Online ISSN : 2249-4596
Print ISSN : 0975-5861

Global Journal

of Researches in Engineering: C

Chemical Engineering

Influence of Scan Rate

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Highlights

Simple Vacuum Distillation

Differential Scanning Calorimetry

Discovering Thoughts, Inventing Future

VOLUME 15 ISSUE 3 VERSION 1.0

Global Journal of Researches in Engineering: C
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Chemical Engineering
Volume 15 Issue 3 (Ver. 1.0)

Open Association of Research Society

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Packaging & Continental Dispatching

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Contents of the Issue

Global Journal of Researches in Engineering: C
Chemical Engineering
Volume 15 Issue 3 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4596 & Print ISSN: 0975-5861

Simple Vacuum Distillation of Vetiver Oil from Smallholders for Quality Improvement

By I Dewa Gede Arsa Putrawan & Eric Farda

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Abstract- This research is aimed to improve the quality of vetiver oils from smallholders in Indonesia by vacuum distillation. The most important parameters of quality mentioned are total vetiverol content and color. It was shown that vetiverol contents could be increased to achieve the required minimum content of 50%. The better the initial sample, the better the distillate obtained. Distillate fractions obeying standard vetiverol content could be obtained with yield of 60%~80%. Although the initial samples were black in color, the distillates had appearance from yellow to reddish brown, as required by the standard, with Gardner scales of color ranging from 10.8 to 14.7. Distillation, however, slightly disturbed the achievement of other parameters including density, acid number and ester number. Lower distillation fractions tend to shift the values of these parameters to out of standards.

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GJRE-C Classification: *FOR Code: 090499*

Strictly as per the compliance and regulations of:

Simple Vacuum Distillation of Vetiver Oil from Smallholders for Quality Improvement

I Dewa Gede Arsa Putrawan [α] & Eric Farda [σ]

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I. Introduction

Vetiver oil is an essential oil extracted from the roots of *Vetiver grass (Chrysopogon zizanioides, Linn Nash)*, a tropical grass growing wild or cultivated. The extraction can be done via water distillation, steam distillation, solvent extraction or expression (Dowthwaite and Rajani, 2000). As an essential oil, vetiver oil will find wider application due to the increasing tendency towards the use of natural materials. The classical roles of vetiver oil are flavor, fragrance, and aromatherapy (Lavania, 2003). It has been observed also that the vetiver oil has activities such as antimicroorganism, antioxidant, insectiside, and sedative.

Indonesia produces 60 to 75 tons of vetiver oil annually and becomes the main vetiver oil suppliers in the words, besides Haiti and La Réunion. About 90% of Indonesia vetiver oil is produced by smallholders in Garut, a small district about 200 km from Jakarta to the south-east. It is produced by water distillation at 4 to 5 bars in the conventional ways. The main equipment consists of a boiling vessel, a burner, and a condenser.Vetiver roots are packed on grids over a layer of water in the base of the vessel. Saturated steam which is produced by direct firing the vessel extracts the essential oil contained in the roots. Essential oil containing steam is then condensed to obtain vetiver oil. Due to primitive equipment and the lack of process control, the quality of most vetiver oil obtained could not satisfy the required standard. Minimum total vetiverol content of 50% is hard to obtain. In addition, the color of the vetiver oil is very dark, from dark brown to black. It is supposed due to overheating and oxidative degradation which results in tar like substances which are hard to separate by physical methods. Standard on vetiver oil requires the color from light yellow to reddish brown. Further treatment, therefore, is necessary to improve such vetiver oils in order to comply with standard qualities.

Many works have been published on vetiver oil. Aggarwal et al. (1998) studied the effects of harvest period, storage period after harvest, roots treatment and distillation time on the yield of extraction by distillation. They found that the extractable oil decreased with harvest delay and storage period. In addition, they found that cutting the roots did not affect oil yield significantly, water distillation gave oil recovery a little bit higher than steam distillation (0.28% vs 0.23%, based on dry root weight). Danh et al. (2010) have studied the extraction by an unconventional method, i.e., an extraction by supercritical CO_2 with ethanol as co-solvent. They found that pressure and ethanol concentration linearly affected oil yield but, in contrary, temperature in the range of 40 to 50 °C did not influence oil yield. It was found also that supercritical fluid extraction did not extract the metals in the vetiver roots giving an advantage for strict applications, such as foods and drugs. The effects of plant environments on the quantity and quality of extracted oil have attracted the attention of researchers. Adams et al. (2004) reported that cleansed vetivers gave lower oil yield with strictly different composition to those of non-cleansed vetivers. They found that the yield of oil from bacteria and fungi free vetivers was almost twenty times lower and contained large amounts of C_{19}-C_{29} alkanes. The roles of microbes were also reported by Pripdeevech et al. (2006) who obtained larger oil yield and higher content of some low molecular weight volatiles from vetiver plants grown in normal soil with added microbes compared to normal soil (0.27% vs 0.18%). Adams et al. (2008), however, reported no correspondence between *arbuscular mycorrhizal fungi* colonization and oil yield, and postulated that the composition of vetiver oils are majorly controlled by vetiver genes. Massardo et al. (2006) reported variations in oil yield and in the contents of isovalencenol and

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khusimol in the obtained oils during growth of roots. The effects of climatic conditions have also been studied by Kotoky et al. (2011) who found that rain-fall played a role on the yield and quality of vetiver oils. Their data indicated that vetiver oil quality is closely related to the metabolism of its root which is influenced by climatic conditions. The complexicity of vetiver oil composition has challenged many researchers in developing qualitative and quantitative analysis, among them are Cazzausus et al. (1988), Weyerstahl et al. (200a, 200b), Paillat et al. (2012) and Filippi et al. (2013). The works of Weyerstahl et al. (2000a, 2000b) and Filippi et al. (2013) are very intensive. In recent years, biological functionalities of vetiver oil have much attention. The antibacterial and antifungal activities of vetiver oil have been reported by Prabuseenivasan (2006), Gupta et al. (2012), and Sangeetha and Stella (2012). The insecticidal activities of vetiver oil have been reported by Zhu et al. (2001a, 2001b). The antioxidant, antinociceptive, and anti-inflammatory activities, the sedative effects, and the toxicity of vetiver oil have also been studied (Kim et al., 2005, Lima et al., 2012, Thubthimthed et al., 2003, Sinha et al., 2014). Apart from the many works, no attention has been paid to upgrade the poor quality of crude vetiver oil which commonly found from smallholders.

II. Materials and Methods

Fig. 1 shows the schematic of the distillation apparatus. The apparatus mainly consisted of a distillation column with insulation, a condenser and a condensate collector. The apparatus was equipped with a cooling water system with controlled temperature and connected to a vacuum system. The pressure inside the apparatus was controlled by a needle valve and was set at 10 mmHg. Boiling points were measured by using a thermometer. Crude vetiver oil of 300 ml was used for each run. Distillates were collected at every 20% recovery until 80% volume. Three vetiver oil samples from small distillers were used. The parameters observed included density, refractive index, acid number, ester number, ester number after acetylation, and vetiverol content. Density was measured by a piknometer. Refractive index was measured using a refractometer. Acid number was measured by titration. Ester number was measured by saponification. Acetylation was done using acetic anhydride. The content of free alcohol as vetiverol was calculated from ester number of original oil and that of acetilated oil. The parameters observed also included color, odor, and solubility in ethanol. Color was observed visually and also measured by a Lovibond Tintometer. Solubility in ethanol was observed as “soluble” or “not soluble” in ethanol at volume ratio of 1:1. All chemicals for analysis were of pro analysis grade.

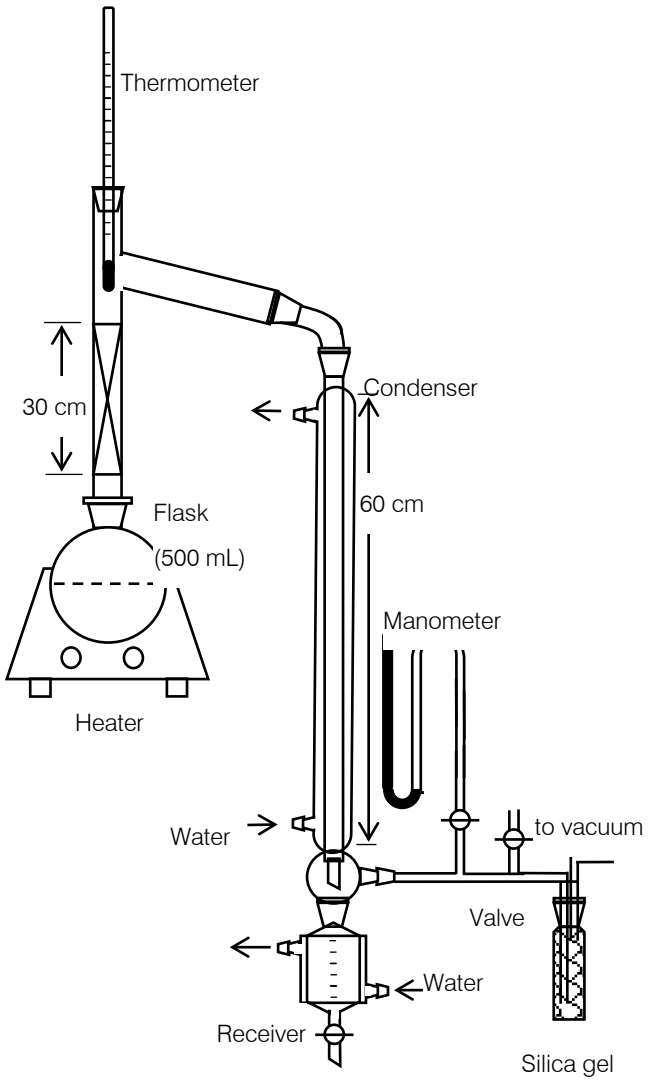

Fig. 1 : Schematic of experimental apparatus

III. Results and Discussion

a) Vetiver Oil Samples

The characteristics of three vetiver oil samples studied here are given in Table 1, columns 3 to 5. The Indonesia standard quality (SNI, 2006) are shown in the rightmost column. As shown in the table, all three samples had vetiverol contents lower than the required standard. Moreover, they smelt smoky and their color were dark black. These three quality parameters are commonly fail to achieve by most smallholders. It is due to poor distillation operation. Many factors affecting the distillation of vetiver root as discussed by Aggarwal (1998) which are not considered in the field. The smoky odor comes from soil materials which were not removed prior to distillation. The vetiverol content appeared to correlate with density. In this case, lighter vetiver oil exhibited higher vetiverol content. All samples were soluble in ethanol at 1:1 volume ratio.

Table 1 also shows the characteristics of Indonesian vetiver oil samples from Guenter [1950], columns 6 to 9. This author procured four samples of vetiver oils from Indonesia in the past, said to be genuine samples, with physicochemical properties as shown in Table 1. The data was completed with vetiverol contents which were calculated in this work from the ester numbers. In regards to vetiverol content, the samples used in this work were inferior to those from Guenter [1950]. The samples used here had higher ester numbers but lower ester numbers after acetylation resulting in lower total vetiverols. In contrast to the samples of this work, the genuine samples from Guenter [1950] complied with the required standards.

Table 1 : Characteristics of vetiver oil samples

#	Parameter	Samples in this work			Guenther (1950)†				Standard‡
		A	B	C	I	II	III	IV	
1	Relative density (20/20 °C)	0.987	0.994	1.024	1.009	1.009	1.007	0.991	0.980 to 1.003
2	Refractive index (20 °C)	1.52	1.52	1.52	1.526	1.5271	1.526	1.5258	1.52 to 1.53
3	Acid number (mg KOH/g)	27	30	34	31.1	32.5	30.2	12.5	10 to 35
4	Ester number (mg KOH/g)	24	22	26	2	12.6	1	4	5 to 26
5	Ester number after acetylation (mg KOH/g)	133	124	117	141.5	150	152.1	129	100 to 150
6	Total vetiverol (%)	46	43	39	61*	60*	67*	54*	Minimum 50%
7	Color	Too dark	Too dark	Too dark	NA	NA	NA	NA	Pale yellow to Reddish brown
8	Odor	Smoky	Smoky	Smoky	NA	NA	NA	NA	Vetiver characteristic
9	Solubility in ethanol 95% (1:1)	Clear	Clear	Clear	NA	NA	NA	NA	Clear
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9	Solubility in ethanol 95% (1:1)	Clear	Clear	Clear	NA	NA	NA	NA	Clear

*†Genuine samples analyzed by Guenther (1950). *Calculated in this work from ester numbers. ‡National Indonesian Standard. NA: not available*

b) Distributions of Physicochemical Properties

Fig. 2 to 7 illustrate the distribution of physico-chemical properties at various distillate recovery. The densities of distillates varied in the range of 0.94 to 1.00, as shown in Fig. 2. As can be expected, lighter distillate distillate had lower relative density. All distillates from samples A and B had density lower than the required minimum density, although their initial sample obeyed the required standard density. The density of sample C was higher than the upper limit of standard density. However, its distillates from 60% to 80% recovery were in the range of standard density. As seen in the figure, the range of standard density is narrow. The deviation in densities were actually not significant, only at the second decimals. The largest was shown by 20% recovery of sample A (a deviation of 0.04 from the lower limit). Fig. 3 shows that the refractive index did not vary significantly. All distillates had refractive index in or close to the range of 1.52 to 1.53, except at 20%-recovery.

In contrast to essential oil in general, vetiver oil exhibited high content of free fatty acid. The contents of free fatty acid as acid numbers are shown in Fig. 4. In general, acid number increased with %-recovery. Lower %-recovery tend to decrease acid number and make the acid number lower than the minimum acid number. The distillation cuts between 40% and 60% and between 60% and 80% had acid numbers significantly larger than the other lighter distillation cuts. This indicated that acid

compounds in vetiver oil concentrate at the heavier fractions.

Fig. 5 and Fig. 6 shows the ester numbers before and after acetylation, respectively. Ester number before acetylation in general increased with %-recovery but ester number after acetylation decreased with %-recovery. Most of distillate obeyed the standard for ester numbers before acetylation. Ester numbers after acetylation of light distillate (20% to 40%) were higher than the maximum allowed numbers. As difference in these two ester numbers indicates vetiverol content, this indicated that the viteverol content should be higher at the lighter distillate.

Fig. 7 shows the distribution of vetiverol content. Vetiverol contents were found to decrease with distilate recovery. The better the sample, the higher the vetiverol content of the distillate obtained. Minimum vetiverol content of 50% could be obtained from samples A and B until 60% recovery and almost achieved at 80% recovery. It means that the vetiverol content of samples A and B could be improved to very close to the required standard at the expense of 20% oil lost. With sample C, however, more quantity have to be sacrificed to achieve standard content of vetiverol. At 80% recovery, sample C gave distillate with a vetiverol content of 42%. It is significantly lower than the required standard, although the vetiverol contents have improved compared to the initial samples. Minimum vetiverol content of 50% could be achieved until 60% recovery for sample C.

