Research Article

Photolysis of Riboflavin in Buffer solution at Different pH

Emad Yousif, Shahbaz Maki and Sura Attwan

Department of Chemistry, College of Science, AL-Nahrain University, Baghdad, Iraq.

ABSTRACT

This work was made to study the photochemistry of riboflavin and the kinetics of riboflavin degradation after exposure to UV-Vis light phosphate buffer solution at different pH interval (2-10) with the same concentration (5×10^{-5}M) was studied by the of UV-Vis spectrophotoscopy analyses, it showed that degradation increase as pH increase from pH (2-7)where the lumichrome a photoderivative forms in the acidic medium. a maximum degradation occurs at neutral pH (7). Then it began to decrease from pH (7-10). Where the lumiflavine (a photoderivative occurs at basic media) formed. We also calculated the quantum yield of this decomposition and found that an increasing occur from pH (2-7) (1.902×10^{-4}-6.020×10^{-4}) then a decrease occur from pH (7-10) (6.020×10^{-4}-1.266×10^{-4}) as well as the reactivity ratio.

INTRODUCTION

A vitamin is an organic compound required as a nutrient in tiny amounts by an organism[1]. Vitamins are classified as either water-soluble or fat-soluble. In humans there are 13 vitamins: 4 fat-soluble (A, D, E, and K) and 9 water-soluble (8 B vitamins and vitamin C). Vitamins have diverse biochemical functions.

Vitamin b2 (Riboflavin) exhibits strong absorption in the ultraviolet and visible region and undergoes degradation by complex photochemical reactions. These reactions involve intramolecular and intermolecular photoreduction, intramolecular and intermolecular photoaddition and intramolecular photodealkylation. The nature and magnitude of photochemical reactions are influenced by many factors including solvent polarity[2,] pH of medium[3-5,] buffer kind and concentration[6], ionic strength[7], oxygen content[8], and light intensities and wavelengths[7]. riboflavin exhibit a photophysical processes when exposed to light The first step of a physical process induced by light involves absorption of a quantum of light by a molecule, producing an electronically excited state. The molecule is said to go from the ground to an excited state. Once in the excited state, the molecule has several available pathways to release the absorbed energy. Different processes may occur: Non radiative decay: the process of vibrational relaxation, in which the excess energy is transferred to the surroundings as thermal motion of the environment (heat) as: Internal conversion, Intersystem crossing (ISC), the other is radiative such as Fluorescence and phosphorescence .the last with a chemical change, when energy results in changes in bonding structures, or a combination of these[9]. Here is some of the Determination methods of riboflavin such as high-speed liquid chromatography[10], isocratic HPLC method[11],elution methods[12] thin-layer chromatography[13], photochemical spectrophotometric method[14], spectrophotometric determination[15], chemiluminescence[16], electrochemical method[17].

MATERIALS AND METHODS

Vitamin B2 (riboflavin) powder given from state company for drugs industries and medical apppliances (samrra/Iraq).

All other chemicals and reagents were obtained from Aldrich chemical company, Inc.

standard solutions of 5×10^{-5}M were prepared at PH range (2,3,4,6,7,8,9,10) using phosphate buffer, and exposed to UV radiation for several hours to measure

the rate of the photodegradation of riboflavin in that pH range.

The absorption spectrum of riboflavin was measured, and then the absorption spectrum of riboflavin at each pH (2.00-10.00) was measured in the range of (300-700) nm. Each half hour the absorbance were measured at λ_{max} and recorded. Distilled water solvent was used as a reference in order to study the kinetic of photodecay of the riboflavin at different pH. The absorbance at infinite time (A_∞) was measured after the solution was irradiated for at least (more than 24 hours). The specific rate constant of the decomposition of the riboflavin (K_d) was determined by the following first order equation [18]

$$\ln(a- x) = \ln a - K_d \quad (1)$$

Where

a = Concentration of riboflavin before irradiation.

x = Concentration of riboflavin after t = time of irradiation.

t = Time of irradiation of riboflavin solution.

A_0 = Absorbance of riboflavin before irradiation.

