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Further Investigations into the Oxidation Chemistry of Adenosine - Effect of Applied Potential on the Products Formation

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The oxidation chemistry of adenosine **(1)** has been studied in Britton-Robinson buffers of pH range 2.2-10.1 at pyrolytic graphite (PGE) and glassy carbon (GCE) electrodes. The oxidation occurred in a single, well-defined peak (I_a) at about 200-250 mV less positive potential in comparison to phosphate medium. The peak potential of peak I_a was dependent on pH and shifted to less positive potential with increase in pH. Under cyclic voltammetric conditions oxidation occurred in Britton Robinson buffer in a 6e, $6H^+$ process in contrast to 5e, $5H^+$ process in phosphate medium and the nature of mechanism is found to be EC in which charge transfer is followed by chemical reactions. The major products of oxidation were found as allantoin, hydroxylated C-O-O-C and C-C dimers and ribose. A comparison of the observed behaviour in B.R. buffers with that observed in phosphate medium has also been presented Tentative mechanism for the formation of products has also been suggested.

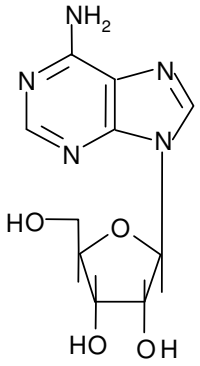
Keywords: Adenosine, electrooxidation, purine nucleosides

1. INTRODUCTION

Adenosine, a ubiquitous nucleoside of adenine is known to be the dephosphorylation product of adenine nucleotide, ATP [1,2]. It is reported to be released by thymocytes [3], endothelial cells and myocytes [4] and acts as a stress hormone and released in the vicinity of the immune cells during both systemic and cellular stress [5]. Besides this, adenosine is also found in both male [6] and female reproductive [7] fluids at high micromolar concentration and rapidly metabolized in the heart tissues [8]. Being, an endogenous signaling nucleoside, adenosine plays a significant protective role in multiple physiological processes such as, neuroprotection and neuromodulation in a variety of central nervous system disorders [9,10] and cardiovascular disorders [11-14].

In a communication from this laboratory, the electrochemical oxidation of adenosine was investigated using pyrolytic graphite electrode in phosphate media [15]. However, due to high

oxidation potential of adenosine the voltammetric peak appeared close to the background discharge potentials, where significant unavoidable water oxidation begins. Under these conditions the formation of only one product was observed at physiological pH, which was characterized as C-O-O-C linked dimer of adenosine. As the water oxidation seems to contribute an important role via hydrogen peroxide or oxygen intermediates during electrooxidation and affects the product distribution [16,17], it was considered worthwhile to study oxidation of adenosine in a medium where, E_p could be shifted to less positive potentials to avoid water oxidation complications. Thus, oxidation studies of adenosine in Britton-Robinson buffer as a supporting electrolyte were carried out. Interestingly the Ep of adenosine shifted to less positive potentials (by 200-250 mV) in comparison to that of phosphate medium. The formation of four products viz. as, allantoin, hydroxylated C-O-O-C and C-C bridged dimers and ribose were noticed at pH 7.0 in contrast to only one product (C-O-O-C dimer) obtained in the phosphate medium. It is expected that the results obtained from these studies will throw light in understanding the biochemical oxidation of this compound.



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2.Materials and Instrumentation

Adenosine, obtained from Merck (Germany) was used as received. N,N-bis(trimethylsilyl)triflouroacetamide (BSTFA) was obtained from Sigma and silylation grade acetonitrile was a product of Pierce Chemical Co., Rockford, IL,USA. Britton-Robinson buffers were prepared by mixing 0.04 mol L^{-1} phosphoric acid, 0.04 mol L^{-1} acetic acid and 0.04 mol L^{-1} boric acid with the appropriate amount of 0.2 mol L^{-1} sodium hydroxide solution as reported in the literature [18]. All other chemical used were of analytical grade.

The details of the pyrolytic graphite electrode (PGE ~ 6 mm^2) and glassy carbon electrode (GCE ~ 7.8 mm^2) used as the working electrodes were essentially same as reported earlier [19,20]. 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 (6×1 cm^2) as working electrode, cylindrical platinum gauze as auxillary electrode and Ag/AgCl electrode at an ambient temperature of 25 ± 2 °C. UV-Vis spectral and kinetics studies of the decomposition of the UV absorbing intermediate were carried using a Perkin-Elmer lambda 35 UV/Vis Spectrophotometer. High performance liquid chromatography (HPLC) was performed on Agilent 1

100 series HPLC system with semipreparative octadecylsilane (C-18) reversed phase column (7.8×300 mm). Gas chromatography/ mass spectrometric analysis (GC-MS) of the silylated samples was performed with Perkin Elmer Clares 500 Spectrometer in EI mode at 70 eV using HP-17 column. FT-IR spectrum of the products was recorded using a Perkin Elmer 1600 series FT-IR spectrophotometer using KBr pellets. The ^{1}H NMR spectrum of the products was recorded in DMSO-d_6 with TMS as internal standard using Avance 500 digital NMR spectrometer from Brucker (500 MHz). Chemical shifts (δ) are reported in parts per million (ppm) of the applied field.

