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Kinetic Potentials of the Effect of Ethanol on Iron Content of Ashed Cow Blood

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Abstract: The kinetic study was carried out to determine the iron content in the ashed cow blood extract. The cow blood was subjected to pretreatment process such as boiling, heating, drying, grinding and ashing. The ashed cow blood was digested in distilled water for 24 hours and then filtered. Few drops of chromic acid was added to the filtrate and left to equilibrate. This was necessary to release iron from the coordinated blood extract. The iron content of the filtered extract was determined spectrophotetrically, using AAS and a concentration of 1.9200 mg/litre of iron was recorded. Kinetic studies were carried out by introducing equal volume of local ethanol into the filtrate and the iron concentration was monitored using AAS at 10 minutes interval. The ethanol led to a gradual decrease of the concentration of iron with increase in time. The average pH of the reaction mixture was 7.5 which was slightly alkaline or near neutral. Other kinetic parameters such as rate of reaction, rate constants, order of reaction, half life and full-life of the kinetics of the effect of ethanol on iron content of the ashed cow blood were determined. The rate of reaction obtained was 0.0215 mg/litre/minute. The rate constant value obtained was 0.010025 $mg^{-}dm^{3}min^{-1}$. The kinetics revealed a second order type as it was found to fit the second order integrated rate law. A half life value of 51.95 minutes was determined which corresponded to a full life of 103.90 minutes. Discussions are made based on the dangers of alcohol intake to the iron contents of the body.

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1.0 INTRODUCTION

1.1 Background of the Study

Chemical research has shifted towards the recovery of waste materials and their conversion to useful materials. For example, Cow blood is wasted in slaughter houses all over the nation, whereas it can be screened and converted into useful material which can be utilized for medical or industrial purposes. Iron play a vital role in engineering structures and it is also very essential to living organisms. Low amount of iron content can cause a lot of damage to an organism.

1.2 Usefulness of Iron in the Body

Iron is essential to living organisms, in particular, to human beings. Iron plays an initial role in supporting life by taking part in oxygen transport and storage, electron transport and energy metabolism iron is important for DNA synthesis. A small amount of iron is present as myoglobin, which acts as an oxygen store in muscle tissue. Iron is the key component of haemoglobin and is useful in cognitive development and immune functioning.
Iron functions in antioxidant activity and is required to breakdown toxic oxygen species in the body. It is useful in the detoxification, neurotransmitters and hormone synthesis (Kinkow, 2002). Literature has shown that there are many food items and organic wastes which contain iron in a reasonable quantity. For example: In 3Oz serving size of liver and 1Oz serving size of pumpkinseeds, roasted, 7.5mg iron and 4.2mg iron is obtained respectively (Monsen, 1988).

1.3 Deficiency of Iron and effect

People with severe iron deficiency suffer from a disease diagnosed as Iron Deficiency Anaemia (IDA). IDA has been identified in large percentage of the world's population with pregnant women, children and teens (Stanislaw, 2011).
Another form of iron deficiency is sickle-cell and genetic disorder. This disease is genetically received, (i.e, one must have inherited in defective copy of the gene from both parents to develop heart disease)
The amount of iron stored in the body can influence a person's potential to develop heart disease. Excess iron is associated with the formation of free radicals which are unstable molecules in the body which may injure vessels supplying blood to the heart. Restless leg syndrome is a form of iron deficiency. It is a neurological movement associated with people with restless leg syndrome. The person experience an uncomfortable

sensation in their arms and legs that result in the need to move and effect sleep patterns. They are linked to iron insufficiencies in spinal fluid or parts of the brain (Cumar, 2012). Restless leg syndrome increases the risks of sepsis and low birth. It also reduces work productivity in adults.

