

## The Factors Effecting the Formation of Curcumin-Al (III) Complexes

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### ABSTRACT

Curcumin has been recognized as a potential natural drug to treat the Alzheimer's disease (AD) by chelating metal ions. In the present paper, curcumin-Al (III) [Cur - Al (III)] complexes were synthesized in aqueous solution and characterized by UV-visible spectroscopy. The formation of complexes between Al (III) and curcumin in acidic condition, by using citric acid as catalyst were studied. The complex molar ratio [Cur - Al (III)] was found to be 2:1 in buffer phosphate of pH 1.0, 1.5, 2.0, 3.0, 3.5 and 4.0. The optimum pH for the complex [Cur - Al (III)] formation was at phosphate buffer of pH=2.5, at this pH the complex [Cur - Al (III)] was more stable than the other pH values (throughout the absorbance calculation at 531nm). About 92% of Al (III) was chelated by curcumin at pH 2.5 as compared with 1.0, 1.5, 2.0, 3.0, 3.5 and 4.0 pH values. Curcumin complexes (at pH 2.5) were thermally stable at 100 °C as compared with 75, 50 and 25°C respectively. The chemical shifts of spectrum shows that the curcumin was strongly interact with Al (III).

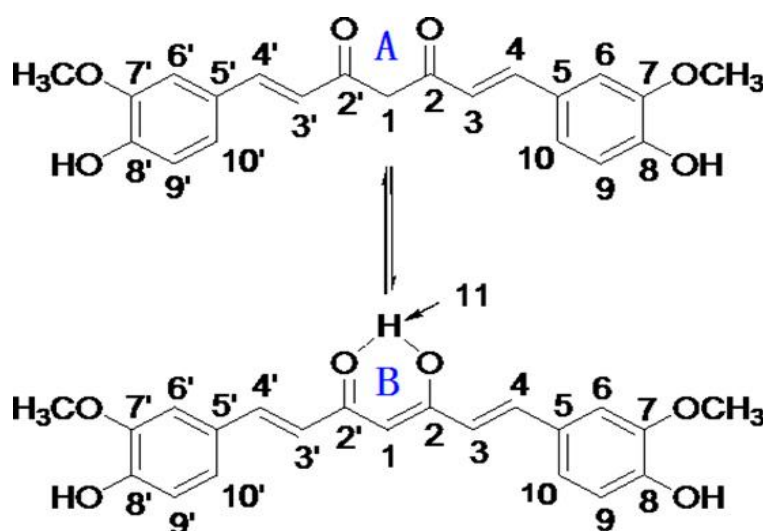
**Keywords:** Cur -Al (III) complex, acidic medium, temperature & time

### 1. INTRODUCTION

Curcumin is a hydrophobic polyphenol derived from rhizome of the herb *Curcuma longa* has a wide spectrum of biological and pharmacological activities<sup>1</sup>. Curcumin ( $MW = 368.4$ ) comprising about 3–5% of turmeric. It has a strong yellow color yet no flavor, and widely used as dietary spice, as a food coloring<sup>2-4</sup>. Curcumin exhibits anti-protozoal, antibacterial, anti-inflammatory, and anti-oxidant activities<sup>5-6</sup>. Curcumin is a strong antioxidant offers a good protection against injuries caused by free radicals as compared with E<sup>7</sup>.

Chemically, curcumin is a bis-R,<sub>2</sub>-unsaturated <sub>2</sub>-diketone [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]<sup>3-8</sup> (commonly called diferuloylmethane) (Fig.1), curcumin exhibits keto-enol tautomerism having a predominant keto form in acidic and neutral solutions<sup>9</sup>. Curcumin at pH 3–7, acts as an extraordinarily potent H-atom donor<sup>10</sup>. Zebib *et al.* was found that, more than 90% of curcumin decomposed rapidly in buffer systems of neutral and basic pH conditions. The stability of curcumin was increased in acidic pH condition<sup>11</sup> contributed to the conjugated diene structure. However, when the pH is adjusted to neutral-basic conditions, the proton was removed from the phenolic group leading to the destruction of this structure<sup>9-12</sup>.

In the keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, and the C-H bonds on this carbon are very weak, due to delocalization of the unpaired electrons on the adjacent oxygen's (Fig. 1)<sup>13</sup>.



**Fig.1:** Di-keto (A) and keto-enolic tautomeric (B) conformers of curcumin. The numbers are symbols of the C or H atom.

