ENERGY CALCULATIONS OF A SALT OF SYNTHETIC NEUROTRANSMITTER (ACETYLCHOLINE CHLORIDE) OXIDATION BY CHLRAMINE-T (A DISINFECTANT)

*WASEEM MAHMUD KAYANI, NEKTAL MADE GOWDA**

ABSTRACT

Acetylcholinestrase inhibitors can have a negative effect on cardiac output. So we have tried to mimic the action of acetylcholinesterase by in vitro degradation of acetylcholine chloride. The activation parameters the energy of activation, enthalpy, Entropy and free energy contents (E_a, Δ H, Δ $S^{\neq}$, $\Delta G^{\neq}$) of the breakdown (oxidation) of acetylcholine chloride by Cholramine-T were calculated at temperature range from 30° C to 60° C. The reaction medium was kept constant.

INTRODUCTION

Note - This research was performed at Western Illinois University U.S.A. in 2003 by the author.

"Acetylcholine release in human atria is controlled by muscarinic M_2-receptors. Blockade of these receptors by atropine doubles the amount of acetylcholine released at a stimulation frequency of 5 Hz. In atria of patients >70 years of age and patients with late diabetic complications, acetylcholine release is reduced. Locally impaired cardiac acetylcholine release may therefore represent a pathophysiological link to sudden cardiac death in elderly and diabetic patients"[1].

"Acetylcholinesterases are the primary targets for organophophate inhibitors because of the irreversible nature of their inhibition, which results in building of acetylcholine concentrations that activate muscarinic and nicotinic receptors and desensitize them, thereby inhibiting respiration. Nevertheless, the high affinities that cardiac muscarinic receptors have for these toxicants point to their extra vulnerability. It is suggested that the success of iv administration of the muscarinic receptor inhibitor atropine in initial therapy of poisoning by OP anticholinesterases may be related in part to the extra sensitivity of M2 receptors to certain Ops"[10].

Due to the diversity of applications of chloramines-T for multiple purposes and its ability as a versatile disinfect and oxidizing agent , we have tried to study the kinetics of the oxidation of other one biologically important compounds namely acetylcholine chloride (a synthetic version of the neurotransmitter acetylcholine).

It is obvious from the their uses and their presence in vivo and in vitro that more knowledge and understanding about the oxidative behavior of acetylcholinechoride is useful as it can further be used for the benefit of humankind. A review of the literature reveals the absence of systematic investigation on the CAT oxidation kinetics of AC.

In the first paper we calculated the rate constants and drew the mechanism of oxidation of acetylcholine chloride by the cholramine-T. In the current paper we calculated the activation parameters the enthalpy, entropy and free energy of AC oxidation by using acidic ($HClO_4$) medium.

Acetylcholine chloride, molecular formula $CH_3CO_2CH_2CH_2N(CH_3)_3Cl$ and M.W. (181.66), is a chloride salt of acetylcholine and is a synthetic version of the central and peripheral neurotransmitter. But it is also used as a vasodilator and a cardiovascular agent. Available via prescription, acetylcholine chloride is used to keep eye pupils dilated during eye surgery and as a bathing solution[2].

Later work showed that acetylcholine binding to acetylcholine receptors on striated muscle fibers, opened channels in the membrane. Sodium ions then

* *Western Illinois University.*

enter the muscle cell, stimulating muscle contraction[3]. Acetylcholine chloride ($CH_3CO_2CH_2CH_2N(CH_3)_3Cl$) is a quaternary ammonium base widely distributed in both animal and plant tissues[2]. It is an essential nutrient to improve the physical performance. Acetylcholine (AC) on enzymatic hydrolysis forms acetic acid and choline[4]. It is a chemical transmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans[5].

Chloramines can be used as bleach, disinfectants and oxidizers/oxidants. Organic disinfectants slowly give off chlorine, causing a slower and less aggressive disinfection than with hypochlorite (OCl^-). Chloramines can be used to improve odor and flavor of the water when chlorine is used as a disinfectant[6]. Solid CAT and its aqueous solutions are stable at temperatures below 60°C. It decomposes slowly in aqueous solutions if temperatures are greater than about 70°C[7].

S. Zhang, H. Zhao and R. John while studying the affect of organphosphorous pesticides on living organisms used the enzyme cholineoxidase along with acetylcholine estrase for the oxidation of the choline.

$$\underset{\text{Acetylcholine (ACh)}}{CH_3COO(CH_2)_2N^+(CH_3)_3Cl^-} + H_2O \xrightarrow{AChE} \underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Choline}}{HO(CH_2)_2N^+(CH_3)_3Cl^-}$$

$$\underset{\text{Choline}}{HO(CH_2)_2N^+(CH_3)_3Cl^-} + O_2 \rightarrow \underset{\text{Betaine}}{HO_2C(CH_2)N^+(CH_3)_3Cl^-} + \underset{\text{Hydrogen peroxide}}{2H_2O_2}$$

As a result of the oxidation of acetylcholine in the presence of oxygen a product betain and hydrogen peroxide are produced[8].

