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Spectrophotometric studies for the interaction of As^{+3} ion with some chelators

دراسة طيفية لتآثر ايون الزرنيخ الثلاثي مع بعض المخلبيات

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Abstract

Arsenic is one of the toxic heavy metal when presented in a high level over it allowable limit. To detoxify this heavy metal a chelators were used as in a chelation therapy. This work studies the complexation of some chelators (EDTA, penicillamine, genistein and qurecetin) with As III ion. A spectrophotometric techniques were applied in water and water-ethanol mixture as a solvent, at four different temperature. A shift in it λ_{max} and decrease in absorbance indicating the complex formation. The stoichiometry of these complexes were determined by a method of the continuous variation (Job's) method, it was found that As-EDTA were (2:1), As-gensitein and As-quercetin (1:1), and As-penicillamine (1:2). These chelator's have a high tendency to complex with As III ion which were reflected by its high values of their equilibrium constants. Thermodynamic parameters indicate a spontaneous interaction (negative free energy change ΔG^o), and the enthalpy changr for As-gensitein complex were positive and the other three complexes have negative ΔH^o which depends on type of the interaction and the structures of the complexes. The kinetic calculations shows a second order interaction.

Keywords: chelating agents, EDTA, penicillamine, genistein, qurecetine, kinetic parameters, thermodynamic parameters and complexation.

الخلاصة

الزرنيخ هو احد العناصر السامة عندما يتواجد بمستوى اعلى من المسموح به صحيا. للتخلص من سمية العناصر يتم استخدام مواد مخلبية كما في العلاج بالاستخلاب.

في هذا البحث تم دراسة تكوين معقدات لبعض المواد المخلبية (EDTA، البنسيل امين، الجنستين والكيورستين) مع ايون الزرنيخ الثلاثي طيفيا، في محيط مائي او مزيج من الماء والايثانول وباربعة درجات حرارية (293-308K) تم الاستدلال على وجود المعقدات من الازاحة في λ_{max} والنقصان في الامتصاصية لطيفية المواد المخلبية المدروسة عند اضافة محلول الايون.

النسب المولية للاتحاد بين هذة المخلبيات والايون تم تعيينها بطريقة التغايير المستمر والمعروفة بطريقة جوب وكانت لAs-EDTA (2:1) ، جنستين-As وكيورستين-As (1:1) و البنسيل امين-As (1:2) واظهرت النتائج ميل كبير لهذه المواد للارتباط بايون الزرنيخ الثلاثي حيث ينعكس ذلك من القيمة الرقمية الكبيرة لثابت الاستقرار لها وبنسبة مختلفة حسب نوع المادة وتركيبها الكيميائي.

الدوال الثرموديناميكية تدل على التآثر التلقائي من خلال القيم السالبة لطاقة جبس الحرة (ΔG^o- كما ان ΔH^o كانت اما سالبة او موجبة حسب نوع التآثر بينهما وان حركيات التعقيد كانت من المرتبة الثانية.

1. Introduction:

Chelation describes a particular way that ions and molecules bind metal ions, chelation involves the formation or presence of two or more separate coordinate bonds between a polydentate ligand and a single central atom. Usually these ligands are organic compounds [1]. Called chelators. A medical procedure that involves the administration of chelators to remove heavy metals from the body called chelation therapy. Detoxification of heavy metal by the administration of chelators, forms a stable complex and prevents the toxic heavy metal species from attacking the biological targets [2].

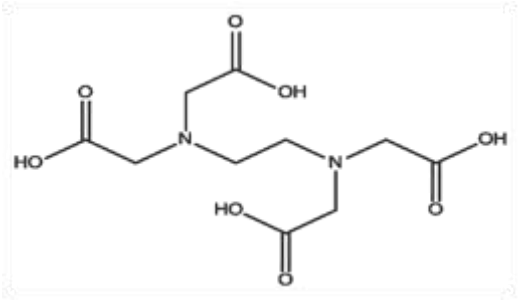
Penicillamine is a well-known heavy metal chelator, classically used in the treatment of Wilson disease, rheumatoid arthritis, and cystinuria. From a dermatologic standpoint, penicillamine was found to be useful in the treatment of systemic sclerosis. The pharmaceutical form is D-penicillamine, it is an α-amino acid metabolite of penicillamine [3].

Quercetin is a flavonoids found in many fruits, vegetables, leaves and grains. It can be used as an ingredient in supplements, beverages, or foods.

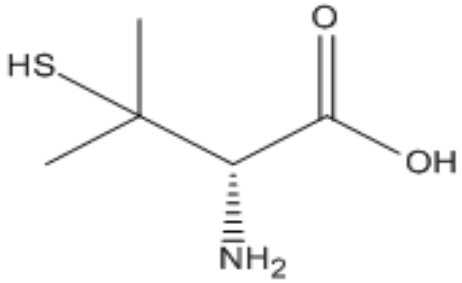
Quercetin widely distributed in nature. The name has been used since 1857, and is derived from quercetum (oak forest), after Quercetin [4]. It is a naturally occurring polar auxin transport inhibitor [5]. Quercetin has anti-oxidant and anti-inflammatory effects, it scavenge damaging particales in the body known as free radicals, which damage cell membranes, tamper with DNA and even causes cell death. Flavonoids were significantly more effectine inhibitors of iron ion-dependent lipid peroxidation systems due to chelating iron ions with the formation of inert iron complexes unable to inhibit lipid peroxidation. At the same time these complexes retained their free radical scavenging activities [6].