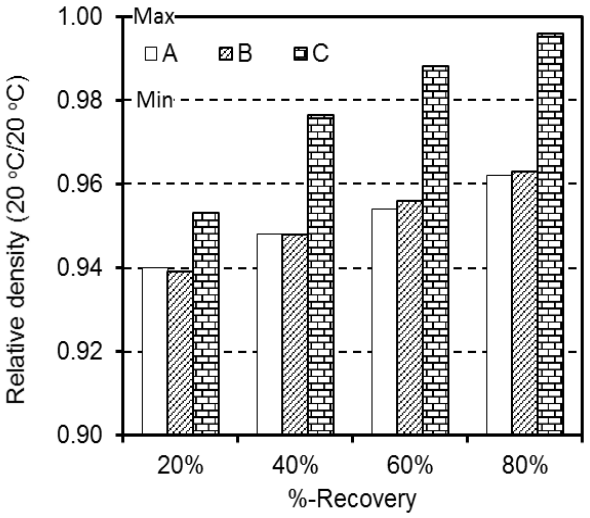

Fig. 2 : Distribution of relative density

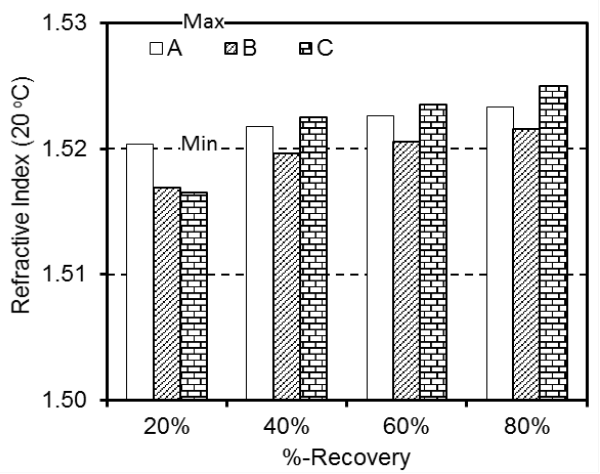

Fig. 3 : Distribution of refractive index

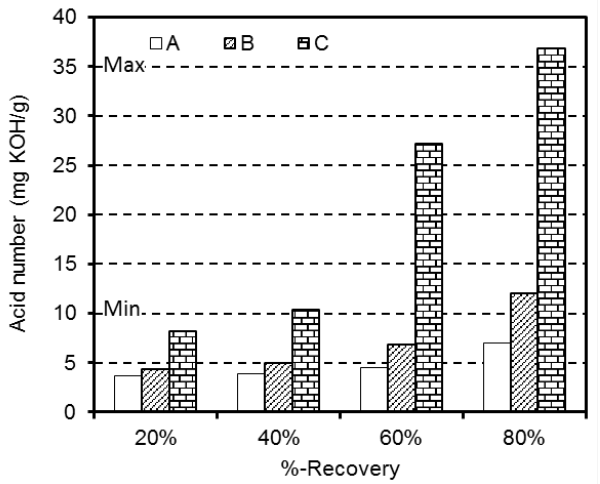

Fig. 4 : Distribution of acid number

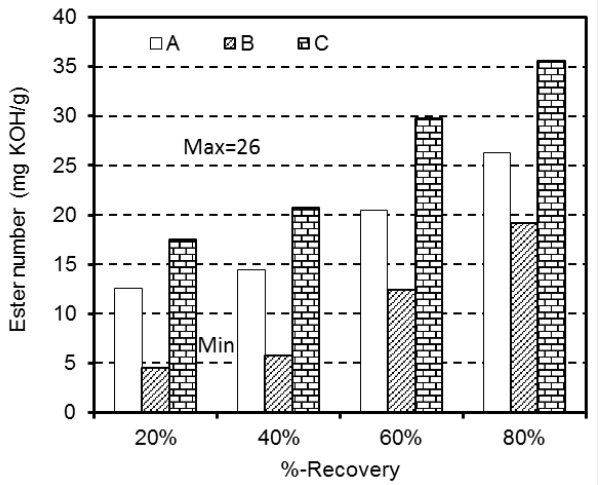

Fig. 5 : Distribution of ester number

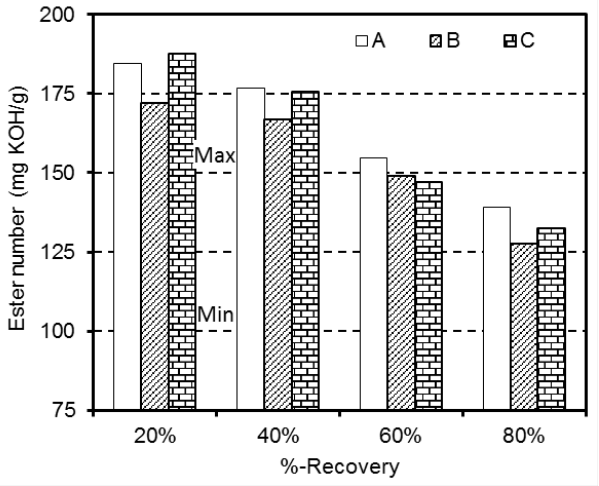

Fig. 6 : Distribution of ester number after acetylation

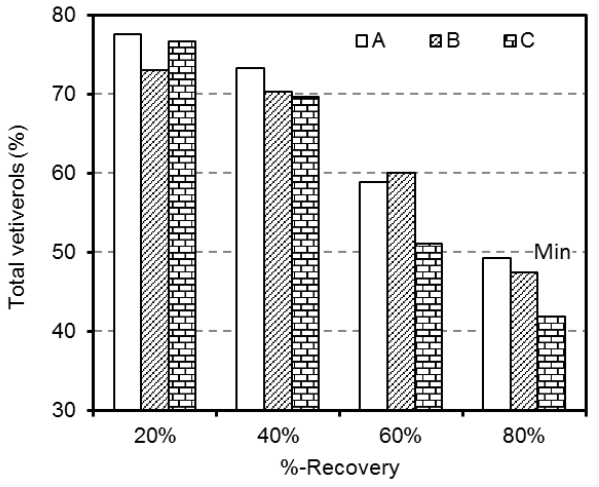

Fig. 7 : Distribution of vetiverol content.

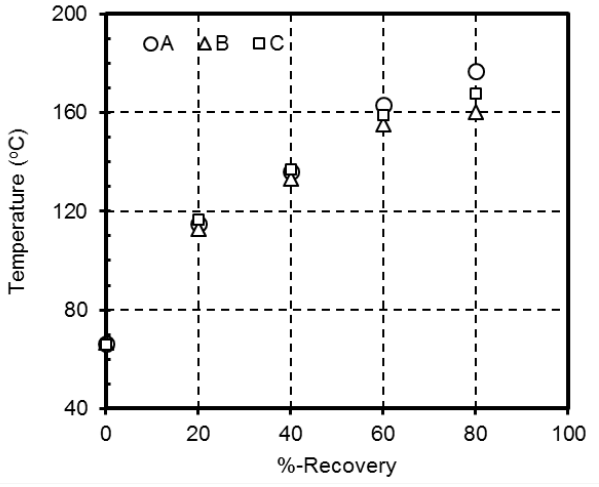

Fig. 8 : Distribution of boiling point

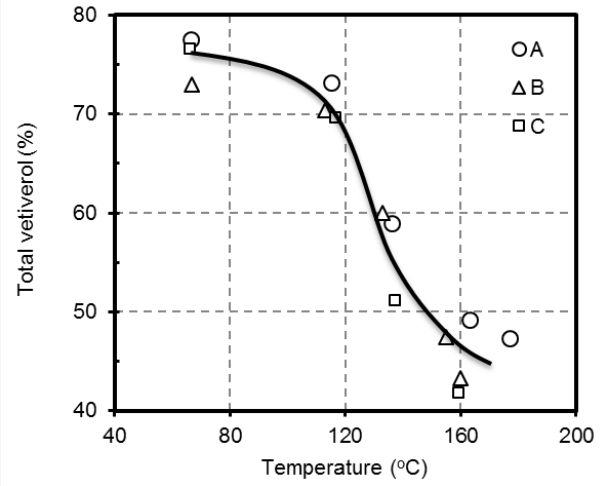

Fig. 9 : Vetiverol content vs boiling point.

Fig. 8 shows the temperature curves of distillation. The boiling temperatures of all samples were close each other, although they were getting away along with temperature. The relationship between vetiverol content and boiling point are shown in Fig. 9. Although the data is rather scattered, they could be approximated by a third order polynomial. More importantly, it clearly shows that lower distillation temperature gave higher vetiverol content. This reveals that the impurities are concentrated at the heavy fractions and distilling the samples under vacuum improved their vetiverol contents.

There are no references for the complete distribution of acid and ester numbers and vetiverol content in respect to vetiver oil distillation cuts. Gildemeister (1913) and Weyerstahl et al. (2000a) presented several distillation cuts of vetiver oils, however, for the purposes of showing the cuts in which individual components are concentrated. The results here showed that all parameters regarding compound groups in vetiver oil were continueously distributed. This indicated that vetiver oil composition is quite complex, consisting of many components with a wide boiling point range. Hundreds of components have been identified (Weyerstahl et al., 2000b and Filippi et al., 2013). However, many components are still unidentified.

c) Color, Odor and Ethanol Solubility

All distillates were soluble in ethanol at volume ratio of 1:1. The Indonesian standard qualitatively requires that vetiver oil has color from pale yellow to reddish brown. None of the vetiver oil samples studied here comply with this color standard. The colors of all samples were dark (almost black). In contrast, all distillates complied with the required qualitative standard. Although there is no quantitative standard for color of vetiver oils, popular color scales for oils and fats or chemicals in general were used in this study to describe the colors of distillates. Sample C was selected to study the color as sample C was the darkest sample.

The colors of distillates from sample C were measured using a Lovibond Tintometer. Table 2 shows the results in the scales of Lovibond RYBN, AOCS RY, FAC, Gardner, and ASTM. Lovibond RYBN scale is based on different densities of red, yellow, blue and neutral. The AOCS-Tintometer Color Scale is a special red and yellow version of Lovibond RYBN. FAC Scale is divided into 5 groups: Scale 1 (1, 3, 5, 7, 9) for lighter colored fats; Scale 2 (11, 11a, 11b, 11c) predominantly for yellow fats; Scale 3 (13, 15, 17, 19) for dark fats (red cast); Scale 5 (31, 33, 35, 37, 39, 41, 43, 45) for very dark fats, predominantly red. The Gardner scale ranges from a pale yellow to a red in shade and is described in terms of the values 1-18. ASTM is a single number, one dimensional, color scale ranging from a pale straw through to a deep red. The Lovibond RYBN and AOCS RY scales showed that the basic colors of distillates from Sample C were red and yellow. In addition, the densities of red and yellow increased with distillate recovery, causing the appearance of distillate is getting reddish brown as distillate recovery increased. Similarly, considering the FAC, Gardner and ASTM scales, it could be seen that the color of distillate became reddish as the recovery increased. Thus, brown become predominant in 80%-recovery, however, it was still reddish brown. The odor, however, could not be removed. All distillates still smell of smoky, thus, the distillates need further treatment for odor removal.

Table 2 : Color of distillates from Sample C

Recovery	Lovibond RYBN				AOCS RY		FAC	Gardner	ASTM	Visual
	Red	Yellow	Blue	Neutral	Red	Yellow				
20%	2.4	39	0	0.1	2.3	32	11	10.8	2.0	Light yellow
40%	3.0	46	0	0.1	2.9	40	11	11.2	2.1	Light yellow
60%	3.3	57	0	0.1	3.3	50	11	11.6	2.3	Light yellow
80%	10.9	70	0	0.1	9.9	70	33	14.7	3.9	Reddish brown

IV. Conclusion

A Simple vacuum distillation has been used to improve the quality of vetiver oils from smallholders. Vetiverol content, which is the difficult quality parameter to achieve could be improved. For all samples, the Indonesian standard on vetiverol content could be obeyed by distilling 60% samples. The better the sample, the less volume of initial oil that have to be sacrificed to achieve the standard vetiverol content. The standard vetiverol content could be achieved from samples A and B, which are better than sample C, until 80% recovery. Although all sample appeared to black and did not meet the standard appearance, standard in color could be achieve by all distillates. The distillate had appearance from yellow to reddish brown, with Gadner scale of color ranging from 10.8 to 14.7. Distillation, however, slightly disturbed the achievement of other parameters including density, acid number and ester number. Lower distillation fraction tend to shift the values of these parameters from their standards. The odor however could not be removed.

V. Acknowledgment

The assistances of color measurement from P.T. Süd-Chemie Indonesia are greatly appreciated.

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Global Journal of Researches in Engineering: C
Chemical Engineering
Volume 15 Issue 3 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4596 & Print ISSN: 0975-5861

Chemical and Electrochemical Study on the Effectively of *Melilotus Officinalis* Extract as Save Corrosion Inhibitor for Aluminium in 1 M Hydrochloric Acid Solutions

By Abd El-Aziz S. Fouda, Rabab Abo-Shohba, Walaa M. Husien & Esraa S. Ahmed
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Keywords: *acidic corrosion, aluminium, melilotus officinalis extract, EIS, EFM, SEM.*

GJRE-C Classification: *FOR Code: 290699*

Strictly as per the compliance and regulations of:

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I. Introduction

Corrosion is a fundamental process playing an important role in economics and safety, particularly for metals. The use of inhibitors is one of the most practical methods for protection against corrosion, especially in acidic media [1]. Most well-known acid inhibitor are organic compounds containing nitrogen , sulfur, and oxygen atoms. Among them, organic inhibitors have many advantages such as high inhibition efficiency and easy production [2-5]. Organic heterocyclic compounds have been used for the corrosion inhibition of iron [6-9], copper [10], aluminum [11-13], and other metals [14-15] in different corroding media. Although many of these compounds have high inhibition efficiencies, several have undesirable side effects, even in very small concentrations, due to their toxicity to humans, deleterious environmental effects, and high-cost [16].

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Plant extract is low-cost and environmental safe, so the main advantages of using plant extracts as corrosion inhibitor are economic and safe environment. Up till now, many plant extracts have been used as effective corrosion inhibitors for aluminium in acidic media, such as: Garlic [17], Black Mulberry [18], Piper Guineense seed [19] Red onion skin [20]. The inhibition performance of plant extract is normally ascribed to the presence of complex organic species, including tannins, alkaloids and nitrogen bases, carbohydrates and proteins as well as hydrolysis products in their composition. These organic compounds usually contain polar functions with nitrogen, sulfur, or oxygen atoms and have triple or conjugated double bonds with aromatic rings in their molecular structures, which are the major adsorption centers.

Melilotus officinalis extract (MOE), belongs to the family Leguminosae (Fabaceae). It exhibits several medicinal properties, this plant is mainly used for agricultural purposes. It is grown as hay despite its toxic properties when moldy. It is considered an excellent green manure. Sweet clover is a major source of nectar for domestic honey bees. Flowers and seeds can be used as flavoring Melilotus officinalis has been used as a phytoremediation—phytodegradation plant for treatment of soils contaminated with dioxins [21].

