 375 λ_{max2} 219 (0.92). FTIR (KBr, Cm⁻¹) ^v**max:** 1520 (C=N, str), 1470 (N=N, str), 1605 (aromatic
- C=C), 850 (CN=NC, str), 1410 (SO₃H, str), 1345 (Ar-NO₂, str), 640 (Metal-O, str). ¹H-NMR (CDCl₃, 400 MHz) δ : 11.35 (s 1H, SO₃H), 6.65-7.92 (m 24H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ : 155.2 (C=N), 149.5 (C=N), 116.8, 117.6, 127.3, 125.2, 126.3, 127.6, 131.8, 132.1, 133.8, 133.4, 135.6, 137.8, 139.2, 140.5, 150.5, 164.7 (Aromatic carbons). Anal. Calc. for C₃₈H₂₆CrN₁₀O₁₂S₂ (MW: 930.799 g/mol): C, 49.03; H, 2.82; O, 20.63; N, 15.05; S, 6.89; Cr, 5.59% and Found: C, 48.95; H, 2.75; O, 20.45; N, 15.01; S, 6.69; Cr, 5.47%.
- 382 3.1.5. [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene
 383 sulfonic acid] nickel II complex (1:1) (16).
- Greenish Brown (Solid), Yield (87%), Melting Point (435°C). λ_{max} in nm (log ε): λ_{max1} 370 384 (1.55), λ_{max^2} 320 (1.25). FTIR (KBr, Cm⁻¹) ^vmax: 1505 (C=N, str), 1370 (N=N, str), 1615 385 (aromatic C=C), 855 (CN=NC, str), 1320 (SO₃H, str), 1340 (Ar-NO₂, str), 635 (Metal-O, str), 386 3450 (O-H, str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.28 (s 1H, SO₃H), 6.90-8.66 (m 12H, Ar-H). 387 ¹³C-NMR (CDCl₃, 75 MHz) δ: 155.2 (C=N), 111.5, 116.56, 117.6, 125.4, 127.3, 127.7, 128.8, 388 131.0, 134.7, 137.0, 139.2, 149.3, 150.5, 159.5 (Aromatic carbons). Anal. Calc. for 389 C₁₉H₁₉NiN₅O₉S (MW: 552.140 g/mol): C, 41.33; H, 3.47; O, 26.08; N, 12.68; S, 5.81; Ni, 390 10.63% and Found: C, 41.19; H, 3.35; O, 25.95; N, 12.56; S, 5.79; Ni, 10.55%. 391
- 392 3.1.6. [4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene
 393 sulfonic acid] cobalt III complex (1:1) (17).

Dark Brown (Solid), Yield (88%), Melting Point (410°C). λ_{max} in nm (log ε): λ_{max1} 345 (1.20), 394 λ_{max^2} 225 (1.17). FTIR (KBr, Cm⁻¹) "max: 1490 (C=N, str), 1330 (N=N, str), 1600 (aromatic 395 C=C), 810 (CN=NC, str), 1220 (SO₃H, str), 1300 (Ar-NO₂, str), 630 (Metal-O, str), 3330 (O-H, 396 str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.19 (s 1H, SO₃H), 6.95-8.70 (m 12H, Ar-H). ¹³C-NMR 397 (CDCl₃, 75 MHz) δ: 155.2 (C=N), 111.5, 116.5, 117.6, 125.4, 127.3, 127.7, 128.8, 131.0, 134.7, 398 137.0, 139.2, 149.3, 150.5, 159.5 (Aromatic carbons). Anal. Calc. for C₁₉H₁₉CoN₅O₉S (MW: 399 552.380 g/mol): C, 41.31; H, 3.47; O, 26.07; N, 12.68; S, 5.68; Co, 10.67% and Found: C, 41.29; 400 H, 3.41; O, 25.93; N, 12.61; S, 5.71; Co, 10.59. 401

