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Synthesis and Characterization of New Azo Dye Derived from Sulfamethaxolevia Diazonium Reaction and Study its Ability as an Acid-base Indicator

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ABSTRACT: ***Background:*** *Most azo dyes are synthesized by diazotization of a primary amine, followed by conjugation with one or more electron-rich nucleophiles.* ***Objective:*** *This study presents the synthesis of a new azocompound by converting sulfamethoxazole to a diazonium salt.* ***Methods:*** *via reaction with hydrochloric acid and sodium nitrite at temperatures between 0 and 5 °C, the resulting salt is then coupled with a 4-methoxyphenol reagent in alkaline medium at the above temperatures to produce an azodye given the IUPAC name of (4-((2-hydroxy-5-methoxyphenyl)diazenyl)-N-(5-methylisoxazol-3yl)benzenesulfonamide). The azo dye precipitate was purified by crystallization methods using a solvent mixture of ethanol water. Characterization was done using different analytical methods such as IR, UV-Vis and H1-NMR in addition to measurements of physical properties.* ***Results:*** *The reddish-pink azo compound gave a maximum absorbance of 495 nm. Acid-base titrimetric analysis was conducted to examine the chemical indicator properties of the azo dye. Clear color differences were obtained between two spaces in the pH range 2-12, where a reddish-pink color was obtained in the basic medium and a yellow color in the acidic medium.* ***Conclusions:*** *A new azo dye successfully presented its properties as an indicator.*

KEYWORDS: *Sulfamethoxazole; 4-methoxy phenol; Indicator; Azo dye; acid-base indicator*

INTRODUCTION

Sulfamethoxazole(SMZ) is a member of the sulfonamide family of antibacterials and the chemical name is 4-Amino-N-(5-methyl-3-isoxazolyl)-benzene sulfonamide [1]. It is one of many antibiotics[2]. A survey of the literature showed that different techniques have been reported for SMZ analysis, some of which include spectrophotometric methods [1], for instance, HPLC [3], electrochemical methods [4] an solid phase extraction [5]. Diazotization method is simple and inexpensive method. Azo dyes are very important dye families, containing the –N=N- band [6], and are the broadest group of dyes for their widespread applications in many areas of textile, medicine, and a variety of paints including artist's paints. It has excellent coloring properties that stabilize dyes in acidic and alkaline media, absorbs visible frequencies of light being colored compounds from the yellow to red range, in addition to color stability, and is a non-toxic and environmentally friendly compound. Dyes are usually large aromatic molecules consisting of many connected rings attached to vinyl groups, which are more stable [7]–[9]. Most azo dyes are synthesized by diazotization of a primary amine followed by conjugation with one or more electron-rich nucleophiles such as amines or hydroxyl [10], [11]. Indicators are acids or weak bases, used to show the end point, equivalence, or neutralization point of a reaction,and added in small quantities to a solution that shows a sharp color change, in response to

a change in pH in a titration (i.e., acid-base) analysis [12]. The analysis is performed by determining the volume of a solution of an accurately known concentration that is required to react quantitatively with the measured volume of a solution of the substance to be determined [13]. Today, more than two hundred known indicators are available that operate within the pH range and range from a strong acid to a strong base in aqueous solutions. Some indicators show only two colors while others exhibit a wide range [14]–[17]. The indicator is of two types;the first, is the natural indicator, which can be isolated from a variety of plants (i.e., fungi and algae). For example, almost all flowers, red, blue or purple, contain a number of organic pigments called anthocyanins that change color with changing pH [18]. The mechanism of action of the color indicator can generally be formulated as follows:

$$\mathrm{HInd} + \mathrm{H_2O} \longrightarrow \mathrm{H_3O^+} + \mathrm{Ind^-} \tag{1}$$

Here HInd stands for the acidic form and Ind− for the basic form, Henderson-Hasselbalch equation can be written as follows [19]:

$$\mathrm{pH} = \mathrm{pK_a} + \log_{10}\left(\frac{[\mathrm{Ind^-}]}{[\mathrm{HInd}]}\right) \tag{2}$$

Secondly, industrial indicators such as phenolphthalein and methyl orange, are expensive and show some toxic effects that cause cancerous diseases and damage to the skin and lungs [20]. The aim of this study was to synthesize azo dye that has indicator properties, easy to prepare, inexpensiveand environmentally friendly, using a pure drug. The synthesized azo dye gave a clear and identical endpoint when compared with standard indicators such as phenolphthalene, and is therefore used for qualitative analysis.