2.1 Procedure for the Electrochemical oxidation

The solutions for the voltammetric experiments were prepared by mixing 2.0 mL of the stock solution (2 mM) of the compound **(1)** with 2.0 mL of Britton-Robinson buffer solution of desired pH. The solutions were bubbled with nitrogen gas for 10-15 min before recording the voltammograms.

The progress of the electrolysis was monitored by recording changes in the UV spectrum at different time intervals. For this purpose, electrolysis was carried out at the peak potential (I_a) and about 2-3 mL of the electrolyzed solution was transferred each time to 1 cm quartz cell to record the spectrum. In the next set of the experiments, the potentiostat was open-circuited when the absorbance at λ_{max} reduced to 50 %. The changes in the UV/Vis spectrum were monitored and the values of the rate constant *k* were calculated from the linear log ($A-A_\infty$) *vs* time plots.

2.2 Procedure for the oxidation product analysis

The products of the electrooxidation of adenosine were characterized at pH 7.0 by exhaustively electrolyzing 20-25 mg of the compound **(1)** by applying a potential corresponding to peak I_a at a large PGE. The progress of the electrolysis was monitored by recording spectra at different time intervals. When the absorbance at the λ_{max} reached to minimal, the exhaustively electrolyzed solution was removed from the cell, lyophilized and extracted with methanol (2×10 mL). The separation of the products was achieved by using HPLC equipped with C-18 reversed phase column and several injections of ~ 500μL solution were made. The mobile phase used was a gradient system of water (Solvent 1) and acetonitrile (Solvent 2) at a flow rate of 2.0 mL/min and the absorbance of the eluents were measured at 260 nm. The gradient system of mobile phase used for the collection of products was, 100 % Solvent 1 for 0-1 min., 90 % Solvent 1 and 10 % Solvent 2 for 2-3 min., 70 % Solvent 1 and 30 % Solvent 2 for 4-6 min. The final composition of the gradient employed was 50 % Solvent 1 and 50 % Solvent 2, which was then maintained for 7-15 min. The column was then equilibrated with 100 % Solvent 1 for 15 min before the next injection was made. The HPLC chromatogram showed four well resolved peaks and after repetitively collecting the volume eluted under the individual HPLC peaks, the solutions were lyophilized and the dried materials obtained were characterized using m.p., FT-IR, ^{1}H NMR and GC-MS. The non-volatile oxidation products were converted to volatile species to determine their molar masses by the formation of trimethylsilyl derivatives. For this purpose, about 100-200 μg of the sample was treated with 100 μL acetonitrile and 100 μL silylating agent bis (trimethylsilyl)triflouroacetamide (BSTFA) in a 3.0 mL vial. The vial was tightly closed and heated in

an oil bath at 110 °C for about 10–15 min with constant shaking [21]. After cooling, to room temperature ~ 2 µL of the sample was injected in GC-MS.

3. RESULTS AND DISCUSSION

3.1 Voltammetric studies

Linear sweep voltammetric studies of 1 mM adenosine exhibit one-well defined anodic peak I_a in the entire pH range of 2.2-10.1 at a sweep rate of 20 mV s^{-1} at PGE and GCE. The peak potential of peak I_a was found to be dependent on pH and shifted to less positive potential with increasing pH. At the GCE the peak potential was at least 25-50 mV more positive than at PGE. The values of the peak current corresponding to peak I_a, however remained constant in the entire pH range. The linear dependence of the peak potential E_p on pH for the peak I_a, can be presented by the relations:

E_p (I_a) [pH 2.2-10.1] = [1399.8 – 49.4 pH pH] mV vs Ag/AgCl --------- at PGE

E_p (I_a) [pH 2.2-10.1] = [1431.8 – 50.6 pH pH] mV vs Ag/AgCl --------- at GCE

having correlation coefficient ~ 0.996 and 0.995 respectively. It was interesting to note that the Ep values were ~ 200 –250 mV less positive in comparison to phosphate medium in the entire pH range.

In cyclic sweep voltammetry, adenosine at a sweep rate of 100 mV s^{-1} exhibited a well-defined oxidation peak I_a, when the sweep was initiated in the positive direction. In contrast to phosphate medium where reduction peak II_c was noticed in the reverse sweep no reduction peak was noticed in the present studies. A comparison of cyclic voltammograms of adenosine at PGE in Britton-Robinson and phosphate buffers at pH 7.0 is presented in Fig.1.