A_t = Absorbance of riboflavin after irradiation time.

ε = Molar extension coefficient

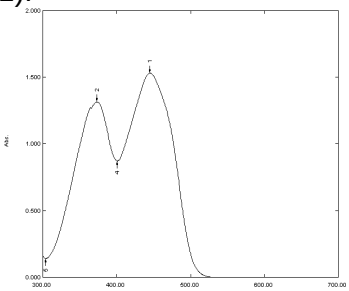
$a = (A_0 - A_\infty) / \varepsilon$, $x = (A_0 - A_t) / \varepsilon$, $a - x = (A_t - A_\infty) / \varepsilon$.

By substitution of a and a - x in equation (1) and rearrangement

$$\ln(A_t-A_\infty)=\ln(A_0-A_\infty)-K_d\, t \quad (2)$$

RESULTS AND DISSCUSION

Riboflavin has showed a maximum absorption at 445, 373 nm when scanning with a range of (300 -700 nm) as shown in Figure (1).



UV-VISIBLE spectrum of riboflavin

Thus a plot of ln $|A_t - A_\infty|$ versus irradiation time (t) gives a line with a slope equal to K_d (S^{-1}). The rate of photodecomposition (R_d) was calculated for riboflavin at each pH using the following equation[18]

$$R_d = K_d \times [\text{concentration of riboflavin}]$$

2.8 Decomposition Quantum yields (Q_d)

After measuring the absorbance of riboflavin at each pH. A knowledge of the incident light intensity (determined by actinometry using the method of Hatchard and Packer [19] and the extinction coefficient of the compound enable the quantum input (I_{abs}) to be calculated.

The quantum yield of photodecomposition (Q_d) is defined by:

$$Q_d = \frac{\text{Rate of photodecomposition}}{\text{Quantum input}}$$

For riboflavin at each pH using the following equation (3)[20]

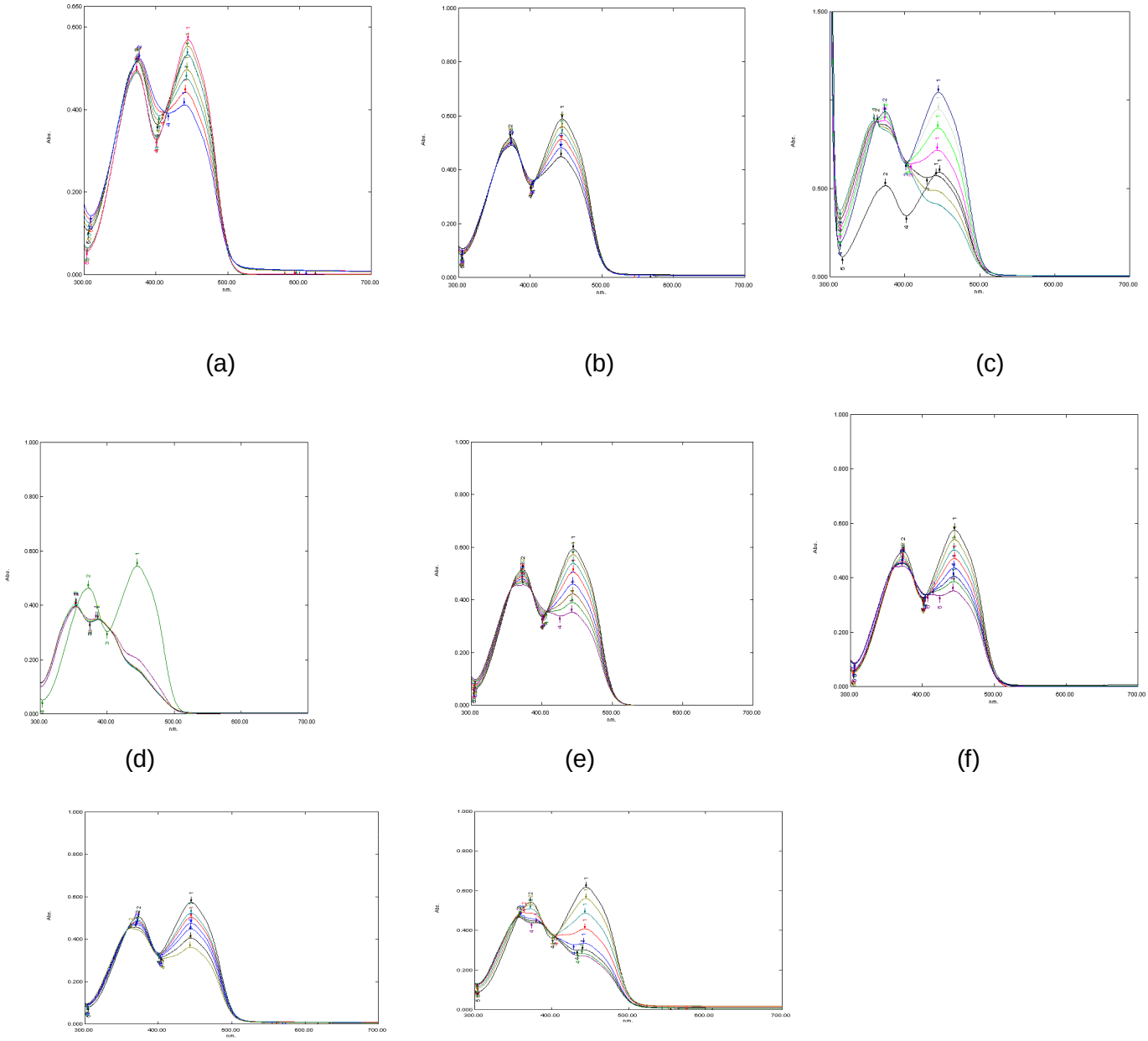
$$Q_d=K_d\times[\text{conc.}] / I_{abs} \quad (3)$$

