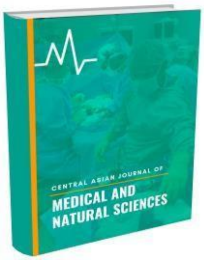

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Article

Comparison Study for Stability Constants of Azo-dyes Produced from The Reaction of Tetracycline with Two Diazotized-reagents at Different pH

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Abstract: Our research focused on a spectroscopic study of the stability constants of the formation of two azo dyes prepared from the reaction of tetracycline with two different diazotized reagents and at three acidic medias: acidic, neutral and basic, and at a constant temperature (288K). We studied the optimal conditions for each prepared azo-dye, as well as the optimal mole-ratios for its components, which were (1:1) for (drug: reagent)".We determined the stability constants of the two prepared azo-dyes at the three acidic media and at the five temperatures (283, 293, 303, 313 and 323K). After that, we studied the factors that affect the values of its stability constants, which are; The effect of temperature: We calculated the stability constants at the five temperatures mentioned above, and it became clear from them that the formation reactions of azo-dyes are spontaneous and exothermic, from the negative values of($\Delta G°$ and ΔH) respectively, and also the negative value of ($\Delta S°$) supports the aforementioned.The effect of the pH-function: We found the stability constants for each of the prepared dyes at each pH and at different temperatures, so we obtained the values of the stability constants, which were evidence of the preparation of stable azo-dyes".The effect of structures: It was found that the difference in the structural formula of the diazotized-reagent has a clear effect on the values of the stability constants for the formation of the prepared azo-dyes, and this was proven through the variation in the values of the stability constants".

Keywords: Tetracycline, pH, 4-aminobenzophenone, Para- nitro aniline and Stability Constants.

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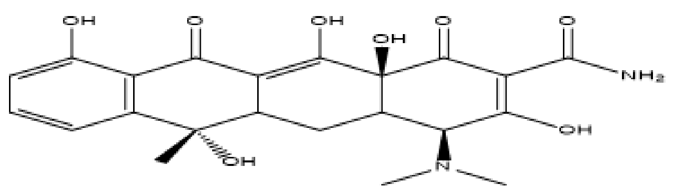
1. Introduction

Tetracycline is a cheap and widely used antibiotic. It is used in the prevention and treatment of human or animal infections. It is also used in animal feed as a growth stimulator. Among the negative effects of tetracycline is that it causes distress in the gastrointestinal tract, nausea, vomiting, and loss of appetite. Lon, Asna: It also inhibits bone growth in children. It also causes toxicity in the liver. In the case of an overdose of tetracyclines, it can lead to liver failure and death [1,2]. Tetracycline has the molecular formula C22H24N2O8. It dissolves hardly in water and is easily soluble in ethanol. Its molecular weight is 444.435 g / mol [3]. Its chemical formula"



(4S,6S,12aS)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide

Tetracycline

Is that it is one of the most important compounds, and the most famous of them in the preparation of azo compounds are diazonio salts. They are very effective and unstable compounds, and the reason for this is that they are rarely isolated, so they are used immediately after preparation. Diazonio salts are prepared through the primary amine reactor with nitrous acid, and the reaction occurs by Primary amine with acid by treating sodium nitrite with a primary amine solution in hydrochloric acid at a temperature of (0−5oC)[4].

The researchers Adel Azouz and Al-Agha (2008) studied the effect of experimental factors on the value of the stability constants of dyes resulting from the reactions of phenolic oximes different with a salt of nitrous sulfaninic acid. They practically concluded that there are three physical variables that have an effect on the value of the stability constant (k), and these variables are the temperature, the acidity function, and the type of isomer in the oxime, and they have proven that this agent is exothermic and spontaneous[5].

"The effect of confined temperatures between (15−55oC) on the value of the stability constants of azo dyes formed through the interactions of electron-donating imines with the sodium salt of nitrous sulfanilic acid as an electron acceptor[6]".