402 **3.2 FTIR Spectral Studies**

Functional groups can be simply recognized in IR spectroscopy. Consequently, IR spectroscopy 403 considered as valuable basis for attaining the structural information of compounds that have 404 organic nature. IR spectra of dyes (12-17) exhibited a sharp peak at 1580-1615 cm⁻¹ that 405 designated the presence of C=C (Aromatic stretching), a small peak at 1490-1540 cm^{-1} indicated 406 the existence of C=N (Stretching) that approves the formation of formazan dye as shown in 407 figure 1 (a). A small peak observed at 1305-1480 cm⁻¹, exhibiting the presence of N=N 408 (Stretching). While a broad peak at 3410 cm⁻¹ showed the N-H stretching in dye **12** as depicted 409 in figure 1 (a). A sharp peak observed at 750-855 cm⁻¹ depicted the existence of C-N=N-C 410 skeleton from which also affirmed the formation of formazan. A peak observed at 1220-1420 cm⁻ 411 ¹, 1300-1395 cm⁻¹, 1130 cm⁻¹ indicated the presence of -S₃OH, Ar-NO₂, and Ar-OH respectively. 412 A sharp and broad peak observed at 3330-3490 cm⁻¹ for OH functional group and small peaks 413 observed at 630-680 cm⁻¹ indicated the formation and presence of metal-oxygen bond in terms of 414 metal-ligand bond to form metal complex formazan dyes as shown in figure 1 (b). Before 415 416 metallization, IR peaks of N-H and Ar-OH only were observed in dye (12) that acts as ligand and

these IR peaks disappear after metallization as in dyes (13-17) as shown in Table 4 and figure 1.
While IR peaks of M-O only observed after metallization of dye (12) with metal like Fe, Cu, Cr,
Ni, and Co in dyes (13-17). And IR peaks for OH only observed in dyes (13, 14, 16 and 17) as
these are (1:1) metal complexes of Fe, Cu, Ni and Co respectively while absent in dye (15) as
this is (2:1) metal complex of Cr. FTIR results of this research work founded to be in close
agreement with the [3].





427 Fig. 1. IR spectra of (a) and (b) for the synthesized formazan dyes 12 and 13 respectively

428 **3.3** ¹H-NMR spectral Studies

The ¹H-NMR data shows that the chemical shift (δ) value of formazan dye 13 shifted to lower 429 field as compared to formazan dye 12. As the chemical shift value (δ) 7.60 is for Ar-H in 430 formazan dye 12 which has been shifted to lower field in formazan dye 13 and has 8.20 chemical 431 shift (δ) values for Ar-H as shown in figure 2 (a) and (b). This is because of the fact that in 432 formazan dye 12, there is two electron donating group such as N-H and Ar-OH is attached to the 433 aromatic ring that shifting the chemical shift (δ) value to higher field. As electron donating 434 groups are responsible for shifting the chemical shift (δ) value to higher field while electron 435 withdrawing group are favorable for shifting to lower field. But in formazan dye 13, both Ar-H 436 and N-H groups are not present. Furthermore this behavior of shifting of chemical shift (δ) from 437 higher field to lower field has been also observed in other formazan dyes (14-17) in contrast to 438 formazan dye 12. This fact is clearly justifiable that except formazan dye 12, both electrons 439 donating group N-H and Ar-H are not present in the structure of other formazan dyes as 440

441 represented figure 2. These results were found in accordance with the previously reported data [3]. ¹H-NMR studies also depicted and confirmed the formation of metal complexes with the 442 formazan dye 12 which also act as ligand. As these two functional groups, N-H and Ar-OH are 443 showing their chemical shift (δ) value in formazan dye 12 but not in others formazan dyes (13-444 17). This fact clearly and justifiably has confirmed the formation of coordinate covalent bond 445 between ligand (Dye 12) and metals Fe, Cu, Cr, Ni, and Co in formazan dyes 13, 14, 15, 16, and 446 447 **17** respectively.



450

(b)

451

452

Fig. 2. ¹H NMR spectra of synthesized formazan dye 12 (a) and 13 (b) measured in CDCl₃ at

453

25°C

454 **3.4 UV-visible spectral studies**

The absorption maxima of the dye 12 that act as ligand and its respective metal complex dyes 13, 455 14, 15, 16 and 17, were recorded in water. Distinguishing three peaks are observed in the 456 spectrum of UV-vis formazans. The value of λ_{max1} is specific for formazans framework. For that 457 reason this research work relates to the value λ_{max1} . Formazan peak value of λ_{max1} is usually 458 experimental at 410 nm to 500 nm and will probably be redirected to 350 nm 600 nm but 459 460 depending on the molecular structure of formazan. These peak absorption is because of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, electronic transition in the framework of the formazan. λ_{max2} values commonly 461 appear in 300-350 nm, that are found to be in accordance with the electronic transitions of -462 N=N- group which is present in the formazan molecule. λ_{max3} values are sometimes experimental 463 in nm 270-300 which is derived from the $n \rightarrow \pi^*$ transition of a -C= N-functional group which is 464 present in the dye molecules. The electronic absorption spectra were recorded in the H₂O. All 465 dyes have same chromophoric functionalities but large change in the λ_{max} has been seen on metal 466 complexation in the form of their bathochromic and hypochromic shift. Results were showed that 467 λ_{max1} values of dyes (12-17) were observed at 470, 380, 340, 360, 370 and 345 nm respectively 468 and the value of λ_{max2} for the dyes (12-17) observed at the wavelength of 340, 280, 218, 219, 320, 469 225 nm respectively as shown in Figure 3. This was indicated that there was found a shift of 470 longer to shorter wavelength (Hypsochromic effect). As the metal act as Lewis acid and attract 471 the electron density from the ligands towards itself that lead to shorter wavelength from longer 472 wavelength. There was found a blue shift of 90 nm for the Fe metal complex dye 13 from its 473

