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# Chalcones as Analytical Reagents of Aluminum: Stability, Thermodynamic and Kinetic Study

**Abstract:** Chalcones, a group of polyphenolic compounds, has been studied for a long time due to their biological properties and their ability as analytical reagents. In this work the stability and thermodynamic parameters concerning the formation in ethanolic medium of two chalcone–aluminium complexes (2',3-dihydroxychalcone-Al(III) and 2',4',3-trihydroxychalcone-Al(III)) were studied and a kinetic study of the formation reaction was also performed. Both systems showed 1 : 1 L : M stoichiometry and stability constants at four temperatures were determined. Thermodynamic parameters indicate that both formation reactions are endothermic and driving force is entropic. Kinetic study revealed that 2',4',3-trihydroxychalcone-Al(III) formation is faster than 2',3-dihydroxychalcone-Al(III), a factor which can be interesting when proposing new analytical reagents.

**Keywords:** Aluminium, Chalcone, Complexes, Kinetic, Stability Constants.

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## 1 Introduction

Chalcones are polyphenolic compounds which belong to the wide family of flavonoids. Chalcones have been studied for a long time due to their ability as

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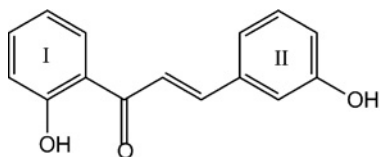
analytical reagents [1–3] and, mainly, due to their biological properties: anti-cancer [4–8] antimicrobial [9–11] and antifungal [12, 13] activity, among others. It has been proposed that biological activities of flavonoids may be enhanced when complexation with metallic ions occurs. A wide number of chalcone-metallic ions complexes have been studied and many authors have reported the synthesis and characterization of these complexes in solid state [7, 14–16]. However, the characterization of these complexes in solution using thermodynamic data is not widely investigated. Another abandoned field of study is the kinetic behavior of the complexes's formation. In some cases the chalcone-metallic ion complex is instantaneously formed [3], and others where kinetic of complex formation requires, at least, several minutes [2]. Either way, no studies concerning kinetic of these complexes are found in literature. This is the main reason why this work was performed: to determine not only the stability and thermodynamic parameters of two chalcone-Al(III) complexes formation, but also to study kinetic parameters of the reaction. In addition, for a further understanding of processes studied, the effect of temperature was evaluated.

Two structurally related chalcones were selected as ligands to achieve our goals: 2',3-dihydroxychalcone (A) and 2',4',3-trihydroxychalcone (B), while Al(III) was selected as metallic ion.

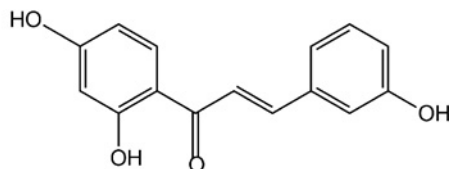
## 2 Experimental section

### 2.1 Reagents

2',3-dihydroxychalcone (A) and 2',4',3-trihydroxychalcone (B) were synthesized as reported elsewhere [3] by condensing, respectively, 3-hydroxybenzaldehyde and 2-hydroxyacetophenone and 3-hydroxybenzaldehyde and 2,4-dihydroxyacetophenone in alkaline medium. Figure 1 shows the structure of both synthesized compounds.



2',3-dihydroxychalcone (A)



2',4',3-trihydroxychalcone (B)

**Figure 1:** Structure of studied chalcones.

Highly concentrated ( $\approx 0.2$  M)  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  (Stanton) solutions were prepared using distilled water as solvent while stoichiometry, stability constants and kinetic experiments were carried out using ethanol spectroscopic grade (Merck) as solvent. A few microliters of  $\text{AlCl}_3$  solutions were used in every experiment in order to keep final concentration of water negligible in reaction mixtures (below 0.5%).

## 2.2 Apparatus

An Agilent 8454 diode-array spectrophotometer provided with an AGILENT 89090A temperature controller was used to record the ligands and their complexes spectra as well as to measure absorbances required.