The reactivity ratio (R_r) (K_2/K_{-1}) was also calculated for riboflavin at each pH using the following equation[20]

$$R_r=Q_d/1-Q_d \quad (4)$$

Spectrophotometeric measurements

On irradiation of 5×10^{-5} M of riboflavin at different pH (2.00-10.00) at room temperature, the riboflavin absorption spectrum changes with the irradiation time. A decrease in the absorbance intensity was observed at wavelength of its maximum absorbencies as shown in Figures (2) for (π→π*) transitions in all prepared solutions. The changes in absorbance (A_t) values with the irradiation time from these changes in absorbance values during irradiation; one could say that photoreduction and photodealkylation reaction occurs predominantly through the excited triplet state of riboflavin.



(a) (b) (c)

(d) (e) (f)

(g) (h)

Fig. 2: Photodegreadation spectra of ($5 \times 10^{-)5}$ M riboflavin at different pH after exposure for (1= 0hr, 2=0.5hr, 3=1 hr, 4=1.5 hr, 5=2hr, 6=2.5 hr, 7=3 hr, 8=3.5 hr). where a) represents pH2, b) pH3 c) pH4 d) pH6 e)pH7 f)pH8 g)pH9 h) pH10

The natural logarithm of each value was taken. To convert the negative values of the resulting logarithm, three was added to each.

Table 1: Natural logarithm of absorbance with irradiation time of riboflavin at

Irradiation time(sec.)	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$
0	0.573	0.561	-0.578	1.421	0.576	0.562	-0.576	1.413	0.583	0.571	-0.560	1.439
1800	0.549	0.537	-0.621	2.378	0.529	0.515	-0.663	1.336	0.521	0.521	-0.652	1.347
3600	0.524	0.512	-0.669	2.330	0.492	0.478	-0.738	1.261	0.478	0.466	-0.763	1.236
5400	0. 502	0.490	-0.713	1.286	0. 457	0.443	-0.814	1.185	0. 442	0.430	-0.843	1.156
7200	0.477	0.465	-0.765	2.234	0.436	0.422	-0.862	1.137	0.396	0.384	-0.957	1.042
9000	0.454	0.442	-0.816	2.183	0.408	0.394	-0.931	1.068	0.366	0.354	-1.038	1.964
10800	0.431	0.419	-0.869	1.130	0.385	0.371	-0.991	1.008	0.333	0.321	-1.136	0.863
12600	0.418	0.406	-0.901	1.098	0.36	0.346	-1.061	0.938	0.310	0.298	-1.210	0.789
	A_∞ = Absorbance at infinite time = 0.012 PH=2				A_∞ = Absorbance at infinite time = 0.014 PH=3				*A_∞ = Absorbance at infinite time = 0.012 PH=4*			

