Research Article

Synthesis, Spectroscopic Characterization, Solvent Effect, and Acid–Base Indicator Properties of Novel Niclosamide-Derived Azo Dyes

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ABSTRACT

Three new azo dyes (1–3) were effectively synthesized by use of a route starting from the anthelmintic drug niclosamide, with reduction of the nitro group followed by diazotization and coupling reaction with 1-naphthol, 2-naphthol, and 2-hydroxybenzoic acid, respectively. FT-IR, ^1H NMR, mass, and UV-visible spectroscopy confirmed the chemical structures of the dyes. The dyes showed maximum absorption wavelengths around 330 nm. It was found that the dye's absorption spectrum is influenced by the solvent polarity significantly. Systematic studies of their acid–base properties over the pH range 2–12 showed spectral shifts in the absorption maxima and isosbestic points associated with protonation/deprotonation. Protonation/ionization constants of the studied compounds were estimated by the half-height method; thereby, the acid–base behavior of the compounds was established. It should be noted that the synthesized dyes were stable in solution for 24 hours and changed their color reversibly depending on the acidity of the medium. Titration of the solutions of these compounds in acidic/alkaline media with HCl and NaOH solutions was performed. It was shown that their color change is appropriate for an acid–base indicator, and it was similar to that of phenolphthalein. These findings demonstrate that the synthesized niclosamide-derived azo dyes are stable, readily prepared, and potentially useful as novel acid–base indicators for analytical applications.

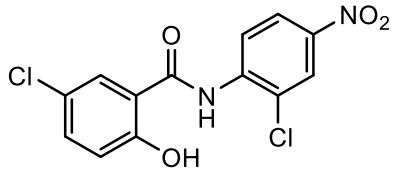
Keywords: Azo dyes, Synthesis, characterization, solvent effect, ionization and protonation constants, Niclosamide.

INTRODUCTION

Azo compounds remain one of the major family of synthetic dyes, renowned by their structural diversity[1, 2], high molar absorptivity, easy preparation methods, and particular physicochemical properties[3]. The existence of the azo bond (–N=N–) in such compounds causes the upsurge of conjugation within molecules, turning their colors strong and modifiable[3–5]. As a result, azo dyes begin to be generally used in various industries, including medicinal chemistry, analytical chemistry, textile coloring, optics, sensors, and as signals of acid-base reactions[6, 7]. In the analytical chemistry domain, azo dyes are becoming particularly important because of their potential to modify the electronic structure of molecules. This possibility allows the realisation of noticeable color changes at different pH values of the solutions[8, 9].

In the field of volumetric analysis, acid–base indicators play a vital role since they provide color changes close to the point of equivalence in acid–base titration[10, 11]. In addition, even though there is an extensive number of artificial indicators available in the market, future research endeavors will seek to discover newer compounds that have better color changes, greater pH ranges, improved stability, and simple and eco-friendly synthetic methods[12, 13]. Substitution of electron-donating/withdrawing groups in azo chromophores can be a promising method to modify their acid-base characteristics[14].

Niclosamide is a known salicylanilide compound that has been in use for many years as an anthelmintic[15–17]. Apart from being used as an effective drug, niclosamide has a chemical structure which contains groups that can be chemically altered, such as a nitro group, which can be easily reduced to give a primary aromatic amine. Reduction to a primary amine allows the compound to undergo a diazotization reaction, followed by azo coupling with phenols to form azo dyes, which have good conjugation with improved optical and analytical properties. Niclosamide has undergone many pharmaceutical studies, but few studies have been conducted on the use of this compound as a precursor for azo dye synthesis[18, 19].



Niclosamide ($C_{13}H_8Cl_2O_4$)

The preparation of azo dyes usually occurs by diazotation of aromatic amines, then electrophilic coupling of the product to an activated aromatic substance such as a phenol or naphthol[20]. The coupling agent is responsible for determining the electronic structure of the azo dye, which affects its absorption spectrum, solvatochromism, protonation-deprotonation reactions, and its color transition characteristics. A comprehensive analysis of these properties is therefore required to evaluate the applicability of new azo dyes to analytical chemistry[21].
In recent years, considerable attention has been directed toward the development of novel azo dyes exhibiting improved spectroscopic characteristics, enhanced stability, and reliable acid–base indicator performance. Nevertheless, information concerning niclosamide-derived azo dyes, particularly their solvent-dependent spectral behavior, protonation and ionization equilibria, and suitability as acid–base indicators, remains limited. Addressing these aspects would contribute to the development of new functional chromophores for analytical chemistry.
In the present research work, three new azo dyes, prepared from the reduced form of niclosamide using the diazotization process coupled to 1-naphthol, 2-naphthol, and 2-hydroxybenzoic acid, were prepared. These new compounds were studied using FT-IR, UV-Vis spectroscopy, ^{1}H NMR spectroscopy, and mass spectroscopic analysis. The studies on electronic absorbance of the compounds in various polarities of solvent systems, their acid-base nature, ionization/protonation constants, and stability of colors were performed. Moreover, the use of dyes as an acid-base indicator was tested using the normal acid-base titration method in comparison with phenolphthalein indicator.

EXPERIMENTAL

The chemicals and reagents purchased from companies such as Merck, BDH, Sigma-Aldrich, and Fluka, were of analytical grade unless otherwise stated. The melting points were measured using a Thermo Scientific SMP10 melting point apparatus, which was not corrected. Fourier transform infrared spectroscopy (FT-IR) spectra were measured on a Shimadzu FT-IR-8400S spectrophotometer with KBr discs over the wave number range 400-4000 cm^{-1} at room temperature. The UV-visible absorption spectra were measured using a Biotech UV-9200 UV/Vis spectrophotometer (Biotech Co. Ltd., UK). The pH values were measured with a BP3001 Professional Benchtop pH Meter.
The proton NMR spectra (^{1}H NMR) were measured by using a Bruker 400 MHz spectrometer using DMSO-d_6 as a solvent with TMS as an internal reference. FT-IR, UV-Vis, pH, and ^{1}H NMR measurements were carried out in the Department of Chemistry, College of Education for Pure Sciences, University of Basra, Iraq. The mass spectra were measured in electron impact (EI) mode at 70 eV on an Agilent 5975C VL MSD at the University of Tehran, Iran.

Reduction of the nitro group in Niclosamide

Niclosamide (pure, 1.60 g) was dissolved in a mixture of ethanol and acetone (1:1 ratio by volume) in 15 mL. The solution was further diluted to 25 mL in a volumetric flask with the same solvent mixture. A portion of the solution (5.0 mL) was placed in a reaction flask. Subsequently, 1.0 g of zinc powder and 5.0 mL of 1.0 M HCl were added to the solution. Heating in a water bath for 15 minutes at 90 °C resulted in full reduction of the compound. After cooling the mixture in an ice bath, the mixture was filtered to obtain zinc as the precipitate, which was then rinsed with distilled water.

Synthesis of Azo Dyes 1-3

Preparation of azo dye 1-3 was achieved using a diazotization-coupling reaction according to the protocol described in the literature, but with slight changes [34]. Briefly, 50 mL of the reduced niclosamide was made acidic by the addition of 1.0 mL of conc. hydrochloric acid, and the reaction was maintained at less than 5 °C with an ice bath. Sodium nitrite (0.003 mol, 0.22 g) in distilled water (5 mL) was added dropwise while constantly stirring. The reaction mixture was maintained at less than 5 °C and stirred for 30 minutes to ensure complete formation of the corresponding diazonium salt.
This newly made diazonium compound was slowly added to an alkaline solution with a suitable coupling agent (0.005 mol) in 100 ml of aqueous sodium hydroxide (0.003 mol, 0.90 g). The following were used as the coupling agents: 1-naphthol as 1, 2-naphthol as 2, and 2-hydroxybenzoic acid as 3. The reaction was allowed to stir for 1 hour, followed by keeping at 4°C for 24 hours. After performing the synthesis process, the product obtained was acidified using dilute HCl for the precipitation of the corresponding azo dyes. The precipitate obtained was

filtered, washed with distilled water, and then purified by recrystallization from a suitable solvent to obtain pure azo dyes 1-3.
The physical characteristics of the synthesized azo dyes are summarized in Table 1, while the synthetic process for the preparation of azo ligands 1–3 is shown in Scheme 1.
(E)-5-chloro-N-(2-chloro-4-((4-hydroxynaphthalen-1-yl)diazenyl)phenyl)-2-hydroxybenzamide. 1
Brown, yield: 63%; 180-182 ºC, IR (KBr) cm^{-1}, v: 3261 OH, 3086 $(C–H)_{Ar}$, 1606 C=C, 1680 C=O, 1508 N=N, 1H-NMR (400 MHz, DMSO-d6) δ (ppm): 9.13 (s, 1H, OH/NH), 9.00 (s, 1H, NH/OH), 8.90-8.87 (d, 1H, Ar-H), 8.36 (s, 1H, Ar-H), 8.21 (d, 2H, Ar-H), 8.03-8.00 (m, 1H, Ar-H), 7.74 (t, 1H, Ar-H), 7.56-7.51 (m, 2H, Ar-H), 7.34 (d, 1H, Ar-H), 7.11 (s, 1H, Ar-H), 6.91 (d, 1H, Ar-H), MS (EI) m/z= 302.31$[M+H]^+$.
(E)-5-chloro-N-(2-chloro-4-((2-hydroxynaphthalen-1-yl)diazenyl)phenyl)-2-hydroxybenzamide. 2
Red-orange, yield: 63%; 170-172, IR (KBr) cm^{-1}, v: 3184 OH, 3093 (C–H)Ar, 1681 C=O, 1606 C=C, 1510 N=N, 1H-NMR (400 MHz, DMSO-d6) δ (ppm): 12.00 (br s, 1H, OH), 8.85 (s, 1H, NH), 8.82 (d, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 8.27 (d, 1H, Ar-H), 7.93 (d, 2H, Ar-H), 7.47 (m, 3H, Ar-H), 7.04 (d, 2H, Ar-H), 6.42 (s, 1H, Ar-H), MS (EI) m/z= 302.31$[M+H]^+$.
(E)-5-chloro-N-(2-chloro-4-((2-hydroxynaphthalen-1-yl)diazenyl)phenyl)-2-hydroxybenzamide. 3
Yellow, yield: 63%; m.p. > 300, IR (KBr) cm^{-1}, v: 3254 OH, 2914 (C–H)Ar, 1627 C=O, 1600 C=C, 1489 N=N, 1H-NMR (400 MHz, DMSO-d6) d (ppm): 8.95 (d, J = 9.4 Hz, 1H, Ar-H), 8.28 (d, J = 2.8 Hz, 1H, Ar-H), 8.13 (d, J = 2.8 Hz, 1H, Ar-H), 7.72 – 7.64 (m, 2H, Ar-H), 7.06 (s, 1H, Ar-H), 2.49 (s, 2H, CH2 or residual solvent peak), MS (EI) m/z= 302.31$[M+H]^+$.