1.4 Consumption of Alcohols in Nigeria

According to World Health Organisation (WHO), Gender Alcohol and Culture; An International Study of (GENACIS) 2013 regional survey; total sample size n = 1949, male n = 1049 and female n = 900. Age range 20 – 64 year. The rate of last year abstainers was 66.3% (Total) 57.1% (male) and 7% (female). Heavy episodic drinking was defined as a consumption of five and more drinks in one sitting at least once a month in the year. The most commonly used alcoholic beverages was palm wine (60.1%) of users; followed by beer (20.8%). Locally fermented wine and locally distilled gin (147%).

1.5 Traditional Alcoholic Beverage

1.5.1 This includes brukutis, a popular alcoholic beverage of a vinegar –like flavor prepared from sorghum grains and fermented guinea corn. It is consumed in the savanna region of Nigeria.

1.5.2 Palm wine is the whitish sap collected in vessel attached to the base of the tree from the source. Fresh palm wine from the source is sweet and contains little alcohol, but with fermentation, the alcohol content increase with time. It is consumed mainly in the southern Nigeria.

1.5.3 Pito is a brown liquid which varies in taste from sweet to bitter. It contains lactic acid, sugars, amino acids and has alcohol content of 3%. It is prepared from the cereal grains (maize, sorghum or a combination of both). It is a traditional drink for Binis in the mid West Africa.

1.5.4 Emu is produced from sugaray palm sap. The most frequently taped palms are raffia and oil palm. These two types contain 5% alcohol.

1.5.5 Ogogoro is a gin-like drink distilled from oil to raffia palm. In Nigeria, the end product is a clear liquid with alcohol content often higher than 40%. It is produced in village across the South (Obot, 2006).

1.6 Effect of alcohol in the body

The effect of alcohol on human capabilities is, of course attracting much contemporary attention; especially in its relation to motoring. Alcohol tends to impair skills and clouds judgment, though the extent of these effects depends on the individual and his alcoholic tolerance. Quantities of alcohol lead to lack of muscular control, e.g the drunken staggar and ultimately, to coma the state of dead-drunk.

A sufficiently large alcoholic consumption taken over a long period causes liver deterioration and accelerate methanol is a more poisonous still: even moderate amount may affect the optic nerve and produce blindness for several days while greater amounts may produce permanent blindness or prove fatal.

Long term alcohol abuse is known to exert harmful effects on a number of the body's organ and systems. Those organs/systems mostly affected by alcohol abuse include:

(i) Alcohol liver disease which leads to liver inflammation
(ii) Heart diseases which include gordiomyopathy, stroke, arrhythmias, and high blood pressure.
(iii) Cancer and pancreatitis
(iv) Alcohol affects the skeletal development causing bone diseases.
(v) It also affects the immune system (Yirmiya *et al.,* 1993).

2.0 LITERATURE REVIEW

2.1 Some studies on iron and ethanol

Schrier, S. L. (2009) studied the body iron and its physiological role. He reported that most well nourished people have 4 to 5 grams of iron in their bodies, the liver's stores ferritin are the primary physiologic source of reverse iron in the body.

Andrew, N. C. (2013) investigated the physiology of iron in human body. He reported that iron is essential to life because of its unusual flexibility to serve a both an electron donor and acceptor.

Jonathan, H. S. (2007), studied iron balance in human body. He reported that the regulation of iron levels is a task of the whole body, as well as for individuals cells, the receptors increase binding of transferring to cells, and therefore stimulating iron uptake.

Brady, P. G. (2007), investigated iron deficiency disorder. He reported that the important cause of iron deficiency anaemia is parasitic infection caused by hookworms, whipworms and round worms, in which intestinal bleeding caused by the worms may lead to undetected blood loss in the stool.

Pietrangelo and Antonello, (2010) studied iron overload in various disease. They reported that iron overload with a hereditary/primary cause from a metabolic order; hereditary haemochromatosis is characterized by an accelerated rate of intestinal iron absorption and progressive iron deposition in various tissues.

