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Chemistry

Highlights

- Rapid & Efficient Method
- Determination of Pregabalin
- Kinetic Empirical Modeling
- Spectrophotometry Analysis & Modeling

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Kinetic Empirical Modeling of Biodiesel Production from *Ricinus Communis* using Refluxed Calcined Snail Shell as Catalyst

By Jibrin, M.S., Muhammad, C., Mukhtar, M., Baki, A.S., Bagudo, B.U. & Idris, M.O
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Abstract- Biodiesel from non-edible oil emerges as one of the most energy-efficient and environmentally friendly options in recent times to fulfill the sustainable future energy needs. Hence, there is a need to produce biodiesel on an industrial scale. In designing a reactor, for a large scale production, the thermodynamic, kinetics and optimization parameters must be studied. In this study, the kinetics of biodiesel production from refined castor seed oil using refluxed calcined snail shell as a catalyst is investigated. The transesterification variables and the level used in kinetics study are obtained from optimization studies carried out as preliminary studies, the experimental conditions of three different runs that gave the highest yield were selected. The kinetics studied of transesterification reaction shows pseudo-first-order kinetics. The rate constants of the reactions at three different runs are 0.03048, 0.03207 and 0.03737 with 496.678 J/Mol and 1652.4263 as activation energy and frequency factor. The lower activation energy may be due to the catalytic activity of refluxed calcined snail shell.

Keywords: *kinetics modeling, castor seed oil, refluxed calcined snail shell and biodiesel.*

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Kinetic Empirical Modeling of Biodiesel Production from *Ricinus Communis* using Refluxed Calcined Snail Shell as Catalyst

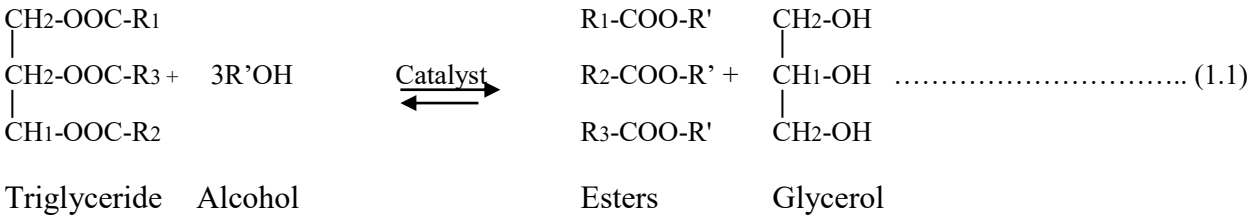
Jibrin, M.S.[α], Muhammad, C.[σ], Mukhtar, M.[ρ], Baki, A.S.[ω], Bagudo, B.U.[¥] & Idris, M.O.[§]

Abstract- Biodiesel from non-edible oil emerges as one of the most energy-efficient and environmentally friendly options in recent times to fulfill the sustainable future energy needs. Hence, there is a need to produce biodiesel on an industrial scale. In designing a reactor, for a large scale production, the thermodynamic, kinetics and optimization parameters must be studied. In this study, the kinetics of biodiesel production from refined castor seed oil using refluxed calcined snail shell as a catalyst is investigated. The transesterification variables and the level used in kinetics study are obtained from optimization studies carried out as preliminary studies, the experimental conditions of three different runs that gave the highest yield were selected. The kinetics studied of transesterification reaction shows pseudo-first-order kinetics. The rate constants of the reactions at three different runs are 0.03048, 0.03207 and 0.03737 with 496.678 J/Mol and 1652.4263 as activation energy and frequency factor. The lower activation energy may be due to the catalytic activity of refluxed calcined snail shell.

Keywords: kinetics modeling, castor seed oil, refluxed calcined snail shell and biodiesel.

I. Introduction

The conventional energy sources are finite and associated with environmental pollution; there is a need to generate a sustainable alternative to support the civilization, i.e. transportation, agriculture, communication, etc. (Hribernik and Kegl, 2007; Ma *et al.,* 1999). Biodiesel emerges as one of the most energy-efficient and environmentally friendly options in recent times to fulfill the future energy needs (Nakarmi and Joshi, 2014; Owolabi *et al.,* 2012). Biodiesel is a renewable diesel substitute that can be obtained by reacting any natural oil or fat with alcohol. There are four well-established liquid biofuels production methods; direct use and blending, micro-emulsions, thermal cracking (pyrolysis), and transesterification (Ma and Hanna 1999). Among these methods, transesterification is one of the most commonly used methods in the biodiesel production industry. Transesterification of vegetable oils and animal fats is the fundamental way to make biodiesel (Encinar *et al.,* 2010). Transesterification is a three-step reversible reaction of vegetable oil or animal fat with short-chain alcohol usually methanol to form fatty acid methyl esters (FAMEs) and glycerol (Encinar *et al.,* 2010), in the presence of a catalyst. The stoichiometric ratio for transesterification, one mole of triglyceride (oil) requires three moles of alcohol as shown in the equation 1.1.Example of alcohols that could be used in the transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol is used most frequently because of its low cost, physical and chemical advantages (polar and shortest chain alcohol). However the molar ratio is associated with the type of catalyst used, and higher molar ratios result in greater ester conversion in a shorter time (Ma and Hanna, 1999). Base catalysts lead to the higher conversion of methyl esters at low temperature, atmospheric pressure and minimum response time, which reduces the cost of the process considerably (Ismail *et al.,* 2016).



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Transesterification reactions are basically heterogeneous because the nonpolar oil phase and the polar alcohol phase are immiscible with each other. Therefore, their overall reaction rates mainly depend on two important factors: the hydrodynamic effect between these two phases and the chemical reaction kinetics. To optimized biodiesel production process and design a high-performance reaction system, the hydrodynamic effect, and chemical reaction kinetics must be entirely understood. Previous kinetic studies on the transesterification reaction were mostly carried out in a pseudo-homogenous reaction system. Sufficient mixing is provided in these systems to eliminate the hydrodynamic effect on the overall reaction rate (Regina, 2013). There are many studies carried out in different approaches to investigate the kinetics of transesterification reaction (Regina, 2013). Among all the approach, it was observed that the transesterification reactions in most cases follow pseudo-first order kinetics (Labib *et al.*, 2013).

In this study, the kinetics of the transesterification reaction of refined castor seed oil using a heterogeneous (refluxed calcined snail shell (CaO)) catalyst was investigated.

II. Methodology

a) Preproduction Process

The castor seed was obtained from Yandodo, Kano State. The seed was sundried to reduce the moisture content. The castor seed was oven dried at 90 °C for 45 minutes. The dried seeds were grounded using mortar and pestle and weighed. Extraction of castor oil was carried out by soxhlet extraction method as reported by Edison *et al.* (2012). The extracted oil was subjected to pretreatments such as water and acid degumming, and neutralization to upgrade it physicochemical properties for efficient transesterification.

The refluxed calcined snail shell (calcium oxide) catalyst that was used in this study was synthesized from snail shell through the hydrothermal method, as reported by Ismail *et al.* (2016).

b) Transesterification Reactions for Kinetics Parameters Determination

The transesterification variables levels (indicated in Table 1) were determined after the optimization study was carried out using Taguchi orthogonal array design (Muhammad *et al.,* 2018). The transesterification reactions runs were conducted according to design through which three runs that gave highest yields; their experimental conditions were used in the kinetics study. The transesterification was carried out in 500 cm3 two necks round bottom flask as reactor equipped with condenser, thermometer, and hotplate magnetic stirrer. During the transesterification reaction, samples were collected from the reaction mixture using 15 cm3 syringe at a different time interval of 30, 60 and 120 minutes, transferred into test tubes, and then immersed in cold water at 4 °C to quench the reaction immediately. The equal volume (5 cm3) of water and n-hexane was immediately added to get clear separation, the top layer sample containing the methyl ester, non reacted triglyceride and some catalyst particle was collected. For better separation, the samples were centrifuged for 5 minutes at 2000 rpm, and, then the top layer sample was collected, washed with distilled water and then sent for GC/MS analysis (Regina, 2013).

Table 1: Transesterification variables and their levels used in kinetics study

S/N	Oil to Methanol Ratio	Catalyst Load (w/w %)	Reaction Temperature (°C)	Reaction Time (Hrs)
1	1: 06	3	60	2
2	1:08	1	70	2
3	1:18	0.5	55	2

c) GC/ MS Analysis of Biodiesel Produced

The oil composition and methyl ester content were assayed using a GC/MS machine in the multi-purpose laboratory of Ahmadu Bello University (ABU), Zaria.

The GC/MS was equipped with an Econo-Cap EC-WAX Capillary Column (30.0 m in length × 250 μm in diameter × 0.25 μm in film thickness). The GC oven was maintained at 50 °C for 3 minutes, and then heated to 210 °C at a rate of 10 °C per minute and held at 210 °C for 9 minutes. The front inlet temperature of the oven was 255 °C (split less-mode). The carrier gas was helium with a flow rate of 12 cm3/min. The analysis of refined castor oil composition and fatty acid methyl ester (FAME) of biodiesel was carried out by injecting 1.0 μL of a sample solution that was prepared by blending the biodiesel sample with a prepared internal standard of GC i.e., methyl heptadecanoate. The FAME content by weight was determined from equation 2.1.

$$wt.\% = \left[\frac{\sum(A_i - A_R)}{A_R}\right]\frac{C_R V_R}{w} \qquad (2.1)$$

Where A_i= the peak area from chromatogram of FAME; A_R= the peak area from chromatogram of the internal standard; C_R= the concentration of the internal standard; V_R= the volume of the internal standard; and W = the total weight of the biodiesel sample (Regina, 2013).

d) Kinetic Modeling

The overall reaction of the transesterification was represented by the following equation 2.2.

$$\text{Triglycerides} + \text{Methanol} \xrightarrow{\text{CaO}} \text{Methylester} + \text{glycerol} \quad \text{.....} \quad (2.2)$$

In developing the description of the overall kinetics, the following simplifying assumptions are made:

1. The presence of excess methanol reactant minimizes the reversibility of the transesterification reaction.
2. The reaction mixture is assumed homogeneous, the solid catalyst is in powder form and at a very low concentration in comparison to the whole volume of the reaction mixture (1- 4 % w/v).
3. Reaction mixture volume is assumed constant being put under reflux.
4. The reaction temperature is kept constant during the whole reaction time.

The overall reaction is assumed to be a single step, represented by equation 2.2, (neglecting intermediate steps) and is considered to develop kinetic rate equations (Pankaj, 2006).

The overall reaction in its simplified form is represented by equation 2.2.1.

$$\text{A (oil)} + \text{B (methanol)} \xrightarrow{\text{CaO}} \text{C (fatty acid methyl ester)} + \text{D (glycerol)} \quad \text{.....} \quad (2.2.1)$$

The concentration of methanol, being in excess, does not influence the global reaction which is finally represented by equation 2.3 which is in accordance with previous studies (Debk, 2003).

$$\text{A (Oil)} \xrightarrow{\text{CaO}} \text{C (fatty acid methyl ester)} + \text{D (glycerol)} \quad \text{.....} \quad (2.3)$$

Because of excess methanol was used to derive the reaction forward, the reverse reaction was ignored. The reaction rate rA can be expressed in equation 2.4:

$$r_A = -\frac{d[A]}{dt} = k_1[A]_t^{\alpha}[B]_t^{\beta} \quad \text{.....} \quad (2.4)$$

Where k_1 = Observed rate of reaction

$[A]_t$ = Concentration of triglyceride (oil) at time t.

$[B]_t$ = concentration of methanol at time t.

While α is the order of the oil and β is the order of methanol in the reaction.

Here, $[B]_t^{\beta}$ could be considered as a constant since methanol is larger than the concentration of triglyceride. Then, the rate could be;

$$r = -\frac{d[A]}{dt} = k_2[A]_t^{\alpha} \quad \text{.....} \quad (2.5)$$

Where $k_2 = k_1[B]_t^{\beta}$

If we assumed reaction order $\alpha = 1$, then

$$-\frac{d[A]}{dt} = k_2[A]_t \quad \text{.....} \quad (2.6)$$

The concentration of triglyceride at time t can be expressed using conversion rate (x) of triglyceride at time t and initial concentration of triglyceride $[A]_0$ as shown in equation 2.7:

$$[A]_t = [A]_0(1-x) \quad \text{.....} \quad (2.7)$$

Thus, the reaction rate can be expressed using the initial concentration of triglyceride and conversion rate as shown in equation 2.8 (Gupta, 2003):

$$r = -\frac{d[A]_t}{dt} = \frac{d\{[A]_0(1-x)\}}{dt} = k_2\,[A]_0\,\frac{dx}{dt} \quad \text{...} \quad (2.8)$$

By integrating equation 2.8:

$$\ln\left(\frac{1}{1-x}\right) = kt \quad \text{.....} \quad (2.9)$$

Where, k is the reaction rate constant at specified time, temperature and catalyst concentration.

From the Arrhenius equation:

$$k = Ae^{-\frac{E_a}{RT}} \quad \text{.....} \quad (2.10)$$

To calculate activation complex Ea and pre-exponential constant A, by taking the natural logarithm of both side to obtain equation (2.11) and then a plot of lnk against 1/T:

$$lnk = lnA - \frac{E_a}{RT} \quad \text{.....} \quad (2.11)$$

III. Results And Discussion

a) Results

The kinetics study of biodiesel production from *Ricinus Communis* using refluxed calcined snail shell

was determined, Values in Table 2, show the conversion (x) of the triglycerides into fatty acid methyl esters at time interval of 30 minutes, 60 minutes and 120 minutes. GC/MS was used to accurately determine the fatty acid methyl esters formed at each stage (appendix I).

Tables 3 to 6, show the kinetic parameters obtained in the experiments. The rate constants (k) of three different reaction conditions are 0.0369, 0.0342 and 0.0312 min-1 as indicated in Table 4. The slight variability of the rate constants (k) might be due to the variability of the experimental conditions used. Their higher correlation coefficients i.e. 0.996, 0.897 and 0.996, validates the pseudo first-order kinetics of the reactions as assumed.

The pre-exponential factor which reflects the collision frequency and orientation of the reactant is 1652.4263 and the activation energy is 496.6784 J/mol (Table 6).

Table 2: Duration time and conversion rate at prescribed conditions

Reaction conditions	Conversion x (%)		
	30 mins	60 mins	120 mins
Oil to methanol ratio 1:6 Catalyst load 3 w/w % Temperature 60 °C	75.5892	90.3251	99.1212
Oil to methanol ratio 1:18 Catalyst load 0.5 w/w % Temperature 55 °C	24.9229	88.7812	96.5280
Oil to methanol ratio 1:8 Catalyst load 1 w/w % Temperature 70 °C	32.6190	80.3271	95.9300

Table 3: Kinetics I

$\ln(\frac{1}{1-x})$	Time (minutes)	Temperature (°C)
1.4101	30	
2.3356	60	60
4.7344	120	
0.2867	30	
2.1876	60	55
3.3604	120	
0.3948	30	
1.6259	60	70
3.2015	120	

Table 4: Calculated rate constant

Rate constant k (min^{-1})	Correlation coefficients (R^2)
0.03737	0.996
0.03207	0.897
0.03048	0.996

Table 5: Rate constants and Temperature in Kelvin

Rate constant k (s^{-1})	lnk	Temperature (Kelvin)	$\frac{1}{T}$
$6.228x10^{-4}$	-7.38123	333	0.00300
$5.345x10^{-4}$	-7.53418	328	0.00305
$5.080x10^{-4}$	-7.58503	343	0.00292

Table 6: Calculated activation complex and frequency factor

Conditions	Pre-exponential factor A	Correlation coefficients (R^2)	Activation complex E_a (J/Mol)
Oil to methanol ratio 1:6 to 1:18, Reaction time 2 hrs Catalyst load 0.5 to 7 wt% Temperature 55 to 70 °C	1652.4263	0.606	496.6784

IV. Discussion

The kinetics of the transesterification of refined castor was discussed below:

a) *Determination of Reaction Rate Constants and Activation Energy*

The GC/MS analysis was used to determine the conversion of triglyceride to methyl ester. The percentage conversion (x) was presented in Table 2 and Appendix I. Based on the previous discussion and results, the experimental setup was designed for studying the reaction kinetics of transesterification. The kinetics study was carried out under different experimental conditions which include catalyst concentrations, reaction temperature, reaction time and oil to methanol ratio as presented in Table 1. The agitation was kept constant at 600 rpm throughout the reaction. The rate constant and activation energy were estimated.

