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Studying the Formation and Stabilization of Cur-Al^{+3} Complexes by Using Tartaric Acid as Catalyst

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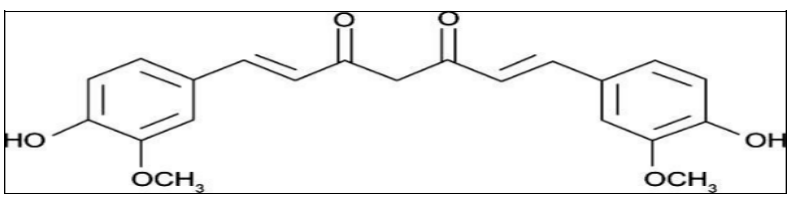
ABSTRACT:

The aluminum cooking wares that were using in many countries, especially the developing countries be a source of free aluminum. Aluminum is recognized as a public health concern because of its potential toxic effects on human health. The present study was undertaken to determine the effectiveness of curcumin (CUR) in reducing the toxicity of leached aluminum through formation stable complexes. The available rhizomes in local markets contain 8.5% of curcumin as crude pigment. The maximum absorbance of curcumin at different pH values was determined. The curcumin-Al^{+3} complex was prepared by using tartaric acid as catalyst, at different pH values which in turn reflect the elimination percentages of Al^{+3} from the solution. The highly complexation was observed at pH 2.5, 3.0, 3.5 and 4.0. The Al^{+3} elimination at these pH values were 61.30,61.95,58.71 and 56.40% respectively. The stability of cur-Al^{+3} complexes at 25, 50, 75 and 100^{o}C for 60 min at pH 2.5 represent 96.94, 96.29, 95.55 and 95.52 % of the initial concentration respectively. The identification of curcumin and its complex with Al^{+3} by using Fourier-transform infrared spectroscopy (FT-IR) was achieved. The leached aluminum from three regions of aluminum cooking wares (local, Syrian and Iranian) were determined at different pH values, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 at boiling point. In general, Iranian cooking ware leached more aluminum comparing with Syrian and local cooking wares. The elimination percentages of leached aluminum by using the most leaching cooking ware (Iranian) were 61.30 and 58.71% at 2.5 and 3.5 pH respectively, which is high comparing with other pH values.

Keywords: *Cur-Al^{+3}, tartaric acid, Formation, Stabilization*

Introduction:

Turmeric (*Curcuma longa*) is a member of Zingiberaceae family and is cultivated in tropical and subtropical regions (Augustine *et al.,* 2017). The pharmacological activity of turmeric has been attributed mainly to curcuminoids consists of curcumin (Ancy and Salini, 2017).



The chemical structure of Curcumin (diferuloylmethane)

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Heavy metals are essential for a wide range of biological processes, but their excessive intake is harmful, specifically, they cause oxidative stress (OS) and generate free radicals and reactive oxygen species (ROS) in metabolism. The accumulation of heavy metals in humans can cause serious damage to different organs, especially respiratory, nervous and reproductive and digestive systems. Biologically, metal chelating therapy is often used to treat metal toxicity. This process occurs through the interaction between ligand and a central metal atom, forming a complex ring-like structure (Ilhami and Alwasel 2022).

Aluminum is widely used in industrial applications as well as in domestic uses. Indirect intake of Al (III) into our bodies cannot be ignored as Al (III) accumulates in brain. The analysis of aluminum, especially in food samples is very critical. The situation can be even worse in case of food prepared in aluminum cooking wares. Regular consumption of food prepared in this way is potentially dangerous as accumulation of Al level in brain cells has been blamed for causingAlzheimer s disease(AD).

The toxic effects of aluminum are attributed to mediation by reactive oxygen species (ROS) generation giving rise to oxidative deterioration of cellular lipids, proteins and DNA, as well as induction of changes in the activities of tissue antioxidant enzymes, altered gene expression, and apoptosis (Mailloux *et al.*, 2011). Evidence indicates that aluminum induced changes in hemato-biochemical parameters, increased lipid peroxidation, and decreased the activities of antioxidant enzymes in plasma and different tissues (AbdelWahab, 2012). The kinetics of aluminum-induced toxicity includes activation of $Fe+^{2}$ and Fe^{+3} ions to cause oxidative damage (XieandYokel,1996). The excessive mitochondrial ROS generation sparks hepatocyte apoptosis and depletes endogenous antioxidant enzymes via activation of the caspases cascade. Consequently, the external supply of antioxidants is important to inhibit caspase activation and also to defend against the injurious effects of oxidative stress (Ozben, T.2007).