Irradiation time(sec.)	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$
0	0.573	0.578	-0.548	1.451	0.62	0.61	-0.494	1.505	0.582	0.57	-0.562	1.437
1800	0.549	0.499	-0.695	1.304	0.547	0.537	-0.621	1.378	0.495	0.483	-0.727	1.272
3600	0.524	0.438	-0.825	1.174	0.473	0.463	-0.770	1.229	0.446	0.434	-0.834	1.165
5400	0. 502	0.376	-0.978	1.021	0.415	0.405	-0.903	1.096	0.394	0.382	-0.962	1.037
7200	0.477	0.332	-1.102	0.897	0.342	0.332	-1.102	0.897	0.356	0.344	-1.067	0.932
9000	0.454	0.283	-1.262	0.737	0.299	0.289	-1.241	0.758	0.321	0.309	-1.174	0.825
10800	0.431	0.252	-1.378	0.621	0.256	0.246	-1.402	0.597	0.280	0.268	-1.316	0.683
12600	0.418	0.229	-1.474	0.525	0.221	0.211	-1.555	0.444	0.250	0.238	-1.435	0.564
	A_∞ = Absorbance at infinite time = 0.013 PH=6				A_∞ = Absorbance at infinite time = 0.01 PH=7				*A_∞ = Absorbance at infinite time = 0.012 PH=8*			

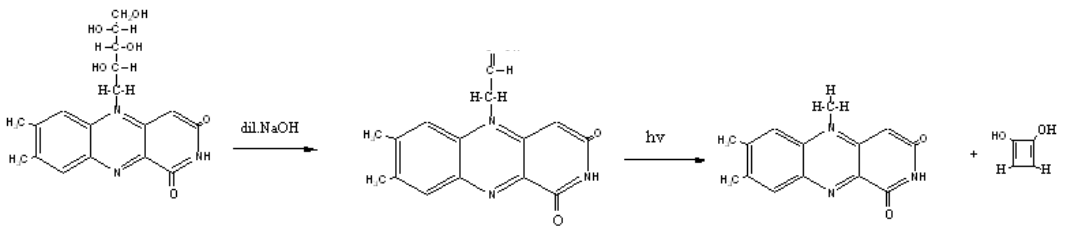
Irradiation time (sec.)	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$	A_t	$(A_t - A_\infty)$	$\ln(A_t - A_\infty)$	$2+\ln(A_t - A_\infty)$
0	0.569	0.555	-0.588	1.411	0.565	0.555	-0.587	1.413
1800	0.526	0.512	-0.669	1.330	0.552	0.539	-0.618	1.382
3600	0. 488	0.474	-0.746	1.253	0.534	0.521	-0.651	1. 349
5400	0. 461	0.447	-0. 805	1.194	0. 518	0.505	-0.682	1.318
7200	0.425	0.411	-0.889	1.110	0.503	0.49	-0.712	1.288
9000	0.398	0. 384	-0.957	1.042	0.482	0.469	-0.757	1.242
10800	0.369	0.355	-1.035	0.964	0.471	0.454	-0.789	1.211
12600	0.451	0.337	-1.087	0.912	0.456	0.443	-0.814	1.185
	A_∞ = Absorbance at infinite time = 0.014 PH=9				A_∞ = Absorbance at infinite time = 0.013 PH=10			

These values were then plotted against irradiation time, as shown in Table (2). The slopes of these plots represent the inverse photodecomposition rate constant (K_d) of riboflavin at each pH.