According to equation 2.9, the relationship between ln $\left(\frac{1}{1-x}\right)$ and time is linear, the value of the rate constant(k) equal to the slope of the linear regression fitted line plot. Thus, ln $\left(\frac{1}{1-x}\right)$ is plotted against time (t) in 3 different experimental conditions as shown in Figure 1- 3. The data from Table 2 was used.

The resulting data fits pseudo- first-order kinetics behaviour. The high correlation coefficients (R^2) of the linear equation as indicated in Table 4 shows that there is a first-order dependence of the transesterification reaction catalyzed by CaO (refluxed calcined snail shell). The rate constants obtained from the fitted plot line are 0.03737, 0.03207 and 0.03048 min^{-1}.

The activation energy and a frequency factor of the transesterification reaction were determined using equation 2.10, by taking the natural logarithm of both sides of equation 2.10 to get equation 3.1.

$$lnk = lnA - \frac{E_a}{RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

Equation 3.1 shows the relationship between lnk and $\frac{1}{T}$ as the linear relationship with a slope of $-\frac{E_a}{R}$ and intercept value of lnA. Since the values of k at a different temperature were determined (as indicated in Table 4). The value of lnk and were calculated as presented in Table 5. By performing a linear regression of lnk versus $\frac{1}{T}$, the activation energy and frequency factor determined from the slope and intercept of the regression trend line respectively as shown in Figure 4.

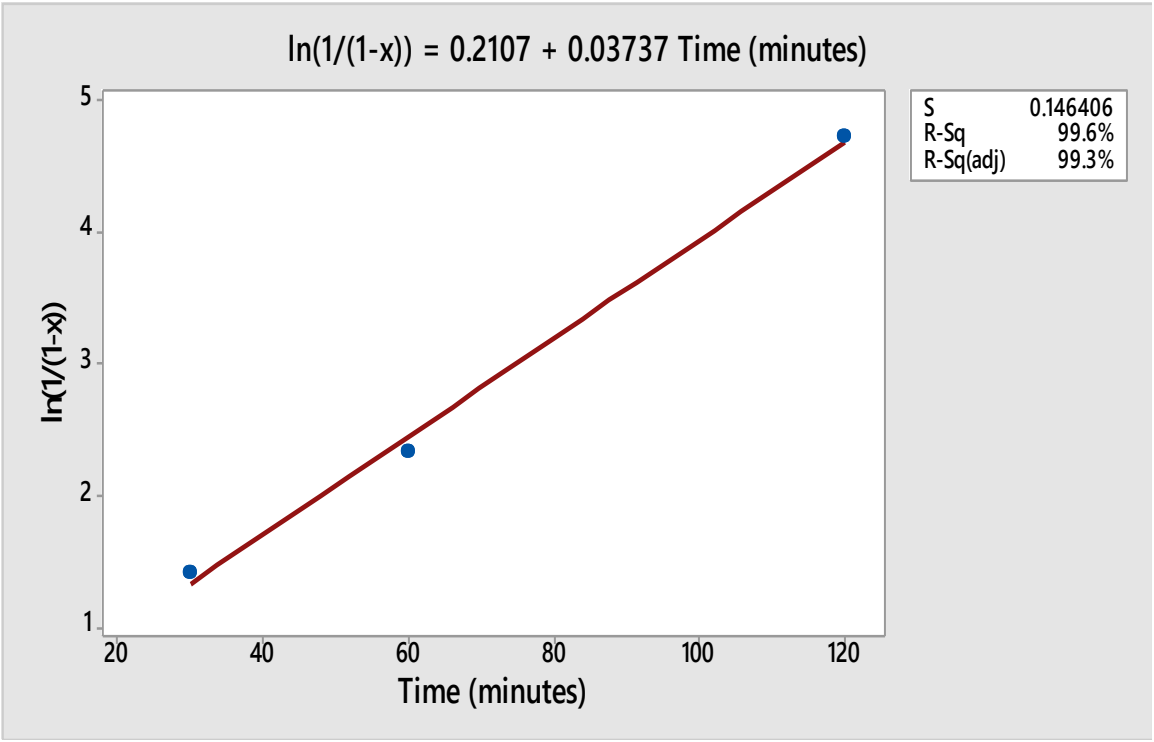

Fig. 1: Reaction rate constant at 60 °C

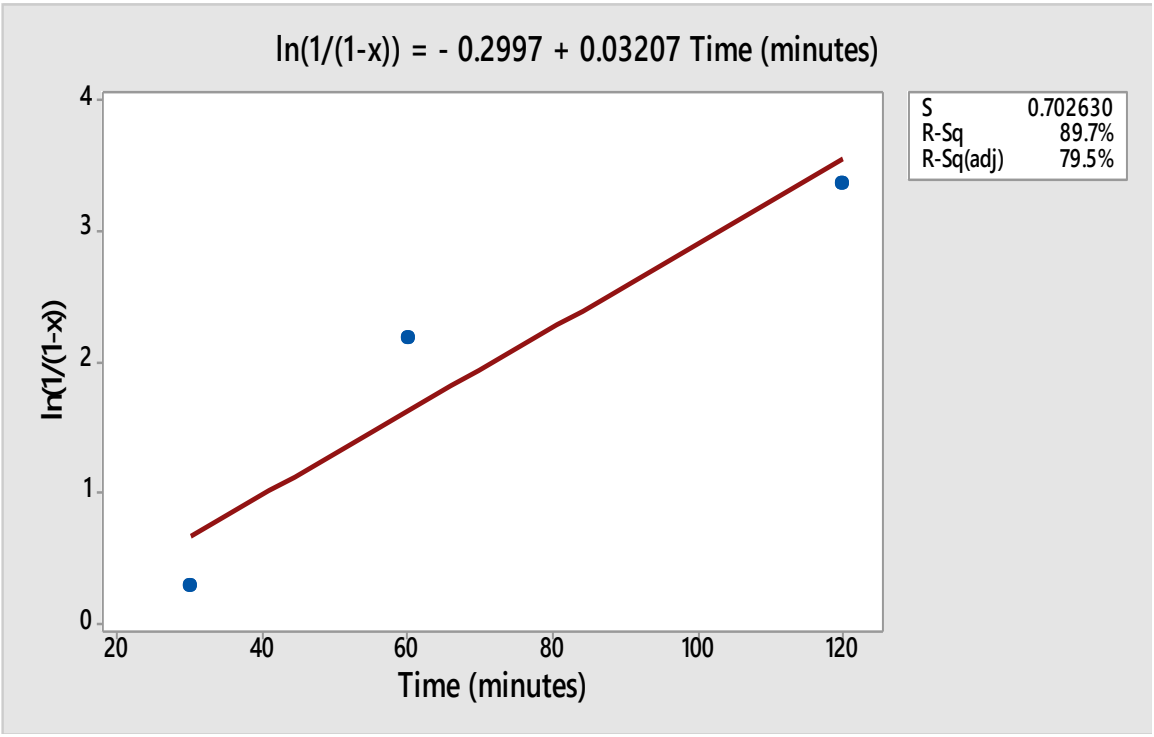

Fig. 2: Reaction rate constant at 55 °C

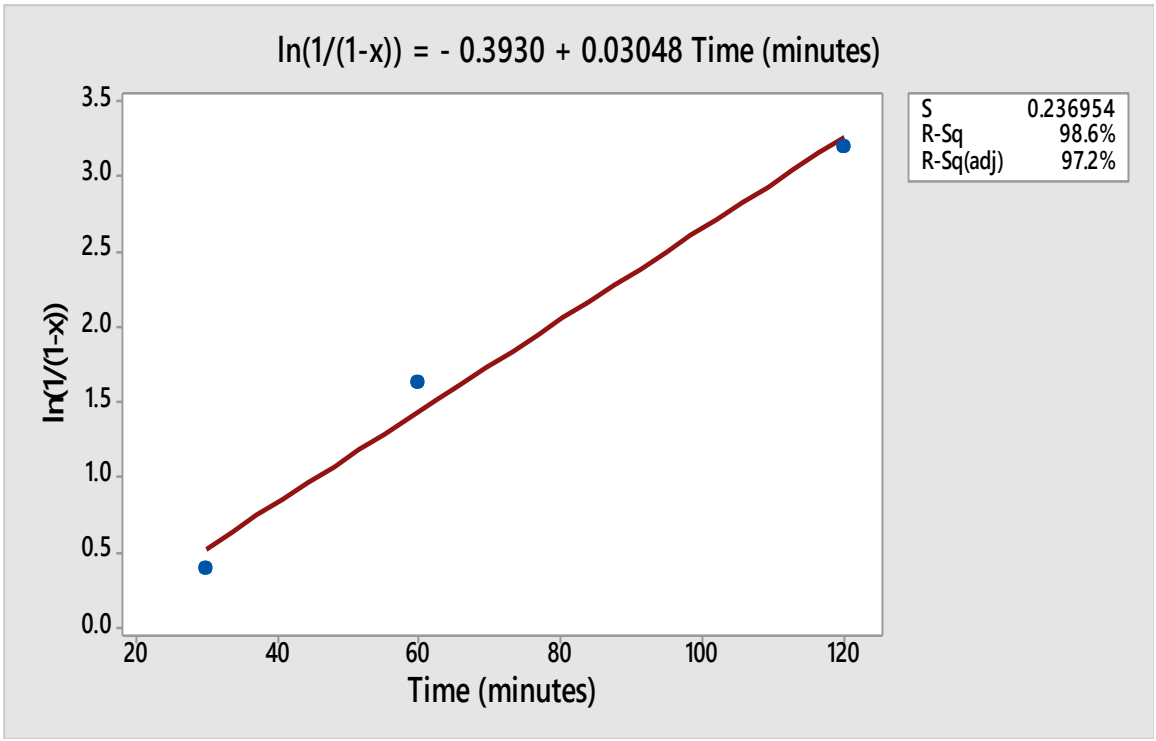

Fig. 3: Reaction rate constant at 70 °C

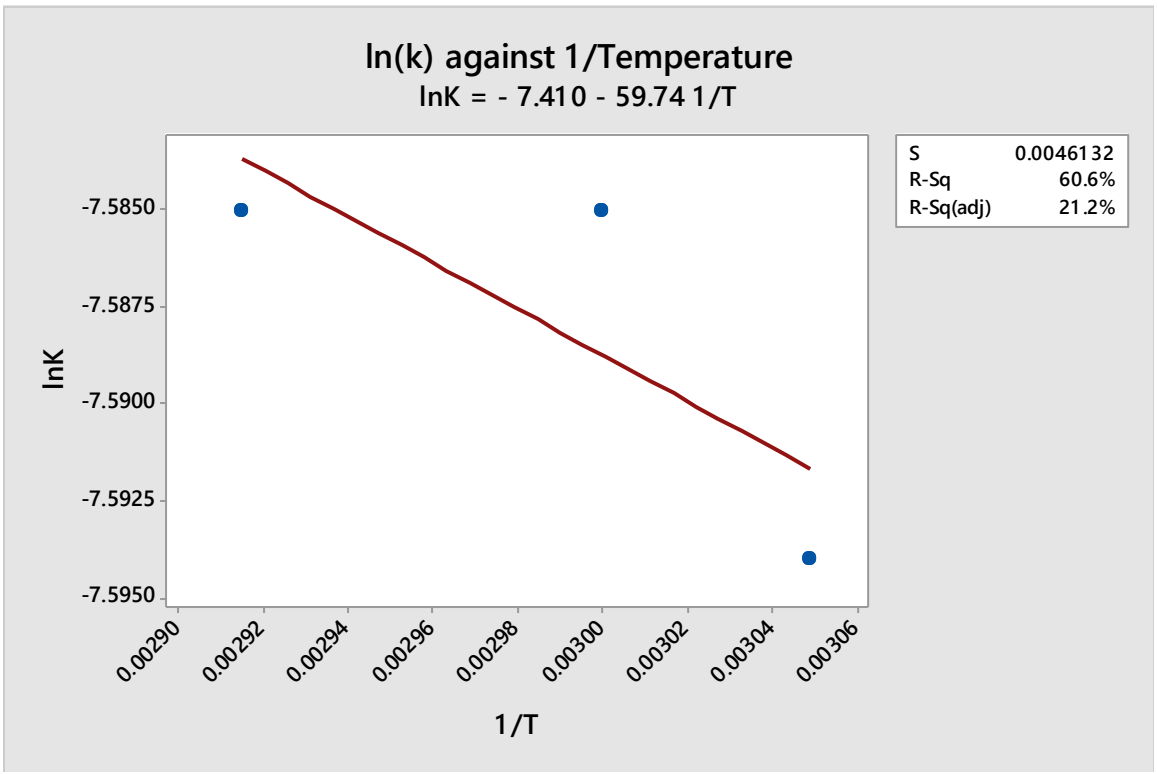

Fig. 4: Fitted line plot of lnk versus 1/T

The results of the experimental condition, activation energy, frequency factor and correlation coefficient (R^2) are shown in Table 6. The activation complex and pre-exponential factor (frequency factor) determined are 496.678 J/Mol and 1652.4263 respectively. Additionally, as the frequency factor increases, the catalyst concentration increases. The high the frequency factor, which is a measure of orientation and collision between reactants, indicates the transesterification reaction is more favoured at 3 wt% than 0.5 and 1 wt%.

Singh and Singh (2010) observed pseudo first-order kinetics for transesterification of palm oil with methanol using H_2SO_4 as a catalyst and determined the activation energy of the reaction as 15.31 KJ/mol, which is high than the one observed in this experiment. Also, the high value of activation energy was determined by Makareviciene *et al.,* (2004) and Supardan (2008), where both observed pseudo- first-order kinetics of the transesterification reaction they conducted with activation energy 13.3 and 30.4 KJ/Mol respectively.

Thiruvengadaravi *et al.* (2009) were also observed pseudo- first-order kinetics for transesterification of *Pongamia* oil with methanol, where 280.1 J/Mol activation energy was determined, which is lower than the one obtained in this experiment.

The activation energy which is the minimum energy required for the reaction to take place, the lower activation energy obtained in this research indicates a significant high catalytic activity of the refluxed calcined snail shell (CaO).

V. Conclusion

The kinetics modelling of transesterification reaction of refined castor seed oil using refluxed calcined snail shell as catalyst shows pseudo-first-order kinetics. The rate constants of the reactions obtained are 0.03048, 0.03207 and 0.03737 with 496.678 J/Mol and 1652.4263 as activation energy and frequency factor. The lower activation energy might be due to improve the catalytic activity of refluxed calcined snail shell or pretreatment of castor oil.

