

Kinetic Potentials of the Effect of Ethanol on Calcium Content of Ashed Cow Bones

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Abstract: The kinetic study was carried out to determine the calcium content in the ashed cow bone extract and to monitor the rate of deterioration of the calcium content in the presence of alcohol. The cow bone was subjected to pre-treatment processes such as drying, grinding, and ashing. The ashed cow bone was digested in distilled water for 24 hours and then filtered. The calcium extract was determined by titration and a concentration of 86.67 mg/l of calcium was recorded. Kinetic studies were carried out by introducing equal volumes of local ethanol into the standard solution of the calcium and measuring the rate of reaction titrimetrically after every 10 minutes interval. The added ethanol led to a gradual decrease of the concentration of calcium with increase in time. The average pH of the reaction mixture was 7.01. Other kinetic parameters such as rate of reaction, reaction constants, order of reaction, half-life and full life of the kinetics of the effect of ethanol on calcium content of the ashed cow bone was determined. The rate of reaction obtained was 0.63 mg/l/min. The rate constant value obtained was 0.00027272 mg/dm³/min. The kinetics revealed a second order integrated rate law. A half-life of 42.3 minutes was determined which is equivalent to a full life of 84.6 minutes. Discussions are made based on the dangers of alcohol intake to the calcium contents of the body.

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1.0 INTRODUCTION

1.1 Background of the Study

The study of chemistry is now shifting emphasis to the scavenging and utilization of waste materials and its conversion to useful substances. Cow bones wasted in slaughter houses all over the nation can be picked, screened and converted into useful substances for medical purposes. Calcium as a nutrient present in cow bones is commonly associated with the metabolism and formulation of bone. Over 99% of the total body calcium is found as calcium hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6(\text{OH})_2$) in bones and teeth, where it provides hard tissues with its strength (Bailey *et al.*, 2010). Calcium in the respiratory system, extracellular fluid, muscle and other tissues is critical for mediating vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signalling and hormonal secretion (Calvo and Maier, 2004).

Calcium metabolism is required in large part by the parathyroid hormone (PTH) - vitamin D and endocrine system, which is characterised by a series of homeostatic feedback loop. The rapid release of minerals from the bone is essential to maintain adequate level of ionized calcium in serum (Rafferty *et al.*, 2007). During vitamin C deficiency state, bone metabolism is significantly affected as a result of reduced active calcium absorption. This leads to increased PTH secretion as the calcium sensing receptors in the parathyroid gland senses changes in circulating ionic calcium (Ca^{2+}).

Studies have shown that alcoholics have multiple risk factors for bone loss, including low dietary calcium and other nutritional deficiencies. Low body weight, apart from resulting from alcohol consumption, may be due to smoking and high caffeine intake (Iaitinen *et al.*, 1991). Although the detrimental effects of alcohol on bone metabolism have been confirmed by animal studies (Peng *et al.*, 1991), most of these studies involved administration of large amount of alcohol. Several studies have suggested that moderate drinking in social settings may be associated with high bone mineral density. The results were somewhat conflicting. However, various problems associated with alcohol intake categories lack adequate proof and are still under investigation.

1.2 Usefulness of Calcium in the Body

The main role of calcium in the body is to provide structure and strength to the skeleton. In the earliest form of skeletons, and in shells, the structural rigidity is usually provided by calcium carbonate. In vertebrates such as reptiles, pisces, mammals, and humans, the structure of the skeleton is usually provided by a form of calcium phosphate called hydroxyapatite crystals, which are found in collagen. Calcium ions on bone surfaces interact with those present in the body fluids, therefore, enabling ion exchange which is essential in maintaining the balance of calcium in the blood and bone.

In the extracellular fluid, calcium serves as a reservoir for circulating calcium. This calcium enters the extracellular fluid via gastrointestinal tract where it is absorbed from the diet. Calcium circulating in the blood is involved in several vital processes including coagulation, nerve signal transmission, and hormone signalling and muscle contraction.

1.3 Food Sources of Calcium

Calcium is the most abundant mineral in the body and is found in a wide variety of foods and beverages and added to many others. Calcium in any form is good for our body. Some of the foods rich in calcium are cheese, yoghurts, milk, sardines, dark leafy greens like spinach, turnips and collard greens, fortified cereals such as total raisin bran, corn flakes (they have a lot of calcium in one serving), fortified orange juice, soybeans, soymilk, enriched breads, grains and waffles.

1.4 Deficiency of Calcium and its Effect

Calcium is an essential element for bones, as a result, deficiency of calcium affects bones the most severely and one of the initial symptoms of calcium deficiency is muscle cramps. This sign of calcium deficiency occurs as the initial alarm towards the decrease of calcium in the body. The muscle ache especially those of the thighs, arm and under arms while walking around may be a sign of calcium deficiency. Tooth decay is another sign of calcium deficiency in the body. There may be a delay in the initial start of tooth formation during early childhood as a result of this. Calcium deficiency results in poor bone density, rickets, weak and brittle nails and late signs of puberty and menstrual cramps in females. Calcium deficiency may also lead to cancer, heart disease, allergies, chronic arthritis and headache. Other symptoms include common cold, flu infections, infertility, low pH, acidic saliva/urine, miscarriage, kidney stone and weak or deformed bones (Bikle, 1993; Krawitt, 1991).

