SYNTHESIS AND CHARACTERIZATION OF NEW AZO DYE (1-(4-SULFONYL PHENYL AZO)-2-(7-CHLORO-4-[{4-(DIETHYL AMINO)-1-METHYL BUTYL}AMINO]QUINDINE FROM CHLOROQUINE DIPHOSPHATE AND STUDY ANTIBACTERIAL ACTIVITY

Layla Addnan

Department of physiology, College of Veterinary Medicinal,University of Basrah .Basrah Iraq.

(Received 14 September 2015, Accepted 2 November 2015)

Keywords; sulfanilic acid, FTIR, Azo dye

ABSTRACT

New azo dye was synthesized by reaction dizonium salt of sulfanilic acid with antimelaria drug (chloro quin diphosphate).This product was characterized by FTIR(Fourier Transform Infrared)andUV.Visible spectrophotometer .The antibacterial activities of the compound was studied and evaluated using gram positive and negative gram stain. The purity of the dye was checked by thin layer chromatography(TLC) using solvent system(sec.Butanol-water-acetic acid)(2:2:1).The melting point of the purified dye was measured in an open capillary tube.

We have been concluded that the prepared azo dye showed antibacterial activity against this micro organism.

INTRODUCTION

Azo dyes contain at least one nitrogen-nitrogen double bond (N=N); however many different structures are possible (1).Mono azo dyes only(N=N) double bond while diazo and triazo dyes contain two and three (N=N) double bonds ,respectively,the azo groups are generally connected to benzene and naphthalene rings ,but can also be attached to aromatic hetro cycles such as chloroquine(2).The side groups are necessary for imparting the color of the dye.Synthesis of most azo dyes involve diazotization of a primary aromatic amine followed by coupling with one or more nucleophiles.Amino and hydroxyl groups are commonly used coupling components(3).

Azo dyes acquired wide interest in application to biological system and indicator in complex ometric titratin of analytical chemistry (4),(5), Aromatic azo compounds especially are used as acid-base indicators , also used in biological strains and commercial colorants for clothing, plastics (6).Color changes are caused by change in extent of delocalization of electrons.More delocalization shifts the obsorption max to longer wave lenghths and makes the light absorbed redder,while less delocalization shifts the absorption max to shorter wave lengths(7).The azo dyes sulfon amide antibacterial drugs were the first effective chemotherapeutic agents that could be used systemically for the cure of bacterial infection in humans .A series of azo dyes containing the sulfonamide functional group were synthesized as potential antimicrobial agents (8).Sulfonamide was classified into three different types ; antibacterials that are aniline-substituted sulfonamide, prodrug that react to generate active sulfanilamide and non aniline sulfonamide.There are also other commonly used drugs that are azo dyes sulfonamides or sulfanilamide,the diuretic chlorthlidone and the oral hypoglycemic drug(tolbutamide).Today,ther are few sulfonamides and especially sulfonamide-trimethoprim combination that are used extensively for apportunistic infection in the patients with AIDS(9).

MATERIAL AND METHODS

Material: Chemicals used in the present studies some were sourced from Merck(India) and other were sourced from Fulka (swiss)

Method

1-Preparation and characterization of azo dye

a-Preparation of dizonium salt According to (10)

1-Place 1g of the sulfanilic acid (0.0058moles) in 5 ml of water and 2.5 ml of Conc HCl in abeaker and shake . Keep the beaker in Ice bath (5C°).

2-Dissolve 1g NaNO2 in 5ml of water and put it in Ice too.Add this solution dropwise to solution of sulfanilic acid keeping the temperature(0-5 C°)

b-Coupling reaction

1-Dissolve 3g of drug(chloroquindiphosphate)(0.005mole) in 10 ml of 10% NaOH.Keep this

solution also in Ice.

2- Now add the diazonium salt slowly to the cold solution of chloroquine raddish-orange dye is produced yield 2.4 g.

3-Fillter on a buchner funnel.The raddish –orange product was characterized by FTIR and UV. Visible spectrophotometer .The purity of the dye was cheeked by TLC using the solvent system (Sec.Butanol-water-acetic acid) (2:2:1).The melting point of the purified dye was measured in an open capillary tube.