ligand (un-metallized dye 12) with the color change from reddish brown to dark bluish brown.
The same phenomenon has been seen in all metal complexes i.e. Cu (14), Cr (15), Ni (16) and
Co (17) with the blues shift of 130, 110, 100 and 125 nm respectively with color change from
reddish brown to blue, bluish beige, greyish beige and greenish blue respectively.



478

479

Fig. 3. Combine UV-Visible spectrum of synthesized formazan dyes (12-17)

480 **3.5 Elemental analysis**

Elemental analysis mostly said to be CHNS analysis. It involves the estimation of the masses of 481 the fractions of C, H, N, heteroatoms i.e. halogens, sulfur etc. and also the masses of the 482 fractions of metal atoms present in a sample. This gives the most useful information which has 483 its most significance in determining the structure of an unidentified chemical compound. It also 484 involved in ascertaining the purity as well as structure of a compound that is being synthesized in 485 research work (Tezcan, 2008). It was evident from the elemental analysis results that there was 486 founded a close agreement between the calculated results of elemental analysis as well as 487 founded results of elemental analysis of synthesized formazan dyes (12-17). Spectral as well 488 elemental analysis data were also confirmed the formazan dyes structures that are presented in 489 scheme 2 for dyes (12-17). 490

491

492 **3.6 XRD Analysis**

Single crystals growth of dye 12 i.e. 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) 493 methylene) hydrazinyl) benzene sulfonic acid which act as ligand from numerous solvents 494 including DMF, ethyl alcohol, chloroform etc. unsuccessful and was characterized by powder X-495 ray diffraction. X-ray powder di raction analysis of the dye 12 and its metal complexes i.e. dye 496 (13-17) was carried out to determine the nature of lattice parameters, crystal system and the cell 497 498 volume. XRD patterns in the XRD spectrum indicates the crystalline nature of dye 12 and its 499 metal complexes as presented in Figure 4. Indexing of the di raction patterns was performed using High Score Plus software. From the indexed data the unit cell parameters were calculated 500 501 and are listed in Table 1. Powder XRD patterns of the metal complexes i.e. dye (13-17) are totally di erent from the un-metallized dye 12, validating the formation of coordination 502 compounds. It is found that dye 12, dye 13, dye 14, dye 16 and dye 17 have monoclinic 503 504 structures while dye (15) has an orthorhombic structure. The crystal structures of similar type of compounds were reported as monoclinic and orthorhombic [32,33,34,31]. Moreover, by means 505 of the di according to according to according to 506 the Scherrer equation (D = 0.9λ ($\beta \cos\theta$), where λ is X-ray wavelength (1.5406 °A), θ is Bragg 507 di \Box raction angle, and β is the full width at half maximum of the di \Box raction peak) [36]. The 508 average crystallite sizes of all the synthesized dyes (12-17) were found to be $\sim 40-70$ nm and the 509 values are given in Table 1. 510

- 511
- 512
- 513
- 514

Dye#	Lattice Parameters			Volume	Crystallite	Crystal	
					(A ³)	size D	system
	a (A)	b (A)	c (A)	β (°)		(nm)	
12	10.0310	16.8315	8.1010	100.0111	1172.6181	59	Monoclinic
13	12.1201	4.0100	9.9989	99.7202	489.86	69	Monoclinic
14	13.9999	10.9870	8.7986	89.99	1399.91	41	Monoclinic
15	13.8901	12.0120	9.9910	109.9990	1499.99	40	Orthorhombic
16	16.0101	7.1021	13.9999	111.2011	1420.20	70	Monoclinic
17	17.0010	7.9898	15.0129	119.9982	1831.90	55	Monoclinic