The present project deals with the kinetic and mechanistic study of oxidation of Acetylcholine by Chloramine–T in $HClO_4$ medium, which is spectrophotometrically monitored at λ_{max} of 256 nm at the temperature range 303-333 K.

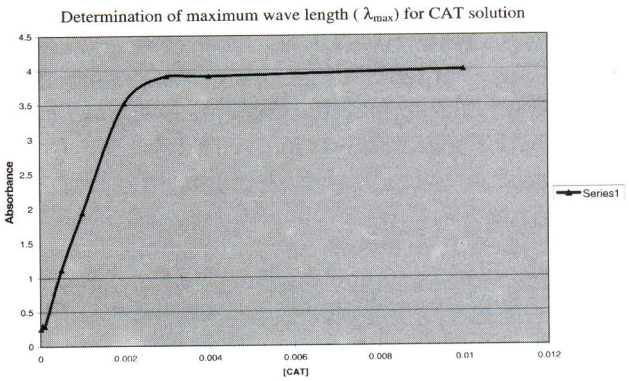
MATERIAL

(Shimadzu UV-1601) UV/Visible high performance spectrophotometer features include photometric, kinetic, spectrum scanning, multiwave length, and quantization capabilities. In the spectrum mode, plots of absorbance *vs* wavelength are taken to determine the λ_{max} value.

11.7M $HClO_4$ (fisher Chemical Co), 0.1 M $HCl\ O_4$, 0.1 N NaOH, 0.05 N KIO_3 solution, 0.05 N $Na_2S_2O_3$, 0.05 M CAT, Acetylcholine chloride 0.1 M.

As it is shown in fig.(1) 256 nm was selected as λ_{max} for monitoring CAT in the reaction mixture.

Fig-1 : A plot of absorbance vs. [CAT] shows that the Beer's law is obeyed in the range, 0-2.00 x 10^{-3} M CAT. Therefore the concentration of CAT in the kinetic run was kept below 2.00 x 10^{-3} M



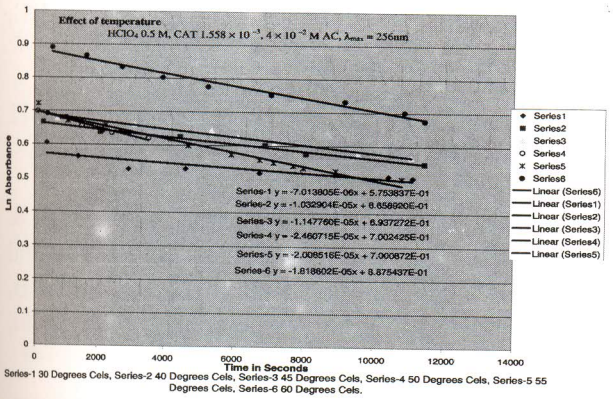
RESULTS

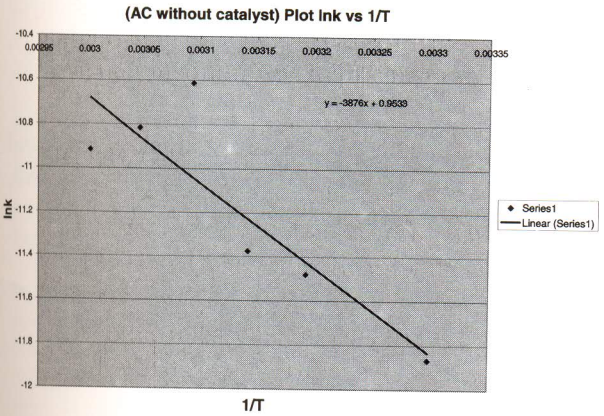
Table-1 : Kinetic data for temperature effect

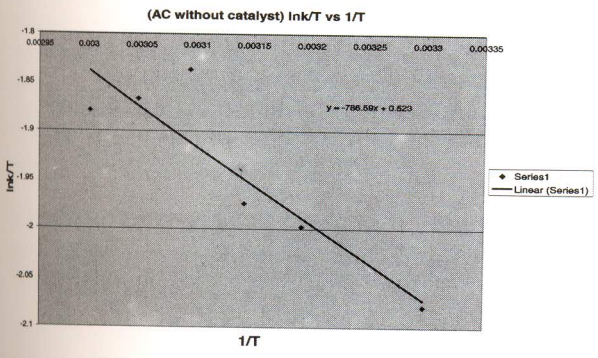
Ks^{-1}	Ln k	T (°C)	T (K)	1/T	Ln (k/T)
7.014×10^{-6}	-11.86	30	303.15	0.0032987	-2.077
1.033×10^{-5}	-11.48	40	313.15	0.0031934	-1.998
1.148×10^{-5}	-11.37	45	318.15	0.0031432	-1.974
2.460×10^{-5}	-10.61	50	323.15	0.0030945	-1.837
2.008×10^{-5}	-10.81	55	328.15	0.0030473	-1.867
1.819×10^{-5}	-10.91	60	333.15	0.0030016	-1.879