MATERIALS AND METHODS

Instruments: The prepared azo dye was characterized using various techniques including uncorrected Electrothermal-9100, Cintra-5- UV-Visible Spectrophotometer, FTIR-8400S SHIMADZU, NMR1.JDX 60.46 MHz Spectrophotometer.

Chemicals: All chemicals were of analytical grade from Merck (Germany). The compound SMZ was supplied by the general company for the manufacture of medical supplies – quality control laboratory (Samarra).

General procedure (Diazotization)[6]: To prepare the azo dye, 1.30 g of SMZ (0.005 mol) was dissolved in 5 mL of distilled water and, to acidify the solution, 0.25 mL of 12 M hydrochloric acid was gradually added and shaken on ice (5 ˚ C).In order to form diazonium salt, 0.35 g $NaNO_2$ dissolved in 5mL of water was then added to the mixture. On the other hand, 0.62 g of 4-methoxyphenol(0.005 mol) was prepared in an alkaline medium of 10% NaOH (0.5 mL) which was added drop-wise to the SMZ solution with continuous stirring and a temperature range of (0-5 ˚ C).The prepared azo dye had a reddish-pink color with maximum absorbance at 495 nm. The precipitate was then filtered, washed with cold water, dried and then purified using a mixture of ethanol-water solvent prior to FT-IR and NMR analyses.

Titration analysis (indicator examination) [21]: In order to examine the properties of the prepared azo dye as an indicator, a solution was prepared by dissolving 50 mg with 10 mL of ethanol and completed with distilled water in a 100 mL volumetric flask. The indicator solution was kept in a dark place, away from light and heat. Two titration analyses were carried out using this indicator, one for a strong acid with a strong base and the other for a weak acid with a strong base (0.1 M HCl versus 0.1 M NaOH, and 0.1 M CH_3COOH versus 0.1 M NaOH) to which 5 drops of the prepared indicator was added. The color changes of the solution are presented in Table 1.

Table 1. Indicator colors during titration analyses

Alkaline medium	Acidic medium
0.1 M NaOH Reddish-pink	0.1 M HCl yellow
0.1 M NaOH Reddish-pink	0.1M CH3COOH yellow

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RESULTS AND DISCUSSION

The azodye synthesis involved diazotizing the SMZ with the phenolic reagent 4-methoxyphenol in an alkaline medium which yielded a reddish-pink dye that gave a absorption at the maximum wavelength of 495 nm. The reaction scheme and spectra of the prepared azodyeare presented in Figure 1 and Figure 2.

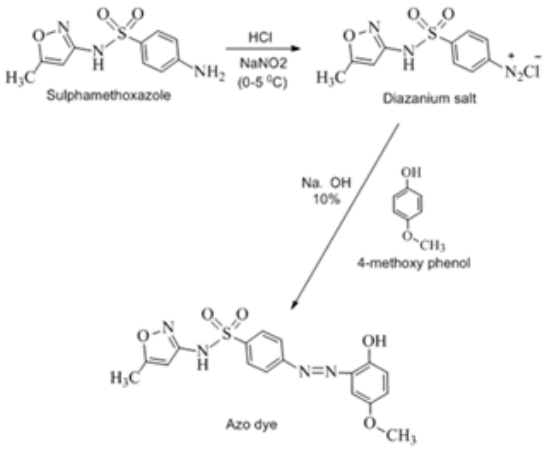

Figure 1. Reaction scheme of the synthesized azo dye

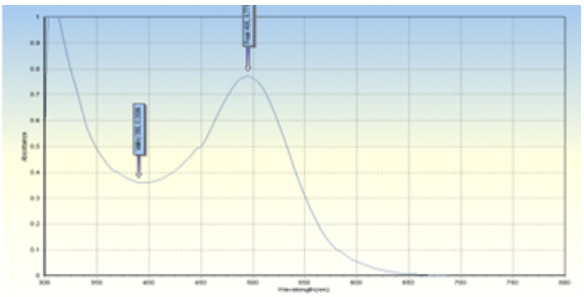

Figure 2. UV-Vis spectrum of the azo dye (50 mg/mL)

The percentage yield of the new reddish-pink dye and the melting point were 84% and 220 ˚C respectively. The physical properties of the synthesized dye are shown in Table 2.