515 Table 1 XRD data of formazan dyes 12-17



517

518



26



- 524 **3.7 Assessment of color properties**
- 525 3.7.1. Fastness properties

526 Valuation of color shade, as well as fastness properties i.e. color change, staining on adjacent
527 fibre, light, wash and perspiration for un-metallized formazan dye (12) and metal complex

528 formazan dyes series (13-17) was checked on goat leather fabric. The detailed properties are described in Table 2. All the synthesized un-metalized formazan dye (12) and metal complex 529 formazan dyes of Fe (13), Cu (14), Cr (15), Ni (16) and cobalt (17) have good penetration on 530 leather and produced color shades of Brown, Beige, Blue, Red, Grey, Black with different tones 531 like Reddish, Greyish, Olive, Bluish and Greenish on leather. Results were demonstrated that the 532 synthesized series of formazan dves (12) have average wash fastness (3-4) but metal complex 533 formazan dyes (13-17) exhibited the good fastness that was from 4-5, 4-5, 5, 4 and 4-5 534 respectively. Wash fastness is the resistance force that is exerted by dyed fabric to retain its color 535 during washing either with detergent and soap. Maximum wash fastness properties were 536 537 demonstrated by chromium complexes (15). Color change, staining on adjacent fiber, Light and perspiration fastness properties were also found to be good to excellent that exhibited by these 538 synthesized un-metalized formazan dyes (12) and metal complex formazan dyes of Fe (13), Cu 539 540 (14), Cr (15), Ni (16) and Cobalt (17) as shown in Table 2. These properties depend upon the physical as well as the chemical interaction of dyes with the applied substrate (leather). There 541 always found a direct relationship between these properties (Color change, Staining on adjacent 542 fibre, Wash, Light, and Penetration) with the physical and chemical interactions of dyes and 543 applied substrate [37]. As the more are the electrostatic interactions then rapid diffusion of dyes 544 takes place to the substrate underneath depth; more will be the penetration level and all dyes (12-545 17) were shown the excellent penetration level [37]. As a result, the synthesized dyes have all 546 properties to be good enough at all tested level. Figure 5 showed the color shades that developed 547 on leather by the application of un-metallized formazan dye (12) and metal complex formazan 548 dyes (13-17). 549

550

		Staining on	Color	Penetration	Wash	Light	Perspiration	
	Dye #	adjacent Fiber	Change		Fastness	Fastness	Fastness	
	12	4-5	4-5	4-5	3-4	3-4	4	
	13	4-5	5	5	4-5	4-5	4-5	
	14	5	4-5	4-5	4-5	4	5	
	15	5	4-5	5	5	4-5	5	
	16	4-5	4-5	4-5	4	4	4-5	
	17	5	4-5	5	4-5	4-5	5	
552								
553								
554	Ι	Dye 12 Dye 13	Dye 14	4 Dye 15	Dye	16	Dye 17	
555		Fig. 5. Application of	f synthesized	formazan dyes (1	12-17) on le	ather at 2%	depth	

551	Table 2 Fastness	properties (of synthesized	formazan dye	es (12-17)

3.7.2. Fixation and exhaustion properties

557 Fixation and exhaustion properties studies were done by the application of synthesized formazan 558 dyes (**12-17**) at the strength of 2% with the reference of dry crust weight of leather. Fixation and 559 exhaustion values are depicted in Table 3. Driving forces for the fixation of these dyes (**12-17**) to 560 the leather fabric are physical interactions (hydrogen bonding) and chemical interactions (ionic

⁵⁵⁶

561 bond) between them while for the exhaustion properties, the concentration of the applied play important role [37]. The percentage of exhaustion and fixation of 2% synthesized formazan dyes 562 (12-17) on leather fabric ranged from 92-97% and 90-98% respectively. Maximum fixation and 563 exhaustion properties (98 and 97 %) were shown by the metal complex dye of Chromium (15) 564 while the minimum was experimental with the un-metallized dye (12) that was 90 and 92% as 565 shown in Table 3. All the dyes were shown the good result as maximum % of fixation and 566 exhaustion properties have been observed. This is indicating that rapid diffusion of synthesized 567 568 formazan dyes molecules have been taken place into the leather fabric under the dying condition and parameters as there was found a strong physical and chemical interactions phenomenon 569 570 between the dye molecules and leather fabric as shown in Figure 6. Strong physical phenomenon was due to the presence of strong polar group i.e. (-SO₃H and -NO₂) within the synthesized 571 formazan dyes molecules (12-17) as shown in scheme 2 that is responsible for making the 572 573 hydrogen bonding with the functionality of leather as chemical nature of leather is a protein, polyamide and nylon [20,37]. As previous studies revealed that a number of polar groups within 574 the dyes molecules are responsible for increasing the physical instruction between dye molecule 575 and leather fiber [31]. While strong chemical interaction was due to the formation of ionic bond 576 between dyes molecule and leather fabric as synthesized formazan dyes (12-17) bearing a 577 negative (anionic) charge while leather carrying a positive (cationic) charge especially during the 578 dyeing process [37]. Ultimately as a result of these two factors, their exhaustion, as well as 579 fixation properties, increased. From the results of the application, it was concluded that formazan 580 metal complex dyes (13-17) have a high fixation and exhaustion properties in contrast to un-581 metallized formazan dye (12). Among metal complexes formazan dyes of Fe (13), Cu (14), Ni 582 (16) and Co (17), chromium metal complex formazan dye (15) shown the best properties. There 583