The present work was designed to study the inhibitory action of Melilotus officinalis for the corrosion of aluminium in 1 M HCl using Chemical and electrochemical techniques, and to study the effect of temperature on the rate of corrosion

II. Experimental Methods

a) Materials and Solutions

Aluminium used has the chemical composition (% weight) 0.30 Si; 0.60 Fe; 0.10 Cu; 1.40 Mn; 0.05 Mg; 0.05 Cr; 0.05 Ti and the rest aluminium. The auxiliary electrode was a platinum wire (1 cm^2), while a saturated calomel electrode (SCE) connected to a conventional electrolytic cell of capacity 100 ml via a bridge with a Luggin capillary, the tip of which was very close to the surface of the working electrode to minimize the IR drop. The aggressive solution used was prepared by dilution

of analytical reagent grade 37% HCl with bidistilled water. The stock solution (1000 ppm) of Melilotus officinalis was used to prepare the desired concentrations by dilution with bidistilled water. The concentration range of MOE used was 50-300 ppm.

b) Preparation of plant extracts

Fresh aerial parts of MOE sample were crushed to make fine powder. The powdered materials (250 g) were soaked in 500 ml of dichloromethane for 5 days and then subjected to repeated extraction with 5× 50 ml until exhaustion of plant materials. The extracts obtained were then concentrated under reduced pressure using rotary evaporator at temperature below 50°C. The dichloromethane evaporated to give solid extract that was prepared for application as corrosion inhibitor. Chemical studies have demonstrated that the main chemical constituents of Melilotus officinalis are the glycosides of coumaric acid, especially melitoside, which by hydrolysis of lactonises gives coumarin. Free coumarin, 3,4-dihydroxycoumarin (melilotin), scopoletin and umbelliferone are also present [22].

c) Weight loss measurements

Seven parallel aluminium sheets of 2×2×0.2 cm were abraded with emery paper (grade 320–500–1200) and then washed with bidistilled water and acetone. After accurate weighing, the specimens were immersed in a 250 ml beaker, which contained 100 ml of HCl with and without addition of different concentrations of Melilotus officinalis. All the aggressive acid solutions were open to air. After 180 minutes, the specimens were taken out, washed, dried, and weighed accurately. The average weight loss of seven parallel aluminium sheets could be obtained. The inhibition efficiency (IE%) and the degree of surface coverage, θ of MOE for the corrosion of Al were calculated as follows [23]:

$$IE\% = \theta \times 100 = \left[1 - \frac{W}{W^{\circ}}\right] \times 100 \qquad (1)$$

where W° and W are the values of the average weight losses without and with addition of the inhibitor, respectively.

d) Gasometric measurements

The gasometric method assembly used for the measurement of hydrogen gas evolution from the corrosion reaction was designed following the method described by Onuchukwu [24]. The gasometric assembly measures the volume of hydrogen gas evolution from the reaction system. Sevenaluminium coupons of dimension 2 x 2 x 0.2cm were used in the experiments for test solutions containing 1 M HCl with the six different concentrations of MOE and the blank at 25°C. A 50ml of each test solution was introduced into the reaction vessel connected to a burette through a delivery tube. The initial volume of air in the burette was recorded. Thereafter, one aluminium coupon was dropped into the corrodent and the reaction vessel quickly closed. Variation in the volume of hydrogen gas evolved with time was recorded every 1min. for 80 min. Each experiment was conducted on a fresh specimen of metal coupon. The hydrogen gas evolved displaced the paraffin water in the gasometric set-up and the displacement representing the volume of hydrogen evolved was read directly. The experiment was repeated in the presence of the six different concentrations of MOE, 50 to 300 ppm asused in the weight loss experiments.

e) Electrochemical measurements

Electrochemical measurements were performed using a typical three-compartments glass cell consisting of the aluminium specimen as working electrode (1 cm^2), saturated calomel electrode (SCE) as a reference electrode, and a platinum wire as a counter electrode. The reference electrode was connected to a Luggin capillary and the tip of the Luggin capillary is made very close to the surface of the working electrode to minimize IR drop. All the measurements were done in solutions open to atmosphere under unstirred conditions. All potential values were reported versus SCE. Prior to each experiment, the electrode was abraded with successive different grades of emery paper, degreased with acetone, also washed with bidistilled water, and finally dried. Tafel polarization curves were obtained by changing the electrode potential automatically from (-0.8 to 1 V vs. SCE) at open circuit potential with a scan rate of 1 mVs^{-1}. Stern-Geary method [25], used for the determination of corrosion current is performed by extrapolation of anodic and cathodic Tafel lines to a point which gives (log i_{corr}) and the corresponding corrosion potential (E_{corr}) for inhibitor free acid and for each concentration of inhibitor. Then (i_{corr}) was used for calculation of inhibition efficiency (IE %) and surface coverage (θ) as in equation 2:

$$IE\% = \Theta \times 100 = \left[1 - \frac{i_{corr\ (inh)}}{icorr\ _{(free\ )}}\right] \times 100 \qquad (2)$$

Where $i_{corr(free)}$ and $i_{corr(inh)}$ are the corrosion current densities in the absence and presence of inhibitor, respectively.

Impedance measurements were carried out in frequency range ($2x10^4$ Hz to $8x10^{-2}$ Hz) with amplitude of 5 mV peak-to-peak using AC signals at open circuit potential. The experimental impedance was analyzed and interpreted based on the equivalent circuit. The main parameters deduced from the analysis of Nyquist diagram are the charge transfer resistance R_{ct} (diameter of high-frequency loop) and the double layer capacity C_{dl}. The inhibition efficiencies and the surface coverage (θ) obtained from the impedance measurements are calculated from equation 3:

$$IE\% = \Theta \times 100 = \left[1 - \left(\frac{R_{ct}^{\circ}}{R_{ct}}\right)\right] \times 100 \qquad (3)$$

Where R^{o}_{ct} and R_{ct} are the charge transfer resistance in the absence and presence of inhibitor, respectively.

Electrochemical frequency modulation, EFM, was carried out using two frequencies 2 and 5 Hz. The large peaks were used to calculate the corrosion current density (i_{corr}), the Tafel slopes (β_a and β_c) and the causality factors CF-2&CF-3 [26]. The electrode potential was allowed to stabilize 30 min before starting the measurements. All the experiments were conducted at 25°C.

All electrochemical measurements were performed using Gamry Instrument (PCI4/750) Potentiostat/ Galvanostat/ZRA. This includes a Gamry framework system based on the ESA 400. Gamry applications include DC105 software for potentio-dynamic polarization, EIS 300 software for electro-chemical impedance spectroscopy, and EFM 140 software for electrochemical frequency modulation measurements via computer for collecting data. Echem Analyst 6.03 software was used for plotting, graphing, and fitting data. To test the reliability and reproducibility of the measurements, duplicate experiments, which performed in each case at the same conditions.

f) Surface morphology

For morphological study, surface features (2 x 2 x 0.2cm) of aluminium were examined before and after exposure to 1 M HCl solutions for 24 hour with and without extract. JEOL JSM-5500 scanning electron microscope was used for this investigation.

III. Results and Discussion

a) Weight loss measurements

Weight loss measurements were carried out for aluminium in 1 M HCl in the absence and presence of different concentrations of Melilotus officinalis and are shown in Figure (1). The inhibition efficiency (IE%) values calculated are listed in Table (1). From this table, it is noted that the IE% increases steadily with increasing the concentration of *Melilotus officinalis* and with increasing temperature from 25-45°C. The inhibition efficiency (IE%) and surface coverage (θ)were calculated by equation (1). The observed inhibition action of the MOE could be attributed to the adsorption of its components on aluminium surface. The formed layer, of the adsorbed molecules, isolates the metal surface from the aggressive medium which limits the dissolution of the latter by blocking of their corrosion sites and hence decreasing the corrosion rate, with increasing efficiency as their concentrations increase [27].

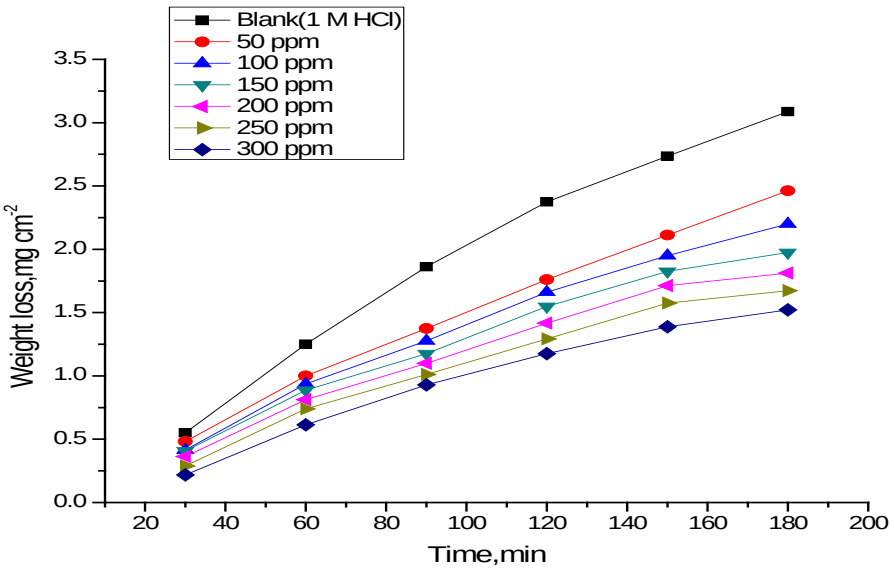

Figure 1 : Weight loss-time curves for the corrosion of aluminium in 1 M HCl in the absence and presence of different concentrations of MOE at 25°C

b) Gasometric measurements

The volume of hydrogen evolved during the corrosion reaction of aluminium in 1 M HCl solutions devoid of and containing different concentrations of extract is measured as a function of the reaction time, and the data are represented graphically in Figure 2. Inspection of the figure reveals that, the hydrogen evolution starts after a certain time from the immersion of aluminum coupon in the test solution. It may be expected thatthis time corresponds to the period needed by the acid to destruct the pre-immersion oxide film before the start of the metal attack, and it is known

as the incubation period. Further inspection of Figure 2 reveals linear relationship between the time of reaction and the volume of hydrogen evolved, in all of the tested solutions. However, the presence of the extract decreases, markedly, the slope of the straight line. Since the slope of the line represents the corrosion reaction rate, it could be concluded that the *Melilotus offecinalis* extract has an excellent ability to inhibit the corrosion of aluminium in the acid solution. The values of inhibition efficiencies of different concentrations of the extract are given in Table 2. Inspection of Table 2 reveals that the IE increases as the concentration of the extract is increased.

Table 1 : Variation of corrosion rate (k_{corr}), surface coverage(Θ)and inhibition efficiency(IE%) with different concentrations of MOE after 120 minutes of immersion in 1 M HCl at different temperatures.

Temp. °C	[Inh] ppm	Weight loss, mg cm^{-2}	k_{corr}, mg cm^{-2} min^{-1}	Θ	
25	Blank	3.24	0.027	-----	-----
	50	1.48	0.012	0.544	54.4
	100	1.46	0.012	0.546	54.6
	150	1.41	0.011	0.560	56.0
	200	1.31	0.011	0.599	59.9
	250	1.30	0.011	0.595	59.5
	300	1.20	0.010	0.623	62.3
30	Blank	7.44	0.062	----	-----
	50	2.55	0.021	0.657	65.7
	100	2.41	0.020	0.674	67.4
	150	2.22	0.020	0.701	70.1
	200	1.81	0.018	0.757	75.7
	250	1.73	0.014	0.767	76.7
	300	1.66	0.013	0.776	77.6
35	Blank	23.67	0.198	----	-----
	50	6.70	0.055	0.717	71.7
	100	6.49	0.054	0.726	72.6
	150	6.10	0.050	0.744	74.4
	200	5.24	0.043	0.778	77.8
	250	4.87	0.040	0.794	79.4
	300	4.39	0.036	0.814	81.4
40	Blank	34.92	0.291	-----	-----
	50	5.82	0.049	0.834	83.4
	100	5.39	0.044	0.845	84.5
	150	4.55	0.037	0.869	86.9
	200	4.10	0.034	0.883	88.3
	250	3.65	0.030	0.895	89.5
	300	3.25	0.027	0.907	90.7
45	Blank	51.96	0.433	-----	-----
	50	8.10	0.068	0.845	84.5
	100	7.35	0.061	0.858	85.8
	150	6.50	0.054	0.874	87.4
	200	5.57	0.046	0.893	89.3
	250	4.75	0.039	0.908	90.8
	300	3.80	0.032	0.926	92.6

Table 2 : Inhibition efficiency obtained from hydrogen evolution method for Al after 80 minutes from immersion in 1 M HCl for various concentrations of MOEat 25°C

[Inh] Ppm	Volume of hydrogen gas evolvedml	IE %
Blank	6.3	-
50	2.7	56.8
100	2.4	61.9
150	2.1	66.6
200	1.8	72.2
250	1.4	77.7
300	1.1	83.3

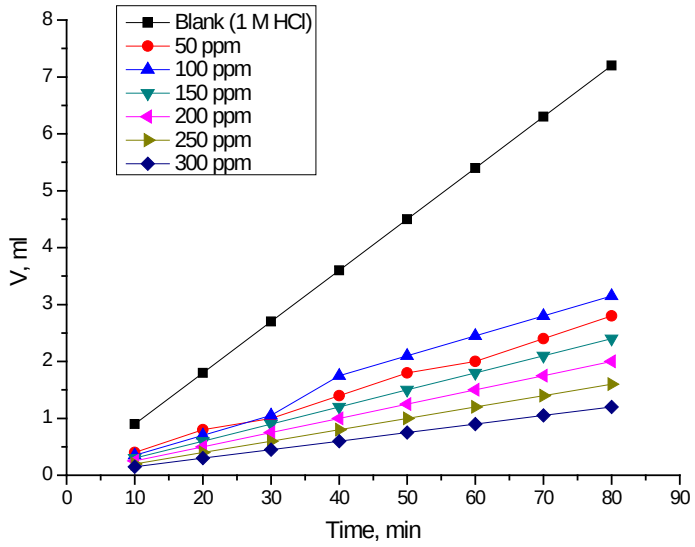

Figure 2 : volume of hydrogen gas evolved during the corrosion of aluminium in 1M HCl in the absence and presence of different concentrations of MOE

c) Polarization curves

Figure 3 shows potentiodynamic polarization curves recorded for aluminium in 1 M HCl solutions in the absence and presence of various concentrations of MOE at 25°C.

Lee and Nobe [28] reported the occurrence of a current peak between the apparent-Tafel and limiting-current regions during potential sweep experiments. The presence of MOE shifts both anodic and cathodic branches to the lower values of corrosion current densities and thus causes a remarkable decrease in the corrosion rate. The parameters derived from the polarization curves in Figure 3 are given in Table 3. In 1 M HCl solution, the presence of MOE causes a remarkable decrease in the corrosion rate i.e., shifts both anodic and cathodic curves to lower current densities. In other words, both cathodic and anodic reactions of aluminium electrode are retarded by MOE in 1 M HCl solution. The Tafel slopes of β_a and β_c at 25°Cdo not change remarkably upon addition of MOE, which indicates that the presence of MOE does not change the mechanism of hydrogen evolution and the metal dissolution process. Generally, an inhibitor can be classified as cathodic type if the shift of corrosion potential in the presence of the inhibitor is more than 85 mV with respect to that in the absence of the inhibitor [29, 30]. In the presence of MOE, E_{corr} shifts to less negative but this shift is very small (about 20-30 mV), which indicates that MOE can be arranged as mixed inhibitor.