" The same researchers also studied the effect of structural bodies on the value of stability constants For the azo dyes resulting from the reaction of imines with the sodium salt of nitrous sulfanilic acid. The researchers calculated the stability constants of the dyes by the photometric method, and we compared the stability constants of the oximine imines that are similar in terms of -sine and anti, as well as Schiff bases that contain compensators CH3,NO2,NH2,OH [7]".

Our current research included finding the conditions, as for the homologous properties, the mole-ratio, the stability constants, and the effect of the acid function of the reaction of the formation of two azo dyes from the reaction of tetracycline with the two reagents azotized 4-aminobenzophenone and azotized para- nitro aniline at the five temperatures (10,20,30,40,50 oC) and at three pH different is (5,7 and 9).

2. Materials and Methods

2.1- Chemicals:

"The chemicals used during the research were obtained from (Switzerland) Fluka '(Spain) PRS and (England) BDH companies, namely: Hydrochloric acid, Sodium carbonate, Sodium nitrite, para- nitro aniline and 4-aminobenzophenone. Tetracycline was obtained from the Nineveh Laboratory"

2.2- Equipment used:

A. pH meter manufactured by (JENWAY) company, Model (3510)
B. Photometric spectrometers

"The following two devices were used in the research: The first is a single-pass device manufactured by the British company (Cecil) (England, Cambridge) and model (1011 / 1000 CE) and within the range of wavelengths (325-1000 nm.) to measure (◎max) of complexes and the organic reagents under study, finding the optimal conditions, and completing the rest of the study. The second device is the two-way photometric spectrometer, which contains a computer manufactured by the Japanese company (Shimadzu), model (UV-l800), produced in the year (2004) to check the Absorbance value of the used reagents or the electronic spectra of the organic compounds under study and draw the different ones with a water solvent and in the confined range is between 190-900 (nm). The cells used in both devices were made of quartz in the invisible region and glass cells in the visible region".

C. Water bath model (D3l65) of the type (Hanigsen) and manufactured by the German company (KOTTERMANN).

2.3- Preparation of para- nitro aniline (p- NA) solution:

"(0.1727 g) of para- nitro aniline is weighed and dissolved in (50 ml) of distilled water, after that (20 ml) of a solution (1M) of hydrochloric acid HCI is added to it, and the solution is heated to dissolve it, after that the mixture is transferred to volumetric flask with a capacity of (250 ml), then it was cooled to a temperature (0-5 ºC) in the ice bath. Then (8.65 ml) of l% sodium nitrite NaNO2 was added to the solution, then stirred, and after five minutes the solution was added. In the volumetric vial with cold water up to the mark, then the solution is kept in a dark and cold place and it is applied immediately every time [8]".

2.4- Preparation of an azotized 4-aminobenzophenone (D4AB) solution:

"Weigh (0.197 g) of 4-aminobenzophenone and dissolve it in (20 ml) of distilled water, after which (8 ml) of a solution (1M) of hydrochloric acid HCl, and stir well until it dissolves, then the mixture is transferred to a volumetric vial of (250 ml) capacity, and prepared to be cooled to a temperature (0-5 ºC) in the ice bath. Then (8.65 ml) of 1% is added to the solution of NaNO2, and it is stirred, after five minutes the solution is completed with cold water to the mark in the volumetric vial, and the solution is kept in a dark and cold place and applied immediately every time[9]".

2.5- Preparation of a solution of (1%) sodium nitrite NaNO2:

Weigh (1 gram) of sodium nitrite and dissolve it with distilled water, then transfer it to a vial. It has a volumetric capacity of (100 ml), and it is supplemented with distilled water to the mark, and it is used for instant diazotization of the reagent.

2.6- Preparing solutions of the two medicinal arrow dyes:

The two azo dyes were prepared at the optimal conditions for each of them, which are tabulated in Table(1) by mixing suitable amounts of (10-3M) of tetracycline solution with (10-3M) of the diazotized reagent and (0.1 M) of the basic sodium carionate salt (Na2CO3), which is useful in adjusting the pH.