Table 1. The physical properties of the prepared azo dyes 1, 2, and 3.

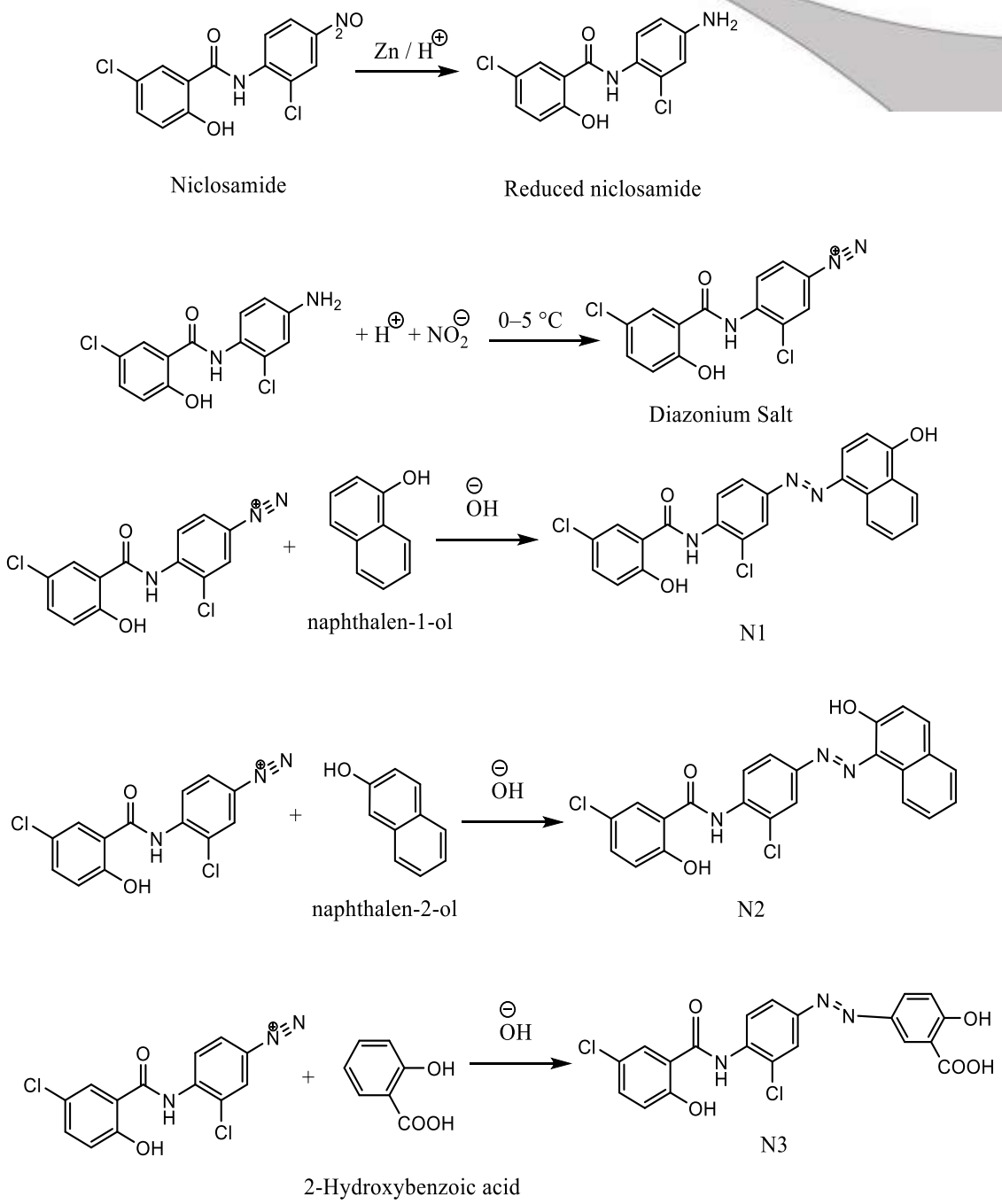
Symbol of Dye	Molecular Formula	Molecular Weight (g/mol)	The Color	Melting Point (^{0}C)	λ max
1	$C_{23}H_{15}Cl_2O_3$	452.29	Brown	180-182	330 nm
2	$C_{23}H_{15}Cl_2O_3$	452.29	Red-orange	170-172	330 nm
3	$C_{20}H_{13}Cl_2O_5$	446.24	yellow	> 300	330 nm

Determination of the solvent effect
The polarity of the solvent effect on the UV-Visible absorption spectrum of 1, 2, and 3 dyes, was assessed by making solutions of each dye in solvents with different polarities, namely 1,4-dioxane, chloroform, methanol, water, acetone, ethanol, and DMSO, at a concentration of 1.0 x 10^{-4} M. The spectra were recorded at wavelengths between 300 nm and 500 nm using a 1-cm quartz cell at ambient temperature. The pure solvent used as the blank for each sample was taken as per the method already reported earlier [22].

Determine the ionization and protonation constants
The values of the ionization constant and the protonation constant, along with the isosbestic points of the dyes 1, 2, and 3, were measured using UV–Vis spectroscopy. A series of universal buffers from pH 2.0 to 12.0 was prepared, and the buffer was added to the dye in order to obtain a final concentration of 1.0 × 10^{-4} M of the dye. The absorbance spectra were recorded in the range of 300–500 nm using a 1 cm quartz cell at room temperature, and the buffer solutions at the respective pH were used as blanks. As described in the literature [23], the half-height (half-absorbance) method were used to calculate the ionization (pKa) and protonation constants.

RESULTS AND DISCUSSION

Chemistry
Three novel niclosamide-derived azo dyes (1–3) were successfully synthesized through a multistep synthetic route involving reduction of the nitro group of niclosamide to the corresponding aromatic amine, followed by diazotization and azo-coupling reactions with 1-naphthol, 2-naphthol, and 2-hydroxybenzoic acid, respectively. The adopted synthetic procedure yielded the desired azo derivatives with good purity, providing an efficient approach for preparing highly conjugated chromophoric systems. As shown in the scheme below:



Scheme 1. Synthesis route of the synthesized azo dyes 1-3

The synthesize dyes chemical structures were successfully established by complementary spectroscopic techniques. FT-IR spectroscopy verified the formation of the azo linkage through the appearance of the characteristic –N=N– stretching vibration together with the expected absorption bands corresponding to the hydroxyl, amide, carbonyl, and aromatic functional groups. The 1H NMR spectra further supported the proposed structures by displaying the characteristic resonance signals of aromatic and phenolic protons in agreement with the expected molecular structures. In addition, mass spectrometry provided molecular ion peaks consistent with the calculated molecular weights of the synthesized compounds, confirming their molecular compositions.

FT-IR spectroscopy was employed to confirm the successful synthesis and identify the functional groups present in the synthesized azo dyes (1–3). The characteristic absorption bands are summarized in Table 2, while the corresponding spectra are shown in Figure 1.

The spectra of all synthesized dyes revealed broad absorption bands within the array of 3184–3261 cm^{-1}, corresponding to the stretching vibration of the hydroxyl (O–H) group, together with weak aromatic C–H

stretching bands at 2914–3093 cm^{-1}. Strong absorption bands observed at approximately 1600–1606 cm^{-1} were assigned to aromatic C=C stretching vibrations, whereas the characteristic bands appearing at 1489–1510 cm^{-1} confirmed the formation of the azo (–N=N–) linkage. In addition, absorption bands in the range of 1627–1681 cm^{-1} were attributed to the amide carbonyl (C=O) group, while bands at 1325–1330 cm^{-1} corresponded to C–N stretching vibrations.

The close agreement of the observed absorption bands for 1, 2, and 3 with the expected functional groups confirms the successful formation of the target azo dyes. Minor variations in the band positions are attributed to differences in the electronic effects of the coupling components, which slightly modify the electron distribution within the conjugated molecular framework.

Table 2. Characteristic FT-IR absorption bands (cm^{-1}) of the synthesized azo dyes (1–3).