Table 2. Physical properties of the synthesized azo dye

Color	Melting point	Yield %	Elemental analysis				Eluent	Suggested formula
			C%	H%	N%	S%		
Reddish-pink	220 °C	84%	43.3 44.1	3.3 3.2	18.9 18.4	14.4 13.9	MeOH-H_2O	C17H14N4SO4

The synthesized azo dye was characterized using spectroscopic methods, including FT-IR analysis. This is utilized to demonstrate the existence of the functional groups as shown in Table 3 and Figure 3.

Table 3. UV-Vis and FTIR analyses of the synthesized azo dye

λ max (nm)	νO-H (cm^{-1})	νN-H (cm^{-1})	νC=C in plane skeletal vibrations (cm^{-1})			νN=N (cm^{-1})	νC=N (cm^{-1})	νC-H (cm^{-1}) Ar	νC-H (cm^{-1}) Al	νC=O (cm^{-1})	S=O
495	3309	3198	1587	1556	1537	1456	1633	3151	2935	1772 1714 1654	1305

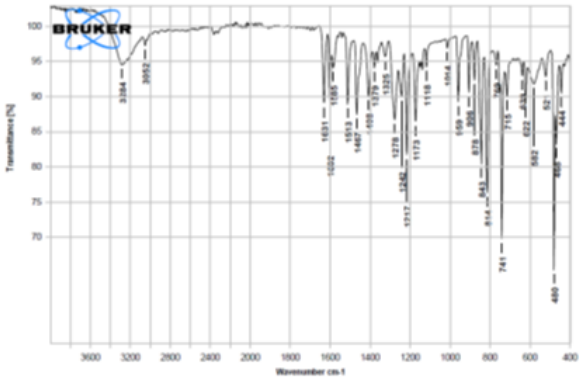

Figure 3. FT-IR spectrum of the synthesized azo dye

As H^1-NMR analysis, the azo dye gave characteristic values as follows: 7.5-8.3 ppm (Multiplet, 8H,Ar-H), 1.9 ppm (Singlet, 3H, Ar-CH3), 2.1 ppm(Singlet, 3H, OCH3), 6.79 ppm (Singlet, 1H, OH), 9.7 ppm (Singlet, 1H, NH), shown in Table 4 and Figure 4.

Table 4. H^1-NMR analysis of the synthesized azo dye

Singlet-ppm	Hydrogen	Assignment
7.55-8.3	8H	Ar-H aromatic
2.1	3H	-OCH3
1.9	3H	Ar-CH3
9.73	1H	N-H
6.79	1H	O-H

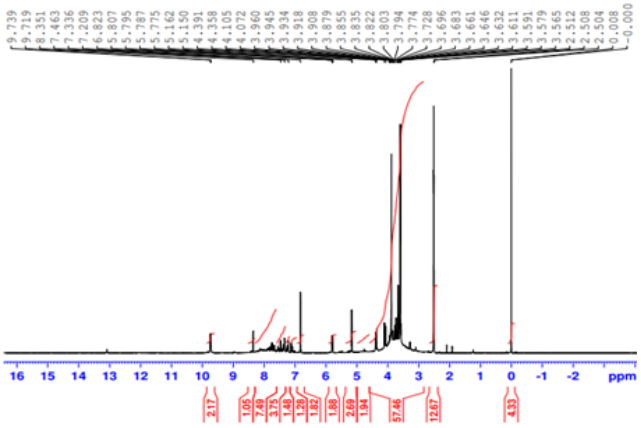

Figure 4. H^1-NMR spectrum of the synthesized azo dye

The curve in Figure 5 represents the absorbance versus pH value of the azo solution at λmax of

495 nm [22].

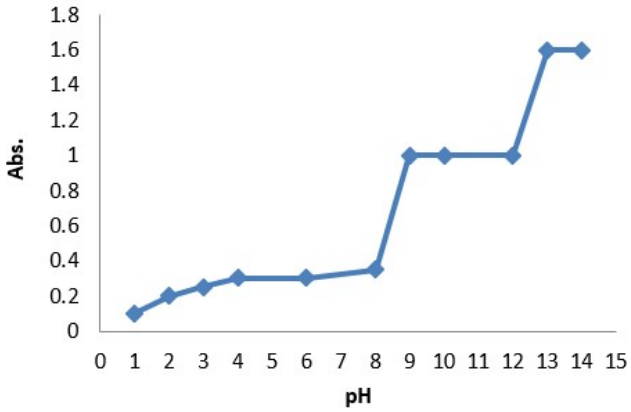

Figure 5. pH effect on the synthesized azo dye

UV-visible spectral measurements of the synthesized azo are considered over a wide pH range (2–12). NaOH, HCl (0.1N) solution were used to adjust the pH value of azo. A series of solutions with different pH are prepared and measurements are made using a UV-Vis spectrophotometer [23], as shown in Figures 6, 7.