Brittenham *et al.* (2005) investigated Assessment of body iron burden. They reported that the availability of a simple assay monitoring non-transferring-bound plasma iron could provide a useful measurement of iron status but has not been applied.

McLennon and Author (2003) studied oxidative stress pathological process. they reported that organisms also key as many iron and copper irons

as possible safely bound in storage or transport protein, there is three times as much transferring iron building capacity in plasma as iron needing to transported so that there are essentially no free irons in the plasma studied.

Kessler *et al.* (2003) conducted their study on wheat grass and iron overload. They reported that humans are unable to eliminate the iron released from the breakdown of transfused red blood cells and the excess iron is deposited and ferritin.

Louis Pasteur (1999) studied how alcohol was converted from sugar. He observed that the breakdown organic compound in absence of oxygen can be used by some organisms as means of obtaining energy; as he put it "Fermentation is life without air". Some strictly anaerobic microorganism, such as butyric acid bacteria, yeast are facultative anaerobic. He showed that sugar is the converted to alcohol and carbon (iv) oxide is the main end product of the aerobic reaction.

Buchner, R. K. (2012) investigated the derivatives of alcohol. He reported that in his attempt to preserve an extract of yeast, prepared by grinding yeast cell with sand, Buchner added a large quantity of sugar to it and was surprised to observed an evolution of carbon iv oxide accompanied by fermentation of alcohol. Recently, the authors have reported on the kinetic potentials of the effect of ethanol on iron content of ashed cow liver (Akpan and Obotowo, 2016).

3.0 MATERIALS AND METHODS

3.1 Collection of the Cow Blood Sample

The cow blood used for this work was collected from Mbak Itam slaughter house in Itu Local Government Area, Akwa Ibom State. The cow blood was heated until it turned from colloid to solid state. The solidified cow blood was grinded in mortar, and a fraction of it put in different crucibles where the crucibles and contents were placed in the MUFFLE furnace for ashing.

3.2 Ashing of Cow blood sample

The crucibles with cow blood were heated to red hot. The ashed cow blood powder was agitated occasionally to bring fresh particles to the interface so as to ensure complete ashing and combustion. The ashing lasted for 6 hours at the temperature of 500^0C. The ashed power was weighed continuously until constant weight was obtained.

3.3 Extraction of iron from the ashed cow blood

The ashed cow blood (56g) was leached with some quantity of distilled water ($200cm^3$) and the volume made up to one litre solution. The leached sample was kept overnight, for 24 hours to ensure equilibrium leaching. The leached suspension was filtered and the filtrate labeled and kept prior to Atomic Absorption Spectrophotometric Measurement (AAS).

3.4 Determination of Iron Present in the ashed cow blood extract

The concentration of iron in the ashed cow blood was determined spectrophotometrically by using solar 969 Atomic Absorption Spectrophotometer and their absorption compared with absorption standard of iron in the calibration curve.

3.5 Equipments/Apparatus:

The following apparatus and equipment were used for this work; Injection syringes, volumetric flask, pH meter, thermometer, glass rod, spatula, wash bottle, filter paper, funnel, beakers, Atomic Absorption Spectrophotometer and a stop watch.

3.6 Kinetic Measurement of the effect of ethanol on the concentration of iron in the ashed cow blood sample.

The percentage purity of the local ethanol obtained was estimated to be 30%. Based on the percentage purity and relative density of ethanol ($789kg/m^3$) the required volume of local ethanol which contained one mole was determined.

Here, ethanol ($194cm^3$) was measured and diluted in about $200cm^3$ of distilled water and the volume made up to $1000cm^3$ of solution. This dilution gave a 1M solution of local ethanol in the solution.

The prepared ethanol solution ($200cm^3$) was measured out. At the same time, the filtered ashed cow blood solution ($200cm^3$) was also measured out.

The equal volumes of both solutions were mixed in a $500cm^3$ beaker and a stop watch started simultaneously.

