

THE DEPENDENCE OF ACID-BASE EQUILIBRIA AND ACIDITY CONSTANTS OF CONGO RED IN BUFFER SOLUTIONS ON THE IONIC STRENGTH

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ABSTRACT

In this study, the spectrophotometric studies of aqueous ethanol solution of Congo red in various buffers solutions at room temperature 25°C and at different ionic strength of 0.05, 0.1, 0.3, and 0.5 KCl mol L⁻¹ were investigated. The acidity constants of Congo red were estimated by three different methods. The two protonation stages were observed in pH range 2 - 4 and 4 - 6 as result as protonation of amino group and azo groups. The dependence of absorption spectra of Congo red on the ionic strength of solutions which exhibited blue shift with increases ionic strength in addition the shape and intensity were varied. Therefore, the acid-base equilibria shifted toward the higher pHs with the rise in ionic strength. Also, the acidity constants were affected by the ionic strength which the acidity constants decrease with increasing ionic strength of solution because the electrostatic interaction force increased.

***Keywords:** Congo red, buffer solutions, ionic strength, blue shift, acid-base equilibria.*

INTRODUCTION

Congo Red [(CR), 1-naphthene sulfonic acid-3,3'-(4,4'-biphenylene bis(azo) bis (4-amino-) disodium salt, Congo red W] is divalent anion at pH above 5.5 and become protonated at lower pH values. It is used as acid-base indicator, red in alkaline solutions and blue in very acidic solutions [1, 2]. This variation in structures with decreasing pH of medium causes change in colour from red into purple and then blue due to delocalization of π -system with decreasing pH [3]. Thus, the characteristic different species are assigned by Pigorsch et al. [4] and Yermiyahu et al. [5].

The pH and pKa are essential parameters to understand the behavior of chemical substance in everyday life. The acid dissociation constant (pKa) as a substance property is recognized as being the most commonly used parameters in modern chemistry. The acid dissociation constant (pKa) is an important parameter refer to the degree of ionization of molecules

in solution at different pH values. The chemical, physical and biological properties for many natural and synthetic compounds are affected by the acidic and basic properties. The pKa in such compounds is controlled in many bioprocesses such as metabolism. So, the study of this parameter is a significant interest in biology, pharmaceuticals, medicine, and other scientific fields [6, 7]. The acid dissociation constant can be obtained using a variety of methods, including potentiometric pH measurement, conductivity measurement and absorption spectrometry techniques [8 - 11]. The spectrophotometric technique is more sensitive and appropriate for studying solution chemical equilibria. For various acid-base indicators, which have different functional groups, there is a marked spectral change with the pH variation of the solution [12 - 14]. Thus, the dependence of pKa constant on different factors including temperature, relative permittivity of solvents and ionic strength [13,14].

The ionic strength is an expression of the effect

of ions in solution on the electrostatic potential and it plays important role in the acid-base equilibria according to the Debye-Huckel theory [15]. Moreover, Media from high ionic strength is used to estimation of stability constants in order to minimize changes during titrations, in the activity quotient of solutes at lower concentration [16]. Also, the effect of change of ionic strength on the acid dissociation constant is reported in many papers [17 - 19]. It is found in the aqueous solution in absent buffer, the ionic strength has strong effect on the electronic spectra of Congo red by Sladewski et al. 2006 [20]. Hence, the acidity constants of some indicators in anhydrous solution are dependent on ionic strength in buffer solutions [21 - 23]. Therefore, the position of the acid - base equilibria for these indicators is shifted.

This work aimed to study the effect of ionic strength on the dissociation constants of Congo red, Fig.1, at room temperature by using spectroscopy technique. Moreover, the shape of spectra, optical density and acid-base equilibria for Congo red were varied with changing the ionic strength. Also, the acid dissociation constants of Congo red in various ionic strength 0.5, 0.3, 0.1 and 0.05 mol L⁻¹ have been estimated by three methods.

