Linear-sweep Voltammetry and the Simultaneous Determination of Purine Bases and Their Nucleosides in the Glassy Carbon Electrode

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(Received March 12, 1977)

Adenine, adenosine, guanine, and guanosine were voltammetrically oxidized in a glassy carbon electrode in aqueous solutions, but at different potentials. In general, the nucleosides were oxidized at more positive potentials than their bases. The bases and their nucleosides were strongly adsorbed on the surface of the glassy carbon electrode at the pH values around neutrality, so that the concentration *vs.* anodic peak current curves were not linear. The adsorption on the electrode was very dependent on the pH, and at pH values below 4 good linear relationships were observed between the anodic peak current and the concentration. The differences between the peak potentials of each of the purine bases and their nucleosides were most pronounced at pH 2—4. Consequently, it was possible to determine simultaneously both in mixtures of the purine bases and their nucleosides by using Britton-Robinson buffer as a supporting electrolyte in the pH range of 2—4. The proposed method is simple and rapid, since no prior treatment or separation procedure is required.

The electrochemical reduction and oxidation of biologically-important compounds have been investigated with much interest. The electrochemical reduction of purine bases, which are important components of nucleic acids, has been investigated in both aqueous[1-3] and nonaqueous solutions.[4] It is known that adenine is reduced and guanine is not at a dropping mercury electrode(DME). Adenosine appears to be reduced by the same mechanism, but, as the half-wave potentials are very close, the polarographic analysis of mixtures of adenine and adenosine is not possible. Also, the ultraviolet absorption spectra of these bases are very similar, so it is very difficult to work out a simple method for the analysis of such mixtures. A paper chromatographic method[5] for the quantitative determination of purine bases and their nucleosides involves extracting the paper chromatogram after separation and determining the amount of each species by UV spectrophotometry. Apart from being time-consuming, this method can be applied only to samples containing relatively high concentrations of the bases and their nucleosides.

A few studies[6-8] on the electrochemical oxidation of the purine bases were done at the pyrolytic graphite electrode(PGE) in aqueous solutions. Dryhurst[9] determined adenine in the presence of fairly large amounts of adenosine by taking advantage of the competitive adsorption of the two compounds on the PGE. However, this method cannot be applied to the simultaneous determination of adenine and adenosine in mixtures of the two compounds, because large amounts of adenosine must be added to test solutions.

In the present work, the oxidation behavior of purine bases and their nucleosides was investigated by linear-sweep voltammetry using a stationary, planar glassy carbon electrode (GCE). Also, in order to develop a satisfactory analytical method it was found necessary to investigate in some detail the adsorption of the purine bases on the GCE. This paper will describe a method whereby adenine, adenosine, guanine, and guanosine in mixtures of these bases can be determined simultaneously by utilizing the voltammetric oxidation peak of each of these bases at the GCE.

Experimental

Chemicals. The adenine, adenosine, guanine, and guanosine were all obtained from the Wako Pure Chemical Co. and were recrystallized two times from distilled water before drying *in vacuo* at 60 °C. The Britton-Robinson buffer used throughout this work was prepared from reagent-grade chemicals.

Apparatus. The polarograms and anodic voltammograms were recorded using a Yanagimoto P-8 type Polarograph at a scan rate of 3.3 mV/s. The three-electrode voltammetric cell maintained at (25 ± 0.1)°C was used for all the experiments. An aqueous saturated calomel electrode(SCE) was used as the reference electrode, and a platinum wire served as the counter electrode.

The DME used had the following characteristics: mercury flow rate, $m=2.28$ mg/s and drop time, $t=3.35$ s at an open circuit with a mercury head of 70 cm in 1 M $HClO_4$.

The working electrode for anodic voltammetry was constructed from a 3-mm glassy carbon rod (Grade GC-20, Tokai Electrode Co.). The rod was cut into a length of 10 mm and was sealed in one end of a glass tube 3 mm in diameter with epoxy cement. The end of the glass tube-carbon rod assembly was polished with 400-grade emery paper until it was quite smooth. Then, by polishing with 1500-grade emery paper and a paste of sedimented calcium carbonate, the surface of the electrode was brought to a mirror finish.