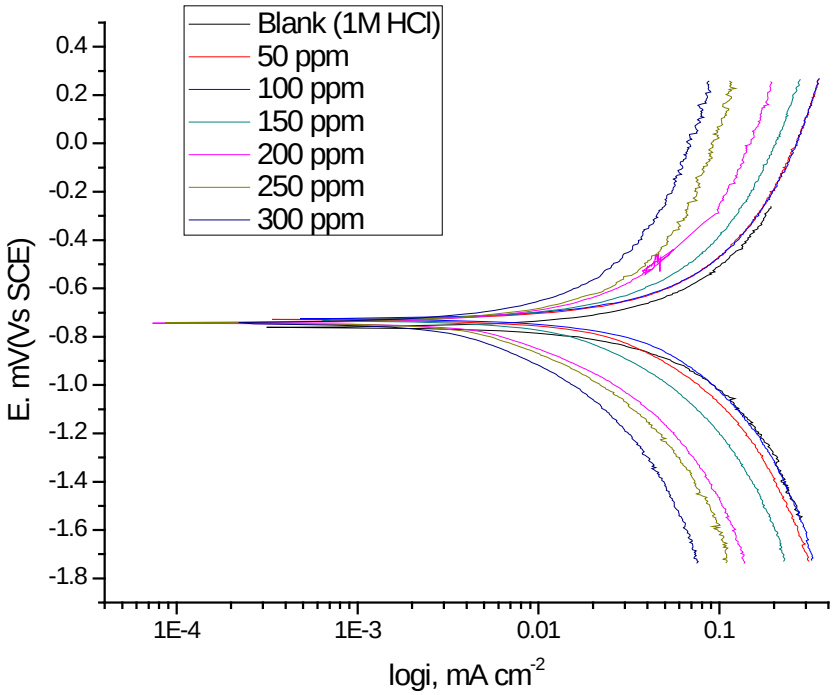

Figure 3 : Potentiodynamic polarization curves for the corrosion of aluminium in 1 M HCl solution without and with various concentrations of MOE at 25˚C

Table 3 : Corrosion potential (E_{corr}), corrosion current density (i_{corr}), Tafel slopes (β_c,β_a), degree of surface coverage (θ), and inhibition efficiency (IE%) of Al in 1M HCl at 25°C

[Inh] ppm	$-E_{corr}$ mV vs SCE	i_{corr} m A cm^{-2}	β_a mV dec^{-1}	β_c mV dec^{-1}	C.Rx10^{-3} mpy	θ	IE%
0	725	275	250	420	164	---	---
50	760	136	150	170	58	0.505	50.5
100	730	127	80	100	57	0.538	53.8
150	732	82	60	100	37	0.700	70.0
200	743	62	30	90	10	0.773	77.3
250	742	56	20	90	5.3	0.796	79.6
300	744	30	30	90	5.0	0.889	88.9

d) Electrochemical impedance spectroscopy (EIS) measurements

Figure 4 shows impedance plots for aluminium in 1 M HClsolution without and with different concentrations of MOE. The impedance spectra consists of a Nyquist semicircle type without appearance of diffusive contribution to the total impedance (Z) indicating that the corrosion proceeds mainly under charge-transfer control and the presence of extract do not alter the mechanism of corrosion reaction.It is found that the obtained Nyquist plots are not perfect semicircle due to frequency dispersion and this behavior can be attributed to roughness and in homogeneities of the electrode surface [31, 32]. When there is non-ideal frequency response, it is common practice to use distributed circuit elements in an equivalent circuit. The most widely employed is the constant phase element (CPE). In general a CPE is used in a model in place of a capacitor to compensate for inhomogeneity in the system [33]. It was found that the diameters of the semicircle increases with increasing the concentration of the investigated extract. This indicates that the polarization resistance of the oxide layer increases with increasing the concentration of MOE and the depressed capacitive semicircle are often referred to the surface roughness and inhomogeneity, since this capacitive semicircle is correlated with dielectric properties and thickness of the barrier oxide film [34]. The data revealed that, each impedance diagram consists of a large capacitive loop with low frequencies dispersion (inductive arc). This inductive arc is generally attributed to anodic adsorbed intermediates controlling the anodic process [35-36]. By following this, inductive arc was disregarded. The electrical equivalent circuit model shown in Figure 5 was used to analyze the obtained impedance data. The model consists of the

solution resistance (R_s), the charge-transfer resistance of the interfacial corrosion reaction (R_{ct}) and the constant phase angle element (CPE). The value of frequency power (n) of CPE can be assumed to correspond to capacitive behavior. However, excellent fit with this model was obtained with our experimental data. The admittance of CPE is described as:

$$Y_{CPE} = Y_o(j\omega)^n \quad (4)$$

where j is the imaginary root, ω the angular frequency, Y_o the magnitude and *n* the exponential term [37].

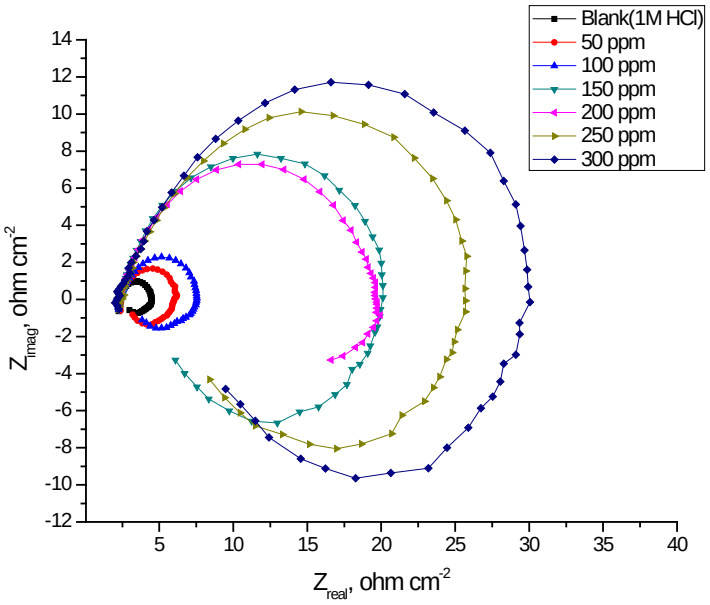

Figure 4 : Nyquist plots for aluminium in 1 M HCl solutions in the absence and presence of various concentrations of MOE at 25°C

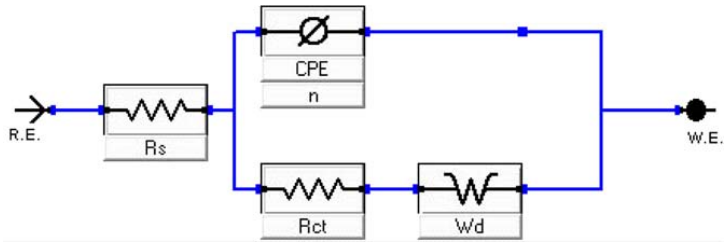

Figure 5 : Equivalent circuit used to model impedance data for aluminium in 1 M HCl solutions

A long Warburg diffusion tail was observed at low frequency values. The tails are inclined at an angle of 45° to the real-axis at the very low frequencies; A diffusion controlled process is therefore exists. Studies reported in the literature [38] showed that the diffusion process is controlled by diffusion of dissolved oxygen from the bulk solution to the electrode surface and the Warburg impedance, which is observed in the low frequency regions, is ascribed to diffusion of oxygen to the alloy surface. This diffusion tail still appears, even in presence of high concentrations of the investigated extract. This means that the corrosion behavior of alloy in the absence as well as in the presence of MOE is influenced by mass transport.

Also, Bode plots for the aluminium in 1 M HCl solution are shown in Figure 6. In which the high frequency limit corresponding to the electrolyte resistance (ohmic resistance) R_Ω, while the low frequency represents the sum of (R_Ω + R_{ct}), where R_{ct} is in the first approximation determined by both electrolytic conductance of the oxide film and the polarization resistance of the dissolution and repassivation process. At both low and high frequency limits, the phase angle between the current and potential (θ), assumes a value of about 0°, corresponding to the resistive behavior of R_Ω and (R_Ω + R_{ct}).

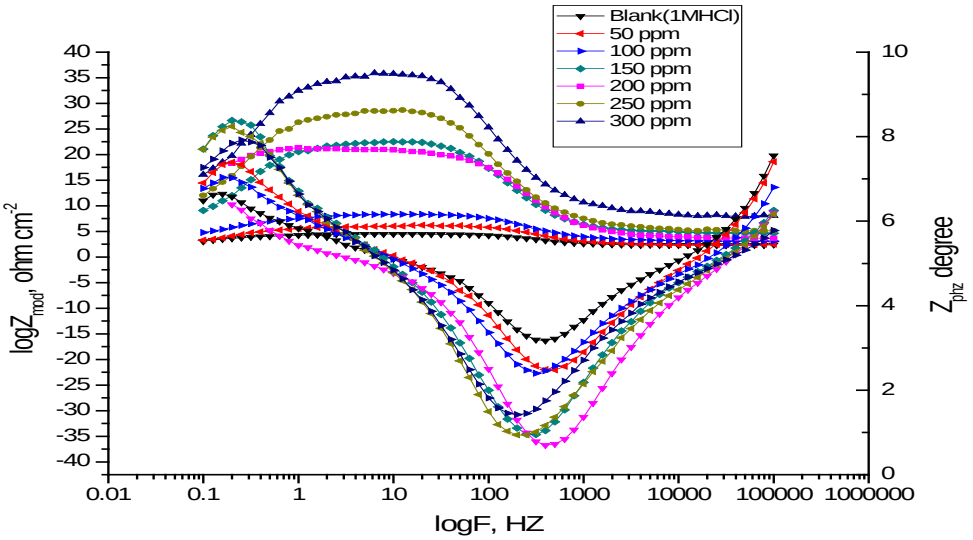

Figure 6 : Bode plots for aluminium in 1 M HCl solutions in the absence and presence of various MOE concentrations at 25°C

The main parameters deduced from the analysis of Nyquist diagram are:

- The resistance of charge transfer R_{ct}(diameter of high frequency loop)
- The capacity of double layer C_{dl} which is defined as :

$$C_{dl} = \frac{1}{2\pi R_{ct} f_{max}} \quad (5)$$

Where f_{max} is the maximum frequency at which the Z_{imag} of the impedance is a maximum. Since the electrochemical theory assumed that ($1/R_{ct}$) is directly proportional to the capacity of double layer C_{dl}, the inhibition efficiency (IE%) of the inhibitor for aluminium in 1 M HCl solution was calculated from R_{ct} values obtained from impedance data at different inhibitor concentration the following equation:

$$IE\% = \left(1 - \frac{R^{\circ}_{ct}}{R_{ct}}\right) \times 100 \quad (6)$$

Where R°_{ct} and R_{ct} are the charge transfer resistance in the absence and Presence of investigated extract, respectively. From the impedance data given in Table 4, we can conclude that the value of R_{ct} increases with the increase in the concentration of the investigated extract and this indicates the formation of a protective film on the Al surface by the adsorption and an increase in the corrosion inhibition efficiency in acidic solution. While the value of C_{dl} decreases with increasing the concentrations of extract in comparison with that of blank solution (uninhibited), as a result from the replacement of water molecules by inhibitor molecules which lead to increase in local dielectric constant and/or an increase in the thickness of the electric double layer formed on the metal surface [39,40].

Table 4 : Electrochemical kinetic parameters obtained from EIS technique for Al in 1M HCl in the absence and presence of different concentrations of investigated plant extracts at 25°C

[Inh] ppm	R_p Ω Cm^2	$C_{dl}x10^{-6}$ $\mu F\ Cm^{-2}$	Θ	IE%
0	1.7	21	---	---
50	2.9	5.9	0.468	46.8
100	3.2	4.4	0.595	59.5
150	4.2	3.2	0.730	73.0
200	6.3	3.0	0.760	76.0
250	7.1	2.5	0.840	84.0
300	10.9	2.3	0.844	84.4

e) Electrochemical frequency modulation (EFM) measurements

EFM is a nondestructive corrosion measurement technique that can directly determine the corrosion current value without prior knowledge of Tafel slopes, and with only a small polarizing signal. These advantages of EFM technique make it an ideal candidate for online corrosion monitoring [41]. The great strength of the EFM is the causality factors which serve as an internal check on the validity of EFM measurement. The causality factors CF-2 and CF-3 are calculated from the frequency spectrum of the current

responses. Figure 7 show the frequency spectrum of the current response of pure Aluminium in 1 M HCl solution, contains not only the input frequencies, but also contains frequency components which are the sum, difference, and multiples of the two input frequencies. The EFM intermodulation spectrums of Aluminium in 1 M HCl solution containing (50ppm- 300ppm) of the MOE extract at 25°C is shown in Figure 7. The harmonic and intermodulation peaks are clearly visible and are much larger than the background noise. The two large peaks, with amplitude of about 200 μA, are the response to the 40 and 100 mHz (2 and 5 Hz) excitation frequencies. It is important to note that between the peaks there is nearly no current response (<100 mA). The experimental EFM data were treated using two different models: complete diffusion control of the cathodic reaction and the "activation" model. For the latter, a set of three non-linear equations had been solved, assuming that the corrosion potential does not change due to the polarization of the working electrode [42]. The larger peaks were used to calculate the corrosion current density (i_{corr}), the Tafel slopes (β_c and β_a) and the causality factors (CF-2 and CF-3).These electrochemical parameters were simultaneously determined by Gamry EFM 140 software, and listed in Table 5 indicating that this extract inhibit the corrosion of aluminium in 1 M HCl through adsorption. The causality factors obtained under different experimental conditions are approxi-mately equal to the theoretical values (2 and 3) indicating that the measured data are verified and of good quality [43]. The inhibition efficiencies IE_{EFM} % increase by increasing the studied extract concentrations and was calculated as follows:

$$IE\ \%_{EFM} = \left(1 - \frac{i_{corr}}{i^{o}_{corr}}\right) \times 100 \quad (7)$$

Where i^{o}_{corr} and i_{corr} are corrosion current densities in the absence and presence of MOE extract, respectively.