Assignment	1 (cm^{-1})	2 (cm^{-1})	3 (cm^{-1})
ν(O–H)	3261	3184	3254
ν(C–H, aromatic)	3086	3093	2914
ν(C=O) (amide)	1680	1681	1627
ν(C=C) (aromatic)	1606	1606	1600
ν(N=N) (azo)	1508	1510	1489
ν(C–N)	1327	1325	1330

The ^{1}H NMR spectra of the synthesized azo dyes (1–3), recorded in DMSO-d_6, were in good agreement with the proposed molecular structures. All spectra exhibited the expected aromatic proton signals within the range of δ 6.42–8.95 ppm, confirming the presence of the aromatic rings and the conjugated azo framework. The amide (–NH–) and phenolic (–OH) protons appeared as characteristic downfield singlets due to the electron-withdrawing effects of the adjacent functional groups and intramolecular hydrogen bonding. In particular, the phenolic proton of dye 2 resonated at δ 12.00 ppm, indicating strong intramolecular hydrogen bonding. Minor differences in the chemical shifts of the aromatic protons among 1, 2, and 3 are attributed to the different coupling components and their electronic effects on the conjugated system. The residual DMSO-d_6 and water signals were observed at approximately δ 2.50 and δ 3.30–3.50 ppm, respectively. Overall, the ^1H NMR data provide strong evidence for the successful synthesis of the target azo dyes and are fully consistent with the proposed molecular structures.

Mass spectrometry was employed to confirm the molecular masses of the synthesized azo dyes (1–3) and to investigate their fragmentation behavior. The mass spectra are presented in Figure 3.

The mass spectra of dyes 1 and 2 exhibited molecular ion peaks at m/z 452, while dye 3 showed a molecular ion peak at m/z 444.8, in good agreement with the calculated molecular weights of the proposed structures. The relatively low intensity of the molecular ion peaks indicates that the molecular ions undergo fragmentation under electron-impact ionization.

Each dye produced characteristic fragment ions that are consistent with cleavage of the azo linkage and fragmentation of the aromatic substituents. The base peaks observed at m/z 115 for 1, m/z 154.9 for 2, and m/z 46.0 for 3 correspond to the most stable fragment ions generated during the fragmentation process. Additional fragment ions were also detected and are fully consistent with the proposed fragmentation pathways of the synthesized dyes.

Overall, the molecular ion peaks together with the characteristic fragmentation patterns provide strong evidence for the successful synthesis of the target azo dyes and support the molecular structures proposed from the FT-IR and ^{1}H-NMR analyses.

Absorption spectra

Absorption spectra of the colored dyes 1, 2, and 3 formed by coupling of diazotized reduced Niclosamide with 1-Naphthol, 2-Naphthol and 2-Hydroxybenzoic acid reagent was recorded against its corresponding reagent blank and show a maximum absorption at 330 nm in contrast to the 1-Naphthol, 2-Naphthol and 2-Hydroxybenzoic acid reagents blank which shows no absorption in the visible region (Figure 4).

Solvent effect on electronic spectra of dyes

The absorption spectra of the synthesized azo dyes (1-3) were studied in various polar solvents such as 1,4-dioxane, chloroform, acetone, ethanol, methanol, water, and DMSO to determine the impact of the properties of the solvent on the spectral characteristics. The absorption spectra of the dyes under study in the various

solvents used in this investigation are shown in Figure 5, while the respective values for the spectral parameters are given in Tables 3-5. The electronic absorption spectra for all the dyes prepared are found to lie within the wavelength region of 330 – 360 nm, typical of π→π* electronic transition of the highly conjugated azo dye moiety. These small changes in both λ_max and molar absorptivity (ε) values prove that the electronic configuration in the azo group is affected by the nature of the solvent medium.

The spectral response of the solutes as a function of solvent could be due to specific as well as non-specific solute-solvent interactions. Specific interactions involve mainly the hydrogen bonding interaction between the hydroxyl group and the amide group of the azo dye with the protic solvents, leading to stabilization of both ground state and excited electronic states. The non-specific interactions involve the electrostatic interactions such as dipole-dipole, dipole-induced dipole, and dispersion interactions, which rely heavily on the dielectric constant of the solvent. In terms of absorbance readings among all the solvents tested, DMSO showed the highest absorbance readings, while 1,4-dioxane was found to have the lowest absorbance readings. This is due to the excellent solvation properties and high dielectric constant that enable DMSO to stabilize the excited state of the dyes. However, in 1,4-dioxane, the low polarity does not help stabilize the excited state, hence leading to low absorbance[24].

In order to probe into the effect of solvent polarity, the wavelengths corresponding to maximum absorption were plotted against the dielectric function, (D−1)/(D+1), where D is the dielectric constant of the solvent. The detected plots (Figure 6) are linear in nature, especially for moderately polar solvents. These findings suggest that the stabilization due to the dielectric effect is the most important mechanism responsible for the solvatochromism of the dyes under study. With an upserge in the dielectric constant, the states are stabilised differentially, giving rise to hypsochromic or bathochromic shifts depending on the relative stabilization of the ground and excited states[25].

The solvent effect was further interpreted using the Gati–Szalay relationship:

$$\Delta\tilde{\nu}=[(a-b)(2-1/22+1)] +b(D-1/D+1)$$

where n is the refractive index of the solvent, D is the dielectric constant, and a and b are constants determined empirically. The described correlation considers the joint effect of the refractive index and the dielectric constant on the shift of the absorption maximum wavelength. The approximately linear dependence of the absorption maxima on the dielectric constant proves that the solvent polarity is the main parameter affecting the electronic transitions of the obtained azo dyes. Some deviations from linearity may be caused by particular hydrogen bonds and different solvent polarizabilities which cannot be considered using only the dielectric constant.

In summary, the findings from the solvatochromic spectral analysis indicate that the azo dyes derived from niclosamide show moderate solvatochromism as a result of the influence of the polarity of solvents on the electronic conjugation system of these compounds. This is beneficial for analytical purposes since it means that the dyes have stable spectra in various solvents.

Table 3. The effect of the dielectric constant function of solvents on the max. wavelength of dye 1

Solvent	Sym.	(D-1)/(D+1)	λmax1 nm	εmax ×10⁴ L·mol⁻¹·cm⁻¹
1,4-Dioxane	1	0.394	330	1.507
Chloroform	2	0.655	340	1.501
Water	3	0.907	360	1.274
Acetone	4	0.92	330	1.461
Ethanol	5	0.913	360	1.286
Methanol	6	0.958	360	1.502
DMSO	7	0.97	330	1.500

Table 4. The effect of the dielectric constant function of solvents on the max. wavelength of dye 2

Solvent	Sym.	(D-1)/(D+1)	λmax1 nm	εmax ×10⁴ L·mol⁻¹·cm⁻¹
1,4-Dioxane	1	0.394	330	1.490
Chloroform	2	0.655	360	1.450
Water	3	0.907	320	1.380
Acetone	4	0.92	330	1.490
Ethanol	5	0.913	330	1.500

Methanol	6	0.958	360	1.500
DMSO	7	0.97	330	1.505

Table 5. The effect of the dielectric constant function of solvents on the max. wavelength of dye 3

Solvent	**Sym.**	**(D-1)/(D+1)**	**λmax1 nm**	**εmax ×10⁴ L·mol⁻¹·cm⁻¹**
1,4-Dioxane	1	0.394	330	1.520
Chloroform	2	0.655	340	1.390
Water	3	0.907	340	1.420
Acetone	4	0.92	330	1.510
Ethanol	5	0.913	330	1.520
Methanol	6	0.958	320	1.460
DMSO	7	0.97	350	1.450

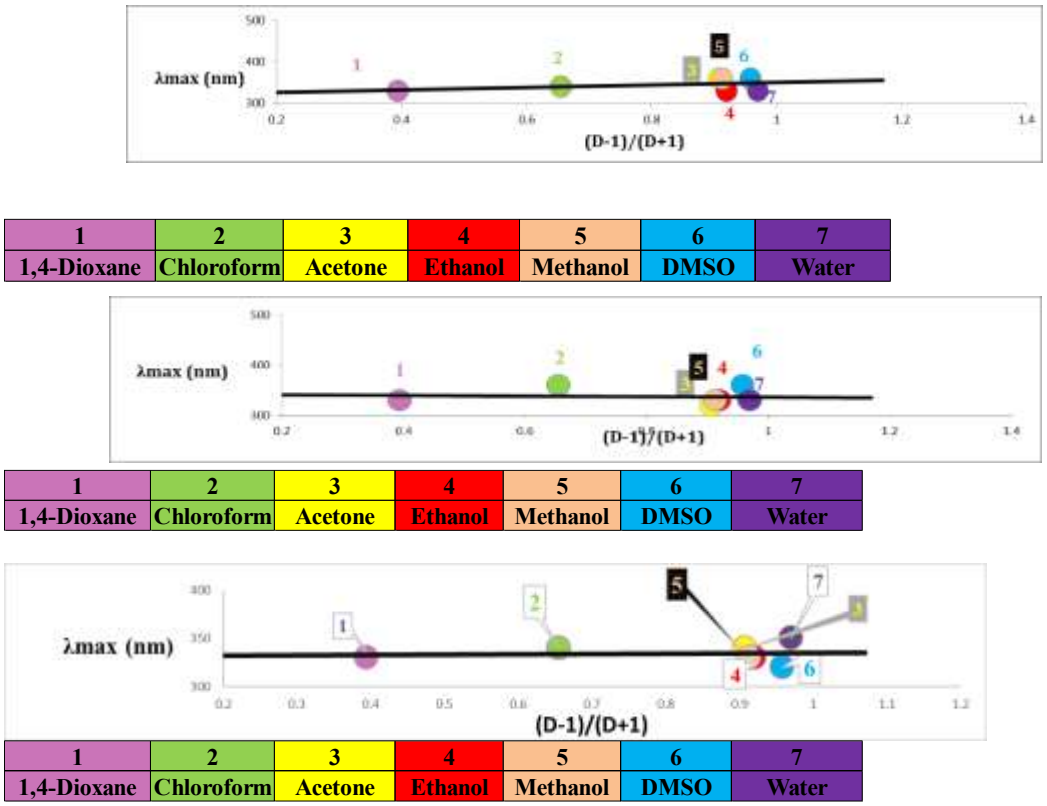


Figure 6. The relationship between the maximum wavelengths of the azo dyes 1-3 and the dielectric constant function in different polar solvents.