2-Study of anti bacterial activity of dye.

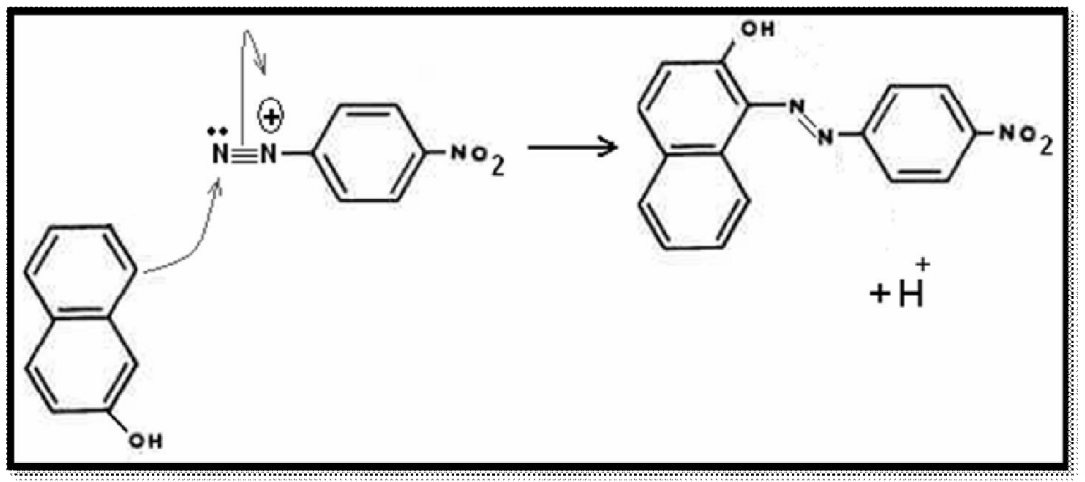
a-Bacterial strains used

Pure cultures of two bacterial strains *Staphylos coccus aureas*,*Echerichia Coli* used in the study were obtained from the culture collection ofMicrobiology Department in College of Veterinary Medicinal,University of Basrah.

b-Anti bacterial activity of dye .Sample was determined by the agar-well diffusion method(11).The test organism was swabbed on to the solidified Muller hinton agar medium there after 6mm dimeter well were punched in the agar plates prepare dye was added to the wells,the plates were then incubated at 37C° for 24h.After incubation the antimicrobial activity was evaluated by measuring the zone of inhibition.

RESULT AND DISCUSSION

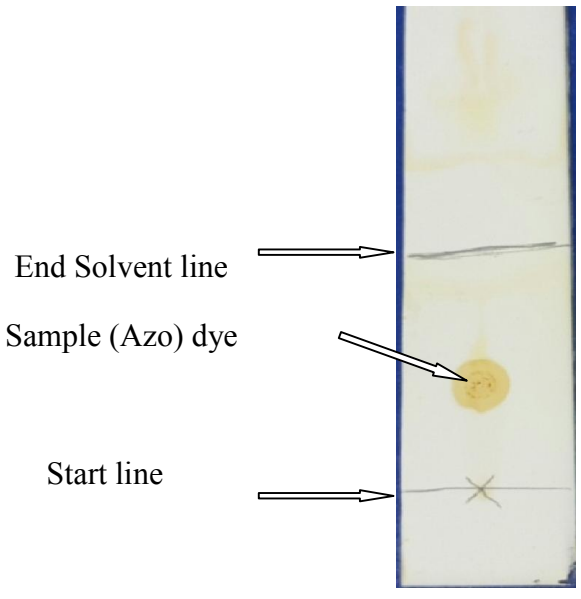
The synthesis of an azo dye requires two organic compounds dizonium salt and coupling component (flouro quine drug). The general synthesis of azo dye is shown below:



Scheme 1: Synthesis of azo dye

The dizonium salt reacts as an electrophile with an electron –rich coupling component,like B-naphthol and quindine derivative through an electrophilic aromatic substitution mechanism. The hydroxyl group (such as B-napthol or chloride\ group (such as chloro quine),direct the aryl diazonium ion to the para site unless that position is occupied, in which case the ion attaches ortho(12) .

