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Study of the chlorination of guanosine monophosphate by hypochlorous acid using a rotating platinum electrode

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Abstract
The in vitro chlorination of the nucleotide Guanosine Monophospate (GMP) by Hypochlorous acid (HOCl) has been herein studied. The in vivo chlorination of nucleobases and nucleotides gives rise to mismatch pairing in genetic coding which in turn results in abnormal protein synthesis and consequently diseases. The reaction studied is found to be rapid and the use of the special kinetic technique - hydrodynamic voltammetry has been adopted. The chlorination results in the formation of the mono-chloro product at position 8 on the substrate nucleotide.

Keywords: Nucleotides, Halogenation, Hypohalous acid, Rotating platinum electrode

Introduction
Nucleic acids are unique molecules due to their ability to transfer hereditary characters from generation to generation [1]. Nucleic acids are biopolymers and each monomer is a nucleotide [1-2]. Nucleobases are heterocyclic molecules attached to a five carbon sugar at the first position in a nucleotide [2]. Nucleobases play an important role in the gene expression as the genetic information stored in the genetic material of the cells is expressed in the form of coding and nucleobases are used to express this information [3]. The sequence of genetic coding is unique and any change in this coding or sequence has a serious impact on protein synthesis and gene expression. DNA has four nucleobases - adenine, guanine, cytosine and thymine. These are used to express genetic information as a genetic code [2, 4].
In vivo halogenations of nucleobases is thought to give rise to various mismatches in the coding sequence of DNA [5, 6]. Halogenations in living cells is thought to be by Hypohalous acids mainly HOCl/HOBr [7]. It is known that Cl^- and Br^- ions are freely available in cellular medium and during invasion of infection myeloperoxidase enzymes generate H_2O_2 as an immunity response. The peroxide generated helps fight the invading microbes but excess peroxide generated damages the host tissue.The peroxide converts the halide ions to Hypohalous acids [8].The Hypohalous acid formed in the cellular medium start attacking the components of the cellular medium like carbohydrates, proteins nucleic acids and others [9].A number of biomarkers of Hypohalous acid degradation for carbohydrates, proteins and nucleic acids have been revealed. Nucleobases are directly attacked by HOCl and are modified into their halogenated form. These halogenated nucleobases are mismatched in DNA coding thereby causing abnormal protein synthesis, which finally leads to various cardiovascular, neurodegenerative and other genetic diseases.
Herein we have studied the in vitro reaction kinetics of the chlorination of Guanosine monophosphate a RNA nucleotide by Hypochlorous acid. The reactions of Hypochlorous acid with nucleobases and nucleotides are rapid and a special technique has been used to observe the reaction kinetics. The technique used is hydrodynamic voltammetry in which a rotating platinum electrode is rotated in the reaction vessel at a high speed with an A.C motor and the fall in current due to the falling chlorine concentration, with the progress of the reaction, is observed by moving coil galvanometer, the reference electrode being the SCE (saturated calomel electrode).HOCl is the only species among the reactants and products that is electro-active yielding the electro-reduction current [10-14].

2. Materials and methods

Instruments: Digital pH meter from Equiptronics, Hydrodynamic voltammetry setup that includes a platinum electrode rotated with an A.C motor, SCE as the reference electrode, moving coil galvanometer, D.C battery, shunt to regulate potential and a measuring scale.

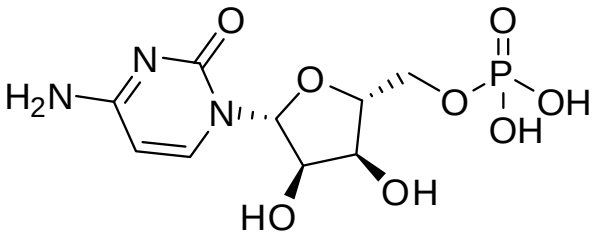
Chemicals: A.R grade Guanosine monophosphate was purchased from Himedia India phosphate buffer components that are sodium dihydrogen phosphate and citric acid was purchased from Merck.

Preparation of solutions: 2×10^{-3}M Guanosine monophosphate solution was prepared by dissolving the nucleotide in double distilled water and shaking the solution till it completely dissolved. The solution was kept in the thermostat. 1M KCl was also prepared. Both the solutions were prepared freshly before use.

Preparation of Aqueous HOCl: Aqueous solution of HOCl was prepared by dissolving concentrated HCl in double distilled water and adding NaOCl, until the pH reached 7. The concentration of HOCl was obtained spectrophotometrically and by iodometry.

Preparation of phosphate buffer: The preparation of the phosphate buffer was obtained by mixing various volumes of (0.2M) of sodium dihydrogen phosphate and (0.1M) of citric acid in double distilled water.