## 2.3 Methods

### 2.3.1 Stoichiometry and apparent stability constants determination

Complexes stoichiometry was determined using Yoe–Jones method [17], preparing a set of solutions where metallic ion concentration is kept constant and ligand concentration is varied within a range. The absorbance of these solutions is measured, at a wavelength where only the complex absorbs, and used to plot a graphic of absorbance vs. ligand/metal concentration ratio,  $L : M$ . The intersection points between straight lines of experimental data indicate the ligand/metal molar ratio. This method was also employed to determine the apparent stability constants using the following expression:

$$K = \frac{[ML]}{[M][L]} = \frac{(A/A_{\text{extr}})C}{[M - (A/A_{\text{extr}})C][L - (A/A_{\text{extr}})C]} \quad (1)$$

where  $M$  and  $L$  are the total analytical concentrations of metal and ligand respectively,  $C$  is the total analytical concentration of the ligand or the metal, whichever is the limiting reactant at the equivalence point,  $A_{\text{extr}}$  is the extrapolated value of the absorbance,  $A$  is the actual value of absorbance and  $K$  is the apparent stability constant. The standard enthalpy associated to the process,  $\Delta_r H^0$ , was determined using Vant'Hoff expression:  $\ln K = -\Delta_r H^0/RT + c$ , while the Gibbs free energy,  $\Delta_r G^0$ , was determined using the equation:  $\Delta_r G^0 = -RT \ln K$ , and the change in entropy,  $\Delta_r S^0$ , was calculated using the expression:  $\Delta_r G^0 = \Delta_r H^0 - T\Delta_r S^0$ , where,  $R$  is the universal gases constant,  $T$  is the absolute temperature and  $c$ , an integration constant.

### 2.3.2 Kinetic study

A kinetic study is aimed to define the rate law of a reaction, which means to determine the rate constant value, the partial orders with respect to each reactant and the overall order, the addition of all partial orders. An overall order of reaction two may be proposed for chalcone-Al(III) complexes formation, which means a partial order one with respect of chalcone and a partial order one with respect of Al(III).

The integrated equation for a second order reaction using the same initial concentrations of both reactants is:

$$\frac{x}{a-x} = a kt \quad (2)$$

where  $a$  is the initial concentration of reactants,  $x$  is the amount of reactants which reacted at time  $t$ ,  $k$  is the rate constant and  $t$  represents time. According to Lambert–Beer law the following equations can be written:

$$t = 0 \quad A_0 = \varepsilon_{\text{ch}} a \quad (3)$$

$$t = t \quad A = \varepsilon_{\text{ch}}(a-x) + \varepsilon_c \cdot (x) \quad (4)$$

$$t = \infty \quad A_{\infty} = \varepsilon_c x_{\text{eq}} \quad (5)$$

and replacing Eq. (3), (4) and (5) in Eq. (2) the following expression can be obtained:

$$\frac{A - A_0}{A_{\infty} - A} = a kt \quad (6)$$

where  $A$  is the absorbance of the reactant mixture at a given time  $t$ ,  $A_0$  and  $A_{\infty}$  are the absorbance of the reactant mixture at the beginning of the reaction and when equilibrium has been reached.

Kinetic constant can be determined through using the expression above, valid for second order reactions when both reactants have the same initial concentration. According to Eq. (6) a plot of  $(A - A_0)/(A_{\infty} - A)$  vs.  $t$  should be linear. If this occurs, then hypothesis of second order reaction is correct and rate constant can be determined from the slope of the straight line.

Kinetic study was performed the following way: ethanolic solutions of chalcone, were added in a quartz cell containing ethanol. This cell was thermostated 5 min and an appropriate amount of  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  aqueous solution was added and mixed, in order to begin the reaction. Absorbance was measured every 10 s for 3 min.  $A_{\infty}$  was measured once the equilibrium was reached.