Genistein is a phytoestrogen and belongs to the category of isoflavones, it was chemically synthesized in 1928 [7]. Genistein influences multiple biochemical functions in living cells: which exhibits antioxidant, antiangiogenic, and immunosuppressive activities. activation of Peroxisome proliferator-activated receptors (PPARs), inhibition of several tyrosine kinases, inhibition of topoisomerase, inhibition of AAAD, direct antioxidation with some proxidative features, activation of Nrf2 antioxidative response, stimulation of autophagy[8], activation of estrogen receptor beta, inhibition of the mammalian hexose transporter GLUT1, contraction of several types of smooth muscles, modulation of CFTR channel, potentiating its opening at low concentration and inhibiting it a higher doses., inhibition of cytosine methylation, inhibition of DNA methyltransferase [9].

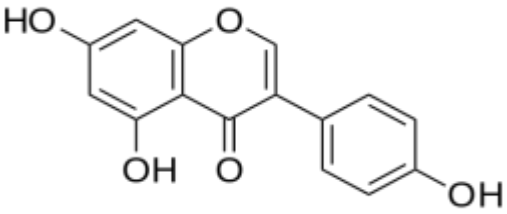
EDTA is an amino polycarboxylic acid and a colourless, water-soluble solid. It is widely used to dissolve limescale. Disodium ethylenediamine tetraacetic acid (Na2EDTA) is the most commonly used chelating agent. It is a derivative of ethylenediamine tetraacetic acid (EDTA); a synthetic polyamino-polycarboxylic acid. Since 1950s it has been one of the mainstays for the treatment of childhood lead poisoning [10] .The drug has been claimed beneficial in vascular disease since 1955. [11]



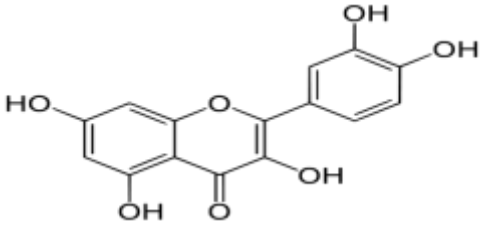
EDTA



Penicillamine



Genstein



Quercetin

Figure (1): Chemical structures of the studied compounds.

Arsenic is a chemical element with symbol As and an atomic number 33. Arsenic occurs in many minerals, usually in conjunction with sulfur and metals, and also as a pure elemental crystal. Arsenic is a metalloid. It can exist in various allotropes, although only the gray form has important use in industry. Arsenic is a common n-type dopant in semiconductor electronic devices, and the optoelectronic compound gallium arsenide is the most common semiconductor in use after doped silicon. Arsenic and its compounds, especially the trioxide, are used in the production of pesticides, treated wood products, herbicides, and insecticides. These applications are declining. Arsenic poisoning is a medical condition caused by elevated levels of arsenic in the body. The dominant basis of arsenic poisoning is from ground water that naturally contains high concentrations of arsenic. A 2007 study found that over 137 million people in more than 70 countries are probably affected by arsenic poisoning from drinking water. Chelating agents that sequester the arsenic away from the blood proteins are used in treating acute arsenic poisoning [12]. The main use of metallic arsenic is for strengthening alloys of copper and especially lead (for example, in car batteries) [13]. I in our present work involves calculation of a thermodynamic parameters and kinetics of the interaction (chelation) of arsenic with some chelators (penicillamine, quercetine, genistein and EDTA) using Uv-Visible spectroscopy.

2. Experimental:

Chemicals and solutions: EDTA was obtained from Labo Chemia, penicillamine Fluka AG. Chem. Fabrik CH-9470 Buchs, genistein from Santa Cruz Biotechnology, and qurecetine from Aldrich chemical company, Methanol from scharlau, Lead nitrate from Hopkin & Williams LTD (CHADWELL HEAT ESSEX ENGLAND).

Way to prepare (EDTA, penicillamine, genistein, qurecetine): The stock solution of EDTA (10^{-2}M) was prepared by dissolving (0.33624 g) in 100mL volumetric flask using distilled water as a solvent. The stock solutions of Penicillamine, genistein (10^{-2}M) were prepared by dissolving (0.2982 g), (0.27 g) respectively in 100mL volumetric flask using 50% ethanol/distilled water mixture as a solvent. The stock solution of Qurecetine (10^{-2}M) was prepared by dissolving (0.3382 g) in 100mL volumetric flask using 80% ethanol/distilled water mixture as a solvent.

As (III) solution: A stock solution of As (III) (10^{-2}M) was prepared by dissolving (0.1299 g) of $NaAsO_2$ sodium arsenite in 100mL volumetric flask using distilled water as a solvent.

Absorption spectroscopy: All spectral measurements were recorded on a double beam UV-Visible spectrophotometric, shimadzue – model - 160A, using a 1cm path length quartz cell.

Absorbance value of EDTA, penicillamine, genistein and qurecetine in the presence and absence of As (III) solution were made in the range of (200-600nm).

Stoichiometry analysis: The stoichiometry of the complexes ligands (penicillamine, quercetine, genistein, and EDTA) with arsenic (III) ion were determined by continuous variation method (Jobs method)[14,15] equimolar concentrations (10^{-4}M) of a ligand and As (III) ion were prepared, and Job's method was applied by placing 1 to 9 mL of (10^{-4}M) ligands solution into a series of 10 mL volumetric flask, this was followed by placing 9 to 1 mL of (10^{-4}M) As (III) ion solution, and the absorbance were measured at the maximum wave length.