Complexation of curcumin with transition metals has attracted much interest over the past years as one of the useful requirements for treatment of (AD) and in vitro antioxidant activity. Among these metals, Al (III) a component in the senile plaques is an important element impacting on the aggregation and toxicity of Aβ peptides (Amyloid beta peptide). Therefore, one of the approach for the (AD) treatment is searching for the agents that can chelate metal ions, preventing metal ions from the interaction with Aβ peptides as well as the redox reaction which leads to the oxidative stress.

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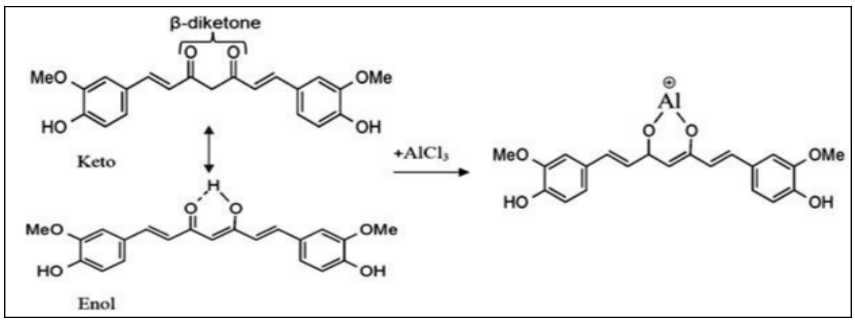

The aim of this paper is to study the conditions that confirm the formation and stabilization of curcumin-

Al^{+3}complex by using citric acid as catalyst, which is attempt to minimize the accumulation of aluminum in human body, that was leached from aluminum cooking wares into foods.

Materials and Methods:

Preparation of curcumin:

The curcumin was prepared by refluxing 100 grams (at boiling point) of turmeric in 500 ml of (96%) ethanol for 2hr .The hot mixture was filtered and evaporated at 50^{o} C till complete dryness .The dry matter (curcumin) was weighed to find out the percentage.

Thecurcumin absorbency measurements:

Electronic absorption spectra of curcumin pigment at 423nm was prepared by using 2 x 10^{-4}M of curcumin in buffer phosphate with different values of pH.

Table 1: Electronic absorption spectra U.V in λ=423 nm of curcumin 2×10^{-4} M at different values of buffer phosphate.

pH of buffer	Abs. maximum of curcumin	Wavelength nm of curcumin
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

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Preparation of curcumin- Al^{+3} complexes by tartaric acid as catalyst:

The complex [Cur.-Al(III)] was prepared in different pH values. 5ml of 2×10^{-4} M curcumin solution was mixed with 5ml of 2×10^{-4} M tartaric acidsolution and 5 ml phosphate buffer of pH, 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. Then 5ml of 1×10^{-4} M $AlCl_3.6H_2O$ was added to each mixture with a molar ratio of [2:1: 2] curcumin: Al(III) : tartaric acid. Five drops of 0.1M sodium potassium tartrate were added to each mixture to inhibit the formation of insoluble $AlCl_3.6H_2O$. The mixtures absorbance was determined at room temperature after 30 min. The absorbance value was determined to find out the wave length of complex absorbance.

Table 2: Electronic absorption spectra U.V of curcumin and complex [Cur.-Al (III)-tartaric acid].

pH of buffer	Maxi. Abs. of complex [Cur-Al^{+3}-tartaric acid]	Wavelength (nm) of complex
1.0	**0.375**	**521**
1.5	**0.303**	**521**
2.0	**0.305**	**522**
2.5	**0.190**	**524**
3.0	**0.191**	**524**
3.5	**0.237**	**526**
4.0	**0.252**	**526**
4.5	**0.256**	**528**
5.0	**0.259**	**528**
5.5	**0.291**	**530**
6.0	**0.385**	**530**
6.5	**0.387**	**530**
7.0	**0.456**	**531**

The stability of cur-Al^{+3} complexes at different temperatures and periods of time:

The complexes of cur-Al^{+3} in buffer phosphate of pH 2.5 by using tartaric acidas catalyst were separately heated in a water bath at 25, 50, 75 and 100°C for 0, 15, 30, 45 and 60 min.The mixtures were measured for their absorbency at 524 nm.