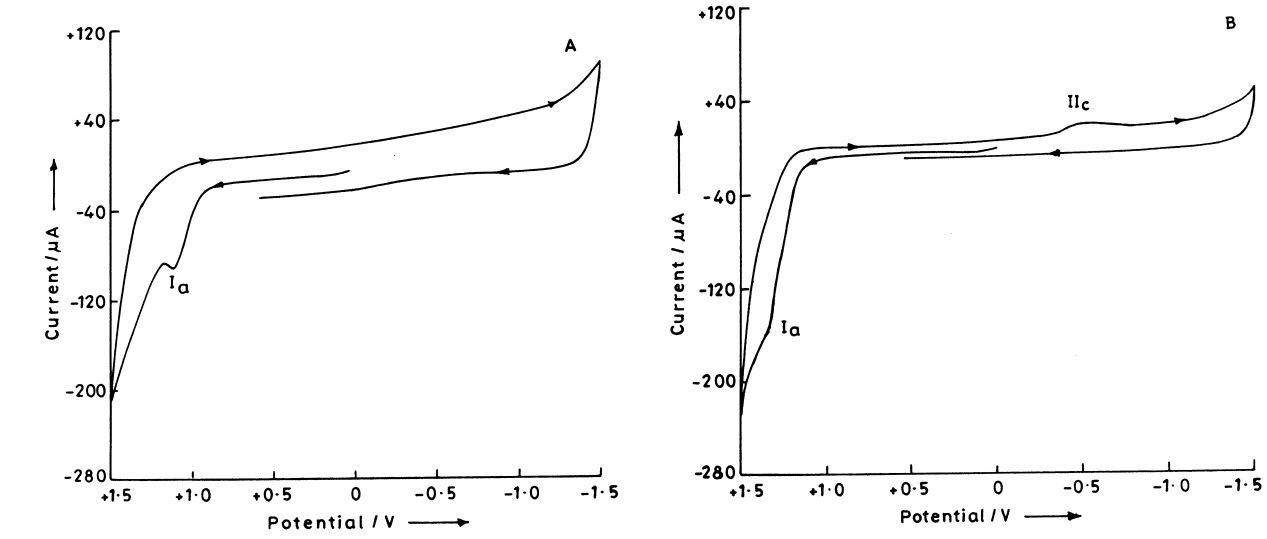


Figure1. A comparison of cyclic voltammograms of 1.0 mM adenosine in Britton-Robinson buffer (A) and phosphate buffer (B) of pH 7.0 at PGE.

The peak current i_p for peak I_a increased with the increasing concentrations of adenosine in the range 0.2–2.0 mM at both the electrodes. The plots between peak current and concentration were linear and can be represented by the equation:

i_p (μA) = 18.9 C ------- at PGE

i_p (μA) = 15.6 C ------ at GCE

where, C is the concentration in mM having correlation coefficient ~ 0.996 and ~ 0.998 respectively. This behavior indicates that the compound **(1)** can be safely estimated in this concentration range at PGE or GCE. The values of the peak current function ($i_p\, v^{-1/2}$) increase with an increase in logarithm of sweep rate (Fig.2) suggesting strong adsorption of the compound **(1)** at the surface of PGE as well as GCE [22].

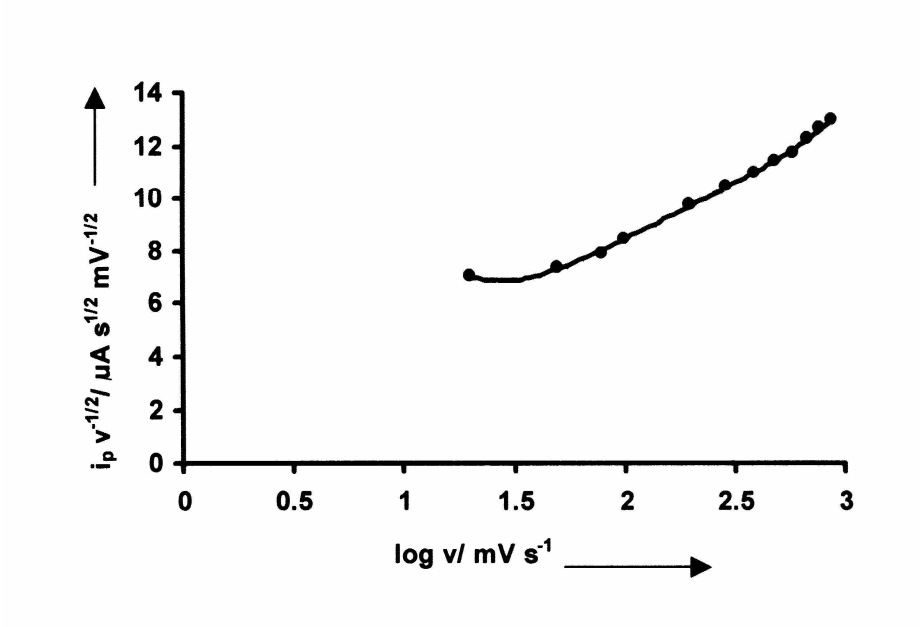


Figure2. Dependence of the peak current function ($i_p/\sqrt{v}$) with the logarithm of the sweep rate (log v) for voltammetric peak I_a of adenosine at pH 7.0, PGE.