Voltammetric Procedure. In order to obtain reproducible results, a standard pretreatment procedure was applied before recording each voltammogram. The GCE was polished for 60 s with 1500-grade emery paper and then for 30 s with a paste of sedimented calcium carbonate. The surface of the electrode was then washed for about 10 s with a fine spray of water. Any water remaining on the electrode surface and the shaft of the electrode was removed by touching the surface very gently with a piece of soft absorbent paper. Test solutions were not deaerated. The voltage scan was commenced after quiescency for 20 s at the starting potential (always 0.0 V *vs.* SCE). At least three replicate voltammograms were recorded for each test solution. A voltammogram of the background solution was recorded in the same way, while the peak current was obtained by arithmetically subtracting the observed background current at the peak potential from that of the test solution.

Results and Discussion

Effect of pH. The half-wave potentials for the polarographic reduction of adenine and adenosine at the DME shifted linearly towards negative potentials with the increase in the pH. The wave-heights for both compounds were relatively constant at pH values below 5, but began to decrease sharply with a further increase in the pH. Adenine and adenosine also had practically identical $E_{1/2}$ values at all the pH values where the polarographic waves were observed, *e.g.*, at the pH 4.0 $E_{1/2}=-1.31$ V for adenine and -1.32 V for adenosine, so that the polarographic analysis of mixtures of the two compounds was not possible. Also, since the polarographic waves of both guanine and guanosine occurred close to the background-discharge potential, it was difficult to measure the limiting current accurately.

Each of the purine bases and their nucleosides gave a well-defined oxidation peak at a stationary GCE. The effect of the pH on the voltammetric peaks was investigated by recording the voltammograms of 0.05 mM purine bases and their nucleosides in Britton-Robinson buffer solutions. Adenine and adenosine both gave the single voltammetric oxidation peaks over the pH range of 2—12. The half-peak potentials ($E_{p/2}$) of the voltammetric oxidation peaks of adenine and adenosine shifted linearly towards negative potentials with the increase in the pH; $E_{p/2}$(V *vs.* SCE) $=1.45-0.064$ pH for adenine and $E_{p/2}$(V *vs.* SCE) $=1.49-0.032$ pH for adenosine (Fig. 1). Both guanine and guanosine also gave single voltammetric oxidation peaks at the GCE. The half-peak potential shifted linearly towards negative potentials with the increase in the pH according to the following equations: $E_{p/2}$(V *vs.* SCE) $=1.13-0.059$ pH for guanine and $E_{p/2}$(V *vs.* SCE) $=1.25-0.046$ pH for guanosine. The analytical significance of these experimental results lies in the facts that the voltammetric oxidation peak-potential for each of the purine bases and their nucleosides is quite different in the pH range of 2—4, and that the oxidation potential for each of the purine bases is more negative than that for each of their nucleosides at all the pH values studied.

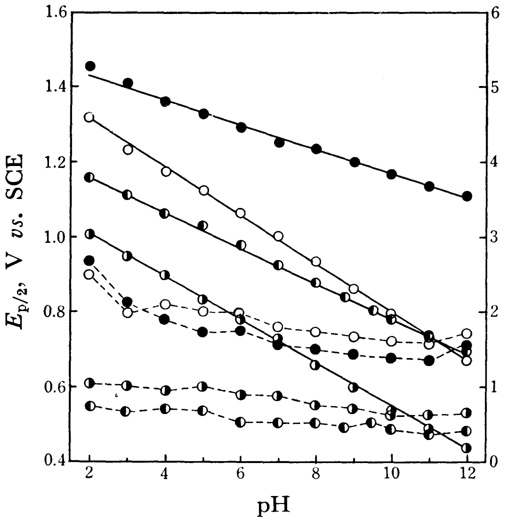

Fig. 1. The effect of pH on the peak current and the half-peak potential of the voltammetric oxidation peaks of 0.05 mM adenine(○), adenosine(●), guanine (◑), and guanosine(◐) in the Britton-Robinson's buffer solutions.
GCE geometric area; 7.1 mm^2, scan rate; 3.3 mV/s.
i_p, (----); $E_{p/2}$, (——).

Table 1. The effect of the pH on the peak current of the voltammetric oxidation peak of 1.0 mM bases
GCE geometric area, 7.1 mm^2; scan rate, 3.3 mV/s.

	Britton-Robinson buffer					0.5 M H_3PO_4
	pH 10	pH 7	pH 4	pH 3	pH 2	
Adenine	4.9[a]	7.9	14.5	14.9	14.9	15.6
Adenosine	6.9	10.5	14.4	14.0	14.2	14.8
Guanine	4.4	7.1	10.0	9.8	10.2	10.5
Guanosine	3.8	6.2	8.5	8.8	9.2	9.1

a) Peak current (μA).