Conflicts of Interest

There are no conflicts of interest to declare.

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Appendix I: Fatty acid methyl ester of biodiesel produced at different stages during kinetics studies of the reactions detected by GC/ MS.

FATTY ACID METHYL ESTER NAME	QUALITY	AREA (%)
A1: Conversion at first 30 minutes of the reaction		
Hexadecanoic acid, methyl ester	90	1.8947
10,13- octadecadienoic acid methyl ester	97	3.0916
Methyl stearate	83	0.3495
9- octadecenoic acid, 12- hydroxyl-, methyl ester [R-(Z)]-	93	70.2534
Total		75.5892
Non-methyl ester		24.4106
A2: Conversion at 60 minutes of the reaction		
Hexadecanoic acid, methyl ester	93	1.9024
9- octadecenoic acid (Z)-, methyl ester	99	9.6731
Methyl stearate	99	1.1755
10,13- Octadecadienoic acid, methyl ester	70	3.1291
9- octadecenoic acid-12- hydroxyl-, methyl ester, [R-(Z)]-	94	74.4450
Total		90.3251
Non-methyl ester		9.6749
A3: conversion at 120 minutes of the reaction		
Hexadecanoic acid, methyl ester	99	1.1912
9- octadecenoic acid [Z]-, methyl ester	99	10.0618
Methyl stearate	99	1.1755
9- octadecenoic acid -12-hydroxy-, methyl ester, [R-(Z)]-	94	86.1838
Oxacyclotetradecane-2,11-dione,13-methyl ester	94	0.5089
Total		99.1212
Non-methyl ester		0.8788

FATTY ACID METHYL ESTER NAME	QUALITY	AREA (%)
B1: Conversion at first 30 minutes of the reaction		
2- Hexyne,4- methyl ester	43	0.055
2H- pyran-3,4- dihydro-6-methyl ester	18	0.1017
2- methyl-Z,Z-3,13- octadecadienoic, methyl ester	90	16.8552
Hexadecanoic acid- 2- hydroxyl-1-(hydroxymethyl), methyl ester	55	7.9110
Total		24.9229
Non-methyl ester		75.0771

B2: Conversion at 60 minutes of the reaction		
Methyl-7-methylhexadecanoate	51	0.1520
Hexadecanoic acid, methyl ester	90	17.1341
Methyl stearate	99	0.9555
2- methyl-Z,Z-3,13- octadecadienol, methyl ester	22	0.2013
9- octadecenoic acid-12- hydroxyl-, methyl ester, [R-(Z)]-	94	70.1682
Total		88.7812
Non-methyl ester		11.2188

B3: conversion at 120 minutes of the reaction		
Hexadecanoic acid, methyl ester	99	1.0701
Methyl-7-methylhexadecanoate	55	10.0618
Methyl stearate	99	0.9555
9- octadecenoic acid -12-hydroxy-, methyl ester, [R-(Z)]-	94	84.3821
9,12- octadecadienoic acid (Z, Z)-, methyl ester	99	4.9006
9- octadecenoic acid (Z)-, methyl ester	99	5.1742
Total		96.5280
Non-methyl ester		3.4720

FATTY ACID METHYL ESTER NAME	QUALITY	AREA (%)
C1: Conversion at first 30 minutes of the reaction		
Methyl-7-methylhexadecanoate	55	0.9821
Hexadecanoic acid methyl ester	67	4.8730
Methyl stearate	77	0.1782
9- octadecenoic acid, 12- hydroxyl-, methyl ester [R-(Z)]-	90	26.5857
Total		32.6190
Non-methyl ester		67.3810

C2: Conversion at 60 minutes of the reaction		
Hexadecanoic acid, methyl ester	80	1.2711
Methyl-7-methylhexadecanoate	30	0.6071
Methyl stearate	77	1.1728
10,13- Octadecadienoic acid, methyl ester	70	17.1079
9- octadecenoic acid-12- hydroxyl-, methyl ester, [R-(Z)]-	90	60.1682
Total		80.3271
Non-methyl ester		19.6729

C3: conversion at 120 minutes of the reaction		
Methyl-7-methylhexadecanoate	99	1.7210
9- octadecenoic acid [Z]-, methyl ester	99	10.7813
Methyl stearate	90	1.0671
9- octadecenoic acid -12-hydroxy-, methyl ester, [R-(Z)]-	95	78.3412
Hexadecanoic acid, methyl ester	88	4.0194
Total		95.9300
Non-methyl ester		4.0700

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Production of Multilayered Nanostructure from Zingiber Officinale by Spray Pyrolysis Method

By S. Kalaiselvan, N Mathan Kumar & J Manivannan
SNS College of Technology

Abstract- This present work describes exploring cost-effective a natural renewable Environment-friendly green precursor for the synthesis of Multi-walled carbon nanotubes (MWCNTs) using methyl ester of Zingiber officinale oil over Fe-Co impregnated alumina support at 850°C under N_2 atmosphere. The characterization of the as-grown carbon materials was analyzed by Scanning electron microscopy (SEM), Highresolution transmission electron microscopy (HRTEM), XRD and Raman spectroscopic analysis. The bimetallic catalyst of Fe and Co supported on alumina gel particles improves the quality and uniformity in diameters of CNTs. The diameters of as-synthesized nanotubes are in the range of 22 nm to 24 nm.

Keywords: *CNTs, spray pyrolysis, SEM, HRTEM, raman & XRD & zingiber officinale oil.*

GJSFR-B Classification: *FOR Code: 250399*

Strictly as per the compliance and regulations of:

Production of Multilayered Nanostructure from Zingiber Officinale by Spray Pyrolysis Method

S. Kalaiselvan [α] N Mathan Kumar [σ] & J Manivannan [ρ]

Abstract- This present work describes exploring cost-effective a natural renewable Environment-friendly green precursor for the synthesis of Multi-walled carbon nanotubes (MWCNTs) using methyl ester of *Zingiber officinale* oil over Fe-Co impregnated alumina support at 850°C under N_2 atmosphere. The characterization of the as-grown carbon materials was analyzed by Scanning electron microscopy (SEM), High-resolution transmission electron microscopy (HRTEM), XRD and Raman spectroscopic analysis. The bimetallic catalyst of Fe and Co supported on alumina gel particles improves the quality and uniformity in diameters of CNTs. The diameters of as-synthesized nanotubes are in the range of 22 nm to 24 nm.

Keywords: CNTs, spray pyrolysis, SEM, HRTEM, raman & XRD & zingiber officinale oil.

I. Introduction

Right from the discovery by Iijima [1], carbon nanotubes attracted significance attention. In general, CNTs are synthesized by arc discharge, laser ablation, and CVD or spray Pyrolysis. Among these method spray pyrolysis method is regarded as a promising method. There are few reports on the synthesis of CNTs from various natural precursors [2-11]. The advantages of the plant derived carbon precursor are that it is a very cheap, renewable biomaterial, green, and abundantly available hence, the resulting product could be commercialized at a lower cost. The present work aims to utilize renewable precursor *Zingiber officinale* oil for the synthesis of CNTs over Fe-Co impregnated Silica support.

II. Experimental Methods

The scheme of our home-made experimental spray-pyrolysis set-up and sprayer used for the synthesis of carbon nanotubes were represented in Figure 1 and 2.

a) *Preparation Catalyst Nanoparticles and Synthesis of Nanotubes*

The bimetallic catalyst of cobalt and iron supported on silica gel particles were used as a catalyst for CNT growth by spray pyrolysis. The mixture of $Co(NO_3)_3$. $3H_2O$ and $Fe(NO_3)_3$. $6H_2O$ were heated at 90 °C to liquefy the Co and Fe nitrates. A one-third weight of alumina gel concerning to the total weight of metal nitrates was added into the liquefied mixture of Co and Fe nitrates and stirred well for 30 min. The mixture of catalyst was cooled down to room temperature and kept in the oven for drying at 60 °C for 24 hours.[12] The complete dried catalyst ground into fine particles in an agate mortar. The fine powder were then calcined for 1 hour at 450 °C and then re-ground before loading into the reactor. The catalyst placed on the quartz boat. The boat placed in the heating furnace. The carrier gas nitrogen (100 ml/min) was flushed out before switch on the reaction furnace to remove air and create nitrogen atmosphere.

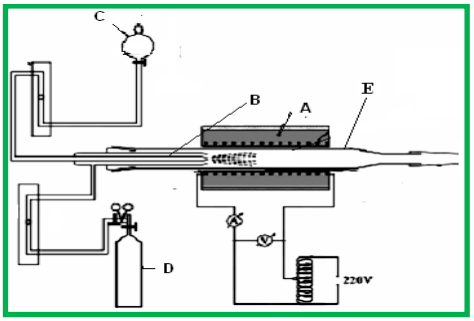

Figure 1: The schematic diagram of the spray pyrolysis set-up. (A) Heating source, (B) Spray nozzle, (C) Carbon feedstock inlet, (D) Nitrogen gas, (E) Quartz tube.

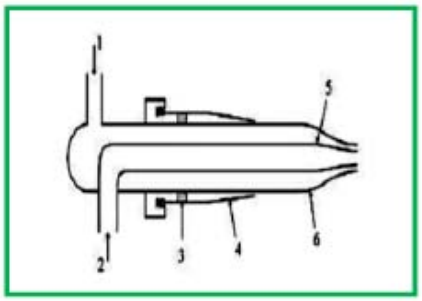

Figure 2: Schematic diagram of the Sprayer 1. Gas inlet; 2. Solution inlet; 3. Tightening; 4. Polished glass-to-glass connection; 5. Inner Pyrex tube; 6. Outer Pyrex tube

The temperature was raised up to the desired growing temperature. Subsequently, *Zingiber officinale* oil introduced into the quartz tube through the spray nozzle, the flow was maintained using the saline tube at the rate of 0.5 ml/min. The deposition time lasted for 45 minutes for each deposition at different temperatures from 550-850°C. The reactor was then allowed to cool to

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room temperature with nitrogen gas flowing. The carbon product on the silica support was collected and characterized by Raman Spectroscopy, X-ray diffraction (XRD), scanning electron microscope and High-resolution transmission electron microscope (HRTEM).

III. Results And Discussions

Fig. 3a and 3b show the scanning electron microscopy image of the as-grown nanostructures over Fe-Co bimetallic catalyst, impregnated in alumina at 850 °C under the flow of nitrogen by CVD assisted spray pyrolysis method. SEM image reveals that MWNTs grew nicely on the surface of the Alumina particles.

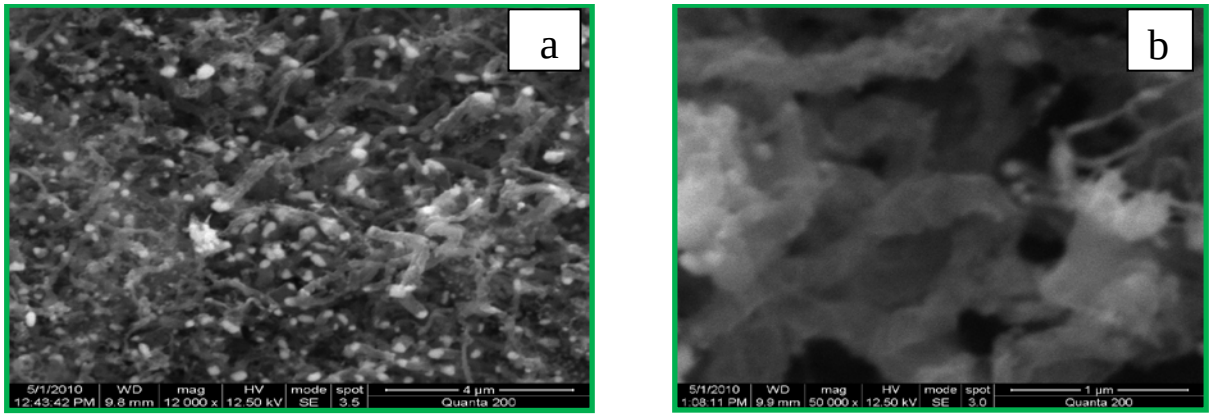

Fig. 3: a and b: SEM images of CNTs grown Fe-Co supported on Alumina at 850 °C

In Fig. 5a and 5b we have presented the high-resolution HRTEM images of MWNTs, grown over Fe-Co bimetallic catalyst impregnated on Alumina support at 850 °C with a flow rate of the methyl ester of *Zingiber officinale* oil at 0.5 mL per minute. Fig. 4a & 4b show that the inner diameters of the nanotubes were in the range of 10-12nm and the outer diameters of the grown nanotubes 22-24nm. The inner core visible (Fig. 4b).

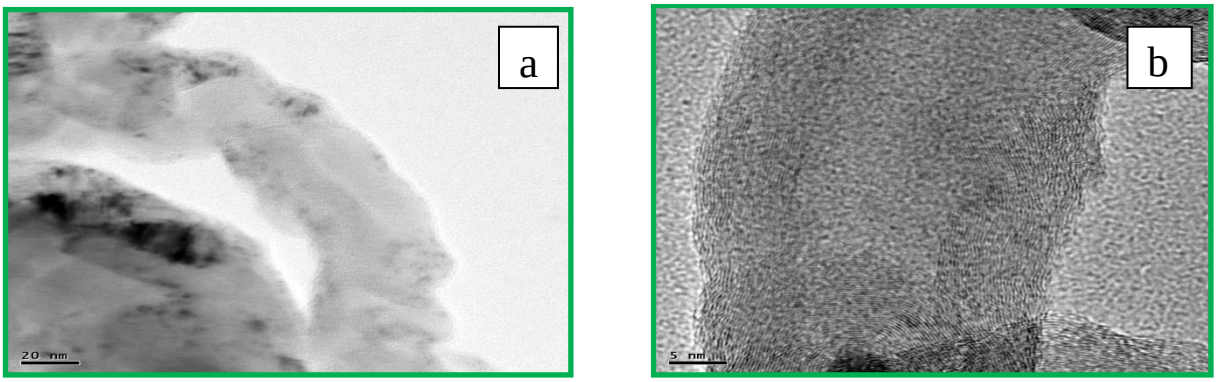

Fig. 4: a and b: HRTEM images of CNTs grown Fe-Co supported on Alumina at 850 °C

Raman Spectroscopy and XRD Analysis

The results of Raman spectroscopy of MWNTs grown on the catalyst surface at 850 °C (Fig-5), indicating two characteristic peaks at 1344.52 cm^{-1} and 1591.20 cm^{-1} which correspond to D and G bands, respectively. The G bands are related to stretching vibration in the basal plane of graphite crystal. The D peaks at 1344.52 cm^{-1} attributed to the defects in the curved graphene sheets. For high-quality samples without defects and amorphous carbon, the I_D/I_G ratio is often less than 3%[13,14]. I_D/I_G ratio was found to be 0.97 indicates lower defects which is in good agreement with theoretical evidence.Fig-6 shows the X-Ray Diffraction pattern of MWNTs. The intense peaks at 26.1° and 44.2° can be as indexed (002) and (101) reflections of hexagonal graphite. Appearance of characteristic peak of graphite shows the presence of Multi-walled carbon nanotubes in the sample [15].

