



Asian Journal of **Biochemistry**

ISSN 1815-9923



Academic
Journals Inc.

www.academicjournals.com

Oxidation Chemistry of 2'-Deoxyinosine at Stationary Solid Electrodes

Rajendra Nath Goyal and Anuradha Tyagi
Department of Chemistry, Indian Institute of Technology Roorkee,
Roorkee-247667, India

Abstract: The oxidation chemistry of 2'-deoxyinosine (1) has been studied in the pH range 2.13-11.06 at pyrolytic graphite and glassy carbon electrodes. The oxidation occurred in a single pH dependent step ($6e, 6H^+, I_a$) at both the electrodes. The initial course of the electrode reaction has been deduced to involve a $2e, 2H^+$ reaction to give 8-hydroxy-2'-deoxyinosine which is further oxidised in a $2e, 2H^+$ reaction to give 2,8-dihydroxy-2'-deoxyinosine. The $2e, 2H^+$ oxidation of dihydroxy derivative then gives a diimine species, which undergoes a series of chemical reactions to give different products. In a parallel pathway initial $1e, 1H^+$ oxidation also occurs to give a free radical, which leads to the formation of dimers. The products of the electrode reaction were separated by HPLC and characterized by m.p., 1H NMR, IR and GC-Mass. A tentative mechanism for the formation of the products has also been suggested.

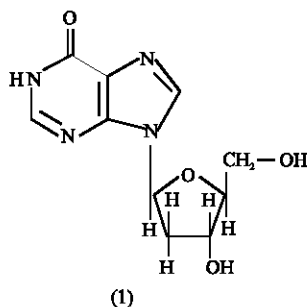
Key words: 2'-Deoxyinosine; electrooxidation; purine nucleosides

Introduction

2'-Deoxyinosine (1) is a nucleoside comprising of hypoxanthine and deoxyribose sugar and formed from inosine by washed cell suspension of a vitamin B₁₂ requiring strain of *Lactobacillus leichmannii* (Miyazawa *et al.*, 1966) and also from 2'-deoxyadenosine in the presence of enzyme adenosine deaminase (Hoffman *et al.*, 1990). It has also been isolated from the butanol fraction of the 95% ethanol extract of the starfish *Astria rollestoni* (Li *et al.*, 2004). 2'-Deoxyinosine and its derivatives play an important role in enhancing the stability of DNA duplexes and triplexes (Kubota *et al.*, 2004; Cubero *et al.*, 2001; Ueno *et al.*, 2004) and also the antiviral drug activity (Ciuffreda *et al.*, 1999). Deoxyinosine has been found to exhibit antiprotozoal activity (Moorman *et al.*, 1991), antitumor activity (Ciccolini *et al.*, 2000; 2001), antileishmanial activity (Wataya *et al.*, 1984) and inhibitory effect on human lymphocytes (Ochs *et al.*, 1979; Carson *et al.*, 1977). It also exhibit antiallergic activity (Kashima *et al.*, 1995) and its derivative 2',3'-dideoxyinosine inhibits the replication of HIV (Human immunodeficiency virus) (Kewn *et al.*, 1999). Catabolism of deoxyinosine occurs in intact WISH cells (cell line derived from human amnion epithelium) (Carta *et al.*, 2001) and also get metabolized in human erythrocyte cells (Lionetti *et al.*, 1966). Deoxyinosine residues deal with the group specific identification of Polioviruses by PCR (Kilpatrick *et al.*, 1996; 1998) have been found to prolong the motility of chick sperm (Sahashi *et al.*, 1968). Deoxyinosine has been found to exhibit selective toxicity against deoxyadenosine sensitive cells (Parsons *et al.*, 1986) and its N-1 adduct plays a role in butadiene induced carcinogenicity (Kowalczyk *et al.*, 2000). Nanomolar accumulations of deoxyinosine have also been identified in uremic patients (Weismann *et al.*, 1984).

Corresponding Author: Rajendra Nath Goyal, Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee-247667, India

As electrochemical oxidation reactions of biomolecules at solid electrodes provide unique insights into the redox chemistry of biomolecules (Goyal, 1989). In view of the limited information available on the oxidation behaviour of 2'-deoxyinosine at solid electrodes, it is considered desirable to study oxidation chemistry of 2'-deoxyinosine to obtain information on the primary electrode reaction. The electrode surface that affects the electron transfer process is a question of fundamental importance in electrochemistry and continues to be the focus of considerable research. Therefore, it is considered necessary to study the electrochemical behaviour of 2'-deoxyinosine at two different carbon electrodes, viz., Pyrolytic Graphite Electrode (PGE) and Glassy Carbon Electrode (GCE). It is expected that the results of these studies will throw light on the mechanism of possible oxidation of 2'-deoxyinosine in biological systems.



Materials and Methods

2'-Deoxyinosine was obtained from Fluka (Buchs, Switzerland) and was used as received. N,O-bis(trimethylsilyl)trifluoroacetamide and silylation grade acetonitrile were obtained from Sigma (USA). The separation of products was achieved by using Agilent 1100 series HPLC with C-18 column (7.8×300 mm column) attached to a precolumn. Linear and cyclic sweep voltammetric studies were performed on a BAS CV 50W voltammetric analyzer. Controlled potential electrolysis was carried out in a three-compartment cell using a pyrolytic graphite plate (area 6×1 cm²) as working electrode, cylindrical platinum gauze as auxiliary electrode and Ag/AgCl as reference electrode. The pyrolytic graphite electrode (PGE) (~6 mm²) was prepared in the laboratory by reported method (Miller *et al.*, 1963). The electrode surface of the PGE was cleaned by rubbing it on an emery paper before recording each voltammogram to remove the adsorbed material. As the surface area available changed each time, an average of at least three runs was taken for determining peak current values. The glassy carbon electrode (GCE) (~7.04 mm²) used for the voltammetric experiments was obtained from BAS, USA and its surface was renewed each time by first washing with 95% ethanol and then with chloroform as suggested by Chan and Fogg (Chan and Fogg, 1979). The surface was then touched onto tissue paper dipped in chloroform and finally dried with clean adsorbent tissue paper.

A stock solution (2 mM) of 2'-deoxyinosine was prepared in twice distilled water. Voltammograms were recorded in phosphate buffers (Christian and Purdy, 1962) of pH range 2.13-11.06. All potentials are reported with reference to Ag/AgCl electrode at an ambient temperature of 25±2°C. UV-Vis spectral studies and kinetic studies of the decomposition of the UV absorbing intermediate were carried using a Perkin-Elmer Lambda 35 UV/Vis Spectrophotometer.

Procedure

The solutions for voltammetric experiments were prepared by mixing 2.0 mL of the stock solution of 2'-deoxyinosine with 2.0 mL of buffer of desired pH ($\mu = 1.0$ M) so that the overall ionic strength of the solution became 0.5 M. The solutions were deaerated by passing a stream of nitrogen gas for 8-10 min before recording the voltammograms. Controlled potential electrolysis was carried out in a three-component cell using a pyrolytic graphite plate as working electrode, cylindrical platinum gauge as auxiliary electrode and Ag/AgCl as reference electrode. The value of 'n' number of electrons involved in oxidation was determined by connecting a coulometer in series.

The progress of the electrolysis was monitored by recording spectral changes at different time intervals. For this purpose, 2-3 mL of electrolyzed solution was transferred each time to a 1.0 cm quartz cell and the spectrum was recorded in the range 200-400 nm. In the next set of experiments, the circuit was opened when the absorbance at λ_{\max} was reduced to $\sim 50\%$. Spectra at different times were then monitored to detect the wavelength region in which the UV-Vis absorbing intermediate was generated. The change in absorbance with time was monitored at selected wavelengths. The values of the rate constant k were calculated from the linear $\log (A-A_{\infty})$ versus time plots.