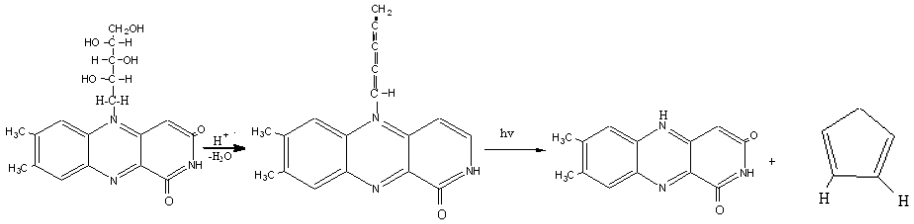
Kinetic of the photodecomposition reactions using UV-VISIBLE spectrophotemetric measurements

The change in the UV-VISIBLE absorptions spectra during irradiation were monitored through the photolysis experiments. The photolysis of riboflavin during the irradiation at 445±1 nm was followed by the change in the vitamin concentration spectrophotometrically. In order to determine the photodecomposition rate of riboflavin from this change, it was found that the value of $(A_t - A_\infty)$ decreased exponentially with irradiation time corresponding to the first

order vitamin decomposition and was consistent with first order reaction.



(5)



(6)

The straight lines are consistent with the first order riboflavin decomposition processes. Therefore, from the slopes of these straight lines, the values of specific rate constants (K_d) were evaluated. Using the value of (K_d), the rate of photodecomposition were calculated (Rate $=K_d$ [Concentration of riboflavin]) and the quantum yield of this process is deduced. The following kinetic equilibrium[18] might be followed for the reactions in equation (5) and equation (6).

$$-\frac{d[RF]^*}{dt} = I_{abs} - K_{-1}[RF]^* \qquad (7)$$

Where I_{abs} is absorbed intensity radiation. I_0 of water was calculated and found to be equal to 1.3891×10^{-5} E $in.l^{-1}.S^{-1}$.[18] respectively. These values were used in the calculation of the quantum yield according to equation (8):

Q_d = rate of photodecomposition / I_{abs} (8)

Since the rate of excited state decomposition can be expressed as in equation (7) is:

$$-\frac{d[RF]^*}{dt} = I_{abs} - K_2[RF]^* - K_{-1}[RF]^* \qquad (9)$$

Assuming that the $[RF]^*$ excited state concentration is fixed, then

$$[RF]^* = \frac{I_{abs}}{K_{-1} + K_2} \qquad (10)$$

The value of excited state concentration, $[RF]^*$, in equation (10) is substituted in equation (7), one can get

$$-\frac{d[RF]}{dt} = I_{abs} - \frac{I_{abs} K_{-1}}{K_{-1} + K_2} = I_{abs}\left(1 - \frac{K_{-1}}{K_{-1} + K_2}\right) \quad (11)$$

Then equation (8) can take the form

$$Q_d = \frac{\text{Rate of photodecomposition}}{I_{abs}} = -\frac{d[RF]}{dt} \Big/ I_{abs} \quad (12)$$

The value of quantum yield of photodecomposition (Q_d) can then be given by equation (13):

$$Q_d = 1 - \frac{K_{-1}}{K_{-1} + K_2} \quad (13)$$

Or

$$Q_d = \frac{K_2}{K_{-1} + K_2} \quad (14)$$

By rearranging equation (13) and equation (14), we can obtain equation (15) for the value of reactivity ratio (K_2 / K_{-1}).

$$\frac{K_2}{K_{-1}} = \frac{Q_d}{1 - Q_d} \quad (15)$$

Equation (15) was used to calculate the reactivity ratio of the photodecomposition of riboflavin at different pH. These values are listed in Table (2).The results of Table (2) for the reactivity ratio indicated that these values began to increase from pH (2.00-7.00) then decreased from pH (7.00-10.00) it may be due to the lack of riboflavin-phosphate divalent ions complex break down preferring photoreduction reaction than the photoaddition reaction [21]. it is also may be due to the presence of HCl with the buffer component (HPO_4^{-2}, $H_2PO_4^{-1}$) that would make the medium to be more acidic (H_3PO_4) and increase the rate of the degradation. While for the photodegradation of riboflavin in the basic medium it may be due to the presence of KOH and that would make salty medium and cause the decrease in the rate of photodegredation rate. The presence of CH_3- group in riboflavin basic derivatives may also be responsible for the decrease in its photodegradation rate.