The 1,3-diketone moiety of curcumin can transform kinetically to a keto-enol tautomeric form, and the later is more stable and can readily chelate the metal ions to form the complexes and scavenge the active free-radicals<sup>14</sup>. Curcumin possesses wide-ranging anti-inflammatory and anti-cancer properties. Many of these activities can be attributed to its

potent antioxidant capacity at neutral and acidic pH, its inhibition of cell signaling pathways at multiple levels, its diverse effects on cellular enzymes and its effects on angiogenesis and cell adhesion<sup>13</sup>.

Aluminum is the second most abundant metal in the earth crust next to iron. It is widely used in industrial applications as well as in domestic uses. Indirect intake of Al (III) into our bodies cannot be ignored as the accumulation of Al (III) in brain, although it has been identified to cause diseases such as Alzheimer's disease (AD) 15<sup>-17</sup>. The analysis of aluminum, especially in food samples is very critical. The situation can be even worse in case of food prepared in aluminum pan with white vinegar added in. Regular consumption of food prepared in this way is potentially dangerous as accumulation of Al in brain cells has been blamed for causing (AD)<sup>18</sup>. The risk increases through drinking of ground water with Al level at 0.10 to 0.20  $\mu\text{g mL}^{-1}$ <sup>19</sup>.

Complexation of curcumin with transition metals has attracted much interest over the past years as one of the useful requirements for the treatment of (AD)<sup>2,8,20</sup> and in vitro antioxidant activity<sup>21</sup>. Furthermore, several metallocomplexes of curcumin have been synthesized, characterized and evaluated for various biological activities<sup>14, 22, 23</sup>.

There are many investigations to demonstrate some metal elements involved in the (AD) development. These metal elements include Pd (II)<sup>24</sup>, Cr (III), Mn (II), Fe (III), Cu (II), Zn<sup>14,25-27</sup> and Al<sup>8,16,28</sup> etc. Among those metals, Al (III) a component in the senile plaques is an important element impacting on the aggregation and toxicity of A $\beta$  peptides<sup>29</sup>. Therefore, one of approaches for the (AD) treatment is searching for the agents that can chelate metal ions<sup>30</sup>, preventing metal ions from the interaction with A $\beta$  peptides as well as the redox reaction which leads to the oxidative stress. So far, some chelating agents and antioxidants have been reported to treat AD<sup>31-32</sup>.

Despite the detailed information from previous studies on curcumin structure and function, the interaction, components and structures of curcumin-Al (III) complexes have not been investigated clearly. Thus the aim of the present is to study the formation of Curcumin-Aluminum (III) [Cur.-Al (III)] in acidic condition in addition to study the factors effecting the complex formation.

## 2. EXPERIMENTAL

### 2.1 Instrumentations and Chemicals

The UV-Visible Spectrophotometer Shimadzu model U.V.-160A and SS-3 pH Meter have been used in the present study. The Chemicals  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ , sodium potassium tartrate and  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  from Fluka, citric acid and ethanol (96%) from BHD and local production deionized water.

### 2.2 Preparation of curcumin

The curcumin (Cur.) was prepared by extraction 5grams of turmeric in 150 ml of (96%) ethanol and refluxed for 2hr at boiling point. The hot mixture was filtered; the filtrate was evaporated at 50°C till complete dryness<sup>33</sup>. The dry matter was weighed to find out the curcumin percentage (it was 25%).

