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Linear-sweep voltammetry and the simultaneous determination of xanthine and xanthosine at the glassy carbon electrode

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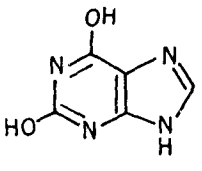
Abstract. Linear sweep voltammetry of xanthine and xanthosine has been studied at a sweep rate of $10\,mVs^{-1}$ in phosphate buffers with different pH values. Based on the linear relation of peak current versus concentration, the simultaneous determination of both the compounds was carried out.

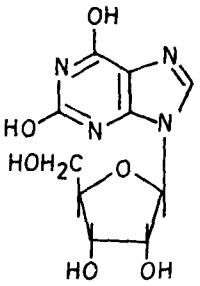
Keywords. Voltammetry; xanthine; xanthosine; purine.

1. Introduction

In recent years electrochemical studies of purines have attracted considerable attention (Dryhurst 1979; March and Dryhurst 1979; Goyal *et al* 1982) owing to their importance as nucleic acid constituents. Nevertheless, in most of the cases reported in the literature attempts were made only to elucidate the mechanism of electrochemical oxidation and reduction of purines; very little information is available on their determination (Yao *et al* 1978). Xanthine and xanthosine are known to be constituents of miscellaneous biological sources (Brown *et al* 1955; Robins 1967; Burian and Sehur 1897). Simultaneous determination of purine bases and their nucleosides in human urine and blood serum is often needed (Dryhurst 1972; Molina 1977; Lewis and Johnson 1978). Literature survey revealed that very little attention has been paid to the determination of xanthine and xanthosine in the presence of each other. This work is concerned with the elucidation of the linear sweep voltammetric behaviour of xanthine (I) and its nucleoside, viz. xanthosine (II), and their simultaneous determination at the glassy carbon electrode.



(I)



(II)

* For correspondence

2. Experimental

Xanthine and xanthosine were obtained from Sigma Chemical Co., USA and were used as received. Phosphate buffers (Christian and Purdy 1962) of different pH values used throughout this work were prepared from reagent grade chemicals. The equipment used for linear sweep voltammetry has been described elsewhere (Goyal *et al* 1984). The glassy carbon working electrode (area 3·2 mm^2) was obtained from PAR, USA and a three-electrode voltammetric cell maintained at $25 \pm 0{\cdot}2°C$ was used for all the experiments. All potentials are referred to the saturated calomel electrode.

2.1 *Voltammetric procedure*

To obtain reproducible results standard pretreatment of glassy carbon electrode was carried out as suggested (Chan and Fogg 1979), before recording the linear sweep voltammograms. The electrode surface was washed with absolute ethanol and then with chloroform. It was then cleaned with a paper tissue soaked in chloroform and dried with dry tissue paper. At least three replicate runs were recorded for each solution. A voltammogram of the background was also recorded in the same way.

3. Results and discussion

Linear sweep voltammetry of xanthine at a sweep rate of 10 mVs^{-1} exhibited a single well-defined anodic peak at the glassy carbon electrode. The peak potential of the anodic peak was dependent on pH (figure 1) and shifted towards less positive potentials with increase in pH. In the phosphate buffers, the peak potential was dependent on pH according to the relation

$$E_p(\text{pH } 2{\cdot}0\text{–}10{\cdot}0) = [1{\cdot}05 - 0{\cdot}055\,\text{pH}]\ \text{volt}.$$

The peak current values were more or less constant in the entire pH range. The peak current values increased linearly with increasing concentration of xanthine up to a concentration of about 0·5 mM. The effect of higher concentratin (> 0·5 mM) could not be elucidated due to the limited solubility of xanthine in aqueous buffers. Thus it was concluded that xanthine can be safely estimated by linear sweep voltammetry at concentrations < 0·5 mM. As the peak current function values ($ip/ACV^{1/2}$) were independent of sweep rate, it was confirmed that xanthine does not get adsorbed (Wopschall and Shain 1967) at the surface of the glassy carbon electrode at concentrations below 0·5 mM.

Xanthosine produces a single voltammetric oxidation peak at the glassy carbon electrode. Below pH 6·7, the oxidation peak was well-defined whereas at pH > 6·7, the peak is not well-defined. The peak potential of this peak was dependent on pH (figure 1), according to the relation

$$E_p(\text{pH } 2{\cdot}0\text{–}10{\cdot}0) = [1{\cdot}190 - 0{\cdot}60\,\text{pH}]\ \text{volt}.$$

In the entire pH range of phosphate buffers studied, the peak potential of xanthosine was about 100 to 150 mV more positive as compared to that of xanthine (figure 1)

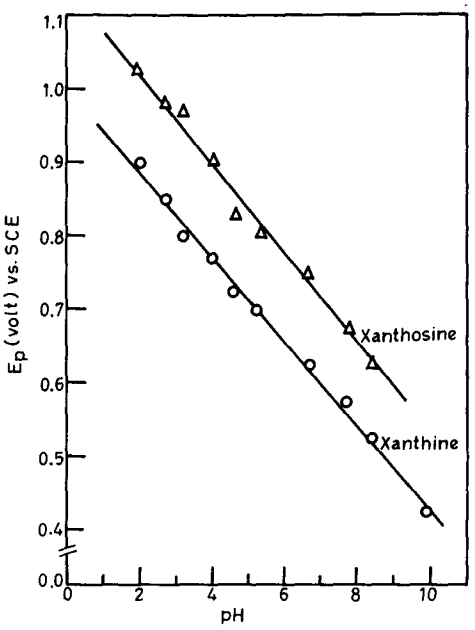


Figure 1. Observed dependence of the peak potential on pH for the voltammetric oxidation peak of xanthine and xanthosine. Sweep rate $10\,mVs^{-1}$.