The peak potential of the peak I_a also shifted to more positive potentials with increasing sweep rate. The plot of E_p vs log v was linear and followed the equation:

E_p (mV) = 602.4 + 236.1 log v, where v is in mV s^{-1}.

with a correlation coefficient of ~ 0.999 at PGE. The nature of the electrode reaction was thus established as EC in which charge transfer is followed by irreversible chemical reactions [23]. The observed voltammetric data of adenosine oxidation in Britton-Robinson buffers is also compared with that observed in the phosphate medium and is presented in Table 1.

Table 1. A comparative data of the voltammetric characteristics for adenosine at pH 7.0 at PGE, in different medium

Medium	dE_p/dpH	E_p (I_a)/ mV	Ep (II_c)/ mV	Linear conc. range/ mM
Phosphate buffer	39.4	~ 1340	- 440	0.05 – 1.0
Britton-Robinson Buffer	49.4	~1064	_	0.20 – 2.0

3.2 *Spectral studies*

The UV-Vis spectra of 0.1 mM adenosine were recorded at different pH. In the entire pH range 2.2-10.1, adenosine exhibited two well-defined λ_{max} at 210 and 260 nm, which are essentially similar to that observed in phosphate buffers. The curve 1 in Fig.3 presents the UV spectrum of adenosine at pH 7.0 just before electrooxidation. Upon application of the potential corresponding to peak I_a potential, absorbance systematically decreased in the regions 200-218 nm and 238-280 nm (curves 2-8). However, the absorbance increased systematically in the regions 218-238 and 280-400 nm (curves 2-8). Two clear isosbestic points were also observed at 218 and 280 nm. The increase in the absorbance in the longer wavelength region 280-400 nm indicates the formation of more conjugated species during oxidation, probably hydroxylated C-C bridged dimer **(10)**. The isosbestic points observed during electrolysis indicate that the intermediates formed during electrooxidation and the starting material has the same absorbance at these wavelengths. In contrast to Britton-Robinson buffer the spectral changes in phosphate medium showed initial decrease followed by an increase in absorbance in the region 215-240 nm. The isosbestic points were also noticed at 245 and 275 nm. The observed differences in the phosphate buffer may be due to very high positive potential (+ 1.4 V vs Ag/AgCl) used for the oxidation, which is close to background discharge.

The kinetics of decomposition of the UV-absorbing intermediate was monitored at 225, 260 and 300 nm. The resulting absorbance versus time profiles showed an exponential decay as shown in Fig.4. The values of the pseudo-first-order rate constant *k* were determined at different pH, using log ($A-A_\infty$) versus time plots (inset in Fig.4). The values of *k* calculated at different pH were in the range (0.46 – 0.51) $\times 10^{-3}$ s^{-1} and these values are similar to the *k* values observed in the phosphate medium.

3.3 Product characterization

The electrooxidation of adenosine in Britton-Robinson buffer (pH 7.0) lead to the formation of four products (allantoin, hydroxylated C-O-O-C and C-C dimers and ribose) in contrast to only hydroxylated C-O-O-C dimer formed in phosphate buffer. The GC-MS chromatogram of the trimethylsilyl derivative of freeze-dried mixture of adenosine showed the presence of four major components having retention times of ~ 8.95, 9.90, 11.93 and 19.26 min. The molar masses

corresponding to these retention times were found to be 518 [M^+, 6.6 %], 908 [M^+, 4.2 %], 438 [M^+, 3.7 %] and 876 [M^+, 5.5 %]. The data giving m/z values and the abundance of higher mass fragments for all the four components have been summarized in **Table 2**.

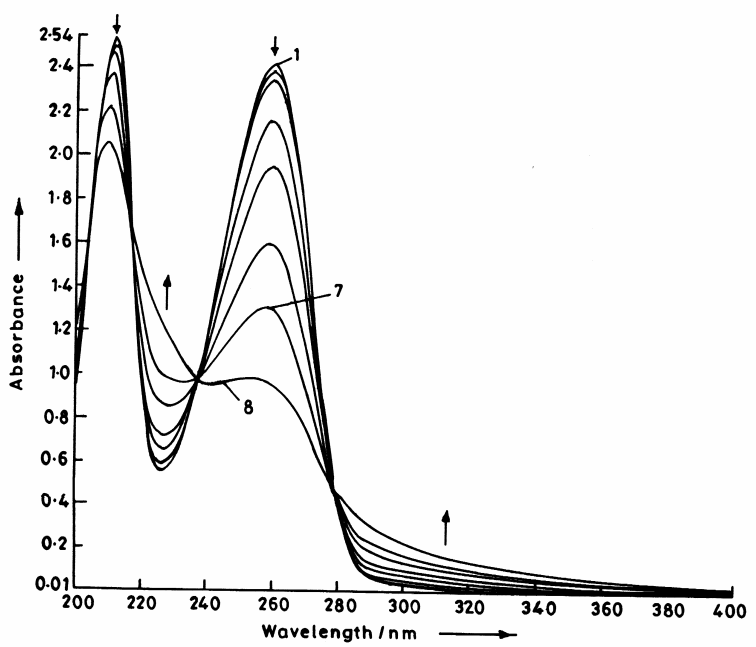


Figure 3. UV spectrum of 0.1 mM adenosine in Britton-Robinson buffer of pH 7.0 at PGE. Curves were recorded at (1) 0 (2) 5 (3) 10 (4) 20 (5) 30(6) 50 (7) 70 (8) 100 min of electrolysis.