Identification the curcumin and the complexes formation by Fourier-Transform Infrared Spectroscopy(FT-IR):

The IR spectra of curcumin and cur-Al^{+3} complex by using citric acid as catalyst were done by using FT-IR to be sure of cur-Al^{+3} formation.

Determination the aluminum leaching by Atomic Absorption spectroscopy:

A different pH solutions (1.5, 2.5, 3.5, 4.5, 5.5 and 6.5) by using tartaric acidwere prepared. A 500 ml of each solution was boiled for one hour in aluminum based cooking wares of three different

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regions (local, Syrian and Iranian). The amount of leached aluminum was determined by using atomic absorption spectroscopy.

Aluminum percentages elimination by the complex formation:
The elimination percentages of leached aluminum from Iranian cooking ware were determined by calculating the amount of pure curcumin which was exhausted by the complexes formation when tartaric acidwas used as catalysts.

Results and discussion:
Preparation of curcumin:
The available turmeric rhizomes in local markets contain 8.5% of curcumin as a crude pigment. Also many solvents have been used to extract the curcumin (since it is a liposoluble compound), like, hexane, acetone, methanol, isopropanol, ethyl acetate and ethanol (Liu *et al*, 2008 and Pawar *et al*, 2018). The present study suggested to use ethanol as a lonely solvent since it is, available, good solvent due to its high solubilization capacity (Sahne et al, 2016) in addition to low cost as compared with other solvents. The present study suggested to use ethanol as a lonely solvent since it is, available, good solvent due to its high solubilization capacity (Sahne et al, 2016) in addition to low coast as compared with other solvents.Curcumin is one of the principal components of turmeric comprising 2-8% of most turmeric preparations (Peter,2013), so curcumin pigment, which was extracted in this study may contain another kinds of phenolic compounds since it was already extracted by ethanol, for this we define the curcumin as a crude pigment. The quality of curcumin and hence the activity of turmeric depends on the quantity of curcumin in it. The findings reveal the curcumin content depend on geographical variation which influences the soil, environment, climatic conditions etc. (Geethanjal *et al*, 2016).

The effect of pH on curcumin absorbency
Fig. 1, shows the electronic absorption spectra of curcumin pigment at 423nm by using different values of pH. As shown there was a proportional relationship between the curcumin absorbance and the pH values. The UV-Visible spectrum of curcumin shows that the maximum absorption was (0.696) at 423 nm by using pH 7.0, while the absorbance was 420 nm by using buffering system of pH 1.0.
Rawa'a *et al*.,2015 found that,the UV-visible spectrum bands of curcumin showed that the maximum absorption at 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 pH values were 424, 423, 423, 423,422, 422 and 415 nm respectively.
Ismail *et al.,*2014 found the UV-Vis spectrum of curcumin ligand showed main absorption bands in the UV– Vis region at 265, 374 (shoulder) and 427 nm. The band at 265 and 374 nm corresponds to a π→π * transition, whereas the band at 427 nm can be due either to an n→π* transition or to a combination of π→π* and n→π* transitions (Balaslasbramanian ,1991). The shifts of these bands in the complexes can be detected on the complexes, indicative of involvement of the carbonyl group of curcumin in metal complexation (Song , 2009).