Analysis of the oxidation products

For product identification, 20-25 mg of 2'-deoxyinosine was exhaustively electrolysed at the desired pH by applying a potential corresponding to the oxidation peak I_a at a large PGE. The progress of electrolysis was monitored by recording spectra at different time intervals. When the absorbance at the maximum in the spectrum completely disappeared, the exhaustively electrolysed solution was removed from the cell and lyophilized. The colourless material obtained after freeze-drying was extracted with methanol (2×10 mL) and the methanolic extract was concentrated to get the products. The TLC of the products at silicagel-G plates using benzene + methanol (80:20) indicated three spots with $R_f \sim 0.30, 0.42$ and 0.48 and thus suggested the formation of three products. High performance liquid chromatography (HPLC) of mixture of electrooxidised products was performed on Agilent 1100 series with C-18 reversed phase column (7.8×300 mm column). The mobile phase used for HPLC experiment was gradient system of methanol (A) and water (B). The following gradient system was used at a flow rate of 1.5 mL min^{-1} , for the time 0-3 min 100% B, 5-6 min 90% B, 8-10 min 80% B, 11-12 min 75% B, 12-15 min 60% B and from 16-19 min 50% B. Three major peaks were observed in the HPLC Chromatogram. Several injections of $500 \mu\text{L}$ were made to collect enough products. After repetitively collecting volume under individual HPLC peaks, the solutions were lyophilized. The components eluted under these peaks were lyophilized and the freeze-dried material obtained were characterized by m.p., IR, $^1\text{H NMR}$. For determining the molar mass of the products they were converted to trimethylsilyl derivatives and the silylated derivatives were then analyzed by GC-MS. For carrying out GC-Mass studies (EI mode: 70 eV), the mixture of products ($50 \mu\text{g}$) was treated with BSTFA-acetonitrile ($100 \mu\text{L}$ each) in a sealed 3 mL vial and heated at 110°C for 10-15 min in an oil bath. The vial was then cooled to room temperature and $2 \mu\text{L}$ of the mixture was then injected in GC-Mass spectrometer. In the second set of silylation, the temperature was increased to 140°C which caused removal of deoxyribose units in the products and the molar mass peaks observed further supported the proposed structure.

The FT-IR spectrum of the products were recorded on a Perkin Elmer 1600 series FT-IR spectrophotometer using KBr pellets and $^1\text{H NMR}$ spectra were measured in appropriate deuteriated solvent ($\text{DMSO-d}_6, \text{D}_2\text{O}$) with TMS as internal standard by using Bruker AC-300F, NMR Spectrometer (300 MHz).

Molecular orbital calculations were performed using the INDO method of Pople and Beveridge (Pople *et al.*, 1970), using the routines provided in the Gaussian'98 (Frisch *et al.*, 2002) programme suite. Initial geometries based on approximate estimates of bond length and angles were input and the geometry optimized during INDO SCF cycles. The values of atomic charges and Hartree-Fock total energies were obtained at the optimum geometry from these calculations.

Results and Discussion

Linear and Cyclic Sweep Voltammetry

Linear sweep voltammetry of 2'-deoxyinosine at a sweep rate of 20 mV s⁻¹, exhibited a single well-defined oxidation peak between pH 2.13-11.06 at pyrolytic graphite electrode (PGE). At glassy carbon electrode (GCE) the oxidation peak was observed only in the pH in the range 2.13-9.12. At pH > 9.12 the oxidation peak I_a merged with the background current at the GCE. The peak potential of the oxidation peak I_a was dependent on pH and shifted towards less positive potential with increase in pH. The plots of E_p versus pH were linear (Fig. 1) and the dependence of E_p on pH for peak I_a at PGE and GCE, respectively, can be expressed by the relations

$$E_p (\text{pH } 2.13-11.06) = [1677.8-53.385 \text{ pH}] \text{ in mV versus Ag/AgCl-----At PGE}$$

$$E_p (\text{pH } 2.13-9.12) = [1550.9-56.47 \text{ pH}] \text{ in mV versus Ag/AgCl-----At GCE}$$

having correlation coefficients 0.981 and 0.983 at PGE and GCE, respectively. The E_p values at GCE were 50-70 mV less positive in the entire pH range studied than observed for PGE. The dE_p/dpH values at both the electrodes were essentially similar and close to 59 mV indicating thereby that the number of protons and electrons involved in the oxidation of 2'-deoxyinosine are same.

In cyclic sweep voltammetry at a sweep rate of 100 mV s⁻¹, a well-defined oxidation peak (I_a) is observed at both the electrodes in the entire pH range studied. However, in the reverse sweep different behaviour was noticed. At the PGE, in the reverse sweep no cathodic peak was obtained, however, further reversal of the sweep direction towards positive potentials a new well-defined oxidation peak (II_a) was observed in the pH range 5.11-9.12. The peak potential of peak II_a was less positive than that of peak I_a. The peak potential of peak II_a was also dependent on pH and shifted to

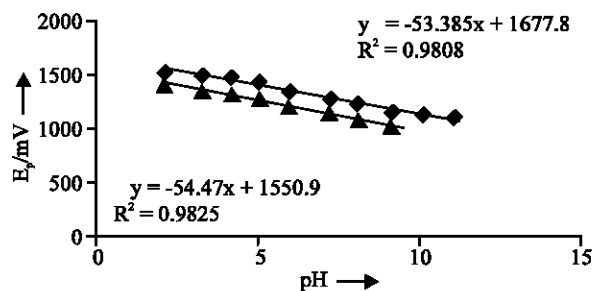


Fig. 1: Observed variation of peak potentials with pH for 1.0 mM 2'-deoxyinosine with a sweep rate of 100 mV s⁻¹ at PGE (◆) and GCE (▲)

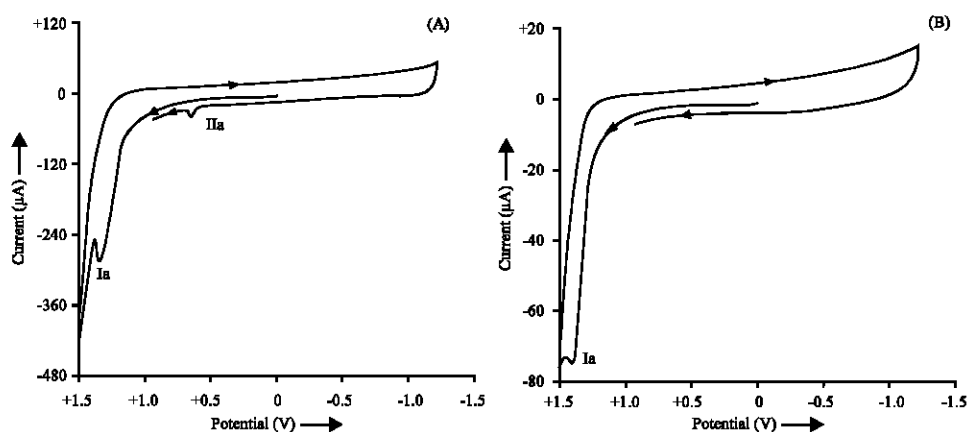


Fig. 2: Cyclic voltammograms of 1.0 mM 2'-deoxyinosine at pH 7.24. Sweep rate 100 mV s⁻¹ at (a) PGE (b) GCE electrode

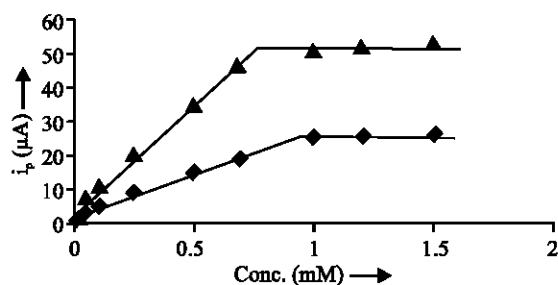


Fig. 3: Observed dependence of peak current (i_p) for the oxidation peak I_a on the concentration of 2'-deoxyinosine at pH 7.24 at PGE (▲) and GCE (◆)

less positive potential with increase in pH. The nature of the plot of E_p vs. pH was linear. The dependence of E_p of peak II_a on pH can be expressed by the relation

$$E_p (5.11-10.07) = [957.22-38.81 \text{ pH}] \text{ in mV versus Ag/AgCl}$$

having correlation coefficient 0.9294. At GCE, in the reverse sweep no cathodic peak was obtained. Further reversal of sweep direction towards positive potential also did not exhibit any new peak. Typical cyclic voltammograms of 2'-deoxyinosine at PGE and GCE at pH 7.24 are shown in Fig. 2. The peak current of peak I_a increased linearly with increase in 2'-deoxyinosine concentration at both the electrodes in the concentration range 0.1 to 0.7 mM and then attained a practically constant value at concentrations greater than 0.7 mM (Fig. 3). This behaviour indicated that 2'-deoxyinosine strongly adsorbed (Wopschall *et al.*, 1967) at the surface of PGE as well as at GCE.

