Simultaneous determination of guanosine and 8-hydroxy-guanosine by differential pulse voltammetry

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Differential pulse voltammetry has been used for the simultaneous determination of guanosine and 8-hydroxyguanosine in phosphate buffers. Well-separated differential pulse voltammetric peaks for guanosine and 8-hydroxyguanosine are observed at pyrolytic graphite electrode. The proposed method is simple, rapid and provides a wide determination range of $0.5 \, \mu M$ to $0.1 \, mM$ with good reproducibility. Interference of ascorbic acid and some guanine related compounds has also been studied and it is found that they do not interfere at very low concentration level.

Purine nucleosides are well known for their metabolic and biological effects in human system. In recent years, the detection and determination of purine nucleosides have become increasingly important in the field of biomedical research. An extensive work concerning the synthesis and applications of purine nucleosides as antiviral agents and antineoplastic agents has appeared in literature. Since purine nucleosides and nucleotides are important in normal cellular function, their analysis is highly desirable. In addition, it has been reported that various pathological conditions induce changes in the level of these purine nucleotides, nucleosides and the corresponding purine bases in body fluids. Hence, monitoring the concentration of such compounds in body fluids has been used as biochemical indicator of various diseases.

Guanosine and 8-hydroxyguanosine are two important purine nucleosides found in nucleic acids. Guanosine and its derivatives have been successfully used for the treatment of various diseases in the last decade, and as biological marker of in vivo oxidative DNA damage. In a communication from this laboratory, it has been reported that the oxidation product of guanosine in albino mice causes nephritis with edema and hence it is toxic in nature. Thus, the quantitative determination of these purine nucleosides is important.

Yonekura et al. have determined guanine and its nucleosides and nucleotides in human erythrocytes and other biospecimens by HPLC and a detection limit of 0.11-1.27 pmol has been suggested. Guanosine and its derivatives have also been analysed by enzyme based techniques, spectrophotometrically and by GC-mass. 8-Hydroxy-2'-deoxyguanosine was determined in human urine by HPLC and electrochemically detected at GCE by Germadnik et al. However, most of these methods are tedious as they generally require sample pre-treatment. The aim of the present work is to develop a simple and reliable voltammetric procedure that could be adopted for the determination of guanosine and 8-hydroxyguanosine simultaneously in a variety of samples. To the best of our knowledge, no information is available on the simultaneous determination of these compounds in literature. As differential pulse voltammetry is one of the popular techniques for carrying out trace analysis in samples of various types due to its high sensitivity and multianalysis capability, this technique has been used in the present investigation. The determination has been carried out at physiological pH utilizing voltammetric oxidation peak of each of these nucleosides at PGE.

**Experimental**

Guanosine was obtained from Sigma Chemical Company, USA and was used as received. 8-Hydroxyguanosine was synthesized in the laboratory by the method reported by Holmes and Robins and its purity was checked by m.p. ($230^\circ C$ dec.), TLC etc. Phosphate buffers were used as supporting electrolyte throughout this study. All the solutions were prepared in doubly distilled water.

Differential pulse voltammetric investigations were carried out using a computer controlled (Cypress System Model CS-1090) electrochemical system. The three-electrode assembly used consisted of a pyrolytic graphite as an indicator electrode, a saturated calomel as the reference electrode and a platinum wire as an auxiliary electrode. Since dissolved oxygen did not interfere with the anodic voltammetry, no deaeration was performed. The pyrolytic graphite electrode (PGE) used in the studies, was prepared by the
previously reported method¹⁸ and had a surface area of 9 mm². The PGE surface was renewed after each voltamogram by rubbing it on Geosyn polishing Aluminium, Grade III (Geologists Syndicate Pvt. Ltd., Calcutta). The electrode was then washed with a jet of distilled water. The effect of pH of the phosphate buffer solutions on the anodic response for a 0.5 mM solution was studied in the pH range 2.5-10.3 by employing the following operating conditions: scan rate = 50 mVs⁻¹; pulse height = 50 mV; pulse width = 40 ms.

The stock solution of guanosine and 8-hydroxyguanosine (1 mM) were prepared in doubly-distilled water. The effect of pH of the phosphate buffer solutions on the anodic response for a 0.5 mM solution was studied in the pH range 2.5-10.3 by employing the following operating conditions: scan rate = 50 mVs⁻¹; pulse height = 50 mV; pulse width = 40 ms.