Reaction rates depend strongly on temperature, being Arrhenius empirical relation the most widely used to describe this dependence. According to this relation:  $\ln k = \ln A - Ea/RT$ , where the quantity  $Ea$  is the activation energy,  $A$

is the frequency factor,  $R$  is the universal gases constant and  $T$  is the absolute temperature. Another way to explain dependence of  $k$  with  $T$  is provided by Collision Theory of Chemical Reactions. According to this theory, the equation that describes dependence of  $k$  with  $T$  is:

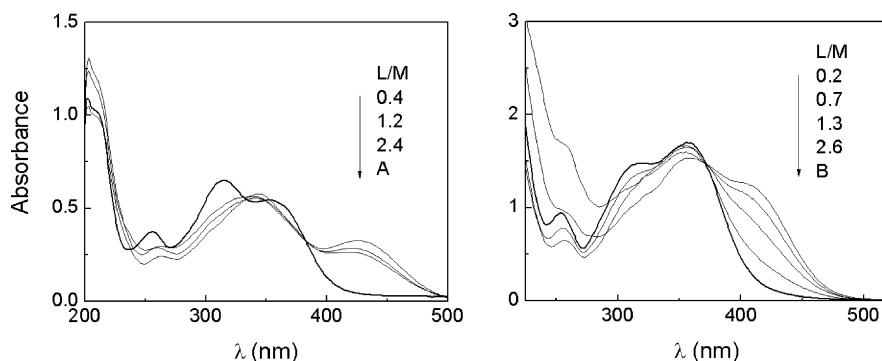
$$\ln \frac{k}{T} = \left( \ln \frac{\kappa k_b}{h} + \frac{\Delta_f S^*}{R} \right) - \frac{\Delta_f H^*}{R} \frac{1}{T} \quad (7)$$

Where  $\Delta_f H^*$  and  $\Delta_f S^*$  are the enthalpic and entropic changes associated with the formation of the activated complex responsible of products generation,  $h$  and  $k_b$  are Planck and Boltzman constants, and  $\kappa$  is the transmission coefficient, in this case  $\kappa = 1$  is considered.

### 3 Results and discussion

In order to determine if complexes between proposed ligands and Al(III) were effectively formed, the following experiment was carried out for each ligand: a set of solutions were prepared where ligand concentration was kept constant and Al(III) concentration was varied in order to obtain different L : M ratio. Spectra of these solutions were registered 24 h after to assure the equilibrium of the reaction was reached. Results obtained are shown in Figure 2, where the thicker lines corresponds to ligands spectra.

In UV-Vis spectra of flavonoids two major absorption bands are displayed: Band I between 320 and 385 nm, related to the cinnamoyl system and Band II, in the 240 to 280 nm range, related to benzoyl system (see Figure 1). Band I absorption spectra in complexes are shifted to higher wavelengths compared to free



**Figure 2:** Spectral changes observed when complexation occurs for 2',3-dihydroxychalcone-Al (left) and 2',4',3-trihydroxychalcone-Al (right), in ethanolic medium.

flavonoids. This red shift is caused by an increased conjugative effect when the complex is formed due to the formation of a new ring involving the metallic ion. Figure 2 shows a bathochromic shift for both studied systems, proving a complex is formed between every ligand and Al(III). Spectra corresponding to A-Al system show an increase in the absorbance of the band centered at 425 nm when metallic ion concentration increases (lower L : M ratio), but no further changes were observed for L : M < 0.4. The isosbestic point at 384 nm indicates the formation of only one species. A similar description can be made for B-Al system.

Spectral changes confirmed formation of complexes of both ligands and Al(III) and the presence of only one complex for each system. The next step in order to characterize these complexes was to determine L : M ratio and stability of each one.

### 3.1 Stoichiometry and apparent stability constants determination

Stoichiometries of complexes were determined by the Yoe-Jones method, previously described. This method was also used to determine the apparent equilibrium constant of both complexes.

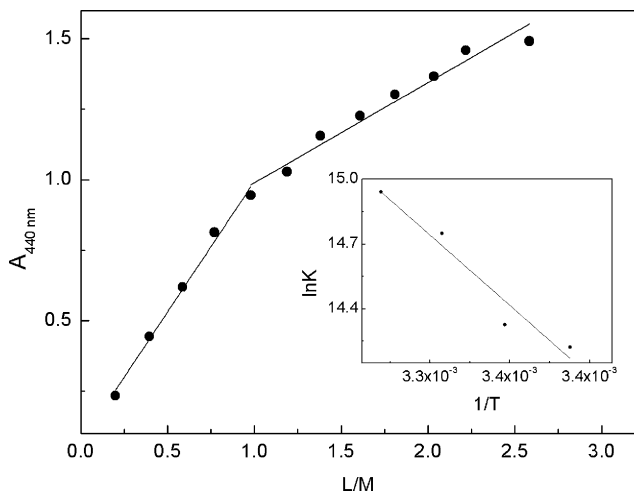
The set of solutions used to determine stoichiometry and apparent stability constants were prepared according to data in Table 1. The absorbance of these solutions was measured after 24 h in order to assure the equilibrium was reached. Figure 3 shows results obtained for B-Al determinations at 25 °C. Similar graph was obtained for A-Al system.