Chlorination of Guanosine Monophosphate by HOCl at a rotating platinum electrode: Guanosine monophosphate is an RNA nucleotide with molecular mass of 363.22 g/mol, solubility in water as 50 mg/ml and the structure as follows.



The kinetic reactivity of this nucleotide has been observed with HOCl at a RPE.

Calibration of diffusion current: Before kinetic measurements, the calibration of diffusion current was performed by observing deflections of the light spot on the scale of varying concentrations of HOCl [13]. The calibration plot is presented in Table 1 and Figure 1.

Table 1: Calibration data of HOCl

HOCl / 10^{-5} M	Galvanometer deflection/ cm
0.25	1.00
0.75	3.00
1.25	5.00
1.75	7.00
2.50	10.0
3.75	15.0
5.00	20.0
7.50	30.0
10.0	40.0

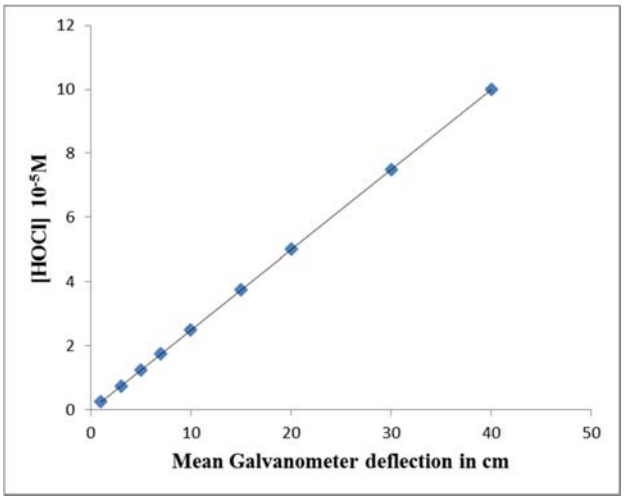


Fig 1: Calibration curve of HOCl versus mean Galvanometer deflection

Kinetic measurements: The solutions were kept in a thermostat to attain the desired temperature. Equal volumes of both the solutions were transferred to a reaction vessel containing both RPE and SCE and a stopwatch was started [12-14]. The readings from the scale were taken every 10 seconds. The scale readings were due to light spot from the moving coil galvanometer. The decreasing diffusion current was due to the falling concentration of HOCl. The data obtained is presented in the form of tables and graph.

Table 2: Kinetic data of Guanosine Monophosphate with HOCl at 8 ^{0}C and 7.4 pH.

Time/s	Mean Galvanometer deflection/ cm	[HOCl]/ 10^{-6} M	$[HOCl]^{-1}/10^4$ M
0	20.0	50.0	2.00
10	9.00	22.5	4.40
20	5.80	14.5	6.80
30	4.00	10.0	10.0
40	3.20	8.00	12.5
50	2.50	6.20	16.1
60	2.00	5.00	20.0

The calibration and kinetic studies are obtained for various temperatures.

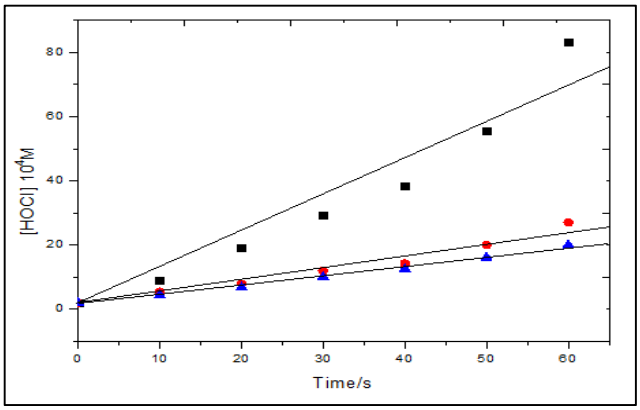


Fig 2: Kinetics of Guanosine Monophosphate at varying temperatures

Calculation of specific reaction rate from slope: The halogenation reaction follows second order kinetics. The slopes of the graph have been calculated for all the

temperatures. These indicate the specific reaction rate at the particular temperature.

Table 3: Specific reaction rates at different temperatures.