### 2.3 Preparation of curcumin-Al complex [Cur.-Al (III)]

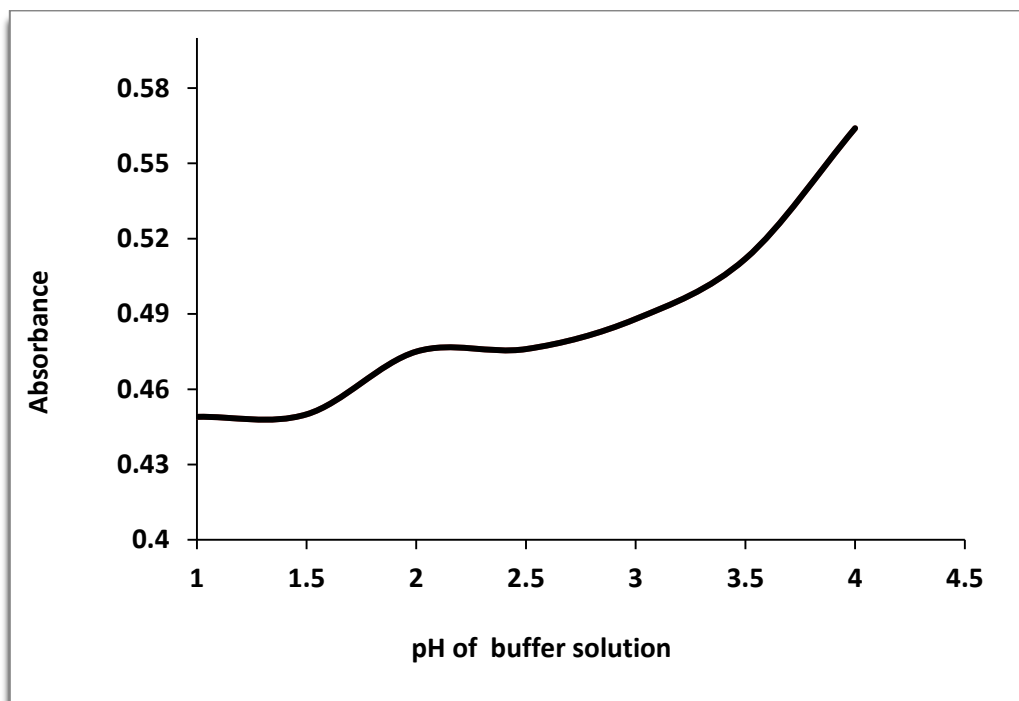
The mixture of complex [Cur.-Al (III)] was prepared in different pH values. 5ml of  $2 \times 10^{-4}$  M (Cur.) solution, 5ml of  $2 \times 10^{-4}$  M citric acid solution (as catalyst), 5 ml of phosphate buffer pH = 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0. 5ml of  $1 \times 10^{-4}$  M  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  was added to the above mixture in molar ratio [2: 1: 2] of curcumin, Al(III) and citric acid respectively; 5 drops of 0.1M sodium potassium tartrate were added to inhibit the formation of insoluble  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ . The mixtures absorbance was determined at room temperature after 30min.

### 2.4 Preparation of [Cur.-Al (III)] complex in different temperatures (25, 50, 75 and 100)°C with time (0, 15, 30, 45 and 60) min. in optimum pH (2.5) of phosphate buffer

The mixture value of [Cur.-Al(III)] complex was 5ml of  $2 \times 10^{-4}$  M (Cur.) solution, 5ml of  $2 \times 10^{-4}$  M citric acid solution, 5 ml of phosphate buffer of pH= 2.5 which was taken as optimum pH according to the previous experiments. 5ml of  $1 \times 10^{-4}$  M  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  was added to the above mixture in molar ratio [2:1: 2, curcumin: Al (III): citric acid] with 5 drops of 0.1M sodium potassium tartrate. The mixture was heated in water bath at 25, 50, 75 and 100°C for 0, 15, 30, 45 and 60 min. The mixtures were subjected to spectrophotometer to record absorbance at 531nm, which is noticed as  $\lambda_{\text{max}}$  for Al(III)-curcumin complex solution.

## 3. RESULTS AND DISCUSSION

As recommended by previous study<sup>11</sup>, we have used an acidic pH conditions which have maintained more than 90% of curcumin, as compared with neutral and basic conditions. The stability of curcumin in acidic conditions may attribute to the conjugated diene structure. In acidic and neutral conditions the proton will be removed from the phenolic group leading to destruction of curcumin structure<sup>11</sup>. Fig (2) shows a direct proportional relationship between pH value and the recorded absorbance.



**Fig.2:** The absorbance (at 423nm) of  $2 \times 10^{-4}$  M curcumin at different phosphate buffer pH values.

We found that the optimum pH for the best complexation [Cur. - Al (III)] was at phosphate buffer of pH=2.5. At this pH, the complexation [Cur - Al (III)] was more stable than the other pH values. It's well known from present study (throughout the absorbance calculation at 531nm), that about 92% of Al (III) was chelated by curcumin at pH 2.5 as compared with 1.0, 1.5, 2.0, 3.0, 3.5 and 4.0 pH values.

