DIFFERENTIAL PULSE VOLTAMMETRIC DETERMINATION OF GUANOSINE AND 8-HYDROXYGUANOSINE

A

REPORT ON

Laboratory Project submitted for the partial fulfilment of the degree

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MASTER OF PHILOSOPHY

in

CHEMISTRY

(Industrial Methods of Chemical Analysis)

By

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesisentitled, "**DIFFERENTIAL PULSE VOLTAMMETRIC DETERMINATION OF GUANOSINE AND 8-HYDROXYGUANOSINE**" in partial fulfillment of the requirement for the award of the degree of **Master of Philosophy** submitted in the Department of Chemistry, University of Roorkee, Roorkee, is an authentic record of my own work carried out during a period of January, 1998 to July 1998 under the supervision of Prof. R. N. Goyal.

The matter embodied in this thesis has not been submitted by me for the award of any other degree.

Deepshikha.

(DEEPSHIKHA)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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CONTENTS

ABSTRACT

Purine nucleosides are well known for their metabolic and biological effects on human beings. The development of sophisticated techniques in electrochemistry and growing importance of oxidation reactions in biological systems facilitated determination of two or more compounds simultaneously. The electrochemical studies of biomolecules provide useful and valuable information about their biological redox reactions. Electrochemical oxidation of guanosine and 8-hydroxyguanosine has been carried out at Pyrolytic Graphite Electrode by differential pulse voltammetry in the pH range 2.5-10.3. I_p versus concentration studies at pH 7 suggested that guanosine and 8-hydroxyguanosine can be determined simultaneously using differential pulse voltammetry in the concentration range of 0.1 to 0.0005 mM.

INTRODUCTION

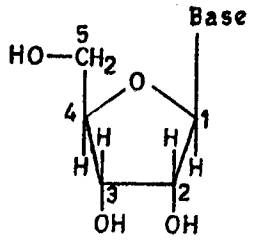
INTRODUCTION

Electrochemistry spans the breadth of chemical science and extends into many other fields such as biology and engineering and it provides invaluable insights into the redox behaviour of biologically important compounds. It has been proved in recent years that electroanalytical techniques provide indispensable, versatile and significant potentialities to extend and deepen the understanding of physico-chemical aspects of the biological changes undergoing in the living systems.

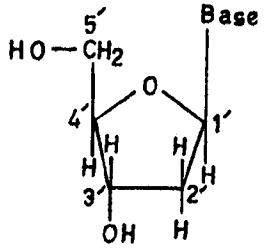
The role played by electrochemistry in the enrichment of other branches of science can not be overlooked whether it is analytical, organic, physical, inorganic chemistry or biological sciences. Various analytical techniques in combination with electrochemical techniques are generally used to explore the fundamental redox chemistry of biomolecules and to assist in understanding biological redox process.

Purine nucleosides are the building blocks of nucleic acid (RNA, DNA) and play a vital role in many biochemical processes in living systems. Nucleic acids (RNA and DNA) [1] are highly polymeric structure of nucleotides which consist of nucleosides of bases (purines and pyrimidines) with sugar. Thus, purines are the basic constituents of RNA and DNA, which are necessary for life. Purine nucleosides are carbohydrate derivatives of purines in which D-ribose or D - 2′ deoxyribose is attached to purine base at N(9) via β-N-glucosidic linkage. As two types of sugar moiety can associate with purine in the nucleosides, there are two types of nucleosides: ribonucleosides and the deoxyribonucleosides. The sugar always

exists in furanose ring form and is normally linked through the group originally at C-1′. The general formulas of ribose and deoxyribose nucleosides are as follows:



D-ribose nucleoside



D-2′-Deoxyribose nucleoside

The purine (and pyrimidine) nucleoside phosphates are widely involved in intermediary metabolism in the form of anhydride derivatives with carboxylic, phosphoric, and sulphuric acids. Such compounds are both chemically and metabolically reactive and are often termed " energy rich" compounds. Probably the most important anhydride compound in metabolism is ATP, which contain two " Pyrophosphate" bounds. ATP is formed from ADP and orthophosphate during the fermentative and oxidative degradation of many foodstuffs. The chemical energy of the foodstuff is thereby stored in the ATP, from which energy may be recovered to initiate many biosynthetic reactions [2].ATP may also serve as a phosphorylating agent: some typical examples are the conversion of ribose-5-phosphate to phosphoribosyl pyrophosphate, of nucleoside to nucleoside-5-phosphate and of the nucleosides monophosphate sequentially to the corresponding disphosphates and triphosphates.

In addition, knowledge of metabolism of these nucleosides often provide useful information regarding their biochemical targets within cells. The early phases of the investigation of purine and purine nucleoside also includes the study of their biochemical effects. Such studies may utilize cells *in vivo* or *in vitro*, whether growing or not, or they may be done using cell extracts or partially or highly purified enzymes. The aim of these studies is to determine the range of biochemical effects that a given analog can or might have, without at this stage to relate these effects to the biological effects of the drug. The biological effects of purine nucleosides can perhaps be discussed most simply with reference to the various aspects of purine nucleoside metabolism and function [3].

Purine nucleosides constitute a large class of pharmacologically active drugs that are of both experimental and clinical significance. Unfortunatly, at the present time we know little regarding the precise mechanism by which such nucleoside inhibit cellular or viral growth or prevent immune responses, and the basis of their selective toxicity are even well understood. Much more study of purine and purine nucleoside analogs is required. Therefore, it is hoped that deeper knowledge of their mechanism of action and bases of selective toxicity will lead to increased clinical usefulness.

In this dissertation an attempt has been made to utilize electrochemical techniques for the determination of some biologically important compounds. The compounds selected for such determination are guanosine and 8-hydroxyguanosine. Both of these compounds are found in the human being in nucleic acid as purine nucleosides

OVERVIEW OF THE TECHNIQUE USED:

The application of electrochemical technique to study biological phenomenon is one of the challenging areas of the modern science [4-5]. As most of the biologically significant molecules, which are involved in the fundamental biological processes, such as energy transformation and storage as well as synthesis and metabolism of essential amino acids, vitamins and hormones etc. are of redox types i.e. capable of transferring electrons. The study of their redox behaviour provides useful insights about their metabolic fate. It has been now a well established fact that electroanalytical techniques have the potentialities to probe and elucidate the mechanistic aspects of such complex biological processes.

Voltammetry is perhaps the most versatile electroanalytical technique so far developed for the rapid diagnosis of sufficiently unstable electroactive intermediate generated either chemically or electroanalytically.

In view of this an attempt has been made in this dissertation to determine biologically important compounds by differential pulse voltammetry.

Differential Pulse Voltammetry (DPV) :

Differential pulse voltammetry can be used both for qualitative and quantitative analysis. In this technique a three electrode system is used.

1) Saturated Calomel Electrode as reference electrode
2) Platinum Electrode as auxiliary or counter electrode
3) Pyrolytic Graphite Electrode as working electrode.

A potential pulse of constant amplitude is applied on linearly increasing d.c. potential (5 mV/s). Pulse of the same amplitude is applied at regular intervals. Differential pulse voltammetric technique takes advantage of computer timing to sample current signal at two points relative to the time of application of a voltage pulse to the electrode. The current is sampled just before the application of pulse and during the last 20 ms of the applied pulse. The difference between the two current values is plotted as a function of the applied d.c. potential to give peaks, rather than voltammetric waves. The major component of this difference of current is the faradic current which flows due to an oxidation or reduction of the electroactive substance. The capacitive current component due to electrical charging of the electrical double layer is largely removed. Resolution and sensitivity of this technique is better than conventional d.c. polarographic technique due to increase in faradic current and decreasing charging current and thus increases the sensitivity of the technique to about 10^{-8}M.