The stock solution of guanosine/8-hydroxyguanosine (2.0 ml) was mixed with 2.0 ml of phosphate buffer of desired pH (µ = 1 M). For studying the effect of concentration, different sets of solution in the concentration range 0.5 µM to 0.5 mM were prepared at pH 7.2 by appropriately diluting the stock solution and voltammograms were recorded.

### Results and discussion

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 the pyrolytic graphite electrode. The peak obtained was sharp in the acidic pH range and became comparatively broader at pH > 6.0. The peak current of the oxidation peak was found to be independent of pH, whereas the peak potential was dependent on pH and shifted to less positive potentials with increase in pH. The peak potential and shape of the peak was found to be independent of pulse height. The \( E_p \) versus pH plot was a straight line and the dependence of the peak potential on the pH could be expressed by the equation:

\[
E_p [pH 2.5 - 10.3] = [1250 - 56.7 \, pH] \, mV \, \text{vs} \, \text{SCE} \quad \text{... (i)}
\]

As most of the biological reactions in human systems occur at pH 7.2, 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 0.5 µM to 0.5 mM at a pyrolytic graphite electrode. In the concentration range 0.5 µM to 0.2 mM, \( i_p \) versus concentration plot was found to be linear with a correlation coefficient of 0.998. However at higher concentrations (> 0.2 mM) the peak current \( (i_p) \) attained practically constant value. This behaviour indicated the adsorption of guanosine at the electrode surface¹⁹ which was further confirmed by the increase in peak current function \( (i_p/N) \) with increase in sweep rate. Thus, it is clear from the \( i_p \) versus concentration plot that the required concentration range for determining guanosine by differential pulse voltammetry is found to be 0.5 µM to 0.2 mM. The standard deviation in this concentration range was ± 4.97%.

Differential pulse voltammetric studies of a 0.5 mM solution of 8-hydroxyguanosine were also carried out in the pH range 2.5-10.3 at PGE. A well-defined peak was observed over the entire pH range studied. The peak potential of the oxidation peak was -450 to 550 mV less positive than the oxidation peak observed for guanosine. Occasionally a small cleft after the peak was noticed, which corresponded to a post adsorption peak due to adsorption of the reactant. Post adsorption peaks in the case of adsorption of reactants are well documented in the literature²¹. The plot between \( E_p \) versus pH was linear and the dependence of \( E_p \) on pH could be expressed by the equation:

\[
E_p [pH 2.5 - 10.3] = [740 - 58.3 \, pH] \, mV \, \text{vs} \, \text{SCE} \quad \text{... (ii)}
\]

The plot of \( i_p \) versus concentration of 8-hydroxyguanosine at pH 7.2 was linear in the concentration range 0.5 µM to 0.5 mM with a correlation coefficient of 0.999. Thus 8-hydroxyguanosine can be determined in the
concentration range 0.5 μM to 0.5 mM. 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.

The differential pulse voltammograms of a mixture of guanosine and 8-hydroxyguanosine showed peaks at characteristic $E_p$ values when guanosine and 8-hydroxyguanosine were subjected to differential pulse individually. At pH 7.2, $E_p$ for guanosine was 873 mV and that for 8-hydroxyguanosine was 313 mV. A typical differential pulse voltammogram of a solution of guanosine and 8-hydroxyguanosine is presented in Fig. 1. As the peak potentials of the two compounds were about 450 to 550 mV apart from each other, it was considered desirable to detect these compounds in their mixture. For this purpose two sets of solutions were prepared.

In the first set of experiments concentration of 8-hydroxyguanosine was kept constant (0.25 mM) and the concentration of guanosine was varied from 0.5 μM to 0.1 mM. The differential pulse voltammograms of these solutions were recorded. A well-defined peak of 8-hydroxyguanosine was observed in all such mixtures. However, variation of guanosine concentration indicated that the peak of guanosine is ill-defined and broad at lower concentrations (20 μM) and was not observed at 0.5 μM. However, the peak became well-defined at higher concentrations of guanosine.