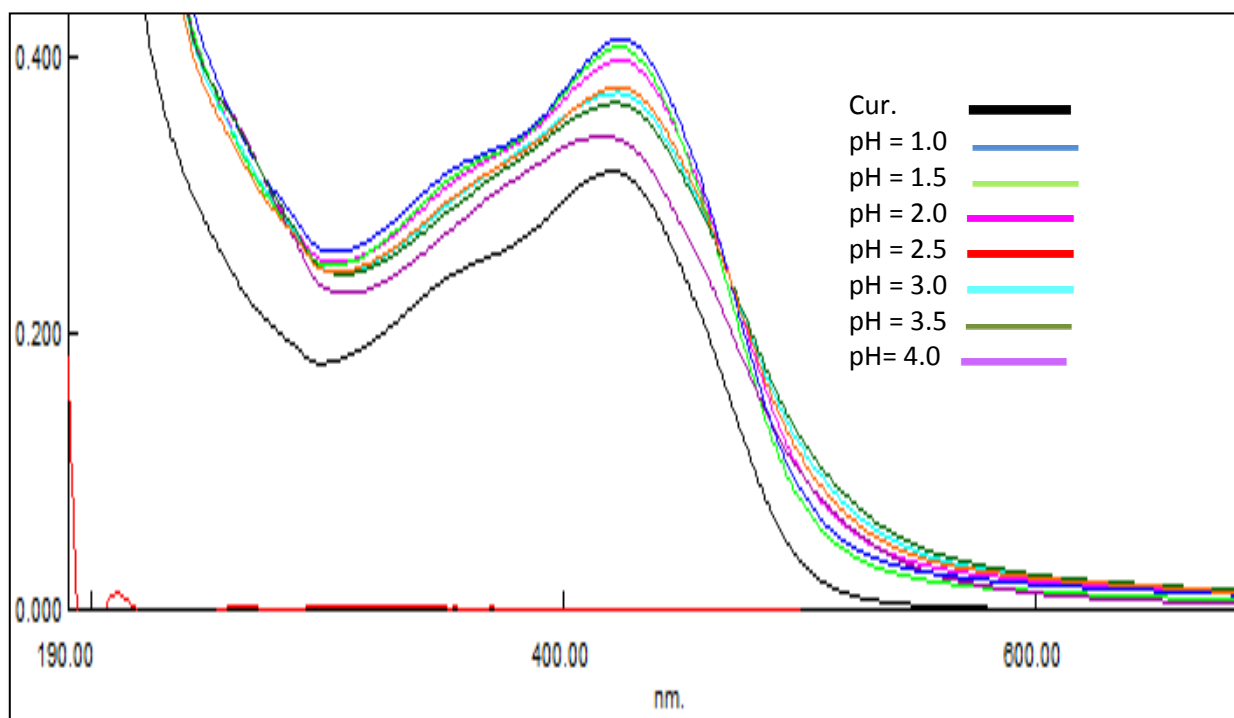
The UV-visible spectrum bands of curcumin showed that the maximum absorption (table 1) in 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 pH values were at 424, 423, 423, 423,422, 422 and 415 nm respectively. These bands assigned to the  $\pi \rightarrow \pi^*$  of curcumin (figure 3)<sup>11,14,24,25</sup>. The UV -visible spectrum bands of curcumin as compared with the complexes of [Cur. - Al (III)] (table 1 & Figure 4) show that the maximum absorption of complexes were shifted to higher wavelengths (table 1) at the same buffer pH respectively.

The interaction between curcumin and metal ions was implied by the change in optical absorbance of curcumin upon mixing with a metal solution<sup>2, 8, 14, 24, 34-36</sup>. The addition of Al (III) to aqueous solution of curcumin increases the absorbance spectrum to a higher wavelength with bathochromic shift of about 107, 109, 75, 108, 80, 80 and 96nm from the original band in the absence of Al(III). It is probable, that the bathochromic shift occurs as a result of coordination by the lone pair electrons on the oxygen (O) donor atoms with the aluminum ion site, thus stabilizing the excited state relative to the ground state and leading to longer wavelength absorption maxima<sup>37</sup>. The observed bathochromic shifts are consistent with the lone pair electrons in the donor atoms (O in curcumin) and participating in (Al ion) coordination which in turn stabilizing the excited state relative to the ground state.