The sweep rate studies were carried in the range of 5-900 mV s⁻¹ and it was found that the peak current of oxidation peak I_a of 2'-deoxyinosine increased with increase in sweep rate at both the electrodes. The peak I_a showed a tendency to merge with the background at a sweep rate

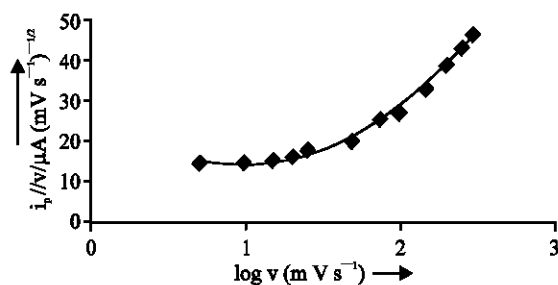


Fig. 4: Observed variation of the peak current function ($i_p v^{-n}$) for peak I_a with the logarithm of the sweep rate for 0.5 mM 2'-deoxyinosine at pH 7.24 at PGE

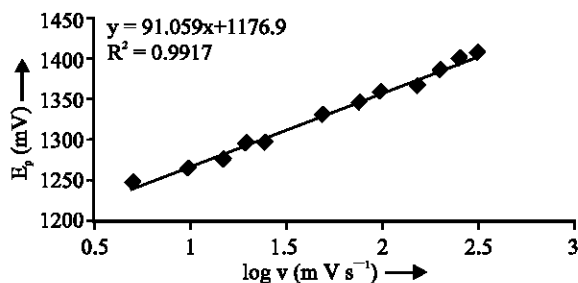


Fig. 5: Observed variation of peak potential (E_p) for the oxidation peak (I_a) with $\log v$ for 1.0 mM 2'-deoxyinosine at PGE at pH 7.24

$>800 \text{ mV s}^{-1}$. A typical plot of i_p/\sqrt{v} versus $\log v$ obtained at PGE is presented in Fig. 4 and indicated that the value of the peak current function increased with an increase in the logarithm of sweep rate. A similar nature of i_p/\sqrt{v} versus $\log v$ was also observed at GCE. This behaviour indicated strong adsorption of the reactant at the surface of PGE as well as at a GCE. However, in both the cases the value of E_p shifted to more positive potentials with the increase in sweep rate. The plots of E_p versus $\log v$ at PGE and GCE were linear with a correlation coefficient of 0.9952 at GCE and 0.9917. A typical E_p versus $\log v$ plot at PGE is depicted in Fig. 5 and this behaviour is consistent with the EC nature of the electrode reaction in which the electrode reaction is coupled with an irreversible follow up chemical steps (Goyal *et al.*, 2001). A plot for surface bound species was plotted between i_p versus v , it was found to be linear with a correlation coefficient of 0.9914.

Coulometric Studies

The controlled potential electrolysis of 2'-deoxyinosine was carried out at a peak potential of 2'-deoxyinosine at pH 7.24. The peak current (i_p) was found to decrease exponentially with time. The plot of $\log i_p$ vs. time was straight line for the first 15 min of electrolysis after which a large deviation from the straight line was noticed. Such a behavior indicated involvement of competitive chemical reactions (Cauquis and Parker, 1973). The experimental value of 'n' for the 2'-deoxyinosine was found to be 5.92 ± 0.40 in the entire pH range.

Spectral Studies

The UV-Vis spectra of 0.2 mM solution of 2'-deoxyinosine were recorded at different pH. In the entire pH range studied, 2'-deoxyinosine exhibited two well defined λ_{\max} at 208 and 250 nm indicating that the same species of 2'-deoxyinosine exhibited in the entire pH range. The progress of electrolysis was monitored by recording UV-Vis spectral changes during electrooxidation of 2'-deoxyinosine at different pH to detect the formation of UV-visible absorbing intermediates. Curve 1 in Fig. 6 is the initial spectrum of 2'-deoxyinosine at pH 7.24 just before electrooxidation. Upon application of a potential corresponding to peak I_a, the absorbance in the wavelength region 235-285 and 200-215 nm decreased while the absorbance in the wavelength region 215-235 nm and 285-400 nm increased as shown by curves 2 to 8 in Fig. 6. Three clear isosbestic points at 215, 235 and 285 nm were noticed. The spectral changes observed during electrooxidation of 2'-deoxyinosine at pH 5.11 and 9.12 were essentially similar to that observed at pH 7.24.

In the second set of experiments, the circuit was opened when the absorbance at λ_{\max} was reduced to ~ 50% (curve 4 in Fig. 6). Spectral changes at different times were then monitored to detect the wavelength region in which the UV-Vis absorbing intermediate is generated. It was found that the absorbance at the wavelength 225 and 250 nm decreased on open circuit relaxation. The resulting absorbance versus time plots were found to be exponential in nature (Fig. 7). The values of the pseudo first order rate constant were determined at different pH, using linear log (A-A_∞) versus time plots (Fig. 7). The values of k calculated at different pHs are presented in Table 1. It was found that the values of k were in the range 0.54-0.59 × 10⁻³ s⁻¹. As the k values did not show significant variation, it is thus concluded that the same UV-Vis absorbing intermediate is generated in the entire pH range.

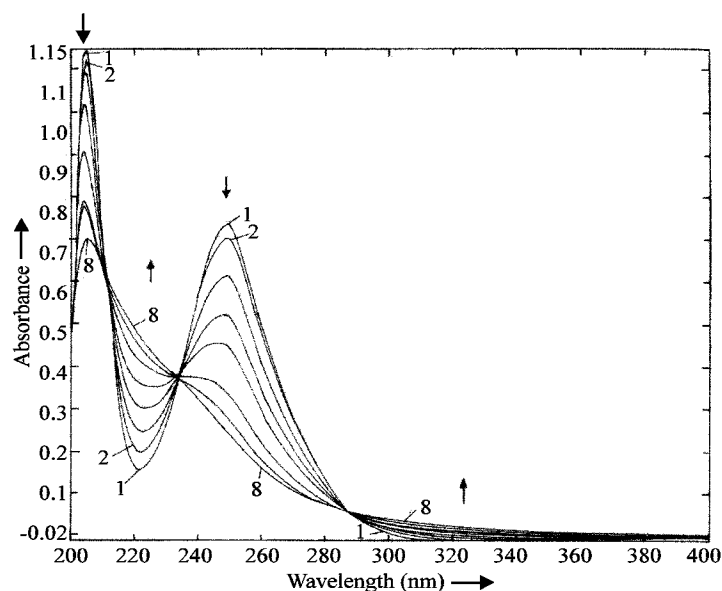


Fig. 6: Observed spectral changes with time during the electrochemical oxidation of 0.2 mM 2'-deoxyinosine at pH 7.24 at E_p; spectra recorded after (a) 0, (b) 5, (c) 10, (d) 15, (e) 15, (f) 30, (g) 30, (h) 60 min of electrolysis

Table 1: Observed pseudo first order rate constants for the decomposition of UV/Vis absorbing intermediate during the electrooxidation of 2'-deoxyinosine

pH	λ (nm)	(k/10 ⁻³) s ⁻¹ *
5.11	225	0.54
	250	0.59
7.24	225	0.57
	250	0.54
9.12	225	0.56
	250	0.56

*average of atleast three replicate determinations

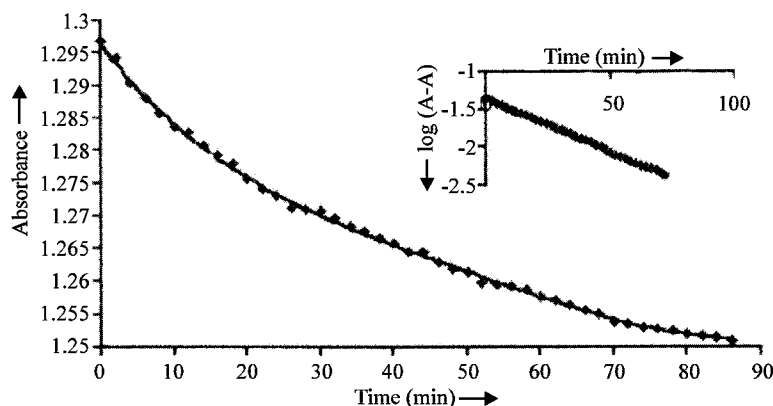


Fig. 7: Observed plots of absorbance versus time and $\log(A-A_0)$ vs. time (inset) for the decay of the UV-absorbing electroactive intermediate generated during the electrooxidation of 0.2 mM 2'-deoxyinosine at pH 7.24

Product characterization

The principal aim of the study reported here was to characterize the intermediates and products and elucidate the mechanism of oxidation of 2'-deoxyinosine at around physiological pH. Therefore, bulk electrolysis of 2'-deoxyinosine was carried at pH 7.24.