Temperature / ^{0}C	Temperature in Kelvin	[T]$^{-1}$/10^{-3}	k (specific reaction rate)/10^4	Log k
8	281	3.55	0.28	3.45
10	283	3.53	0.38	3.58
17	290	3.44	1.30	4.12

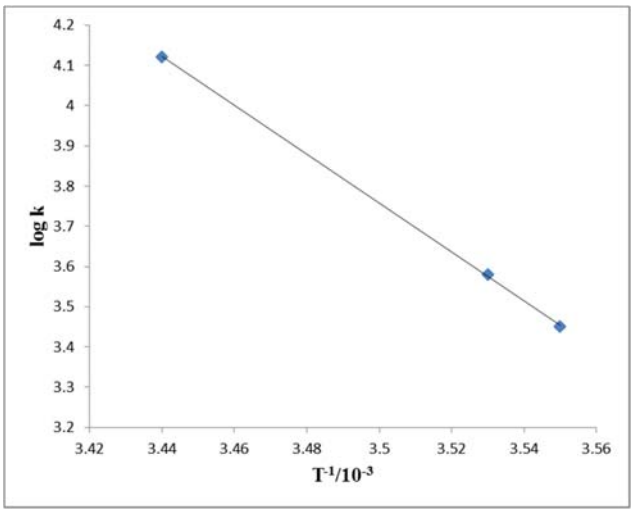


Fig 3: Variation of log k versus T^{-1}.

Calculation for Activation energy (Ea) at 17^{0}C

Slope calculated from Arrhenius plot

Slope = (-6250)

Activation energy (Ea) = -2.303 (slope) ×R×T

Where R is gas constant and T temperature in Kelvin

Ea = -2.303× (-6250) ×8.314×290

Ea = 119669.63 J

Ea = 119.66 kJ.

Calculation for frequency factor (A) at 17 ^{0}C

Frequency factor calculated from the equation $k = A\, e^{-(Ea/RT)}$

Where k is specific rate constant,

Ea is activation energy in joules,

R represents gas constant and

T is temperature in Kelvin.

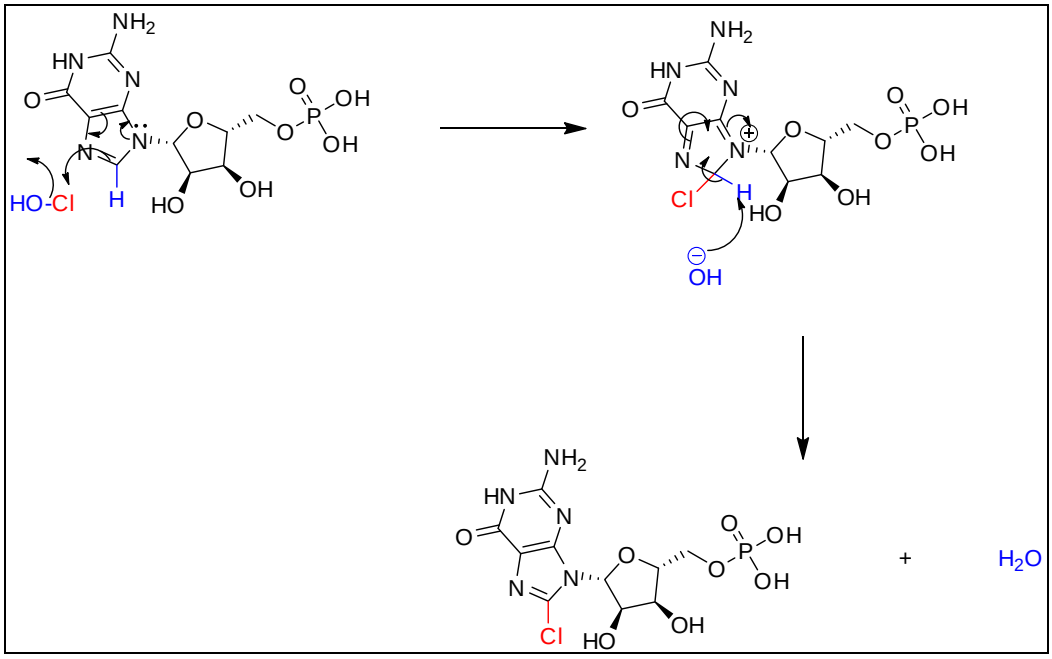
$k = A\, e^{-(119669.63/8.314\times290)}$

$A = 4.76\times10^{25}$

Table 4: Kinetic data for chlorination of Guanosine monophospate at 17 ^{0}C.

Kinetic parameter	Value
Specific reaction rate /M^{-1} s^{-1}	1.3×10^4
Activation energy /kJmol^{-1}	119.6
Frequency factor /$M^{-1}s^{-1}$	4.76×10^{25}

Probable reaction mechanism



3. Conclusion

The in vitro study at biological pH observed is fast and follows second order kinetics. It was not possible to study this reaction by conventional methods; hence hydrodynamic voltammetry was used to study this reaction. The probable mechanism deduced indicates the chlorination of the nucleotide at position 8 which is the most permeable position. The formation of the mono-chloro product is ascertained from NMR investigations. The kinetic and related thermodynamic parameters-specific reaction rate, activation energy and frequency factor, for this chlorination study have been evaluated.

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