At the interval of 10 minutes, the reaction mixture was gotten out using injection syringe and the sample was analyzed spectroscopically.

The pH of the reaction mixture was also taken at every 10 minutes interval also.

Table 4.1: Variation of Concentration of Iron in the ashed cow blood extract in reaction with ethanol at various time intervals.

Time (mins)	0	10	20	30	40	50	60	70	80
pH	7.83	7.53	7.51	7.51	7.51	7.51	7.51	7.51	7.51
$[Fe^{2+}]$mg/l	1.9198	1.8831	1.8097	1.7713	1.6172	1.2277	1.1688	1.1047	0.1978

Table 4.2: Variation of $Na_o(a_o-x)$ versus time for the kinetics of the reaction of ashed cow blood extract with ethanol

Time(mins)	0	10	20	30	40	50	60	70	80
$[Fe^{2+}]$mg/l	1.9198	1.8831	1.8097	1.7713	1.6172	1.2277	1.1688	1.1047	0.1978
$\frac{x}{a_o(a_o-x)}$	0	0.0102	0.0317	0.0437	0.0975	0.02936	0.3347	0.3843	0.45352

Table 4.3: Data of variation of rate of reaction with concentration of iron with local ethanol

Time (mins)	0	10	20	30	40	50	60	70	80
$[Fe^{2+}]$mg/l	1.9198	1.8831	1.8097	1.7713	1.6172	1.2277	1.1688	1.1047	0.1978
Rate	-	0.0037	0.0073	0.0038	0.0154	0.0390	0.0056	0.0064	0.0907
Log R	-	-2.4353	-2.2343	-2.4157	-1.8122	-1.4095	-2.2299	-2.1931	-1.0424
Log C	0.2833	0.2749	0.2576	0.2483	0.2088	0.0891	0.0677	0.0432	-0.7038

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The results obtained for the kinetics of the reaction of ethanol with iron extract are presented in Table 4.1 to 4.3. Relevant figures are plotted in figure 4.1 to 4.3.

4.2 DISCUSSION

4.2.1 Effect of ethanol on iron content

Table 4.1 and figure 4.1 record the effect of ethanol on the concentration of iron extract. The Table reveals a gradual decrease in iron concentration in the extract with time. This shows that ethanol actually reduces the quantity of iron present in a system and there by weakens the strength of the biological system. The variation of the concentration of iron with time in the kinetic system studied is presented in fig. 4.1 below:

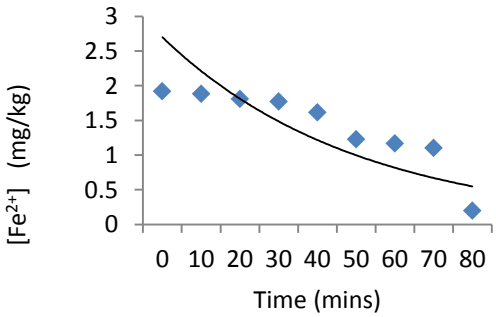

Fig. 4: Plot of Concentration of iron (mg/l) against time (mins)

It was observed that the original iron content in the ashed cow blood extract was 1.9198mg/l. After 10 minutes of the reaction of local ethanol with the iron extract, the iron content reduced to 1.8831 mg/l. At 30 minutes interval, the concentration of the iron was found to reduce to 1.7713 mg/l. The decrease in the iron content continued gradually with increasing time interval as the ethanol persisted in the solution. At one hour (60 minutes), the original content 1.9198 mg/l had reduced to 1.1658 mg/l. At the end of 80 minutes of the measurement interval, the iron content left in the reaction mixture was 0.978mg/l.

The gradual decrease in the iron content could be used to estimate the half-life and rate constant of the kinetics of the effect of alcohol on iron content in the biological system. The full life of the iron content in a biological system with the interaction of alcohol can also be estimated.