The prepared dye [1-(4-sulfonyl phenyl azo)-2-(7-chloro-4-[{4-(di ethyl amino)-1-methyl butyl}amino]quindine] was obtained as amorphous powder raddish –orange color yield2.4g.The melting point of dye was estimated at 253-254 c°.The TLC results figure(1) showed that only single spot was observed that have rate flow(Rf) value 0.4.



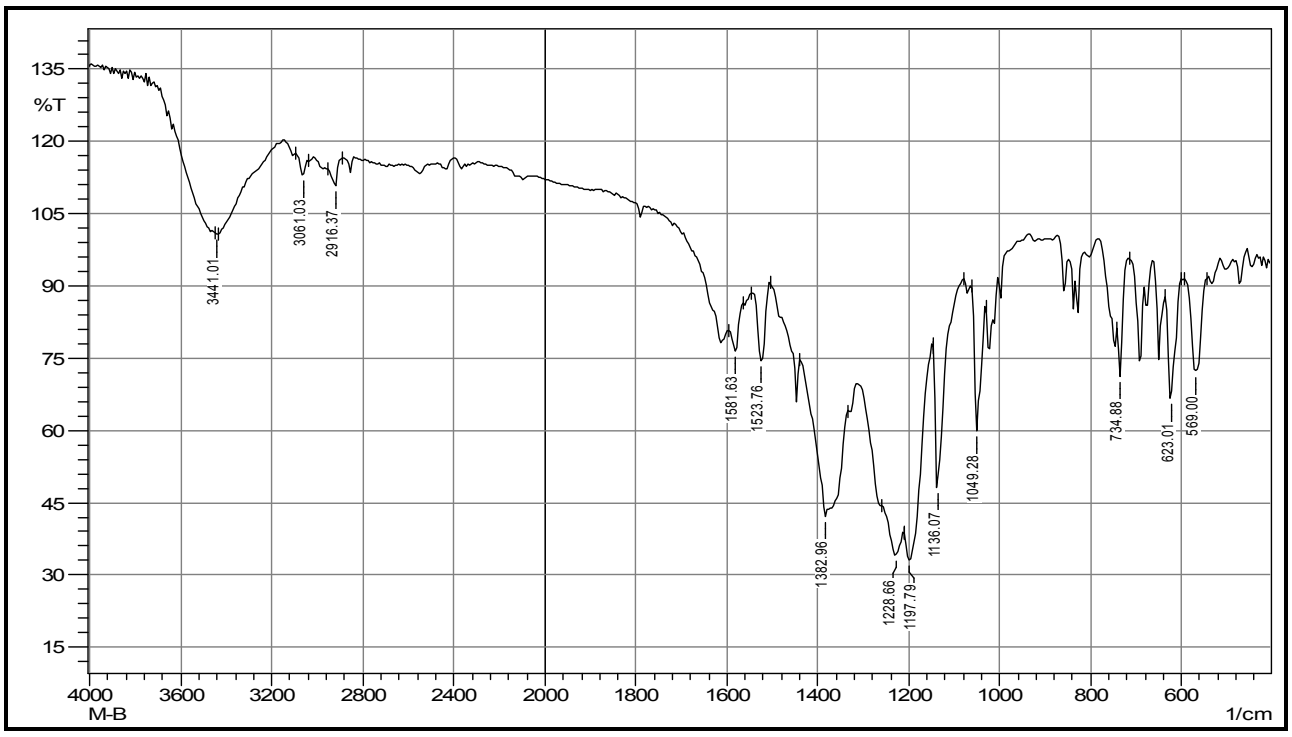
Figure(1) :Thin layer chromatography of prepared azo dye

The prepared product as azo dye was characterized by various available techniques.The infrared spectroscopy is one of the efficient techniques used in the characterization of organic compounds.IR spectrophotometers as FTIR which was used in the present study to confirm the presence of functional groups as following distinguish stretching vibration band of azo group (N=N) at 1523 cm^{-1}(13) as shown in figure(2).The stretching vibration of NH group appear at 3441.01cm^{-1}. The asymmetry stretching vibration of S-O(SO3-H) group appearance at 1197.79cm^{-1} position,while symmetry at 1049.28cm^{-1} position.Other peaks of the principle bonds are shown in Table(1) and figure(2).