Time-Dependent Stability Analysis

The kinetic stability of the studied azo dyes was systematically studied (1, 2, and 3) with time. The initial stability profile was obtained by monitoring the spectral variations at short time intervals immediately after mixing (1, 2, 5, 10, and 20 min) using UV-Vis spectroscopy. The monitoring period was then extended to long-term intervals (1 h and 24 h) to fully capture the full time-dependent stability behavior of the dyes (Figure 17). [6]

Acid-Base Properties:

The acid–base behavior of the synthesized azo dyes (1–3) was investigated by recording their UV–Visible absorption spectra over the pH range of 2–12 using buffer solutions. The dye concentration was maintained at 1×10^{-4} M, and the spectra were recorded between 300 and 500 nm to evaluate the influence of pH on the electronic structure of the dyes and to determine their protonation and ionization constants[26, 27].

As shown in Figure 8, all three dyes exhibited considerable pH-dependent spectral changes. In the pH range 2–8, the spectra exhibited a dominant absorption band centered at about 340 nm with the maximum of absorption at pH 4. This band is attributed to the protonated (cationic) form of the dyes, due to the protonation of the azo and other basic sites of the molecules. Protonation decreases the extent of π-electron delocalization, and thus preserves the absorption at relatively shorter wavelengths.

In alkaline media (pH 9–12), a second absorption band appeared at longer wavelengths, reaching approximately 380 nm for dye 1 and 370 nm for dyes 2 and 3. This bathochromic shift is attributed to the deprotonation of the phenolic hydroxyl group, resulting in the corresponding phenoxide ion. The negative charge generated upon ionization becomes delocalized over the aromatic–azo conjugated system, increasing the degree of conjugation and lowering the energy required for the electronic transition. Consequently, absorption shifts toward longer wavelengths[28].

A well-defined isosbestic point was observed at approximately 360 nm for all synthesized dyes, indicating a clean and reversible equilibrium between the protonated and deprotonated species without the formation of detectable intermediate products. The presence of this isosbestic point confirms that the acid–base transition involves two predominant absorbing species in solution.

The protonation (pK_p) and ionization (pK_a) constants were determined from the absorbance–pH curves (Figures 21–23), and the calculated values are summarized in Table 8. The ionization constant (pK_a) corresponds to deprotonation of the phenolic hydroxyl group, Whereas the protonation constants (pK_{p1} and pK_{p2}) are associated with protonation of the azo nitrogen (–N=N–) and the amide nitrogen atoms, respectively. The proposed protonation and ionization mechanisms are illustrated in Schemes 2–4.

The equilibrium constants were calculated using the half-height method according to the following equations[29]:

pK = pH (at A1⁄2) …….. (1)

A1⁄2 = (Amax + Amin)/2 …….. (2)

Where:

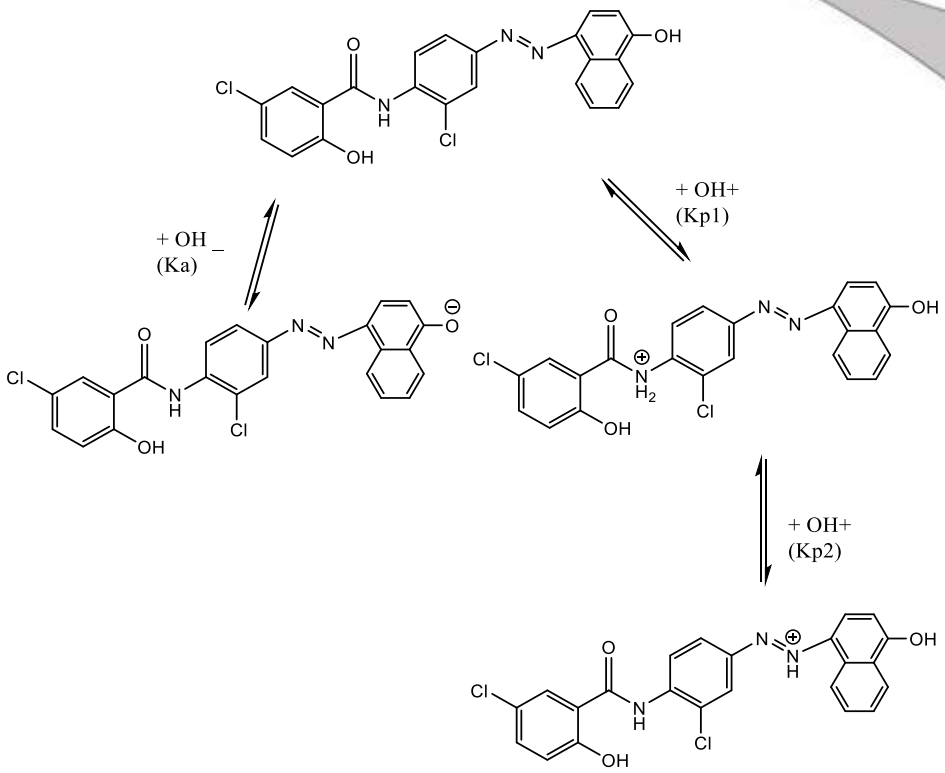
Amax: maximum absorption,

Amin: minimum absorption

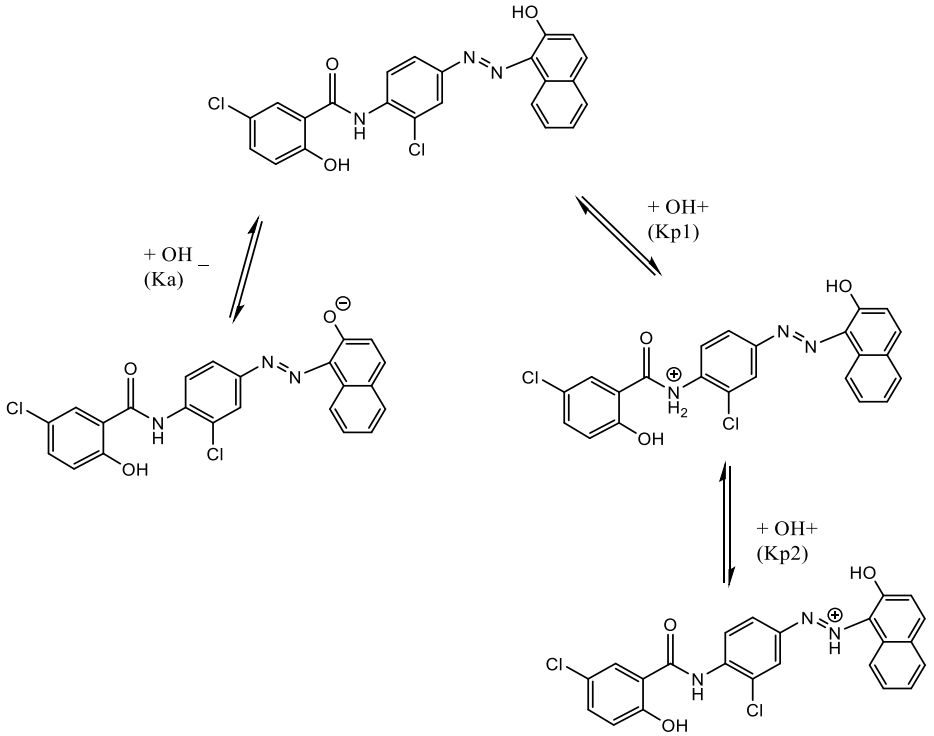
where A_{max} and A_{min} represent the maximum and minimum absorbance values, respectively. The obtained pK values demonstrate that the synthesized dyes undergo well-defined and reversible protonation–deprotonation equilibria, which are consistent with the observed spectral changes.

Table 6. The half-height method parameters for calculation of protonation and ionization constants of azo dyes

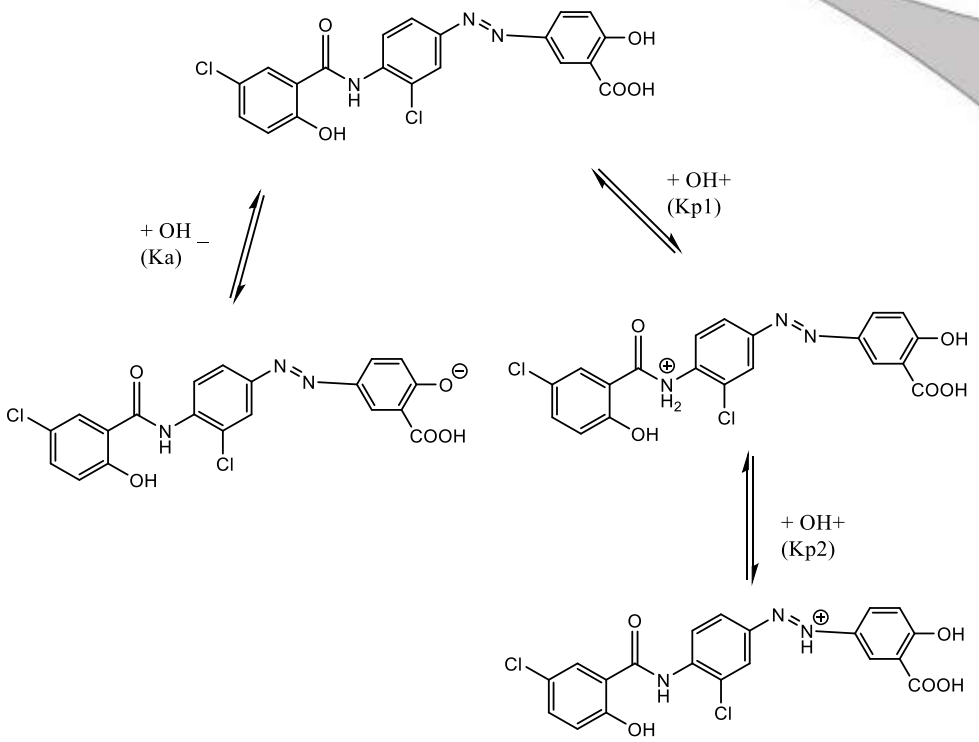
Dye name	**Abs.at**		**Abs.at**	
Dye 1	**(PH6-PH7) A1/2**	**Pka1**	**(PH8-PH9) A1/2**	**Pkp1**
λmax 330nm	0.99	6.5	0.865	8.5
Dye 2	**(PH 2-PH4)**	**PKa1**	**(PH6-PH9)**	**PKp1**
λmax 330 nm	1.118	3	1.226	6.3
Dye 3	**(PH 3-PH7)**	**PKa1**	**(PH9-PH10)**	**PKp1**
λmax 330 nm	1.245	5	1.066	9.5



Scheme 2. The suggested mechanism for ionization and protonation of the 1 dye.



