Determination of Ethyl Vanillin by a Using Briggs-Rauscher Oscillator

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A study on the determination of an antioxidant ethyl vanillin (EV) built on its perturbation effects on a Briggs-Rauscher (BR) oscillator was reported in this paper. A tetraazamacrocyclic Ni(II) complex, $[NiL](ClO_4)_2$, was used as a catalyst in such a oscillator, and the ligand L in the complex is 5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-4,11-diene. The pH value for the BR reaction was kept at 2, which is analogous to the pH of the fluids of the human stomach. It has been concluded from the experimental data that the addition of EV into BR system could cause the temporary cease in the oscillation after inhibition time (t_{in}) depending on the concentration of EV added. It was found that, as the concentration of EV were increased, the t_{in} were increased. Thus, a polynomial regression curve was achieved over a range 7.5×10^{-6} to 3.5×10^{-5} M of EV with a correlation coefficient of 0.98. The obtained RSD from six measurements of 2.5×10^{-5} M of EV is 0.159%. On the basis of the FCA model, reaction mechanism involving HOO$^\bullet$ radical has been proposed.

1. INTRODUCTION

Oxidation process is a chemical phenomena causing loss of electrons during which free radicals could be generated. Such radicals promote chain reactions within cell, which could induce the cell and tissue damages in the human body. Antioxidants have the ability to terminate these chain reactions through eliminating free radical intermediates and constrain some oxidation reactions through self-oxidation. Therefore, antioxidants are usually considered as reducing agents. Thiols, ascorbic

acid and polyphenols are some good examples of them. Deficiency in standard of antioxidants could lead to oxidative stress and may damage or slay cells [1]. Oxidative stress play a substantial role in various human illnesses, such as cancers [2]. Consumption of food antioxidants, dietary supplements reduce the oxidation stress by increasing the level of antioxidant capacity within human body [3]. On the basis of occurrence, antioxidants can be classified into two kinds: natural and synthetic. The natural antioxidants are mainly founds in fruits, vegetables and beverages in a form of vitamin C & E, polyphenols and carotenoids [4]. Both natural and synthetic antioxidants have the ability to lower the level of auto-oxidation of lipids [5]. The side effects of natural antioxidants is less in the human body as compare to synthetic antioxidants [6] and natural antioxidants has more application in the medicine and food industry [7, 8].

The influences of antioxidants on the Briggs-Rauscher (BR) oscillator using metal ion and macrocyclic catalysts have been recently reported [9-14]. The BR oscillator [15] was discovered by two science instructors in San Francisco by replacing bromate (BrO_3^-) in the BZ oscillator with iodate and adding hydrogen peroxide. When antioxidants were put into the active BR system, the oscillation could be quenched and regenerated after inhibition time (t_{in}). Damping in the oscillation is due to the free radical intermediates present in the course of reaction. Such damping in oscillation cause by antioxidant on the BR system using macrocyclic Ni (II) catalyst are well explained on basis of FCA mechanism.

In this article we described a new technique for the determination of an antioxidant, Ethyl vanillin (EV) based on inhibitory effect on the BR oscillator in the presence of macrocyclic Ni (II) catalyst ($NiL(ClO_4)_2$), where ligand L is 5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-4,11-diene. The addition of EV into the active BR caused immediate ceasing and regeneration of oscillation after t_{in} dependent on the concentration of EV. Reaction temperature was set at $4 \pm 0.5\ ^{\mathrm{O}}C$ and pH value was kept at 2 which is similar with the pH of human stomach fluid.

EV (Scheme 1) is a synthetic compound having chemical formula $(C_2H_5O)\,(HO)C_6H_3CHO$, flavor agent derived from vanillin. It is available as fine white or slightly yellow crystal and is slightly soluble in water. EV has much stronger flavor than vanillin. It is widely used in various industries like food manufacturing, beverages, pharmaceutical products etc. Different methods were reported for the determination of EV, including spectrophotometry [16], liquid chromatography [17], liquid chromatography with mass spectrometric [18] and liquid chromatography with ultraviolet detection [19]. We contribute a novel quantitative analytic technique for the determination of EV by using BR, which has some advantages over instrumental techniques i.e. simplicity in setup, ease for the procedure, larger linear range and lower limit of detection.
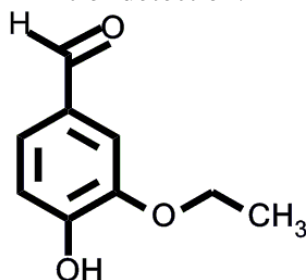

Scheme 1. Structure of ethyl vanillin.

2. EXPERIMENTAL

2.1. Reagents

All the reagents, malonic acid (MA), H_2SO_4 (Aldrich, 98%), KIO_3 and H_2O_2 (30%), and EV were used of analytical grade. Whereas tetraazamacrocyclic complex, $[NiL](ClO_4)_2$, was synthesized [20, 21] and identified by IR spectrum and elemental analysis. Its structure is shown in Scheme 2. Solution of 2.5×10^{-2} M H_2SO_4 was made from 98% of H_2SO_4, and 2.00 M malonic acid, 0.014 M KIO_3, 1.73×10^{-2} M $[NiL](ClO_4)_2$, 4.00 M H_2O_2 were prepared within such a H_2SO_4 solution. Solutions of different concentration of EV were also prepared in acidic solution of H_2SO_4.
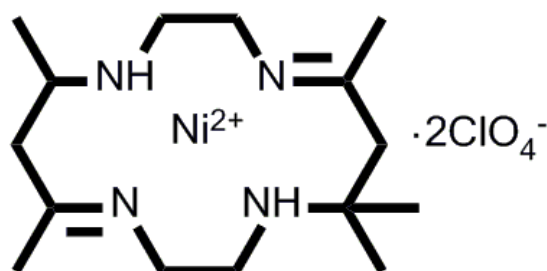
Scheme 2. Structure of macrocyclic Ni complex, $[NiL](ClO_4)_2$.