4.2.2 The Rate of Reaction of Ethanol with the Iron Content of the Ashed Cow Blood Extract

Table 4.3 and figure 4.3 reveal the various rates of reaction obtained at each time interval for the reaction of ethanol with the iron content of the ashed cow blood extract. The values obtained show that the rate was progressively faster at the beginning of the experiment, then reduced and afterwards it increased gradually as the experiment progressed with time.

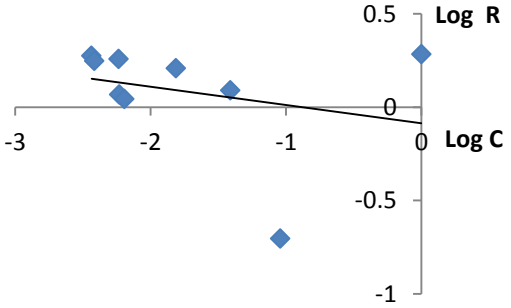

Fig. 4.3: Plot of the Logarithm of the rate of reaction versus the logarithm of concentration of iron for the reaction of iron extract with the local ethanol.

During the first 10 minutes interval, the rate obtained was 0.0037mg/l.min. This value increased to 0.0073mg$L^{-1}min^{-1}$ at 20 minutes interval. During 30 minutes, it decreased as the rate obtained was 0.0038mg$L^{-1}min^{-1}$. At 60 minutes and 80 minutes interval, the rates were 0.0059mg$L^{-1}min^{-1}$ and 0.0907mg$L^{-1}min^{-1}$ respectively. The average rate of reaction obtained for the reaction over 80 minutes interval was 0.0215mg$L^{-1}min^{-1}$.

4.2.3 pH of the Reaction Mixture

The reaction mixture, was slightly alkaline due to the hydroxyl group in ethanol molecule. The alkalinity reduced negligibly in 20 minutes to a constant value throughout the experiment.

4.2.4 Order of the Reaction of Local Ethanol and the Iron Extract

The concentration-time data obtained from the Atomic Absorption Spectrophotometric measurement was subjected to various integrated rate laws fitted for zero, first, second and third order reaction kinetics. The integrated rate equation tested were as follows:

$k = a_o - a/t$ for zeroth order reaction.

$k = 2.303 \log \frac{(a_o)}{a_o - x}$ for the first order reaction,

$k = x/t\ a_0\ (a_o - x)$ for second order reaction and

$k = \frac{½t\ (2a_0\ 2a_0\ x - x^2)}{a_o^2(a_o - x^2)}$ for third reaction

For the integrated rate equations, it was discovered that the concentration –time data fitted into the second order kinetic model which resulted in an approximately equal and constant value of the various rate constants. The kinetic test confirms that the reaction of the local ethanol with the iron extract obtained from ashed cow blood follows a second order reaction.

4.2.5 Rate Constant of the Reaction of the Ethanol with the Iron Extract

The various rate constants obtained from the experimental fittings of the kinetic data into the integral rate laws were obtained and their average was calculated.

The average rate constants obtained for the hydrolysis of the iron extract with the ethanol was $0.010025 mg^{-1}dm^3min^{-1}$. This value confirms that the rate of the reaction of the alcohol molecule with iron is moderately fast and portends a quick danger at high consumption of alcohol.

The graph of $\frac{x}{a_o\,(a_o - x)}$ versus time (mins) for the reaction ethanol with cow blood extract is presented below in fig. 4.2. The slope gave a rate constant value of $0.010025 mg^{-1}dm^3min^{-1}$

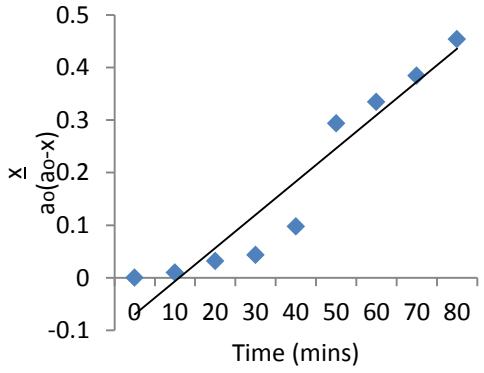

Fig. 4.2: Plot of $\frac{x}{a_o\,(a_o - x)}$ versus time (mins) for the reaction of ethanol with cow blood extract

4.2.6: Half life of the concentration of [Fe^{2+}] with Respect to the reaction of Local ethanol

The kinetic reaction was found to follow the second order integrated rate law.