EXPERIMENTAL

Reagents

The Congo red and reagents used in this work were purchased from Sigma - Aldrich, BDH and Merck companies. All chemical and reagents were HPLC grade and have been used without further purification with highly purity (> 96 %).

Preparation of stock solutions

25 mg was weighed separately and dissolved in some quantity of 50 % aqueous ethanol before making up to 25 mL volume with the same solvent to give 1.4×10^{-3} mol L⁻¹ stock solution of Congo red. The working solution 1.4×10^{-5} mol L⁻¹ prepared by dilute 0.1 mL of stock solution in 10 mL volumetric flask. The pH control was achieved using modified universal buffer solutions [8].

Preparation of buffer solution

The universal buffer utilized in this work was prepared by mixing 0.04 mol L⁻¹ of H₃BO₃, H₃PO₄ and glacial CH₃OOH acids and including the desired volume

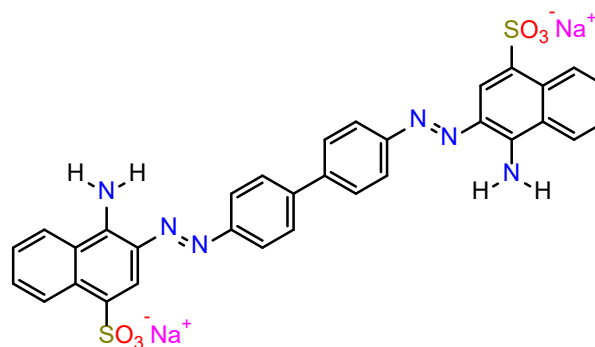


Fig. 1. Structure of Congo red (CR).

of 0.2 mol L⁻¹ NaOH (CO₂free) to grant the specified pH. The ionic strength of the considered solution was balanced by including 0.5, 0.3, 0.1 and 0.05 mol L⁻¹ solution of KCl. All solutions were prepared with de-ionized and CO₂-free water. The distilled water was obtained by using Gambro distilled water device (AK95 S, Sweden).

Instrumentation

The electronic absorption spectra in solutions were recorded with CECIL-CE 7400 (S.n.146368, England) UV-Visible Spectrophotometer model cell, covering the wavelength range 250 - 700 nm with a 1 cm path length quartz cell at room temperature. The pH measurements were carried out using Precise pH-benchmeter Model PHS-3C accurate to ± 0.01 pH unit, where the pH meter was standardized with standard buffer solutions (pH = 7 and 10) at room temperature 25°C.

Calculation of the pKa Values from spectrophotometric data.

The dissociation constant of the Congo red was estimated from the data obtained within the pH range 2 - 12. Three different spectrophotometric methods are applied to calculate the pKa values. The half-curve height, the modified limiting absorption and Colleter methods [24].

The half-curve height method:

The pK was evaluated at a constant wavelength from the half height of the A_s versus pH curves as follows:

$$\text{pH} = \text{pKa} + \log \frac{(A_s)_{1/2}}{(A_s)_{\text{max}} - (A_s)_{1/2}} + \log \gamma_i \quad (1)$$

where γ_i = activity coefficient terms and controlled by ionic

strength of supporting electrolyte, at $(A_s)_{1/2} = \frac{1}{2}(A_s)_{\max}$

then $\frac{(A_s)_{1/2}}{(A_s)_{\max} - (A_s)_{1/2}} = 1$. Hence, $pK_a = pH$ at half height

of the curve, where the pK_a values are calculated. The activity coefficient of molecular species was calculated according to the Debye-Huckel limiting law:

$$\log \gamma_i = -A(Z_+ Z_-) \sqrt{I} \quad (2)$$

where A is constant (0.509) at room temperature 25°C, Z is charge of ion species and I is ionic strength [25].