2.2. Apparatus

The instrumental setup for the BR mainly consists of a glass reactor (50 ml) and a potential measurement system. The glass reactor was equipped with a thermostat model (DZCS-IIC, Nanjing Dazhankejiao Institute of Instrument, China). A 79-3 magnetic stirrer (Jiangsu, China) was used to homogenize the reaction solution by kept its stirring rate at 500 rmp, and a temperature controller to keep reaction temperature at 4 ± 0.5 ^{O}C. The potential measurement system consists of two electrodes. A platinum electrode (model 213 Shanghai, China) was applied as working electrode while a saturated calomel electrode (SCE) (Model 217 Shanghai, China) via a salt bridge containing 1 M Na_2SO_4 acted as reference electrode, both of which were immersed into the glass reactor. The changes in potential *Vs* time were recorded in PC through an Amplifier and a GO!Link sensor interface, both of which were purchased from Vernier Software Technology, USA, with a Logger Lite data-acquisition program.

2.3. Procedure

All the reagents were put into the glass reactor in the following sequence, 15 ml of 2.5×10^{-2} M H_2SO_4 solution, 6 ml of 1.4×10^{-1} M KIO_3 solution, 2 ml of 1.73×10^{-2} M $[NiL](ClO_4)_2$ solution, 3 ml of 2.00 M MA solution , 14 ml of 4.00 M H_2O_2 solution. The glass reactor was than kept in ice bath and a temperature controller was used to keep reaction temperature at 4 ± 0.5 ^{O}C. The solution was homogenized by continues stirring at 500 rmp. The two electrodes, platinum and SCE were put into

the glass reactor and potentials v.s. time were recorded in a PC through an Amplifier and a Go!link sensor interface with logger lite data acquisition program. After a short interval of time, the oscillation was started. The different concentrations of EV were used to perturb the BR oscillator at the base of potentiometric cycle. As a result, oscillations could be quenched and then restarted with an inhibition time (t_{in}) being noticed. The t_{in} is the interval of time in sec before oscillation regains.

3. RESULT AND DISCUSSION

The typical oscillation profile was shown in Figure 1a, which was obtained by direct mixing of the above described reagents. The solution color was noticed to change from yellow to brown then back to yellow due to the one electron transfer process between $[NiL]^{2+}$ and $[NiL]^{3+}$ for catalyst during oscillation.

$$[NiL]^{2+}(yellow) \Leftrightarrow [NiL]^{3+}(green)$$
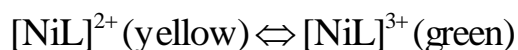
During oscillation, I_2 was formed and dissolved into the oscillating solution, and that is why the appearance of the solution color was observed brown rather than green [22]. However, in Mn^{2+}-catalyzed BR oscillator, continuous change in color from colorless to yellow to blue was noticed in starch indicator [23].
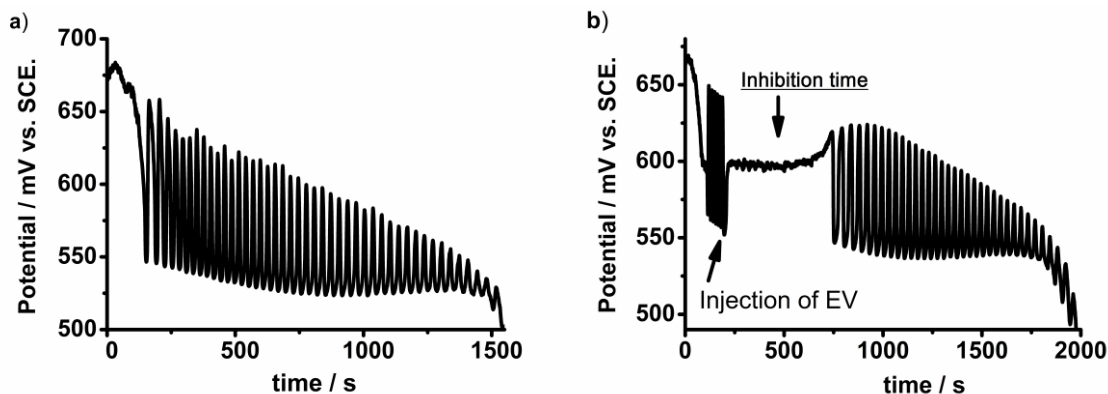

Figure 1. The oscillation profile for the proposed BR oscillator in the absence and presence of ethyl vanillin perturbation using Pt vs SCE electrodes. a): [EV] = 0 M; b): [EV] = 2.5×10^{-8} M; Common condition: $[H_2SO_4] = 9.375 \times 10^{-3}$ M; $[KIO_3] = 2.1 \times 10^{-2}$ M; $[[NiL](ClO_4)_2] = 8.65 \times 10^{-4}$ M; [MA] = 1.5×10^{-1} M; $[H_2O_2] = 1.4$ M; t = 4 ± 0.5 ^{O}C

The oscillating features in present oscillation catalyzed by a macrocyclic Ni complex is quite different from that in Ce^{3+}/Mn^{2+}-catalyzed oscillators both in duration of oscillation and in number of oscillation cycle [10, 24-26]. For example, the present typical oscillation profile obtained through macrocyclic Ni complex-catalyzed BR oscillator has an oscillation duration of 1500 sec with 44 oscillation cycles. Meanwhile, the typical oscillation recorded through Mn^{2+}-catalyzed BR oscillator has a duration of 240 sec with only 22 oscillation cycles [10].