The exhaustively electrolysed solution of 2'-deoxyinosine at pH 7.24 on freeze-drying gave a colourless material. The lyophilized material was analysed by GC-Mass after silylation at 110°C. The GC-MS of the electrooxidised product of 2'-deoxyinosine showed the presence of three major components having retention times at 4.12, 5.03 and 6.12 min. The molar masses corresponding to these peaks were found to be 707 (M+H⁺), 878 (M⁺) and 966 (M⁺), respectively.

The molar mass of $m/z = 707$ (M+H⁺, peak at R_t 4.12 min) indicates the formation of allantoin (12) having deoxyribose unit attached to it. The molar mass of this compound was further confirmed by carrying out silylation at higher temperature (~ 140°C) at which the deoxyribose unit underwent hydrolysis. The intense molecular ion peak observed in GC-MS at $m/z = 519$ (M+H⁺, 6.80%), further supported the formation of allantoin as one of the products of oxidation of 2'-deoxyinosine and removal of deoxyribose unit was confirmed by the appearance of peak at $m/z 351$ (M+H⁺; 2.49%)

The molar mass corresponding to $m/z = 878$ (M⁺, peak at R_t 5.03 min) indicates the formation of C-O-N dimer (18) of 2'-deoxyinosine in which oxygen atom at position 8 of one moiety is linked

to N₁' of the second unit. This dimer (18) has five silylable sites, which can undergo silylation with BSTFA/Acetonitrile. The replaceable hydrogens on silylation in this molecule will lead to a molar mass of $m/z = 878$. The molar mass of 878 was further confirmed by carrying out silylation of the mixture of products of exhaustively electrolyzed solution of 2'-deoxyinosine at 140°C. The deoxyribose unit hydrolyses at this temperature and a molecular ion peak corresponding to molar mass of $m/z = 503$ ($M+H^+$, 2.45%), was clearly noticed. This experiment further supported that the molar mass of peak at R_t 5.03 min is 878. The relative abundances of various high mass peaks observed is presented in Table 2 and their formation in the fragmentation pattern is depicted in Scheme 3.

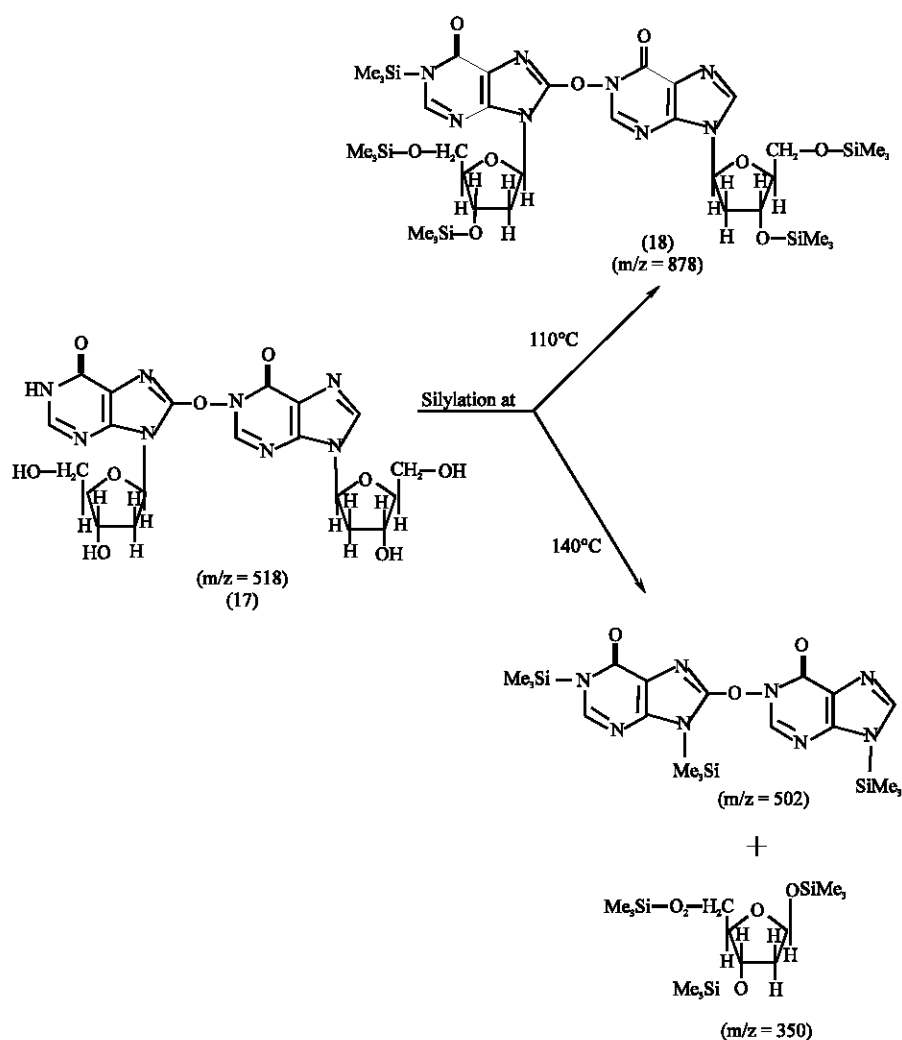


Table 2: Relative abundances observed for various fragments of the peaks observed in the GC-MS

R _t	m/z	Relative abundance of different fragments	Compound identified
4.12	707 (M+H ⁺) (3.72%)	640 (4.12), 606 (4.91), 559 (1.27), 512 (4.12), 464 (4.91), 433 (7.68), 386 (7.38)	(12)
5.03	878 (M ⁺) (1.55%)	822 (1.01; M ⁺ -2(CO)), 520 (2.74; 822-C ₅ N ₃ O ₃ H ₅ (SiMe ₃) ₂), 483 (1.71; M ⁺ -C ₁₀ N ₄ O ₄ H ₆ (SiMe ₃) ₂), 410 (6.20; 483-SiMe ₃), 395 (1.54; M ⁺ -C ₁₀ N ₄ H ₅ O ₃ (SiMe ₃) ₃), 354 (2.48; 410-2(CO)), 134 (5.91; 395-C ₃ H ₇ O ₃ (SiMe ₃) ₂)	(18)
6.12	966 (M ⁺) (2.36%)	938 (3.72; M ⁺ -CO), 866 (3.72; 938-H ₂ C=SiMe ₂), 800 (3.04; 938-C ₃ N ₂ H(SiMe ₃)), 785 (2.36; 800-CH ₃), 677 (3.04; 938-C ₃ H ₇ O ₃ (SiMe ₃) ₂)	(15)