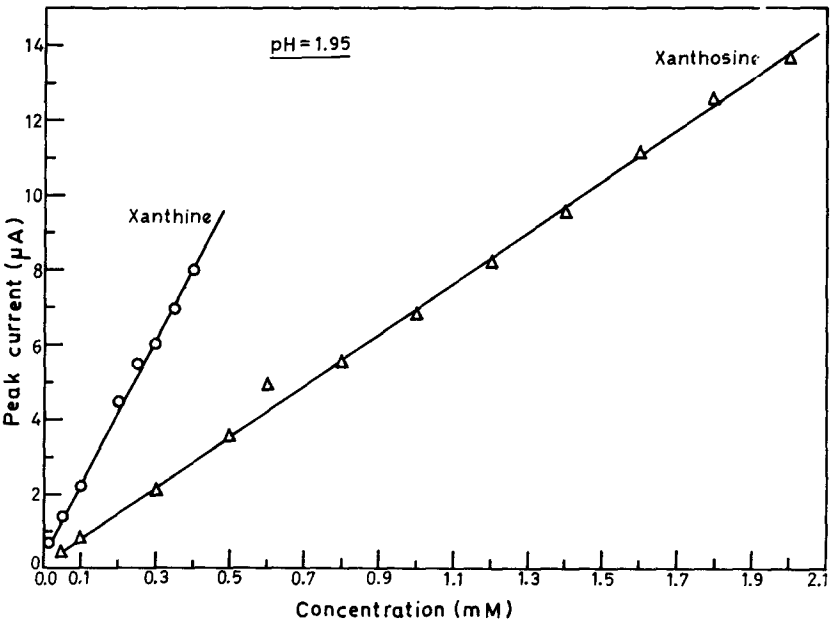


Figure 2. Effect of concentration on peak current values of xanthine and xanthosine in phosphate buffer of pH 1·95.

and hence it was considered worthwhile to estimate the two compounds simultaneously in a mixture. The plot of peak current versus concentration was linear for xanthosine in the concentration range 0·1 to 2 mM (figure 2). The values of $ip/ACV^{1/2}$ were practically independent of sweep rate values and hence it was concluded that xanthosine does not undergo adsorption (Brown and Large 1974) at the surface of the glassy carbon electrode in the above concentration range and thus the electrode reaction is diffusion controlled.

3.1 *Simultaneous determination*

As the oxidation peak for xanthosine was well-defined in the pH range 2·0–6·3, the quantitative determination of xanthine and xanthosine was carried out in this pH range. The anodic voltammograms for the mixture of xanthine and xanthosine were recorded in phosphate buffers of different pH values. At all pH values two well-defined oxidation peaks were observed, however, the separation of the two peaks was maximum below pH 3·2 and at higher pH values the difference was reduced to about 100 mV. Thus the pH range 1·9 to 3·2 was selected for the simultaneous determination of xanthine and xanthosine.

In order to make quantitative determination, linear sweep voltammograms of the mixture of xanthine and xanthosine were recorded at pH 1·95 at a stationary glassy carbon electrode. The concentrations of xanthine and xanthosine were determined from the calibration curves (figure 3) by using the peak current values. The coefficient of variation for the determination of xanthine or xanthosine (10 determinations) at 0·1 mM level was 1%. It is thus clear that this method is readily capable of

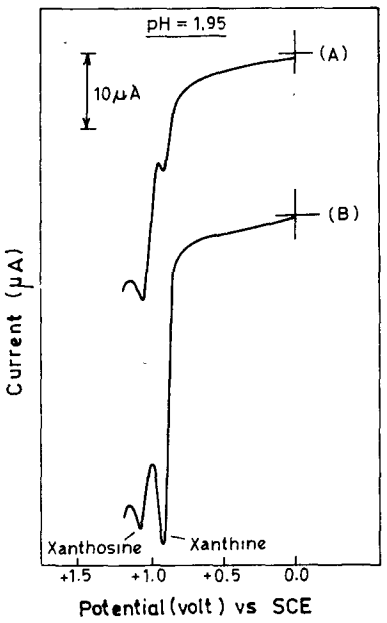


Figure 3. Typical linear sweep voltammograms obtained for a mixture of 0·05 mM xanthine and 0·40 mM xanthosine in phosphate buffer of pH 1·95 (sweep rate 10 mVs^{-1}) (A), and 0·4 mM xanthine with 0·4 mM xanthosine (B).

quantitatively analysing purine bases and their nucleosides in a mixture, and is much superior to the paper chromatographic method suggested earlier (Gerlach *et al* 1965).

The principal advantage of the method is that no prior treatment is necessary and as analysis is based on voltammetric oxidation peaks, deaeration of the sample is not required.

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