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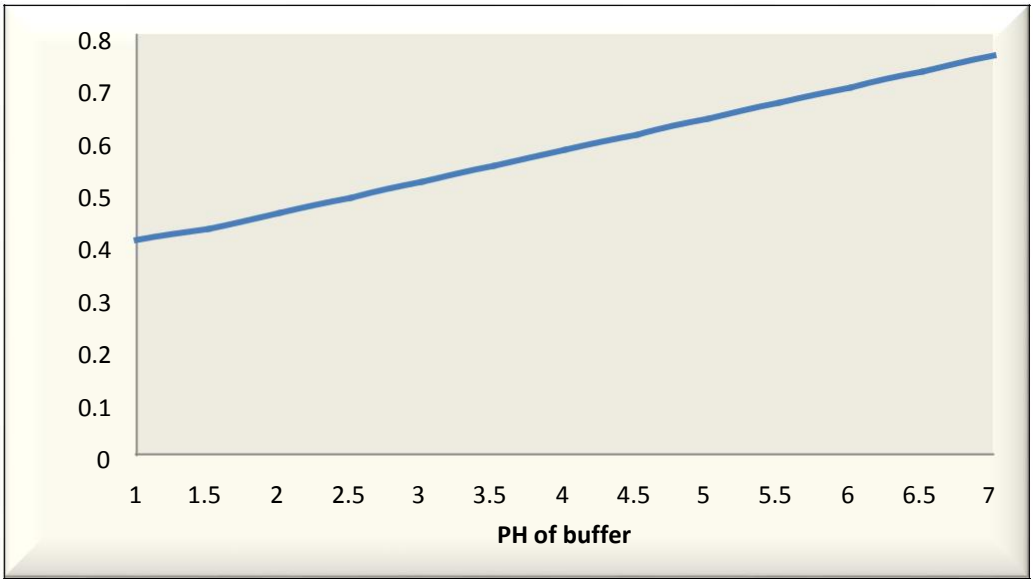

Fig. (1): The absorbance curve at 423 nm at different pH values.

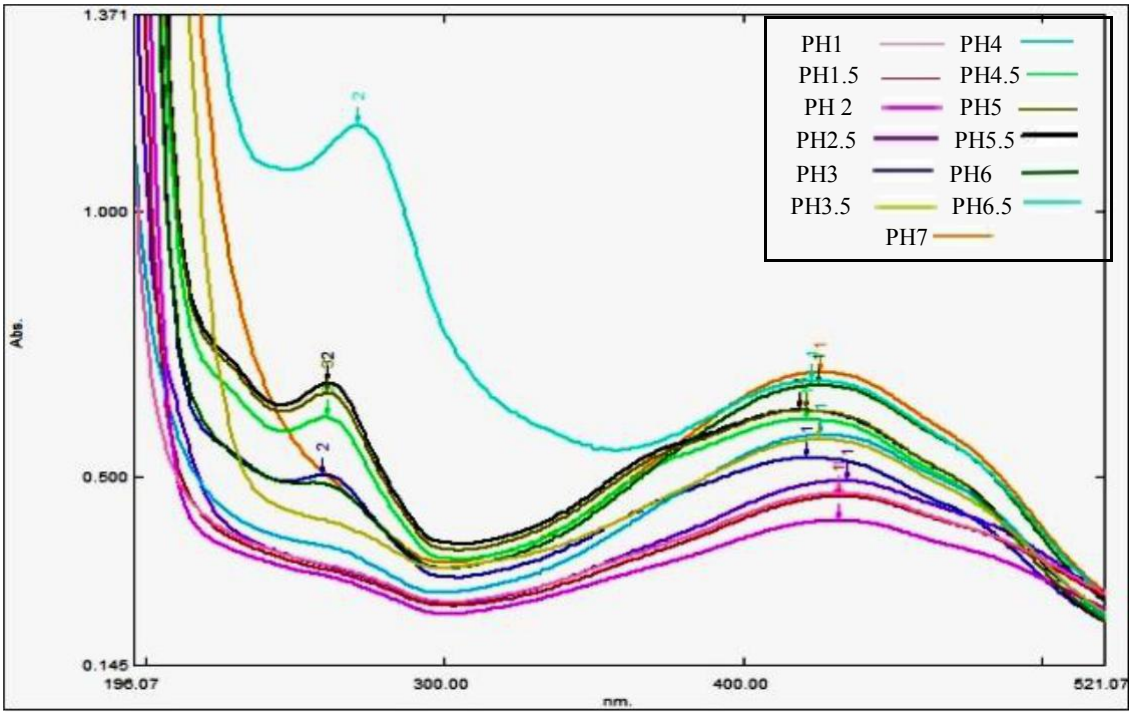

Fig. 2: Electronic absorption spectra at 420 nm of curcumin (2×10^{-4} M) at different pH values.

Curcumin appears to have a brilliant yellow hue at pH values from 2.5-7 and red color at pH > 7 (Sharma *et al*, 2005 and Goel *et al*, 2008).When curcumin is exposed to weakly basic conditions, its chemical structure experience a subtle change from a diketone form to a keto-enol form. This

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change causes the compound to change colour from yellow to brown. Moreover, when exposed to strongly basic conditions the molecule ionises, changing from a brown to a reddish-brown colour (Priyadarsini, 2014).