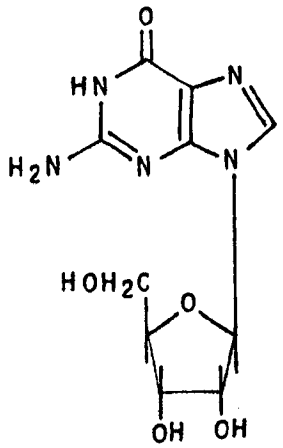
LITERATURE SURVEY

The turn of the last decade has seen an enormous literature on studies on purines and their derivatives. Ogorevec et al. [6] presented a comprehensive review on square wave voltammetry, cyclic voltammetry and linear sweep voltammetry in the determination of biologically important compounds. In the present studies differential pulse voltammetry has been used for the simultaneous determination of guanosine and 8-hydroxyguanosine.

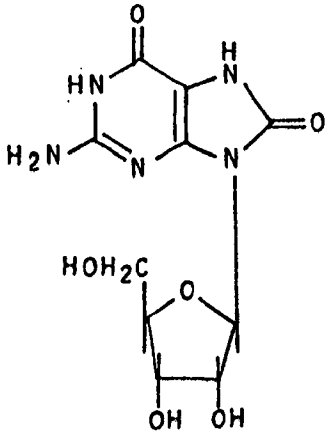
Guanosine and its derivatives play an important role in various biological processes and are useful precursors in the synthesis of a wide variety of antiviral, anticancer and antitumor drugs [7]. Bonnet et al [8] have reviewed the clinical use of guanosine analogous as antitumour and antiviral agents involving a modulation of leukocyte genetic expression. Guanosine derivatives, viz., 8-hydroxy-2-deoxyguanosine has been claimed useful as a biological marker of *in vivo* oxidative DNA damage [9].

The parent bases of guanosine, guanine (2-amino-6-hydropurine), is one of the two important purine bases commonly found in the nucleic acids and hence is intimately involved in protein synthesis and transfer of genetic information. Guanosine and its derivatives have been sucessfully used for the treatment of various diseases in the last decade.

As determination of purines in biosystem provides information on depletion of nucleosides, hence two important purine nucelosides namely guanosine and 8-hydroxyguanosine are selected in the present dissertation.



Guanosine



8-hydroxy guanosine

Germadnik et al. [10] used high performance liquid chromatography with electrochemical detection for the determination of urinary 8-hydroxy-2-deoxyguanosine. Detection was made at a glassy carbon electrode operated at +0.6V vs Ag/AgCl. Calibration graph was found linear in the range 55-440 nM, with the detection limit of 0.9 nM.

Quantitative determination of 8-hydroxyguanine and guanosine was done using isotope dilution mass spectrometry by Hamberg and Zhang [11]. In this technique 8-[4,5,6,8-$^{13}C_4$]- hydroxyguaunine and [4,5,6,$^{13}C_3$]- guanine were used as internal standards in the determination of guanine and 8-hydroxyguanine after trimethylsilylation of pyrimidine intermediates at room temperature by GC-MS on a phenylmethyl-silicone capillary column. The ratio between guanine and its 8-hydroxyderivative could be directly estimated and method was applied to determine guanine in calf thymus DNA.

Long et al. [12] used near infra-red fourier transform surface enhanced Raman scattering of guanine, guanosine and their derivatives in aqueous solutions at concentration of 0.01-0.1 mg/l. Results indicated that at these low concentrations interference of fluorescence via their adsorption on the surface of Ag film was not observed. Separation of cyclic GMP and

cyclic AMP has been attempted by Villegas and Brunton [13] by extracting with TCA and H_2O. cGMP and cAMP were separated on small columns(0.8cm i.d.) containing 2 ml of Dowex-1 formate. Elution of cAMP was effected with 2N-formic acid and cGMP with 1N-HCL with recovery 95-100%. Derivative spectrophotometry was used for the simultaneous determination of flavour enhancers ionosine 5′-monophosphate in food preparations by Duran-Meras and coworkers [14]. The simultaneous determination of ionosine- 5′-monophosphate(I) and guanosine-5′- monophosphate (II) was possible at 5-40 μg/ml in the presence of ≤ 5000 μg/ms of monosodium glutamate. Detection limts for I and II were 0.1 and 0.4 μg/ml respectively and recoveries were 95-111%.

Yang and coworkers [15] made a study of the fluorescence system deoxyguanilic acid- terbium (III) and determination of deoxyguanilic acid. The sample solution was treated with Tb solution and 1ml of 10% hexamine buffer solution. The calibration graph for emission at 545 nm was linear for 1-10 μm 2′-deoxyguanosine 5′-monophosphate. Recoveries from standard mixtures of nucleotides were > 94%. Similarly liquid -chromatography and infra-red studies on ethylenedi-amine platinum (II) guanosine complexes were carried out by Goetze and coworkers [16]. The experimental data was analysed by theoritical methods such as molecular modelling and normal co-ordinate analysis; seven Pt-en-Gua complex, three monofunctional (N_1,N_3 and N_7), two bifunctional (N_7-N_1) and two adducts (N_7-N_3 and N_7-N_7) were identified. Pt complexes of this type are of interest in cancer research and chemotherapy. Selective fluorimetric sensing system was developed for guanidium ion in the presence of primary ammonium ions by Takeshita and Shinkai[17]. Shubietah et al.[18] reported the determination of nanomolar

levels of guanine by differential pulse adsorptive cathodic stripping voltammetry of its copper-complex. Chemiluminescense determination of guanine and its nucleosides and nucleotides, using phenyl glyoxal has been reported by Kai and coworkers [19]. A portion of reaction mixture was analysed by HPLC on a 5μm TSK gel ODS-120T column (15 x 4.6 mm i.d.) The detection limits for guanine and its nucleosides and nucleotides were 4-53 and 180-445 p mol/ml for chemiluminescence and fluorescence detecton, respectively. The calibratian graph (chemiluminecense detection) for guanosine - 5′- monophosphate (GMP), guanosine -5′- triphosphate (GTP) guanine and guanosine was linear upto 100 n mol/ml. The RSD(n=20) for 50 n mol/ml - GMP was 5.7%. Analysis of 8-hydroxy-2′- deoxyguanosine in rat urine and liver DNA by stable isotope dilution-gas chromatography mass spectrometry was reported by Teixeira and coworkers [20]. The calibration graph was linear for 2.3 pmol-0.47nmol in urine and 88 fmol-1.8 pmol in DNA. The detection limit was 9.2 pm in urine and for 8-hydroxy-2′-deoxyguanine per 10^6 deoxynucleosides in 30μg of DNA. Purine nucleotide diphosphate was determined by the use of pyruvate kinase and lactate dehydrogluase by Shimofurya et al. [21].The analysis range for these nucleoside diphosphate was 4 m mol to 300 n mol

Yonekura and coworkers [22] determined guanine and its nucleosides and nucleotides in human erythrocytes by high performance liquid chromatography with post column fluorescence derivatization using phenyl-oxal. The calibration graphs for guanine and its nucleosides and nucleotides were linear from 20-500 p mol and the detection limits were 3.2 - 10 p mol/ 20 μl. Rush and coworkers [23] determined the cellular levels of guanosine - 5′- diphosphate - mannose based on a weak interaction with

concavadin at a low pH.Detection was made at 254 nm. This method was used to follow the incorporation of [2-^{3}H] mannose into GDP-mannose pools of cultured cells.

An attempt was made by Wroblewski and Bilewiez [24] for the determination of purine derivatives i.e. adenine, guanine and DNA (isolated from Escherichia coli) based on mercury accumulation using reverse-pulse stripping voltammetry by dissolving in Na_2HPO_4/ NaH_2PO_4 buffer solution of pH 5.6-8. Micellar electrokinetic capillary chromatography of 8-hydroxy guanosine and other oxidized derivatives of DNA was done by Guarnieri and coworkers [25]. Detection was made at 254 nm. Enzyme immuno assays for the estimation of adenosine 3′-5′ cyclic mono- phosphate and guanosine 3′,5′ cyclic monophosphate in biological fluids was reported by Harton and coworkers [26].The assays were capable of detecting levels as low as 2 fmol of cAMP and cGMP and good correlation were obtained between values generated by EIA and RIA. Lu et al. [27] reported the separation of biopurine bases by high-performance liquid chromatography and their detection was done by adsorptive cathodic stripping voltammetry.