Results and Discussion:

Absorption spectroscopy: The optimized solvent mixture (ethanol/water) was obtained by measuring the Uv-Vis absorption spectra of EDTA, penicillamine, genistein and qurecetine in various mixture compositions as shown in Table (1). This Table shows EDTA, penicillamine, genistein and qurecetine absorption band. The band do not exhibit any significant changes in λ_{max} with the variation of solvent composition, whereas the absorbance does. Figures (2, 3, 4 and 5) and Table (1)

Table (1): Optimized condition for the absorbance of the ligands

No.	ligands	Ethanol%	Wave length (nm)		Absorbance	
			λ_{II}	λ_{I}	II	I
1	(10^{-4}M) Quercetin at λ_{max} = 372nm in ethanol/water mixture	40	254	372	2.128	2.327
		50	254	372	2.127	2.232
		60	254	372	2.129	2.296
		70	254	372	2.13	2.266
		80	254	372	2.136	2.353
2	(10^{-4}M) EDTA at λ_{max} = 241nm in ethanol/water mixture	0	241	-	0.099	-
		40	241	-	0.088	-
		50	241	-	0.037	-
		60	241	-	0.033	-
		70	241	-	0.088	-
		80	241	-	0.015	-
3	(10^{-4}M) Penicillamine at λ_{max} = 194nm in ethanol/water mixture	0	194	-	0.522	-
		40	194	-	0.504	-
		50	194	-	0.594	-
		60	194	-	0.592	-
		70	194	-	0.265	-
		80	194	-	0.436	-
4	(10^{-4}M) Genistein at λ_{max} = 261nm in ethanol/water mixture	40	261	-	3.075	-
		50	261	-	3.103	-
		60	261	-	2.736	-
		70	261	-	2.493	-
		80	261	-	3.101	-

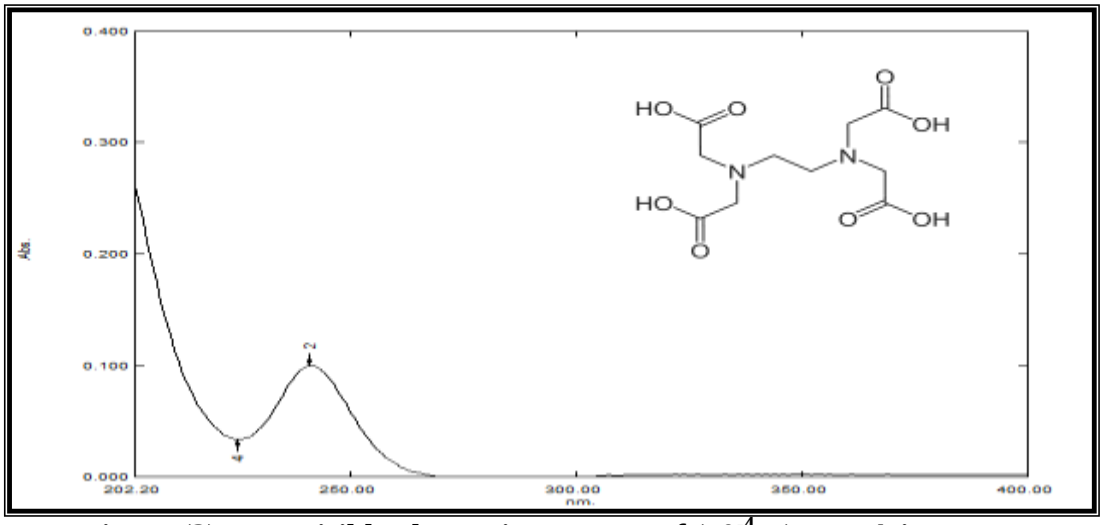


Figure (2): UV-Visible absorption spectra of (10^{-4}M) EDTA in water.

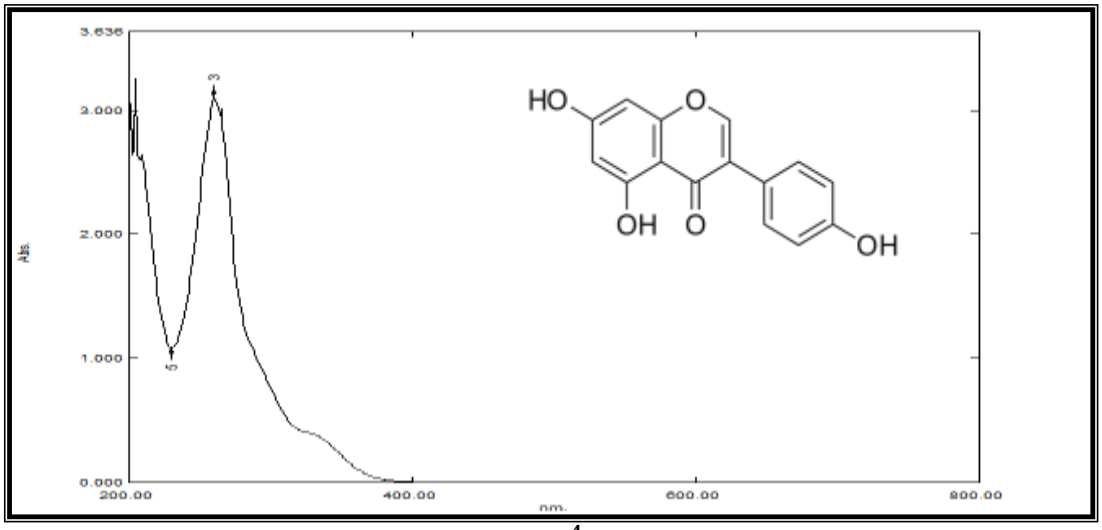


Figure (3): UV-Visible absorption spectra of (10^{-4}M) genistein in 50% ethanol/water mixture.