Active BR oscillator was perturbed by 40 μL of different concentration of EV at the bottom of potentiometric cycle. The oscillation, after temporary cease upon perturbation, could be regenerated with inhibition time (t_{in}), as shown in Figure 1b. In order to make precise and accurate measurements, numerous injection points were tested. From experimental data it was notice that the t_{in} is dependent on the concentration of EV added. As the concentration of EV increased, the t_{in} was increased. Thus a new way for the determination of EV could be anticipated to be applied from above-mentioned behavior. This analytical technique, has more significance over instrumental method as its shows a good limits of detection and precision, which will be discussed in determination section.

3.1. Influence of experimental variables

To guarantee the extreme conceivable sensitivity and precision for determination of EV, the effects of MA, sulfuric acid, potassium iodate, hydrogen peroxide, $[NiL](ClO_4)_2$ were studied, by keeping the concentration of one reactant variable while the concentration of others reagents were kept constant. The t_{in} was selected as measured parameter, because the addition of EV in the active BR caused the temporary cease in oscillation.

The influence of H_2SO_4 was tested over a range $8.75 \times 10^{-3}\,M - 3.75 \times 10^{-2}\,M$. It was notice that as the concentration of H_2SO_4 increased the t_{in} were decreased, as shown in Figure 2a. A concentration $2.5 \times 10^{-2}\,M$ was selected at optimum, because oscillations run uniformly.

The influence of changing KIO_3 concentration on the t_{in} over a range of $2.1 \times 10^{-2}\,M - 4.2 \times 10^{-2}\,M$ was studied. It was found that, as concentration of KIO_3 was increased, the t_{in} of the system decreased, shown in Figure 2b. A concentration $2.1 \times 10^{-2}\,M$, the top concentration point, was chosen because more stable oscillations over time could be obtained at such a concentration of KIO_3.

The influence of changing concentration of $[NiL](ClO_4)_2$ was similar to the effect of H_2SO_4 and KIO_3 over system. In the $[NiL](ClO_4)_2$ concentration range of $6.49 \times 10^{-4}\,M$ to $1.73 \times 10^{-3}\,M$, it was notice that as the concentration of catalyst was increased, the t_{in} was decreased, shown in Figure 2c. The $8.65 \times 10^{-4}\,M$ of $[NiL](ClO_4)_2$ was selected as optimum concentration for catalyst as system oscillated stably.

The effect of H_2O_2 was studied over range of 1 M to 1.6 M. The t_{in} was increased when concentration of the H_2O_2 was increased from 1 M up to 1.2 M but t_{in} was decreased when concentration of H_2O_2 dropped from 1.2 to 1.6 M, as shown in Figure 2d. Thus 1.4 M of H_2O_2 was chosen as best concentration because at this concentration the oscillation was more stable.

The influence of MA shows similar effect like that of H_2O_2. The effect was tested over a concentration range of 0.1 M to 0.225 M. The t_{in} was increased as concentration of MA was increased from 0.1 M up to 0.15 M, but t_{in} was decreased below 0.15 M of MA, shown in Figure 2e. Thus $1.5 \times 10^{-1}\,M$, the middle highest concentration point, was selected because at this concentration the oscillation was more stable.

The oscillating system (BR) based on $[NiL]^{2+}$-catalyst has strongly affected by the influence of temperature. The influence of temperature had studied over a temperature range between 4 ^{O}C and 30 ^{O}C. It was notice that, with the increase in temperature (4 - 25 ^{O}C) the oscillating parameters like, t_{in} ,

amplitude of oscillation (A), oscillation period (T), life of oscillation were decreased while above 25 ^{O}C the oscillation had ceased. Thus 4 ± 0.5 ^{O}C was selected as optimum value for working condition.

The oscillating system is highly affected by foreign species. We examine the effects of a few foreign species on $[NiL]^{2+}$-catalyzed chemical oscillator. The value of interference producing inaccuracy below 5% in the determination of 1×10^{-5} M EV was consider as tolerance range. The data are mention in Table 1. It was notice that, no influences of Na^+ and other common ions were observed while few species like I^- and Br^- have immense effect on the determination of EV.

