

# STUDYING THE FORMATION AND STABILIZATION OF CUR-AL<sup>+3</sup> COMPLEXES BY USING CITRIC ACID AS CATALYST

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## ABSTRACT

Cooking ware that is made of aluminum is used in different nations, particularly developing nations, and are a free source of aluminum. Due to its possible harmful effects on human health, aluminum is acknowledged as a public health problem. The goal of the current investigation is to evaluate curcumin's (CUR) capacity to decrease aluminium's toxicity by forming stable complexes. Rhizomes that are sold in the local marketplaces have an 8.5% crude pigment content of CUR. At various pH levels, CUR's maximum absorbance was identified. Citric acid was used as a catalyst to create the CUR-Al<sup>+3</sup> combination at various pH levels, which correspond to the rates at which Al<sup>+3</sup> was eliminated from the solution. At pH 2.5, 3.0, 3.5, and 4.0, the highly complexation was seen. At such pH levels, the Al<sup>+3</sup> elimination was 79.22, 78.28, 79.26, and 79.41%, respectively. The CUR-Al<sup>+3</sup> complexes' stability represent, respectively, 96.94, 96.29, 95.55, and 95.52% of the initial concentration at 25°, 50°, 75°, and 100° C for 60 min at pH value of 2.50. Using Fourier-transform infrared spectroscopy (FT-IR), it has been possible to identify CUR and its complex with Al<sup>+3</sup>. At varied pH levels, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 at boiling point, the leached aluminium from 3 regions of the aluminium cooking ware (Syrian, Iranian and local) was specified. Comparatively to Syrian and local cooking wares, Iranian cooking ware generally leached more aluminum. In comparison to other pH values, the percentages of the elimination of the aluminum that is leached by utilizing the most leaching cookware (Iranian) have been 79.23 and 79.26% at pH values of 2.50 and 3.50, respectively.

Keywords: cooking wares, aluminum, catalyst, elimination.

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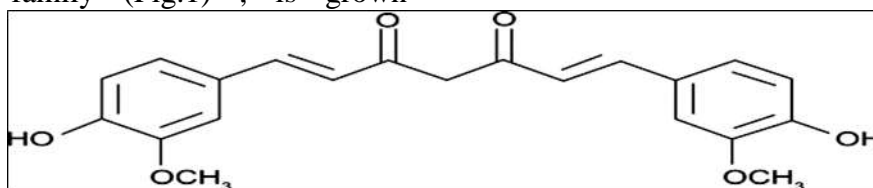
## المستخلص

تستعمل قدور الطبخ المصنوعة من الألمنيوم في العديد من الدول وبخاصة الدول النامية وتعد مصدرا للألمنيوم الحر. تم تشخيص الألمنيوم على انه يمتلك تأثيرات سمية مهمة على صحة الإنسان. شرعت الدراسة الحالية لتقدير فعالية الكركمين في تقليل سمية الألمنيوم الناضج من خلال تكوين معقدات ثابتة. تحتوي درنات الكركم المتوفرة في الأسواق المحلية على 8.5% من الكركمين كصبغة خام. تم تقدير أعلى امتصاصية لصبغة الكركمين في قيم رقم هيدروجيني مختلفة. تم تحضير معقد كركمين-المنيوم باستعمال حامض الستريك كعامل مساعد في قيم رقم هيدروجيني مختلفة والذي بدوره سيشير الى النسب المئوية لإزالة ايون الألمنيوم من المحلول. لوحظ إن تكوين المعقد كان واضحا في قيم من الرقم الهيدروجيني مقدارها 2.5, 3.0, 3.5 و 4.0. وإن نسب إزالة ايون الألمنيوم عند قيم الرقم الهيدروجيني تلك كانت 79.22, 78.28, 79.26 و 79.41% على الترتيب. تمت دراسة ثباتية معقدات كركمين-المنيوم في درجات حرارة 25, 50, 75 و 100 درجة مئوية لمدة 60 دقيقة في رقم هيدروجيني 2.5 وكانت تمثل 96.94, 96.29, 95.55 و 95.52% من التركيز الأولي على الترتيب. تم تشخيص الكركمين ومعقده مع الألمنيوم باستعمال طيف الأشعة تحت الحمراء. تم تقدير نضوج الألمنيوم باستعمال ثلاث مناشئ لقدور الطبخ المصنوعة من الألمنيوم (محلي، سوري و إيراني) في قيم رقم هيدروجيني 1.5, 2.5, 3.5, 4.5, 5.5 و 6.5 وفي درجة الغليان. في العموم فإن قدور الطبخ الإيرانية نضجت ألمنيوم أكثر مقارنة مع قدور الطبخ السورية والمحلية على الترتيب. وكانت النسب المئوية لإزالة الألمنيوم من أكثر القدور نضوحا (القدور الإيرانية) بواقع 79.23 و 79.26 في قيم رقم هيدروجيني 2.5 و 3.5 على الترتيب والتي كانت عالية مقارنة بباقي قيم الأرقام الهيدروجينية.