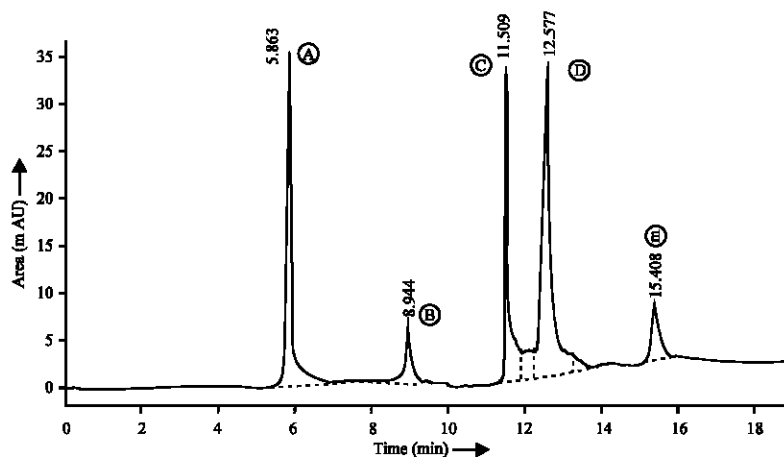
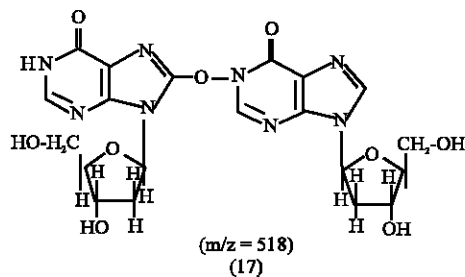


Fig. 8: HPLC chromatogram of the product mixture obtained following controlled potential electrooxidation of 2'-deoxyinosine at pH 7.24

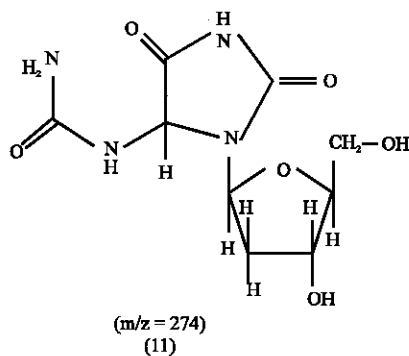
The molar mass of $m/z = 966$ (M⁺, peak at R_t 6.12 min) indicates the formation of O-O-(peroxide) linked dimer (15). The presence of peroxide group in the product was also confirmed by the test suggested in the literature (Vogel's textbook 1994; Swern, 1971) in which 1.0 mL of 10% acidified potassium iodide solution was added to a small amount of the product. A few drops of starch solution were then added and a blue black colour was observed in ~1 min. This dimer is likely to be formed by the dimerisation of C-8 oxygen free radical. Such a structure has six silylable sites in which two are present in hypoxanthine part of the dimer and four are present in two deoxyribose sugar moieties. The replaceable hydrogen on silylation in this molecule will lead to a molar mass of $m/z = 966$ as observed for peak at R_t 6.12 min. To further confirm that the molar mass of this peak is 966, silylation at 140°C was carried out. An intense molecular ion peak at $m/z = 591$ (M+H⁺, 7.80%), due to the hydrolysis of two deoxyribose units in the dimer 15 together with a peak of deoxyribose ($m/z = 350$) supported the proposed molar mass of dimer 15. The relative abundance of high mass peaks observed is presented in Table 2 and is in accordance with the fragmentation pattern expected.

For further characterization of the products of electrooxidation of 2'-deoxyinosine the lyophilized material obtained after exhaustive electrolysis was separated by using HPLC. The HPLC chromatogram showed three major peaks A, C, D at $R_t \sim 5.86, 11.51, 12.58$ with two minor peaks at 8.94 and 15.41 min respectively as shown in Fig. 8. In order to collect sufficient amount of products, the repetitive 500 μL injections of the products solution were made and the volume eluted under the peaks was collected and lyophilized.

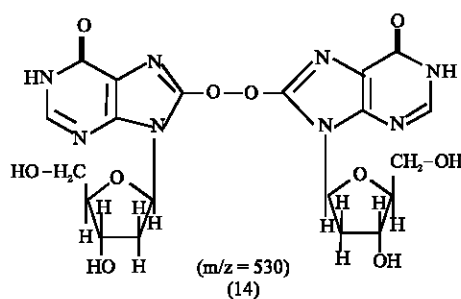
The volume collected under liquid chromatography component A was a white powder and had a m.p. of 276°C . The dimer exhibited a clear molecular ion peak at $m/z = 518$ (4.89%). GC-MS of this component after silylation with silylating reagent BSTFA/Acetonitrile gave a single peak in a chromatogram at $R_t \sim 5.76$ min, which corresponded to intense molecular ion (M^+) peak $m/z = 878$ (7.20%) [C-O-N linked dimer (18 in reaction scheme) calculated $m/z = 878$]. The IR spectrum of this material showed important bands at 3700 (N-H); 3426 (O-H); 1688 (cyclic $>\text{C}=\text{O}$) actual IR stretching for cyclic $>\text{C}=\text{O}$ appear at 1715 cm^{-1} but the stretch is low because the carbonyl belongs to an amide; 1478 (C-H); 1256 (C-C); 1022 (C=N) and 805 cm^{-1} (C-N). The ^1H NMR spectrum of this material exhibited signals at $\delta = 7.16$ (d, 1H), 7.20 (s, 1H), 7.24 (s, 1H) and 7.46 (d, 1H) and multiplets are obtained in the range of $\delta = 1.68\text{-}2.22$ and $3.77\text{-}4.64$ corresponding to the 18 H's (protons) of deoxyribose units of dimer (18) and thus, confirmed the product as C-O-N dimer.



The component C eluted in HPLC at $R_t \sim 11.51$ min was white coloured powder. Thin layer chromatography (TLC) of the component B in benzene-methanol (80:20) exhibited a single spot with $R_f \sim 0.30$. This material had a m.p. 232°C and ^1H NMR spectrum of the material exhibited signals at $\delta = 5.28$ (d, 1H); 5.8 (s, 2H); 6.89 (d, 1H); 8.04 (s, 1H) and multiplets are obtained in the range of $\delta = 1.46\text{-}2.17$ and $3.80\text{-}4.66$ corresponding to the 9 H's (protons) of deoxyribose unit of (12) (structure in reaction scheme) hence suggested the structure as allantoin containing deoxyribose moiety.



The volume collected under liquid chromatography component D was white powder and had a m.p. of 282°C. GC-MS of this component after silylation with silylating reagent BSTFA/Acetonitrile gave a single peak in a chromatogram at $R_t \sim 6.65$ min, which corresponded to intense molecular ion (M^+) peak of $m/z = 966$ (5.62%) [C-O-O-C linked dimer (15 in reaction scheme) calculated $m/z = 966$]. The IR spectrum of this material showed important bands at 3705 (N-H); 3428 (O-H); 1676 (cyclic $>C=O$) actual IR stretching for cyclic $>C=O$ appear at 1715 cm^{-1} but the stretch is low because the carbonyl belongs to an amide; 1460 (C-H); 1250 (C-C); 1020 (C=N) and 826 (C-N) cm^{-1} . The $^1\text{H NMR}$ spectrum of this material exhibited signals at $\delta = 7.18$ (d, 1H, $2 \times C_2\text{H}$), 7.49 (d, 1H, $2 \times N_1\text{H}$) and multiplets are obtained in the range of $\delta = 1.43$ -2.13 and 3.78-5.09 corresponding to 18 H's (protons) of deoxyribose units of dimer (15) and thus, confirmed the product as C-O-O-C linked dimer.



The HPLC peaks B and E were relatively minor component and the material collected under them was never enough to permit their characterization.