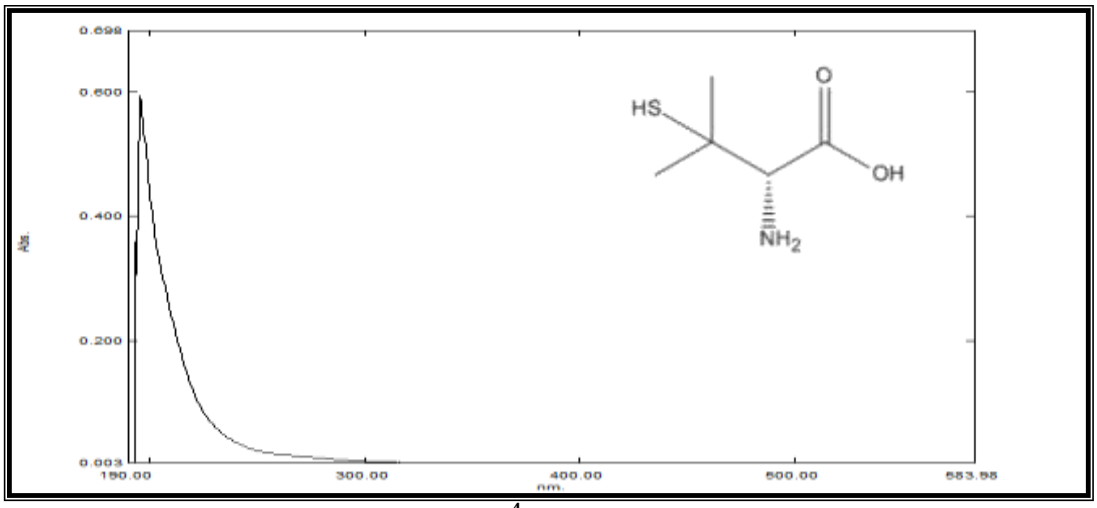


Figure (4): UV-Visible absorption spectra of (10^{-4}M) penicillamine in 50% ethanol/water mixture.

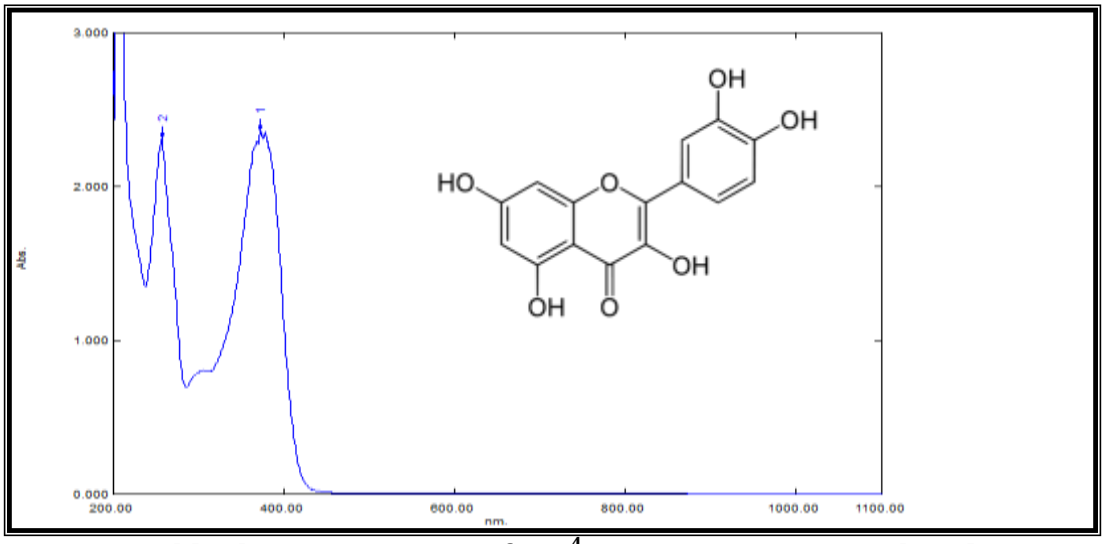


Figure (5): UV-Visible absorption spectra of (10^{-4}M) quercetin in 80% ethanol/water mixture.

Upon addition of Arsenic (III) solution to (10^{-4}M) chelating agents solutions, significant changes were observed in the electronic spectra, as shown in Table (2). This Table shows that the electronic spectra shifts λ_{max} to a longer wave length (bathochromic shift) upon addition of As (III) ion and a decrease in absorbance, these two evidence indicate a complex formation between the studied chelators and As (III) ion.

Table (2): electronic spectral data of (10^{-4}M) As (III) with the chelators.

Compound	λ_{max} nm	Absorbance	Assignment
EDTA	241	0.035	
As(III) – EDTA	241	0.019	n ⟶ π*
Genistein	330 260	0.392 3.103	
As(III) – genstein	325 261	0.3 2.173	n ⟶ π* π ⟶ π*
Penicillamine	194	0.593	
As(III) – Penicillamine	212	0.762	n ⟶ π*
Qurecetine	372 256	2.353 2.306	
As(III) – Quercetine	376 256	0.625 0.605	n ⟶ π* π ⟶ π*

Stoichiometry of the formed complexes:

The stoichiometric ratio of As (III) to chelating agents (EDTA, penicillamine, genistein and qurecetine) in the complexes were determined by Jobs method of equimolar solutions. The curve displayed maxima absorbance at mole fraction X_{max}, which indicates the formation of complexes with metal ion to ligands ratio, figures (6-9).

$n = X_{max} / 1 - X_{max}$, n represent coordination number of the complexes, X_{max} represent mole fraction corresponding to the maxima absorbance.