الكلمات المفتاحية: أدوات طبخ، المنيوم، عامل مساعد، إزالة.

## INTRODUCTION

In subtropical and tropical areas, turmeric (*Curcuma longa*), member of the Zingiberaceae family (Fig.1), is grown

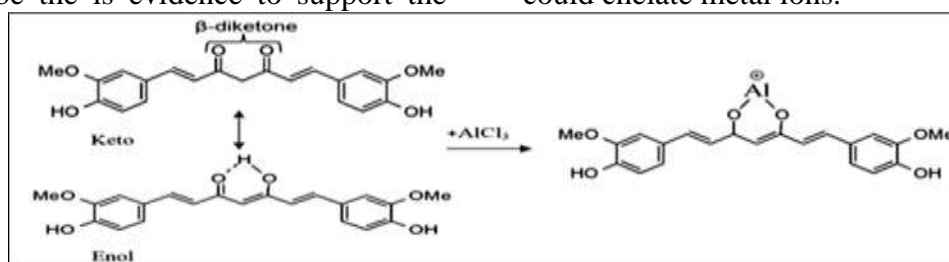


**Fig. 1. Curcumin's chemical structure (diferuloylmethane).**

Although heavy metals are necessary for many biological functions, an excessive intake of them can be hazardous because they increase oxidative stress (OS), which in turn produces reactive oxygen species (ROS) and free radicals in body's metabolism. Heavy metal accumulation in humans might seriously harm several organs, including the respiratory, reproductive, neurological, and digestive systems. Metal chelating treatment is frequently utilized for treating metal toxicity biologically. A complex ring-like structure is formed (Fig. 2) as a result of interactions between ligand and the central metal atom in this process (12). Both for home and industrial purposes, aluminum is frequently used.  $Al^{+3}$  accumulates in the brain, hence it is impossible to ignore indirect  $Al^{+3}$  intake. Aluminum analysis is crucial, particularly when it comes to food samples. When food is prepared in aluminum cooking wares, the situation may even get worse. Regular consumption of food prepared in this manner could be hazardous because Alzheimer's disease (AD) was linked to Al accumulation in brain. ROS formation, which causes oxidative damage to cellular lipids, DNA, and proteins, along with changes in activity of tissue antioxidant enzymes, apoptosis, and altered gene expression, are thought to be the evidence to support the

(5,20). Curcuminoids, which include curcumin, have been primarily blamed for the pharmacological activity of turmeric(4,19).

claims that aluminum promoted lipid peroxidation, altered hemato-biochemical parameters, and diminished activity of antioxidant enzymes in the plasma as well as the other tissues (1). Due to increased mitochondrial ROS production, which results in triggering the apoptosis of the hepatocytes and depletes the endogenous anti-oxidant enzymes by activating caspases cascade, aluminum-induced toxicity has a kinetics that includes the activations of the ions of  $Fe^{+3}$  and  $Fe^{+2}$  to result in oxidative damages (34). As a result, it's critical to have an external source of antioxidants to both prevent caspase activation and protect against the damaging consequences of oxidative stress (22). Throughout the last several years, the complexation of the CUR with the transition metals had drawn a lot of attention as a very important prerequisite for the AD treatment and in vitro antioxidant activities. Al (III), which is a component that is found in the senile plaques, is one of such metals that has a significant effect on the toxicity and aggregation of the  $A\beta$  peptides (i.e. the Amyloid beta peptide). In order to prevent metal ions from interacting with the  $A\beta$  peptides and from causing a redox reaction that results in oxidative stress, one strategy for treating (AD) is to find medicines that could chelate metal ions.