The modified limiting absorption method:

In the modified limiting absorption method, the pK_a values were evaluated by applying the subsequent equation:

$$pH = pK_a + \log \gamma_i + \log \frac{(A_s) - (A_s)_{\min}}{(A_s)_{\max} - (A_s)} \quad (3)$$

where $A_{s_{\max}}$ is that the maximum absorption, $A_{s_{\min}}$ is that the minimum absorption, A_s is the absorption at any pH and γ_i is activity coefficient term. By plotting the log absorbance ratio versus pH, a straight line was obtained with a slope, when log absorbance ratio term equals zero, $pK_a = pH$.

The Colleter method:

In this method, the pK_a values were evaluated, where three different concentrations of hydrogen ions were selected and their absorbance values got, $[H^+]_1 > [H^+]_2 > [H^+]_3$ and $A_{s1} > A_{s2} > A_{s3}$. The acid dissociation

constant is calculated:

$$K = \frac{[H^+]_2 - M[H^+]_3}{M - 1} \quad (4)$$

$$M = \frac{A_{s3} - A_{s1}}{A_{s2} - A_{s1}} * \frac{[H^+]_1 - [H^+]_2}{[H^+]_1 - [H^+]_3} \quad (5)$$

Then, the average pK_a values were calculated by adding three values for the pK_a in three methods and then dividing on the count by those methods and the results were listed in the Table 1.

RESULTS AND DISCUSSION

The influence of pH on the electronic absorption spectra of Congo red.

The electronic absorption spectrum of Congo red indicator is investigated in various pH's values at room temperature 25°C. In Fig. 2 it could be seen that the intensity and also the band position are pH-dependence. An isosbestic point is observed according to the rearrangement of the molecule and also the ionization of the groups, Fig. 2. The formation of an isosbestic point refer to the existence of acid-base equilibria between two absorbing species [26]. In aqueous solution, which the molecular structure is influenced by alterations in the pH leading to changes in the absorption characteristics and color is associated to the modification of the π system delocalization pattern. Different positions are available for protonation such as azo group $-N=N-$ to give protonated form $H-N^+=N-$. The azo-hydrazo

Table 1. Acidity constants of Congo red (1.4×10^{-5} mol mL⁻¹) in numerous solutions of 50 % water-ethanol in different ionic strength at room temperature 25°C.

Ionic strength	Method 1		Method 2		Method 3		Average pKa		λ_{\max}	Ref.
	pka ₁	pka ₂	pKa ₁	pKa ₂	pKa ₁	pKa ₂	pKa ₁	pKa ₂		
-	-	-	-	-	-	-	4.1	-	-	31
0.00	-	-	-	-	-	-	3.60	-	494nm	32
0.05	3.95	11.55	4.05	11.30	4.19	-	4.08 ± 0.12	11.43 ± 0.18	490 nm	this work
0.10	4.40	11.40	4.49	11.30	4.43	11.55	4.42 ± 0.05	11.35 ± 0.15	490 nm	„
0.30	4.05	10.85	4.31	10.90	4.18	10.99	4.18 ± 0.13	10.91 ± 0.07	485 nm	„
0.50	4.20	11.20	4.09	11.22	3.62	10.62	3.97 ± 0.30	11.01 ± 0.34	480 nm	„

*Method 1: half-curve height method, Method 2: limiting modified absorption method, Method 3: Colleter method.