The integrated rate for the second order reaction is:

$$1/a - 1/a_0 = K_2 t$$

At half life, a = $a_0/2$ *and* $t = t_{\frac{1}{2}}$

Therefore by appropriate substitution, the half life,

$$t_{\frac{1}{2}} = \frac{1}{k_2 a_0}$$

Half life value obtained for the reaction of the local ethanol with the iron extract was 51.95 minutes. This value showed that if the original iron concentration in the body was 22.6131mg/litres in the body, by consuming $200cm^3$ of 1 molar local ethanol, it will take approximately 52 minutes for the original iron concentration to reduce to half of the concentration. Signifying that at 103.9 minutes interval after consumption of the ethanol, the iron in the blood would have been completely depleted by the alcohol leading to complete exhaustion as experienced by most alcohol addicts.

4.2.7 Effect of Alcohol on Mammalian iron Content

The effect of alcohol on human capabilities is attracting much contemporary attention, especially in its relation to driving. Alcohol tends to impair skills and cloud judgment, though the extent of these effects depends on the individual and his alcoholic tolerance. Quantities and indiscriminate consumption of alcohol leads to lack of muscular control, e.g the drunken stagger, and ultimately to coma, the state of dead-drunk. Recent report has it that excessive consumption of alcohol has sapped the body nutrients to lethal level and has caused many to die unexpectedly.

This study reveals that alcohol has deleterious effect on the iron content of the organs in the mammalian body. It reduces the level of iron with time as it persists in the body. The study reveals that the order of the reaction of the alcohol with the concentration of the iron depends on the pH of the body. At near neutral pH, the reaction of follows a second order kinetics.

In basic medium, the study reveals that first order kinetic is favoured.

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The kinetic potentials of the effect of ethanol on iron content of ashed cow blood has been studied.

The ashed blood extract has been found to be rich in iron content up to 1.9198mg/l for the concentration studied. The original iron content of the ashed cow blood extract has been observed to decrease with increase in time with the addition of local ethanol. The average pH of the reaction mixture was 7.5 showing that the reaction of the ethanol with the cow blood extract progressed at near neutral or slightly alkaline condition.

The average rate of reaction was found to be $0.0215 mg^{-1}min^{-1}$ showing that the reaction of ethanol with the cow blood extract was progressively moderate. The rate constant of the kinetic was obtained to be $0.010025 mg^{-1}dm^3min^{-1}$.

The interaction of the ethanol with the ashed cow blood extract revealed a half life of 51.95 minutes, leading to a full life of 103.90 minutes. The full life

value can be used to predict the deleterious effect of the alcohol to the iron content of the body.

By the use of integrated rate laws, the kinetics of reaction of local ethanol with the ashed cow blood extract was determined to follow a second order kinetics. Intake of alcohol has been observed to reduce the available iron content in the body.

5.2 Recommendations and Suggestion for Further Studies

This study recommends that a kinetic study be carried out on other iron-containing waste materials such as pumpkin seeds, dried beans, grains cereals etc. Apart from local ethanol the effect of aromatic hot drinks (dry gin) such as schnapps, whisky and brandy should be studied on the content such that their effect could be compared.

It is suggested that the reaction should be carried out under acidic and alkaline medium by adjusting the pH. It is suggested that the effect of tobacco extract on the iron content of the cow blood extract be studied. The kinetic study should be carried out using charred cow blood extract with the ethanol to compare the difference.

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