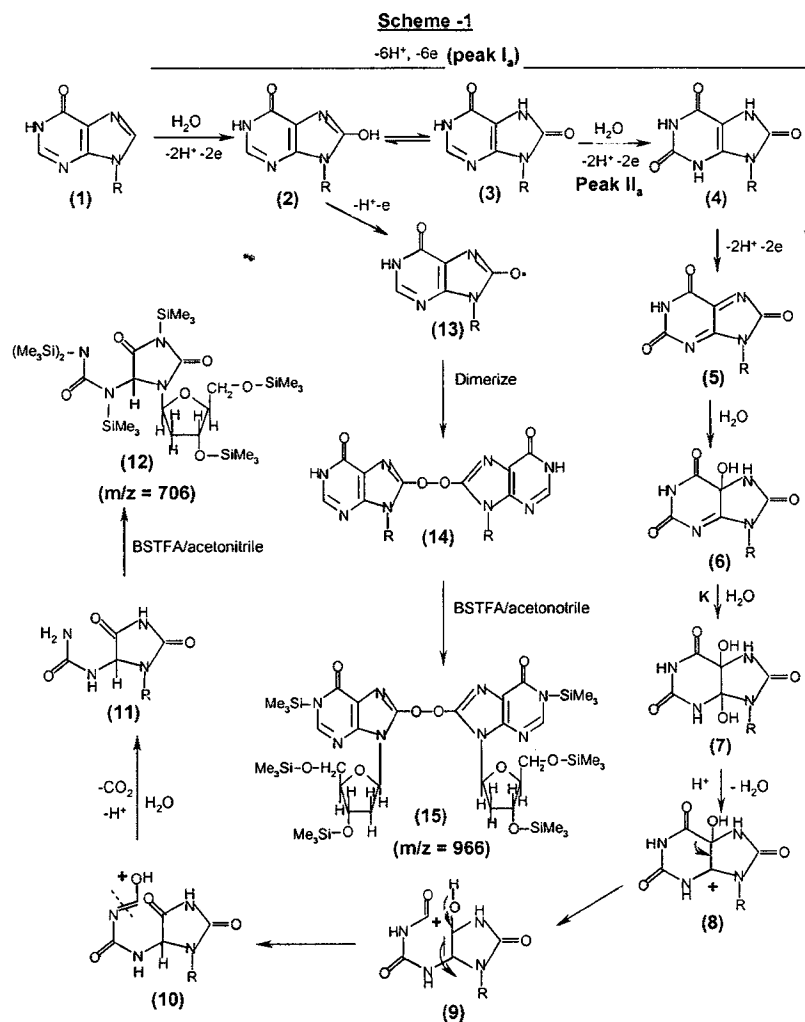
Intermediate characterization

The intermediates generated after 1, 2 and 3 h of electrolysis of 2'-deoxyinosine were also characterized at pH 7.24 by carrying out GC-MS studies of the silylated samples. For this purpose about 2.0 mL of the solution during bulk electrolysis was taken out from the electrolysis cell at desired time and immediately lyophilized. All the samples gave three peaks in GC-MS at $R_t \sim 4.31$, 5.76 and 6.78 min which corresponded to parent compound i.e., 2'-deoxyinosine (1) (molar mass of $m/z = 468$, $M+H^+$, 2.30%), either 2-hydroxy- or 8-hydroxy-2'-deoxyinosine (2) (molar mass of $m/z = 556$, $M+H^+$, 3.74%) and 2,8-dihydroxy-2'-deoxyinosine (4) (molar mass of $m/z = 644$, $M+H^+$, 7.90%). Thus, it is clear that the oxidation of 2'-deoxyinosine proceeds through the formation of 2,8-dihydroxy-2'-deoxyinosine.

Reaction scheme

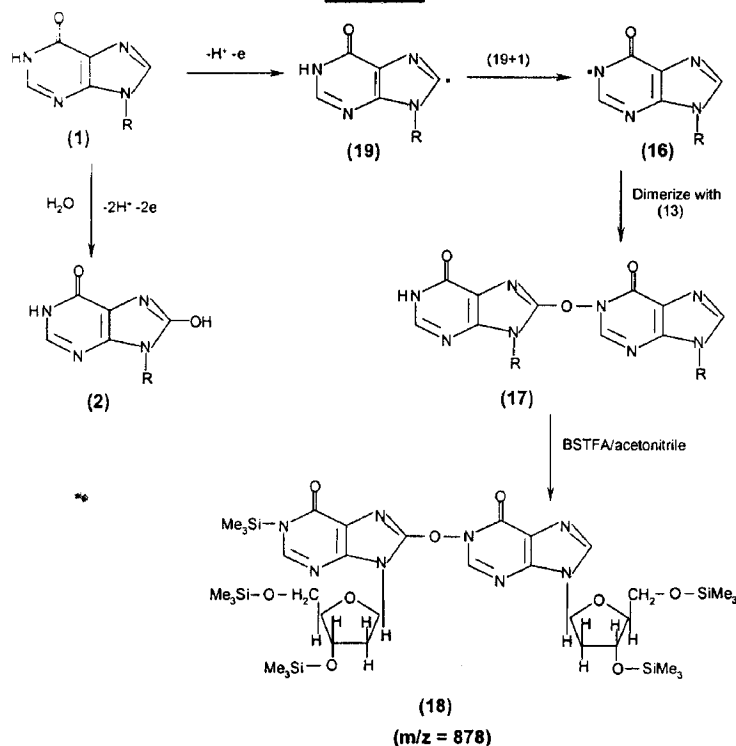
The results presented in Schemes 1 and 2 indicate that the oxidation of 2'-deoxyinosine proceeds in a mechanism involving close to $6H^+$, $6e$. The formation of the observed products can be explained by two different pathways. Scheme 1 suggests the breakdown of pyrimidine ring leading to the formation of allantoin whereas alternate pathway involves the free radical formation and combination of several radicals lead to the dimers.

The initial oxidation of 2'-deoxyinosine (1) in a $2H^+$, $2e$ reaction can give either 8-hydroxy-2'-deoxyinosine (2) or 2-hydroxy-2'-deoxyinosine. It is difficult to say at this stage with certainty that which of these products is formed. However, on the basis of oxidation of hypoxanthine where initial $2H^+$, $2e$ oxidation is reported to proceed through the formation of 8-hydroxyxanthine (Conway *et al.*, 1981), it seems reasonable to conclude that initial oxidation would occur at positions



to give 8-hydroxy-2'-deoxyinosine. The oxidation potentials of hydroxypurines have been found to be less positive in comparison with simple purines (Yao *et al.*, 1978). The 2H⁺, 2e oxidation of 8-hydroxy-2'-deoxyinosine will give 2,8-dihydroxy-2'-deoxyinosine (4). The appearance of peak II_a in the second cycle in the CV is due to this oxidation. The oxidation of 2,8-dihydroxy-2'-deoxyinosine would be easier due to presence of two hydroxy groups. Thus further 2H⁺, 2e oxidation then gives a diimine species (5). One would expect that 2e, 2H⁺ oxidation peak of 2,8-dihydroxy-2'-deoxyinosine should also be observed in the CV. However, no peak for this oxidation step was noticed. One of the probable reasons for this behaviour is that due to too positive potential of 2,8-dihydroxy-2'-deoxyinosine it will be easily oxidizable and hence will not be available at the surface of electrode. The formation of diimine species is common and has been reported during oxidation of several purines (Brajter-Toth *et al.*, 1981). The increase in absorbance in the region 285-400 nm during spectral study