1.5 Effect of Alcohol in the Body

The effect of alcohol on human capability is of course attracting much contemporary attention, especially to its relation to motoring. Alcohol tends to impair skills and cloud judgement, though the extent of these depends on the individual and his alcoholic tolerance. Large intake of alcohol leads to lack of muscular control, e.g. the drunken stagger and ultimately to coma – the state of dead drunk. A sufficiently large alcoholic consumption taken over a long period causes liver deterioration and accelerates death. A survey of literature reveals that much work has been carried out on the effect of calcium content of bones. It has been recorded that alcohol intake disrupts hormonal balance (Sampson, 1997), causes a condition known as

hypocalcaemia [7], reduces bone formation and induces bone desorption (Chappard *et al.*, 1991), causes osteoporosis and estrogen deficiency (Gavaler and Thiel, 1992; Diamond *et al.*, 1989). Men associated with heavy drinking have decreased levels of the male steroid hormone – testosterone and female alcoholic addicts experienced increased metabolic conversion of testosterone (produced in the ovaries and adrenal glands) to the female steroid hormone; estradiol (Hogan *et al.*, 1997). Long term alcoholic consumption results in reduced osteoblast activity and retarded bone growth (Dyer *et al.*, 1998; Sampson *et al.*, 1996; Poccock *et al.*, 1987), low level of vitamin D (Diaz *et al.*, 1997; Hanan *et al.*, 2000), vertebral deformity (Turner *et al.*, 2001). The present authors had carried out intensive works on the kinetic potentials of the effect of ethanol on calcium content of ashed palm bunch and also on ashed cow liver. The present work is to further investigate the effect of alcohol on calcium content using ashed cow bones to lend credence to our postulates.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and preparation

The cow bones used for this study were collected from the abattoir in Itam Village in Itu Local Government Area of Akwa Ibom State, Nigeria. The cow bones were screen and scrubbed thoroughly to remove all bits of flesh. They were oven dried for hours and the dried samples were crushed to powder. The powdered cow bones were then ashed in a crucible for six hours at 500°C.

2.2 Extraction of Calcium from the Ashed Cow Bone

The ashed cow bone (40g) was leached with 200 cm³ of distilled water and the volume was made up to 1 litre. The leached sample was kept overnight for 24 hours to ensure equilibrium leaching. The leached suspension was then filtered and the filtrate labelled and kept prior to titration measurements.

2.3 Determination of Calcium present in the Ashed Cow Bone Extract

The cow bone extract (10 ml) was measured into a 500 ml conical flask. Ammonia buffer solution (2 ml) and a tablet for total hardness was added to the extract and a purple colour solution was produced (blank). The mixture was titrated against 0.02N EDTA to give a blue colour end point. The concentration of calcium was determined using the following formula:

$$\text{Total hardness} = \frac{V_2 \times 10^3}{V_1} \text{ -----(1)}$$

Where V_2 = average titre

V_1 = Volume of ashed cow bone extract

Since total hardness is made of Mg^{2+} and Ca^{2+} , and Ca^{2+} is $2/3$ of total hardness, the concentration of calcium at every 10 minutes interval was determined using the following formula:

$$[Ca^{2+}] = \frac{2}{3} \times \text{Total hardness} \text{ -----(2)}$$

2.4 Kinetic Measurements of the Effect of Ethanol on the Concentration of Calcium in the Ashed Cow Bone Extract

Ethanol (194 cm^3) was measured and diluted in 200 cm^3 of distilled water and the volume made up to 1000 cm^3 . This dilution gave $1M$ solution of ethanol in the solution. Equal volumes of the prepared ethanol solution and the ashed cow bone solution were measured and mixed in a 500 cm^3 beaker and a stop watch started simultaneously. At every 10 minutes, the reaction mixture was bled out using a syringe and the bled sample was determined by titration. The pH of the reaction mixture was also taken at every 10 minutes interval.

3.0 RESULTS AND DISCUSSION

3.1 Effect of Ethanol on Calcium Content

The results obtained for the kinetics of the reaction of ethanol with calcium extract is represented in Table 1. The Table reveals a gradual decrease in the concentration of the calcium in the calcium extract.

The kinetics of the reaction revealed that the rate was progressively faster at the beginning of the experiment and then reduced gradually with increasing time and reached equilibrium at 90 minutes as is shown in figure 1. It is expected that the gradual decrease in calcium content could lead to the estimation of the half life and rate constant of the kinetics of the effect of alcohol on calcium content in the biological system. The full life of the calcium content in a biological system with the interaction of ethanol can also be estimated. This shows that ethanol actually reduces or destroys the quantity of calcium present in a system and thereby weakens the strength of the biological system.