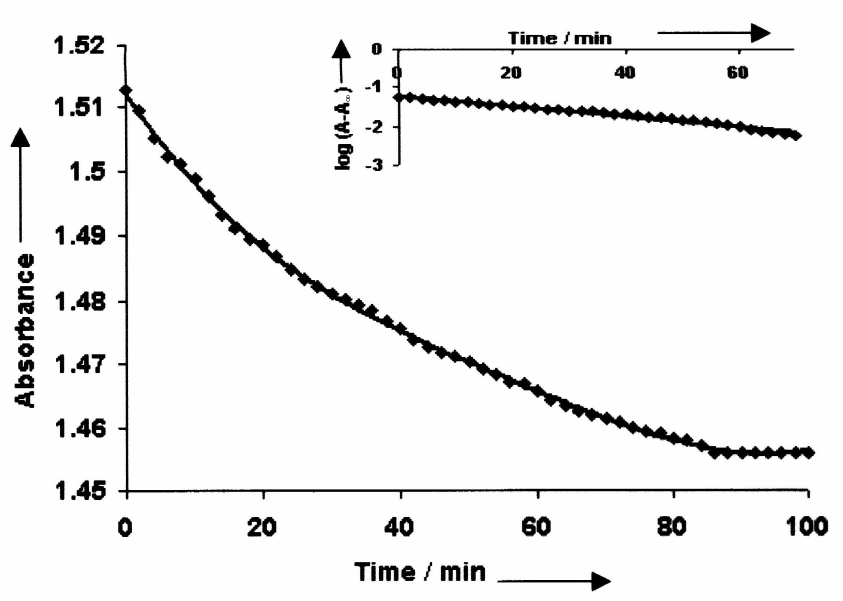


Figure 4. Observed changes in absorbance versus time and plot of log ($A-A_\infty$) vs. time (inset) plot measured at 260 nm for UV-absorbing intermediates generated during electrooxidation of adenosine at PGE, pH 7.0.

Table 2. Relative abundance of the peaks observed in GC/MS chromatogram of electrooxidized adenosine at pH 7.0.

R_t/min	m/z	Abundance of different fragments (%)	Relative Area	Product
8.95	518 (M+, 6.6 %)	504 (6.1), 503 (40.2), 490 (5.7), 446 (4.6), 341 (82.1), 327 (38.4), 243 (3.2), 202 (4.8)	11480872	Pentasilylated allantoin
9.90	908 (M+, 4.2 %)	898 (3.8), 839 (5.2), 835 (4.3), 603 (6.6), 454 (8.8), 381(2.8), 365 (4.2), 294 (5.5)	2180791	Hydroxylated C-O-O-C dimer
11.93	438 (M+, 3.7 %)	433 (2.8), 432 (2.1), 431 (61.6), 429 (94.2), 415 (16.8), 401 (21.3), 366 (4.8), 361 (27.2)	2355030	Tetrasilylated ribose
19.26	876 (M+, 5.5 %)	861 (4.1), 803 (5.4), 773 (3.8), 692 (2.2), 604 (5.8), 551 (2.5), 507 (4.7), 438 (14.6)	1884913	Hydroxylated C-C dimer

The molar mass of 518 corresponds to pentasilylated allantoin **(8)**, formed due to the rupture of the pyrimidine ring. The two components of molar mass 908 and 876 have been identified as octasilylated hydroxylated C-O-O-C **(14)** and C-C **(11)** bridged dimers having OH groups. The component corresponding to the molar mass of 438 was identified as tetrasilylated D-ribose, which clearly suggests the hydrolysis of the ribose groups from the electrolyzed products either during electrooxidation or silylation step.

High-performance liquid chromatography was used for the separation and further characterization of the components. Fig.5 presents the HPLC chromatogram observed for the mixture of products and shows four well resolved peaks at retention time (R_t) ~ 3.22 (P_1), 5.24 (P_2), 7.96 (P_3) and 9.27 (P_4) min respectively. The volume eluted under HPLC peaks were collected separately, lyophilized and further characterized using FT-IR and ^{1}H NMR.