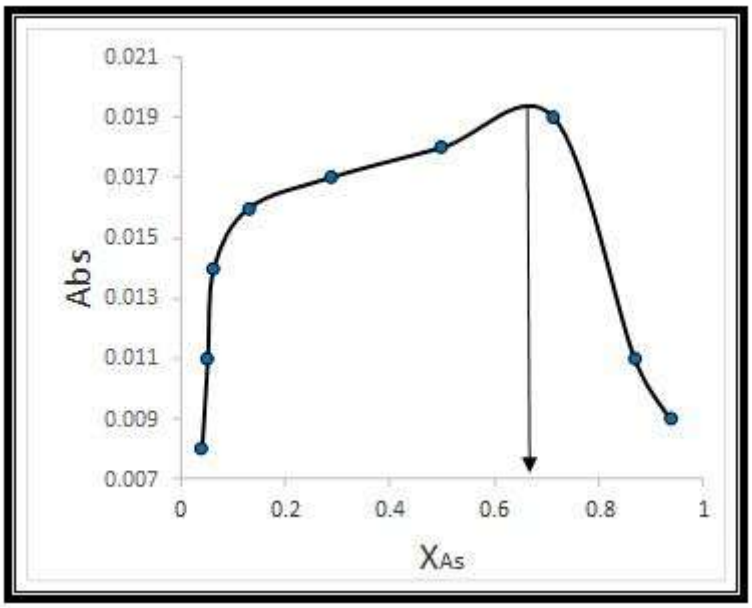


Figure (6): Job's plot for the composition of As (III) - EDTA complex at λ = 241nm.

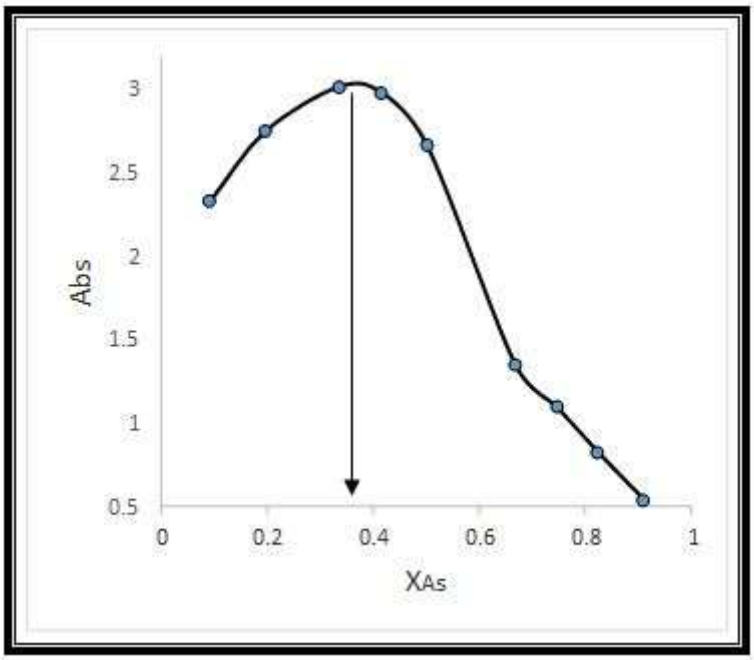


Figure (7): Job's plot for the composition of As (III) - gensitein complex at λ = 261nm.

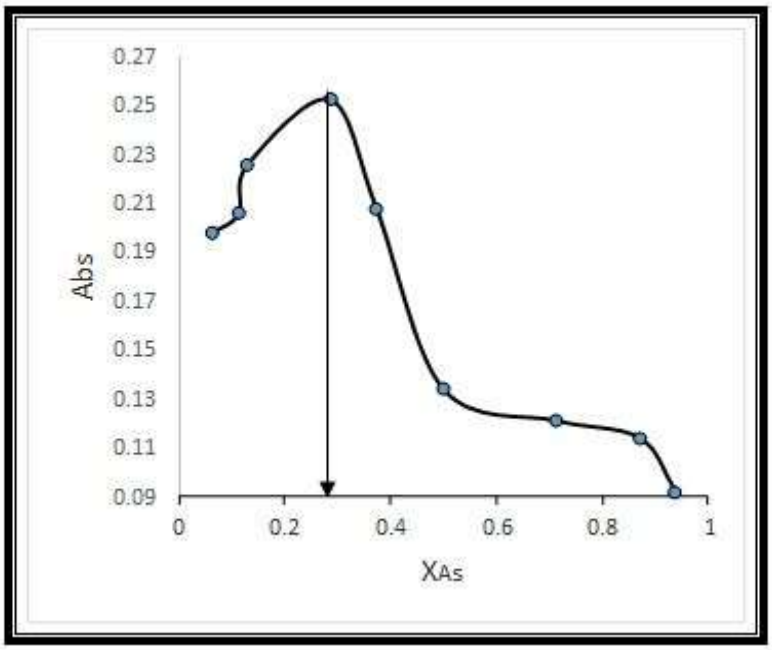


Figure (8): Job's plot for the composition of As (III) - penicillamine complex at λ = 215nm.