One of the voltammetric technique i.e. stripping voltammetry with adsorption accumulation for trace determination of some thioguanine derivatives has been reported by Ahmed et al.[28]. Analysis was performed at 22 °C, with a hanging - Hg-drop electrode on Ag/AgCl reference electrode and a Pt-auxilliary electrode. Differential pulse stripping voltammetry was carried out at 20 mV/s, with a pulse amplitude of 100 mV. Surfactants like Triton X -100 decreased the sensitivity by inhibiting the adsorption, but this could be partly overcome by standard additon methods. Zilberberg and coworkers [29] observed the effect of cisplatin binding on guanine in

nucleic acid.Binding of cisplatin to the 06 site of guanine breaks the corresponding H-bond between guanine and cytosine which, in turn, can cause the point mutation. Jocob et al. [30] reported the direct determination of cyclic guanosine monophosphate in plasms. Plasma cGMP concentration could be used to moniter patients heart failures, as an alternative to measuring atrial natriuretic peptide (ANP) concentration. This would overcome problems due to ANP degradation.c GMP was measured by RIA (Amersham) with and without prior ethanol extraction of EDTA treatment plasma from healthy donors and patients with heart failure or chronic renal failure.

Anastassopoulou and coworkers [31] reported FT-Raman spectra of metal guanosine - 5′- monophosphate complexes. The metal: ligand ratio of the complex formed was 1:1. The mangnese ions replaced the sodium ions from the phosphate group. Adsorption - voltammetric determination of guanine, guanosine and adenosine with capilliary zone electrophoresis separtoin has been reported by Jin et al. [32] Comparative study of the voltammetric behaviour of guanine at carbon - paste and glassy [vitreous] - carbon electrode and its determinations in purine mixtures by differential pulse voltametry has been reported by Gilmartin and Hart [33]. Calibration graphs were rectilinear from 0.75 to 10 μm and 0.1 to 10 μm guanine with use of a vitreous - carbon and a carbon- paste electrode respectively.

High performance liquid chromatographic determination of guanosine 3′, 5′ - cyclic mono phosphate in human urine with fluorescence detection using 3,4- dimethyloxyphenyl glyoxal has been reported by Ohba et al. [34] Calibration graphs for guanine and its nucleosides and nucleotides were linear for 12.5-250 pmol injected, with detection limits of 4-14 p mol.

EXPERIMENTAL

EXPERIMENTAL

Material Used :

Guanosine was obtained from Sigma Chemical Company, USA and was used as recieved. 8-Hydroxyguanosine was synthesized in the laboratory by the method reported by Holmes and Robins [35] and its purity was checked by m.p. (230 ^{0}C dec), TLC (Thin layer chromatography) etc. Chemicals used for phosphate buffers preparation viz. disodium hydrogen phosphate, sodiumdihydrogen phosphate (AR) were obtained from E. Merck. Agar agar (BDH), Potassium chloride (E. Merck). Mercurous chloride (Fluke) and Mercury (E. Merck) were used. All the solutions were prepared in double distilled water.

Equipment Used :

All experiments were carried out in phosphate buffer of pH-7. The pH of the buffer solution was measured using Century digital pH meter Model CP- 901-P.

Differential pulse voltammetric investigations were carried out using computer controlled Cypress Systems Model CS-1090 electrochemical system. This system combines the power and versatility of a personal computer with a specially designed potentiostat. The system had data aquistion capacity for the differential pulse voltammetry and consisted of four major components.

(1) The personal computer

(2) The data aquistion control interface card

(3) The potentiostat and

(4) The computer software.

Electrochemical cell used had three electrodes-Pyrolytic graphite electrode as working electrode; Saturated calomel electrode as reference electrode and a Platinum electrode was used as counter electrode.

Pyrolytic graphite pieces was obtained as a gift from pfizer, USA and electrode was prepared as follows -

A Pyrex glass tube of suitable length and diameter was cleaned up thoroughly and dried. Epoxy resin (Araldite) was then inserted inside the one end of the glass-tube. The pyrolytic graphite piece was then slided carefully into the tube from the other end and pushed with the help of a wire. It was made sure that the electrode piece got covered with epoxy resin to avoid air pocketing between the tube and the graphite piece and allowed to dry for 24 hrs. at room temperature. A sufficient amount of mercury was then placed into the glass tube and copper wire of suitable length was inserted to make proper electrical contact of the electrode to the outer circuit.

The pyrolytic graphite electrode surface was renewed after each voltammogram by rubbing it on Geosyn polishing Aluminium, Grade III (Geologists Syndicate Pvt. Ltd.) The electrode was then washed with a jet of distilled water and then electrode surface was dried completly by touching it on the tissue paper.This procedure resulted in a significantly new surface area for each run and gave different peak current in repeated runs.For determining the peak current values an average of at least three runs were taken.

PROCEDURE

Stock solution of guanosine (1mM.) was prepared in double distilled water. For studing effect of concentration, different sets of solution of concentration range 0.01 mM to 0.5 mM of guanosine were prepared at pH 7.0 by appropriately diluting the stock solution and voltammograms were recorded.

Effect of pH on Ep and Ip was observed at 0.5 mM concentration by mixing 2.0 ml. of the stock solution of guanosine with 2.0 ml of buffer of desired pH.

Similar procedure was followed for 8- hydroxyguanosine.

RESULTS AND DISCUSSION

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GUANOSINE

The differential pulse voltammograms of 0.5 mM guanosine, at pulse height 50 mV and pulse width 40 ms, in the pH range 2.5-10.3 exhibited one well defined oxidation peak at pyrolytic graphite electrode. The peak obtained was sharp in acidic pH range and became broad at pH >6.0. Some of the typical differential pulse voltammograms of guanosine are presented in Fig.1 and 2. The peak current was found to be independent of pH, whereas the peak potential of peak was dependent on pH and shifted towards less positive potential with increase in pH. The E_p versus pH plot at PGE was a straight line (Fig-3) and the dependence of the peak potential (Ep) on pH can be expressed by the equation:

$$E_p \text{ [pH 2.5 - 10.3] = [1250 - 56.7 pH] mV vs SCE} \qquad (1)$$

As most of the biological reactions in human system occur at pH 7, hence the determination of guanosine and 8-hydroxyguanosine was carried out at physiological pH. The effect of concentration of guanosine on peak current was studied in the concentration range 0.01 to 0.5 mM at pyrolytic graphite electrode. In the concentration range 0.01 to 0.2 mM Ip versus concentration plot was linear. However at higher concentration (>0.2 mM) the peak current (Ip) attained more or less constant value (Fig-4). This behaviour indicated the involvement of adsorption at the electrode surface[36,37].

Thus, it is clear from Ip versus concentration plot that the useful concentration range for determining guanosine by differential pulse voltammetry is 0.01 to 0.2 mM.The standard deviation in this concentration range was ± 11.97% .The peak potential and shape of the peak was found to be independent of pulse height.

8-HYDROXYGUANOSINE

Differential pulse voltammetric studies of 0.5 mM solution of 8-hydroxyguanosine were carried out in the pH range 2.5-10.3 at PGE. A well defined peak was observed in the entire pH range studied. The shape of the peak indicated a small clink after the peak which corresponded probably to adsorption contribution of the reactant. Post adsorption peaks in the case of adsorption of reactants are well documented in literature[38]. The plot between E_p versus pH was a straight line (Fig.5). Some of the typical voltammograms of 8-hydroxyguanosine are shown in (Fig.6 and 7) The dependance of E_p on pH can be expressed by the equation:

$$E_p \text{ [pH 2.5 - 10.3] } = \text{ [740 - 58.3 pH] mV vs SCE} \qquad (2)$$

The effect of concentration on peak current was studied in the concentration range 0.01- 0.5 mM at pyrolytic graphite electrode. The plot of Ip versus concentration was linear in the entire concentration range used (Fig.8).Thus 8-hydroxyguanosine can be determined in the concentration range 0.01 to 0.5. The standard deviation in this concentration range was ± 5.15%. The peak potential and shape of the peak of 8-hydroxyguanosine was also found to be independent of pulse height used.

SIMULTANEOUS DETERMINATION

For the simultaneous determination of guanosine and 8-hydroxyguanosine two sets of solutions were prepared.