Table(1) Major stretching vibration of absorption bonds by FTIR spectroscopy

Compound	Wave numbers(Cm^{-1})										
	V C=C aromatic	V S-O sym	V S-O asy	V.s CH alephatic	V.s N=N	V.s C-C aromatic	V.s C-N	V.s C-H aromatic	V.b NH	V.s NH	V.b C-H aromatic
Azo dye	1581.63	1049	1197.79	2916.37	1523	1382	1228.66	3061.63	623	3441	734

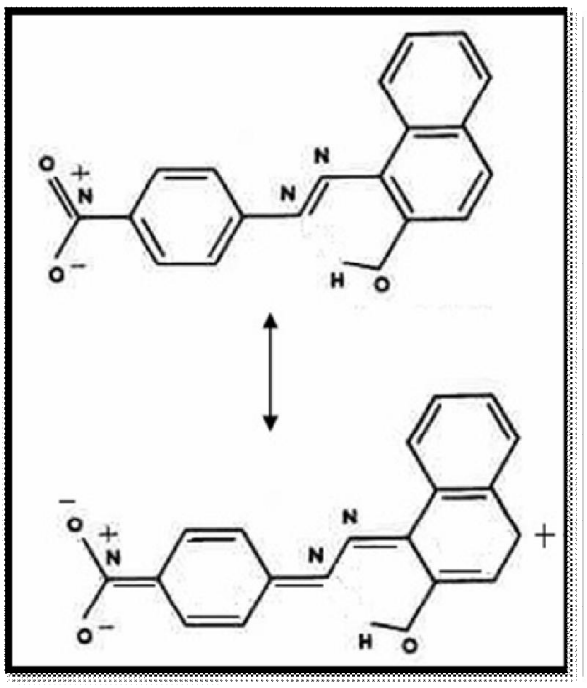
V= vibration ,s=stretching, sym=symmetric,asy=asymmetric,b=bending



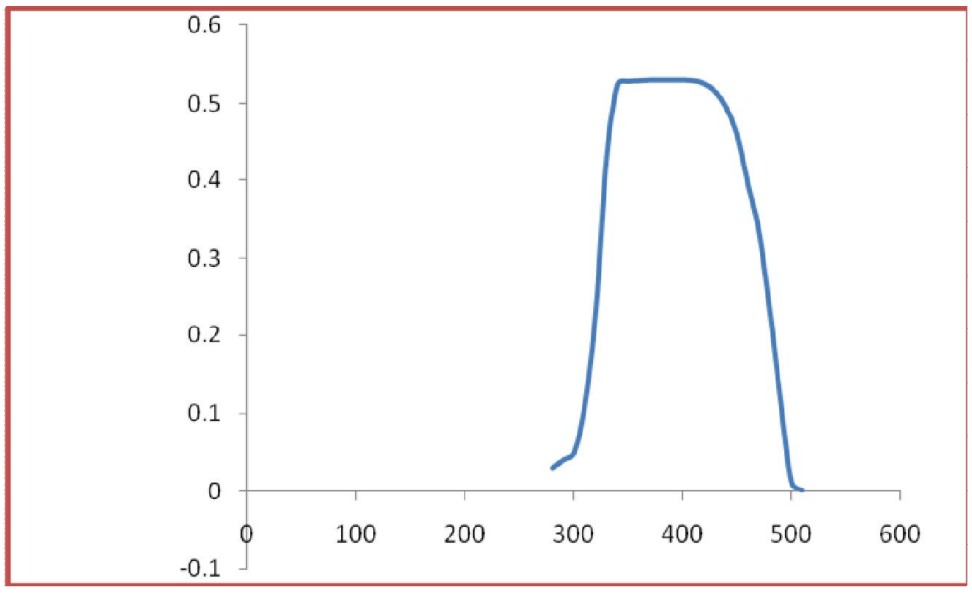
Figure(2):FTIR spectroscopy for azo dye compound