**Fig.2. The complex form between curcumin and aluminum.**

In an effort to reduce accumulations of the aluminium in the human organism which results after the aluminium cookware being

leached to the foods, the goal of this research is investigating circumstances that confirm production and stabilizations of the  $CUR-Al^{+3}$

complex with the use of citric acid as a catalyst.

## MATERIALS AND METHODS

**Curcumin Preparations:** CUR has been made through the refluxing of 100g of turmeric (at the boiling point) in 500 ml of (96%) of the ethanol for two hours. The heated mix has been filtered then dried completely by evaporation at 50 Celsius. Weighing the dry matter (CUR) to determine the percentage.

### The curcumin absorbency measurements

Utilizing phosphate buffer solutions with pH ranges between 1 and 7,  $2 \times 10^{-4}$  M of CUR was used to create the electronic absorption spectra of the CUR pigment at 423 nm

### Preparing complexes of curcumin - $Al^{+3}$ through the citric acid as the catalyst

At various pH levels, the complex [Cur.-Al (III)] was produced. 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 phosphate buffers were combined with 5ml of a  $2 \times 10^{-4}$  M CUR solution, 5ml of a  $2 \times 10^{-4}$  M citric acid solution, and 5ml of the phosphate buffer. After that, 5ml of  $1 \times 10^{-4}$  M  $AlCl_3 \cdot 6H_2O$  in the following molar ratios: CUR: Al (III): tartaric acid: was added to each combination. In order to prevent the production of insoluble  $AlCl_3 \cdot 6H_2O$ , each combination received five drops of 0.10M sodium potassium tartrate. After 30 minutes, the mix's absorbance has been assessed at room temperature. The absorbance value was calculated for determining the complex absorbance's wave length.

### Stability of cur- $Al^{+3}$ complexes at a variety of the temperature degrees and time periods

pH were individually heated in water bath at 25°, 50°, 75°, and 100° C for 0 min, 15min, 30 min, 45 min, and 60 min. At 531 nm, the mixes' absorbency was evaluated.

**Identifying curcumin and complex formations by the FT-IR:** To confirm production of the cur- $Al^{+3}$ , infra-red spectra of the CUR and the cur- $Al^{+3}$  complex were performed with the use of FT-IR.

**Determining aluminium leaching by Atomic Absorption spectroscopy:** Citric acid was used to create various pH solutions (1.5, 2.5, 3.5, 4.5, 5.5, and 6.5). In three distinct regions' (local, Syrian and Iranian) aluminum-based cooking wares, 500 ml of every one of the solutions has been boiled for 1 hr. Atomic

absorption spectroscopy was used to calculate how much aluminum was leached.

**Aluminum elimination percentages by the complex formation:** Calculating the amount of pure CUR consumed by the complexes formed when citric acid was utilized as a catalyst allowed researchers to quantify the removal percentages of leached aluminum from Iranian cooking.

## RESULTS AND DISCUSSIONS

**Preparations of the curcumin:** Turmeric rhizomes that are sold in local marketplaces have an 8.50% crude pigment content of CUR. Since CUR is a liposoluble substance, it has been extracted using a variety of solvents, including acetone, hexane, isopropanol, methanol, ethyl acetate, and ethanol (17,23). The current work recommended using ethanol as sole solvent because it's readily available, a good solvent because of its high capacity of solubilisation (27) and a cost-effective alternative to other solvents. Given that CUR is a main component of turmeric and makes up 2-8% of the majority of the turmeric preparations (24), the CUR pigment used in this work may also contain other phenolic compounds because ethanol was used to extract it. For this reason, we refer to CUR as a crude pigment. The amount of CUR in turmeric determines its quality and its effectiveness. The findings of Geethanjali et al. (10) show that geographic variance, which affects the environment, soil, meteorological conditions, etc., affects the CUR content.