Curcumin rapidly degraded by hydrolysis under basic pH conditions (Fig.3). Suresh *et al,* 2013 found that, curcumin is unstable at higher pH conditions, more than 90% of curcumin decomposed rapidly in buffer system at neutral basic condition. The increased stability of curcumin at acidic pH may be contributed by the conjugated diene structure. However when the pH is adjusted to neutral basic condition, proton removed from the phenolic group leads to the destruction of this structure. in the absence of light or in dark the degradation of the curcumin was much lower in the pH range from 1 to 6.8 as compared to the buffers in the presence of light. The curcumin degradation in the pH of 1.2 at the time of 6 hrs was less than 1% of the total curcumin. This result shows that the degradation of the curcumin was much higher in the presence of light. However, the degradation of curcumin is extremely slow at pH 1-6, as normally encountered in the stomach (Wang *et al*, 1997 and Tønnesen, 2006). Naksuriya *et al* . 2017, investigated the kinetic degradation of curcumin from a natural curcuminoid mixture under various conditions (pH temperature, and the dielectric constant of the medium). The results showed that increasing pH, temperature, and the dielectric constant of the medium resulted in an increase in the degradation rate.

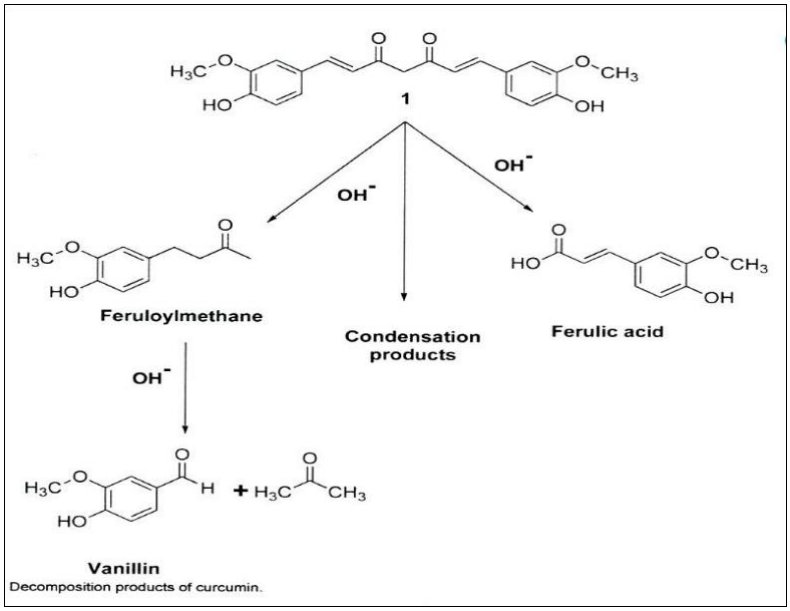

Fig. 3: The degradation of curcumin in basic conditions.

The [Cur-Al (III)] complex by using tartaric acid as catalysts:

Curcumin as a natural colorant product from turmeric used in food industry has low stability. Curcumin is easily oxidized due to light, oxygen, or high temperature. One of the methods that can be used to increase its stability is by complexation process using metal ions (Alvin and Reggie, 2021).

Curcumin usually reacts with metals through the β-diketone moiety to generate metal–curcumin complexes. It is well established that curcumin strongly chelates several metal ions (Sahdeo et al,

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2021) including aluminum.Curcumin is a beta-diketone where feruloyl groups replace two hydrogens in the structure. It is known to exist in at least two tautomeric forms, keto and enol. The keto form of curcumin exists in acidic and neutral pH media, while the enol form exists in alkaline pH medium (Aggarwal *et al*, 2015 and Petchana et al, 2020). Structurally, curcumin is comprised of a seven-carbon linker and three major functional groups.

These functional groups include an α, β-unsaturated β-diketone moiety, an aromatic O-methoxy-phenolic group, and a seven-carbon linker molecule (Fig. 4, A). Two α, β-unsaturated carbonyl groups connect the aromatic rings of curcumin. The diketones easily deprotonate themselves and form enolates (Fig. 4, B), whereas the α, β-unsaturated carbonyl acts as a Michael acceptor and undergoes nucleophilic addition (Priyadarsini, 2014**)**. The antioxidant activity of curcumin is caused by its phenolic group, whereas the carbon linker molecule provides hydrophobicity (Salem et al, 2014) (Fig. 4, C). As curcumin is hydrophobic in nature, curcumin has been structurally modified to increase its biological activity.