Table 5 : Electrochemical kinetic parameters obtained from EFM technique for aluminium in 1M HCl in the absence and presence of different concentrations of MOE

[Inh] ppm	i_{corr} m A cm^{-2}	β_a mV dec^{-1}	β_c mV dec^{-1}	CF-2	CF-3	CRx10^{-3} mpy	θ	IE%
0	1100	182	195	1.1	2.3	667	---	---
50	400.9	32	102	2.0	2.7	238	0.642	64.2
100	348.8	31	66	1.1	2.9	207	0.688	68.8
150	345.7	34	55	1.7	2.2	204	0.691	69.1
200	342.8	24	36	1.8	2.5	146	0.694	69.4
250	235.5	19	33	1.2	2.3	140	0.789	78.9
300	214.3	18	25	2.0	2.3	127	0.809	80.9

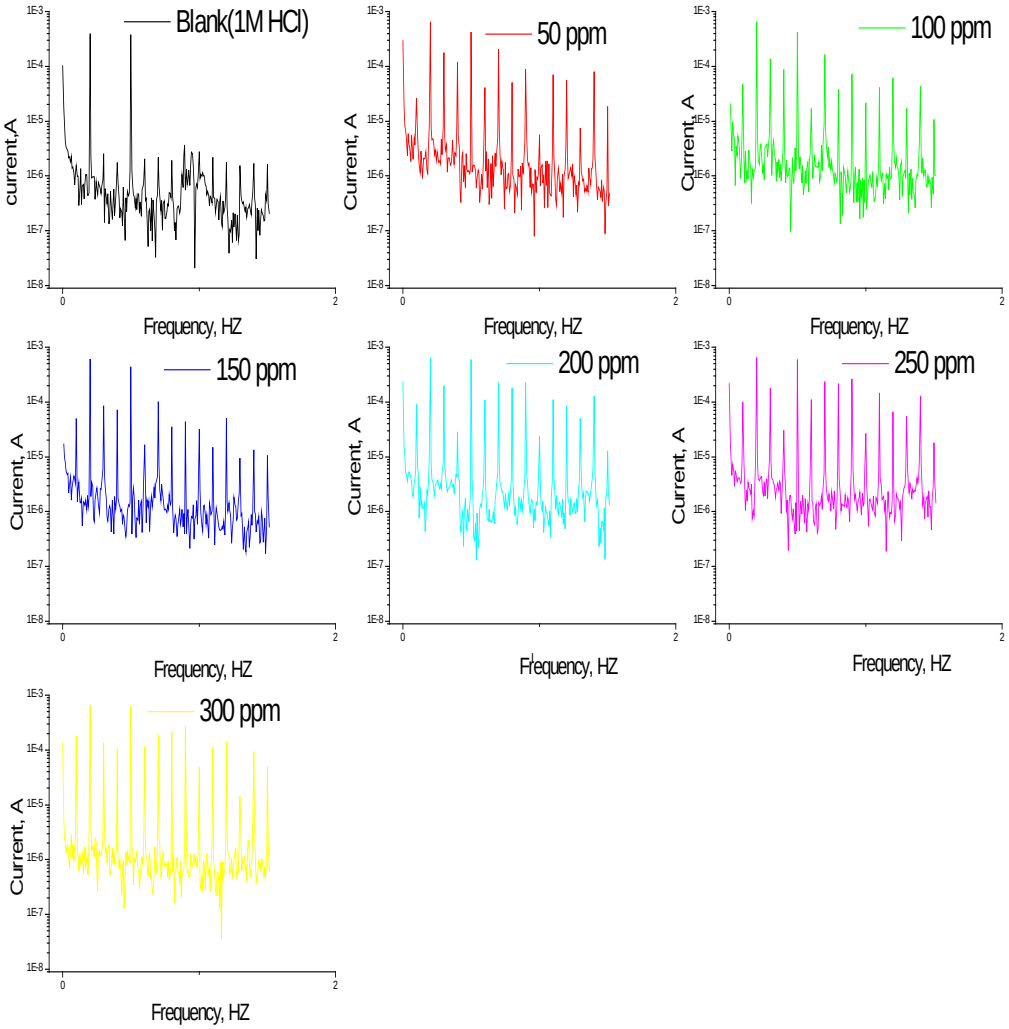

Figure 6 : Intermodulation spectrums for the corrosion of aluminium in 1 M HCl without and with various concentrations of MOE at 25˚C.

f) Adsorption isotherms

The mode and interaction degree between an inhibitor and a metallic surface have been widely studied with the application of adsorption isotherms. The adsorption of an organic molecule occurs because the interaction energy between an inhibitor and a metallic surface is higher than that between water molecules and metallic surface [44, 45]. To obtain the adsorption isotherms, the degree of surface coverage (θ) obtained from weight loss method was determined as a function of inhibitor concentration. The values of θ were then plotted to fit the most suitable model of adsorption [46]. Attempts were made tofit experimental data to various isotherms including Frumkin, Langmuir, Temkin, Freundlich, isotherms. By far the results were bestfitted by Temkin adsorption isotherm as seen in Figure 7 [47].

$$a/2.303\Theta = \log K_{ads} + \log C \qquad (9)$$

The equilibrium constant of adsorption K_{ads} obtained from the intercepts of Temkin adsorption isotherm is related to the free energy of adsorption ΔG° ads as follows:

$$K_{ads} = 1/55.5 \exp[(-\Delta G^{\circ}_{ads})/ RT] \qquad (8)$$

where 55.5 is the molar concentration of water in the solution in M^{-1}.The values obtained are given in Table 6.

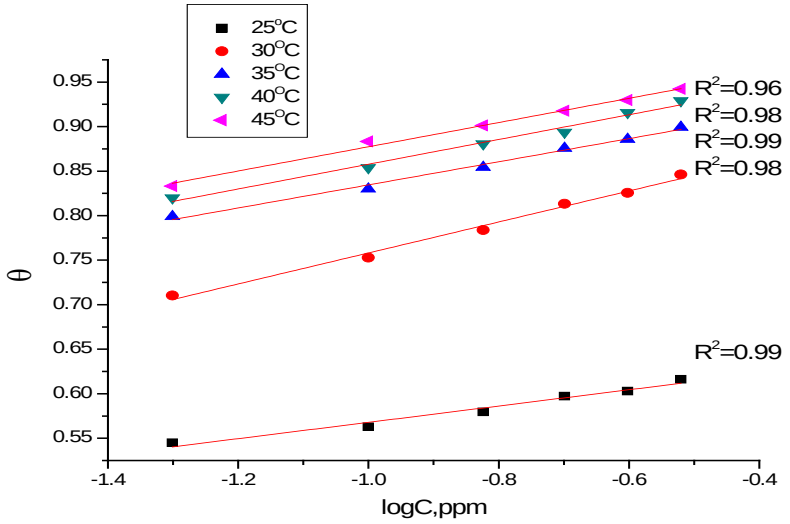

Figure 7 : Temkin adsorption plots for aluminium in 1 M HCl containing various concentrations of MOE at 25°C

Plot of (ΔG^{o}_{ads}) versus T (Figure 8) gave the heat of adsorption (ΔH^{o}_{ads}) and the entropy (ΔS^{o}_{ads}) according to the thermodynamic basic equation 5:

$$\Delta G^{o}_{ads} = \Delta H^{o}_{ads} - T\,\Delta S^{o}_{ads} \quad (5)$$

Table 6 clearly shows a good dependence of ΔG^{o}_{ads} on T, indicating the good correlation among thermodynamic parameters. The negative value of ΔG^{o}_{ads} reflect that the adsorption of studied inhibitors on aluminium surface from 1 M HCl solution is spontaneous process and stability of the adsorbed layer on the aluminium surface. Generally, values of ΔG^{o}_{ads} around -20 kJ mol^{-1} or lower are consistent with the electrostatic interaction between the charged molecules and the charged metal (physical adsorption); those around -40 kJ mol^{-1} or higher involves charge sharing or transfer from organic molecules to the metal surface to form a coordinate type of bond (chemisorption) [48]. From the obtained values of ΔG^{o}_{ads} it was found the existence of chemical adsorption). The values of thermodynamic parameter for the adsorption of inhibitors Table 6 can provide valuable information about the mechanism of corrosion inhibition. Endothermic adsorption process ($\Delta H^{o}_{ads} > 0$) is attributed unequivocally to chemisorption [49], an exothermic adsorption process ($\Delta H^{o}_{ads} < 0$) may involve either physisorption or chemisorption or mixture of both processes. In the presented case, the calculated values of ΔH^{o}_{ads} for the adsorption of extract in 1 M HCl indicating that this extract may be chemically adsorbed. The values of ΔS^{o}_{ads} in the presence of extract is large and positive that is accompanied with endothermic adsorption process. This indicates that decrease in disorder takes places on going from reactants to the metal-adsorbed reaction complex [50].

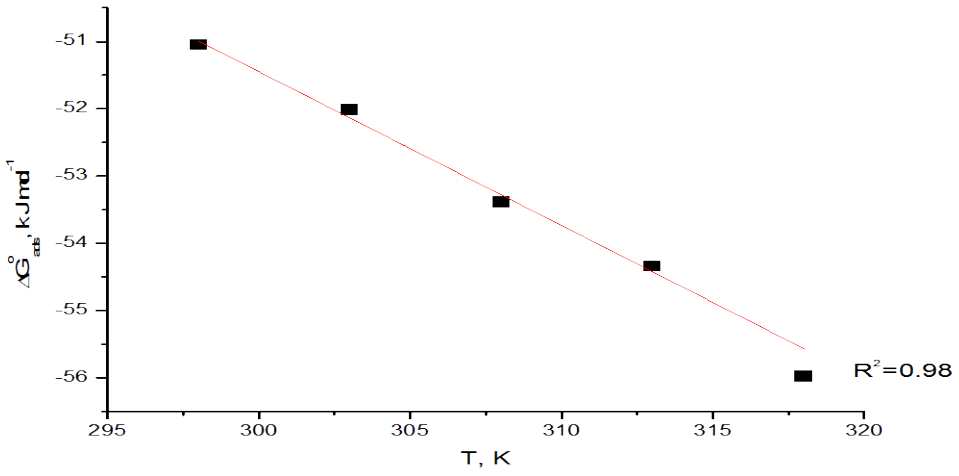

Figure 8 : Variation of ΔG^{o}_{ads} versus T for the adsorption of MOE on aluminium surface in 1 M HCl at different temperatures

Table 6 : Thermodynamic parameters for the adsorption of MOE on aluminium in 1MHCl at different temperatures.

Temp. °C	$K_{ads}x10^{-6}$ M^{-1}	$-\Delta G^{o}_{ads}$ k J mol^{-1}	$-\Delta H^{o}_{ads}$ k J mol^{-1}	ΔS^{o}_{ads} J mol^{-1} k^{-1}
25	12.2	50.4	17.2	226.7
30	15.6	51.8		227.8
35	20.6	53.4		229.2
40	26.9	54.9		230.5
45	33.9	56.5		231.6

g) Kinetic-thermodynamic corrosion parameters

Weight loss method was carried out at different temperature (25°C–45°C) in the presence of different concentration of MOE. It has been found that he corrosion rate decreases with the increase in temperature for MOE (Table 1). The corrosion rate of aluminium in the absence of MOE increased steeply from 25 to 45°Cwhereas; in the presence of MOE the corrosion rate decreased slowly. The inhibition efficiency was found to increase with temperature. The corrosion parameter in the absence and presence of extract in the temperature range 25–45°C has been summarized in (Table 1).The apparent activation energy (E^{*}_{a}) for dissolution of aluminium in 1 M HCl was calculated from the slope of plots by using Arrhenius equation:

$$\log k = \frac{-E_a^*}{2.303\ R\ T} + \log A \qquad (9)$$

where k is rate of corrosion, E^{*}_{a} is the apparent activation energy, R is the universal gas constant, T is absolute temperature and A is the Arrhenius pre-exponential factor.

By plotting log k against 1/T the values of activation energy (E^{*}_{a}) has been calculated (E^{*}_{a} = (slope) 2.303 x R) (Figure 9). Activation energy for the reaction of aluminium in 1M HCl decreases in the presence of extract (Table 7). This decreas indicates the formation of chemical bonds were strengthen by increasing the temperature. However, the extent of the rate increment in the inhibited solution is lower than that in the free acid solution. Therefore, the inhibition efficiency of the MOE increases markedly with increasing temperature. This result supports the idea that the adsorption of extract components on the aluminium surface may be chemical in nature. Thus, as the temperature increases the number of adsorbed molecules increases leading to an increase in the inhibition efficiency. This could be done by adsorption on the aluminium surface making a barrier for mass and charge transfer. However, such types of inhibitors perform a good inhibition at high temperature with considerable increase in inhibition efficiency at elevated temperatures [51]. Moreover, the relatively high value of activation energy in presence of MOE suggests a chemical adsorption process.

The values of change of entropy (ΔS^{*}) and change of enthalpy (ΔH^{*}) can be calculated by using the formula:

$$k = \left(\frac{RT}{Nh}\right) exp\left(\frac{\Delta S^*}{R}\right) exp\left(\frac{\Delta H^*}{RT}\right) \qquad (10)$$

where k is rate of corrosion, h is Planck's constant, N is Avogadro number, ΔS^{*} is the entropy of activation, and ΔH^{*} is the enthalpy of activation. A plot of log (k/T) vs. 1/T (Figure 10) should give a straight line, with a slope of (ΔH^{*}/2.303R) and an intercept of [log (R/Nh)+ΔS^{*}/ 2.303R], from which the values of ΔS^{*} and ΔH^{*} can be calculated (Table 7). The positive value of ΔS^{*} for the extract indicates that activated complex in the rate determining step represents a dissociation rather than an association step, meaning that an increase in disorder takes place during the course of transition from reactant to the activated complex [52] The positive sign of ΔH^{*} indicates that the adsorption of extract molecules is an endothermic process. Generally, an endothermic process signifieschemisorption process.

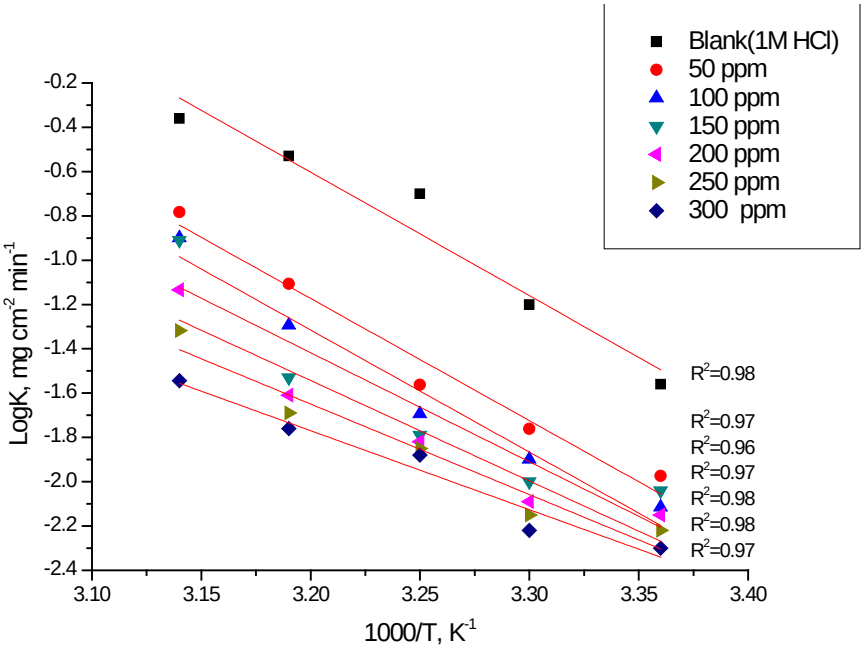

Figure 9 : log k (corrosion rate) – 1/T curves for aluminium in 1 M HCl in the absence and presence of different concentrations of MOE