### Effects of the value of the pH on the absorbency of the curcumin:

The electronic spectra of the absorption of the CUR pigment at 423 nm for various pH levels are shown in Fig. 1 and Table 3. As demonstrated, there has been a direct correlation between pH values and absorbance of the CUR. UV-Vis spectrum of the CUR reveals that absorbance was (0.417) at 420nm with the use of a buffering system of pH 1.0, whereas maximal absorption has been (0.696) at 423nm with a pH value of 7. Rawaa *et al.*, (26) have discovered that, UV-vis. spectrum bands of Cur had shown that maximal absorption at 1, 1.50, 2, 2.50, 3, 3.50 and 4 pH values have been 424nm, 423nm, 423nm, 423nm, 422nm, 422nm and 415nm respectively. Ismaeil *et al.*, (13) have discovered UV-Visible curcumin ligand

spectrum had shown main bands of absorption in UV–Visible range at 265nm, 374nm (shoulder) and 427nm. The band at 265nm and 374nm is corresponding to  $\pi \rightarrow \pi^*$  transition, while the band at 427nm may be a result of either  $n \rightarrow \pi^*$  transitions or combinations of the

$n \rightarrow \pi^*$  transition and  $\pi \rightarrow \pi^*$  transition (6). Shifts of those bands in complexes may be found on complexes, which is an indication of the involvement of curcumin's carbonyl group in the metal complexations (29).

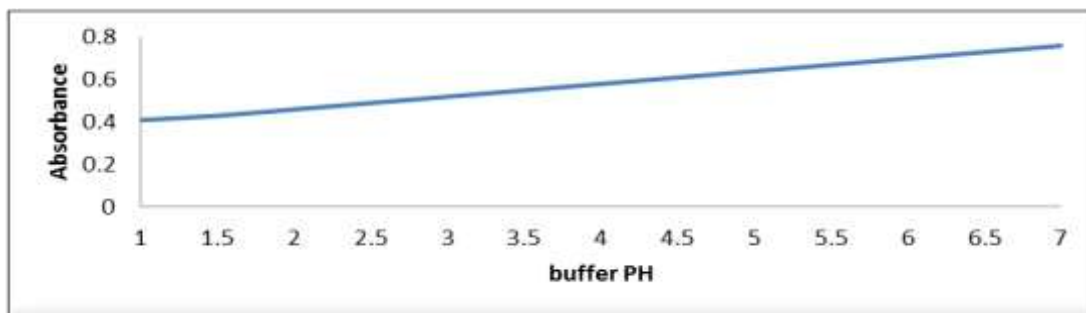


Fig.3. curve of absorbance of the curcumin at 423nm at various pH levels

Table1. Electronic absorption spectra UV in  $\lambda=423\text{nm}$  of the curcumin  $2 \times 10^{-4}\text{M}$  at different pH values of phosphate buffer

Buffer PH	Max absorption of the curcumin	Curcumin Wave-length
1.0	0.417	420
1.5	0.462	420
2.0	0.468	422
2.5	0.491	422
3.0	0.502	422
3.5	0.574	421
4.0	0.578	418
4.5	0.607	420
5.0	0.624	421
5.5	0.625	419
6.0	0.679	422
6.5	0.680	422
7.0	0.696	423