Scheme 3. The suggested mechanism for ionization and protonation of the 2 dye.



Scheme 4. The suggested mechanism for ionization and protonation of the 3 dye.

The pH-dependent structural transformations were accompanied by sharp and reversible color changes between acidic and alkaline media (Figure 10). The distinct color transitions enabled the synthesized dyes to function effectively as acid–base indicators. The analytical performance of the sensors was further assessed by titration of 0.1 M HCl with 0.1 M NaOH using phenolphthalein as the reference indicator. The synthesized dyes produced sharp and accurate end points which were in good agreement with those obtained with phenolphthalein. These results are summarized in Table 7 and Figure 11 and exhibit the potentiality of the synthesized dyes as reliable acid base indicators for volumetric analysis[30, 31].



Figure 11. Color of the synthesized azo dye as an indicator in different media: (a) alkaline, (b) natural, and (c) acid

Table 7. Titration results using HCl (0.1M) against NaOH (0.1M).

Comp.	Volume of HCl (mL)	Mean Volume of NaOH(mL)	Color in acid	Color in base
1	8	7.0	Light orange	yellow
2	8	6.7	Maroon	Light yellow
3	8	6.9	Colorless	Light yellow
Ph ph	8	7.9	Colorless	Dark pink

Ph.ph. =Phenolphthalein

Conclusion

Three novel niclosamide derived azo dyes (1–3) were synthesized by diazotization and azo-coupling reactions and characterized thoroughly using FT-IR, UV–Vis, 1H-NMR and mass spectrometry, which confirmed their proposed molecular frameworks. The synthesized dyes showed intense absorption bands at about 330nm with a clear solvent dependence of the spectra, indicating a significant effect of the solvent polarity on the electronic transitions via both specific and non-specific solute–solvent interactions. The dyes were also characterized by well-defined acid–base properties in the pH range of 2–12. Isosbestic points were observed, indicating the clean interconversion between the molecular species, due to reversible protonation and deprotonation processes, which resulted in different spectral and color changes. The protonation and ionization constants obtained by half height method further confirmed the synthesized compounds acid-base equilibrium behavior.

Moreover, all dyes were spectroscopically stable in solution for at least 24 h, confirming their appropriateness for methodical applications. The end points obtained were comparable to those obtained using phenolphthalein and successfully demonstrated their performance as acid–base indicators in the HCl–NaOH titrations.

In conclusion, the simple synthesis, good spectroscopic stability, reversible pH-responsive behavior, and reliable indicator performance of these niclosamide-derived azo dyes indicate their potential as promising candidates for analytical acid–base indicators and provide a useful platform for the future development of functional azo chromophores with tunable optical properties.

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Figure's

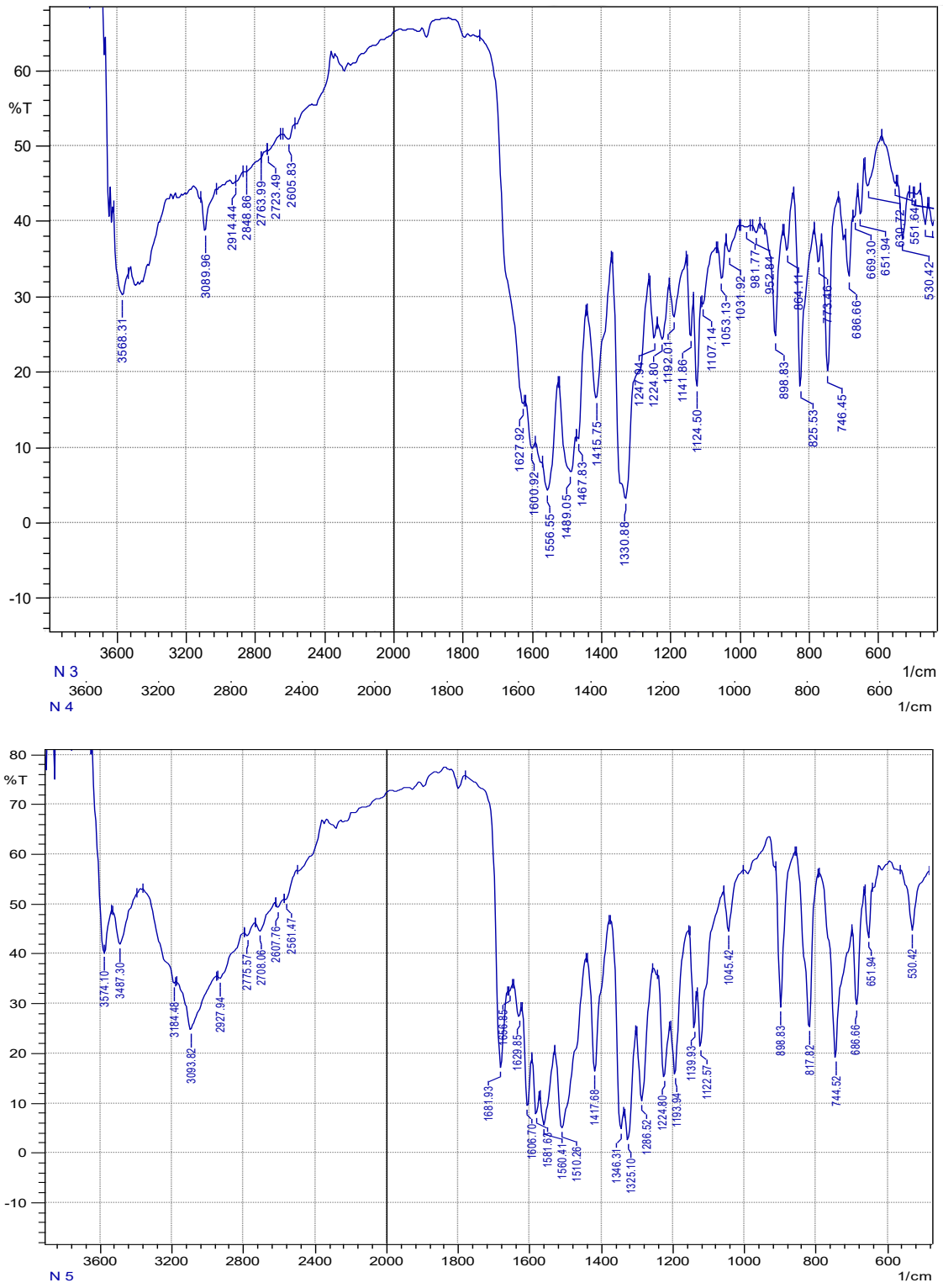
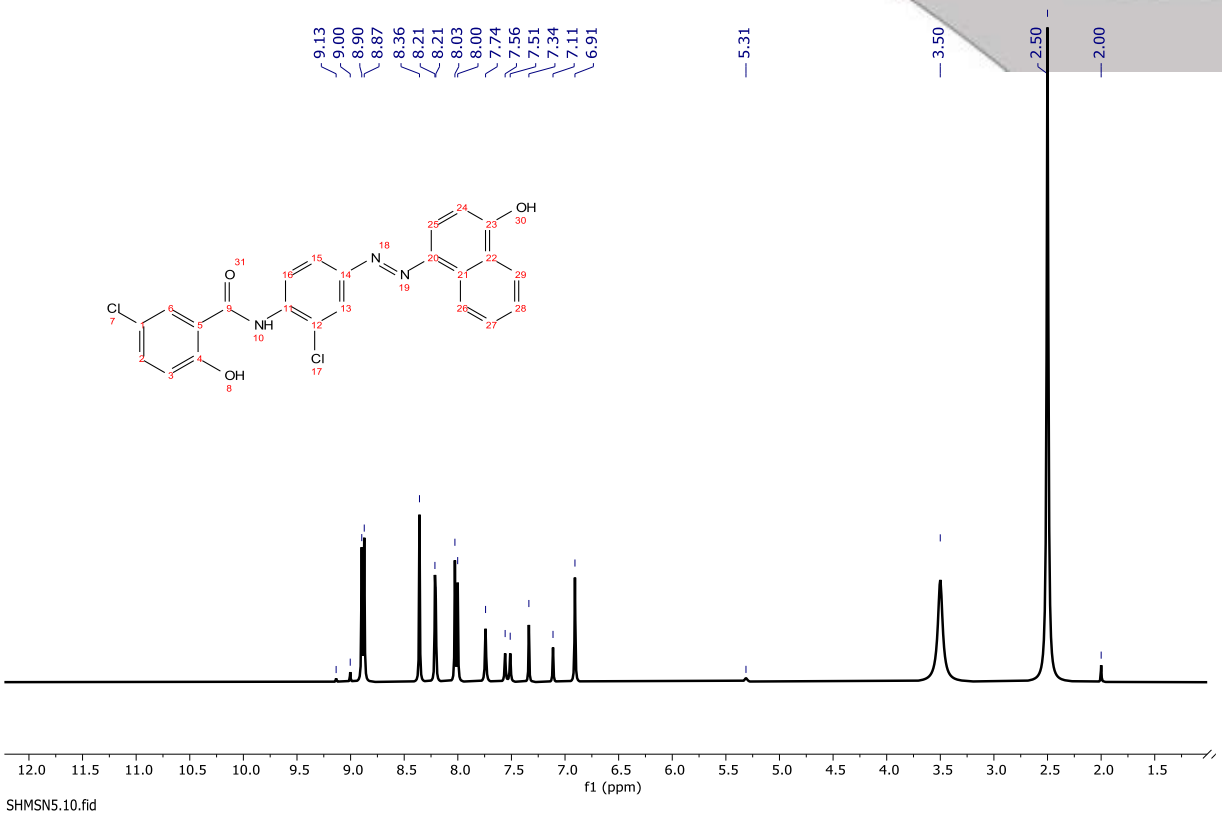
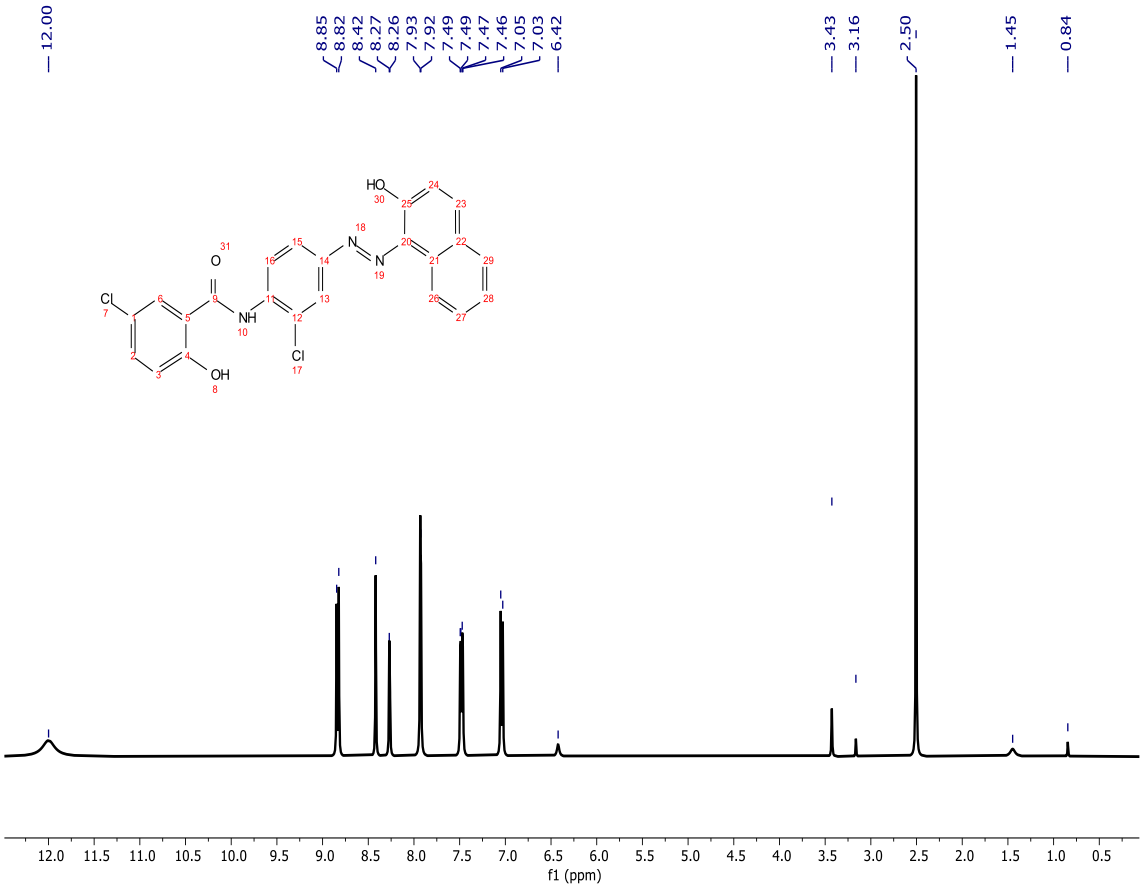