Table 1. Influence of foreign ions and species on the determination of 1×10^{-5} M EV*

Foreign ion and species	Tolerated ratio
Br^- , I^-	0.2:1
Ag^+, Fe^{2+}, Cl^-	2:1
F^-	10:1
H_2PO^-, Co^{2+}	200:1
Ni^{2+}, Li^+	1000:1
Na^+, K^+, Al^{3+}, Zn^{2+}	2000:1

* Common conditions: $[H_2SO_4] = 9.375 \times 10^{-3}$ M; $[KIO_3] = 2.1 \times 10^{-2}$ M; $[[NiL](ClO_4)_2] = 8.65 \times 10^{-4}$ M; $[MA] = 1.5 \times 10^{-1}$ M; $[H_2O_2] = 1.4$ M; $t = 4 \pm 0.5$ ^{O}C
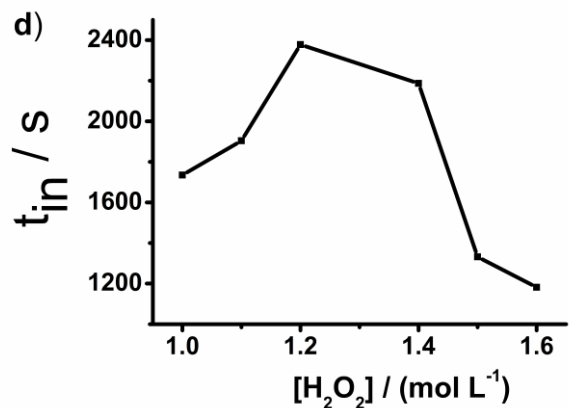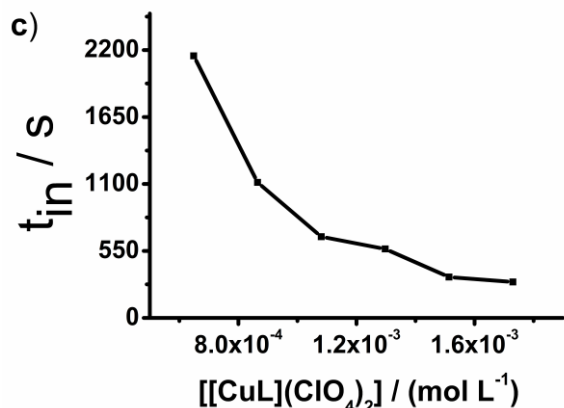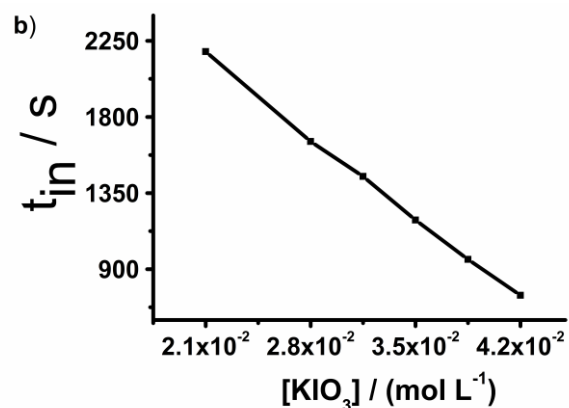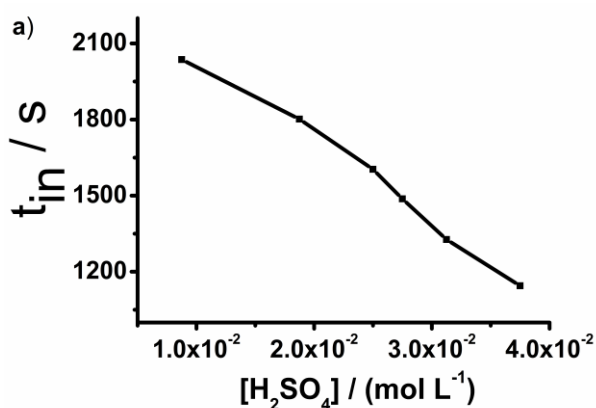
Figure 2. Influence of the concentrations of reactants on the inhibition time: (a) H_2SO_4; conditions: $[KIO_3] = 2.1 \times 10^{-2}$ M; $[[NiL](ClO_4)_2] = 8.65 \times 10^{-4}$ M; $[MA] = 1.5 \times 10^{-1}$ M; $[H_2O_2] = 1.4$ M; $t = 4 \pm 0.5$ ^{O}C. $[EV] = 2.5 \times 10^{-8}$ M. (b) KIO_3; conditions: $[H_2SO_4] = 9.375 \times 10^{-3}$ M; $[[NiL](ClO_4)_2] = 8.65 \times 10^{-4}$ M; $[MA] = 1.5 \times 10^{-1}$ M; $[H_2O_2] = 1.4$ M; $t = 4 \pm 0.5$ ^{O}C. $[EV] = 2.5 \times 10^{-8}$ M.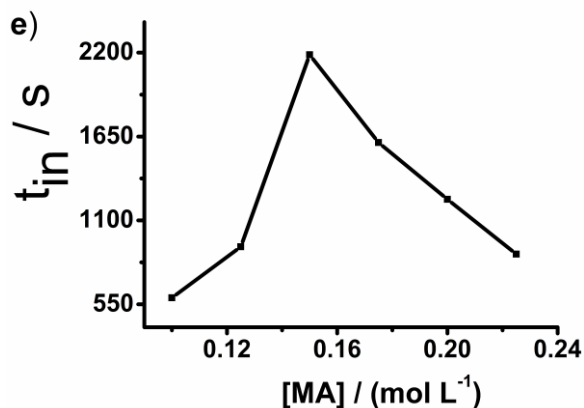 (c) $[NiL](ClO_4)_2$; conditions: $[H_2SO_4] = 9.375 \times 10^{-3}$ M; $[KIO_3] = 2.1 \times 10^{-2}$ M; $[MA] = 1.5 \times 10^{-1}$ M; $[H_2O_2] = 1.4$ M; $t = 4 \pm 0.5$ ^{O}C. $[EV] = 2.5 \times 10^{-8}$ M. (d) H_2O_2; conditions: $[H_2SO_4] = 9.375 \times 10^{-3}$ M; $[KIO_3] = 2.1 \times 10^{-2}$ M; $[[NiL](ClO_4)_2] = 8.65 \times 10^{-4}$ M; $[MA] = 1.5 \times 10^{-1}$ M; $t = 4 \pm 0.5$ ^{O}C. $[EV] = 2.5 \times 10^{-8}$ M. (e) MA, conditions: $[H_2SO_4] = 9.375 \times 10^{-3}$ M; $[KIO_3] = 2.1 \times 10^{-2}$ M; $[[NiL](ClO_4)_2] = 8.65 \times 10^{-4}$ M; $[H_2O_2] = 1.4$ M; $t = 4 \pm 0.5$ ^{O}C. $[EV] = 2.5 \times 10^{-8}$ M.