The U.V-Visible spectrophotometer study was showed position of transition in 430 cm^{-1}

Figure(3).The broader transition may be due to hyperconjugation system of the molecule:



Scheme 2: hyperconjugation system of azo dye



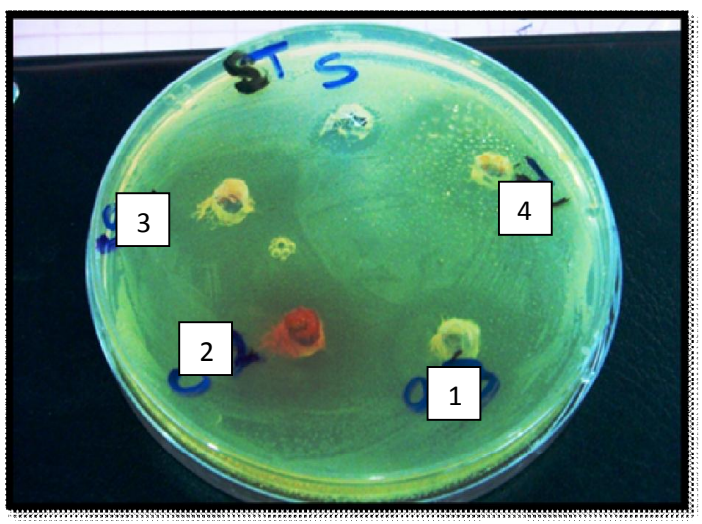
Figure(3): UV-Visible curves of azo dye

Anti bacterial activity of prepared dye.

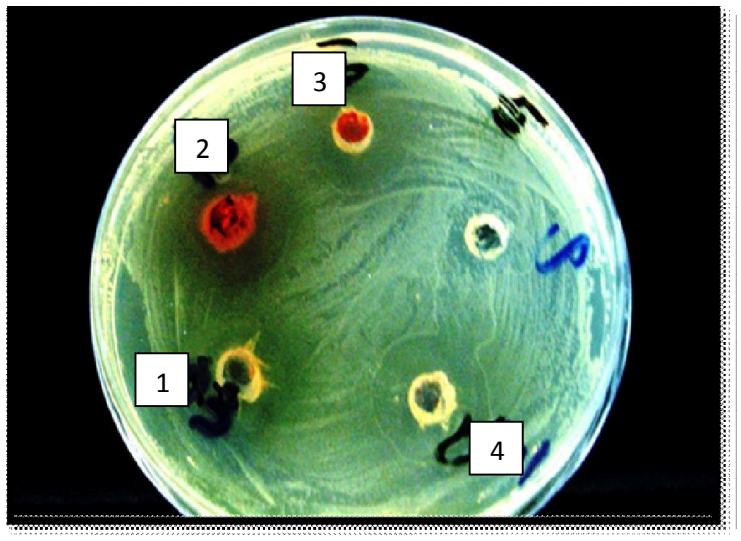
The biological activity against two types of both gram positive (*Staphylo coccus aureus)*and gram negative (*E.Coli*) micro organism were studied *Staphylo Coccus aureus* shows maximum zone of inhibition (14mm) followed by E.Coli (13mm) that are shown in figures (4),(5) and table (2).In the study of Saranya Devi ,2014 (14) ,showed the anti microbial activity of azo dye with inhibition zone(20mm)against gram positive which have highest antibacterial activity than gram negative (18mm).