Figure 1. IR Spectra of the synthesized azo dyes 1-3

SHMSN4.10.fid
N4



SHMSN5.10.fid
N5



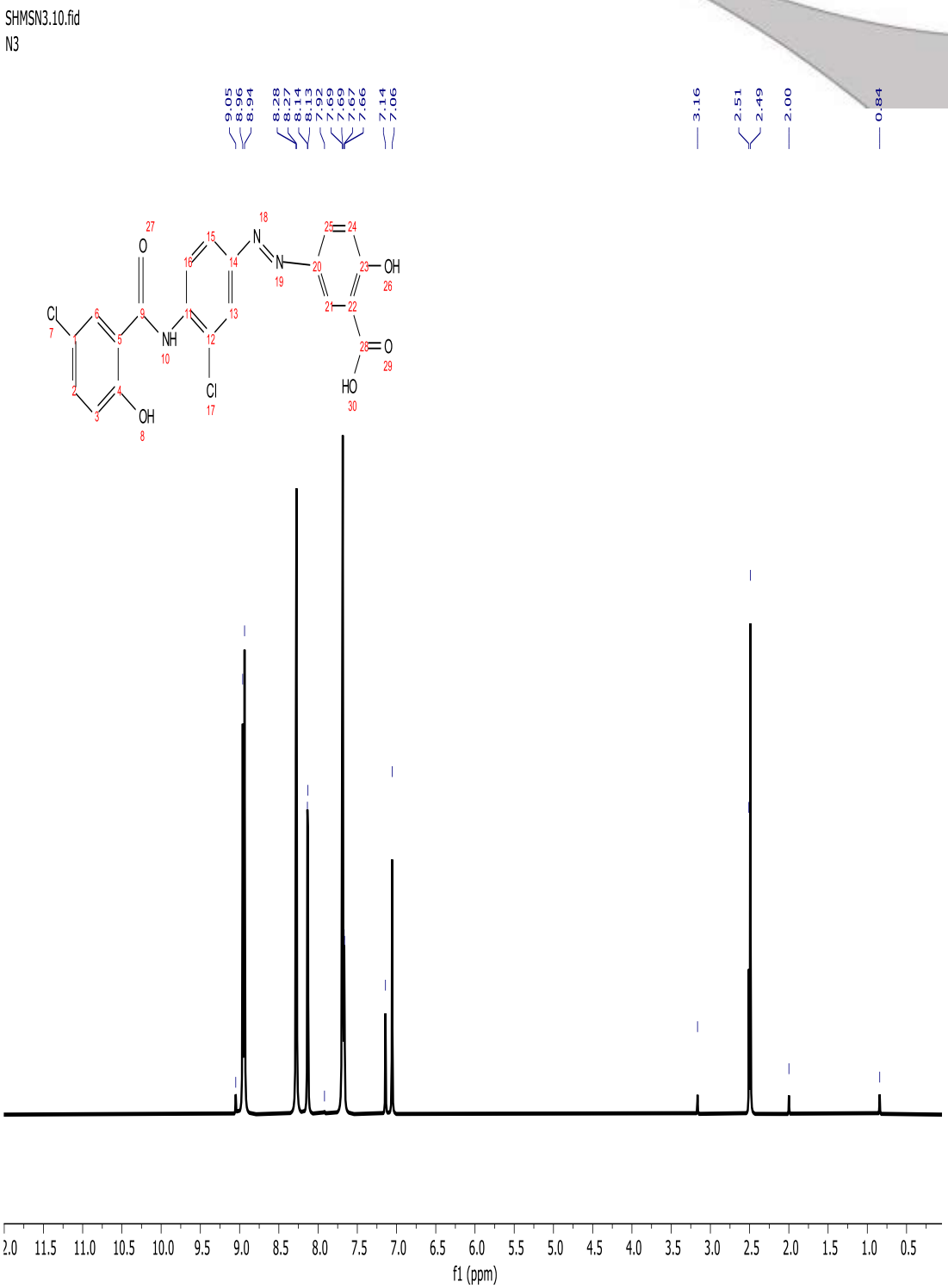


Figure 2. 1H-NMR spectra of the synthesized azo dyes N-3

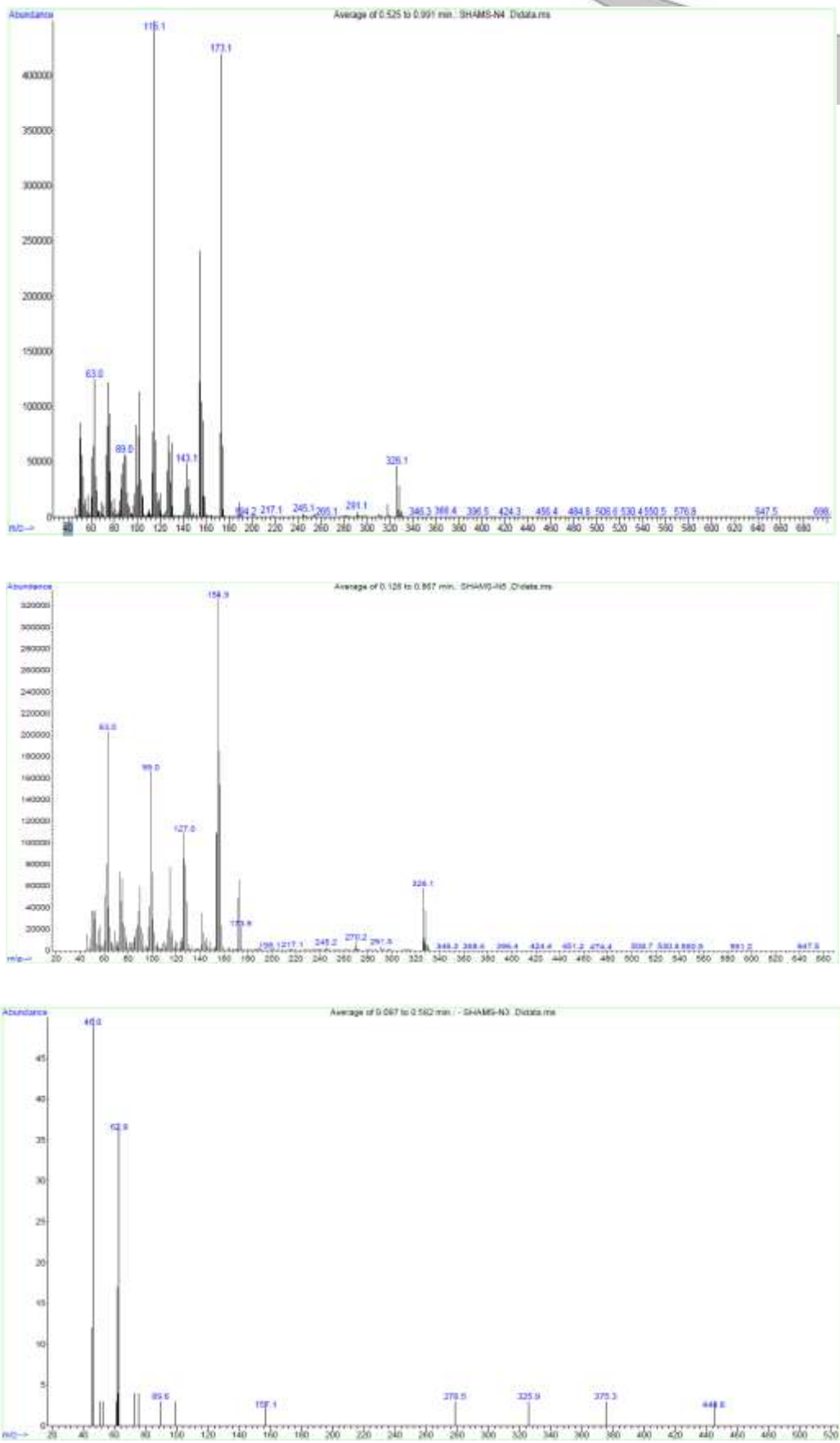


Figure 3. Mass spectra of the synthesized azo dyes 1-3