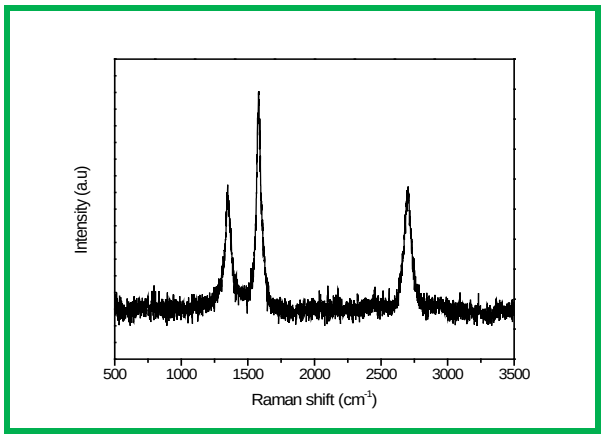

Fig. 5: Raman Spectrum of grown CNTs at 850°C

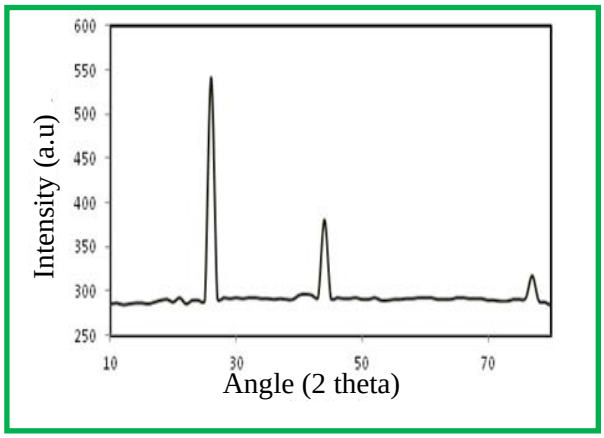

Fig. 6: XRD spectrum of grown CNTs at 850°C

IV. Conclusion

We accomplished the synthesis of MWCNTs at a temperature of 850°C using methyl esters of *Zingiber officinale* oil a renewable natural precursor, as a carbon source. CNTs morphology and structure were investigated by SEM, HRTEM, XRD and Raman spectroscopy. It was found that temperature was enough to transform hydrocarbon source into carbon nanotubes with heterogeneous diameter, good quality and yield at this temperature. The experiment has also succeeded in minimizing I_D/I_G ratio of about 0.97. The highest yield of 68% was obtained at the same temperature.

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A Rapid and Efficient Method for Purification of Peroxidase from *Opuntia-Ficus Indica* Stem with Decolorization Effect

By Fatih Uckaya & Meryem Uckaya
Alanya Alaaddin Keykubat University

Abstract- Peroxidase enzyme was purified from *Opuntia ficusindica* stem for decolorization some synthetic dyes including congo red, methyl red, indigo carmine, crystal violet and ponceau red by TPP. For the purification of the enzyme, the stem of a fresh plant was first homogenized by using phosphate buffer, and subsequently, supernatant was filtered. The homogenate was mixed with ammonium sulfate at room temperature, and *t*-butanol was added. The intermediate phase was separated and optimized according to different process parameters as homogenate/t-butanol ratio (1:0.5), ammonium sulfate concentration (40%), pH (8), temperature (30°C) and values of K_M (4.8 mM) and V_{max} (0,005 U/mL.min) with 2, 25-fold purification and 106, 64% recovery. The molecular weight of partitioned enzyme was found about 28 kDa compared with protein standard. TPP was found to be an easy and effective applicable technique with utilization for extraction and purification of peroxidase enzyme and the purified enzyme decolorized the tested synthetic dyes, successfully. The most important advantage of the method is a high recovery of peroxidase enzyme without requirement any column.

Keywords: decolorization, opuntia-ficus indica, peroxidase, synthetic dyes, three-phase partitioning.

GJSFR-B Classification: FOR Code: 030599

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Keywords: decolorization, opuntia-ficus indica, peroxidase, synthetic dyes, three-phase partitioning.

I. Introduction

The purification of proteins and enzymes is a complex procedure. Development of a new process requires the method increases purity and yield, decrease the time spent. Three-phase partitioning (TPP) is a simple and economical method for purification of enzymes and separation of oils. The technique is based on the hydrophobicity of proteins. Cosmotropy, electrostatic forces, protein hydration shifts and conformation tightening have been proposed of the physicochemical base for the protein precipitation. TPP uses ammonium sulfate and *t*-butanol to precipitate proteins in convenient aqueous solutions. The solution is separated into three phases after the centrifugation. These phases are an aqueous phase, *t*-butanol phase and intermediate precipitate phase. *t*-Butanol stabilizes protein structure extraordinarily. It also inhibits the enzyme activities and protein/protein interactions. The solubility of protein depends on the salt concentration of the solution. The salt used in TPP keeps the charged groups on a protein molecule at low concentrations. Then the protein is pushed into the solution and solubilized. This is "salting-in" mechanism. In addition to this, when the salt concentration is increased, it is reached to maximum solubility point of protein. The water available is finished by increasing the salt concentration. Finally, protein begins to precipitate due to not sufficient interaction between water and protein molecules. This precipitation in the presence of excess salt is called as "salting-out". Several salt types have been employed to increase separation and purification of protein by salting-out mechanism (Harde and Singhal 2012).

Opuntia ficus-indica (L.) Miller, called prickly pear, is a cactus species largely used in semi-arid areas as a fruit and forage crop. Cactus pear fruits and stems were described as a source of nutrients and vitamins (Saenz 2000). It is a kind of plant which can be adapted to the climate condition in different regions of the world. It is quite rich in vitamins, minerals, waters, sugars, and fibers about many plants. The plant has been used in the treatment of a wound, common cold, hyperlipidemia, obesity, ulcer, allergic reaction and prostate hypertrophies (Teixeira et al. 2000). Fruit ovoid, 5-10 cm long, red, yellow or purplish, bearing glochids in the areoles but no spines. In Turkey, *O. ficus-indica* is cultivated locally in South and West Anatolia (Köyceğiz, Antalya, Alanya, Anamur, Mersin, İskenderun for its fruit (prickly pear, in Turkish "frenk inciri") and sometimes used as a hedge plant. It is easily propagated vegetatively using joints (Matthews, 1972).

The enzyme peroxidase (E.C. 1.11.1.7) is widely distributed in nature. They are used for determination of pH, DNA, zinc, copper. They can use an indicator for reactive oxygen species in food processes as well as a catalyzer for the synthesis of resin (Aghelan and Shariat 2015). The peroxidase enzyme is used in the field of health, immune assay kits and clinical diagnosis of laboratory experiments. Also, peroxidases function as free radical scavengers in biotherapy. Furthermore, it is used as a detergent additive recently (Humaira and Qayyum, 2010).

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The demand is increasing in the industrial area of cosmetic, paper, textile and pharmaceutics for synthetic dyes. But these dyes lead to serious pollution on the environment, and also most of them have toxic, carcinogenic and mutagenic effects. The recent studies in this field are namely the enzymatic treatment of wastewater and soil. The advantages of the enzymatic decolorization consist of the application of non-soluble materials, process on high and low waste concentrations at a large pH, temperature and amount of the salt. Recently, utility of enzymes attracts considerable attention in the removal of synthetic dyes from aqueous solutions. Since enzymes interact with recalcitrant pollutants and remove them to another product through precipitation or transformation (Duran and Esposito 2000).

The present study includes purification and recovery of peroxidase from the cactus stem by using a rapid and cheap method called TPP which not requires the use of any column. Several parameters such as a concentration of *t*-butanol, salt, pH and temperature were optimized beside values of K_M, and V_{max} was calculated. Furthermore, decolorization of some synthetic dyes has been performed with purified cactus stem peroxidase. The method used is a single step and promising for application in obtaining enzyme at industrial scale.

II. Materials And Methods

a) Plant and chemicals

Opuntia ficus-indica stems were collected from Antalya, Turkey. The stems stored -80°C deep freezer until they were used. The chemicals, ammonium sulfate, t-butanol, Coomassie Brillant Blue G-250, Bovine Serum Albumin, phenol, pyrogallol, hydrogen peroxide glucose diagnostic kit and horseradish peroxidase were purchased from Sigma-Aldrich (St. Louis, MO, USA).

b) Preparation of Homogenate

10 g of the cactus stem were weighed, ground in a mortar until completely crushed. It was homogenized in 100 mL phosphate buffer (100 mM, pH 7) using a magnetic stirrer. The obtained homogenate was centrifuged at 20.000×g for 20 min. The resulting precipitate and supernatant were separated. The studies of peroxidase activity and protein content were performed on the supernatant.

c) Three-phase Partitioning of Peroxidase

The TPP was employed with a slight modification of the method of Roy and Gupta (2002). The cactus homogenate adding to *t*-butanol at ratios of 1:0.5, 1:1, 1:1.5, and 1:2 (v/v) was studied in a glass reactor. 4 g of ammonium sulfate was gradually added over 10 ml of homogenate at room temperature. The ammonium sulfate was completely dissolved in water and then mixed with *t*-butanol. The reaction mixture was stirred at 200 rpm for 60 min.; it was then centrifuged at 10.000×g for 20 min. The upper phase was discarded. The intermediate protein precipitate and lower aqueous phase were separated carefully. Protein precipitate in intermediate phase was dissolved in 100 mM phosphate buffer of pH 7. The obtained intermediate and bottom phases of TPP were dialyzed for 12 h against 100 mM phosphate buffer at pH 7 and protein content, and peroxidase activity was measured of both dialyzers.

The ammonium sulfate concentrations were defined according to Roy and Gupta 2002. At the concentrations of 20, 30, 40, and 50% (w/v) were evaluated by using 1:0.5 (v/v) ratio of homogenate:*t*-butanol on intermediate phase. The lower aqueous phase and the intermediate protein precipitate were separated carefully and dialyzed 12 h against 100 mM pH 7, phosphate buffer at 4°C. After the dialysis, the assays of peroxidase activity and protein content were applied to obtained fractions. The phases obtained from partitioning systems were checked against by gel electrophoresis to determine the best combination for yielding the highest peroxidase recovery and purification fold.

d) Protein Content

Protein concentration was analyzed according to the method developed by Bradford. In the method, Coomassie Blue G-250 main reagent and Bovine Serum Albumin (BSA) were used as standard (Bradford 1976). The mechanism of this reaction is that Coomassie Blue G-250, which has a negative charge, binds to positively charged proteins.

e) Peroxidase Activity Assay

The activity of peroxidase enzyme was determined with a colorimetric assay, depends on the absorbance at 420 nm (Ebiloma et al. 2011). The method based on forming the purpurogallin from pyrogallol in the presence of hydrogen peroxide (H_20_2) by the catalysis peroxidase at 25^0C.

f) SDS-PAGE Analysis

SDS-PAGE analysis of the homogenate and purified enzyme was performed according to the method developed by Laemlii (1970).

g) Determination of Optimum pH, Optimum Temperature, K_M and V_{max} Values

Optimum pH studies were carried out from pH 3 to 10. The different buffer solutions which are acetate buffer, citrate buffer, phosphate buffer and tris/HCl buffer were used. The enzyme activities of the samples at each pH were measured at 420 nm, and a figure was drawn from obtained results. For optimum temperature, the obtained peroxidase has incubated at 0, 10, 20, 30, 40, 50, 60, 70 and 80°C about 10 minutes. The peroxidase activities of the samples at each temperature were measured at 420 nm, and a figure was drawn from obtained results. Pyrogallol was used a as substrate for

the determination of K_M and V_{max} values in the presence of H_2O_2. Substrate solutions were prepared at different concentration (50-500 μmoles). Then the peroxidase activities of each sample were measured at 420 nm, and a figure was drawn about results.

h) Decolorization Activity Assay

Partitioned cactus peroxidase was used individually for decolorization of synthetic dyes congo red, methyl red, indigo carmine, crystal violet and ponceau red. The activity of decolorization was determined by a slight modification of Kumar et al. (2012). A calibration curve prepared at different concentrations of dye solutions at 497 nm, 520 nm, 610 nm, 592 nm and 510 nm, respectively. Dye concentrations determined according to this curve. All experiments were carried out in 3 ml cuvettes and 2 ml final reaction volume. Reaction mixture included 100 mM phosphate buffer at pH 7, 100 ppm dye concentration and 20 U/ml purified peroxidase. When the partitioned peroxidase was added, the reaction started, and it was incubated at 30°C. Finally, the absorbance of dyes was measured using UV-Vis spectrophotometer at 10 min on appropriate wavelengths for each of them. Blanks included all components of the reaction mixture except the dyes.

III. Results And Discussion

There is an increasing demand for new methods for purification of the enzyme including peroxidases, with the purpose more economically and efficiently uses of them. The TPP is an effective method for purification of proteins, where significant recovery of the various enzymes reported (Azmi et al. 1998, Shaffiqu et al. 2002). In the present study, we have used TPP for single-step purification of peroxidase from the cactus stem and the purification results showed in Table 1. TPP can be affected by various conditions, such as salt concentrations, solvent ratio, and pH. Therefore, different ratios of *t*-butanol, different concentrations of salt and various pH values were performed in this study.

a) Effect of Homogenate to t-Butanol Ratio on TPP

Correlation between *t*-butanol and ammonium sulfate was used for concentration of enzymes at an interface. *t*-Butanol has selected because of various advantages over the other organic solvents. It has not lead to denaturation of the purified enzyme due to its high molecular size and no ability to penetrate of the three-dimensional structure of a protein. The ratio of homogenate: *t*-butanol was studied in the range of 0.5 to 2 (v/v) in the presence of 40% ammonium sulfate. Purity and activity recovery of obtained peroxidase increased from 0.5 to 2 (v/v) homogenate: *t*-butanol ratio as shown in Figure 1. As a result, maximum purity and recovery of cactus peroxidase were obtained as 2, 25 and 106, 64%, respectively.

b) Effect of Ammonium Sulfates Concentration on TPP

Ammonium sulfate has an important role in TPP system due to its relation with protein-protein interaction and precipitation. It uses mechanism of salting out for precipitation of protein at an interface. The salt ions affect water molecules using strong protein-protein interactions, and the protein molecules are coagulated by hydrophobic interactions (Roy and Gupta 2002). Ammonium sulfate saturations of 20%, 30%, 40% and 50% were studied with 1:0.5 homogenate to *t*-butanol ratio. 40% ammonium sulfate showed the best activity recovery result as can be seen in Figure 2.

c) Effect of pH on Partitioned Peroxidase

The pH is an important parameter ionization of amino acids which are acidic or basic. The effect of pH on partitioned peroxidase was investigated on values from 3 to 10 about purity. Different pH values (3-10) was investigated for 60 min, at 40% ammonium sulfate concentration with 1:0.5 (v/v) homogenate:*t*-butanol ratio at 25°C, as seen in Figure 3. In this study, the optimal pH value of cactus peroxidase was found like 8.

d) Effect of Temperature on Partitioned Peroxidase

Temperature is a significant factor which affects the configuration and stability of the enzyme. The temperature optimization assay was performed between 0°C and 80°C while keeping all other parameters constant. Figure 4 showed that the temperature effect on a characteristic of peroxidase in the TPP system. The degree of purity of the enzyme has increased to about 30°C. After this temperature, the activity started to decrease. This decrease in activity at high temperature can result from the thermal deactivation of the enzyme. The possible reason behind this may be the *t*-butanol has important cosmotropic and aggregation effects at 20-30°C. Consequently, the optimal temperature of partitioned peroxidase was found at 30°C. Vetal and Rathod (2015) found the similar result in their work for peroxidase enzyme from orange peel.

e) Effect of Kinetic Constants (K_M and V_{max})

Influence of substrate concentration on peroxidase activity was performed with the beginning concentration of pyrogallol ranging from 1 to 6 mM at pH 8 and 30°C. Values of K_M and V_{max} for pyrogallol were calculated from Lineweaver-Burk plot as 12.5 mM and 9.2 U/ml.min, respectively. The plot was composed of 1/V against 1/[S] for system determining the Michaelis-Menten equation. Results were indicated in Figure 5. K_M value of peroxidase obtained in this study which is in agreement with those presented in the literature (Triplett et al. 1992).

f) SDS-PAGE Analysis

Molecular weight and purity of partitioned peroxidase were estimated by SDS-PAGE. Molecular mass was found to be nearly 28 kDa which was similar to the previous results by Vetal and Rathod (2015)

purified peroxidase from orange peel, and they found molecular weight as 26 kDa. Figure 6 showed the bands of the homogenate and purified peroxidase with TPP. Minor stain on the peroxidase bands indicated the purity of the purified enzyme. Many major bands of the homogenate were not seen in the TPP fractions. SDS-PAGE showed a substantial purification of peroxidase from the cactus stem.

g) Decolorization Effect of Partitioned Peroxidase

Effect of decolorization of various synthetic dyes was analyzed using partitioned peroxidase from the cactus stem. Synthetic dyes are recalcitrant compounds (Azmi et al. 1998). The cactus peroxidase decolorized more than 80% synthetic dyes within a short time. These dyes have electron donating methyl and methoxy groups. Thanks to these groups they could bleach fast and efficiently. As follows from Figure 7 indigo carmine (84.12%) was the most sufficiently decolorized through enzymatic treatment, indicating the exact removal of color in the treated samples. Following indigo carmine, congo red and ponceau red were decolorized at the ratio of 35.16% and 30.93%, respectively. While crystal violet (22.23%) was less decolorized compared to the above three dyes. Methyl red (17.4%) had the least decolorization effect among treated samples. Results in the present study were in agreement with the literature (Shaffiqu et al. 2002). When the work investigated from an industrial point, it could be easier to add a solution of peroxidase partitioned with the TPP directly into the wastewater. Consequently, TPP peroxidase obtained from cactus stem should be used for decolorization of synthetic dyes present in the industrial wastewater.