The apparent equilibrium constant determination was carried out at four different temperatures so thermodynamic parameters could be calculated. Data obtained at every temperature are shown in Table 2.

According to data above, both ligands form 1 : 1 L : M complexes and showed high enough  $K$  values ( $K \sim 10^6$ ) to be considered analytical reagents of Al(III). Nevertheless, no significant differences in  $K$  values were observed in order to select one over the other.

**Table 1:** Ligand concentrations ( $L$ ), metallic ion concentrations ( $M$ ) and wavelength ( $\lambda$ ) used in stoichiometry determination.

System	$L$	$M$	$\lambda$
2',3-dihydroxychalcone-Al(III)	$9.8 \times 10^{-5}$ – $9.7 \times 10^{-4}$ M	$3.6 \times 10^{-4}$ M	425 nm
2',4',3-trihydroxychalcone-Al(III)	$2.0 \times 10^{-5}$ – $3.8 \times 10^{-4}$ M	$1.2 \times 10^{-4}$ M	440 nm



**Figure 3:** Yoe–Jones graphical representation for determination of stoichiometry and apparent equilibrium constant of 2',4',3-trihydroxychalcone-Al(III) in ethanolic medium at 25 °C. Inset: Determination of thermodynamic parameters for 2',4',3-trihydroxychalcone-Al(III) formation.

**Table 2:** Determination of apparent equilibrium constants for 2',3-dihydroxychalcone-Al(III) and 2',4',3-trihydroxychalcone-Al(III).

2',3-dihydroxychalcone-Al(III)							
$T$	$A_{\text{extr}}$	$A$	$L : M$	$M \times 10^4$ (M)	$L \times 10^4$ (M)	$K \times 10^{-6}$	$\ln K$
288	1.706	1.657	1.03	2.85	3.09	1.05	13.86
293	1.789	1.743	1.10	2.83	3.08	1.21	14.00
298.6	1.447	1.425	1.06	2.82	3.06	2.22	14.61
303	1.746	1.665	1.02	2.82	2.73	5.36	15.49
2',4',3-trihydroxychalcone-Al(III)							
$T$	$A_{\text{extr}}$	$A$	$L : M$	$M \times 10^5$ (M)	$L \times 10^5$ (M)	$K \times 10^{-6}$	$\ln K$
293	0.989	0.920	0.98	9.89	9.68	1.50	14.22
298	0.983	0.918	0.99	9.83	9.62	1.67	14.33
303	1.018	0.944	1.06	9.78	9.57	2.54	14.75
308	0.963	0.898	0.99	9.73	9.52	3.08	14.94

A study on thermodynamic parameters could be a way to find which ligand is more suitable for Al(III) complexation. Table 3 shows thermodynamic data determined for both formation reactions. A-Al parameters values are larger than those of B-Al, explaining the mayor temperature dependence of  $K$  for this system, but still no significant differences can be noticed between both systems. Nevertheless,

**Table 3:** Thermodynamic parameters determined for formation reaction of both studied complexes.

Parameter	A–Al	B–Al
$\Delta_r H^0$ (kJ mol <sup>-1</sup> )	77.81	38.55
$\Delta_r S^0$ (J K <sup>-1</sup> mol <sup>-1</sup> )	382.06	248.49
$\Delta_r G_{25}^0$ (kJ mol <sup>-1</sup> )	-36.27	-35.50

this table gives information about complexation reactions: both are endothermic, have positive entropy and are spontaneous at room temperature. This means driving force in both systems is entropic.