The peak current of the voltammetric oxidation peaks of purine bases and their nucleosides increased gradually with the decrease in the pH, as is shown in Fig. 1. This experiment was undertaken at a very low concentration (0.05 mM) of the purine bases. When the depolarizer concentration was fairly high (1.0 mM), the pH values were found to have a pronounced effect on the peak current of the purine bases and their nucleosides. This result is shown in Table 1. The peak current increased sharply with the decrease in the pH and remained almost constant at pH values below 4. The same result was obtained in other buffers (acetate buffers and chloride buffers) and acid solutions (1 M HCl, H_2SO_4, $HClO_4$, and H_3PO_4) as supporting electrolytes; in electrolytes containing chloride ions the oxidation peaks of both adenine and adenosine were masked by the oxidation of the chloride ion in the background electrolytes. Also, in the acid solutions the oxidation peak of adenosine occurred close to the background discharge potential, so that the accurate measurement of the peak current was impossible.

Effect of the Scan Rate. In order to study the adsorption of the purine bases and their nucleosides on the stationary GCE, the effect of the voltage scan rate on the peak current of these compounds was examined.

The electrochemical oxidation of the purine bases and their nucleosides was irreversible, as was shown by cyclic voltammetric experiments in which no cathodic peak corresponding to the reduction of the oxidation product was observed at any scan rate.

For a linear diffusion-controlled irreversible peak, the peak current function, $i_p/ACV^{1/2}$, shoud be a constant,[10] and a plot of this function *vs.* $V^{1/2}$ should be a straight line parallel to the $V^{1/2}$ axis, where A is the area of the electrode surface; C, the bulk concentration of depolarizer; and V, the scan rate. At pH values below 4, the peak current functions of both adenine and adenosine were almost independent of the scan rate. At pH values above 4, however, the peak current functions increased markedly with the increase in the scan rate. This behavior is typical of an electrode process where the

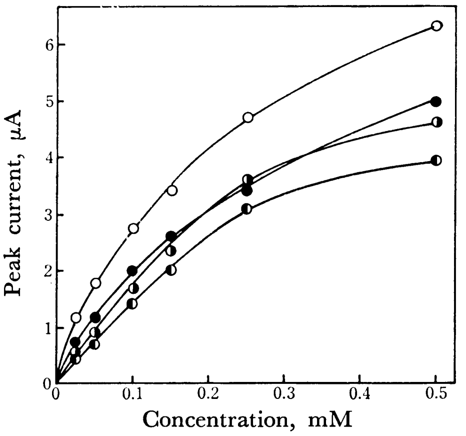

Fig. 2. Peak current-concentration curves for the voltammetric oxidation of the purine bases at the stationary GCE in the Britton-Robinson's buffer (pH 7.0).
○, Adenine; ●, adenosine; ◑, guanine; ◐, guanosine. GCE geometric area; 7.1 mm^2, scan rate; 3.3 mV/s.

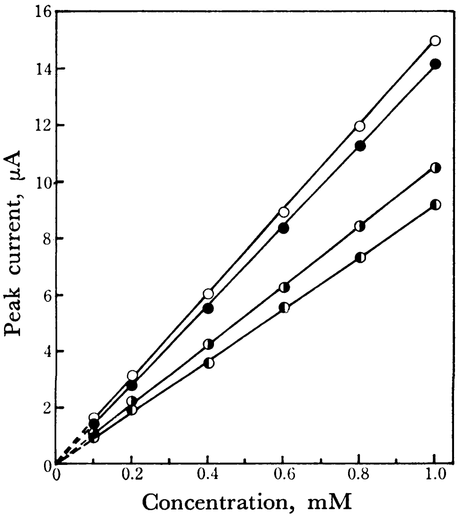

Fig. 3. The relationships between peak current and concentration for the purine bases in the Britton-Robinson's buffer at pH 3.0.
○, Adenine; ●, adenosine; ◑, guanine; ◐, guanosine. GCE geometric area; 7.1 mm^2, scan rate; 3.3 mV/s.

reactant is adsorbed on the electrode.[11] In the cases of both guanine and guanosine, also, similar effects of the pH and the scan rate on the peak current function were observed.

Effect of the Concentration. In the Britton-Robinson buffer with pH values above 4, each of the purine bases studied showed a non-linear relationship between the peak current and the concentration, as is shown in Fig. 2. Such curves clearly indicate that the adsorption of the depolarizer takes place on the surface of the GCE. Also, the peak potential of the voltammetric oxidation peak for each of the purine bases and their nucleosides was dependent on the concentration and slightly shifted to more positive values with an increase in the concentration. Over the concentration range of 0.05—0.5 mM this shift was about 30—50 mV for all the purine bases.