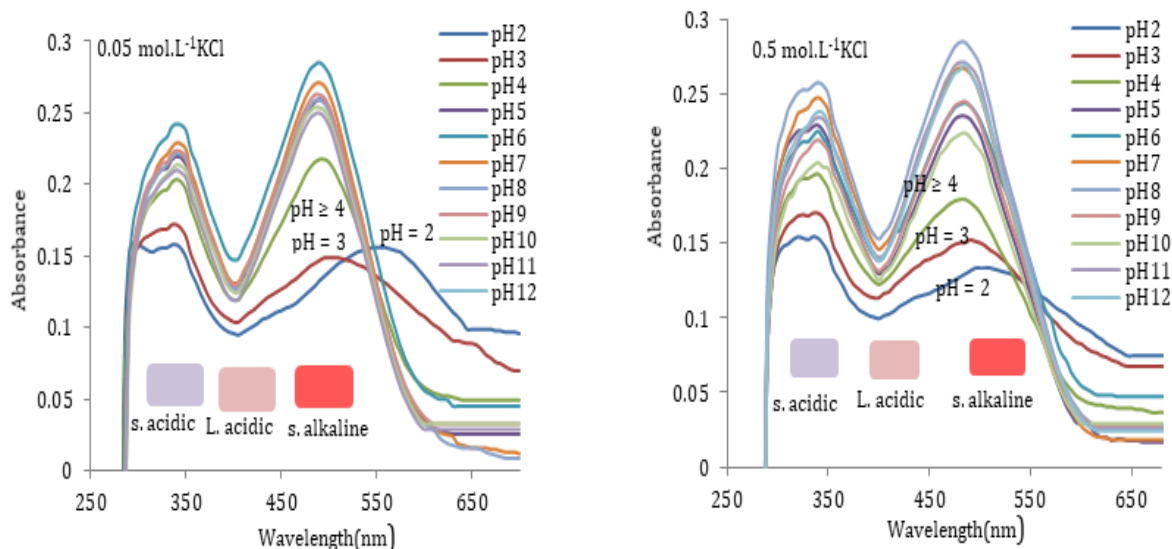
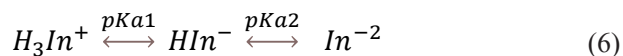


Fig. 2. The electronic absorption spectra of $1.4 \times 10^{-5} \text{ mol L}^{-1}$ solution of Congo red in 50 % aqueous ethanol at different pH's.

tautomerism can be strongly influenced by the synergetic tautomerism in another portion of the molecule [13, 14, 24]. Three stages as possible to protonation of Congo red are proposed by Yermilahu et al. at the pH ranges 5.5 - 3.6, 3.6 - 1.1 and at pH < 1 and at pH ≥ 5.5 the unprotonated form is formed [5].

The electronic spectra of $1.4 \times 10^{-5} \text{ mol L}^{-1}$ solution of Congo red in 50 % (v/v) ethanol-water solution within the pH range 2 - 12 at 0.05 mol L^{-1} KCl as for example show in Fig. 2. The electronic absorption spectra showed two well-defined bands, the first bands at $\lambda_{\text{max}} = 340 \text{ nm}$ are assigned to $n-\pi^*$ transitions of $\text{N}=\text{N}$ group [14] and other bands centered at $\lambda_{\text{max}} = 490 \text{ nm}$ are assigned to $n-\pi^*$ transition with as possible CT nature formed through the conjugation between the aromatic rings systems via azo linkage [13, 24]. The intensity of first absorption bands at $\lambda_{\text{max}} = 340$ is lowest in addition to no change in position with decreasing the pHs. On other hand, the second bands at $\lambda_{\text{max}} = 490 \text{ nm}$ for buffer solutions with the pH ≥ 6 that remain at the identical position with hyperchromic shift in intensity. In contrast, the acidic solutions with pH ≤ 5 , which the position of second bands is changed and become very broad this suggesting the protonation reaction with as possible formation of different resonance structures, Fig.3. The proton dissociation scheme can be represented as below:



At alkaline pH, the Congo red solution is red, at neutral is purple and which turns gradually to violet-blue when the pH is lowered, Fig. 3. In this study, at pH values ≥ 6 , the unprotonated anionic form is present. In acidic solution occur protonation on two sites in Congo red, which the first position is one amino group and second position is azo groups, this result is accordance with results reported by Yermilahu et al. [27] at pH ≤ 5 the protonated CR consists of three structures: two tautomeric forms by Yermilahu et al. [27] and Panczyk et al. [28] (Fig. 3 (Ia and Ib)). Accordingly, the color of the solution changes to purple due to the formation of the protonated form of the CR molecule, which is exist as a resonance hybrid between its quinone diimine form [Ia] and azonium [Ib] structures [29, 30]. Consequently, with increasing the pH, the protonation sited of the Congo red molecule containing the ammonium group is also possible, Fig. 3 [II] which the unprotonated form Fig. 3 [III] is observed in highly basic medium more than pH ≥ 6 . The spectrum of solutions forming an isosbestic points at 535 nm indicating the equilibrium between over one species of the protonated kinds of CR, Fig. 2. [13, 14, 23].