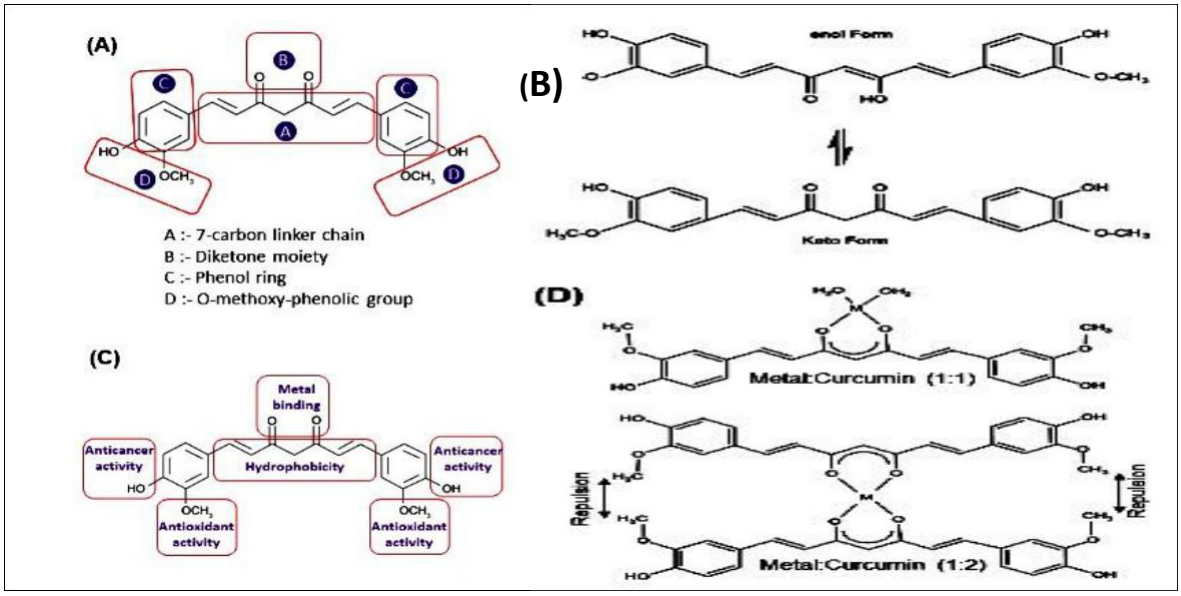

Fig. 4: (A) The basic structure of curcumin. (B) Existence of curcumin keto-enol tautomeric forms. (C) Groups of curcumin responsible for its biological properties . (D) Structure of 1:1 and 1:2 metal:curcumin complexes showing the β-diketone moiety of curcumin as the metal binding site.(Sahdeo et al, 2021).

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Table 3: The elimination percentages of leached aluminum by using Curcumin and tartaric acid as catalyst.

pH of buffer	Maxi. Abs. of complex [Cur-Al^{+3}-tartaric acid]	% elimination of aluminum
1.0	0.375	10.07
1.5	0.303	34.42
2.0	0.305	34.83
2.5	0.190	61.30
3.0	0.191	61.95
3.5	0.237	58.71
4.0	0.252	56.40
4.5	0.246	57.82
5.0	0.259	58.50
5.5	0.291	53.44
6.0	0.385	43.30
6.5	0.387	43.08
7.0	0.456	34.48

High elimination percentage of aluminum (as $AlCl_3$) were shown at pH between 2.5 – 5.5. As mentioned previously, the acidic conditions encourage the formation of complex.

The stability of cur-Al^{+3} complexes at different temperatures and periods of time:

Table 4, show the stability of complexes by using different temperature degrees (25, 40, 75 and 100^{o}C) for various periods of time (0, 15, 30 and 60 min.) by applying 531nm wave length. Table 3, shows the absorption of cur-Al^{+3} by using pH 2.5 at different temperature.