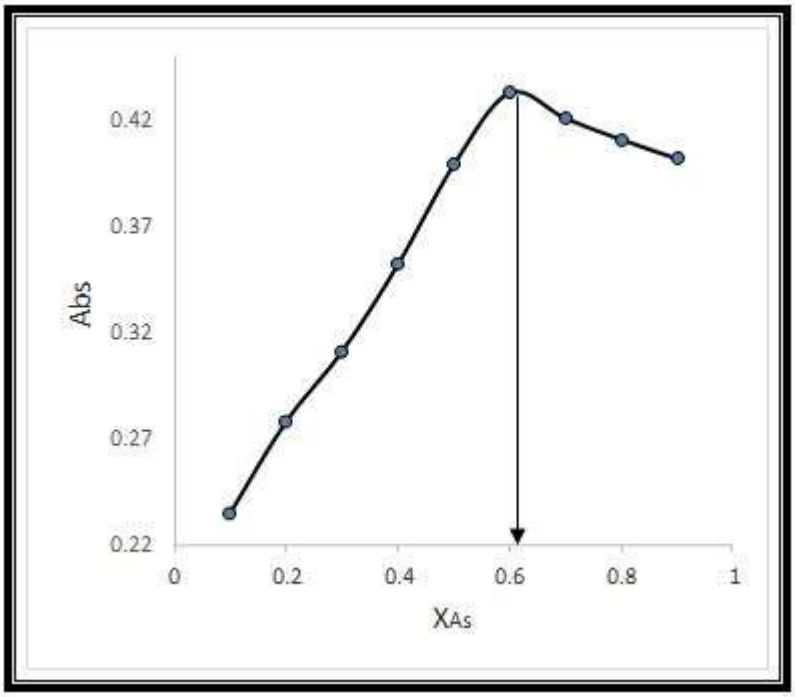


Figure (9): Job's plot for the composition of As (III) - quercetine complex at λ = 376nm.

Stability constant (K_{eq}): The equilibrium constant can be calculated using the continuous variation method [16].

$$As(III) + chelating\ agents$$

$$K_{eq} = \frac{[(\text{As III})_n\text{-(chelators)}_m]_{complex}}{[\text{As III}]_{eq}\,[\text{chelators}]_{eq}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$K_{eq} = \frac{\left[\frac{A_{max}}{\varepsilon l}\right]}{\left[C_{As} - \frac{A_{max}}{\varepsilon l}\right]\left[C_{chal.} - \frac{A_{max}}{\varepsilon l}\right]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

A_{max} = the maximum absorbance of the complex
ε = molar absorptivity of the complex (L. mole^{-1}. cm^{-1})
l = path length. cm.
C_{As} = Initial concentration of the Arsenic
$C_{chel.}$ = Initial concentration of chelating agents.

$$[(\text{As III})_n\text{-(chelators)}_m]_{complex} = \frac{\text{Absorbance}(max)}{\varepsilon\, l} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$[\text{As III}]_{eq} = [\text{As III}]_o - [(\text{As III})_n\text{-(chelators)}_m]_{complex} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$[\text{chelator}]_{eq} = [\text{chelator}]_o - [(\text{As III})_n\text{-(chelators)}_m]_{complex} \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The molar absorptivities of the complexes were calculated by recording the absorbance of a various concentration of the complexes at its stoichiometric values of each complexes and plotting of the absorbance of the complexes against concentration given a straight line with the slope equal to ($\mathcal{E}$) L . Mole^{-1}. Cm^{-1} as shown in tables (3, 4, 5 and 6).

The values of K_{eq} obtained by the continuous variation method were determined in five temperatures (293 - 308K) as shown in Tables (3, 4, 5 and 6), then allows us to calculate ΔG^o at different temperatures [17] [18]. $\Delta G^o = -RT \ln K_{eq}$ …….. (6)

Thermodynamic parameters: table (3, 4, 5 and 6) reported the thermodynamic parameters of the complexation of As (III) with studied chelators.

The enthalpy change were calculated by substituting the values of the slope of vant Hoff plot (log K_{eq} vs 1/T) as in equation (7) and figure (10, 11, 12 and 13)

$$\ln Keq = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \ldots\ldots\ldots (7)$$

Slope = -ΔH/R, R = gas constant

Entropy change for the system can then be calculated from:

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad \ldots\ldots\ldots\ldots\ldots (8)$$