The influence of ionic strength on the electronic absorption spectra in different pHs

Herein, it noted that the ionic strength affected on the acid-base equilibria, which the electronic absorption spectra of Congo red in presence of various ionic

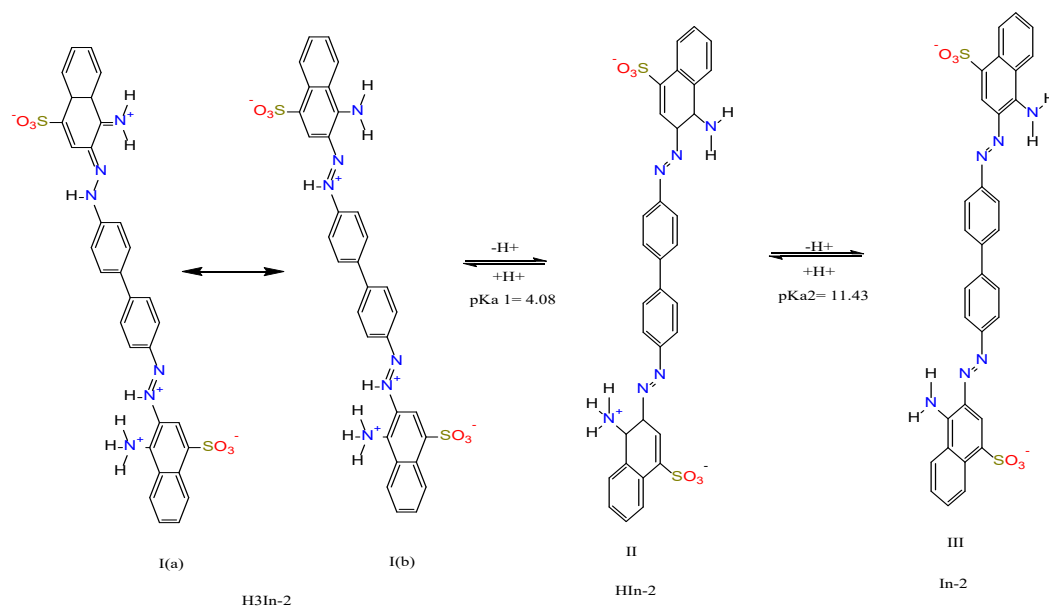


Fig. 3. The dissociation mechanism of Congo red with changing the pHs.

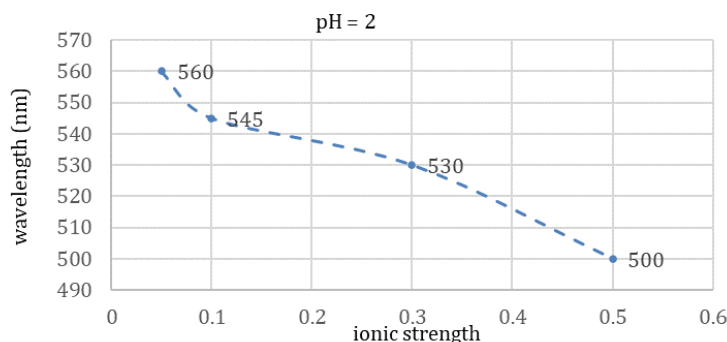


Fig.4. The change of wavelength with increasing the ionic strength in solutions.

strength is blue shifted with the rise in ionic strength, see Fig. 2. The intensity of solutions colour is decreased with growth of ionic strength. Therefore, the change in the shape of the spectrum is obtained for the indicator solution as a function of ionic strength is shown in, Fig. 2. These findings agreed with results reported by Sladewski et al [20]. Moreover, the effect of ionic strength is evident for acidic solution, which the shift in the λ_{max} for pH = 2 solution is increases with increasing ionic strength, Fig. 4, toward the blue shift in order λ_{max} at $0.05 < 0.1 < 0.3 < 0.5$. Also, the optical density of Congo red in various buffer solutions decreases with the rise in ionic strength and the shape of curve change when ionic strength increased show in Fig. 5. Finally, from Fig. 2 the position of acid-base equilibria shifted toward the higher pHs with increasing the ionic strength.