### 3.1.1 Kinetic study

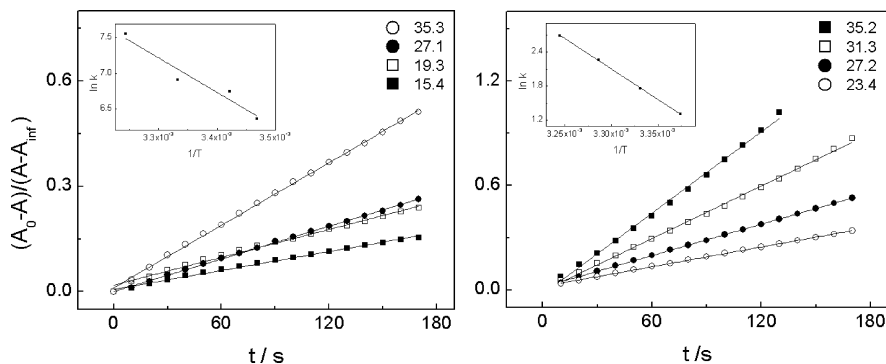
Thermodynamic characterization data was not sufficient to distinguish which system was more suitable for Al(III) complexation, so a kinetic study was performed. It was proposed a second overall order for each complex formation reaction. According to this, graphic representation of Eq. (6) should be a straight line and constant rate might be calculated from the straight line slope. Figure 3 shows results obtained. For both systems at each temperature of work the graphic correspond to a straight line, indicating that proposed overall order is correct for both systems.

It was possible to determine kinetic parameters of complexation reaction using data in Figure 4. Table 4 shows determined rate constants, considering  $a = 9.3 \times 10^{-5}$  M for A-Al system and  $a = 1.0 \times 10^{-4}$  M for B-Al system, as well as activation energy calculated through Arrhenius law and thermodynamic parameter for activated complex formation, determined using Eq. (7).

Table 4 shows, as expected, an increase of rate constant values with temperature for both systems, but  $k$  values for B-Al are larger than those of A-Al, meaning the formation of B-Al is faster.  $E_a$  values were determined for both systems using Arrhenius equation and are in concordance with usual values. Thermodynamic parameters of activated complex formation indicate the reaction is endothermic for both systems. A-Al system exhibits a very small and negative  $\Delta_r S^*$  while B-Al system has a large positive  $\Delta_r S^*$ . Since this parameter is related with disorder and disorder increases with temperature, this could explain the enhancement of kinetic rate value for B-Al system.

According to these kinetic results, B could be selected as a more suitable analytical reagent for Al(III), while considering just stability and stoichiometry both ligands are equally suitable.





**Figure 4:** Left: Constant rate determination at different temperatures for 2',3-dihydroxycyclacone-Al (left) and 2',4',3-trihydroxychalcone-Al (right) systems. Insets (left and right): Graphical representation of Arrhenius equation for each system.

**Table 4:** Experimental parameters determined for both complex formation reactions.

	A-Al				B-Al			
$T$ ( $^{\circ}\text{C}$ )	15.4	19.3	27.1	35.3	23.4	27.2	31.3	35.2
$k$ ( $\text{min}^{-1}\text{M}^{-1}$ )	578.0	850.4	998.6	1913.4	1323.2	2083.5	3424.2	5194.3
$E_a$ ( $\text{kJ mol}^{-1}$ )	40.5				91.7			
$\Delta_r H^*$ ( $\text{kJ mol}^{-1}$ )	38.0				89.0			
$\Delta_r S^*$ ( $\text{J K}^{-1}\text{mol}^{-1}$ )	-4.0				170.2			
$\Delta_r G^*$ ( $\text{kJ mol}^{-1}$ )	39.2				38.3			

## 4 Conclusions

In this work the formation of two structurally related chalcones, 2',3-dihydroxycyclacone and 2',4',3-trihydroxychalcone, and Al(III) complexes in ethanolic medium was studied. Stoichiometry determined for both systems was 1 : 1 L:M and stability constants values were high enough ( $K \sim 10^6$ ) to consider both ligands as possible analytical reagent for Al(III). Thermodynamic parameters of complexes reactions indicate that both formation reactions are endothermic and driving force is entropic. Kinetic study showed that 2',4',3-trihydroxychalcone-Al(III) formation is slightly faster than 2',3-dihydroxycyclacone-Al(III), a factor which can be interesting when proposing new analytical reagents.

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