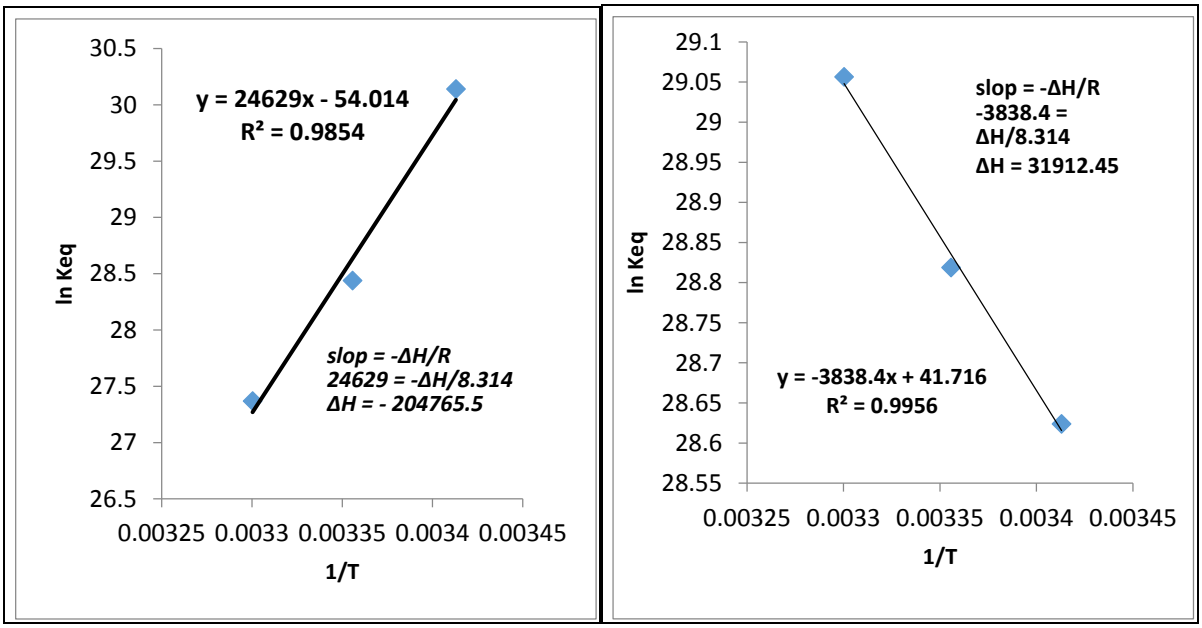


Figure (11): Vant Hoff plot for interaction of:
A/ EDTA – 2As (III). **B**/ gensitein – As (III). **C**/ 2penicillamine – As (III). **D**/ quercetine – As (III).

Table (3): Thermodynamic parameters for 2As (III) - EDTA complex.

T(K)	Keq	ΔG^0 (J/mole)	ΔH^0 (J/mole)	ΔS^0 (J/mole)	ε
293	1.23×10^{13}	-73422.6	-204766	-448.26	445.71
298	0.2245×10^{13}	-70461.4		-450.68	537.14
303	0.077×10^{13}	-68948		-448.24	525.71
308	0.0609×10^{13}	-69485.1		-439.22	828.57

Table (4): Thermodynamic parameters for As (III) - gensitein complex.

T(K)	Keq	ΔG^o (J/mole)	ΔH^o (J/mole)	ΔS^o (J/mole)	ε
293	2.699×10^{12}	-69727.9	31912.45	346.895	92079
298	3.28×10^{12}	-71400.8		346.688	91900
303	4.16×10^{12}	-73197.5		346.897	91577
308	7.83×10^{12}	-76024.9		350.44	89793

Table (5): Thermodynamic parameters for As (III) - 2penicillamine complex.

T(K)	Keq	ΔG^o (J/mole)	ΔH^o (J/mole)	ΔS^o (J/mole)	ε
293	5.91×10^{13}	-77246.23	-35035.2	144.06	3786.4
298	1.1587×10^{13}	-74526.1		132.52	3798.8
303	0.996×10^{13}	-75396.9		133.2	3835
308	0.731×10^{13}	-75848.96		132.51	3878.6

Table (6): Thermodynamic parameters for As (III) - quercetine complex.

T(K)	Keq	ΔG^o (J/mole)	ΔH^o (J/mole)	ΔS^o (J/mole)	ε
293	1.082×10^{8}	-45064.8	-62658.5	-60.04	26954
298	0.267×10^{8}	-42366.9		-68.09	27334
303	0.1712×10^{8}	-41958.2		-68.31	27482
308	0.1175×10^{8}	-41686.8		-68.08	27548

These tables' shows that the equilibrium constant values decrease with increase in temperature of As (III) with (EDTA, penicillamine, and qurecetine) and increase with increase in temperature for As (III) with gensitein. The negative value of Gibbs free energy for these interaction indicate the spontaneous process in the direction of equilibrium. The positive value or negative value of enthalpy and entropy change refers to the type of interaction between As (III) and these chelators. For As (III)-EDTA complexes ΔH^o were negative and ΔS^o also negative that means the process were enthalpy driven and the interaction may be ionic interaction. As (III)-gensitein have positive ΔH^o that means endothermic process and suggest a week hydrophobic interaction, and positive ΔS^o, so spontaneously are entropy driven. As (III)-penicillamine complexes have negative ΔH^o, positive ΔS^o which indicate an enthalpy and entropy deriven and have a strongest complexes. Finally As (III)-quercetine complexes have negative ΔH^o and ΔS^o that means enthalpy deriven. The different in their behavior due to its different in their structures.

Interaction Kinetics: In order to investigate the interaction kinetic of As (III) ion with chelators, the absorbance of complexes were followed with time at a certain wave length.
The first order rate equation and the second order rate equation were applied.

$$A+B \longrightarrow C+D$$

k : rate constant for the reaction which is independent of the concentration but depends on the temperature.
First order reaction: The first order rate law for the consumptive of a reaction A:

$$\frac{dA}{dt} = -K[A] \quad \text{..... (9)}$$

$$ln\left(\frac{[A]}{[A]_o}\right) = -Kt \quad \text{..... (10)}$$

$$ln\, A - ln\, A_o = -Kt \quad \text{..... (11)}$$

Second order reaction: The second-order rate law**.**

$$\frac{d[A]}{dt} = -K[A]^2 \quad \text{..... (12)}$$

$$\frac{1}{[A]} - \frac{1}{[A]_o} = Kt \quad \text{..... (13)}$$

A= Absorbance of complex (As (III)-chelator) with deferent time.
$\mathbf{A_o}$ = Absorbance of complex (As (III)-chelator) in time zero.

Table (7, 8, 9, and 10) shows the absorption of complex As (III) with (EDTA, penicillamine, genistein and qurecetine) all of each with Time (0-30) min.

Table (7): Data for application the second order equation for (2:1) As (III)-EDTA complex, at 293K, λ_{max} = 241 nm.

Time	Abs	1/Abs
0	0.018	55.55556
5	0.017	58.82353
10	0.015	66.66667
15	0.012	83.33333
20	0.01	100
25	0.009	111.1111

Table (8): Data for application the second order equation for (1:1) As (III)-gensitein complex, at 293K, λ_{max} = 261 nm.

Time	Abs	1/Abs
0	2.46	0.406504
5	2.44	0.409836
10	2.432	0.411184
15	2.42	0.413223
20	2.41	0.414938

Table (9): Data for application the second order equation for (1:2) As (III)-penicillamine complex, at 293K, λ_{max} = 212 nm.