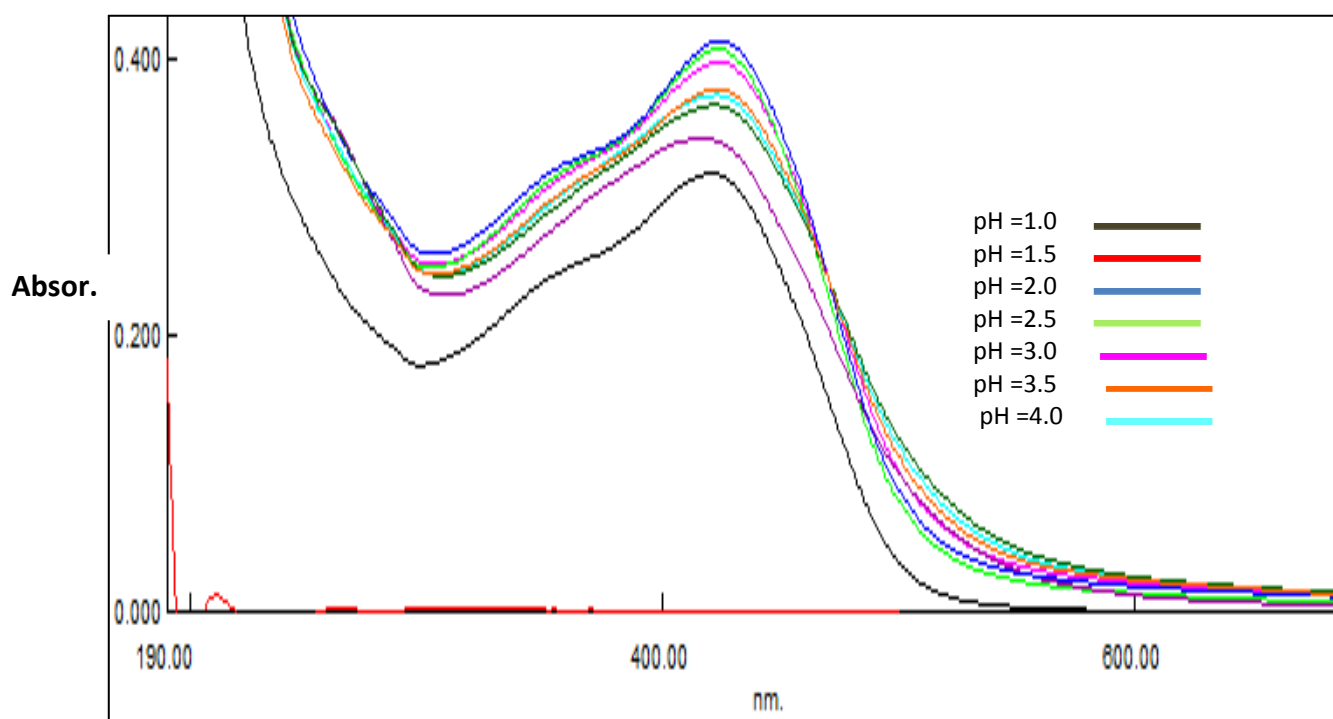
Since the curcumin and its complexes were thermally stable up to  $160^\circ\text{C}$ <sup>11,38</sup>, the effect of 25, 50, 75 and  $100^\circ\text{C}$  for 0, 15, 30, 45 and 60 min with a molar ratio of 2:1:2 (curcumin: Al: citric acid) were studied. Table (2) and Fig (5) show that the complex [Cur. - Al] was highly stable at  $100^\circ\text{C}$  up to 60 min (the yellowish color disappear due to the chelation activity of curcumin).

**Table-1:** Electronic absorption spectra U.V of curcumin and complex [Cur.-Al (III)]

pH of buffer	Abs.maximum of curcumin	Wavelength nm of curcumin	Abs. maximum of complex[Cur.-Al(III)]	Wavelength nm of complex[Cur.-Al(III)]
1.0	0.415	424	0.046	531
1.5	0.408	423	0.034	532
2.0	0.399	423	0.045	498
2.5	0.337	423	0.027	531
3.0	0.375	422	0.063	502
3.5	0.367	422	0.063	502
4.0	0.343	415	0.048	511