IV. Conclusions

Recovery activity of peroxidase purified from the cactus stem by using the TPP was found to be higher than the other plant peroxidases studied. The main advantages of the purified enzyme are to be stable for several days having optimal pH and temperature values, one step and cheap purification procedure with high recovery activity which make this technique good choice for several industrial applications. Also, the purified peroxidase showed a significant decolorization activity on the synthetic dyes without any redox mediators. It is noticeable that cactus peroxidase enzyme is fairly effective in removing textile wastewaters.

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Declaration of interest

The authors declare that they have no conflicts of interest to disclose.

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Table 1: Three-phase partitioning of peroxidase from the stem of cactus.

Samples	Total activity (U)	Total protein (mg)	Spesific activity (U/mg)	Purification fold	Activity recovery (%)
Homogenate	262,61	0,141	1863,71	1	100
TPP intermediate phase	280,04	0,067	4200,61	2,25	106,64
TPP bottom phase	1,62	0,021	77,76	0,04	0,62

**The experiments were carried out in triplicate for purification steps and the difference in the readings was less than ±5%.*

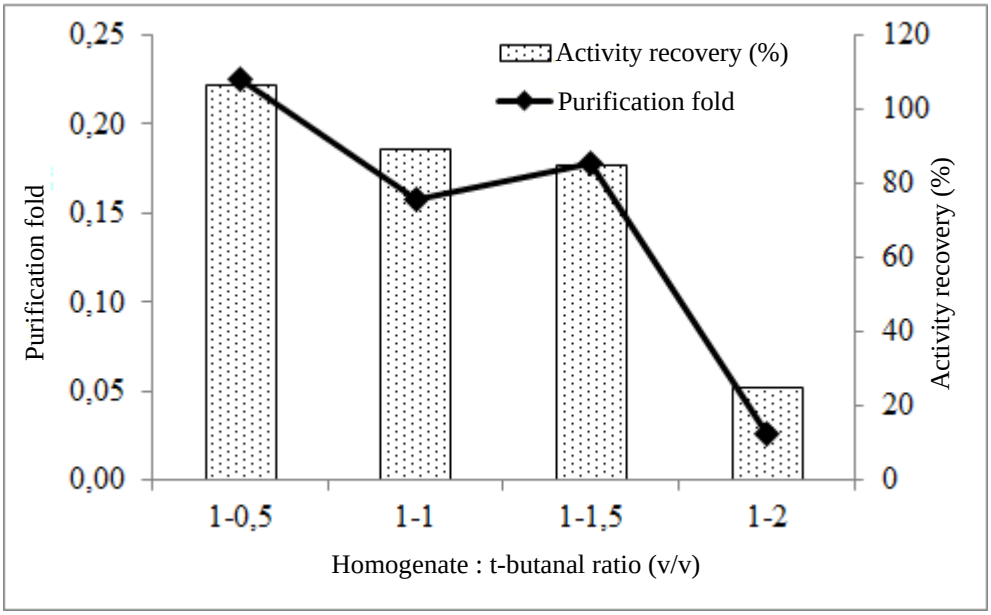

Figure 1: Effect of homogenate: *t*-butanol ratio (v/v) on purification fold and activity recovery of peroxidase from the stem of cactus.

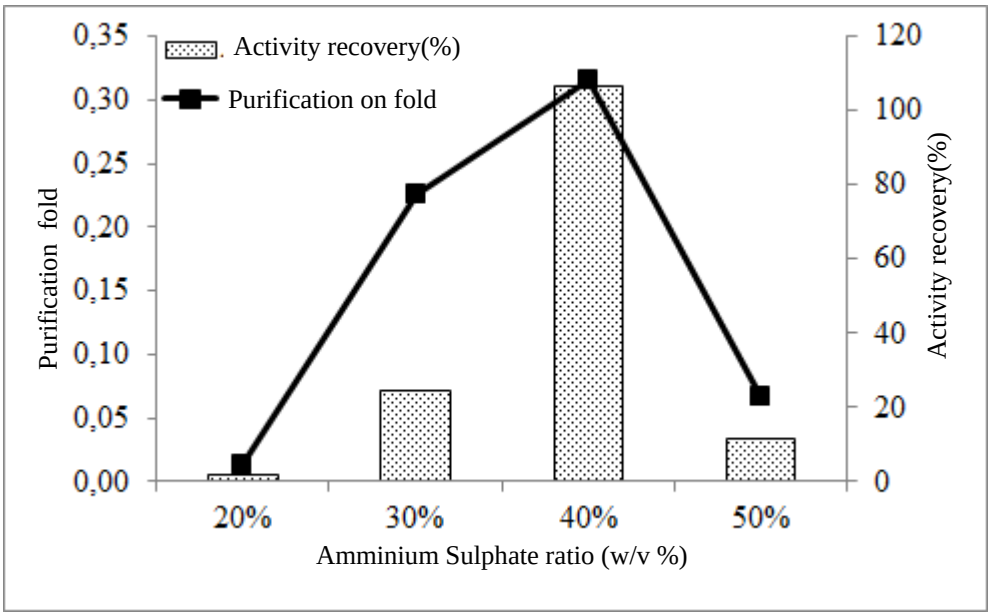

Figure 2: Effect of the concentration of ammonium sulfate (%, w/v) on purification fold and activity recovery of peroxidase from the stem of cactus.

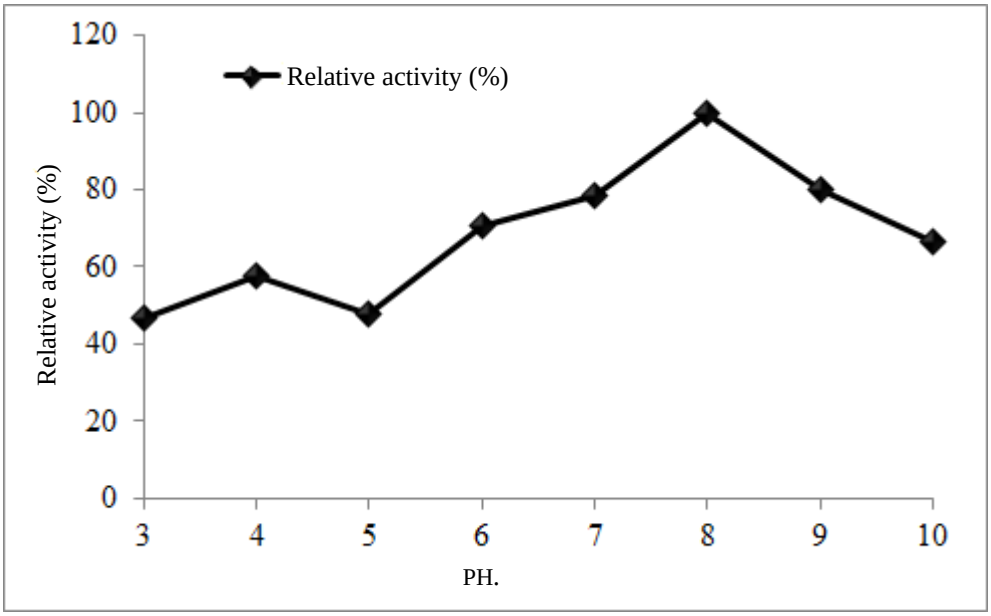

Figure 3: Effect of pH on the activity of peroxidase from the stem of cactus.

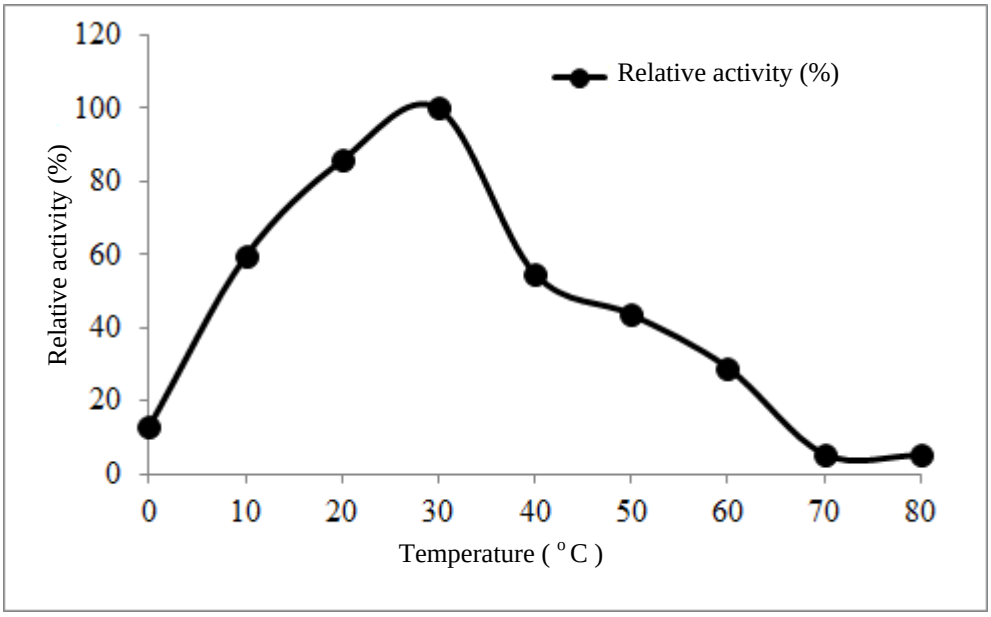

Figure 4: Effect of temperature on the activity of peroxidase from the stem of cactus.

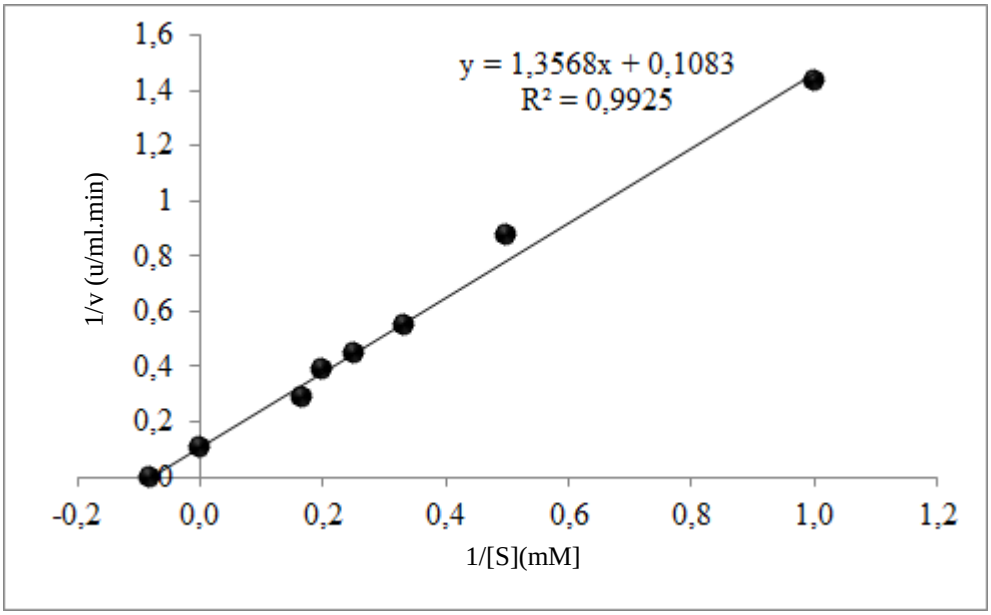

Figure 5: Lineweaver-Burk plot of substrate of peroxidase from the stem of cactus.

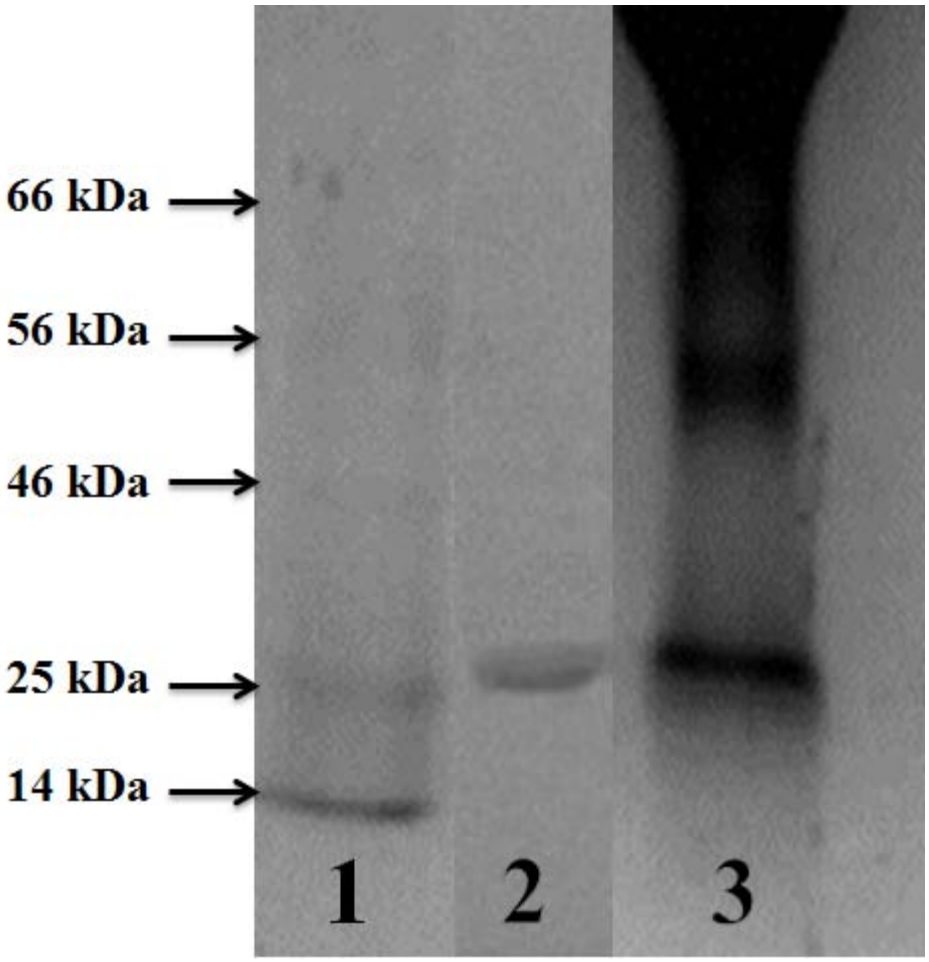

Figure 6: SDS-PAGE pattern of the purified peroxidase. Lane 1: Molecular weight marker, Lane 2: TPP intermediate phase, Lane 3: Homogenate.