3. Results and Discussion

The development in scientific research and in all fields of life, including the field of chemistry, led to an in-depth study and preparation of several types of complexes of great importance from a life and industrial medical point of view, known as complexes (donor-accepter) depending on spectral techniques such as electronic spectra.

"Our research focuses initially on finding the optimal conditions for the preparation of the two azo dyes under study from the reaction of tetracycline conjugation with the two diazotized reagents (4-aminobenzophenone and para- nitro aniline) at five temperatures (10,20,30,40,50 ºC) and at different acidity functions (5,7 and 9). It was (1:1) for both the diazotized reagent and the tetracycline and included the optimal conditions for dye formation: the initial optimum wavelength, optimum volume of the diazotized reagent, the optimum volume of basic salt, the optimum order of addition, and the final optimum wavelength ( ) for dye formation at the optimal conditions for its formation, as mentioned in our study. [10-12] and the following table shows the final optimal conditions for the formation of the two azo dyes at a temperature of (288 K), which were prepared at three different pH (5,7 and 9)":

Table 1:The final optimal conditions for the two drug dyes, at a temperature of (15 ºC) and at different pH levels.

No.	pH	Optimal conditions for azo dyes	λ_{max} (nm.)	Stability time
Tetracycline + D4AB	5.0	ml Reagent 4 ml Na_2CO_3 + 0.150. + 0.5ml (Tetracycline)	380	70
	7.0	ml Na_2CO_3 + 2ml Reagent +0.40. 0.5ml (Tetracycline)	382	50
	9.0	ml Na_2CO_3 + 6ml Reagent +0.40. 0.5ml (Tetracycline)	384	40
Tetracycline + p- NA	5.0	ml 5ml Na_2CO_3+ 0.220. ml Reagent 4 0. Tetracycline +	391	115
	7.0	ml 5ml Na_2CO_3+ 0.420. ml Reagent4 0. Tetracycline +	421	70
	9.0	ml Reagent 4 ml Na_2CO_3+ 0.750. ml Tetracycline50. +	425	35

3.1- Calculating the stability constants of the two azotized dyes :

According to the mole-ratio method, the arrow dye (DA) is formed by the reaction of tetracycline (A) with the azotized reagent.(D) with a ratio of (1:1) and as shown in the following equation:

$$A+D \rightleftharpoons AD \quad ......(1)$$

It is possible to write an equation to calculate the stability constant as follows: (13)

$$K = \frac{[DA]}{[D][A]} \quad ....(2)$$

The value of the degree of association of the dye (◎) can be found from the following relationship:

$$\alpha = \frac{[E_m - E_s]}{[E_m]} \quad ....(3)$$

Es - Absorbance of a solution of the formed formula containing equidistant concentrations of the diazotized reagent and the drug under study.

Em - Absorbance of the dye solution formed under optimal conditions for the formation of the dye under study.

And [$(1-\alpha)C$] represents the concentration of the formed azo dye, and the equation for the stability constant (K) can be written as follows:

$$K = \frac{(1-\alpha)C}{[\alpha C][\alpha C]} \quad ....(4)$$

$$K = \frac{1-\alpha}{\alpha^2 C} \quad ....(5)$$

"Then find the value of (◎) from Equation No. (3), and the value of (K) can be calculated from Equation No. (5). The stability constant of the formed azo dye was found by the following method":-

"A solution of the drug azo dye is prepared that contains a ratio of (1:1) from tetracycline to the reagent. Under these conditions, the dye is formed relatively with little absorbence, in which we find (E_s). A solution similar to the first solution, but with optimal conditions and based on table (1), and in this case the resulting dye is more stable and has a higher absorbance in which we find (E_m), and on the condition that it is measured (E_s and E_m) for each solution against its stock solution, after that Equation (3) applies the calculation of the value of (◎), and then equation (5) the calculation of the value of (K)".