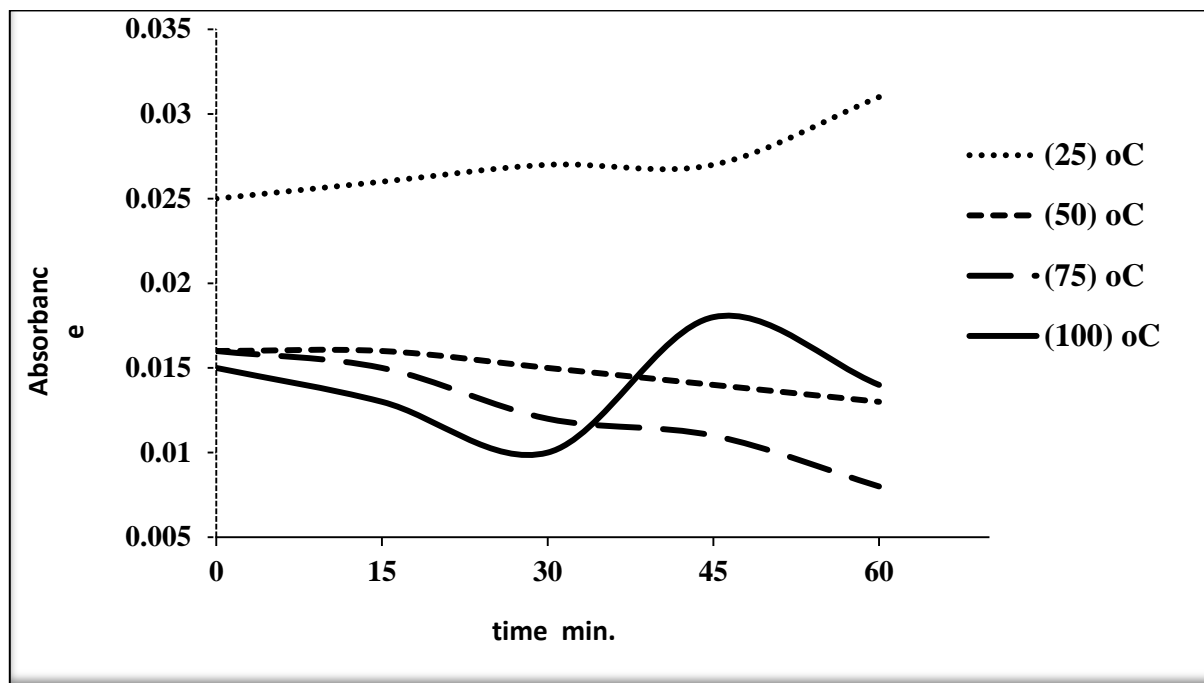
**Fig-3:** Electronic absorption spectra U.V of curcumin  $2 \times 10^{-4}$  M and citric acid  $2 \times 10^{-4}$  M at different values of buffer pH= 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 without Al(III).



**Fig-4:** Electronic absorption spectra U.V of complex of [curcumin  $2 \times 10^{-4}$  M and citric acid  $2 \times 10^{-4}$  M] in different values of buffer pH= 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 with Al(III)  $1 \times 10^{-4}$  M]

**Table-2:** Electronic absorption spectra U.V of complex of curcumin  $2 \times 10^{-4}$  M and citric acid  $2 \times 10^{-4}$  M in optimum pH (2.5) of phosphate buffer with Al (III)  $1 \times 10^{-4}$  M in different temperature (25, 50, 75 and  $100^\circ\text{C}$ ) with time (0, 15, 30, 45 and 60) min. in  $\lambda = 531$  nm.

time min.	Abs. at $25^\circ\text{C}$	Abs. at $50^\circ\text{C}$	Abs. at $75^\circ\text{C}$	Abs. at $100^\circ\text{C}$
0	0.025	0.016	0.016	0.015
15	0.026	0.016	0.015	0.013
30	0.027	0.015	0.012	0.010
45	0.027	0.014	0.011	0.018
60	0.031	0.013	0.008	0.014



**Fig-5:** Electronic absorption spectra U.V in  $\lambda = 531 \text{ nm}$  of complex of curcumin  $2 \times 10^{-4} \text{ M}$  and citric acid  $2 \times 10^{-4} \text{ M}$  in optimum pH (2.5) of phosphate buffer with Al(III)  $1 \times 10^{-4} \text{ M}$  in different temperature (25, 50, 75 and 100)°C with time (0, 15, 30, 45 and 60) min.

#### 4. CONCLUSION

The optimum pH, for the best complex [Cur - Al (III)] formation was at phosphate buffer of pH=2.5 (92% of Al (III) was chelated by curcumin). The Al (III) caused a bathochromic shift of the visible absorption bands in curcumin solution. The absorbance show that the complex of [Cur. – Al (III)] was very stable in higher degree of temperature (100 °C) until 60 min. All chemical shifts of spectrum pointed that the curcumin was interact strongly with Al (III) ion under different pH values and temperature degrees.

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