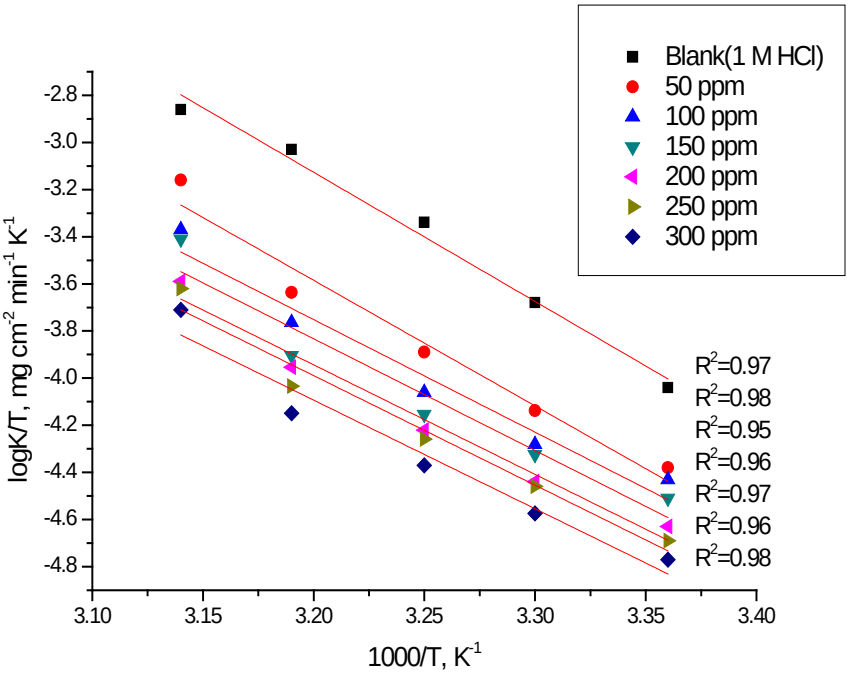

Figure 10 : log k (corrosion rate) /T – 1/T curves for aluminium in 1 M HCl in the absence and presence of different concentrations of MOE

Table 7 : Activation parameters for dissolution of aluminium in the absence and presence of different concentrations of MOE in 1 M HCl

Conc. ppm	E_a^*, kJ mol^{-1}	ΔH^*, kJ mol^{-1}	ΔS^*, J mol^{-1}K^{-1}
1 M HCl	106.6	45.5	78.1
50	105.5	44.3	60.1
100	105.3	39.7	23.2
150	94.2	39.4	19.4
200	86.7	38.6	11.7
250	78.1	38.5	10.7
300	68.1	38.2	6.1

h) Surface analysis by scanning electron microscopy

Figure11shows an SEM photograph recorded for aluminium samp-les polished (a) and exposed for 24 h in 1 M HCl solution without (b) and with (c) 300 ppm of MOE at 25C^0. A photograph of the polished aluminium surface before immersion in 1 M HCl solution is shown in Figure 11a. The photograph shows the surface was smooth and without pits. The SEM micrographs of the corroded aluminium in the presence of 1 M HCl solution are shown in Figure 11b. The faceting seen in this figures was a result of pits formed due to the exposure of aluminium to the acid. The influence of the inhibitor addition 300 ppm on the aluminium in 1 M HCl solution is shown in Figure 11c. The morphology in Figure 11c shows a rough surface, characteristic of uniform corrosion of aluminium in acid, as previously reported [52], that corrosion does not occur in presence of inhibitor and hence corrosion was inhibited strongly when the inhibitor was present in the hydrochloric acid, and the surface layer is very rough. In contrast, in the presence of 300 ppm of MOE, there is much less damage on the aluminium surface, which further confirm the inhibition action. Also, there is an adsorbed film adsorbed on aluminium surface (Figure 11c). In accordance, it might be concluded that the adsorption film can efficiently inhibits the corrosion of aluminium.

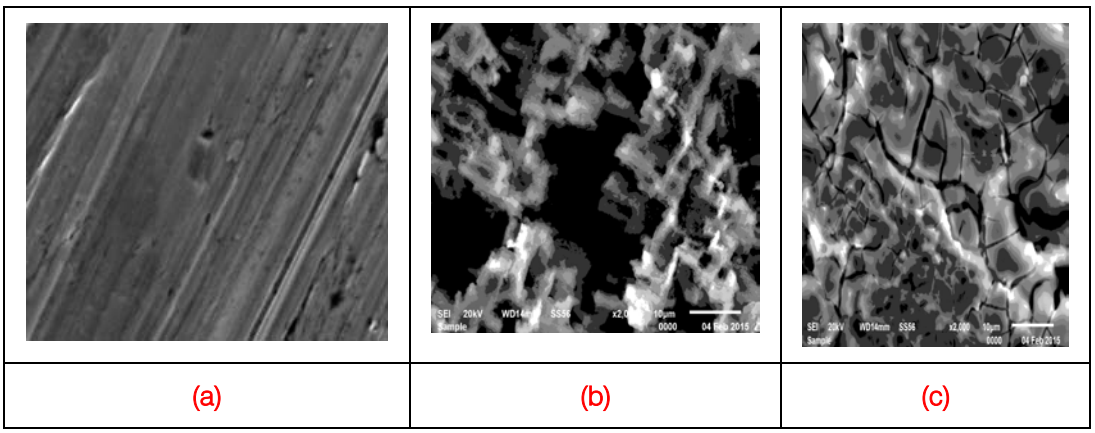

Figure 11: SEM micrographs of aluminium surface (a) before of immersion in 1 M HCl, (b) after 24 h of immersion in 1 M HCl and (c) after 24 h of immersion in 1 M HCl + 300 ppm of MOE at 25°C

i) Mechanism of the corrosion inhibition

The adsorption of organic compounds can be described by two main types of interactions: physical and chemisorptions adsorption. In general, physical adsorption requires the presence of both the electrically charged surface of the metal and charged species in solution. The surface charge of the metal is due to the electric field existing at the metal/solution interface. A chemisorption process, on the other hand, involves charge sharing or charge transfer from the inhibitor molecules to the metal surface to form a coordinate type of a bond. This is possible in case of a positive as well as a negative charge of the surface. The presence of a transition metal, having vacant, low-energy electron orbitals and an inhibitor with molecules having relatively loosely bound electrons or heteroatoms with a lone pair of electrons is necessary for the inhibiting action [53]. Generally, two types of mechanisms of inhibition were proposed. One was the formation of polymeric complexes with aluminium ions (Al^{3+}) depending on the applied conditions[54, 55]. The other was the chemical adsorption of MOE components on aluminium surface

[56, 57]. The inhibition action of MOE does not occur by the simple blocking at the surface of aluminium, especially at high temperature. This might be attributed to the different adsorption capacities of the MOE extract on the aluminium surface at different temperatures. It has been studied that with the increase in temperature, the desorption effect of MOE on aluminium surface increased. Some of the hydrophilic groups with positively charged atoms (O^+) desorbed from the surface of aluminium and did more work to prevent the H^+ from getting nearer to the metal surface. Therefore, MOE preferentially inhibited both cathodic and anodic corrosion processes at high temperature.

IV. Conclusions

From the overall experimental results the following conclusions can be deduced:

1. The MOE shows good performance as corrosion inhibitor in 1 M HCl.
2. The results obtained from weight loss showed that the inhibiting action increases with the MOE concentration and also increase with the increasing in temperature.
3. Double layer capacitances decrease with respect to blank solution when the plant extract is added. This fact confirms the adsorption of plant extract molecules on the aluminium surface.
4. The MOE inhibits the corrosion by getting adsorbed on the metal surface following Temkin adsorption isotherm.
5. The inhibition efficiencies determined by weight loss, potentiodynamic polarization and EIS techniques are in reasonably good agreement.

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Global Journal of Researches in Engineering: C
Chemical Engineering
Volume 15 Issue 3 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4596 & Print ISSN: 0975-5861

Influence of Scan Rate on Simulation of Differential Scanning Calorimetry Profiles of Protein Denaturationt

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GJRE-C Classification: FOR Code: 030599, 030505

Strictly as per the compliance and regulations of:

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Maryam Ghadamgahi [α] & Davood Ajloo [σ]

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Keywords: carbonic anhydrase, melting temperature, molecular dynamics simulation, scan rate, simulated annealing.

I. Introduction

Carbonic anhydrase (CA) is a clinically relevant and biochemically well-characterized protein. It catalyzes hydration of carbon dioxide to carbonic acid and is involved in vital physiological processes such as pH and CO_2 homeostasis, transport of bicarbonate and CO_2, biosynthetic reactions, bone resorption, calcification, tumorigenicity, and other physiological or pathological processes. Therefore, this enzyme is an important target for inhibitors with clinical applications, primarily for use as antiglaucoma agents but also for the therapy of various pathologies such as epilepsy and Parkinson's disease. Many groups have used carbonic anhydrase—both bovine and human—as a model protein for studies of folding and unfolding.[1-3]

Differential scanning calorimetry (DSC) is a technique able to study thermally induced transitions and particularly, the conformational transitions of biological macromolecules (for example between the folded and the unfolded structure of a protein). It measures the excess heat capacity of a solution (C_p) of the molecule of interest as a function of temperature and has been extensively used to study protein thermal denaturation.[4] A variety of techniques have evolved which can be used to gain structural information on protein stability. DSC has become one of the key physicochemical methods to study the stability of protein biopharmaceuticals.[5,6] In experimental study of carbonic anhydrase unfolding by DSC the enthalpy of unfolding in the temperature range of 39 to 72 °C by carrying out DSC experiments at various pH was determined.[7] T_m (effectively the transition peak) is defined as the temperature at which 50% of the protein molecules are unfolded or as a midpoint in a thermal ramp and represents a temperature where the free energy of the natives and nonnative forms are equivalent. Protein melting temperatures can be determined by numerous methods, including differential scanning calorimetry and optical methods (circular dichroism, fluorescence or absorbance spectroscopy). These techniques have low throughput, are time consuming, and require significant amounts of protein and, thus, are not generally utilized when testing the large numbers of compounds generated during drug development.

Scan rate dependence was determined using the methods described by Sanchez-Ruiz et al.[8] The scan-rate-dependent shift in T_m for denaturation was fitted to the equation:

$$\frac{scan\ rate}{T_m^2} = \frac{AR}{E_a} e^{-E_a/RT_m} \qquad (1)$$

Such that a plot of ln(scan rate/T_m^2) against $1/T_m$ yields a slope– E_a/R, where E_a is the activation energy for denaturation, R the gas constant and A the pre-exponential factor in the Arrhenius equation. The effects of scan rate on the DSC profiles are given. These results are expressed in the form of DSC profiles (excess C_p, vs. T).

The calorimetric transitions for carbonic anhydrase denaturation are highly scanning-rate dependent, which indicates that the thermal denaturation is under kinetic control.[9-12] All above results are confirmed by a MD simulation study and a good agreement was found for the DSC data with experimental values.

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Finally, according to the two-state irreversible model, the scanning rate effect on the DSC transitions is given by:[13]

$$ln\left(v/T_m^2\right) = const - E/RT_m \quad (2)$$

Scan rate and pH dependence of carbonic anhydrase stability were determined by measuring the T_m values at various scan rates and pH to generate a DSC curve. Models relating the scan rate-dependent increase in protein thermal stability to association constants require an accurate knowledge of the thermodynamics of protein stability. Thus, carbonic anhydrase stability was studied by MD simulation of differential scanning calorimetry (DSC), giving a complete thermodynamic description of the Gibbs free energy, calorimetric enthalpy, and heat capacity of unfolding .[14-20]

We chose to conduct the study on carbonic anhydrase—a protein commonly used as a model for biophysical and physical-organic studies by us and others.[21-24]

Thermal denaturations of carbonic anhydrase have been examined using simulation of differential scanning calorimetry by molecular dynamic simulation. Thermal denaturation has never been directly examined previously theoretically. Carbonic anhydrase have been examined as a function of scan rate. In this work we have found denaturation of protein. Finally, it is noteworthy that CA could become important biotechnological materials. Therefore investigation of thermal stability of the CA has not only academic, but also applied, interest. This model correctly predicts scan rate and pH-dependent changes in T_m of BCA. These T_m values are then compared to those obtained by experimental methods that are in good agreement.

II. Experimental

a) Molecular dynamics simulations

All MD simulations were carried out using the GROMACS 4.5.0 package together with the GROMOS96 force field in parallel by the BirgHPC. The starting structure of bovine carbonic anhydrase was constructed based on the X-ray crystal structure of BCA (PDB ID: 1CA2, Fig. 1). The simple point charge (SPC) model was used to describe water. A different time step was used to integrate the equations of motion with the Verlet algorithm. A non bond pair list cutoff of 0.9 nm was used. Temperatures and pressures were controlled by a Nose-Hoover thermostat and Parrinello-Rahman barostate with coupling constants of 0.1 and 0.5, respectively. For all simulations, the atomic coordinates were saved every 50 ps for analysis. A cubic simulation box of the volume 321 nm^3 was made and then water molecules were randomly added into the simulation box and initial configurations were minimized using steepest descent algorithm with 5000 integration step. BirgHPC (Bioinformatics Research Group High Performance Computing) which is a free Linux Live CD distribution based on Pelican HPC and Debian Live including 56 processors was also used for our simulations. BirgHPC has been developed to create high-performance clusters for bioinformatics and molecular dynamics studies using any Local Area Network (LAN)-networked computers. The latest versions of GROMACS 4.5.0 was run in parallel by the birgHPC.

Variations of temperature were adjusted in mdp file. In order to study thermal denaturation, temperatures varied in the range of 273 to 405 K to calculate the stability of the protein.

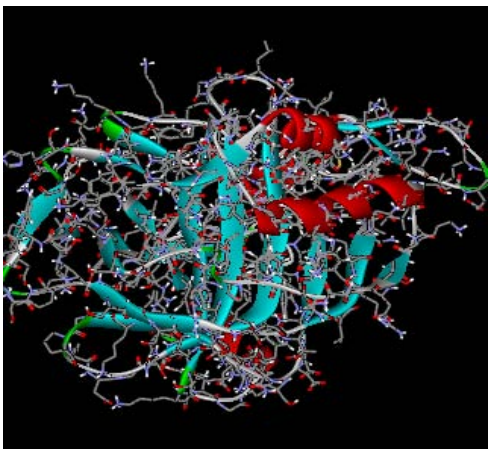

Fig. 1 : Protein structure (1CA2) taken from protein data bank www.RCSB.org

Simulated annealing is a special case of MD or MC simulation, in which the temperature is gradually reduced during the simulation. Often, the system is first heated and then cooled. Thus, the system is given the opportunity to surmount energetic barriers in a search for conformations with energies lower than the local-minimum energy found by energy minimization. One of the applications of MD is involved in utilization of MD, often with simulated annealing protocols, to determine or refine structures with data obtained from experiments.[25]

Creation of different scan rates by variation of temperature in each step was adjusted in mdp file by simulated annealing. DSC calculations were performed, keeping a constant pressure of 1 atm over the simulation. Different scanning rates within the range 0.010-0.02 K/ps were employed. In order to simulate scan rate of 0.01 K/ps, temperature increased from 250 to 450 K during 20 ns. It means that in each 500 step temperature increased 5 K. For the scan rate of 0.0125, initial and final temperature was 225 to 475 and in each 2000 ps step temperature increased 25 K, for scan rate of 0.015, initial and final temperature was 270 to 570 and in each 1000 ps step temperature increased 15 K and for scan rate of 0.02, initial and final temperature was 150 to 545 K and in each 250Ps step, temperature increased 5 K.