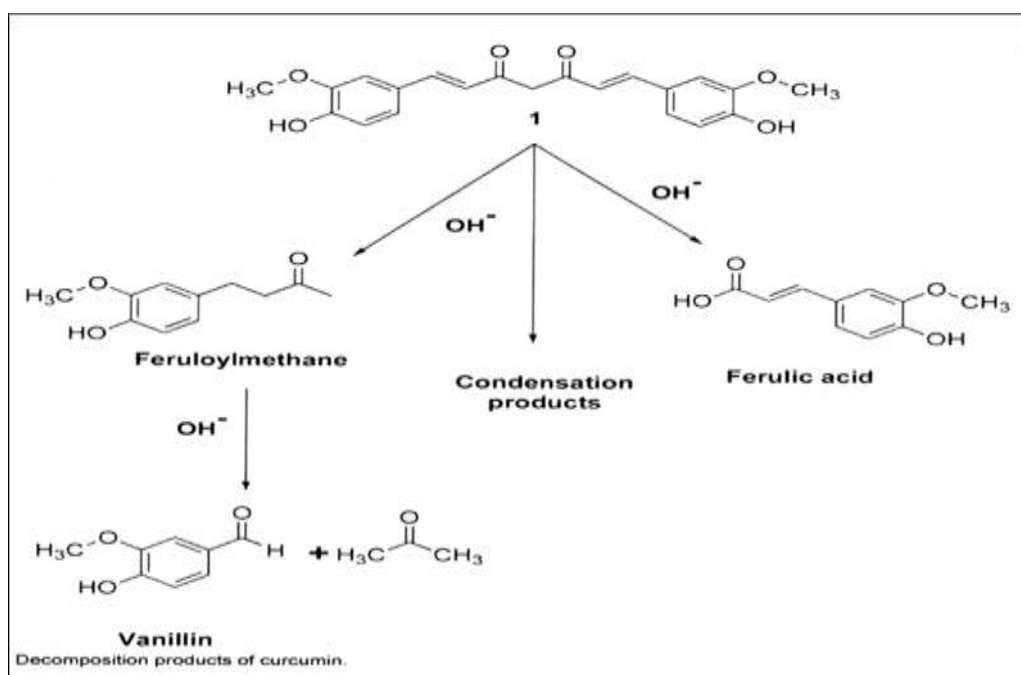


Fig. 4. Curcumin degradation in basic conditions (31).

At pH ranges between 2.5-7, CUR seems to have a brilliant yellow hue, whereas pH > 7 results in a red color (11,28). Weakly basic circumstances cause CUR's chemical structure to subtly alter from diketone to keto-enol forms. The compound's color shifts from yellow to brown as a result of this modification. Additionally, the molecule ionizes under strongly basic circumstances, changing from brown to reddish-brown in color (25). Under basic pH circumstances, hydrolysis of CUR causes fast degradation (Fig.4). According to Suresh et al. (31) over 90% of the CUR quickly disintegrated in a buffer system under neutral basic circumstances, indicating that CUR is unstable under higher pH levels. The conjugated diene structure might play a role in the improved CUR's stability at the acidic pH. The disintegration of this structure, on the other hand, occurs in the case where pH has been brought to a neutral basic condition and the proton from the group of phenolic is removed. Contrary to buffers in presence of the light,

CUR degradation was substantially slower in the pH range of 1 to 6.8 when it was dark or in the absence of light. Less than 1% of the entire amount of CUR was degraded in the pH of 1.2 after 6 hours. This outcome demonstrates that the rate of CUR degradation was significantly higher when light was present. But at pH 1-6, which is often found in the stomach, the degradation of CUR is incredibly slow (32,33). In a variety of the settings (pH, temperature, and dielectric constant of medium), Naksuriya et al investigation's of kinetic degradation of CUR from a combination of the compounds of the natural curcuminoid (21). The findings demonstrated that raising the medium's temperature, pH, and dielectric constant increased the rate of degradation.

#### [Cur-Al (III)] complex utilizing citric acid as catalysts

In order to specify the wave length of complex absorbance, the absorbance value was calculated. The high absorbency was discovered to be at 531 nm, as shown in table 2.

**Table 2. Electronic absorptions' spectra UV of the curcumin and the [Cur.-Al (III)-citric acid] complex**

Buffer pH	Max. Abs. of the complex [Cur-Al <sup>+3</sup> -Citric acid]	Complex Wave-length (nm)
1.0	0.216	521
1.5	0.182	521
2.0	0.213	522
2.5	0.102	524
3.0	0.109	524
3.5	0.119	526
4.0	0.135	526
4.5	0.149	528
5.0	0.157	528
5.5	0.161	530
6.0	0.229	530
6.5	0.238	530
7.0	0.240	531

As shown in table 3, high elimination percentages of aluminum (as AlCl<sub>3</sub>) was at pH between 2.50-5.50. As it has been mentioned

earlier, acidic conditions encourage enol form formation, which in turn leads to complex formation.