In the first set of experiment concentration of 8- hydroxyguanosine was kept constant (0.025 mM) and concentration of guanosine was varied from 0.1 mM to 0.005 mM. The differential pulse voltammograms of these solutions were recorded. As the current at pyrolytic graphite electrode was not very reproducible (standard deviation ± 11.97%), at least five to six voltammograms were recorded and an average value of current was determined.

The I_p value observed from these voltammograms were used for calculating the observed concentration from calibration plot of guanosine (Fig.9). The values of concentration taken and observed from this plot are presented in Table 1. An examinaiton of Table 1 indicates the error obtained in the concentration range 0.005 to 0.1 mM was -0.5 to +10%. The maximum error of 10% was obtained at 0.005 mM of guanosine concentration.The above results were reproducible in the concentration range studied. In all the cases the peak current for 8-hydroxyguanosine was practically constant and showed a variation of ± 2%.

In the second set of experiment the concentration of guanosine was kept constant (0.25 mM) and concentration of 8-hydroxyguanosine was varied. The working solutions were prepared as in previous case with effective concentration of 8-hydroxyguanosine ranging from 0.1 mM to 0.0005 mM. Differential pulse voltammograms were recorded under exactly identical conditions.

In this case also at least five to six voltammograms were recorded and average value of current was determined. The I_p values observed from these

voltammograms were then used for calculating the observed concentration from the calibration plot of 8-hydroxyguanosine (Fig. 10). The values of concentration taken and observed are summarized in Table 2 .It is interesting to observe that the maximum error obtained was only ± 5% which is much lower than that observed in the case of guanosine. Moreover, 8-hydroxyguanosine can be determined upto ten times lower concentration (0.0005 mM) than guanosine (0.005 mM). Thus, the above studies show that guanosine and 8-hydroxyguanosine can be determined simultaneously at pyrolytic graphite electrode by differential pulse voltammetry upto concentration of 0.005 mM and 0.0005 mM respectively.

The method has an advantage that no prior treatment is required and deaeration is not needed because determination is based on oxidation peak. Also the analysis can be achieved using low cost instrument.

Initial E	(mV)	= 0
Final E	(mV)	= 1200
Pulse Height	(mV)	= 50
Step Height	(mV)	= 10
Cycle Period	(ms)	= 200.00
Pulse Width	(ms)	= 40.00
Sample Time	(ms)	= 35.00
Current Range	(fA)	= 500
Noise Filter	(fs)	= 500

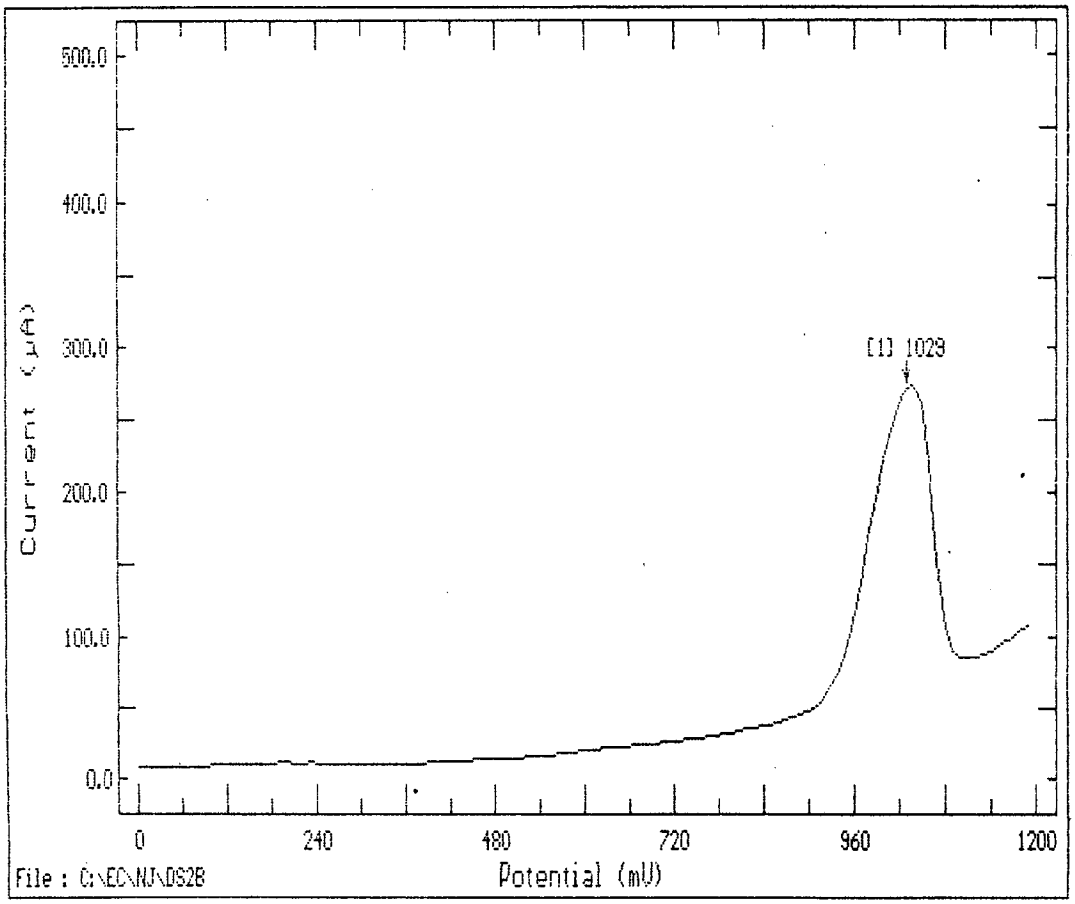

Fig.1 DIFFERENTIAL PULSE VOLTAMMOGRAM OF 0.5mM GUANOSINE AT pH-3.0

Initial E	(mV)	= 0
Final E	(mV)	= 1200
Pulse Height	(mV)	= 50
Step Height	(mV)	= 10
Cycle Period	(ms)	= 200.00
Pulse Width	(ms)	= 40.00
Sample Time	(ms)	= 35.00
Current Range	(fA)	= 1000
Noise Filter	(fs)	= 500