Table 2: The values of the stability constants (K) for the two azo dyes prepared at different pH values, at different temperatures, and at the optimal conditions for each of dyes and within the times of their stability

No.	Symbol of Drug-Dye	pH	T (K)	E_s	E_m	α	K
1	**Tetracycline + D4AB**	5.0	283	0.403	0.418	0.036	14973689
			293	0.4	0.429	0.068	4080856
			303	0.395	0.433	0.088	2368906
			313	0.391	0.441	0.113	1379448
			323	0.382	0.442	0.136	938022.2
		7.0	283	0.43	0.447	0.038	13301730
			293	0.424	0.459	0.076	3177404
			303	0.417	0.455	0.084	2627909
			313	0.412	0.494	0.166	605377.8
			323	0.409	0.513	0.203	387975.2
		9.0	283	0.399	0.414	0.036	14683200
			293	0.433	0.462	0.063	4757337
			303	0.439	0.477	0.08	2900319
			313	0.431	0.481	0.104	1658488
			323	0.429	0.489	0.123	1165450
2	**Tetracycline + p- NA**	5.0	283	0.17	0.234	0.274	9.718
			293	0.144	0.212	0.321	6.605
			303	0.101	0.152	0.336	5.905
			313	0.089	0.139	0.36	4.950
			323	0.08	0.132	0.394	3.907
		7.0	283	0.365	0.517	0.294	8.172
			293	0.356	0.506	0.296	8.011
			303	0.335	0.492	0.319	6.69
			313	0.319	0.481	0.337	5.849
			323	0.298	0.461	0.354	5.173
		9.0	283	0.398	0.493	0.193	21.770
			293	0.38	0.476	0.202	19.651
			303	0.357	0.454	0.214	17.245
			313	0.333	0.431	0.227	14.959
			323	0.311	0.416	0.252	11.744

2.3- Effect of temperature and thermodynamic parameters on the formation of two azo dyes:

"The researchers found that there are several effects of temperature in many chemical reactions, and in this paragraph we will address the effect of temperatures in various reaction systems that contain an azo group (-N=N-) in the azo dyes under study, due to its direct relationship to the research. Various studies have confirmed that the effects of temperature and thermodynamic parameters were evident in several interactions of Schiff bases and oximes, such as the pka of acids and bases, tautomeric and agglomerating, and research groups recently conducted a thermodynamic study of the formation reactions of azo dyes, and based on this [13-15] The stability constants of the two drug azo dyes formed from tetracycline reactions were calculated with the two azotized reagents (D4AB and p- NA). And as a result of the change in the values of the azo stability constants formed and related to temperature change, we studied these interactions thermodynamically, Any extraction of parameters ΔG°, ΔH, ΔS° The thermodynamic parameter (ΔH) has been calculated from the Vant Hoff integrative equation formula:"

$$\ln K = \text{constant} - \frac{\Delta H}{RT} \quad \text{.............................} \quad (6)$$

Since: K = the stability constant of the formed pharmaceutical azo dye.

ΔH = enthalpy formation reaction for a pharmaceutical azo dye.

"Equation (6) is used to calculate the reaction enthalpy (ΔH), and by drawing the relationship between the Ln K against the reciprocal of the absolute temperatures, you will get straight lines for the azo dyes, with a correlation coefficient (R) within the range (0.9542- 0.9697) and with different acidic values, and as in Figures (1 and 2)"

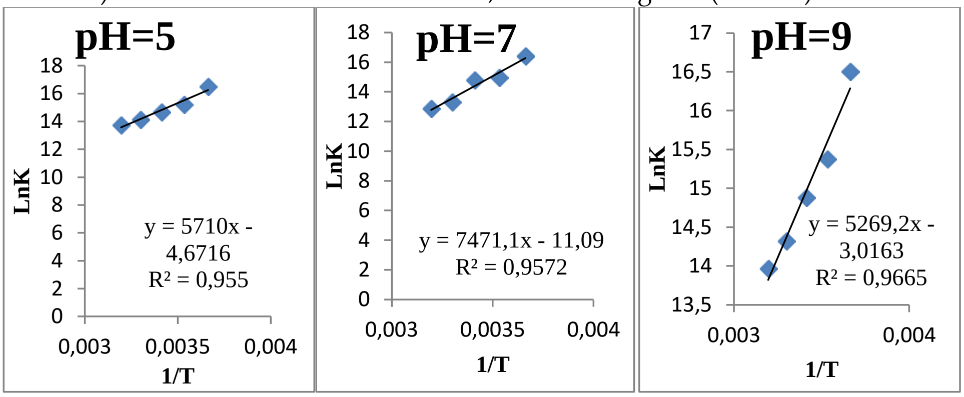


Figure 1: The effect of temperature on the pharmaceutical azo dye formed from the reaction of Tetracycline with the diazotized (D4AB) reagent at different pH levels.