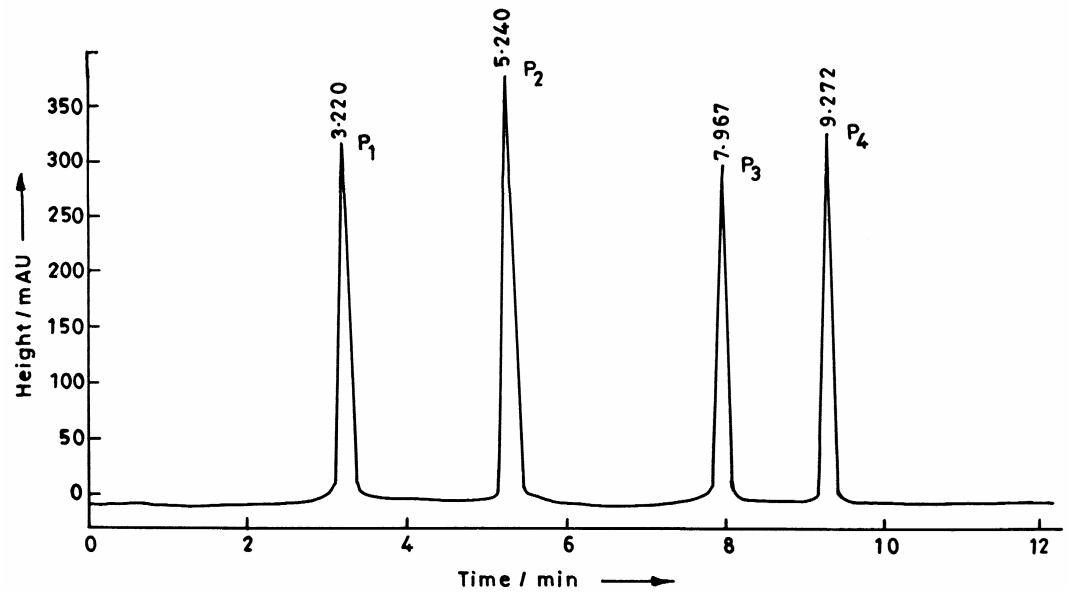


Figure 5. A typical HPLC chromatogram observed for the products of adenosine in Britton-Robinson buffer of pH 7.0.

HPLC Peak (P_1):

The colourless material obtained under this peak had a m.p. 259 °C and showed prominent bands in FT-IR spectrum at ν_{max}/ cm $^{-1}$ 3441, 3437, (NH2), 3284, 3278 (OH), 1686, 1671, 1625, 1618, 1591, 5127 (C=N) and (C=C). The mass spectrum of the material exhibited a clear molecular ion peak at m/z 908 (M^+, 4.2 %) and indicated it to be octasilylated C-O-O-C dimer with peroxide linkage **(14)**. The ^{1}H NMR spectrum of the material exhibited signals at δ_H (500 MHz; DMSO-d_6; Me_4Si) 8.22 (4H, s, 2 × NH_2), 8.05 (2H, s, 2 × OH), 6.98 (2H, s, 2 × NH). Hence, the material was concluded to be hydroxylated C-O-O-C bridged dimer with peroxide linkage **(13)** as one of the products of electrooxidation due to the rapid dimerization of the radical species **(12)** generated in the electrode reaction. The presence of the peroxide group in the compound was also confirmed by the test suggested in the literature [25,26] 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 color was observed in 1 min. This product was also obtained during oxidation of adenosine in phosphate buffer.

HPLC peak (P_2):

The white material obtained under the chromatographic peak P_2 had a m.p. 230 °C and its mass spectrum exhibited a clear molecular ion peak at m/z 518 (M^+, 6.6 %) along with a large peak at m/z 503 (M^+-CH_3, 40.2 %), suggesting it to be pentasilylated allantoin **(8)**. The ^{1}H NMR spectrum of the product exhibited signals at δ_H (500 MHz; DMSO-d_6; Me_4Si) 5.23 (1H, d, *J* 10.0), 5.79 (2H, s), 6.90 (1H, d, *J* 10.0), 8.15 (1H, s) and 10.53 (1H, s) and hence indicated the product as allantoin **(7)**.

HPLC peak (P_3):

The lyophilized material eluted under this peak had a m.p. 267 °C. The FT-IR spectrum exhibited bands at ν_{max}/ cm $^{-1}$ 3432, 3428 (NH_2), 3226, 3220 (OH), 1666, 1636, 1624, 1611, 1587, 1501, 1478 (C=N) and (C=C). The mass spectrum of the material exhibited a clear molecular ion peak at m/z 876 (M^+, 5.5 %) and indicated it to be octasilylated hydroxylated C-C dimer **(11)**. The ^{1}H NMR of the material exhibited signals at δ_H (500 MHz; DMSO-d_6; Me_4Si) 8.47 (4H, s, 2 × NH_2), 7.22 (2H, s, 2 × OH), 6.61 (2H, s, 2 × NH) and further confirmed the formation of hydroxylated C-C bridged dimer **(10)** The formation of such dimer can be accounted for on the basis of a rapid coupling of free radical species **(1_d)** with a subsequent attack of two water molecules and a loss of 4e, $4H^+$ to form hydroxylated C-C linked dimer **(10)**.