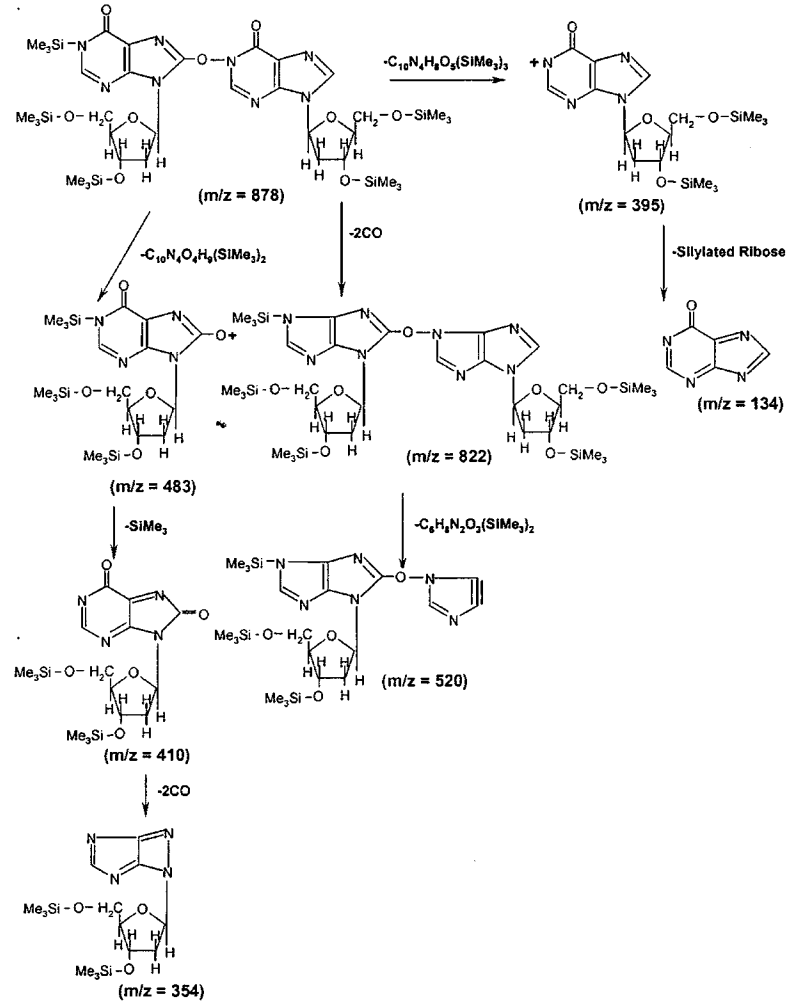
Scheme - 2



seems to be due to the formation of highly conjugated diimine species. It has been already reported in the literature that diimine generated during oxidation of purines had short half life (~ 50 ms) at different electrodes (Goyal *et al.*, 1982; Teresa *et al.*, 1988). Hence, it is expected that the diimine formed from 2,8-dihydroxy-2'-deoxyinosine will be unstable and readily attacked by the water molecules to give 4,5-diol species (7). The attack of first water molecule on the diimine (Goyal *et al.*, 1991) would occur almost instantly because of the unstable nature of diimine to give imine alcohol (6). However, the attack of second water molecule would be slow and the decay of imine alcohol (Roth *et al.*, 1998) to give 4,5-diol has been monitored spectrophotometrically and the value of k represents this step. Simultaneous attack of H⁺ and loss of H₂O molecule from (7) leads to the formation of carbocation (8). The breaking of bond and migration of H⁺ will then generate a protonated isocyanate specie (10). Further attack of water will lead to decarboxylation and generate the ultimate product allantoin (11). The formation of allantoin has been observed in GC-MS after silylation corresponding to m/z = 706, at R_t ~ 4.12 min. along with CO₂.

Oxidation of 2'-deoxyinosine also follows a parallel pathway in which 1H⁺, 1e oxidation of (2) produces an oxygen free radical (13), which rapidly undergoes dimerization and leads to the formation of peroxide linked dimer (14). The dimer (14) on silylation exhibited a molar mass peak at m/z = 966, (R_t ~ 6.12 min). The dimer (17) can be obtained by 1H⁺, 1e oxidation of 2'-deoxyinosine (1) to give C₈ radical (19). Such a removal of an electron from position-8 is well documented during oxidation of purine nucleoside (Subramanian *et al.*, 1987). The free radical (19) on further reaction with (1) at electrode surface generates N₁ radical (16), which combines with radical (13) to give C-O-N linked

Scheme-3



dimer (17). The formation of dimer (17) was confirmed by its silylation. The silylated (17) eluted at R_f 5.03 min in GC-MS and exhibited a molar mass at $m/z = 878$.

Conclusions

The present studies reveal that the electrooxidation of 2'-deoxyinosine occurred in a single 6e, 6H⁺ peak. The peak potential was dependent on pH. The oxidation proceeds in two parallel pathways leading to the formation of O-O-linked and C-O-N-dimers and allantoin riboside which have been characterized by IR, ¹HNMR, m.p. and GC-MS. A comparison of electrooxidation behaviour of inosine and 2'-deoxyinosine has also been made. In both the cases oxidation occurred in a single oxidation peak and the products allantoin, O-O-linked and C-O-N linked dimers were obtained. However, several tetramers were obtained as products in the case of inosine, whereas in the case of

2'-deoxyinosine no tetramers were obtained. Thus, it appears that the free radical reactions are more prominent in inosine leading to the formation of tetramers along with dimers and allantoin riboside. In the case of 2'-deoxyinosine oxidation stops at the dimer stage. This difference in behaviour is probably due to the applied potential during Controlled Potential Electrolysis (CPE). In case of inosine, slightly higher potential than peak potential was applied to achieve faster electrolysis, whereas, in 2'-deoxyinosine the peak appeared close to background current and hence the applied potential was selected as peak potential. Thus, it appears that the applied potential plays a role in product distribution.

Another reason for the behaviour could be the presence of ribose or 2'-deoxyribose at N₉ position. This fact has been further confirmed by the molecular orbital calculations. The atomic charge values calculated for N₉ position of inosine and 2'-deoxyinosine by INDO method have been found as -0.291304 and -0.287396 respectively. These values indicated that electron density in case of inosine is more than 2'-deoxyinosine hence, it seems that free radical pathway followed to much more extent in case of inosine in comparison to 2'-deoxyinosine. Thus, it can be concluded on the basis of these investigations that 2'-deoxyinosine and related nucleosides are also oxidised in biosystems leading to the formation of several dimeric products. It is likely that these dimers may exhibit toxicity as has been reported for O-O-linked dimer of guanosine (Goyal *et al.*, 1997) and may lead to DNA damage. It must be realized that Scheme 1 and 2 depict one of the most probable routes for the formation of products out of several possible pathways.

Acknowledgements

One of the authors (A. Tyagi) is grateful to the Council of Scientific and Industrial Research New Delhi, for the award of a Junior Research Fellowship. Partial financial assistance for this work was provided by CSIR, New Delhi vide grant No. 01(1815)/02/EMR-II.

References

- Brajter-Toth, A., R.N. Goyal, M.Z. Wrona, T. Lacava, N.T. Nguyen and G. Dryhurst, 1981. Electrochemical and enzymic oxidation of biological purines. *Bioelectrochem. Bioenergetics*, 8: 413-435.
- Carson, D.A., J. Kaye and J.E. Seegmiller, 1977. Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency: Possible role of nucleoside kinase(s). *Proc. Natl. Acad. Sci. USA.*, 74: 5677-5681.
- Carta, M.C., A. Mattana, M. Camici, S. Allegrini, M.G. Tozzi and F. Sgarrella, 2001. Catabolism of exogenous deoxyinosine in cultured epithelial amniotic cells. *Biochimica et Biophysica Acta*, 1528: 74-80.
- Cauquis, G. and V.D. Parker, 1973. *Methods for the Elucidation of Organic Electrochemical Reactions in Organic Electrochemistry*, (Ed.), Baizer M M and Marcel Dekker, New York, pp: 134.
- Chan, H.K. and A.G. Fogg 1979. Determination of the phenolic analgesics, cirmadol, meptazinol, dezocine and pentazocine in pharmaceutical dosage forms by linear-sweep voltammetry at a glassy carbon electrode. *Anal. Chem. Acta*, 105: 423-428.
- Christian, G.D. and W.C. Purdy, 1962. Residual current in orthophosphate medium. *J. Electroanal. Chem.*, 3: 363-367.