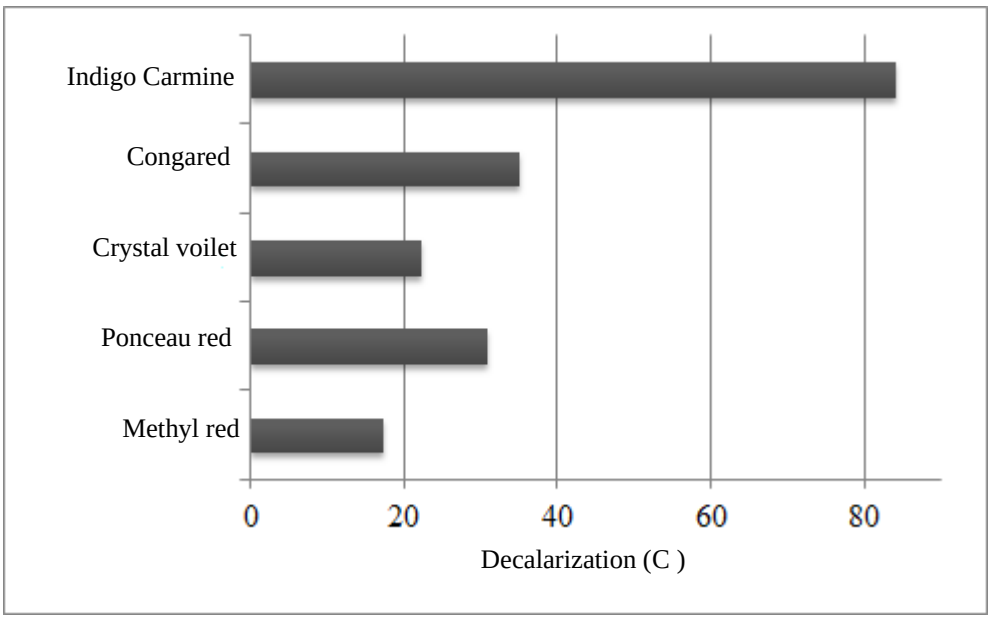

Figure 7: Decolorization activity of peroxidase from the stem of cactus.

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1.10-Phenanthroline as Anti-Radiation UV Agent: Spectrophotometry Analysis and Modeling

By Khusna Arif Rakhman, Ahmad Muchsin Jayali, Muhammad Ikhlas Abdjan, Andi Udin Ahmad & Chairil Anjasmara Robo Putra

Universitas Khairun

Abstract- Determination of the activity of a 1.10-Phenanthroline compound as an agent of anti UV was measured using a UVVis spectrophotometer. The study of the electronic transition of 1.10-Phenanthroline compounds was carried out computationally with the semi-empirical of ZINDO/s method. The results of determining the potential of the 1.10-Phenanthroline compound as an anti UV radiation agent showed the lowest%Te value of 0.102%, the highest SPF value of 2.328 and the lowest%Tp value of 0.509%. The type of transition found in 3 peaks in the 1.10-Phenanthroline compound shows the transition types; n to π^* and π to π^*. Whereas, molecular orbitals (MO) involved in electron excitation were: 32 (HOMO-1), 33 (HOMO), 34 (LUMO) and 35 (LUMO + 1) with the smallest energy difference value being 33 to 34 excitation of 7.78 eV.

Keywords: the 1.10-phenanthroline, anti-UV radiation agent, UV-vis spectrophotometer, ZINDO/s.

GJSFR-B Classification: FOR Code: 030199

Strictly as per the compliance and regulations of:

1.10-Phenanthroline as Anti-Radiation UV Agent: Spectrophotometry Analysis and Modeling

Khusna Arif Rakhman [α], Ahmad Muchsin Jayali [σ], Muhammad Ikhlas Abdjan [ρ], Andi Udin Ahmad [ω] & Chairil Anjasmara Robo Putra[¥]

Abstract- **Determination of the activity of a 1.10-Phenanthroline compound as an agent of anti UV was measured using a UV-Vis spectrophotometer. The study of the electronic transition of 1.10-Phenanthroline compounds was carried out computationally with the semi-empirical of ZINDO/s method. The results of determining the potential of the 1.10-Phenanthroline compound as an anti UV radiation agent showed the lowest%Te value of 0.102%, the highest SPF value of 2.328 and the lowest%Tp value of 0.509%. The type of transition found in 3 peaks in the 1.10-Phenanthroline compound shows the transition types; n to π^* and π to π^*. Whereas, molecular orbitals (MO) involved in electron excitation were: 32 (HOMO-1), 33 (HOMO), 34 (LUMO) and 35 (LUMO + 1) with the smallest energy difference value being 33 to 34 excitation of 7.78 eV.**

Keywords: the 1.10-phenanthroline, anti-UV radiation agent, UV-vis spectrophotometer, ZINDO/s.

I. Introduction

The 1.10-Phenanthroline (Fig. 1)is one ofthe organic compounds which are soluble in water and organic solvents such as alcohol and acetone [1,2,3]. 1.10-Phenanthroline is the white crystalline powder that has a melting point of 93-94 °C with a molecular weight of 180.20 grams/mol. The structure of the compound 1.10-phenanthroline (C12H8N2) consists of 3 benzene rings with two nitrogen atoms which have a quiet electron pair in the compound [5,6,7].

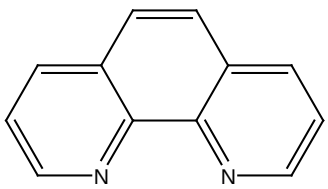

Figure 1: Molecule of 1.10-phenanthroline

1.10-phenanthroline compounds are often used as ligands that are used as catalysts, complexation of transition metal ions and various biological activities [8-11].Research on 1.10-phenanthroline compounds has been carried out such as spectroscopic characterization, molecular structure studies, anti-cancer / tumor, anti-microbial and so on [12-17]. 1.10-Phenanthroline includes derivatives of aromatic compounds (3 benzene) with conjugated bonds [18, 19]. Aromatic compounds have the potential as anti-UV radiation, where aromatic derivatives such as chalcone and anthocyanin show good results as anti-UV agents in UV-B (290-320 nm) and UV-A (320-375 nm) [20, 21]. This article reports the activity of 1.10-phenanthroline as an anti UV agent and an electronic transition model using the ZINDO/s semi-empirical method.

II. Methodology

a) Measurement of Pigmentation Percent (% Tp), Percent Erythema (% Te) and SPF

Percent pigmentation measurements, percent erythema, and SPF were carried out in wavelength ranges of 290-375 nm with 5 nm intervals using variations in concentrations of 0.1, 0.5, 1, 5 and 10 ppm. Percent pigmentation (% Tp), percent erythema (% Te) and SPF can be determined based on the absorbance value of the UV-Vis spectrophotometer. [22, 23].Determination of AUC Area Value (Area under Curve) is determined based on the absorbance value of each wavelength and SPF (Sun Protection Factor) value determined in each concentration variant.

b) 1.10-Phenanthroline Molecular Geometry Modeling

Optimization of 1.10-Phenanthroline molecular geometry aims to find the optimal molecular structure with the smallest energy value [24]. Modeling molecular geometry and molecular energy calculations can be determined using the semi-empirical method of PM3 [25]. Optimization of 1.10-Phenanthroline molecular geometry uses the semi-empirical method PM3 with a gradient limit of change of 0.001 kcal/(Å.mol) reaching the near gradient limit based on the Polak-Ribiere method.

c) Electronic Transition Studies

Modeling the structure of geometry optimization results from the semi-empirical method of PM3, followed by the semi-empirical method of ZINDO / s to produce electronic transition spectra data. The transition calculation criteria use single point configuration interaction (CI) calculations with a singly excited-CI excitation limit with HOMO-LUMO orbitals respectively 2. The electronic transition of 1.10-phenanthroline was analyzed from a discontinuous spectral diagram of the modeling results.

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III. Results And Discussion

a) Potential 1.10-Phenanthroline as an Anti-Radiation Agent for UV

The measurement of the absorbance value at 1.10-Phenanthroline shows that there is a decrease in the consecutive absorbance of the UV-B wavelength to UV-A (Figures 2 and 3). The higher absorbance value of UV-B of 1.10-phenanthroline compounds showed that UV-B deterrent activity was better than UV-A. The potential of the 1.10-Phenanthroline compound as an anti-UV radiation agent was reviewed based on percent pigmentation (% Tp), percent erythema (% Te) and SPF values.

The measurement results of absorbance of 1.10-phenanthroline compounds in the UV-B wavelength range (290-320 nm) showed that the optimum concentration was at a concentration of 10 ppm with an absorbance value of 0.512 precisely at a wavelength of 290 nm. Whereas, the results of absorbance measurements at UV-A wavelength (325-375 nm) showed that the optimum concentration was at a concentration of 10 ppm with an absorbance value of 0.074 at a wavelength of 325 nm.

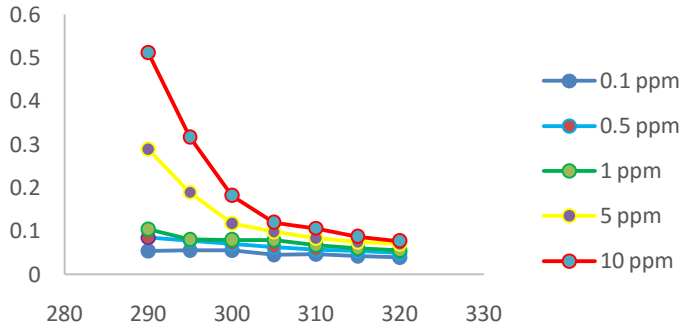


Figure 2: Absorption Graph of 1.10-Phenanthroline in the UV-B Area

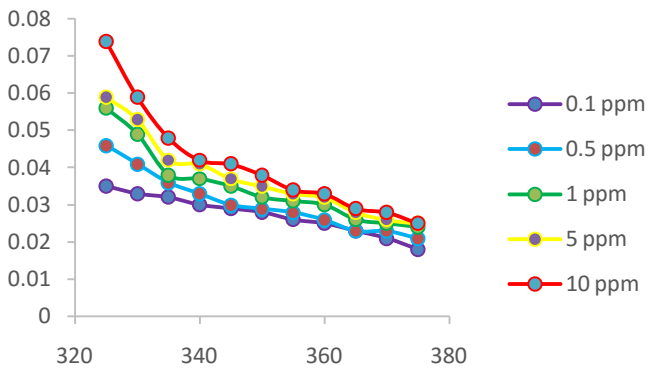


Figure 3: Absorption Graph of 1.10-Phenanthroline in the UV-A Area

The resulting test of thepercent of erythema (% Te) and SPF of compound 1.10-Phenanthroline was carried out in the UV-B area with the results showed that the% Te value with the lowest value was at a concentration of 0.1 ppm and the highest SPF was at a concentration of 10 ppm (Table 1). The lower of erythema shows good potential as anti-UV, where the average %Te shows the sunblock protection category. The greater the SPF shows good ability as an anti-UV agent [26], and the optimum SPF at 10 ppm 1.10-Phenanthroline shows a minimum UV protection category.

Table 1: Percent of Erythema and SPF 1.10-Phenanthroline

C (ppm)	absorbance							%Te	SPF
	290	295	300	305	310	315	320		
0.1	0.054	0.056	0.056	0.045	0.047	0.042	0.039	0.102	1.265
0.5	0.085	0.078	0.07	0.063	0.057	0.053	0.049	0.134	1.364
1	0.104	0.081	0.08	0.079	0.068	0.061	0.056	0.156	1.431
5	0.289	0.189	0.117	0.098	0.083	0.074	0.07	0.245	1.759
10	0.512	0.317	0.182	0.12	0.106	0.088	0.077	0.367	2.328

The percentage of pigmentation (%Te) was measured at the wavelength of the UV-A area, where the lower %Te showed better anti-UV activity. The results of the percent pinging test showed that the lowest%Tp value was at a concentration of 0.1 ppm (Table 2). The average yield of 1.10-Phenanthroline compound pigmentation value based on concentration variants shows the sunblock category. The greater %Te and %Tp in skin care products can cause skin irritation. [27].

Table 2: Percent of Pigmentation of 1.10-Phenanthroline

C (ppm)	absorbance											%Tp
	325	330	335	340	345	350	355	360	365	370	375	
0.1	0.035	0.033	0.032	0.03	0.029	0.028	0.026	0.025	0.023	0.021	0.018	0.509
0.5	0.046	0.041	0.036	0.033	0.03	0.029	0.028	0.026	0.023	0.023	0.021	0.514
1	0.056	0.049	0.038	0.037	0.035	0.032	0.031	0.03	0.026	0.025	0.024	0.520
5	0.059	0.053	0.042	0.041	0.037	0.035	0.033	0.032	0.028	0.026	0.025	0.525
10	0.074	0.059	0.048	0.042	0.041	0.038	0.034	0.033	0.029	0.028	0.025	0.532

b) Geometry Optimization and Electronic Transition

Optimization of 1.10-Phenanthroline molecular geometry was carried out using the semi-empirical PM3 method found in the *Hyperchem* 8.0.10 application [28]. One parameter for predicting a structure that has the optimum geometry is to have the smallest total energy which is the total amount of binding energy, and heat of formation. The geometry optimization results of the 1.10-Phenanthroline molecule involved 22 atoms, and 66 electrons with measurement parameters including total energy, binding energy, and formation heat (Table 3).