3.2. Determination of Ethyl vanillin (EV)

Under the optimal condition as described above, we performed the perturbation experiment, by injecting different concentrations of EV which resulted in variation in t_{in}. It was notice that as the concentration of EV was increased the t_{in} was increased. Thus polynomial regression curve was achieved in a range between 7.5×10^{-6} to 3.5×10^{-5} mol/L of EV with a correlation coefficient of 0.98, as shown in Figure 3a. However, the calibration data achieved follow following equation:

$t_{in} = 470.55265 - 6.36091 \times 10^7 * EV + 2.94766 \times 10^{12} * EV^2$ (r = 0.98).

The precision relative standard deviation (RSD), calculated from six perturbation of 2.5×10^{-5} M EV, was 0.159%. The limit of detection obtained was 5×10^{-6} M. Such precision is quite acceptable.

Several injection points were tested. It was notice that when the same amount (40 µL) of the same amount (1.5×10^{-5} M) of EV were injected in increasing number of cycle, the t_{in} of the system were increased. For example, the t_{in} (120 second) was obtained when injection was carried out at the third cycle. However, t_{in} (672 second) was observed when injection was carried at the number 21 cycle. This is due to the fact that, as the oscillation proceed the concentration of the reactant was consumed more and more at every cycle. For example, as the reactant concentration was higher (at cycle 3), it took less time to consumed EV. Similarly, when the reactant concentration had been reduced (at cycle 21), then it took longer time to consumed EV. This phenomenon support that, t_{in} is depending on the reactant concentration as well as on the scheduled time commencing perturbation performance (counting from on setting of the oscillation). Linear regression curve for t_{in} *v.s.* ordinal number of cycle

was achieved by injecting same amount of same concentration at different ordinal cycle i.e. (3 to 21), as shown in Figure 3b.
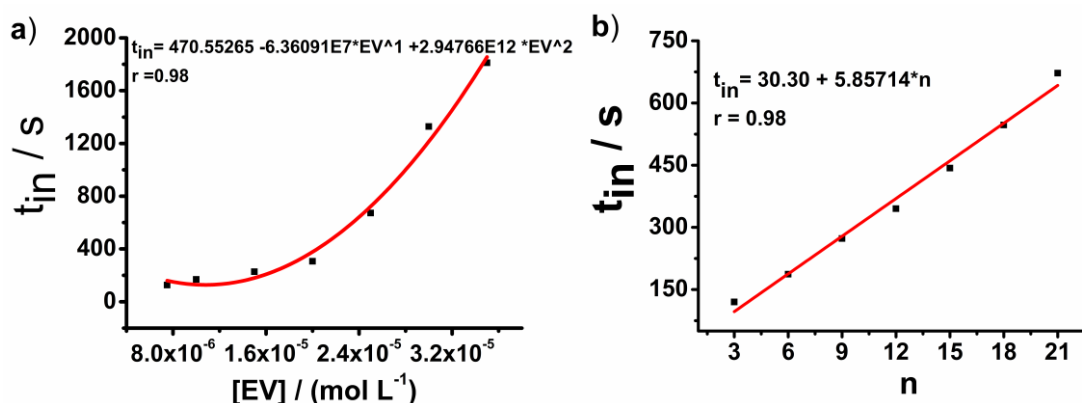

Figure 3. (a). The polynomial regression curve between t_{in} and concentration of EV in the range of 7.5 × 10^{-6} to 3.5 × 10^{-5} M. b). t_{in} Vs scheduled time commencing perturbation performance (n: ordinal number of cycle for the oscillations) in the presence of 1.5 × 10^{-5} M EV. Common condition: $[H_2SO_4]$ = 9.375 × 10^{-3} M; $[KIO_3]$ = 2.1 × 10^{-2} M; $[[NiL](ClO_4)_2]$ = 8.65 × 10^{-4} M; $[MA]$ = 1.5 × 10^{-1} M; $[H_2O_2]$ = 1.4 M; t = 4 ± 0.5 °C

Table 2. Determination results and recovery for EV*

Sample	Known concentration / 10^{-5} M	Added concentration/10^{-5} M	Found concentration/10^{-5} M	Recovery %
1	0.9	0.3	1.19	99
2	1.25	0.3	1.47	94
3	1.50	0.2	1.55	91
4	1.60	0.2	1.66	92
5	1.65	0.2	1.78	96
6	1.75	0.2	1.79	91
7	2	0.2	2.18	99
8	2.5	0.2	2.66	99