**Table 3. leached aluminium elimination percentages with the use of the curcumin and the citric acid as a catalyst**

pH of buffer	Maxi. Abs. of complex [Cur-Al <sup>+3</sup> - Citric acid]	% elimination of aluminum
1.0	0.216	48.20
1.5	0.182	60.60
2.0	0.213	54.48
2.5	0.102	79.22
3.0	0.109	78.28
3.5	0.119	79.26
4.0	0.135	79.41
4.5	0.149	75.45
5.0	0.157	74.83
5.5	0.161	74.24
6.0	0.229	66.27
6.5	0.238	65.00
7.0	0.240	65.51

**Cur-Al<sup>+3</sup> complex stability at a variety of the temperature degrees and time periods**

Table 4, lists the values of complex stability with the use of different temperatures (25°, 40°, 75° and 100°C) for a variety of the time

periods (0min, 15min, 30min and 60min.) through the application of 531nm wavelength. Table4, lists cur-Al<sup>+3</sup> absorption at a pH value of 2.50 and various temperatures (25,50,75, and 100 °C).

**Table 4. cur-Al<sup>+3</sup> complexes' stability at various temperatures and for a variety if the time periods (pH= 2.5 at 531nm).**

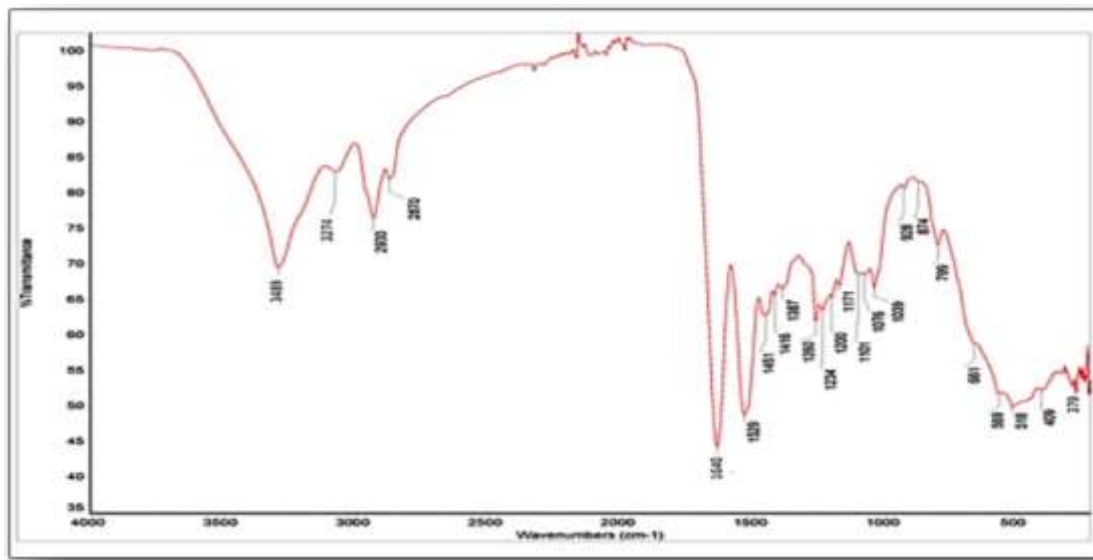
Min	Abs. At 25°c	Stabilization%	Abs. At 50°c	Stabilization%	Abs. At 75°c	Stabilization%	Abs. At 100°c	Stabilization%
0	0.149	100.00	0.135	100.00	0.135	100.00	0.134	100.00
15	0.148	99.32	0.135	100.00	0.134	99.25	0.132	98.50
30	0.146	97.99	0.134	99.25	0.132	97.78	0.131	97.76
45	0.145	97.32	0.133	98.52	0.131	97.04	0.129	96.27
60	0.144	96.64	0.130	96.29	0.129	95.55	0.128	95.52

Cur-Al<sup>+3</sup> complexes' stability 60min later at 25°, 50°, 75° and 100°C are representing 96.94, 96.29, 95.55 and 95.52 % of initial concentration levels (at 0 time) respectively. Kummar *et al.*, (15,35) have discovered that curcumin and curcumin complexes have been stable thermally until up to 160°C.