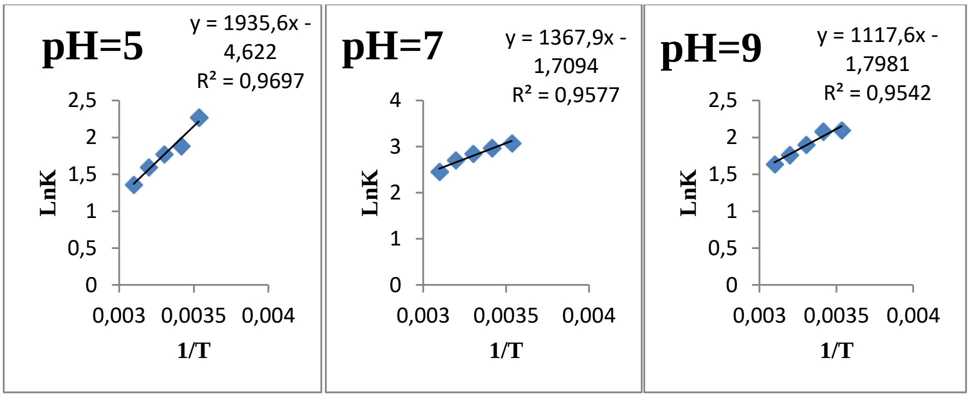


Figure 2: The effect of temperature on the pharmaceutical azo dye formed from the reaction of Tetracycline with the diazotized (p- NA) reagent at different pH levels.

Table 3: Study of the effect of temperature and thermodynamic parameters on the reaction of the formation of the two pharmaceutical azo dyes at different pH.

Comp . No	Symbol of Drug-Dye	pH	T (K)	lnK	ΔG^0 (KJ. mo1^{-1})		ΔH (KJ. mo1^{-1})		ΔS^0 (J. mo1^{-1}. K^{-1})	
1	Tetracycline + D4AB	5	283	16.522	-37.500	-36.094	-48.103	-47.473	-38.840	-38.840
			293	15.222	-35.815		-46.806		-38.840	
			303	14.678	-35.755		-47.136		-38.840	
			313	14.137	-35.614		-47.382		-38.840	
			323	13.752	-35.785		-47.942		-38.840	
		7	283	16.403	-37.231	-35.098	-62.402	-62.115	-92.202	-92.202
			293	14.972	-35.226		-61.319		-92.202	
			303	14.782	-36.008		-63.024		-92.202	
			313	13.314	-33.539		-61.476		-92.202	
			323	12.869	-33.488		-62.347		-92.202	

			283	**16.502**	**-37.455**		**-44.302**		**-25.078**	
			293	**15.375**	**-36.176**		**-43.273**		**-25.078**	
		9	**303**	**14.880**	**-36.249**	**-36.462**	**-43.596**	**-43.808**	**-25.078**	**-25.078**
			313	**14.321**	**-36.078**		**-43.676**		**-25.078**	
			323	**13.969**	**-36.350**		**-44.200**		**-25.078**	
			283	**2.274**	**-5.35**		**-16.225**		**-38.427**	
			293	**1.888**	**-4.599**		**-15.858**	**-16.093**	**-38.427**	**-38.427**
		5	**303**	**1.776**	**-4.473**	**-4.449**	**-16.117**		**-38.427**	
			313	**1.599**	**-4.162**		**-16.19**		**-38.427**	
			323	**1.363**	**-3.659**		**-16.071**		**-38.427**	
	Tetracycline		**283**	**2.101**	**-4.943**		**-9.173**		**-14.949**	
			293	**2.081**	**-5.069**		**-9.449**	**-9.2923**	**-14.949**	**-14.949**
2	**+**	**7**	**303**	**1.901**	**-4.788**	**-4.762**	**-9.318**		**-14.949**	
			313	**1.766**	**-4.596**		**-9.276**		**-14.949**	
	p- NA		**323**	**1.643**	**-4.413**		**-9.242**		**-14.949**	
			283	**3.081**	**-7.248**		**-11.27**		**-14.212**	
			293	**2.978**	**-7.255**		**-11.419**	**-11.373**	**-14.212**	**-14.212**
		9	**303**	**2.848**	**-7.173**	**-7.066**	**-11.48**		**-14.212**	
			313	**2.705**	**-7.04**		**-11.488**		**-14.212**	
			323	**2.463**	**-6.615**		**-11.206**		**-14.212**	