Table(2) – Effect the azo dye against two types of bacteria

bacteria	Inhibition zone diameter(mm)			
	Concentration of azo dye (mg/ml)			
	300	200	100	50
Staph.aureus	14	12	9	3
E.coli	13	13	5	3



Figure(3) plate showing antibacterial activity by agar well diffusion method of dye against Staph .aureus at follow concentration:1=300mg/ml ,2=200mg/ml ,3=100mg/ml ,4=50mg/ml



Figure(4) plate showing antibacterial activity by agar well diffusion method of dye against E.coli at fallow concentration:1=300mg/ml ,2=200mg/ml ,3=100mg/ml ,4=50mg/ml

تحضير وتشخيص صبغة ازوية جديدة (1-(4- ازو سلفونات البنزيل)-2-(7- كلورو-4-[{-4(ثنائي اثيل امين)-1- بيوتيل المثيل} امين] كوينيدين من كلوروكوين ثنائي الفوسفيت ودراسة فعاليتها الضد بكتيرية.

ليلى عد نا ن

فرع الفسلجة،كلية الطب البيطري،جامعة البصرة.البصرة،العراق.

الخلاصة

تم تحضير صبغة ازوية جديدة عن طريق مفاعلة أملاح الدايزونيوم لحامض السلفانيليك مع عقاركلوروكوين ثنائي الفوسفيت المستخدم لعلاج مرض الملاريا . تم التشخيص الطيفي للصبغة المحضرة بمطيافية الاشعة تحت الحمراء ومطيافية الاشعة المرئيةوفوق البنفسجية. تم دراسة الفعالية المضادة للجراثيم للصبغة المحضرة بأستخدام الجراثيم الموجبة لصبغة غرام والسالبة لصبغة غرام .

تم التأكد من نقاوة الصبغة المحضرة بواسطة كروموتوغرافيا الطبقة الرقيقة وبأستخدام نظام التصعيد (البيوتانول الثانوي-الماء-حامض الخليك) وبنسبة(2:2:1). تم قياس درجة الانصهار للصبغة النقية بأستخدام انبوبة شعرية مغلقة من طرف واحد.

نستنتج من الدراسة ان الصبغة المحضرة ابدت فعالية بايولوجية تجاه الجراثيم قيد الدراسة.

REFERENCES

1-Hao OJ ,Kim H,Chang PC, Decolourization of waste water. Critical Rev.Environ. Sci.Technol.30,2000,PP:449-505.

2-K.Venkataraman,the chemistry of synthetic dyes,New York and London:Academic Press(1970).

3-J.Griffitths Recent developments in Colour and Constitution of Organic dyes Rev.Prog.Color.,Volume 11,1981,PP.37-57.

4-Hunger,K.,ed.Industrial Dyes.Chemistry,properties Applications,Weinheim:Wiley –Vch.2003.

5-Golkak,Koppss,Myslak ZW Carcinogenicity of azo Colorants:influence of solubility and bio avoil ability151(1),2004,PP203-210.

6-Zollinger,H.,.Color chemistry synthesis, properties and Application of organic dyes and pigment,VCH Publishers,New York,1987,92-102.

7-Zolinger H.,. Color chemistry Syntheses properties ,Application of organic dyes and pigments ,VCH Wiley, (2003), thirdrevised edtion.

8-Wilson and Gisvolds,Text book of organic medicinal and pharmaceutical chemistry,11th Ed.,Lippincott,USA,2004,269.

9-Dixit,B.C.,Patel,H.M.and Desai,D.J.2007.,Synthesis and application of New mordant and disperse azo dyes on 2,4-dihydroxy benzophenone,J.Serb.Chem.Soc.,72(1),110-127.

10-M.Gur,H.Kocaokutgen and M.Tas,Dyes pigments,72(2007)101.

11-Chung,K.Tand Cerniglia,C.E.,Mutagenicity of azo dyes:Structure-activity relation ships.Mutat.Res, 77, 1992 ,201-220.

12-KucharskiSandJanikR,1999.,Trans-Cis Isomerisation of azobenzene amphiphiles containing asulfonyl group ,New.J chem,23,765.

13-Silverstein RM and Webster FX,Spectrometric Identification of organic compounds,6th Ed. John Wiley &Sons.Inc.,1999.

14-Saranya Devik,Sruthy.P.B.Anjance.J.C,J.Rathinamala and S.Jayashree (2014).Study on antibacterial activity of natural dye from the bark of Araucaria Columnaris and its' application in Textile cotton fabrics.USA.J.Microbiol,Biotech .Res,4(3):32-35.