was found a close agreement between the results of fastness properties, fixation and exhaustion properties. As between these properties, there is found a direct relationship that depends upon the electrostatic interactions that is the factor by which rapid diffusion of dyes takes place to the substrate underneath depth [31,37]. More is the fixation and exhaustion, more will be the fastness properties and poor is the fixation and exhaustion, lower will be the fastness properties.

589 Table 3 Fixation and exhaustion properties of synthesized formazan dyes 12-17

	Shade on	$\lambda_{max}(nm)$	Fixation	Exhaustion
Dye #	Leather	in ethanol	%(C)	%(C)
12	Reddish Brown	440	90	92
13	Dark Bluish Brown	460	92	91
14	Blue	410	94	94
15	Bluish Beige	435	98	97
16	Greyish Beige	425	97	96
17	Greenish Blue	415	96	95



590

Fig. 6. Interaction phenomenon between synthesized formazan dyes (**12-17**) and leather fabric

592 **3.8 Antibacterial activity**

593 3.8.1 Antibacterial activity of synthesized formazan dyes (12-17) in solution

Synthesized formazan dyes (12-17) were screened for the determination of their antibacterial 594 potential against different bacterial strains (Gram positive and gram negative bacteria). 595 Preliminary results were indicated that all the synthesized formazan dyes (12-17) were effective 596 and strongly inhibited the growth of all bacterial strain. The clear zones of inhibition (ZOIs) for 597 the synthesized formazan dye 15 were shown in Figure 8 and their ZOIs values in against off all 598 bacterial strains (E. coli, S. aureus, Klebsiella and B. subtilis) are presented in Figure 7. 599 Synthesized un-metallized formazan dye (12) was shown the effective antibacterial activity 600 against all strains that was found in close agreement with the ZOIs value of standard drug 601 (Cephradine) but lesser in contrast to metal complex formazan dyes (13-17). As all metal 602 complexes formazan dyes (13-17) were manifested the significant and greater antibacterial 603 activity in comparison to employed standard drug and their respective un-metal complex dye 604 (12) as shown in Figure 7. Against of all tested bacterial strains, maximum antibacterial activity 605

606	was demonstrated by chromium complex formazan dye (15) with the ZOIs $(19\pm0.05 \text{ mm})$,
607	25±0.07 mm, 23±0.09 mm, 27±0.03 mm) among all formazan dyes complexes of Fe (12), Cu
608	(13), Ni (16) and Co (17). Their minimum inhibitory concentration (MICs) values were also
609	calculate in (mg/mL) against each bacterial strain and depicted in Table 4. Synthesized formazan
610	dye 15 was exhibited the 0.25, 0.07, 0.10 and 0.06 mg/ml of MICs against E. coli, S. aureus,
611	Klebsiella and B. subtilis respectively. All the dyes were shown the MICs value that was
612	comparable with the standard drug. From the ZOIs and MICs, it was evident that synthesized
613	formazan dyes are bactericidal as well as bacteriostatic in nature.