In order to simulate lower pH (acidic form), all carboxyl groups (COO-) were protonated and converted to COOH by definite tools implemented in GROMACS. In addition, at neutral pH, we used the crystal structure (pdb code; 1CA2) downloaded from the protein data bank. All MD simulations for comparison of pH effect were carried out for three scan rates of 0.01, 0.0125 and 0.015 K/ps during 20 ns. Variations of RMSD, $CD_{222,nm}$, hydrogen bond (HB), solvent accessible surface (sas) area, radius of gyration and Hamiltonian energy were also calculated.

b) Analyses

The conformational changes of the protein during MD simulations were monitored by the root-mean-square derivations (RMSD) with its X-ray structure as a reference. The RMSD value, a measure of molecular mobility, is calculated by translating and rotating the coordinates of the instantaneous structure to superimpose the reference structure with a maximum overlap. The RMSD is defined as

$$RMSD = \sqrt{\frac{\sum_{i=1}^{N} m_i (r_i - r_i^0)}{\sum_{i=1}^{N} m_i}} \quad (3)$$

Where m_i is the mass of atom i. r_i and r_i^o are the coordinates of atom i at a certain instance during MD simulations and at its reference state, respectively. RMSDs were calculated, for the trajectories, from the starting structures of carbonic anhydrase as a function of time. In the all systems, RMSDs reach a stable value within the first nanosecond of all the analyses.

The simulation trajectories were analyzed using several auxiliary programs provided with the GROMACS package. The programs include g_energy that calculate all energies such as Hamiltonian, total pressure, box volume etc. and displays averages. Calculation of the heat capacity at constant pressure (C_P) can be used to directly compare with experimental DSC results. In general, a straightforward but difficult method to accomplish this is to use the trajectory energy fluctuations to determine the C_P, directly. From a trajectory, one can determine the trajectory average energy and the enthalpy of each step i H_i. to determine the heat capacity:

$$H = E + PV \quad (4)$$

$$C_P = \frac{\langle H^2 \rangle - \langle H \rangle^2}{RT^2} \quad (5)$$

$$\langle H \rangle = \frac{\sum_V \sum_j H_i e^{-E_{Vj}/kT} e^{-pV/kT}}{\sum_V \sum_j e^{-E_{Vj}/kT} e^{-pV/kT}} \quad (6)$$

$\langle H \rangle$ is the average value of enthalpy, H_i is enthalpy of ith state and k is bultzman constant. It is important to note that in all calculations of DSC profiles, heat capacity has been subtracted from solvent heat capacity and it has been done for all scan rates calculation.[26-30]

III. Results and Discussion

a) Thermal denaturation (Tm of carbonic anhydrase)

We used the MD simulation of differential scanning calorimetry (DSC) to monitor the thermal unfolding of BCA. The melting temperature of BCA in temperature range from 273 to 405 K was about 345 K which is near the experimental value.[7, 31-32] Fig. 2 shows DSC profile or representative thermo gram in several temperatures for unfolding of CA protein. Thermodynamic of CA unfolding was studied by DSC using various temperature conditions previously demonstrated to give T_m using MD simulation. This observation was consistent with experiments, where the T_m for BCA was estimated to be 343 K.

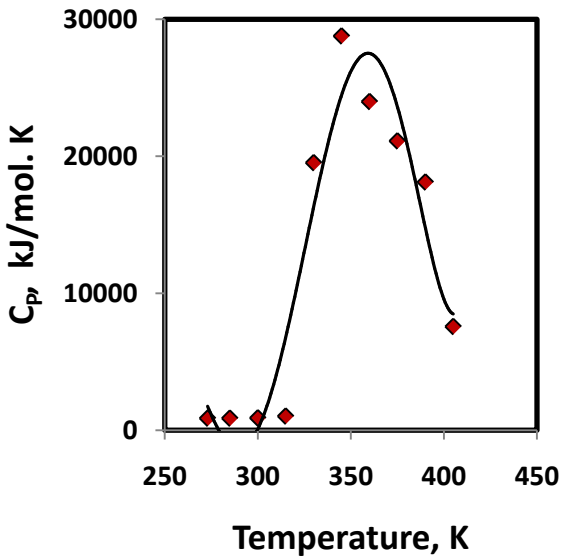

Fig 2 : DSC profile for the unfolding of BCA protein

Here, we describe simulations of CA at various temperatures, focusing on the unfolding process. Increasing temperature accelerates protein unfolding without changing the pathway of unfolding. Temperature is believed to alter the structure of hydrogen bonds network of protein in water and increase the SAS and protein size and decrease the intermolecular hydrogen bond, electrostatic and hyrophobic interactions of proteins. Structure parameters were obtained from MD simulation for each temperature and results were averaged. The structure information such as solvent accessible surface, inter molecular hydrogen bonding (HB) between CA and solvent molecules, gyrate radii (Rg), $CD_{222,nm}$ and RMSD were obtained and averaged at each temperature. Fig. 3 show parameters of total SAS, hydrophobic SAS, radius of gyration and RMSD

that increase by temperature due to unfolding of protein while intermolecular hydrogen bond, hydrophilic SAS and $CD_{222,nm}$ decrease with temperature.

The variation of surface area during 20 ns time evolution was significant and obtained. Fig. 3a shows the averaged value of total solvent accessible surface area of CA in 20 ns time interval in the temperature ranged from 273 to 405. These figure shows increase of surface by increasing temperature. This proves that the CA structure has been unfolded and it is obvious that surface area of CA in system with 273 K is less than higher temperatures. Fig. 3b and 3c show average solvent accessible surface area of hydrophobic and hydrophilic part for CA in 20 ns time interval vs. temperature respectively. Solvent accessible surface area of hydrophobic part and total surface area of CA is more in the presence of higher temperatures. This proves that the CA structure has been unfolded more in the presence of higher temperature so the surface areas of protein increase due to unfolding process. Totally temperature cause more interaction and structural change in CA and this result is in good agreement with experiment data.

Root mean square deviation (RMSD) of the CA for all temperatures was obtained. Fig. 3d shows the average of CA RMSD in the 20 ns time interval in all temperatures. The figure shows that CA has more structural changes (RMSD) in the higher temperature. Fig. 3e shows the RMSD of CA in the 20 ns time interval for 273 K which has been selected randomly. It shows that the system reaches a stable state after about 5 ns.

Fig. 3f shows the average values of radius gyration of CA in 20 ns time interval vs. temperature. This figure shows increase of radius gyration of CA in the presence of higher temperature. This result is in good accordance with increase of hydrophobic and total surface area of CA. This proves that the CA structure has been unfolded and it is obvious that surface area and therefore radius gyration of CA in system with 405 K temperature is increased more due to more increase of CA surface area. This proves that the CA structure has been unfolded more in the higher temperatures.

Fig. 3g shows averaged value of intermolecular hydrogen bond of CA in 20 ns time interval vs. temperature. Reduction of this parameter proves that the CA structure has been unfolded in high temperatures and this result is in good agreement with above results. The number of intermolecular hydrogen bonds decreased in the process of increasing temperatures and protein structure is unfolded and denatured. The number of hydrogen bonds between protein-solvent is also increased due to unfolding of protein in high temperatures and is not shown here. In some of the temperatures these trends are reverse due to formation of helix and beta sheets. Decrease of SAS may be due to formation of beta sheet and helix structures.

Fig. 3h shows averaged $CD_{222,nm}$ of CA by molecular dynamics vs. temperature this figure shows the helicity as $CD_{222,nm}$ which decreases in the high temperatures due to unfolding and denaturation of protein.

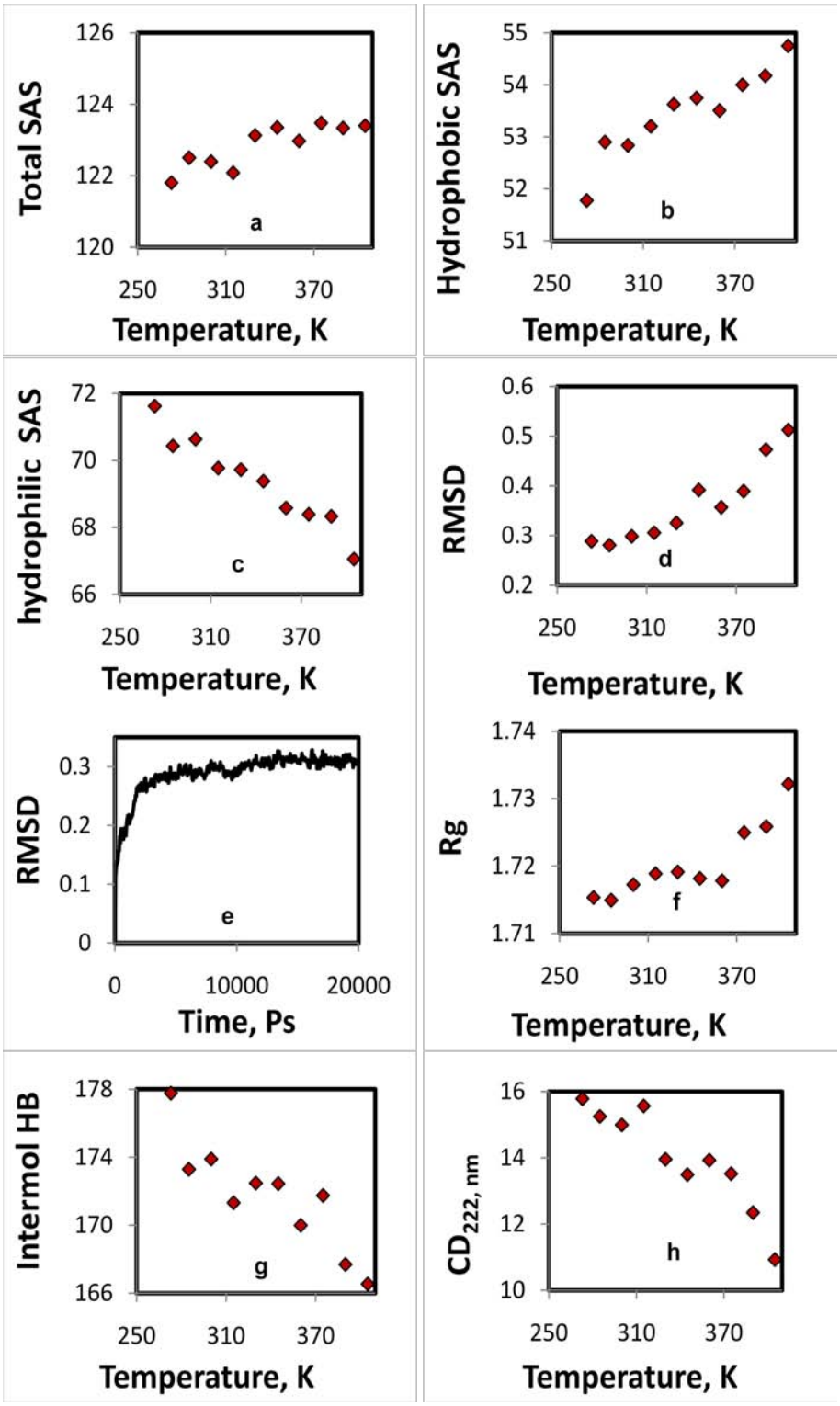

Fig. 3 : a) averaged total solvent accessible surface area of CA by molecular dynamics b) and c) averaged solvent accessible surface area of hydrophobic and hydrophilic part of CA by molecular dynamics, respectively d) averaged RMSD by molecular dynamics for CA e) Calculated RMSD by molecular dynamics for CA in the 273 K f) averaged radius gyration of CA by molecular dynamics g) averaged inter molecular hydrogen bond of CA by molecular dynamics h) averaged CD222,nm of CA by molecular dynamics vs.temperature

b) Scan rate dependence

In the case of the thermal denaturation of CA, however, the DSC transitions are strongly scanning-rate dependent. This indicates that the state of the transitions at any given temperature (within the denaturation range) depends on the time required to reach that temperature; therefore, the thermal denaturation of CA is under kinetic control and the DSC transitions are distorted by changing scan rates. The thermal denaturation of several soluble proteins such ac CA has been found to conform to this model. Fig. 4 shows temperature-dependence of excess heat capacity for CA at several scan rates.

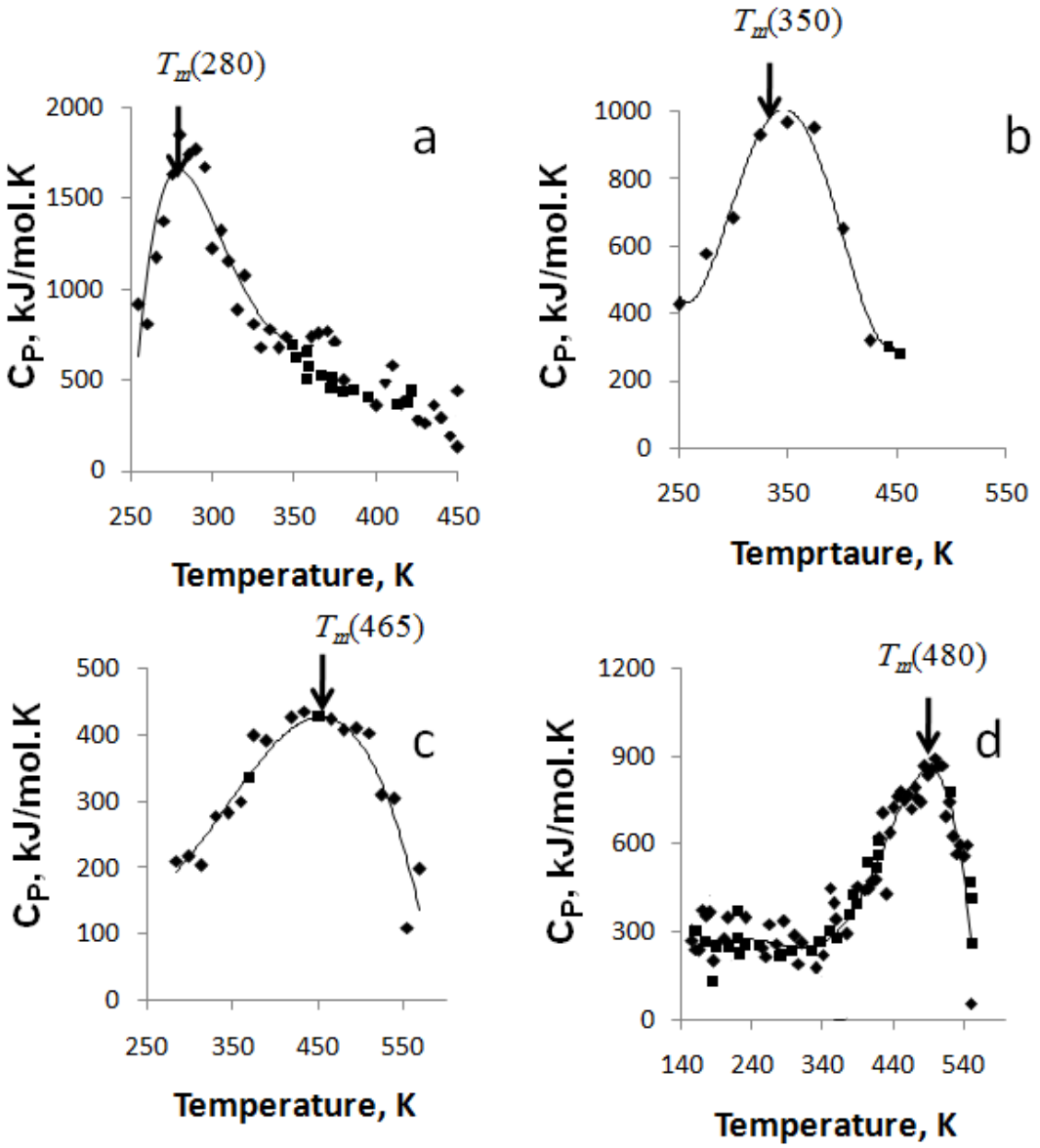

Fig. 4 : Characteristic heat-capacity curves for CA, with four scan rates a) 0.01, b) 0.0125, c) 0.015 and d) 0.02 K/ps

As it may be seen in this figure the traces were scan rate-dependent. It may be concluded, therefore, that the thermal denaturation of CA is kinetically controlled under the conditions employed. The denaturation shapes of the transitions agree in general with the results of Brouillette et al.[19] The results displayed in this figure show, however, that the DSC transitions are highly scanning rate-dependent. The curves of C_P, vs T are shifted to higher temperature with increasing scan rate (Fig. 3). This illustrates the importance of varying the scan rate or the rate of unfolding on the profiles. The temperature of maximum C_P, (T_{max}) obeys the equation (2). As scan rate increases further, the profile shifts to higher temperature which is obvious in our results. Fig. 5 shows scan rate dependence of CA denaturation.