HPLC peak (P_4):

The colorless material obtained under peak P_4 had a m.p. 88 °C and exhibited prominent bands in FT-IR spectrum at ν_{max}/ cm $^{-1}$ 3910, 3774, 3666, 3470, 3304, 3093, 3047, 2843, 2603, 2332 and 1636. Its mass spectrum exhibited a molecular ion peak at m/z 438 (M^+, 3.7 %) and is characterized as ribose. A further confirmation of its formation was made by recording the IR spectrum of the authentic ribose. The IR spectrum of the authenthic D-ribose was practically superimposable on the IR spectrum obtained for the material.

3.4 Redox mechanism

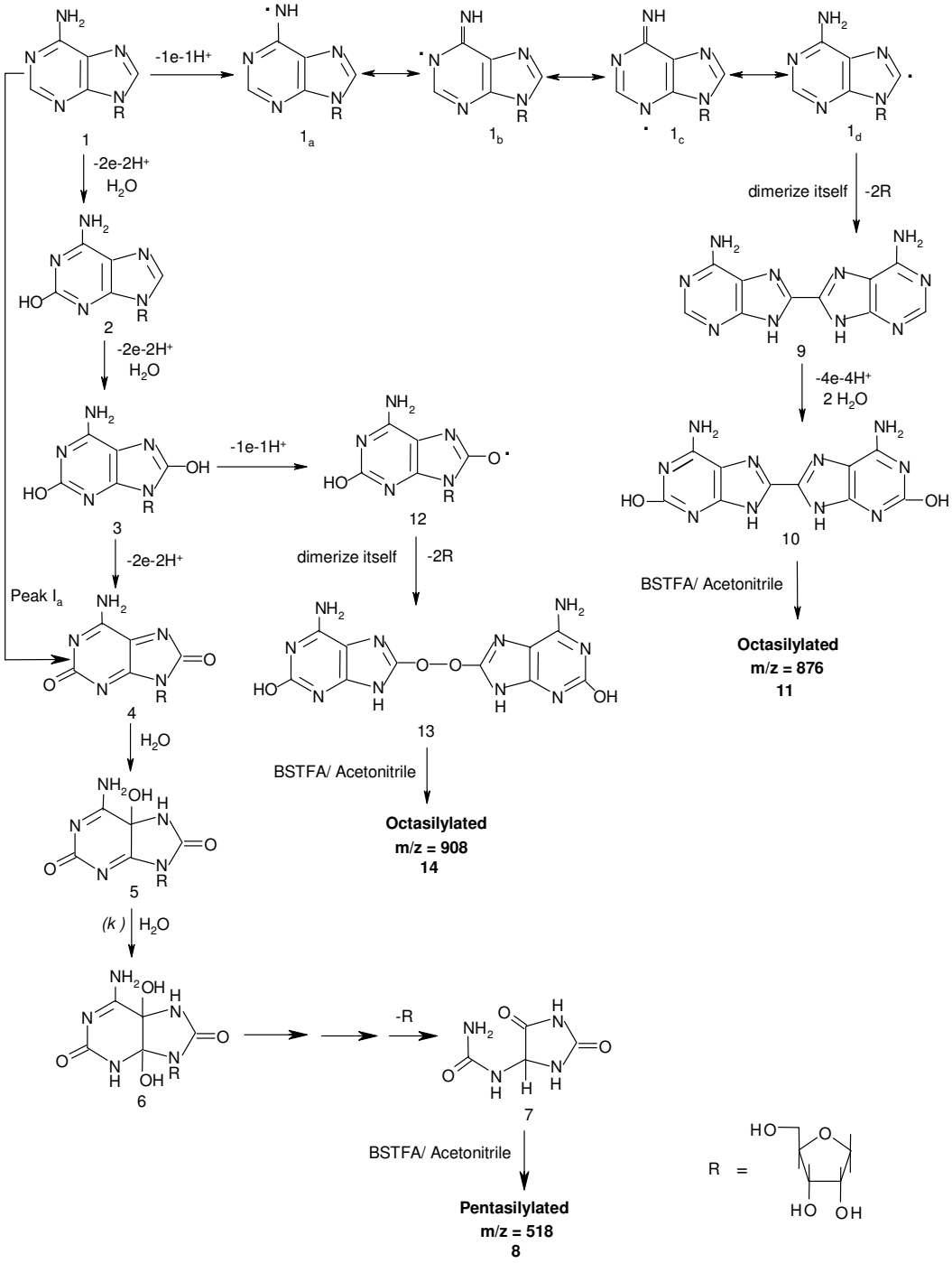
The results obtained above indicate that the electrooxidation of adenosine **(1)** occurs by a mechanism involving a 6e, $6H^+$ process to form allantoin, hydroxylated C-O-O-C and C-C bridged dimers in Britton-Robinson buffers. Their formation can be explained as follows-