On scrutiny the results in Table.1, the calculations of ionization constants of Congo red at different ionic strength by using the three mentioned spectrophotometric methods reveal two acidity constants (pK_{a} 's). The $\text{pK}_{\text{a}1}$ value is attributed to the dissociation the proton from azo groups, H_3In^{-2} . However, the other $\text{pK}_{\text{a}2}$ value is attributed to the dissociation of proton of the amino group, HIn^{-2} and formation neutral form, ($\text{HIn}^{-2} \leftrightarrow \text{In}^{-2}$), this pK_{a} is around 10 [13]. These acidity constants are different in values as results as change the ionic strength of solution. This fact explained by the Debye-Huckel theory based on the relationship between concentration and activity, which the ions in solution interact with each other via coulomb forces. These ions are not separately treated in solutions because of these interactions in equilibrium state. It is clear from Fig. 6 that an increase

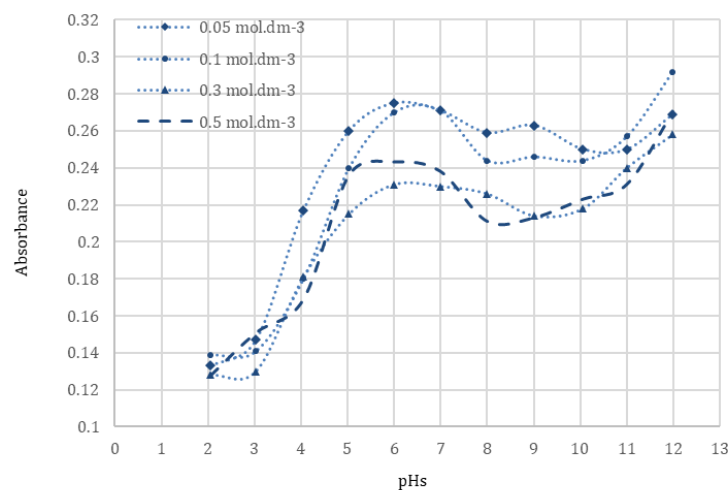


Fig. 5. This plot represents the half-height method; absorbance versus pH curve of Congo red and at the same time represent the change in the optical density with increasing ionic strength.

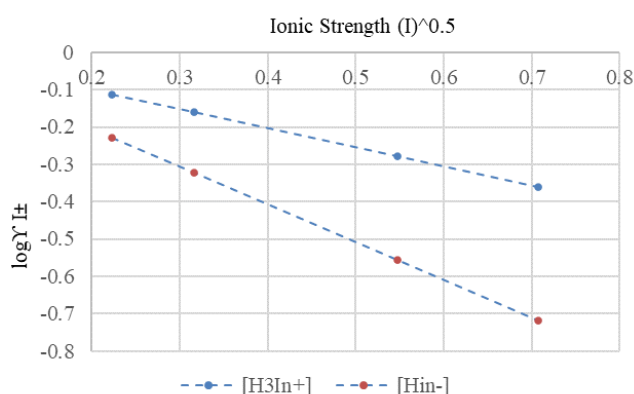


Fig. 6. The mean activity coefficient against square root of ionic strength for molecular species.

in the ionic strength resulted a decrease in the mean ionic activity of coefficient leading to decrease in the acidity constants, this result is discordance with result reported previously by Naumova et al. [33], this different in findings due to different the pH range, dye concentration, experimental method and method of analysis.