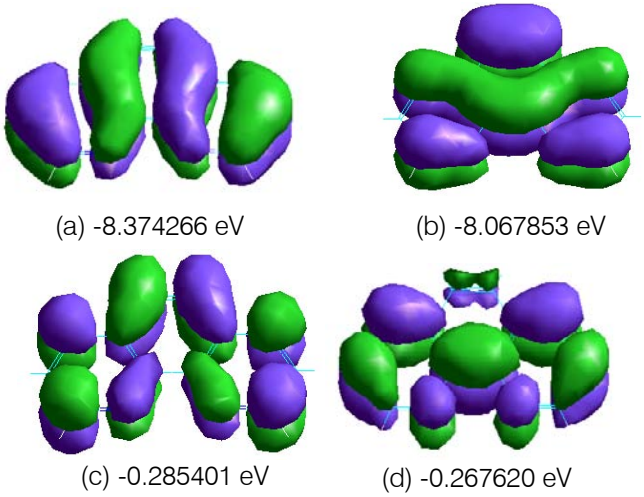
Table 3: Geometry Optimization of 1.10-Phenanthroline Using the PM3 Method

No.	Parameter	value
1.	Energy Total (kcal/mol)	-43084.3805
2.	Binding Energy (kcal/mol)	-2622.1956
3.	Heat Formation (kcal/mol)	71.3004

The electronic transition study was carried out using the ZINDO/s semi-empirical method by looking at the transition spectrum modeling [29]. Where the data were taken in the form of nm wavelength, oscillator strength, molecular orbital (MO) level, HOMO energy and LUMO energy to study the transition type of compound 1.10-Phenanthroline. Based on table 5 data obtained three peak wavelengths with oscillator strength values are in the UV region (Table 4). These results show the ability of 1.10-Phenanthroline compound through the simulation of ZINDO/s semi-empirical modeling with the optimum wavelength value at 250.50 nm. Also, the transition type at each peak in compound 1.10-Phenanthroline shows the transition type, namely n to π^* and π to π^*. Electronic transition activity at 1.10-Phenanthroline, when exposed to UV light, is affected by the π conjugated bond in the aromatic ring and the lone pair electron in the Nitrogen atom.

Table 4: ZINDO/s Method Simulation of 1.10-Phenanthroline

λ (nm)	Osc.	MO level	ΔE_g (eV)	Transition Judgments
278.9	0.0617	33→34 32→35	7.78 8.10	n→π*
250.5	1.8804	33→34 32→35	7.78 8.10	π→π*
234.7	0.9577	33→35 32→34	7.80 8.08	π→π*



(a) -8.374266 eV (b) -8.067853 eV

(c) -0.285401 eV (d) -0.267620 eV

Figure 4: MO level of complex 1.10-Phenanthroline (a), (b) HOMO 32, 33 and (c), (d) LUMO 34, 35

Electronic transition modeling using semi-empirical methods involves 64 molecular orbitals with different MO levels. The molecular orbitals(MO) levels involved in the electron excitation process are MO 32 (HOMO-1), 33 (HOMO), 34 (LUMO) and 35 (LUMO +1). The reactivity of a compound to UV light can be seen from the difference in HOMO to LUMO energy in the excitation of electrons [30, 31]. The electron excitation at MO 33 to 34 has the smallest energy difference, and MO 32 to 35 has the greatest energy difference of HOMO-LUMO (ΔEg) at the 1.10-Phenanthroline compound.

IV. Conclusion

The results of the activity of the 1.10-Phenanthroline compound as an anti-UV radiation agent showed that the highest absorption was in the UV-B wavelength region precisely at a concentration of 10 ppm of 0.512The highest SPF is 2,328 at a concentration of 10 ppm. The lowest%Te value obtained was 0.102% and the lowest %Tp obtained was 0.509%. The results of the 1.10-Phenanthroline molecular geometry optimization using PM3 semi-empirical method get an optimal structure with a total energy of -43084 (kcal/mol). Electronic transition modeling using the ZINDO/s semi-empirical method shows that there are three peaks with peak height at a wavelength of 250.50 nm (1.8804) and involving four molecular orbitals, namely HOMO (32.33) and LUMO (34.35). The type of transition found in 3 peaks in compound 1.10-Phenanthroline shows the transition types n to π^* and π to π^*.

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Determination of Pregabalin in Bulk Drug and Pharmaceutical Formulations using Validated Stability-Indicating Spectrophotometric Methods

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Abstract- The present study describes the development and subsequent validation of stability-indicating, accurate, reliable, and sensitive spectro- photometric methods for the determination of Pregabalin in presence of its degradation products, including Dual wavelength and Ratio derivative after derivatization with vanillin reagent. With the Dual wavelength technique, Pregabalin could be determined in the range of 40-160 µg/mL at 390nm and 395.8nm. With the Ratio derivative technique, it could be determined in the above ranges at 401.6nm. All the methods were validated according to the International Conference on Harmonization guidelines and successfully applied to determine Pregabalin in pure form, laboratory-prepared mixtures, and pharmaceutical formulation. The obtained results were statistically compared with reported methods of analysis and there were no significant differences with respect to accuracy and precision of the adopted techniques.

Keywords: fibromyalgia; pregabalin; spectrophotometric methods; dual wavelength; ratio derivative; derivatization; vanillin reagent.

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Determination of Pregabalin in Bulk Drug and Pharmaceutical Formulations using Validated Stability-Indicating Spectrophotometric Methods

Maha F. Abdel-Ghany [α], Omar Abdel-Aziz [σ] & Eman W. E. Farag [ρ]

Abstract- The present study describes the development and subsequent validation of stability-indicating, accurate, reliable, and sensitive spectro- photometric methods for the determination of Pregabalin in presence of its degradation products, including Dual wavelength and Ratio derivative after derivatization with vanillin reagent. With the Dual wavelength technique, Pregabalin could be determined in the range of 40-160 µg/mL at 390nm and 395.8nm. With the Ratio derivative technique, it could be determined in the above ranges at 401.6nm. All the methods were validated according to the International Conference on Harmonization guidelines and successfully applied to determine Pregabalin in pure form, laboratory-prepared mixtures, and pharmaceutical formulation. The obtained results were statistically compared with reported methods of analysis and there were no significant differences with respect to accuracy and precision of the adopted techniques.

Keywords: fibromyalgia; pregabalin; spectro-photometric methods; dual wavelength; ratio derivative; derivatization; vanillin reagent.

I. Introduction

Pregabalin (Figure 1) is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures. It has also been found effective for generalized anxiety disorder.

Recent studies have shown that pregabalin is effective at treating chronic pain in disorders such as fibromyalgia and spinal cord injury.

No spectrophotometric methods were reported in major pharmacopeias like USP, EP and BP for determination of Pregabalin. Literature survey revealed many analytical methods for estimation of Pregabalin [1 - 13], However, no stability-indicating spectrophotometric method has been developed for bulk and pharmaceutical formulations.

The proposed methods were found to be easier than published HPLC methods for the determination of Pregabalin, for there is no need to use an internal standard, gradient elution, and time programming to adjust wavelengths. Moreover, the proposed methods are the first spectrophotometric methods for the determination of these drugs in presence of their degradation products. The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, less expensive, and less time-consuming than other published LC methods.

a) *Theoritical Background*

i. *Dual wavelength* [14]

This technique is used for binary mixtures for determination of one component without interference from the other. Two wavelengths are selected where the difference in absorbance of one component at these selected wavelengths is found to be zero, so, the difference in absorbance reflects only the concentration of one of the two components in the mixture.

ii. *Ratio Derivative Spectrophotometric method* [15, 16]

Salinas et al. proposed the spectrophotometric method termed ratio-derivative spectrophotometry, for the simultaneous determination of two compounds in binary mixtures. Their method is based on the derivative of the ratio spectra for a binary mixture. The absorption spectrum of the mixture is divided by the absorption spectrum of a standard solution of one of the compounds and the first derivative of the ratio spectrum is obtained. The concentration of active compounds are then determined from the calibration graphs obtained by measuring the amplitudes at points corresponding to the minimum or maximum wavelengths.

II. Materials and Methods

a) *Chemicals, Pharmaceutical Formulations and Reagents*

(a) *Pregabalin.:* Obtained from Optimus Drugs Ltd (Hyderabad, India).

(b) *75 and 150 mg capsules of Pregabalin (Irenypathic®).:* Produced by Amoun Pharmaceuticals Inc. (Cairo, Egypt).

(c) *Methanol, hydrochloric acid, sodium hydroxide, potassium permanganate, sulphuric acid, sodium sulphite, disodium hydrogen phosphate, citric acid anhydrous, ethyl alcohol (96%) and vanillin.*— Analytical reagent grade were purchased from Scharlau (Scharlab S.L, Sentmenat, Spain)

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(d) *Double distilled water.:* Prepared by using Millipore Milli-Q plus water purification system (Millipore Corp., Billerica, MA).

b) Equipments

(a) *Double-beam UV-Vis spectrophotometer (Shimadzu 1650 PC)* connected to a computer fitted with UVPC personal spectroscopy software version 2.42 (Shimadzu) was used to process absorption and derivative spectra.: (Shimadzu Corp., Kyoto, Japan).

(b) *Hotplate (WiseStir) with temperature controller*.: Used to carry out degradation studies for all solutions (Daihan Scientific Co. Ltd, Korea).

(c) *pH-meter (Orion).:* Equipped with combined glass electrode for pH adjustment (Thermo Scientific).

(d) *Ultrasonic bath.:* Elma (Danbury, CT).

c) Preparation of solutions

ICH guidelines Q1A_R2 (2.1.2) and Q2_R1(part II - 1.2) don't mention specified conditions or reagents for stress testing of drug substance. So, mild conditions (0.1N HCl or 0.1N NaOH) were used at first but didn't give complete degradation. So, drastic conditions were used to achieve complete degradation. $KMnO_4$ was used instead of hydrogen peroxide because it gave better results and the degradation reaction could be controlled or stopped on the contrary with hydrogen peroxide where the reaction could not be stopped.

1) *Stock standard solution.:* Stock standard solution of Pregabalin (5 mg/mL) was prepared by dissolving 500 mg of the drug in water, sonicated and completed to volume with the same solvent in a 100 mL volumetric flask. Then, 5 mL were further diluted to 50 mL with water. The required concentrations were prepared by serial dilutions.

2) *Oxidative-induced forced degradation of Pregabalin (1000 µg/mL). :* In a conical flask, 10 mL 0.1N $KMnO_4$ were added on 10 mL from stock standard solution (5mg/mL), the conical flask was covered with a funnel, heated on a hot plate - adjusted at 140 ^{0}C - for 60 min, cooled, then 0.5 mL of 4.5M H_2SO_4 and 1M sodium sulphite solution were added till discoloration, excess sulphuric acid was neutralized with 1M NaOH using a calibrated pH meter, and completed to 50 mL with water. Complete degradation was checked using HPLC.

Pregabalin is only sensitive to oxidative degradation. Acid, base, dry heat degradation and photo-degradation were tried but no significant change in the peak area appeared, indicating stability of Pregabalin to acid, alkaline, thermal and Photo-degradation.

3) *Sample preparation.:* Separately, the contents of 75 mg and 150 mg capsules were mixed, an amount equivalent to 500 mg of Pregabalin was accurately weighed, volume was completed to 100 mL with water then sonicated for 15 minutes and filtered. Then, 1 mL was further diluted to 50 mL with water.

4) *Vanillin reagent.:* Two grams of vanillin were weighed, volume was completed to 50 mL with ethyl alcohol (96%)

5) *Buffer pH 7.5.:* Prepared by mixing 35.5 mL of 0.2M disodium hydrogen phosphate anhydrous with 64.5 mL of 0.1M citric acid anhydrous, pH adjusted to 7.5 with 1M NaOH.

d) Procedures

The absorption spectra of the intact drug and its degradates are strongly overlapped, so application of the traditional spectral techniques failed to resolve this problem (Figure 2). On the other hand, this spectral overlapping was sufficient to demonstrate the resolving power of the proposed methods.

Pregabalin exhibits a very low UV absorption and as a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric methods. Pregabalin contains a primary aliphatic amino group, which is known to react with many color reagents as vanillin. Literature [34] shows that maximum absorbance intensities were achieved using 2 mL of buffer at pH 7.5. It was also found that 2 mL of Vanillin reagent was sufficient for production of maximum and reproducible color intensity. Time required for complete reaction at room temperature was 40 min. Heating leads to decrease in absorbance so reaction was done at room temperature.

This was further applied to perform the below mentioned methods under [*(2.4.1)* and *(2.4.)*] to determine pregabalin in presence of its degradation products.

i. *Dual wavelength (DWL)*

Laboratory prepared mixtures of different concentrations of the intact drug and its degradation product were recorded against blank in the range from 390nm to 430nm for Pregabalin (Figure 3). Determine the absorbance at 390nm and 395.8nm.

The concentrations of Pregabalin in each mixture was determined by calculating the difference in absorbance measured at these wavelengths.

ii. *Ratio Derivative Spectrophotometric method (RDer)*

The absorption spectra recorded in the previously mentioned method *(2.4.1)* was divided by its divisor of oxidative induced degradate spectrum and the first derivative of the absorption spectra obtained was computed. Pregabalin concentrations were determined in each mixture from the absorbance at the amplitudes 401.6nm (Figure 4).

e) Method validation

1. *Linearity.:* Accurately measured aliquots of stock standard solution *(2.3.1)* were separately transferred

into a series of 25 mL volumetric flasks, to produce 40 to 160 µg/mL, on each flask, 2.5 mL of oxidative-induced degradate *(2.3.2)* were added to produce 100 µg/mL, 2 mL of Vanillin reagent *(2.3.4)*, 2 mL of buffer pH 7.5 *(2.3.5)*, flasks were left in dark at room temperature for 40 minutes and then completed to volume with water. Each of these solutions was measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*.

2. *Accuracy.: Assay of drug in bulk powder.*— The mentioned procedures under *(2.4.1)* and *(2.4.2)* were repeated by measuring 80, 100, 120 µg/mL Pregabalin standard solutions, prepared from stock standard solution *(2.3.1)*, in triplicate after reaction with Vanillin reagent *(2.3.4)* in presence of buffer pH 7.5 *(2.3.5)* and the concentrations of Pregabalin were calculated by the corresponding regression equation.
3. *Specificity.:* In three separate flasks, accurately measured aliquots of standard stock solution *(2.3.1)* were transferred into a series of 25 mL volumetric flasks to produce 100 µg/mL each, accurately measured aliquots of oxidative-induced degradates *(2.3.2)* were added to produce 80, 100, 120 µg/mL each on a flask, and treated as mentioned under *(2.5.1)*. Each of these solutions was measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*.
4. *Precision.:* Six replicates of same concentration (100 µg/mL) were checked for repeatability. The intraday and interday variation for the determination of Pregabalin was carried out at three different concentration levels of 80, 100, 120 µg/mL as mentioned under *(2.4.1)* and *(2.4.2)* after treatment as mentioned under *(2.5.1)*.
5. *Assay of pharmaceutical dosage forms.:* For determination of Pregabalin in 75 mg or 150 mg capsules, from the sample solutions *(2.3.3),* aliquots were transferred to 25 mL volumetric flasks, treated as mentioned under *(2.5.1)* to produce 100 µg/mL and then measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*].

Further, standard addition technique was followed:

In three separate flasks, accurately measured aliquots of a previously analyzed sample solution of 75 mg capsules *(2.3.3)* were transferred into a series of 25 mL volumetric flasks to produce 100 µg/mL each, accurately measured aliquots of standard stock solution *(2.3.1)* were added to produce 20, 40, 60 µg/mL each on a flask, treated as mentioned under *(2.5.1)* and then measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*.

The same steps were repeated with sample solution 150 mg capsules *(2.3.3)*.

III. Results and Discussion

a) Method validation

Method validation was performed according to the ICH guidelines for the suggested spectrophotometric methods.

Linearity.: was evaluated by analyzing different concentrations of Pregabalin in the ranges of 40 to 160 µg/mL. The analysis was performed according to the experimental conditions previously mentioned in *(2.4.1) and (2.4.2)*. Results are summarized in (Table 1).

Accuracy.: The accuracy of the results was checked by applying the proposed methods for the determination of % recoveries of 3 different concentrations of the drug in bulk powder. The concentrations were obtained from the corresponding regression equation, and the recoveries were calculated (Table 4).