* Common conditions: $[H_2SO_4]$ = 9.375 × 10^{-3} M; $[KIO_3]$ = 2.1 × 10^{-2} M; $[[NiL](ClO_4)_2]$ = 8.65 × 10^{-4} M; $[MA]$ = 1.5 × 10^{-1} M; $[H_2O_2]$ = 1.4 M; t = 4 ± 0.5 °C

In order to found the accuracy of the proposed technique, to the standard solution of EV known amounts of EV were added (as listed in Table 2), and the same process was repeated. The recovery experiments show that the accuracy of the proposed technique with the obtained percentage recovery of EV lies in range between 91 to 99%, which is quite better than the reported standard methods such as, liquid chromatography [27] (85 to 95 %), mass spectrometry [16] (88 to 99%), LC / MS [28] (89.8 to 95.6%), HPLC [29] (97.5 to 103%) as indicated in Table 3. Furthermore, the linear range and limit

of detection of EV which was obtained by our oscillating techniques were compared with the linear range and limit of detection of EV obtained through other instrumental techniques as shown in Table 3. It is clear that, from the above and below (Table 3) experimental data, this method is suitable for practical analysis.

Table 3. Comparison of EV determination by BR chemical oscillator with some instrumental techniques.

Techniques	Linear Range	Percentage recovery (%)	Limit of Detection
BR Chemical Oscillator (this work)	7.5×10^{-6} to 3.5×10^{-5} M	91 to 99%,	5×10^{-6} M
Liquid Chromatography [27]	0.1 – 1.22 g / 100 ml	85-95	0.10 g / 100 ml
Mass Spectrometry [16]	1-20 mg / L	88-99	0.38 mg / L
LC/MS [28]	10- 100 ug/Kg	91-99	1.5 ug / kg
HPLC [29]	0.5- 200 mg / L	97.5-103	0.04 mg / L

For comparison with present method, EV was determined by UV spectroscopy (standard method). For UV experiments, equal amount (200ul) of different concentration of EV in the range between 2×10^{-4} M to 3.17×10^{-4} M were put into the mixture of 25 ml of 2.5×10^{-2} M H_2SO_4 and 5 ml of 1.4×10^{-1} M KIO_3.
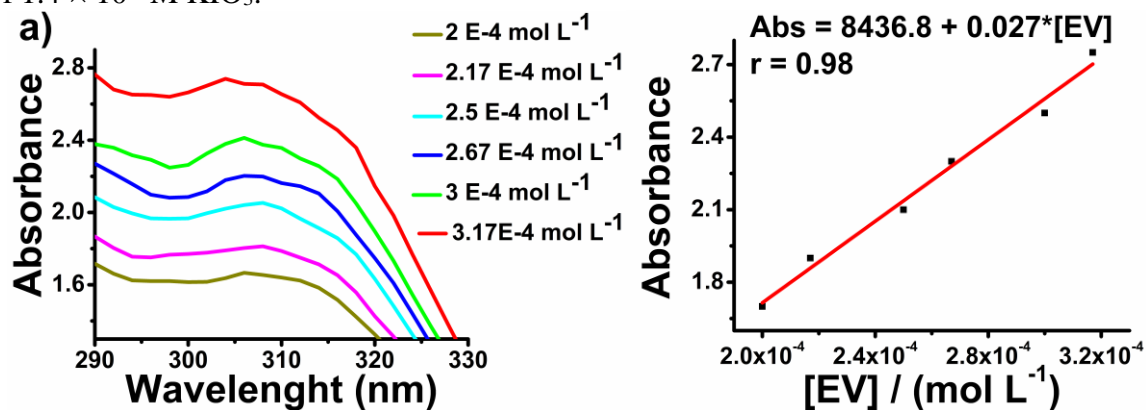

Figure 4. (a). The UV spectrum of EV in the presence of H_2SO_4 and KIO_3, conditions: $[EV] = 2 \times 10^{-4}$ to 3.17×10^{-4} M, $[H_2SO_4] = 2 \times 10^{-2}$ M, $[KIO_3] = 2.3 \times 10^{-2}$ M, (b). The linear regression curve between absorbance and concentration of EV in the range of 2×10^{-4} to 3.17×10^{-4} M.

The maximum absorbance was obtained at wavelength of approximately 310 nm (as shown in Figure 4a) which is in agreement with the maximum absorbance for EV in the literature [16]. We further examine that as the concentration of EV was increased the absorbance increased. Thus, linear

regression curve was achieved over a range 2×10^{-4} to 3.17×10^{-4} M of EV with a correlation coefficient of 0.98 (as shown in Figure 4b).

3.3. Mechanism

3.3.1. Cyclic voltammetry (CV)

In the presence and absence of EV, the experiments were performed to the below listed media to clarify which reagent react with EV:

1).H_2SO_4 + KIO_3, 2) H_2SO_4+H_2O_2, 3) H_2SO_4 + M.A, 4) H_2SO_4 + $[NiL](CLO_4)_2$

It may be concluded from the result of cyclic voltammetry that, the EV can be oxidized by KIO_3 as indicated in the Figure 5. It was observed that, from the Figure 5, as the amount of EV increases, the reduction current decreases while oxidation current increases. This phenomena indicate the redox reactions that exit between the KIO_3 and EV.