DISCUSSION

"Acetylcholinestrase inhibitors can increase activity in both sympathetic and parasympathetic ganglia supplying the heart and at the acetylcholine receptors







Arrhenius Equation $\ln(k) = (-Ea / RT) + \ln A$ (1)
Plot ln k vs 1/T and Fig.2 gives
Slope = $- E_a / R$
And Ea = Slope x R = 3876 x 8.3145 J K^{-1} mol^{-1}
= 32227 J K^{-1} mol^{-1}

Eyring Equation $\text{Ln}(k/T) = -\Delta H / RT + \Delta S / R + \text{Ln}(k/h)$ (2)
Plot of Ln (k / T) vs 1/T and Fig.3 gives
Slope = $-\Delta H^{\neq} / R$ (3)
Intercept = $\Delta S^{\neq} / R + \text{Ln}(k/h)$ (4)
k is the Boltzman's constant and h is Plank's constant
Using Eq (4) Slope = Slope = -786.59

$\Delta H^{\neq}$ = -786.59 x 8.3145 J K^{-1} mol^{-1} = 6540 J K^{-1} mol^{-1}
Using Eq (4) Intercept = 0.9533
$R(I - \text{Ln } k/h) = \Delta S^{\neq}$
$\Delta S^{\neq}$ = - 193.2 J K^{-1} mol^{-1}
$\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$ (5)
$\Delta G^{\neq}$ = 64.142 J K^{-1} mol^{-1}

on neuroeffector cells (cardiac and vascular smooth muscles) that receive cholinergic innervation. The cholinesterase inhibitors such as edrophonium, physostigmine, or neostigmine mimic the effect of vagal nerve activation on the heart. Negative chronotropic, dromotropic, and inotropic effects are produced, and cardiac output falls"[9].

There is a attenuation of the parasympathetic control in heart failure (HF). Cholinestrase inhibitors can induce muscarinic blockade which can further reduce the R-R interval by 308 ms by controls but only 32 ms in heart failure, indicating low levels of resting vagal tone. Vagomimetic doses of atropine sulfate prolonged the R-R interval by 109 ms in controls and increased standard deviation of the R-R interval by 66 ms but only by 46 and 16 ms, respectively, in HF. Bradycardia elicited by electrical stimulation of the vagus nerve was also attenuated in the HF group. Conversely, muscarinic receptor activation by bethanechol, and indirectly by neostigmine, elicited exaggerated R-R interval responses in HF. Muscarinic receptors are upregulated and acetylcholinesterase is reduced in the sinus node in HF. Therefore, reduced vagal control in HF is most likely due to changes of presynaptic function (ganglionic), because postsynaptic mechanisms augment vagal control in HF[11].

CONCLUSION

From the calculations of activation parameters it is obvious that in vitro oxidation of the acetylcholine chloride reaction with chloramine-T is not spontaneous and it needs an input of energy. Reaction rate as it is evident from table.1 and fig.1 increases with an increase in reaction temperature. The E_a value is indicative of the high energy barrier that the reaction has to overcome. The negative value of entropy translates into converging behavior and shows that reactions is not spontaneous and needs an impetus. So in living systems or in biological conditions if we want to design in future some advanced pharmacodynamics which can block the acetylcholine inhibitors actions, we assume that these activation parameters knowledge might play a key role because it provides an insight of the thermodynamics of acetylcholine breakdown.

REFERENCES

1. Vitus O., Eckhard S., Tobias R., Friedhelm B., Lars C. R., Am Heart Asso. Cir. 103; 1638-1643 (2001).

2. Pryde B.G. and Soper F.G., J. Chem. Soc., 1582 (1926); 1514 (1931).

3. Gowda B. T., Pardasaradhi V. and Ramachandra P., Oxidation. Commun., 14(3-4), 163 (1991).

4. Singh J.P, Singh.N. and Rajivkumar., Asian J. Chem., 4(4), 823(1992).

5. Rao R.V. and Gowda B.T., J. Indian Chem. Soc., 68, 210(1991)

6. Singh B, Singh A.K, Singh N.B and Saxena B.B.L., Tetrahedron., 40, 5203 (1984).

7. Nair C.G.R., Kumari R.L. and Senan P.I., Talanta., 25, 525 (1978).

8. Afanos'ev B.N., Zadvodskaya Lab., 16, 1011 (1950); 15, 1271 (1949).

9. Achilles J. P., cholinoceptor activating & cholinoestrase inhibiting drugs. In Basic and clinical Phamacology: Betram G. K., Susan B. M., Anthony J. T: Tata Mcgrew Hill New Delhi, 2009; pp 95-11.

10. Carmen L. P. Silveira1, Amira T. E., and Mohyee E. E., Toxicology and Applied Pharmacology, 103, 3, 474-481. (1990)

11. Mark E. D., Steve B., Terrone L. Rosenberry, and Paul E., Am J Physiol Heart Circ Physiol., 285, 632-640, (2003).