- Ciccolini, J., P. Cuq, A. Evrard, S. Giacometti, A. Pelegrin, C. Aubert and J.P. Cano, 2001. Combination of thymidine phosphorylase gene transfer and deoxyinosine treatment greatly enhances 5-fluorouracil antitumor activity *in vitro* and *in vivo*. *Molecular Cancer Therapeutics*, 1: 133-139.
- Ciccolini, J., L. Peillard, A. Evrard, P. Cuq, C. Aubert, A. Pelegrin, P. Formento and G. Milano, 2000. Enhanced antitumor activity of 5-fluorouracil in combination with 2'-deoxyinosine in human colorectal cell lines and human colon tumor xenografts. *J. Catalin. Clin. Cancer Res.*, 6: 1529-1535.
- Ciuffreda, P., S. Casati and E. Santaniello, 1999. Lipase-catalyzed protection of the hydroxy groups of the nucleosides inosine and 2'-deoxyinosine: a new chemoenzymatic synthesis of the antiviral drug 2',3'-dideoxyinosine. *Bioorganic Med. Chem. Lett.*, 9: 1577-1582.
- Conway, A.C., R.N. Goyal and G. Dryhurst, 1981. Electrochemical oxidation of hypoxanthine. *J. Electroanal Chem.*, 123: 243-264.
- Cubero, E., R. Guimil-Garcia, F.J. Luque, R. Eritja and M. Orozco, 2001. The effect of amino groups on the stability of DNA duplexes and triplexes based on purines derived from inosine. *Nucleic Acids Res.*, 29: 2522-2534.
- Gaussian 98, Revision A.11.4, Frisch M J, Trucks G W, Schlegel H B, Scuseria G E, Robb M A, Cheeseman M A, Zakrzewski V G, Montgomery J A, Stratmann Jr R E, Burant J C, Dapprich S, Millam J M, Daniels D A, Kudin K N, Strain M C, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pomelli C, Adamo C, Clifford S, Ochterski J, Petersson G A, Ayala P Y, Cui Q, Morokuma K, Rega, Salvador P, Dannenberg J J, Malick D K, Rabuck A D, Raghavachari K, Foresman J B, Cioslowski J, Ortiz J V, Baboul A G, Stefanov B B, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin R L, Fox D J, Keith T, Allaham M A, Peng C Y, Nanayakkara A, Challacombe M, Gill P M W, Johnson B, Chen W, Wong M W andres J L, Gonzalez C, Head-Gordon M, Replogle E S and Pople J A (2002), Gaussian Inc., Pittsburgh PA.
- Goyal, R.N., 1989. Electrochemical oxidation of xanthine at solid electrodes. *Ind. J. Chem.*, 28A: 467-471.
- Goyal, R.N., N. Jain and D.K. Garg, 1997. Electrochemical and enzymic oxidation of guanosine and 8-hydroxyguanosine and the effects of oxidation products in mice. *Bioelectrochem. Bioenerg.*, 43: 105-114.
- Goyal, R.N., A. Kumar and A. Mittal, 1991. Oxidation chemistry of adenine and hydroxyadenines at pyrolytic graphite electrodes. *J. Chem. Soc. Perkin Trans.*, 2: 1369-1375.
- Goyal, R.N., A. Rastogi and A. Sangal, 2001. Electro-oxidation of 6-mercaptopurine riboside with special emphasis on the stability of the dimer in aqueous solutions. *New J. Chem.*, 25: 545-550.
- Goyal, R.N., A.B. Toth and G. Dryhurst, 1982. Electrochemical and peroxidase-catalyzed redox chemistry of 9-methyluric acid. *J. Electroanal Chem.*, 133: 287-297.
- Hoffman, E.A., W.C. Kopp and A.G. Moat, 1990. Enhanced antibody response in the presence of partial adenosine deaminase inhibition. *Proc. Soc. Exp. Biol. Med.*, 194: 36-42.
- Kashima, M., K. Tsuji and K.T. Koho, 1995. Allergy inhibitors containing inosine or 2'-deoxyinosine, Application: JP 93-192671 19930708 CAN 122: 230780, AN., 1995: 503298.
- Kewn, S., P.G. Hoggard, J.S. Henry-Mowatt, G.J. Veal, S.D. Sales, M.G. Barry and D.J. Back, 1999. Intracellular activation of 2',3'-dideoxyinosine and drug interactions *in vitro*. *AIDS Res. Human Retroviruses*, 15: 793-802.

- Kilpatrick, D.A., B. Nottay, C.F. Yang, S.J. Yang, M.N. Mulders, B.P. Holloway, M.A. Pallansch, and O.M. Kew, 1996. Group-specific identification of polioviruses by PCR using primers containing mixed-base or deoxyinosine residue at positions of codon degeneracy. *J. Clin. Microbiol.*, 34: 2990-2996.
- Kowalczyk, A. and T.M. Harris, 2000. Synthetic Studies of Deaminated N1 Adduct of Deoxyadenosine Adduct of Butadiene Monoxide, Book of Abstracts, 219th ACS National meeting, San Francisco TOXI-103.
- Kubota, M. and A. Ono, 2004. Stabilization of DNA triplexes by dangling aromatic residues. *Tetrahedron Lett.*, 45: 1187-1190.
- Kilpatrick, D.A., B. Nottay, C.F. Yang, S.J. Yang, E.D. Silva, S. Penaranda, M. Pallansch and O. Kew, 1998. Serotype-specific identification of polioviruses by PCR using primers containing mixed-base or deoxyinosine residues at positions of codon degeneracy. *J. Clin. Microbiol.*, 36: 352-357.
- Li, G., Z.W. Deng, J. Li, H.Z. Fu and W.H. Lin, 2004. Chemical constituents from starfish *Asterias rollestoni*. *J. Chinese Pharmaceutical Sci.*, 13: 81-86.
- Lionetti, F.J. and N.L. Fortier, 1966. Metabolism of deoxyinosine by human erythrocyte ghosts. *Biochimica et Biophysica Acta*, 119: 462-469.
- Miller, F.J. and H.E. Zittel, 1963. Fabrication and use of pyrolytic graphite electrode for voltammetry in aqueous solutions. *Anal. Chem.*, 35: 1866-1869.
- Miyazawa, E., A. Maekawa, T. Suzuki and Y. Sahashi, 1966. Formation of deoxyinosine from inosine by washed cell suspension of a vitamin B12-requiring strain of *Lactobacillus leichmannii*. *J. Vitaminol.*, 12: 80-87.
- Moorman, A.R., S.W. Lafon, D.J. Nelson, H.H. Carter, J.J. Marr and R.L. Berens, 1991. Antiprotozoal activity of 3'-deoxyinosine. Inverse correlation to cleavage of the glycosidic bond. *Biochem. Pharmacol.*, 42: 207-212.
- Ochs, U.H., S.H. Chen, H.D. Ochs, W.R.A. Osborne and C.R. Scott, 1979. Purine nucleoside phosphorylase deficiency: a molecular model for selective loss of T cell function. *J. Immunol.*, 122: 2424-2429.
- Parsons, P.G., E.P.W. Bowman and R.L. Blakley, 1986. Selective toxicity of deoxyadenosine analogs in human melanoma cell lines. *Biochem. Pharmacol.*, 35: 4025-4029.
- Pople, J.A. and D.L. Beveridge, 1970. Approximate Molecular Orbital Theory. Mc Graw-Hill, New York, pp: 214.
- Roth, J.S., J. Jr. Ford, M. Tanaka, H. Mitsuya and J.A. Kelley, 1998. Determination of 2'- β -fluoro-2',3'-dideoxyadenosine, an experimental anti-AIDS drug, in human plasma by high-performance liquid chromatography. *J. Chromatogr. B: Biomed. Applied*, 712: 199-210.
- Sahashi, Y., T. Suzuki, S. Choki and F. Takeuchi, 1968. Inosine related compounds and their physiological activities. I. Metabolic activities of 2'-deoxyinosine and 2'-deoxyadenosine. *Amino Acid Nucleic Acids*, pp: 18-24.
- Subramanian, P. and G. Dryhurst, 1987. Electrochemical oxidation of guanosine. Formation of some novel guanine oligonucleosides. *J. Electroanal Chem.*, 224: 137-162.
- Swern, D., 1971. Organic Peroxy Acids as Oxidizing Agents. Epoxidation, Organic Peroxides, Wiley Interscience, New York.
- Teresa, E., A. Peterson and A.B. Toth, 1988. Electrochemical and photochemical oxidation of the deazapurine nucleotide drug tubercidin-5'-monophosphate. *J. Electroanal Chem.*, 239: 161-173.
- Ueno, Y., A. Shibata, A. Matsuda and Y. Kitade, 2004. Thermal stability of triple helical DNAs containing 2'-deoxyinosine and 2'-deoxyxanthosine. *Bioorganic Med. Chem.*, 12 : 6581-6586.

- Vogel's Textbook of Practical Organic Chemistry, ELBS with Longman, 1994, UK, pp: 402.
- Wataya, Y. and O. Hiraoka, 1984. 3'-Deoxyinosine as an antileishmanial agent: The metabolism and cytotoxic effects of 3'-deoxyinosine in *Leishmania tropica promastigotes*. Biochem. Biophys. Res. Comm., 123: 677-683.
- Weismann, W.P. and H.K. Webster, 1984. Impaired metabolism of deoxyadenosine in uremic erythrocytes. Adv. Exp. Med. Biol., 165A: 359-362.
- Wopschall, R.H. and I. Shain, 1967. Effects of adsorption of electroactive species in stationary electrode polarography. Anal. Chem. Soc., 39: 1514-1527.
- Yao, T., Y. Taniguchi, T. Wasa and S. Musha, 1978. Anodic voltammetry and its analytical application to the detection and simultaneous determination of hypoxanthine, xanthine and uric acid. Bull. Chem. Soc. Jpn., 51: 2937-2941.