Here it is noticed that in oscillating system the concentration of EV is less (trigger in micro-molar level or less) as compare to the concentration of KIO_3. So EV would quickly be consumed if it reacts directly with KIO_3. Due to this respect it is hard to clarify the t_{in} by considering the direct reaction between KIO_3 and EV. Here we demonstrated that the t_{in} was cause by the reaction of antioxidant (EV) with intermediate species (HOO$^{\bullet}$) as indicated in below reactions (13 & 14).
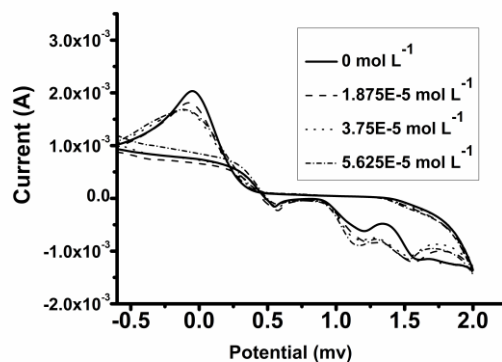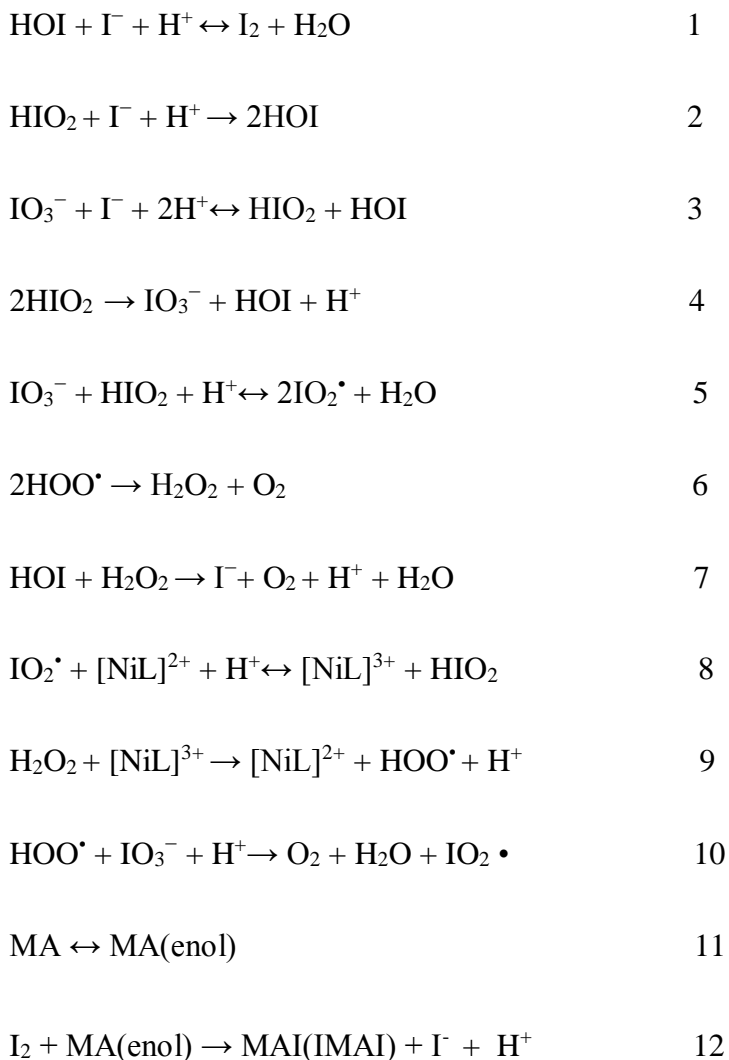

Figure 5. Cyclic Voltammogram of the reaction between EV and the reactants obtained in the idant and prooxidant action of same concentration of EV, $[KIO_3] = 2 \times 10^{-2}$ M, Common condition; $[H_2SO_4] = 2.50 \times 10^{-2}$ M. Scan rate = 100 mV / s.

3.3.2. Interpretation of Mechanism

Oscillating chemical reactions has highly complex mechanisms which contain numerous kinetic steps and independent variables. In 1982, Noyes and Furrow et al developed the first oscillating mechanism with the name of NF model [30] which give some basic features on oscillation system. In the same era, a quantitative mechanism named DE model [31] was designed by De Kepper and Epstein. In 1996, Sorensen et al [32] put forward a mechanism for oscillation that well professes the

experimental outcomes from flow and batch reactors. Furrow et al [13, 33] proposed a new mechanism called FCA model which was derived from the NF and DE models. The FCA mechanism is based on the important role played by $HOO^{\bullet}$ radical (intermediate species which was generated during oscillatory reactions). Based on FCA model, the oscillatory mechanism for $[Ni]^{2+}$ complex-catalyzed system consists of 12 oscillatory reactions as below.