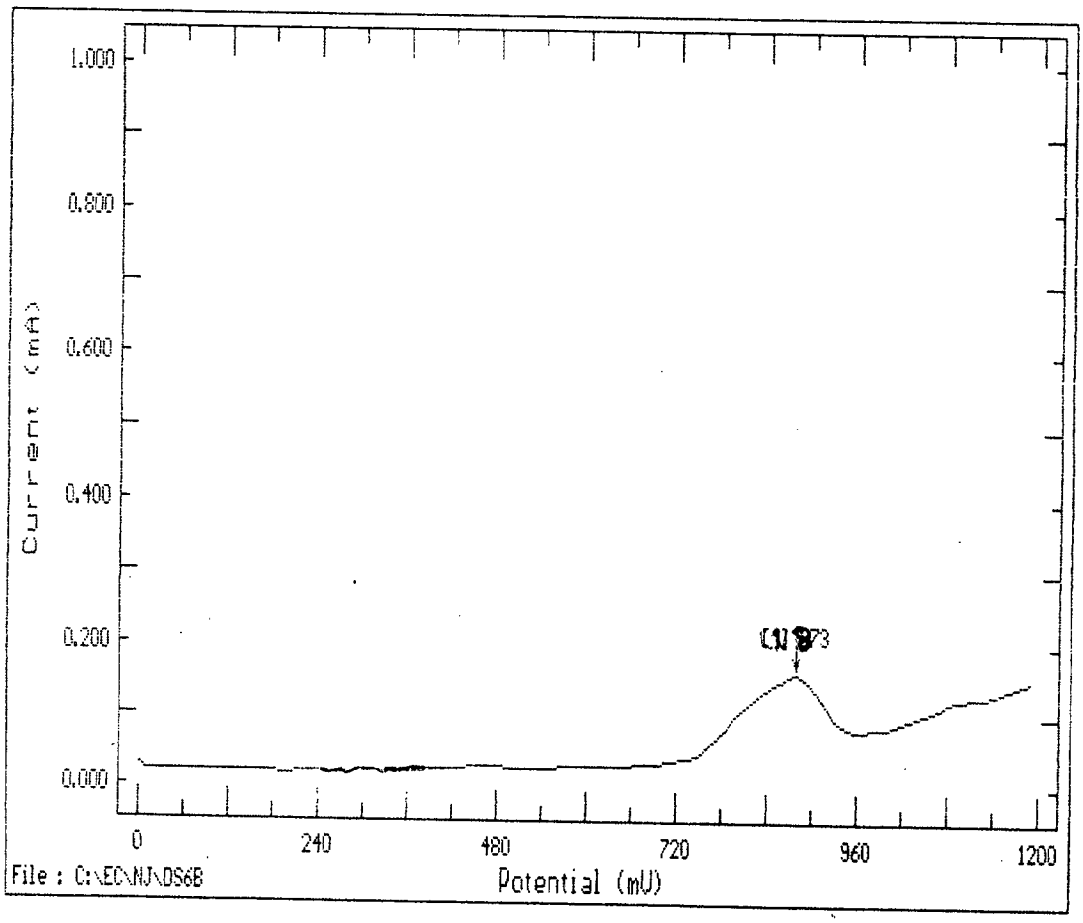

Fig.2 DIFFERENTIAL PULSE VOLTAMMOGRAM OF O.5 mM GUANOSINE AT pH-6.8

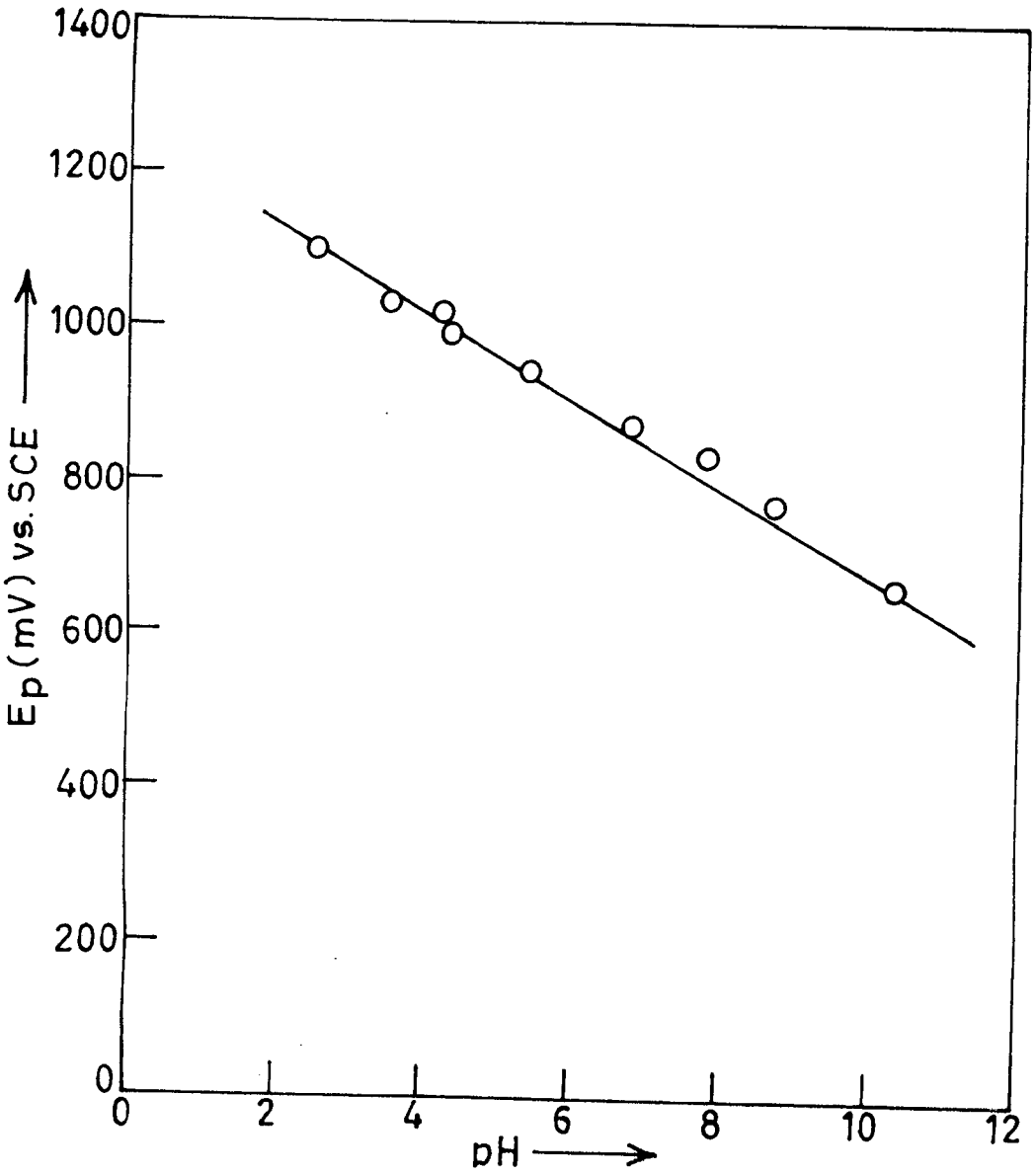

Fig.3 OBSERVED DEPENDENCE OF Ep ON pH FOR THE OXIDATION PEAK OF GUANOSINE.

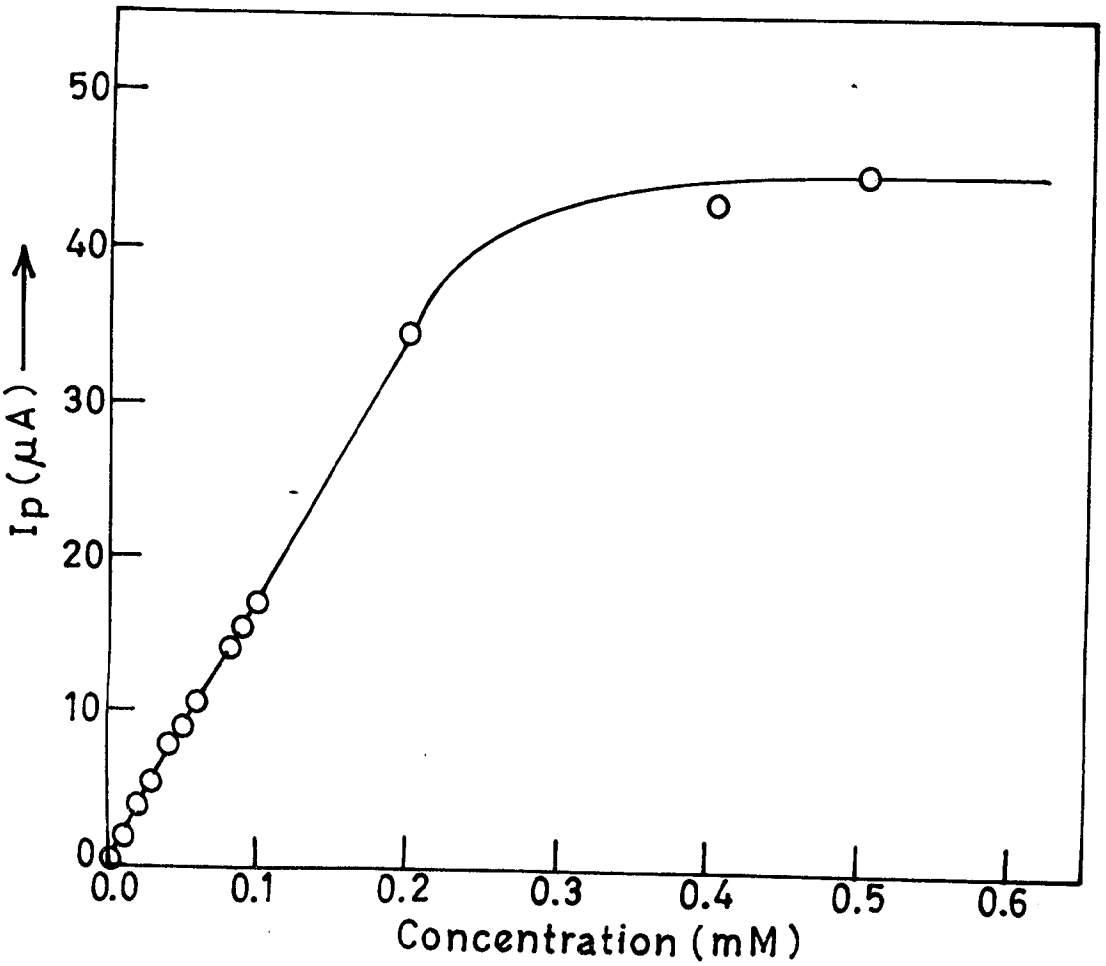

Fig.4 PEAK CURRENT VERSUS CONCENTRATION PLOT FOR GUANOSINE IN THE CONCENTRATION RANGE 0.01 TO 0.5mM.