Table 4 :The stability of cur-Al^{+3} complexesat different temperature and for various periods of time (pH= 2.5 at 531 nm).

time min.	Abs. in T 25 ^{0}C	Abs. in T 50 ^{0}C	Abs. in T 75 ^{0}C	Abs.inT 100 ^{0}C
0	0.227	0.218	0.218	0.217
15	0.228	0.218	0.217	0.215
30	0.229	0.217	0.215	0.214
45	0.229	0.216	0.214	0.212
60	0.230	0.212	0.212	0.211

The stability of cur-Al^{+3} complexes after 60 min at 25, 50, 75 and 100^{o} C represent 96.94, 96.29, 95.55 and 95.52 % of the initial concentrations of at zero time respectively. Kumar *et al*.,2008; Zebib *et al*.,2010 found that the curcumin and its complexes were thermally stable up to 160^{0}C.

Identification the curcumin and the complexes formation by using Fourier-transform infrared spectroscopy (FT-IR):

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The IR spectra of curcumin, Fig. 5, shows stretching vibrations at 1640 cm^{-1}attributed predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Infrared of curcumin ligand show a sharp peak at 3489 cm^{-1} indicating the phenolic O-H stretching with a broad band at a range from 3200-3500 cm^{-1}, which is due to the ν(OH) group (in enol form). stretching vibration at 1451 cm^{-1} and high intensity band at 1529 cm^{-1} attributed to the mixed vibrations

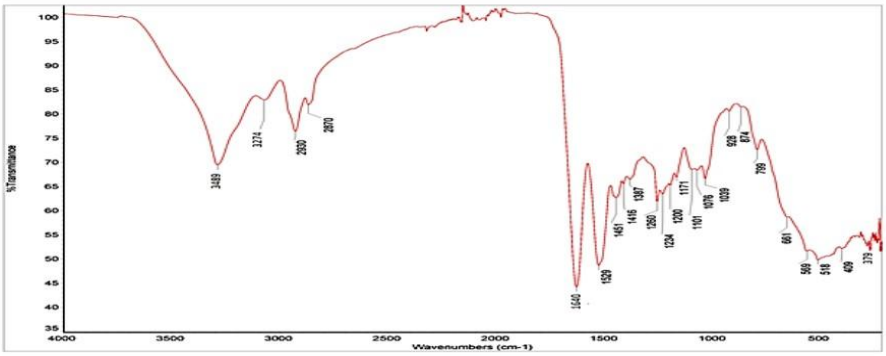

Fig.5:The FTIR spectra of curcumin

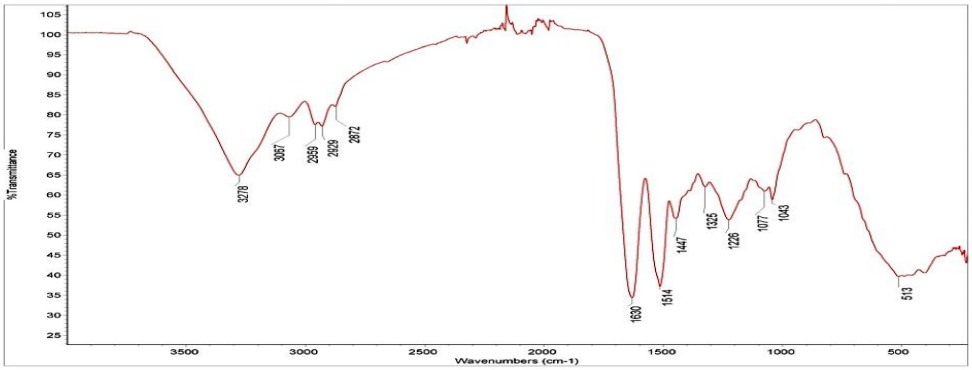

Fig. 6: The FTIR spectra of[cur.- Al(lll)- tartaric acid] complex curcumin

inclu ding stretc hing carbo nyl bond vibrations γ(C=O), in plane bending vibrations around aliphatic δ CC-C, δ CC=O and in planebending vibrations around aromatic δ CC-H of keto and enol configurations and stretching vibrations around aromatic νCC bonds of keto and enolic form of curcumin (Kolev *et al*.,2005). Furthermore, significant intense band at 1260 cm^{-1} attributed to the bending vibration of the n(C-O) phenolic band. The band at 886 cm^{-1}, belongs to the C-H out-of-plane vibration of aromatic rings, could be described as pure vibrations. The IR bands at 874 cm^{-1} are assigned to the highly mixed γ(C-H) and aromatic γ(CCH). The out-of-plane vibrations of both –OH groups are found at 451 cm (Kolev *et al*., 2005; Song *et al*.,2009) .