Precision.: Precision of the obtained results of three concentrations of Pregabalin (80, 100, 120 µg/mL) were evaluated by three replicate determinations to estimate the intraday and interday variations. Then, the RSD% was calculated (Table 1).

LOD and LOQ.: Approaches based on the SD of the intercept and the slope were used for determining the LOD and LOQ, where

LOD = 3.3 x SD/slope and LOQ = 10 x SD/slope

These were determined experimentally for the proposed methods and are presented in Table 1.

Specificity.: The developed methods were found to be specific and selective. The intact drug can be detected without interference from its degradation products and formulation excipients. Recovery and relative standard deviations were calculated. Results are summarized in Table 2.

b) Analytical applications

The proposed methods were successfully applied to commercial preparations, and the standard addition technique was performed. The concentrations were calculated using the corresponding regression equation. Results are summarized in Table 3.

c) Statistical analysis

A statistical comparison of the results obtained by the proposed methods and the reported method [3] for determination of Pregabalin was done. The significant difference between groups was tested by *t*-test as shown in Table 5. The test ascertained that there was no significant difference with respect to accuracy and precision between the proposed methods and the reported method [3].

IV. Conclusion

The paper describes simple, inexpensive, precise, accurate, and sensitive methods for determination of Pregabalin in bulk drug as well as in

pharmaceutical dosage forms, also, can separate the drug from its degradation products, so, can be described as stability-indicating assay methods. The minimum sample preparation and the speed of analysis are the main advantages of these methods over other analytical procedures, unlike the HPLC procedures, the instrument is simple and inexpensive using a small quantity of reagents, thus, cost and time saving.

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Table 1: Calibration data for the determination of Pregabalin by the proposed methods

Method	Range µg/mL	Regression equation (Y = bC + a)[a]	r^2	LOD, µg/mL	LOQ, µg/mL	Repeatability RSD%	Intraday RSD%	Interday RSD%
DWL	40 - 160	Y = 0.0 C - 0.021	0.9998	3.092	9.369	0.573	0.862	0.803
RDer	60 - 140	Y = 0.012 C + 0.069	0.9992	6.022	18.25	0.139	0.117	0.206

aa = Intercept, b = slope, and C = concentration of drug in µg/mL
P+OD= Pregabalin and oxidative degradates
DWL= Dual wavelength, RDiff= Ratio difference, RDer= Ratio Derivative

Table 2: Specificity results of Pregabalin using the proposed methods

Method	Claimed conc. (µg/mL)	Imp. conc. added (µg/mL)	Recovery %	Av. recovery ±RSD%	Method	Recovery %	Av. recovery ±RSD%
DWL	100	80	100.96	100.32 ±0.735	RDer	101.66	101.23 ±1.024
		100	99.52			100.05	
		120	100.48			101.98	

Table 3: Application of standard addition technique for the determination of Pregabalin in formulations using the proposed methods

Method	Claimed conc. (µg/mL)	Std conc. added (µg/mL)	Recovery % (dosage form 1)	Recovery% (dosage form 2)	Method	Recovery % (dosage form 1)	Recovery % (dosage form 2)
DWL	100	20	100.00	100.00	RDer	98.14	98.14
		40	98.84	100.00		98.45	98.39
		60	101.55	99.22		98.18	98.43
		Av. recovery ±RSD%	100.13 ±1.359	99.74 ±0.448	Av. recovery ±RSD%	98.25 ±0.171	98.32 ±0.159

Table 4: Results of recovery studies of Pregabalin

DWL		
Theoritical conc. (µg/mL)	Actual conc found (µg/mL)	Recovery%
80	78.97	98.71
100	97.28	97.28
120	100.76	120.91
RDer		
Theoritical conc. (µg/mL)	Actual conc found (µg/mL)	Recovery%
80	79.85	99.81
100	100.37	100.37
120	120.38	100.32

Table 5: Statistical comparison between the results of the proposed spectrophotometric methods and the reported method for determination of Pregabalin

Statistical term	Reported method	DWL (P+OD)	RDer (P+OD)
Accuracy			
mean recovery	100.258	100.13	98.25
Variance	0	1.852	0.028
t-test	2.776	0.164	-20.58
	(t-tabulated)	(t-calculated)	(t-calculated)
Precision - intraday			
mean recovery	100.059	100.36	99.25
Variance	0.241	0.918	0.986
RSD% mean	0.723	0.862	0.117
t-test	2.776	0.487	1.258
Precision - interday			
mean recovery	100.02	100.14	99.09
Variance	0.207	1.089	0.845
RSD% mean	0.762	0.803	0.206
t-test	2.776	0.18	-1.56

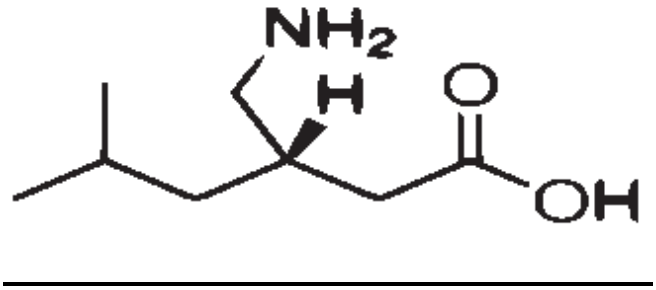

Figure 1: Pregabalin [(*S*)-3-(aminomethyl)-5-methylhexanoic acid] intact drug

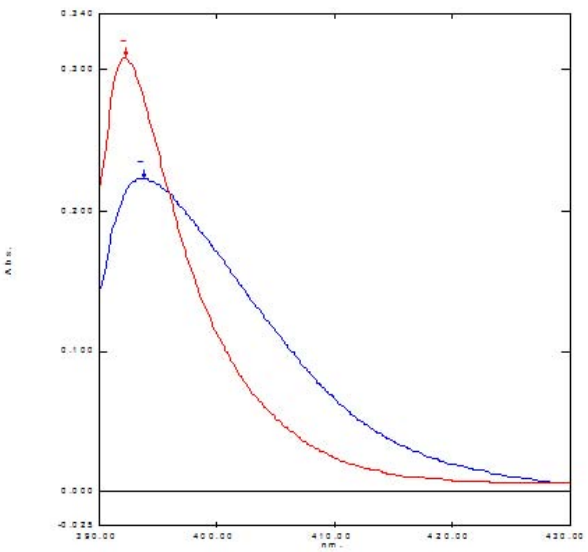

Figure 2: Pregabalin 0.1mg/ml (blue) + oxidative degradation 0.1mg/ml (red)

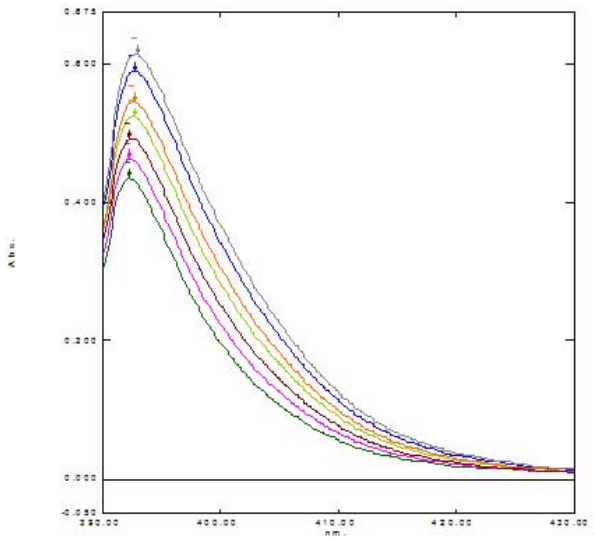

Figure 3: Pregabalin (40 - 160μg/ml) + oxidative degradation (100μg/ml) by Dual wavelength

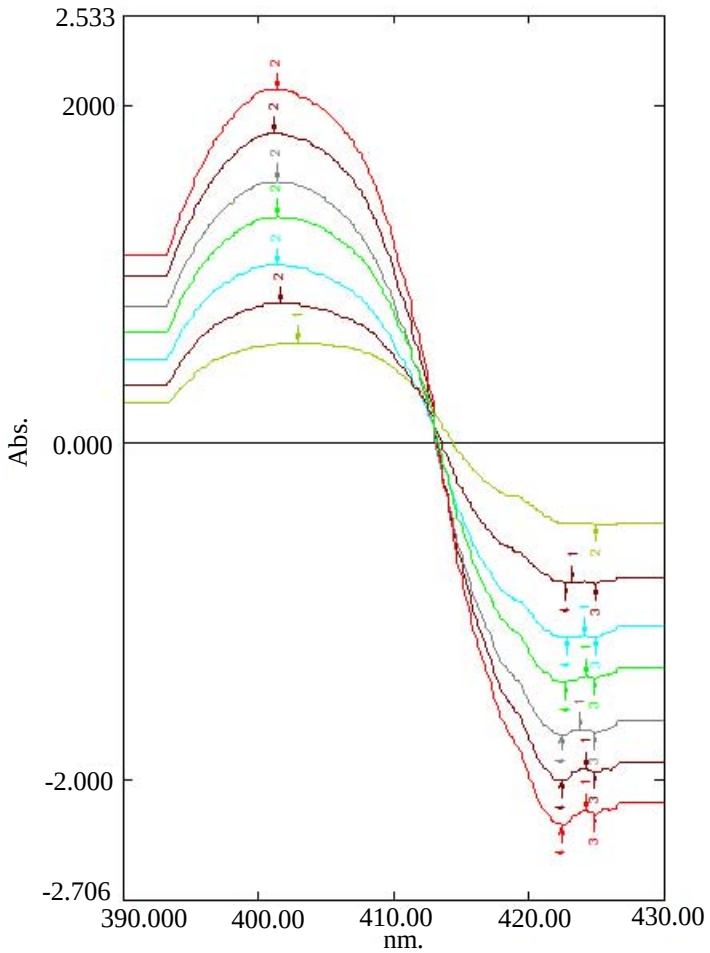

Figure 4: Pregabalin (40 - 160μg/ml) + oxidative degradation (100μg/ml) by Ratio derivative

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Investigation of Pesticidal Remainings in Environmental Matricies

By K. Sivasankar & Thommandru Raveendranath Babu
Rayalaseema University

Abstract- Determination of pesticidal recidues was made in this approach. To determine the persistence of pesticides with various activities present in environmental samples electro chemical techniques such as adsorptive stripping voltammetry, cyclic volatametry was applied. Carbon nano tubes paste electrode was used as working electrode. Average amounts for ten replicates founded by using standard addition method. Statistical concepts such as standard deviation and correlation coefficient successfully applied for calculations. In all the findings in this approach all the possible errors are minimised and accuracy is maximised. Water samples of a variety of areas collected and investigated for pesticide residues before and after the application of pesticides.

Keywords: *pesticides, adsorptive stripping voltammetry, carbon nano tubes paste electrodes, water samples.*

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Investigation of Pesticidal Remainings in Environmental Matricies

K. Sivasankar [α] & Thommandru Raveendranath Babu [σ]

Abstract- Determination of pesticidal recidues was made in this approach. To determine the persistence of pesticides with various activities present in environmental samples electro chemical techniques such as adsorptive stripping voltammetry, cyclic volatametry was applied. Carbon nano tubes paste electrode was used as working electrode. Average amounts for ten replicates founded by using standard addition method. Statistical concepts such as standard deviation and correlation coefficient successfully applied for calculations. In all the findings in this approach all the possible errors are minimised and accuracy is maximised. Water samples of a variety of areas collected and investigated for pesticide residues before and after the application of pesticides.

Keywords: pesticides, adsorptive stripping voltammetry, carbon nano tubes paste electrodes, water samples.

I. Introduction

When residual pesticides get into the environment they can remain poisonous and active for many years. If applied incorrectly or used in the wrong place, these chemicals may spread to other land areas and possibly to the water supply. There are good reasons (advantage spesticides are very effective. This means that nearly all the target pests which come in contact with these pesticides are killed Results are quick. This means the pests are killed within a very short time.

Using pesticides can be an economical (cheap) way of controlling pests. Pesticides can be applied quickly and there is not the high labour cost which might apply to other methods of control, such as removing weeds by hand [1-7].

If pesticides are not used correctly, they can affect human health or cause serious injury or death to the pesticide operator, other people or household pets. Pesticides can also directly affect other non-target animals. For example, a gardener spraying his garden to kill caterpillars will probably also kill harmless lady bird beetles and praying mantises. If pesticides are used incorrectly or applied wrongly, they may find their way into places where they are not wanted, for example, they might be washed into rivers or into the soil. In this article an elctroanalytical method voltammetry supported by statistical findings was applied.

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a) Apparatus and chemicals

Electro analytical investigations performed with a model meterohm Auto Lab 101 PG stat (Netherlands). Carbon nano tubes paste electrodes used as working electrode for determinations. pH measurements carried out with an Eutech PC_510 cyber scan. Meltzer Toledo (Japan) Xp26 delta range micro balancer were used to weigh the samples during the preparation of standard solutions. All the experiments were performed at 25^{0C}.

Reagents used are analytical reagent grade. Double distilled water was used entire the analysis. In the present investigation universal buffer of pH 4.0 was used as supporting electrolyte.

b) Outcome and deliberations

Single Well resolvable and reproducible peak obtained for every sample is useful for the analysis of water samples. The optimum pH to get well defined peak for the detection is found to be 4.0. The peak current is found to vary linearly with the concentration of the pesticide over the range 1.03×10^{-5}M to 1.06×10^{-9}M. The lower detection was limit found to be 1.07×10^{-9}M. The correlation coefficient and relative standard deviation (for 10 replicates) obtained using the above procedure [8-15].

c) Recovery experlments

A stock solution (1.0×10^{-3} M) of each sample is prepared in dimethyl formamide. In voltammetric cell, 1 mL of standard solution is taken and 9 mL of the supporting electrolyte (pH 4.0) is added to it. Then the solution is de aerated with nitrogen gas for 10 min. after obtaining the voltammogram, small additions of standard solution are added and the voltammograms are recorded under similar experimental conditions. The optimum conditions for analytical estimation at pH 4.0 are found to be pulse amplitude of 25 mV, applied potential of -0.35V and scan rate 40 $mVs.^{-1}$.

Water samples are collected from paddy fields which sprayed by the pesticides under investigation 48 hours after spraying the pesticides. These samples were filtered through a Whatman No.41 filter paper and Aliquots of water samples were taken in a 25mL graduated tube, to it buffer solution was added and analyzed as described above. The recoveries of samples obtained in water samples ranged from 51.00 to 57.00% and the results are summarized in Table 1.0.

Table 1.0: Recoveries of pesticides in water samples

Name of the pesticide	Amount added (mg/L)	Amount found (mg/L)	*Recovery (%)	Standard deviation
1. valone	8.0	4.15	51.75	0.04
2. monalid	8.0	4.36	54.50	0.07
3. Fentrieanil	8.0	4.31	53.87	0.12
4. Dinosam	8.0	4.25	53.25	0.07
5. crotametone	8.0	4.10	51.25	0.05
6. flurethrin	8.0	4.18	52.25	0.06
7. dinitramine	8.0	4.22	52.75	0.05
8. oxabetrinill	8.0	4.26	53.25	0.03

**Average of 10 replicates*

II. Conclusions

In this paper voltammetry for the determination of pesticide residues satisfactory applied to detect residual pesticides without considerable errors. And during the estimations pollution arises due to heavy metal electrodes such as mercury electrodes is avoided by using carbon electrodes.

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- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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