The $i_p$ values observed from these voltammograms were used for calculating the observed concentration from a calibration plot of guanosine. Some typical analytical results observed are presented in Table 1. An examination of Table 1 indicates that the error obtained in the concentration range 5 μM to 0.1 mM was −0.5 to +10%. The maximum error of 10% was obtained at a 5 μM concentration of guanosine. 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 experiments the concentration of guanosine was kept constant (0.25 mM) and concentration of 8-hydroxyguanosine was varied. The working solutions were prepared as in the previous case with an effective concentration of 8-hydroxyguanosine ranging from 0.3 μM to 0.1 mM and the voltammograms were recorded under exactly identical conditions.

In this case also at least five to six voltammograms were recorded and an average value of current was determined. The peak of 8-hydroxyguanosine was well-defined in all the voltammograms whereas the peak for guanosine oxidation was clearly observed only in the concentration range 2.5 μM to 0.1 mM of 8-hydroxyguanosine. At 8-hydroxyguanosine concentrations <2.5 μM, the peak of guanosine was broad in shape and ill-defined. The $i_p$ values observed from these voltammograms were used for calculating the observed concentration from the calibration plot of 8-hydroxyguanosine. The values of concentration taken and observed are summarized in Table 1. It is interesting to observe that the maximum error

<table>
<thead>
<tr>
<th>Concentration</th>
<th>$i_p^*$ (μA)</th>
<th>Concentration</th>
<th>Error (%)</th>
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<tr>
<td>(taken) mM</td>
<td>G&quot;</td>
<td>HG&quot;</td>
<td>G&quot;</td>
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<tr>
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<td>—</td>
<td>0.1</td>
<td>—</td>
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<tr>
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<td>—</td>
<td>0.5</td>
<td>—</td>
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<tr>
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<td>0.0995</td>
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</table>

*Data corresponding to set-2 when G concentration was constant (0.25 mM).

Table 1—A comparison of concentration taken and concentration observed of guanosine (G) and 8-hydroxyguanosine (HG)
that observed in the case of guanosine. Moreover, 8-hydroxyguanosine can be determined simultaneously at a pyrolytic graphite electrode by differential pulse voltammetry up to concentrations of 0.5 mM and 0.5 μM respectively.

As the interest in the present work was in exploring the possibility of applying the DPV in biotransformations, the interference of ascorbic acid and several guanine derivatives, viz., guanine, 8-hydroxyguanine, deoxyguanosine, and guanosine monophosphate was studied. Differential pulse voltammograms of all these compounds were recorded separately at different concentrations. It was found that all the five compounds exhibited well-defined oxidation peaks with \( E_p \) values close to either guanosine or 8-hydroxyguanosine and thus cause interference in the determination. To determine the interference of these compounds, the DPV of guanosine and 8-hydroxyguanosine were recorded in their presence. Table 2 presents the tolerance limit of ascorbic acid, guanine, 8-hydroxyguanine, deoxyguanosine and guanosine monophosphate. At concentrations greater than the tolerance limit, a serious interference in determination due to these compounds was noticed.

Determination of guanosine in presence of guanine and adenine has been suggested by Yao et al.\(^{25}\) using linear sweep voltammetry. However, the \( pH \) and non-linear relationship between \( i_p \) vs concentration limited the range for the determination of guanosine as 0.05-0.5 mM only. The possibility of guanosine determination in presence of uric acid was also suggested by Gilmartin and Hart\(^{23}\) at carbon paste electrode; however its actual determination and the range of determination was not reported. Thus, the present investigation demonstrates the possibility of using differential pulse voltammetry for the simultaneous determination of guanosine and 8-hydroxyguanosine at PGE at about 1000 times lower concentrations than reported earlier. Purines are usually excreted in human system in micromolar amounts and a variety of disorders have been found associated with purine metabolism. The present analysis based on DPV described a method which can be used in biomedical analysis. The method developed is simple and showed excellent sensitivity and selectivity. It also provides a wide determination range of 0.5 μM to 0.1 mM. Furthermore advantages of present method are that it is rapid and does not require deaeration or time consuming predetermination step. Although the oxidation potentials of guanine related compounds studied are very close to either guanosine or 8-hydroxyguanosine, it is reasonable to expect that low concentration of these compounds will cause no interference in the determination of guanosine and 8-hydroxyguanosine at physiological \( pH \).

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**References**