The initial 2e, $2H^+$ oxidation of adenosine **(1)** in presence of water can occur at position 2 or 8 to give either 2-hydroxy- or 8-hydroxyadenosine. However, on the basis of the studies reported in the literature [27], on the oxidation of adenine it seems reasonable to conclude that the first 2e, $2H^+$ oxidation will lead to the formation of 2-hydroxyadenosine **(2)**. The further 2e, $2H^+$ oxidation of 2-hydroxyadenosine will form 2,8-dihydroxyadenosine **(3)**, which rapidly oxidizes in a 2e, $2H^+$ step to give corresponding diimine species **(4).** Thus, these mechanistic steps are similar to that reported earlier for adenosine oxidation in phosphate medium. The diimine **(4)** has been reported to be unstable due to the presence of two C=N bonds and therefore attack of a water molecule rapidly forms imine alcohol **(5)**. The attack of a second water molecule on **(5)** will be a slow process [28] and leads to the formation of diol **(6),** as presented in **Scheme 1**. The *k* values monitored represent the decay of imine alcohol **(5)** to diol **(6)** in a pseudo first-order reaction. The formation of allantoin **(7)** then takes place in a series of reactions involving the cleavage of pyrimidine ring as already reported by many workers [29]. Allantoin possesses five silylable sites and GC-MS data clearly indicates that all the reactive hydrogen atoms are replaced with trimethyl silyl groups with BSTFA/ acetonitrile corresponding to m/z 518 at R_t ~ 9.91 min **(8)**. In phosphate medium the k values are reported at pH 7.0 at wavelength 232 and 280 nm inspite of the fact that only C-O-O-C dimer is reported as the final product [15]. Such a dimer formation does not require hydrolysis step and hence k values should not have been noticed. It appears that more products are formed in earlier studies [15] which could not be separated probably due to the use of gel permeation chromatography.

The formation of dimers can be explained by radical-radical coupling reactions. In the side reactions 1e, $1H^+$ electrochemical oxidation of adenosine **(1)** also appears to take place at amino group as reported earlier for adenine N1-oxide [30]. Such an oxidation will give a free radical species **(1_a)**, which can exhibit several resonating forms **(1_a-1_d)** as shown in **Scheme 1**. The formation of hydroxylated C-O-O-C bridged dimer **(13)** can be explained on the basis of 1e, $1H^+$ oxidation of 2,8-dihydroxyadenosine **(3)** to give a radical species **(12)**. Such an oxidation at position 8 in 1e, $1H^+$ step has been well documented in the literature [31,32]. The radical species **(12)** undergoes rapid dimerization to form C-O-O-C bridged dimer **(13)**. The ^{1}H NMR data of the characterized dimer **(13)** clearly depicts the removal of the ribose moieties. One of the reasons for the removal of ribose is the steric hindrance in the dimer **(13)** during its formation. The removal of ribose units due to steric hindrance has been already reported during oxidation of guanosine and similar purine nucleoside [33]. The molecular ion peak having m/z 908 in GC-MS data clearly indicates that all the reactive hydrogen atoms are replaced with trimethylsilyl units in C-O-O-C dimer **(13).** The dimer **(13)** was obtained as the only product during oxidation of adenosine in phosphate medium.

The formation of other hydroxylated C-C bridged dimer **(10)** can be accounted for, on the basis of the dimerization of radical species **(1_d)** generated in the oxidative pathway to give a stable dimer **(9)**. The ultimate product, however, is a dihydroxy dimer **(10)** formed on further 4e, $4H^+$ oxidation of

dimer **(9)** and having two hydroxyl groups at position C_2 and C'_2. The presence of hydroxyl groups is indicated by a sharp FT-IR band at around 3226 and 3220 cm^{-1} and was also confirmed by a signal at δ 7.22, corresponding to two protons in ^1H NMR spectrum of the product. The molar mass of 876 of the octasilylated dimer **(11)** clearly indicates the hydrolysis of ribose units during electrooxidation as for the C-O-O-C bridged dimer **(13).** The removal of ribose units was further confirmed on the basis of ^1H NMR data for this product.



Scheme 1: A tentative mechanistic pathway of electrooxidation of adenosine at pH 7.0.

4. CONCLUSION

The results presented in the current study on the electrochemical oxidation of adenosine in Britton-Robinson buffer as a supporting electrolyte clearly demonstrate that the oxidation of adenosine occurs at ~ 200–250 mV less positive potential in Britton-Robinson buffers in comparison to phosphate medium. The low peak potential resulted in application of less positive potential in CPE and which seems to influence the product distribution during electrooxidation. Though, a comparison of rate constant (*k*) values indicated the generation of same intermediates during the electrochemical oxidation. The primary electrode reaction leading to the formation of allantoin appears to be the major route in Britton-Robinson buffers. The secondary electrode reactions leading to the formation of several novel dimeric products appear to be due to radical species generated. The formation of dimer **(13)** as the only product in phosphate medium clearly suggests that the applied potential in CPE being close to background discharge simply permitted free radical pathway. It is quite likely that ribose might have been formed in phosphate medium, however, could not be detected due to gel-permeation separation technique used in the investigations. Thus, if an applied potential is close to water oxidation potential in CPE, the oxygen intermediates formed favor free radical pathway in the case of adenosine oxidation. Whereas in other cases allantoin is formed as the major product. However, it must be realized that more than one pathways are always possible for the formation of these products.

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