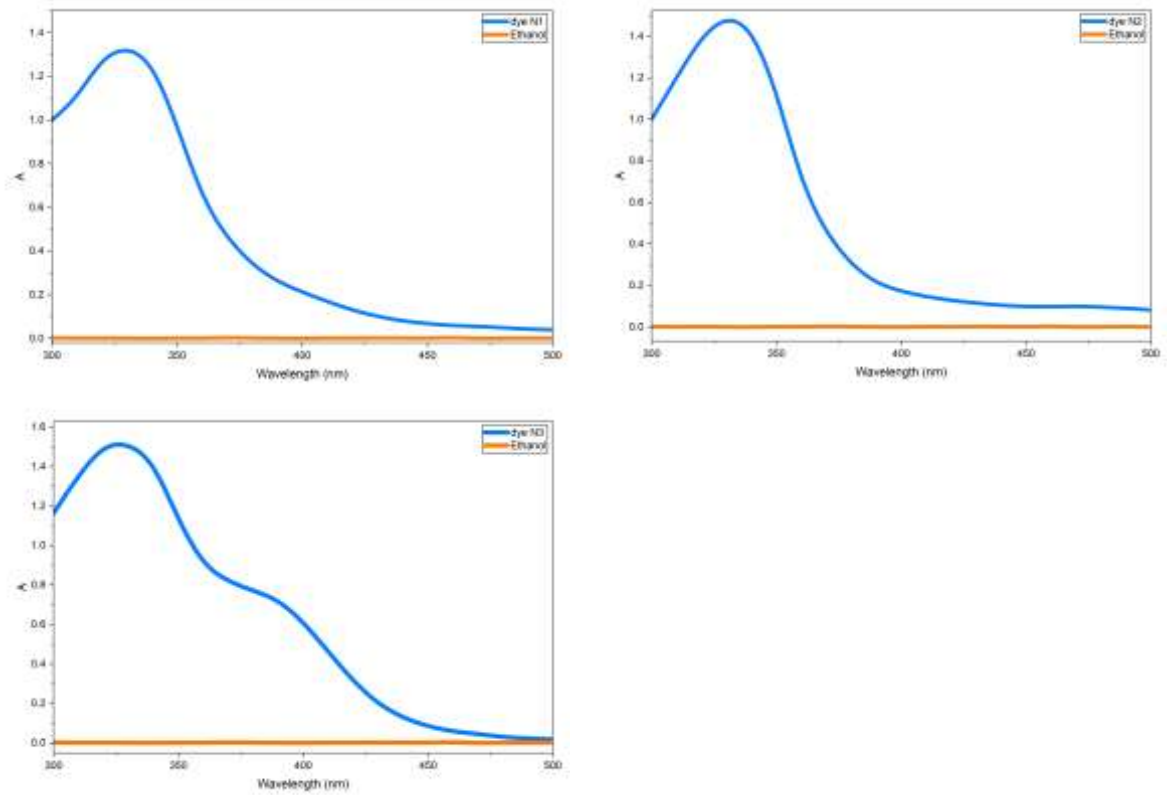


Figure 4. Absorption spectra of 1x10^{-4} M 1, 2 and 3 azo dye solution measured against blank

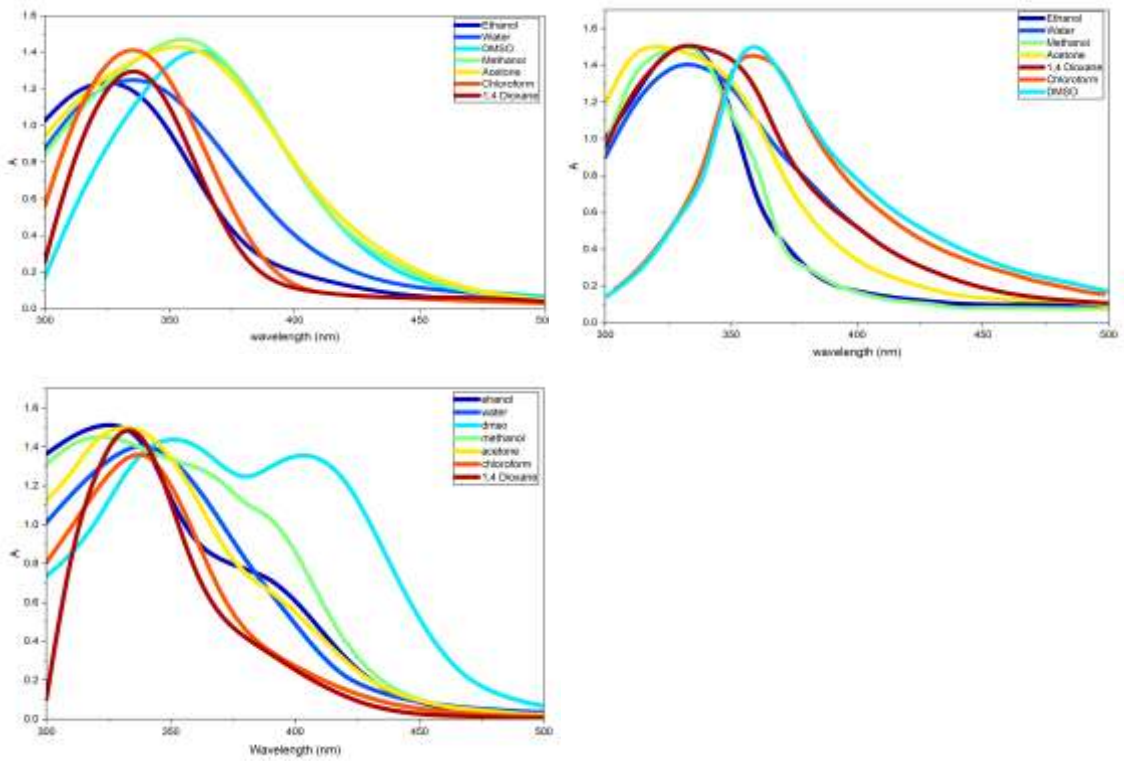


Figure 5. The visible absorption spectra of the dyes 1-3 in different polar solvents.