From Table (3) on the public shows a decrease in the values of stability of stability of the arguments resulting from increasing temperature and increasing the degree of disintegration (◍) and described in table (2) proof. Table 3 shows the various enthalpy (ΔH) negative sign and Within the temperatures that gave values of stability constants. The negative enthalpy values (ΔH) indicate that the formation reactions of the two azo dyes are exothermic reactions.

As for values (ΔG) for the above reactions has been found from the following mathematical relationship:-

$\Delta G^{o} = - RT \ln K$ ………………………… (7)

Since The values (ΔG) negative sign indicated in table (3) confirm that the Pharmaceutical azo dye formation reactions is an interaction spontaneous.

while the change in the value of antropy reaction (ΔS) has been calculated from the following known equation: -

$\Delta G^{o} = \Delta H - T.\ \Delta S^{o}$ …………………………(8)

as the negative signal for the value of the antropy (ΔS) means that the value S_1 larger From S_2 and that the value (ΔS) is in theory should be negative signal, this is in line with the studied vehicles and at different pH. Finally, the different values for both ΔG , ΔH and ΔS that were obtained was due to the different structural bodies of the compounds under study, and that is expected and compatible with many studies [16-25]

3.3- The effect of the acidic function on stability constants for prepared dyes:

from Table (3), we infer the following:

A. The values of stability constants for a single pigmentation are changed when changing acid circles to configure them. as the dye (tetracycline + D4AB) was more stable at pH=9 and has the values of constants stabilized according to the following sequence[26-30]: pH=7 < pH=5 < pH=9
B. The dye (tetracycline + p- NA) was more stable at pH=9 too, Then at 7 PH, then at 5-PH.

4. Conclusion

We can conclude from this study, the following :

A- The study confirmed the formation of colored azo dyes from the interaction of tetracycline with the azotized reagent, and the appearance of a spectral peak in the electronic spectrophotometer of the formed dye at a wavelength higher than the wavelengths of the spectrum of tetracycline alone and the two azotized reagents each alone".

B- It was found that the molae-ratio at optimal conditions for both azo dyes formed is (1:1) for each of (drug: azotized reagent).

C- It was found that the (ΔH) values are negative in sign, which indicates that the formation reactions of the two azo dyes are exothermic reactions. And the negative (ΔG) values of the sign confirm that the reaction of the azo dye formation is spontaneous, in addition to that the negative sign of the thermodynamic (ΔS) variable is consistent with the formation of the two dyes.

D- The change in the values of the stability constants of the one dye formed when the acid media changes to form an evidence of the effect of changing the pH on the values of the stability constants.

E- The change in the values of the stability constants of the one dye formed when the reagent constituting it changes, is evidence of the effect of changing the structures of the azotized reagent on the values of the stability constants.

F- The change in the values of the stability constants of one dye formed when the temperature of its formation reaction changes, evidence of the effect of the reaction temperature on the values of the stability constants. As when the temperature is increased, the stability constant of the formed dye decreases, and this conclusion was scientifically supported by the emergence of negative enthalpy values, which indicate that the reaction is exothermic. All of the above conclusions are expected and consistent with many previous studies .

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