Table 2: Specific rate constant (K_d), photodecomposition constant (R_d), the quantum yield (Q_d) and the reactivity ratio (K_r) for riboflavin at different pH in water (Irradiation wavelength 445 ± 1)

riboflavin	Concentration 10^{-5}M	r r	K_d 10^{-5} s^{-1}	R_d $10^{-9}s^{-1}$M	Q_d 10^{-4}	K_r 10^{-4}
pH2	5.000	0.999	0.00003	1.5	1.902	1.902
pH3	5.000	0.9979	0.00004	2	2.560	2.560
pH4	5.000	0.999	0.00005	2.5	3.205	3.206
pH6	5.000	0.9985	0.00007	3.5	4.548	4.550
pH7	5.000	0.999	0.00009	4.5	6.020	6.024
pH 8	5.000	0.9987	0.00007	3.5	4.507	4.509
pH9	5.000	0.9991	0.00004	2	2.542	2.542
pH10	5.000	0.9986	0.00002	1	1.266	1.266

From the results shown in Table (2), one could notice that K_d and Q_d values were dependent on the presence of CH_3- group in the compound. The photodecomposition decreased as the CH_3- group existed in the compound.

REFERENCES

1. Shari Lieberman and Nancy Bruning. The Real Vitamin and Mineral Book", NY: Avery Group, 2nd edition. USA, 1990.
2. Schmidt H, Brown EB, Schwaller B and Eilers. J Biophys J. 2003a; 84(4):2599–2608.
3. Carr DO and Metzler DE. Biochim Biophys Acta. 1970;205:63- 71.
4. Schachman HK. Method Enzymol. 1975;4(32):99-103.
5. Ahmad I and Rapson HD. J Pharm Biomed Anal.1990; 8:217-223.
6. Ahmad. J Phys Chem. 2012A;116(4): 1199–1207.
7. Kenji Sato, Hiroyasu Taguchi, Tomoko Maeda, Hironori Minami, Yuji Asada, Watanabe Y and Yoshikawa K. J Invest Dermatol. 1995;105:608–612.
8. Tread well, Schuman Jorns and Ahmad. 1975;2004a
9. Nelson NL and Cox MM. Lehninger Principles of Biochemistry, 3rd edition New York: Worth Publisher. 2000;1150.
10. Wittmer D and Haney Jr WG. Journal of Pharmaceutical Sciences. 1974;63(4):588–590.
11. Ashoor Erich GJ, Monte WC and Welty J. Journal of Food Science. 1983;48(1):92–94.
12. Anna Gliszczyn´ska-S´wigło and Anna Koziołowa. Journal of Chromatography.2000;881:285–297.
13. Diaz M, Luiz M, Bertolotti SG, Miskoshi S and Garcia NA. Can J Chem. 2004;82:1752-1759.
14. Tomás Perez-Ruiz, Carmen Martínez-Lozano, Virginia Tomás and Otilia Val. Analyst. 1994;119:1199-1203.
15. Aberásturia. Can J Chem. 2002; 82:1752-1759.
16. Zhenghua S and Lin W. Analyst. 2001;126:1393-1398.
17. Zhang H, Jinsheng Z, Houting L, Huaisheng W, Renmin Liu and Jifeng Liu. Int. J. Electrochem Sci. 2010;,52:95 – 301.
18. Maki S Yousif E and Salman E. Photolysis of Tris (ethylxanthato) Chromium (III) complexes in DMSO", Germany:LAP LAMBERT Academic publishing, 2011.
19. Hatchard CG and Parker CA. proc Roy Soc A. 1956;235:518.
20. Al-Lamee KG. Thesis Baghdad univ. Baghdad.1976.
21. Aliwi S and Abduallah S. polm Interat. 1995;35:375-380.