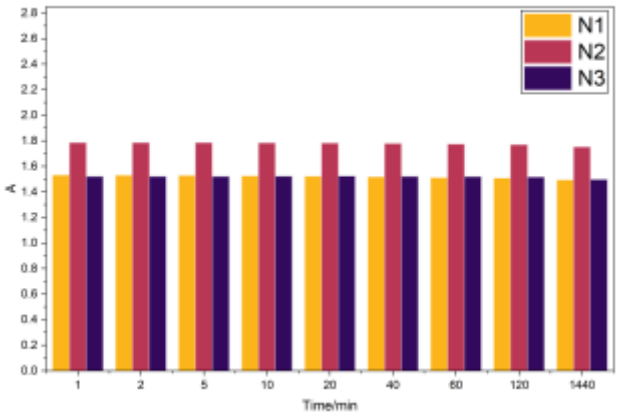


Figure 7. Time-dependent UV-Vis absorbance profiling of Dye 1, 2 and 3

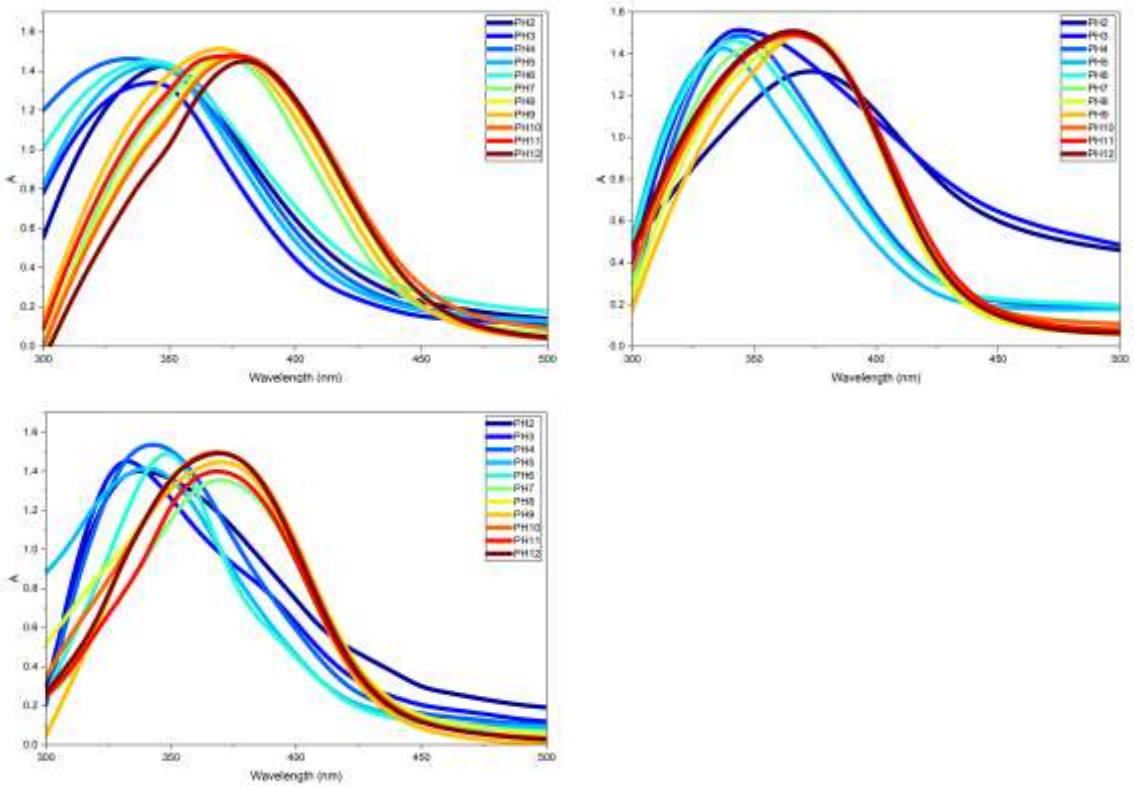


Figure 8. The visible absorption spectra of the 3 dye in different pH solutions

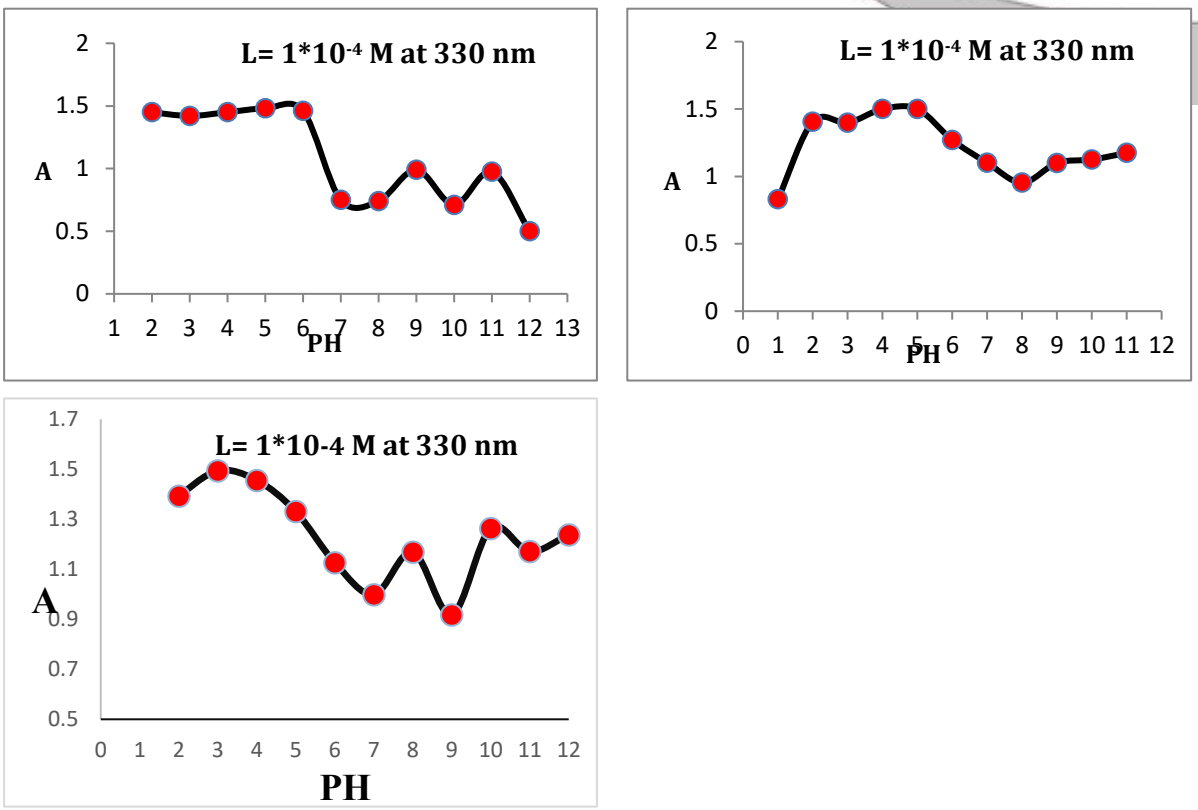


Figure 9. The absorption curve of the 1-3 dyes at the maximum wavelength (330 nm)

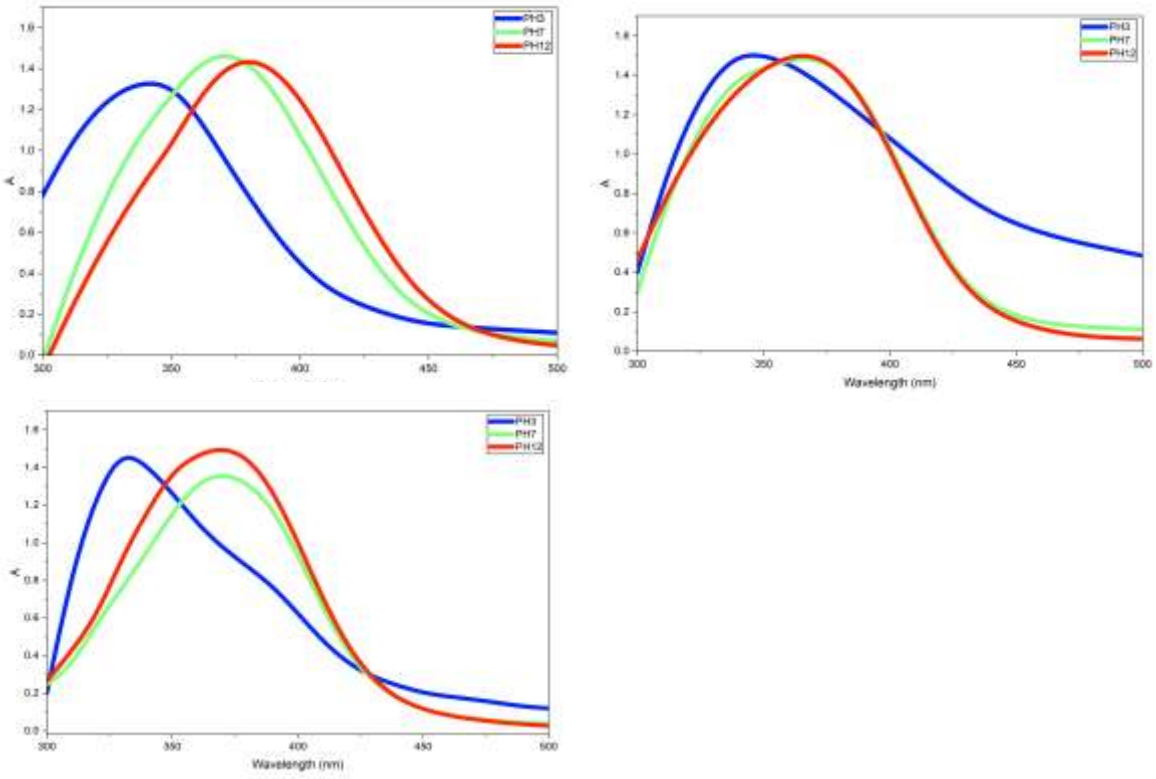


Figure 10. The absorption spectra of 1-3 at pH (3,12 and 7)