$$HOI + I^- + H^+ \leftrightarrow I_2 + H_2O \qquad\qquad 1$$

$$HIO_2 + I^- + H^+ \rightarrow 2HOI \qquad\qquad 2$$

$$IO_3^- + I^- + 2H^+ \leftrightarrow HIO_2 + HOI \qquad\qquad 3$$

$$2HIO_2 \rightarrow IO_3^- + HOI + H^+ \qquad\qquad 4$$

$$IO_3^- + HIO_2 + H^+ \leftrightarrow 2IO_2^{\bullet} + H_2O \qquad\qquad 5$$

$$2HOO^{\bullet} \rightarrow H_2O_2 + O_2 \qquad\qquad 6$$

$$HOI + H_2O_2 \rightarrow I^- + O_2 + H^+ + H_2O \qquad\qquad 7$$

$$IO_2^{\bullet} + [NiL]^{2+} + H^+ \leftrightarrow [NiL]^{3+} + HIO_2 \qquad\qquad 8$$

$$H_2O_2 + [NiL]^{3+} \rightarrow [NiL]^{2+} + HOO^{\bullet} + H^+ \qquad\qquad 9$$

$$HOO^{\bullet} + IO_3^- + H^+ \rightarrow O_2 + H_2O + IO_2 \bullet \qquad\qquad 10$$

$$MA \leftrightarrow MA(enol) \qquad\qquad 11$$

$$I_2 + MA(enol) \rightarrow MAI(IMAI) + I^- + H^+ \qquad\qquad 12$$

BR mechanism based on FCA usually consists of important intermediates namely I_2, I^-, oxyidodine species HOI, HOIO, $IO_2^{\bullet}$ and $HOO^{\bullet}$ radical [10,11]. Franz [34] observed the inhibition effect by putting the superoxide dismutase pellet (SOD) into the active BR oscillator. As SOD is $HOO^{\bullet}$ radical scavenger, he claimed that inhibition time was due to $HOO^{\bullet}$ radical. Later, R. Cervellati et al [10] not only successfully proved Franz's claim but also published a lot of work based on the inhibitory effect caused by $HOO^{\bullet}$ radical [11,12, 23]. In both Mn^{2+}-catalyzed BR oscillator [10, 35-40] and macrocyclic complex-catalyzed BR oscillator [9, 41, 42], the inhibitory effects caused by some antioxidants have recently been reported. In these publications, the authors have claimed that hydroperoxyl radical ($HOO^{\bullet}$) is responsible for the temporary cease in the oscillations. In this article, we have reported that the perturbation of antioxidant (ethyl vanillin) on active BR could cause the

inhibition time. It is our claim that such an inhibition time was due to the involvement of HOO$^{\bullet}$ radical with antioxidant (ethyl vanillin). The detail explanations are as below.
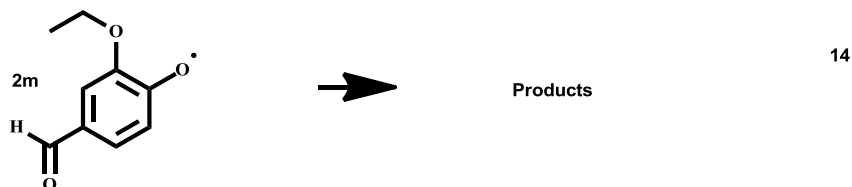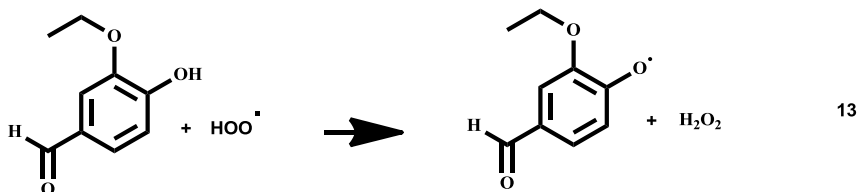
(Where m stands for integer)

Reactions 13 and 14 show that EV reacts with HOO$^{\bullet}$ radicals to generate EV radical and EV radical reacted together to form products. Both reactions 13 and 14 should be included to FCA Model [43].

The HOO$^{\bullet}$ radical were consumed both in reactions 10 and 13, whereas consumption in reaction 13 is faster, that's why reaction 13 become dominated in consuming HOO$^{\bullet}$ radicals. The deficiency in HOO$^{\bullet}$ radicals caused the decrease in the $IO_2^{\bullet}$ radicals via reaction 10. The $IO_2^{\bullet}$ radical responsible for the oxidation of $[NiL]^{2+}$ into $[NiL]^{3+}$ via reaction 8. The Insufficiency of $IO_2^{\bullet}$ radical led to the decreased in the $[NiL]^{3+}$ thus the reduction of $[NiL]^{3+}$ into $[NiL]^{2+}$ was stop via reaction 9. So in short the oscillation between $[NiL]^{2+}$ and $[NiL]^{3+}$ were terminated. Therefore the inhibition time were noticed. When EV was completely consumed in reaction 13, the reaction 8, 9, and 10 become predominate again and the oscillation regenerated.

4. CONCLUSIONS

In this paper, we presented the quantitative method for determination of EV (antioxidant) by using a Briggs-Rauscher oscillator. The oscillator was catalyzed by a tetraazamacrocyclic complex with ligand L being 5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-4,11-diene. The addition of small amount of EV in the BR system temporarily ceased and regenerated oscillation after inhibition time (t_{in}). The t_{in} was dependent on the concentration of EV. When the amount of EV increased, inhibition time increased. Thus polynomial regression curve was achieved over a range of 7.5×10^{-6} to 3.5×10^{-5} M with a correlation coefficient of 0.98 of EV. Although the cyclic voltammetry results showed the redox reaction between the KIO_3 and EV, the inhibition time was actually caused by the reaction between EV and HOO$^{\bullet}$ intermediate species.

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