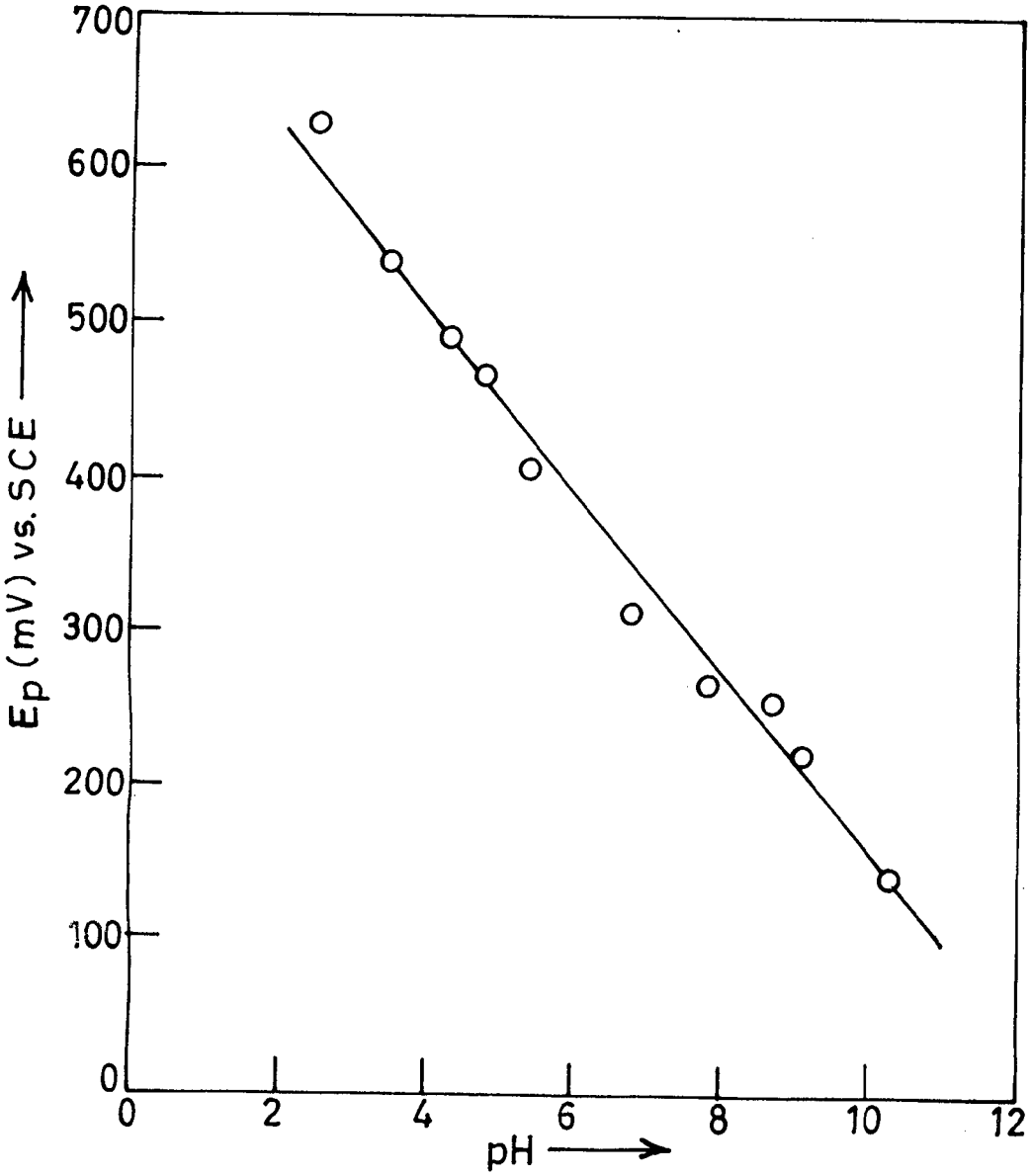

Fig.5 VARIATION OF Ep ON pH FOR THE OXIDATION PEAK OF 8-HYDROXYGUANOSINE.

Initial E	(mV)	= 0
Final E	(mV)	= 1200
Pulse Height	(mV)	= 50
Step Height	(mV)	= 10
Cycle Period	(ms)	= 200.00
Pulse Width	(ms)	= 40.00
Sample Time	(ms)	= 35.00
Current Range	(fA)	= 1000
Noise Filter	(fs)	= 500

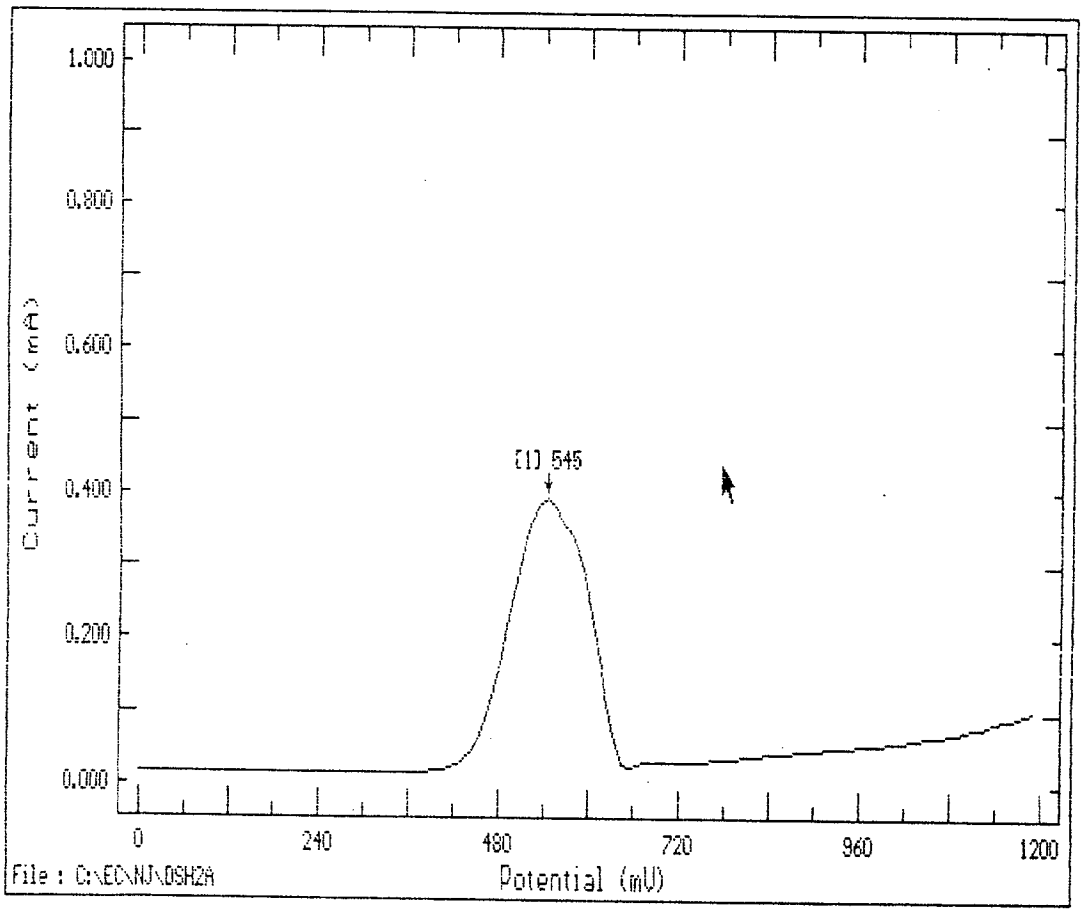

Fig.6 DIFFERENTIAL PULSE VOLTAMMOGRAM OF 0.5 mM 8-HYDROXYGUANOSINE AT pH-3.5.