**Identifying curcumin and complex formations by the use of the FT-IR**

Infra-red curcumin spectra, Fig5, illustrates the stretching vibration values at 1640cm<sup>-1</sup> which predominantly results from carbonyl (C=O) and alkenes (C=C) character overlapping stretching vibrations. Infra-red spectrum of the curcumin ligand exhibits sharp peak at 3489cm<sup>-1</sup>, which is an indication of phenolic stretching of the O-H with broad band at the range between 3,200 and 3,500cm<sup>-1</sup> that results from ν(OH) group (in the form of the enol). The stretching vibration at 1451cm<sup>-1</sup> and band

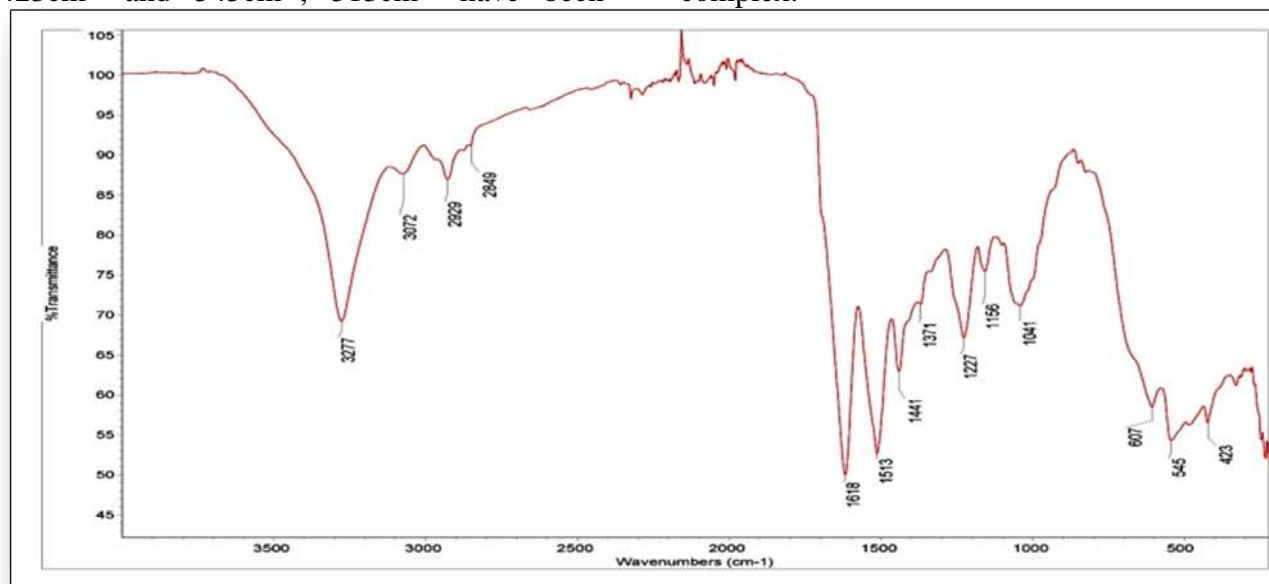
of high intensity at 1529cm<sup>-1</sup> which results from mixed vibrations that include the stretching carbonyl bond vibrations γ(C=O), in the plane bending vibrations around the aliphatic δCC-C, δCC=O and in the plane bending vibrations around aromatic δCC-H of the keto and the enol configurations and the stretching vibrations around aromatic ν CC bonds of curcumin's keto and enolic forms (14). In addition to that, considerable intense band at 1260cm<sup>-1</sup> resulted from bending n(C-O) phenolic band's vibration. A band at 886cm<sup>-1</sup>, is part of C-H out-of-plane vibration of the aromatic rings, may be considered as pure vibrations. Infra-red bands at 874cm<sup>-1</sup> have been assigned to highly mixed γ(C-H) and aromatic γ(CCH). Out-of-plane vibrations of the two groups of the - OH have been noticed at 451cm<sup>-1</sup> (14,29).