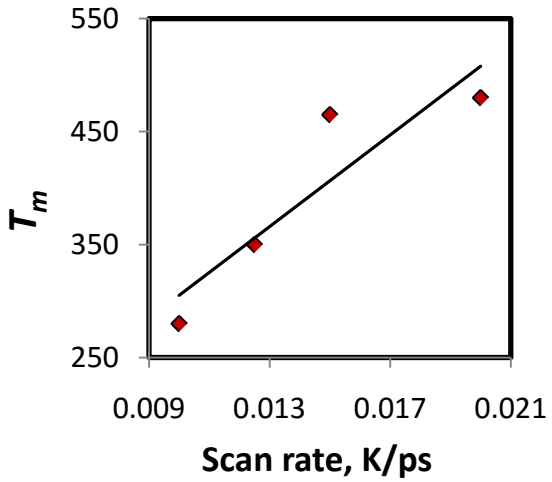

Fig. 5 : Scan rate dependence of thermal denaturations of CA

Calculated RMSD by molecular dynamics for CA for studied scan rates is depicted in Fig. 6. It shows that all of the systems reach a stable state.

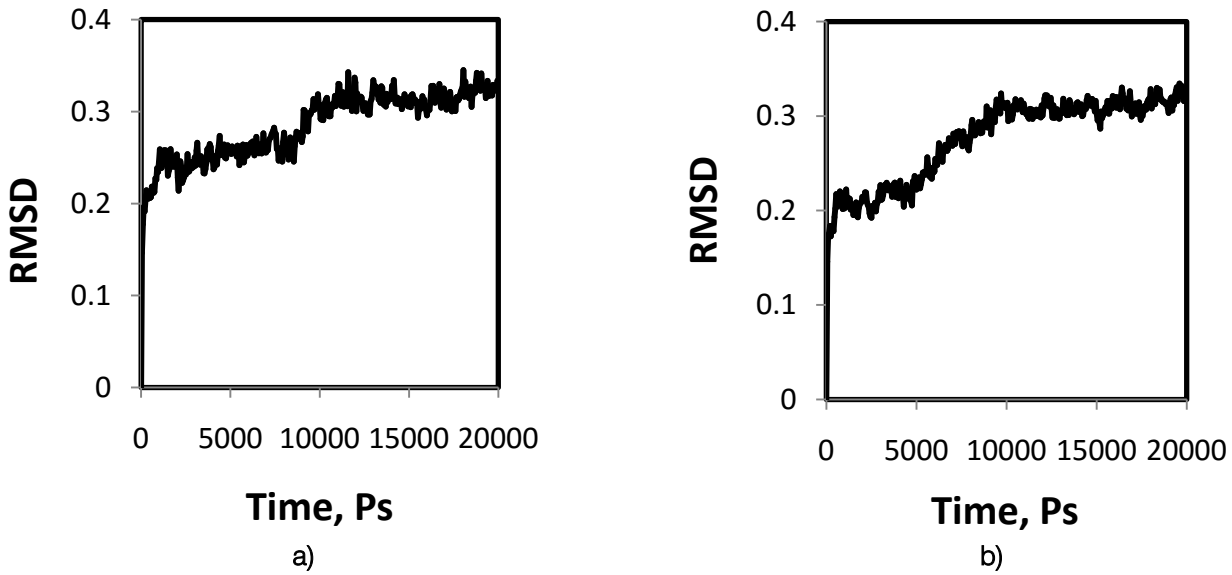

Fig. 6 : Calculated RMSD by molecular dynamics for CA for scan rates of a) 0.01 and b) 0.0125K/ps

The area under a DSC curve normally yields the calorimetric enthalpy of denaturation of the protein, ΔH. The position of the peak yields the T_m for denaturation. CA thermally denature as a single peak in DSC. The fitted values for the calorimetrically determined apparent thermodynamic parameters for the denaturation of the CA in each scan rate are shown in Table 1.

Table 1 : T_m and thermodynamic parameters of CA denaturation in different scan rates.

Scan rate	ΔH(neutral)(kJ/mol)	ΔS(kJ/mol)	T_m(neutral)	T_m(acidic)
0.01	62207	222.17	280	278
0.0125	76986	219.96	350	341
0.015	78914	169.71	465	415
0.02	79638	165.91	480	Not done

Where ΔS_{Tm} is the entropy of unfolding at the melting temperature obtained by:

$$\Delta S_{T_m} = \frac{\Delta H}{T_m} \quad (7)$$

Since the Gibbs free energy of unfolding at T_m is equal to zero: $\Delta G_{T_m} = 0$

The enthalpy of melting ΔH was determined by integrating the area under the peak. It is common practice to determine the enthalpy of protein unfolding by differential scanning calorimetry (DSC). This approach limits the available data to higher temperatures where most proteins denature, ranging from about 273 to 405 K.

Proteins have to be artificially destabilized to reduce their melting temperature. One of the most common means to destabilize a protein is to reduce the pH. It was shown using CA as a model protein that the enthalpy of unfolding determined by simulated DSC at various pHs is equal to the enthalpy of unfolding determined by isothermal calorimetric titration of the protein with acid.[9] The free energy of denaturation at a reference temperature *T* was calculated.

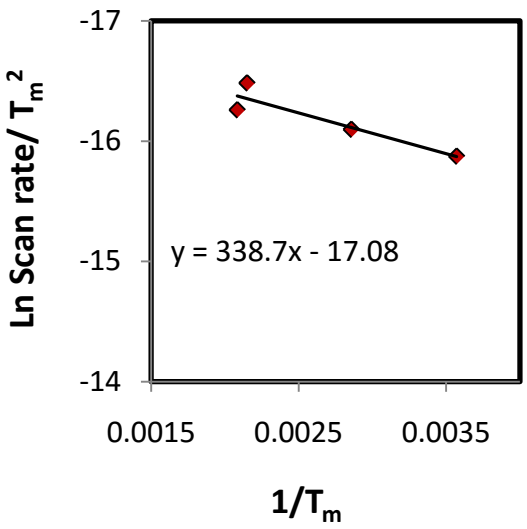

Fig. 7 : Plot of Ln(V/T_m^2) *Versus* 1/T_m, where each data point refers to one of the four scan rates used

Fig. 7 shows Arrhenius plots of the scan-rate dependent changes in T_m. The slopes of these lines provide the apparent activation energies of denaturation.[31]

Since Sanchez-Ruiz and co-workers and other authors [33, 34] reported more or less equivalent activation energies with each of the different Arrhenius-based analysis methods, we have demonstrated only one of these methods here (in Figure 7). T_m values for CA were found to be scan-rate-dependent. Kinetic activation energies for irreversible denaturation were derived from the scan rate dependence of the DSC transitions using Arrhenius plots as described by Sanchez-Ruiz et al., [18] and these plots of ln(scan rate/T_m^2) against 1/T_m are also given in Fig. 7. The slopes of these plots provide the activation energies for irreversible denaturation, which are 338.7/R kJ/mol for CA which R is gas constant.

c) pH Effect

Further exploration of the denaturation of CA was also performed as a function of scan rate in acidic pH. Protein stability could be altered dramatically by changing pH or by changing pH in a scan rate mode. For example, lowering the pH to acidic value lowered the T_m by 8, 16, or 25 °C experimentally.[9] Here we used the MD simulation and showed thermal denaturation of the sample was pH and scan-rate dependent. We have made DSC studies into the thermal stability of CA within different pH at neutral and acidic pH values and different scan rates and compared these calculation results to those of the native protein in experimental condition. Effects of the pH on T_m of both scan rate and thermal denaturation can be taken as an interesting result. The scan rates used were 0.01, 0.0125 and 0.015 K/ps in a lower acidic media which all carboxyl groups (COO^-) were protonated. Fig. 8 shows a typical DSC profiles for a protein in three different scan rates and acidic media. Melting temperature as a function of pH for different scan rates was obtained. T_m values in each scan rate for acidic condition are presented in Table 1.

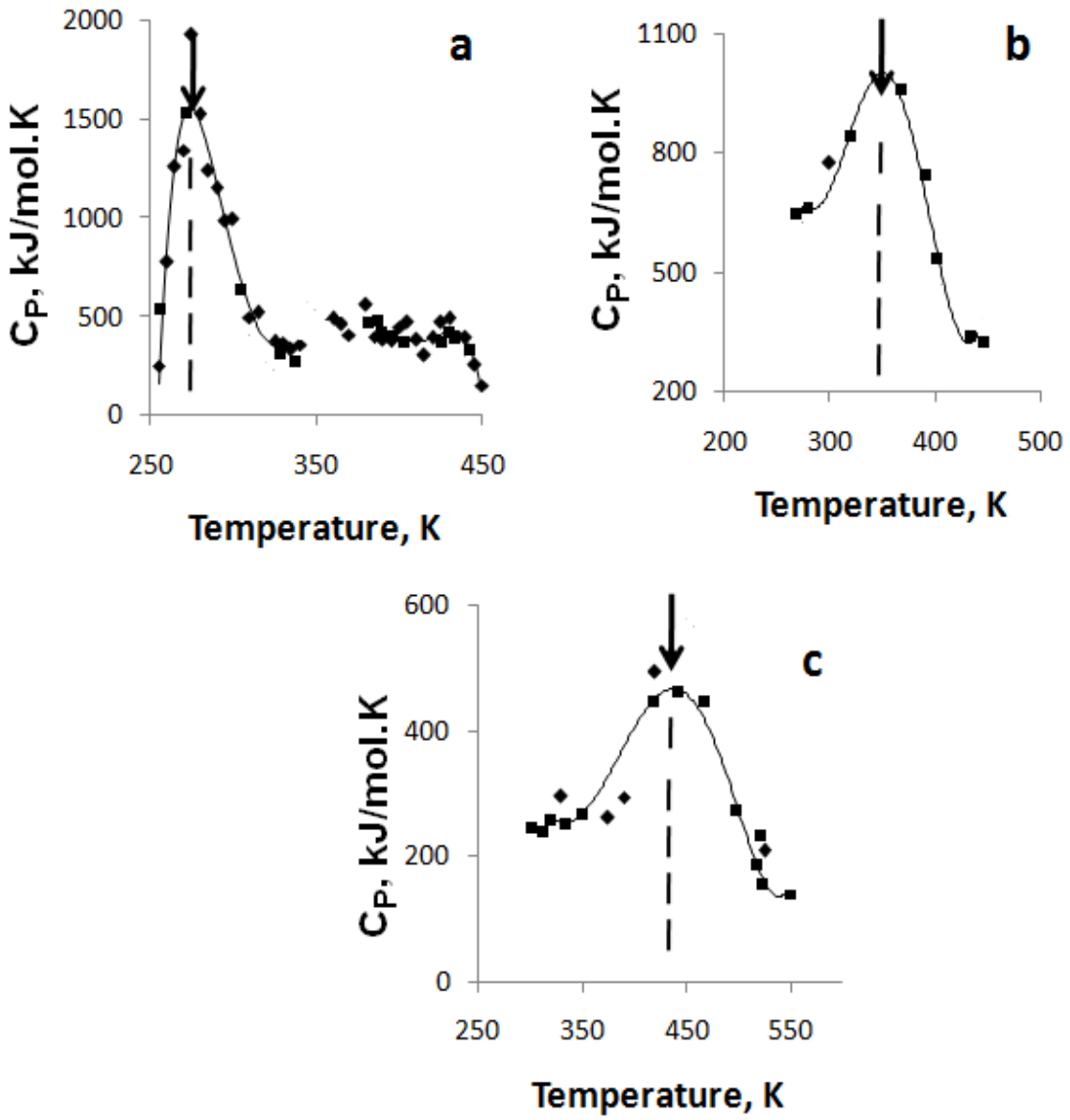

Fig. 8 : DSC profiles for CA in three different scan rates in created acidic media

Thermal stability was essentially pH dependent. In lower pH, the T_m gradually decreased as the pH became more acidic, as expected for a protein that binds protons more tightly in the non-native state.[15]

The calorimetric enthalpy of unfolding was calculated as the area of the unfolding peak, normalized to the molar protein concentration. The unfolding enthalpy was linearly proportional to T_m, and the slope of H vs T_m yielded C_p.

IV. Conclusion

BCA is a protein that is commonly used as a model for biophysical studies, and this work provides additional information on the effect of different denaturants of scan rate, pH and temperature on the stability of BCA. It demonstrates that increasing the scan rate in acidic media reduces protein stability. Increasing the scan rate in neutral media improves the stability of BCA. Calculations were performed using molecular dynamics simulation with four scan rates. The calorimetric traces were found to be scan-rate-dependent under the conditions employed.

V. Acknowledgments

The financial support of Damghan University is acknowledged.

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FELLOWS

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- The "FARSE" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSE or William Walldroff, M.S., FARSE.

FARSE accrediting is an honor. It authenticates your research activities. After recognition as FARSE, you can add 'FARSE' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

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We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

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The FARSE will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSE member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSE, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

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The FARSE member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

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The 'MARSE ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The "MARSE" is a dignified ornament which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., MARSE or William Walldroff, M.S., MARSE.

MARSE accrediting is an honor. It authenticates your research activities. After becoming MARSE, you can add 'MARSE' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSE designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSE, you will be given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSE member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSE, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

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Institutional Fellow of Open Association of Research Society (USA)-OARS (USA)

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The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

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After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

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The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

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(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

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- Font type of all text should be Swis 721 Lt BT.
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- Author Name in Font Size of 11 with one column as of Title.
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- Two Column with Equal Column with of 3.38 and Gaping of .2
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You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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2) Drafting the paper and revising it critically regarding important academic content.

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All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

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Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

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Complete support for both authors and co-author is provided.

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Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(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, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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

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

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

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All manuscripts submitted to Global Journals Inc. (US), ought to include:

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Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

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

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin 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 research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

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Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

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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.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

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The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

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You must strictly follow above Author Guidelines before submitting your paper or else we will not at all be responsible for any corrections in future in any of the way.

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. 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.

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

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. 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 to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After 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 to 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 in 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 criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of 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 the 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 evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose 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 sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, 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 the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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

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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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ISSN 9755861