Initial E	(mV)	= w
Final E	(mV)	= 1000
Pulse Height	(mV)	= 50
Step Height	(mV)	= 10
Cycle Period	(ms)	= 200.00
Pulse Width	(ms)	= 40.00
Sample Time	(ms)	= 35.00
Current Range	(fA)	= 1000
Noise Filter	(fs)	= 500

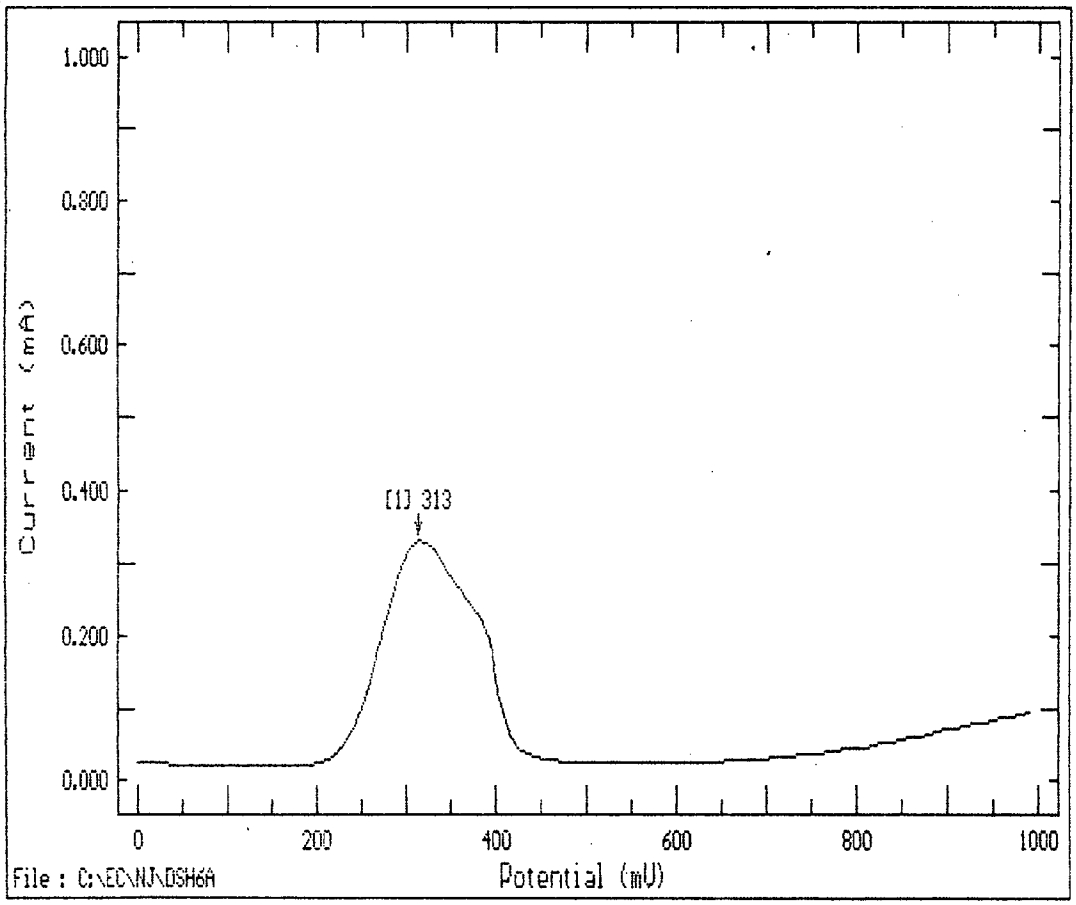

Fig.7 DIFFERENTIAL PULSE VOLTAMMOGRAM OF 0.5 mM 8-HYDROXYGUANOSINE AT pH-6.8

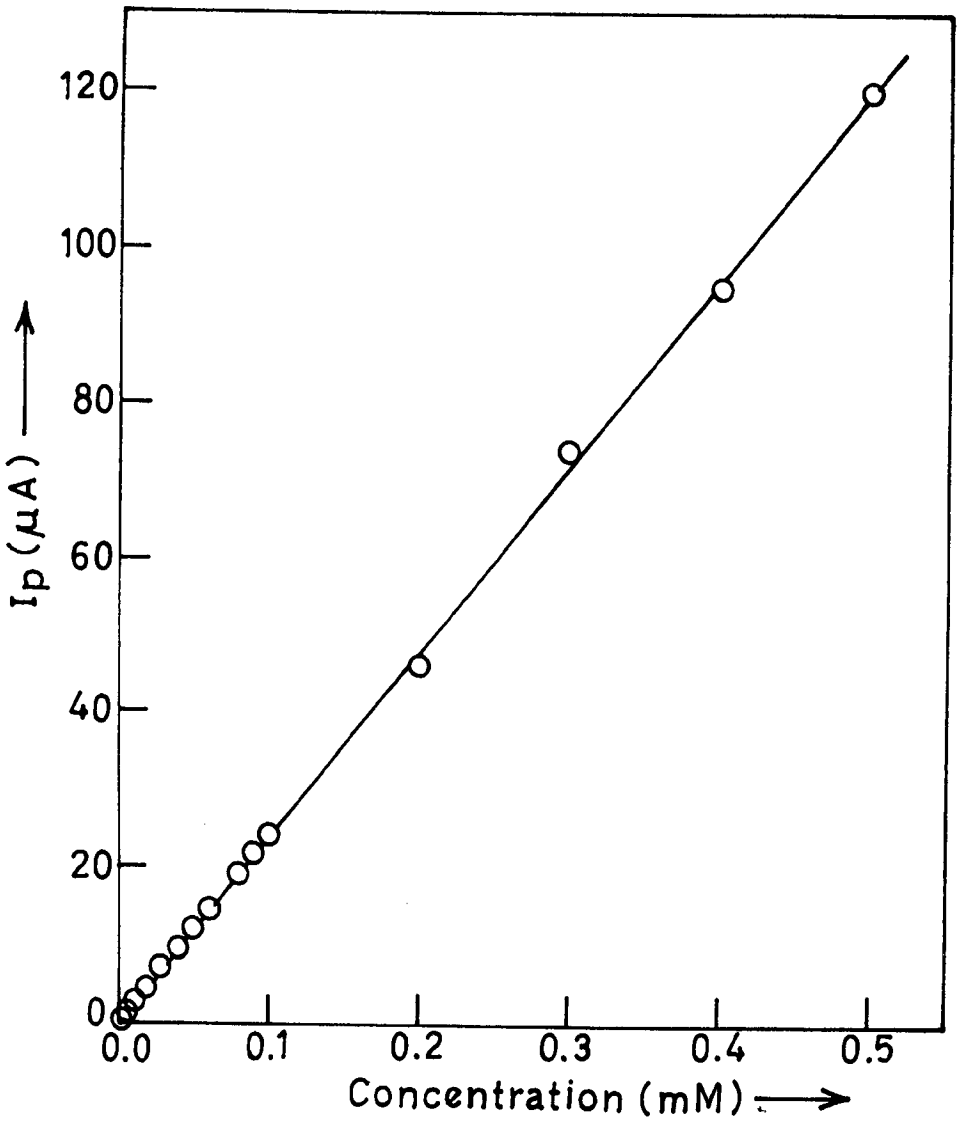

Fig.8 PEAK CURRENT VERSUS CONCENTRATION PLOT OBSERVED FOR 8-HYDROXYGUANOSINE IN THE CONCENTRATION RANGE 0.01 TO 0.5mM.