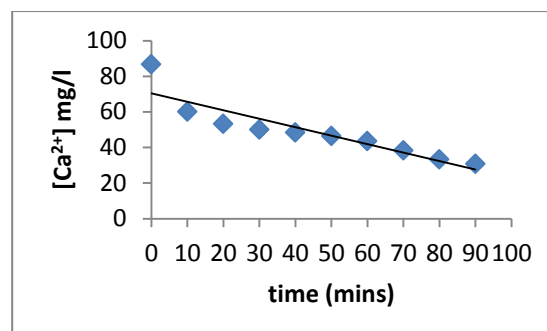


Figure 1: variation of the concentration of calcium in the ashed cow bone extract in reaction with ethanol at various time intervals

3.2 Order and Rate Constant of the Reaction.

The concentration-time data obtained from the experimental measurement was subjected to various integrated rate laws fitted for zero, first, second, and third order reaction kinetics. From the integrated rate reactions, it was discovered that the concentration-time data fitted into the second order kinetic model and that the reaction of local ethanol with the calcium extract obtained from ashed cow bone followed a second order reaction as given by the equation below:

$$k = \frac{x}{ta_0(a_0 - x)} \text{ -----(3)}$$

The various rate constants obtained from the experimental fittings of the kinetic data into the integrated rate laws were obtained and their average was calculated. The average rate constant obtained for the hydrolysis of the calcium extract with the ethanol was $0.000272 \text{ mg/dm}^3/\text{min}$ as shown in Figure 2. This value confirms that the rate of the reaction of the alcohol molecule with calcium is moderately fast and portends a quick danger at a high rate of consumption of alcohol.

By proper substitution, the half-life, $t_{1/2}$ for the second order reaction is given as:

$$t_{1/2} = \frac{1}{k_2 a_0} \text{ -----(5)}$$

Table 1: Kinetic data for the reaction of ethanol with ashed cow bone extract

Time (mins)	0	10	20	30	40	50	60	70	80	90
pH	8.02	6.80	6.87	6.80	6.92	6.88	6.92	7.05	6.92	6.93
[Ca ²⁺] mg/l	86.67	60.00	53.33	50.00	48.33	46.47	43.47	38.33	33.30	30.80
pH	8.02	6.80	6.87	6.80	6.92	6.88	6.92	7.05	6.92	6.93
x	-	0.005	0.007	0.008	0.009	0.012	0.011	0.015	0.018	0.020
a ₀ (a ₀ - x)										
Log R	-	-0.40	0.2	0.50	0.80	0.80	0.50	0.30	0.30	0.50
Log C	-	1.80	1.70	1.70	1.70	1.70	1.60	1.60	1.50	1.50

Half-life of the concentration of calcium (Ca^{2+}) with respect to the reaction of local ethanol
The integrated rate law for the second order reaction is given as

$$K_2t = \frac{1}{a} - \frac{1}{a_0} \text{-----(4)}$$

The half-life value obtained for the reaction of the local ethanol with the calcium extract is 24.57 minutes. The value shows that if the original calcium concentration in the body was 22.613 mg/l in the body, by consuming 200 cm³ of 1M local ethanol, it will take approximately 25 minutes for the original calcium concentration to reduce to half of the concentration.

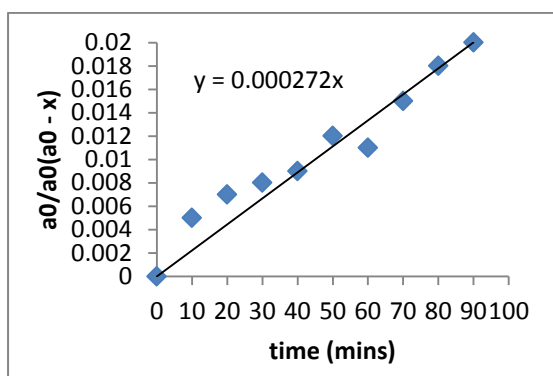


Figure 2: variation of $\frac{x}{a_0(a_0 - x)}$ against time for the reaction of ethanol with the ashed cow bone extract

CONCLUSION

The kinetics of the effect of ethanol on calcium content of ashed cow bone revealed that the cow bone extract is rich in calcium content up to 86.67 mg/l. the calcium content was observed to decrease with increase time on addition of local ethanol. The average kinetic rate constant was obtained to be 0.00027272 mg/dm³/min, revealing a half-life of 42.3 minutes. By use of integrated rate law, the kinetics of the reaction of local ethanol with the ashed cow bone was determined to follow a second order kinetics. This study has further confirmed that intake of alcohol reduces the available calcium content of the body.

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