Time	Abs	1/Abs
0	0.502	1.992032
5	0.493	2.028398
10	0.478	2.09205
15	0.46	2.173913
20	0.453	2.207506
25	0.434	2.304147
30	0.425	2.352941
35	0.411	2.43309
40	0.393	2.544529
45	0.379	2.638522
50	0.367	2.724796

Table (10): Data for application the second order equation for (1:1) As (III)-quercetine complex, at 293K, λ_{max} = 376 nm.

Time	Abs	1/Abs
0	1.21	0.826446
5	1.158	0.863558
10	1.155	0.865801
15	1.153	0.867303
20	1.149	0.870322
25	1.144	0.874126

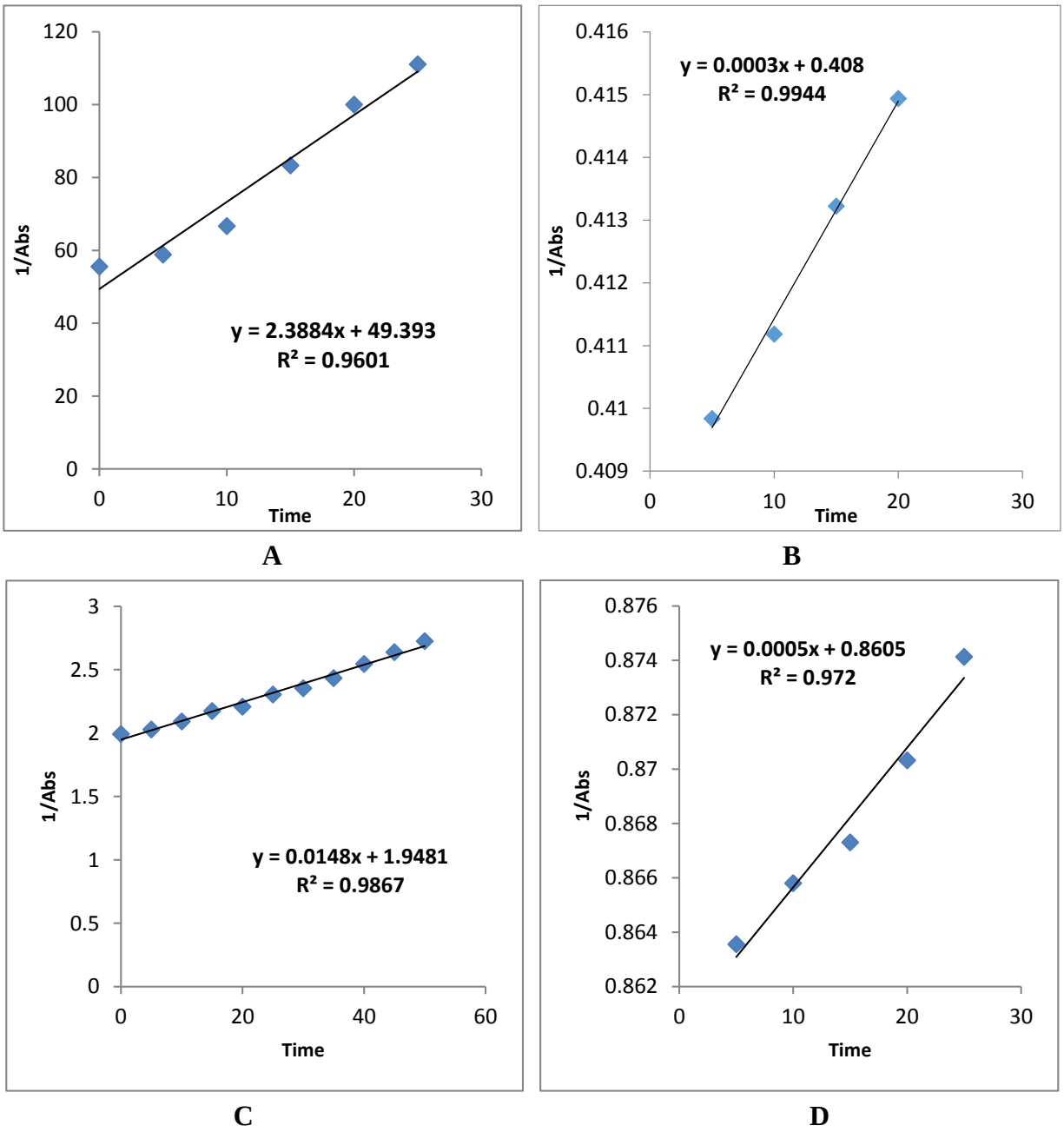


Figure (12): The application of the second order reaction equation for complex of As (III) with: A/ EDTA. B/ Genistein. C/ Penicillamine. D/ Quercetin.

Table (11): Rate constant of the second order reaction for complex As (III) with chelators.

Complex Title	Second order rate constant k($M^{-1}.min^{-1}$)
As(III) - EDTA	2.3884
As (III) - Genistein	$3*10^{-4}$
As (III) - Penicillamine	$1.48*10^{-2}$
As (III) - Quercetin	$5*10^{-4}$

Conclusion

The complex of the chelating agents (EDTA, penicillamine, genistein and qurcetin) with Arsenic (III) shows a high tendency of these antioxidants to As (III), this were obvious from the values of their equilibrium constant with the comparison with EDTA which were considered as a good complexing agent used .The thermodynamic parameter shows that this complexation is a spontaneous and may be entropy or enthalpy driven or both, depending a chelator's structures.

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