**Fig.5.The FTIR spectra of curcumin**

Infra-red spectra of the [Cur.- Al (III)-citric acid] Fig.6, shows that, a strong stretching peak of C=O has been observed for the curcumin at  $1640\text{cm}^{-1}$  had shown a blue shifting in the metal complex and assigned value has been  $1618\text{cm}^{-1}$  for the [Cur.- Al (III)-citric acid]. Infra-red data of complex suggested typical mode of chelation where ionic form of the enol is chelated by the metal. Such chelation type has been reported for the Pb (II) and Cd (II) complex of the curcumin (3,7). Infra-red spectra of [Cur.- Al (III)-citric acid] complex had shown new band values at  $423\text{cm}^{-1}$  and  $545\text{cm}^{-1}$ ,  $513\text{cm}^{-1}$  have been

assigned to the  $\nu$  (M-N) and the  $\nu$  (M-O) stretching frequencies respectively (25,29,35). This work has been in line with findings that have been presented by Sobhan *et al.* (30) that have prepared curcumin and metal complexes (M = Eu, La, Ce, Y, Pd, Cr) and have identified them by the infra-red and UV-Visible spectroscopy. Their results have shown that the curcumin had coordinated with the ions of the metal in the bi-dentate mode in the de-protonated form (30). Which is why, FT-IR spectral study had led to a conclusion that protective film includes  $\text{Al}^{+3}$ -Curcumin complex.



**Fig. 6. FT-IR spectra of[cur.-Al(III)-citric acid] complex curcumin**

#### Determining aluminium leaching by the Atomic Absorption spectroscopy

Applying citric acid, several pH solutions (1.5, 2.5, 3.5, 4.5, 5.5, and 6.5) were used. Atomic

absorption spectroscopy was used for determining the amount of aluminium that had leached(Table5)

**Table 5. Determining leached aluminium of the cooking ware from 3 regions (as ppm) with the use of the atomic absorption spectroscopy**

pH	Local cookware ppm	Iranian cookware ppm	Syrian cookware ppm
1.50	402.30	493.20	465.10
2.50	399.00	491.90	446.80
3.50	73.880	164.00	90.310
4.50	16.900	52.830	28.470
5.50	2.4870	9.6160	5.6590
6.50	2.0110	3.9600	2.7640

This table demonstrates a negative correlation between the pH value and the amount of aluminum that has leached. Compared to Syrian and local cooking wares, Iranian cooking utensils leak more aluminum, as evidenced. The thickness and quality could be the cause of the variations in aluminum leaching amounts under the same conditions.

This same experimentation under same conditions has been carried out with the addition of the curcumin for the purpose of giving molar ratio 2 curcumin: 1 Al for encouraging the formation of the complex with the use of the most leaching cookware. Table 6, lists effects of the curcumin in the reduction of leached aluminium's quantity.

**Table 6. percentages of the elimination of the leached aluminium utilizing the Iranian cookware and citric acid as a catalyst at 531nm**

pH	Pure Curcumin Absorbance	Abs (Cur-Al) complex utilizing the citric acid as the catalyst	% eliminations of aluminium
1.50	0.4620	0.182	60.60
2.50	0.4910	0.102	79.23
3.50	0.5740	0.119	79.26
4.50	0.6070	0.149	75.45
5.50	0.6250	0.161	74.24
6.50	0.6800	0.238	65.00

The findings of the present work are consistent with those of (9,10), who efficiently employed CUR as a green metal ion inhibitor. According to Abdul-Halim et al. (2), CUR's capacity to form a compound with  $Al^{+3}$  allows it to effectively eliminate aluminum's toxic action. CUR was successfully employed by Layla, (16), as green corrosion inhibitor at quasi-cooking temperatures (90°C) in order to prevent the leaching of the aluminium in various meat and vegetable solutions.

### CONCLUSIONS

When used as chelation agent, CUR creates a complex with aluminum ions ( $Cur-Al^{+3}$ ) that removes the leached aluminum from aluminum cooking wares used for food preparation. Citric acid acts as a catalyst to speed up the formation of complexes by preventing the  $Al^{+3}$  ion from oxidizing and precipitating and instead allowing it to interact with curcumin.

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