- **Table 4** Minimum inhibitory concentration (MICs) of synthesized formazan dyes (**12-17**) against
- 615 each bacterial strain

Bacterial	Minimum inhibition concentration (mg/mL)						
Strains	Standard	Dye 12	Dye 13	Dye 14	Dye 15	Dye 16	Dye 17
E. coli	0.30	0.35	0.27	0.28	0.25	0.29	0.27
S. aureus	0.10	0.12	0.09	0.10	0.07	0.08	0.11
Klebsiella	0.14	0.16	0.13	0.12	0.10	0.11	0.13
B. subtilis	0.08	0.09	0.07	0.06	0.06	0.07	0.08

616 Values are mean ± SD triplicate assays.



617

Fig. 7. Antibacterial activity of synthesized formazan dyes (**12-17**) in solution against different

619

bacterial strains



620

Fig. 8. Antimicrobial activity of synthesized formazan dye 15 determined by an agar well
diffusion method. Picture shows the inhibition zone produced by synthesized formazan dye 15
against *Klebsiella* (A), *B. subtilis* (B), *E. coli* (C) and *S. aureus* (D). N= Neutral, P=Sample and
T= Standard drug

3.8.2 Antibacterial activity of synthesized formazan dyes (12-17) on substrate (Leather fiber)

As the synthesized formazan dyes (12-17) have been shown the significant and imperative antibacterial activity in solution, it was thought worthwhile to study their antibacterial potential on dyed leather fabric. The leather fiber dyed with the synthesized formazan dyes (12-17) were

630 employed as model system. The results are shown in Figure 9. Maximum reduction in bacterial growth on the dyed leather fiber was observed with formazan dye of chromium complex (15) 631 while minimum was observed with the un-metallized dye (12) but all synthesized dyes (12-17) 632 were shown the reduction in bacterial growth comparable to that of standard drug, % reduction in 633 bacterial growth (56±0.03%, 65±0.07%, 70±0.05%, and 72±0.08%) observed by synthesized 634 formazan dye (15) while % reduction of (50±0.07%, 60±0.06%, 65±0.09%, and 68±0.05%) was 635 manifested by standard drug against E. coli, S, aureus, Klebsiella and B. subtilis respectively. 636 637 There is found a close agreement in the results of antibacterial activities in solution as well as on dyed leather by synthesized formazan dyes (12-17) and their findings are found to supportive to 638 639 each other. These results are confirmed that synthesized formazan dyes (12-17) are antibacterial as well as bacteriostatic in nature as they strongly inhibited the growth of tested bacteria in both. 640





642

Fig. 9. % reduction in bacterial growth by the synthesized formazan dyes (12-17)

The structural moiety which made the synthesized formazan dyes (**12-17**) to show significant and effective antibacterial activity is the presence of electron withdrawing group on the *para* position in the structure of these dyes as shown in scheme in 2. As previously studies revealed

that antibacterial activity depending on the presence and position of functional group within the molecular structure [38]. Electron withdrawing group enhances the antibacterial potential of the molecule. *Ortho* and *para* position are more favorable towards enhance antibacterial activity rather than *meta* position [39]. In our current research work, electron withdrawing groups (-SO₃H and -NO₂) are functionality that are present on *ortho* position within the structure of dyes molecules (**12-17**). As a result of this all dyes exhibited strong and significant antibacterial potential against all bacterial strains.

653 **4. Conclusion**

This study has successfully synthesized the un-metalized (12) and metal complex formazan dyes 654 (13-17) in water without using buffer and different solvents. Hence, color shades that developed 655 on leather fabric by novel formazan dyes (12-17) are intense and had excellent color properties 656 like color change fastness, staining on adjacent fiber fastness, light fastness, wash fastness, 657 perspiration fastness and better penetration to leather. Thus these dyes have such light properties 658 and enormous potential to be commercialize. Another significant finding of this study is the 659 remarkable antibacterial potential that was manifested by all the synthesized formazan dyes (12-660 17) is solution and on substrate. This study has also established that these synthesized novel 661 formazan dyes itself enhance the life of leather fabric by preventing it from bacterial effects and 662 663 no need to use addition antibiotics along with these dyes. Finally, we report that this research work will prove beneficial for the development of new industrial products. 664

665 **DECLARATIONS**

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669 *Conflict of interest*

670 No conflict of interest associated with this work.

671 *Contributions of authors*

The authors declare that this work was done by the authors named in this article. The authors Shakeel Ahmad Khan and Sadia Kanwal have done the experimental work and Sammia Shahid supervised the whole research work. Ghulam Hussain assisted in the characterization of new synthesized formazn dyes. All liabilities pertaining to claims relating to the content of this article will be borne by them.

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Metal Complexes of 4-(2-(((2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid based formazan dyes were synthesized with Cr (III), Co (III), Fe (II), Cu (II) and Ni (III).

The synthesized formazan dyes produced different coloration on leather from Blue, violet, Green, Brown to Red.

Excellent fastness, fixation and exhaustion properties were observed by the synthesized formazan dyes

Antibacterial activity of synthesized formazan dyes was determined in solution and on leather by agar well diffusion method.