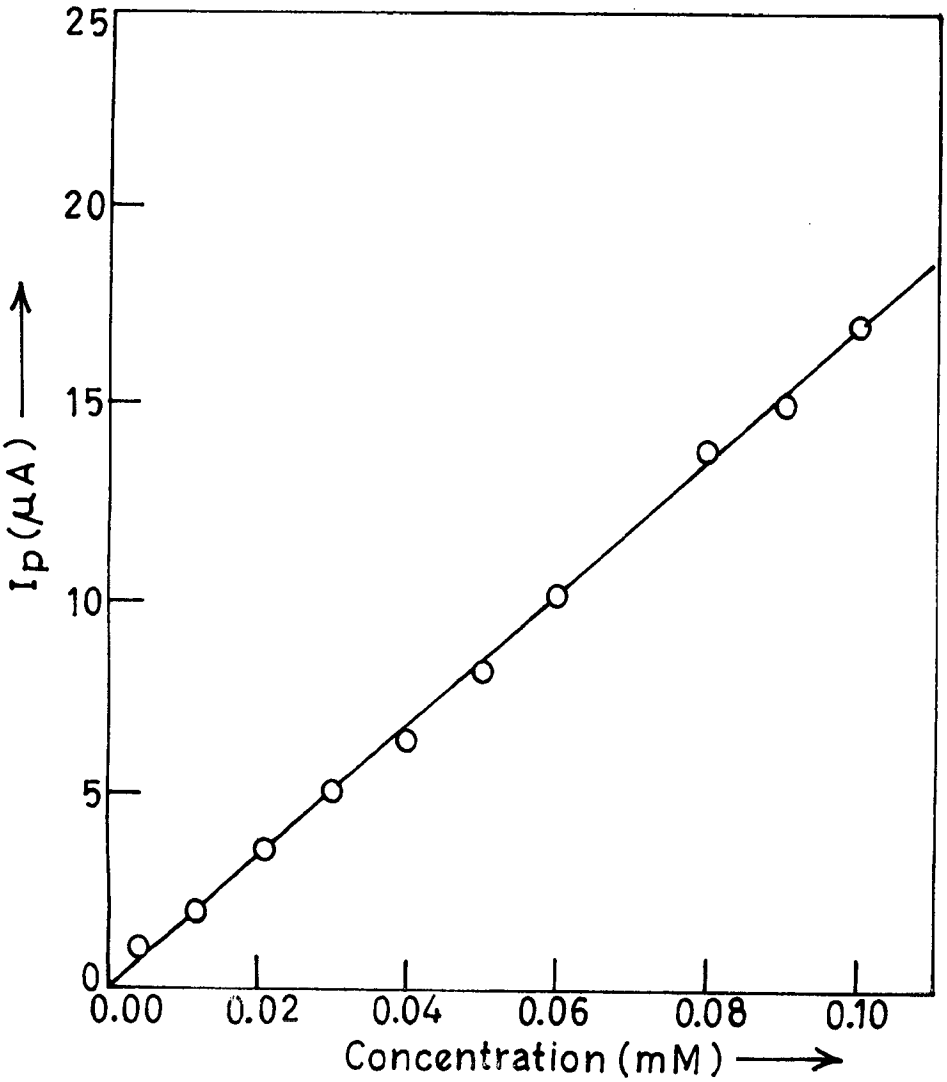

Fig.9 CALIBRATION PLOT USED FOR THE DETERMINATION OF GUANOSINE IN THE CONCENTRATION RANGE 0.005 - 0.1mM.

Table -1 **A Comparison of Actual Concentration and Observed Concentration of Guanosine**

Concentration(taken) mM	Ip, μA	Concentration (observed) mM	Error (%)
0.005	0.9	0.0055	+10·00
0.010	1.7	0.0105	+ 5·00
0.020	3.4	0.0200	0.0
0.030	5.1	0.0305	+1.66
0.040	6.8	0.0405	+1.25
0.050	8.4	0.050	0.0
0.060	10.2	0.060	0.0
0.080	13.6	0.081	+1.25
0.090	15.2	0.090	0.0
0.100	16.9	0.0995	- 0.50

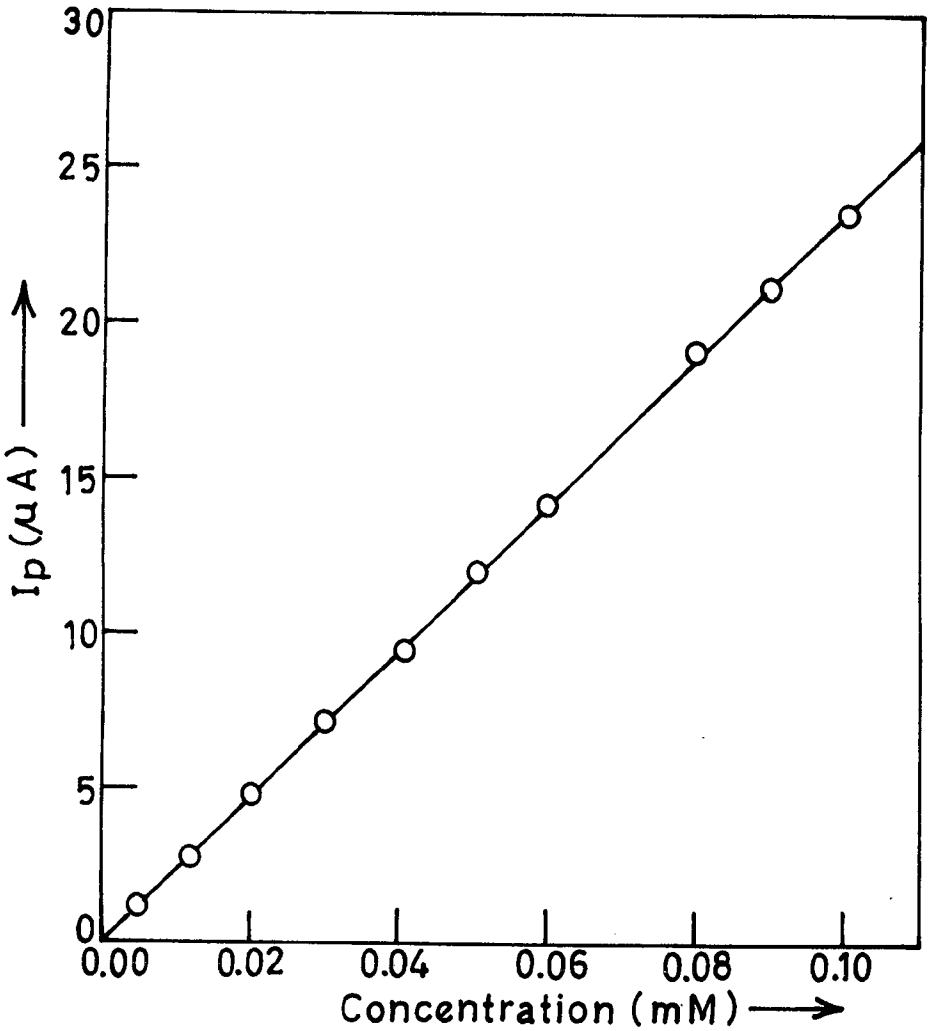

Fig.10 CALIBRATION PLOT USED FOR THE DETERMINATION OF 8-HYDROXYGUANOSINE IN THE CONCENTRATION RANGE 0.0005 - 0.1mM.

Table - 2 **A Comparison of Actual Concentration and Observed Concentration of 8-hydroxyguanosine**

Concentration(taken) mM	Ip,μA	Concentration (observed) mM	Error (%)
0.01	2.5	0.0105	+5.00
0.02	4.9	0.0205	+2.50
0.03	7.2	0.0305	+1.66
0.04	9.6	0.0405	+1.25
0.05	12.0	0.0505	+1.00
0.06	14.3	0.0600	0.0
0.08	19.1	0.0800	0.0
0.09	21.5	0.0900	0.0
0.10	23.8	0.1050	+5.00
0.005	1.25	0.0050	0.0%
0.0025	0.50	0.0025	0.0%
0.0005	0.10	0.0005	0.0%

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