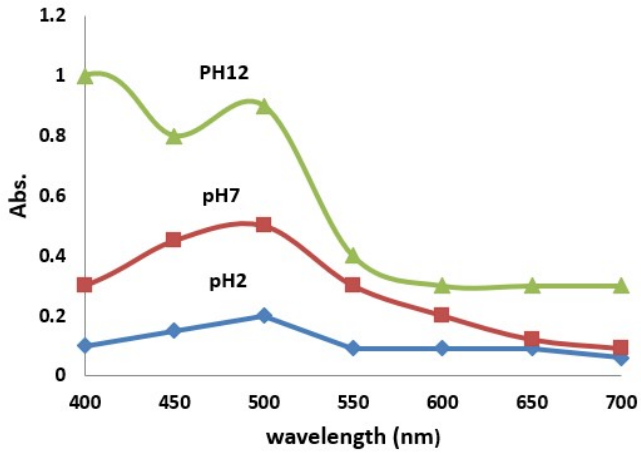

Figure 6. UV-Vis spectrum of the synthesized dye at different pH

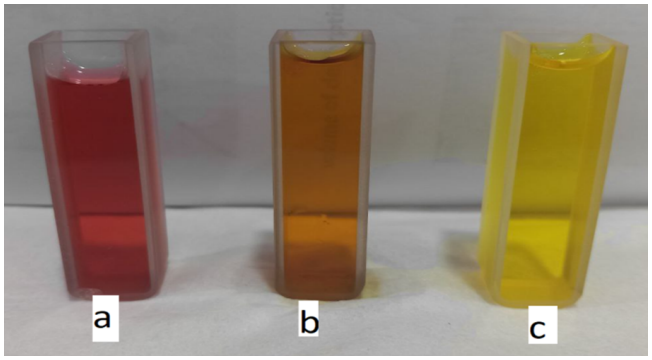

Figure 7. Color of the synthesized azo dye as an indicator in different media: (a) alkaline, (b) natural and (c) acid

Similarly to the phenolphthalein indicator, the synthesized azo dye does not cause an obvious color change when titrating a weak acid against a weak base due to its short reaction time, as shown in Figure 8.

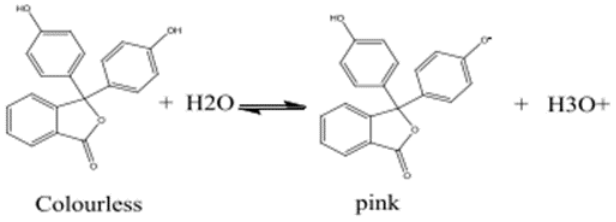

Figure 8. Ionization of phenolphthalein as indicator in acidic and alkaline media

CONCLUSION

The indicator is one of the most important chemical compounds that has important uses in determining and estimating concentrations of unknown chemicals. In this study, an environmentally friendly, simple, and inexpensive green method for preparing a new azo dye was performed using the diazonium reaction of SMZ with a phenolic dye reagent that yielded a colored dye compound that absorbed at 495 nm. Various analytical techniques have been applied to characterize the newly synthesized azo dye including UV-Vis, FT-IR, H^1-NMR in addition to other physical properties measurements. The new dye had a reddish-pink color in an alkaline medium and yellow in an acidic medium. The prepared dye was then applied as an indictor to test 20 samples by titration analysis (acid-base titration). The results showed that the newly synthesized dye gave accurate measurements as compared to a standard indicator. It was measured in different ways, including UV-Vis , FTIR, H^1-NMR. New dye gave a reddish -pink color in an alkaline media and yellow in an acidic media. The new azo dye has an important practical effect in the industrial and practical fields, where a test for this indicator was carried out using 20 samples by titration of the most important reactions (acid-base titration), which is an essential point for knowing and determining the concentrations of the chemicals being dealt, also it gave high conformity and efficiency with the standard indicator.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology and software Marwa Sabar Falih; validation, formal analysis, and investigation Neda Ibrahim Mahdi; resources, data curation and writing original draft preparation Ala'a Abdull wahid Jasim; writing review, editing, visualization, supervision, and project administration Ruba Fahmi Abbas ; funding acquisition Abdul Adheem Abdul Abbas Rahi.

FUNDING

None.

DATA AVAILABILITY STATEMENT

Data is available in the article.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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