The IR spectra of [Cur.- Al (III)- tartaric acid] Fig.6, show that, the strong C=O stretching peak was observed for curcumin at 1640 cm^{-1} showed a blue shift in metal complex and the value assigned 1630 for [Cur.- Al (III)- Tartaric acid] complex. The IR data of the complex suggest a typical chelating mode where the ionic enol form is chelated with metal. This type of chelating is reported for Cd (II) and Pb (II) complex of curcumin (Daniel *et al*.,2004).

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The IR spectra of the [Cur.- Al (III)- tartaric acid] complexe exhibited new bands at 423 and 545cm^{-1}, 513 cm^{-1} are assigned to υ (M-O) and υ (M-N) stretching frequency respectively (Mohammadi *et al*.,2005; Song *et al*.,2005 sand Zhao *et al*.,2010). The present study in agreement with the findings of Subhan *et al*. 2014 who prepared curcumin and metal complexes (M = Eu, Ce, La, Y, Cr, Pd) and characterized them by IR and UV-Vis spectroscopy. Their results showed thatcurcumin coordinated with metal ions in bidentate mode in deprotonated form (Subhan *et al*.,2014). Thus, the FTIR spectralstudy leads to the conclusion that the protective film consists of the Al^{+3}- Curcumin complex.

Determination the aluminum leaching by Atomic Absorption spectroscopy:

A different pH solutions (1.5, 2.5, 3.5, 4.5, 5.5 and 6.5) by using tartaric acid were applied. A 500 ml of each pH solution was boiled for one hour in aluminum based cooking wares of different regions (local, Syrian and Iranian). The amount of leached aluminum was determined by using atomic absorption spectroscopy (Table 5).

Table 5: Determination leached aluminum of three regions (as ppm) by using atomic absorption spectroscopy.

pH	Local cooking ware ppm	Syrian cooking ware ppm	Iranian cooking ware ppm
1.5	**402.3**	**465.1**	**493.2**
2.5	**399.0**	**446.8**	**491.9**
3.5	**73.88**	**90.31**	**164.0**
4.5	**16.90**	**28.47**	**52.83**
5.5	**2.487**	**5.659**	**9.616**
6.5	**2.011**	**2.764**	**3.960**

The table show that there was a reverse relationship between the quantity of leached aluminum and pH value. As shown the Iranian aluminum cooking wares leaching more aluminum comparing with Syrian and local. The differences in aluminum leaching quantity at the same conditions may due to the quality and the thickness.

The same experiment under the same condition was done by adding curcumin to give the molar ration 2 curcumin: 1 Al to encourage the complex formation by using the most leaching cooking ware. Table 6, shows the effect of curcumin in reducing the quantity of leached aluminum.

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Table 6:The elimination percentages of leached aluminum by using Iranian cooking ware and tartaric acid as catalyst at 531 nm.

PH	Pure Curcumin Absorbance	Abs (Cur-Al) complex by using tartaric acid as catalyst	% elimination of aluminum
1.5	**0.462**	**0.303**	**34.42**
2.5	**0.491**	**0.190**	**61.30**
3.5	**0.574**	**0.237**	**58.71**
4.5	**0.607**	**0.256**	**57.71**
5.5	**0.625**	**0.291**	**53.44**
6.5	**0.680**	**0.387**	**43.10**

The results of this study in agreement what were found by Elmsellema et al, 2014 and Fouda & Elattar,

2012, whom used successfully the curcumin as green inhibitor for many metals ion. Abdul-Halim *et al*, 2011, stated that curcumin is effective in eliminate the poisonous activity of aluminum through its ability to form complex with Al^{+3}. Layla, 2016, used curcumin successfully as a green corrosion inhibitor at quasicooking conditions (90˚C) to inhibit leaching of Aluminum in different vegetable and meat solutions.

Conclusion: Curcumin as a chelating agent is very effective to make a complex with aluminum ions ($CurAl^{+3}$) which eliminates the leached aluminum during use aluminum cooking wares for food preparations. Using tartaric acid as catalyst facilitate the formation of complex through holding the Al^{+3} ion to be react with curcumin instead of oxidizing and precipitation.

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