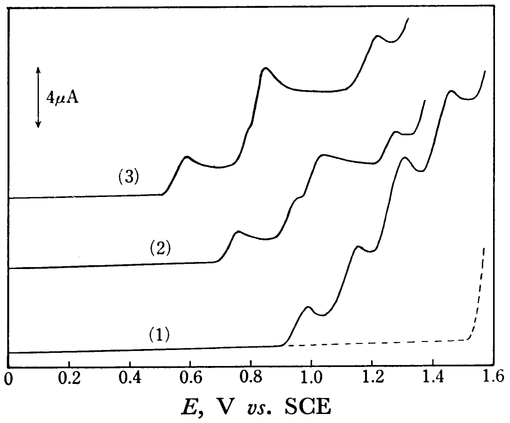

Fig. 4. Anodic voltammograms for the mixtures of adenine, adenosine, guanine, and guanosine.
Concn of each of adenine, adenosine, and guanosine : 0.4 mM, Concn of guanine : 0.2 mM. pH(Britton-Robinson's buffer); (1): 3.0, (2): 7.0, (3): 10.0.

TABLE 2. VOLTAMMETRIC RESULTS FOR THE DETERMINATION OF EACH COMPONENT IN MIXTURES OF PURINE BASES AND THEIR NUCLEOSIDES

Taken (mM)				Found (mM)			
A	B	C	D	A	B	C	D
0.20	0.20	0.20	0.20	0.18	0.18	0.20	0.22
0.20	0.40	0.20	0.40	0.19	0.38	0.22	0.44
0.20	0.80	0.20	0.80	0.21	0.83	0.20	0.86
0.40	0.20	0.40	0.20	0.37	0.19	0.40	0.21
0.40	0.40	0.40	0.40	0.42	0.42	0.36	0.41
0.40	0.80	0.40	0.80	0.41	0.85	0.38	0.77
0.80	0.20	0.80	0.20	0.77	0.19	0.75	0.18

A, Guanine; B, guanosine; C, adenine; D, adenosine. Supporting electrolyte; Britton-Robinson's buffer pH 3.0. GCE geometric area, 7.1 mm^2; scan rate, 3.1 mV/s.

In equimolar solutions (pH 7.0) of adenine and guanine, guanine showed a non-linear relationship between the peak current and the concentration similar to that shown in Fig. 2, but the peak current of adenine decreased in concentrations more than 0.25 mM in spite of the increase in the adenine concentration. However, in the Britton-Robinson buffer at pH 3 these purine bases showed a linear relationship between the peak current and the concentration, as is shown in Fig. 3.

These effects of the concentration and the pH and the scan rate on the peak current imply that the adsorption of the depolarizer on the GCE is very dependent on the pH, but in the electrolytes at pH values below 4 the voltammetric oxidation is a diffusion-controlled process.

The Simultaneous Determination of the Purine Bases and Their Nucleosides. The anodic voltammograms for the mixtures of adenine, adenosine, guanine, and guanosine were recorded in Britton-Robinson buffer solutions of various pH values (Fig. 4). At pH values below 4 four successive well-defined oxidation peaks were observed, but a further increase in the pH made the voltammetric peaks sensitive to the adsorption of depolarizer on the GCE. Also, the differences between the peak potentials of each of the purine bases and their nucleosides were most pronounced at pH 2—4. Accordingly, for the quantitative determination of the purine bases and their nucleosides a Britton-Robinson buffer in the pH range of 2—4 is suitable.

In order to test the quantitative method developed here, the voltammograms for some mixtures of four purine bases were recorded in the Britton-Robinson buffer of pH 3.0 at a stationary GCE. The concentration of each of the purine bases was determined by the calibration-curves method from the respective heights of the resulting voltammetric oxidation peaks. Some typical analytical results are shown in Table 2. Clearly this method is readily capable of determining simultaneously each component in mixtures of the purine bases and their nucleosides.

The principal advantage of the proposed method is the rapidity of analysis. The speed of analysis is aided by the fact that no prior treatment or separation procedure is needed. Furthermore, as dissolved oxygen does not interfere with the anodic voltammetry, no deaeration is required. One disadvantage is that the electrode must be pretreated before every voltammogram in order to ensure reproducibility. In addition, the method is of only moderate sensitivity, and traces (sub ppm) of the purine bases cannot be detected. It would be possible to improve the sensitivity by utilizing a pulse voltammetric method.

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