Hence, the effect of ionic strength on the acidity constants is investigated and the results obtained are given in Table 1. The acidity constants of Congo red are mainly affected by the electrostatic interactions as result as the ionic strength that affecting on the inter- and intra-molecular interactions. It is seen from Table 1, the first acidity constant pK_{a1} is decreases with increasing ionic strength in the range (0.1 - 0.5 mol L⁻¹ KCl). In contrast,

the second pK_a is decreased with increasing the ionic strength in the range (0.05 - 0.3 mol L⁻¹ KCl). Further, the increase of K⁺ ion concentrations is responsible to the lower in acidity constant as at high concentration due to increasing electrostatic shielding effect [16, 34].

Distribution of species at different pH values

In the distribution diagrams, a plot the mole fraction of an acid species versus that mole fraction varies with pH is made, Fig. 7. The variation of the species is due to the acid dissociation shifting as pH changes [35]. CR has two pK_a values and is considered as a diprotic acid. The concentration of distribution diagrams shows that the ligand is protonated giving H_3In^+ species at pH 2. Upon increase of pH the concentration of this species decrease and the mono-protonated species HIn^- starts to form and reach its maximum concentration of 100% in the pH range 6 – 9, on further increase of pH the concentration of mono-protonated species decrease and the unprotonated species In^{2-} start to form and reach its maximum concentration of 100 % in the pH \geq 11. It is concluded that the presence of three species varied as pH changes. In acidic medium, the predominant species is H_3In^+ , in basic medium, the predominant species is In^{2-} and in neutral medium, the predominant species is HIn^- . This means the Congo red in acidic medium liberate one proton from amino group and in neutral medium the Congo red liberate two protons from azo group.

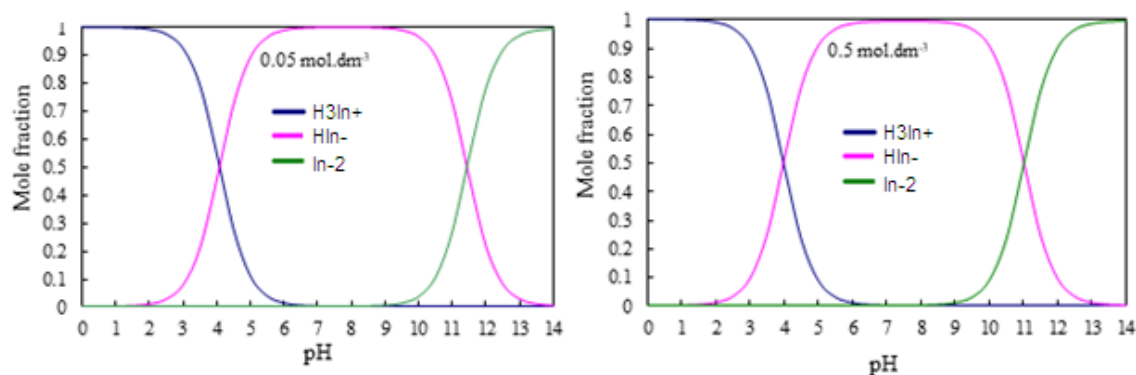


Fig.7. Distribution diagram of the acid species of Congo red in % 50 (v/v) water-ethanol at different pH's at room temperature 25°C.

CONCLUSIONS

Herein, the electronic absorption spectra of Congo red in different pHs solutions at room temperature was measured. In addition, the influence of ionic strength on the acid-base equilibria and acidity constants was investigated. The acidity constants of Congo red were determined by three methods from the spectrophotometric data and produced results with good similarity. Therefore, the ionic strength was affected on the acid-base equilibria this fact confirmed by blue shift with increasing the ionic strength. The acidity constants pK_{a1} and pK_{a2} values of Congo red were affected by ionic strength, which the pK_{a1} values decreases with increasing ionic strength from 0.1 into 0.5. In contrast, the pK_{a2} values decreases with the rise in ionic strength from 0.05 into 0.3, which this difference in pK_a 's values were assigned to strength of electrostatic interaction in solutions.

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