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Determination of paracetamol in tablets and blood plasma by differential pulse voltammetry*

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Introduction

Paracetamol, also known as acetaminophen (APAP) (*N*-acetyl-*p*-aminophenol), is a widely used non-narcotic analgesic and antipyretic agent. The determination of paracetamol is important for assessing stability for therapeutic monitoring and for possible toxicological effects. Among the numerous methods that have been reported for the determination in biological fluids or pharmaceutical preparations are spectroscopic methods which usually involve extractions and/or reaction to obtain a coloured derivative [1–5]. The interference of other phenolic compounds, including 4-aminophenol (the product of paracetamol hydrolysis), is a disadvantage. This interference is overcome by gas-liquid chromatographic methods, some of which involve the preparation of derivatives [6–8]. Several HPLC methods offer increased selectivity and sensitivity [9–18]. However, these methods require expensive equipment and some of them make use of the electrochemical properties of paracetamol. Although the electrochemical oxidation of the drug has been previously recognised as being useful for its analytical determination [19], direct electrochemical methods are rare and laborious [20–22].

In this paper the oxidation of paracetamol on a carbon paste electrode is studied by differential pulse voltammetry (DPV) and the optimum conditions are used for the determination in tablets and in serum.

Experimental

Reagents and materials

Paracetamol from Sigma was used as received. All the other chemicals were reagent grade. Panadol (500 mg) tablets were purchased locally.

Aqueous buffer solutions were prepared from phosphoric acid (pH 1, 2, 3 and 6), acetic acid (pH 5) or boric acid (pH 8). The buffer concentration was 0.1 M and the ionic

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strength was adjusted to 1 M with sodium nitrate. Stock solutions of paracetamol were prepared in water.

Apparatus

A home-made microcomputer-controlled electrochemical system was used [23, 24], consisting of an Apple II Plus microcomputer, a fast potentiostat and high-speed A/D and D/A converters. In order to get high sensitivity without serious loss of resolution, pulses of 50 or 100 mV and 40 ms were applied with a delay of 100 ms.

Thermostated electrochemical cells from Metrohm and Bioanalytical Systems were used. The carbon paste electrode was from Bioanalytical Systems or one prepared following Adams' specifications [25] from carbon powder and liquid paraffin, mounted on a cylindrical glassy support (4 mm internal diameter). In both cases, the reproducibility of the electrode response was checked in a solution of the drug by cyclic voltammetry. A platinum and a calomel electrode from Ingold or Bioanalytical Systems were used as auxiliary and reference electrodes. In the analysis of serum samples the cell solutions contain perchloric acid from the deproteinisation treatment. Consequently a saturated solution of sodium chloride was intercalated between the reference electrode and the cell solution to avoid precipitation of potassium perchlorate.

Procedure

Solid dosage forms. Twenty tablets were accurately weighed and powdered. One hundred millilitres of water were added to an accurately weighed portion of powder containing about 100 mg of paracetamol, shaken for 10 min and filtered. A 20 ml aliquot was transferred to a 100-ml volumetric flask and diluted to volume with water.

Spiked serum samples. Blood from rats was immersed in a thermostated bath (37°C) for 1 h. It was then centrifuged at 2800 rpm for 10 min. Perchloric acid solution (0.33 M) was added to the serum and the proteins were separated after further centrifugation. The samples prepared in this way were spiked with a standard solution of paracetamol in the therapeutic range.

The working solutions were obtained by adding a fraction (500 μl–1 ml) of the spiked serum to 10 ml of buffered (1 M) aqueous solutions.

Administration to rats. Rats were given paracetamol dissolved in water by intragastric administration in doses of 200 mg kg^{-1}. Successive extractions of blood were carried out at different times after the administration, and the serum was treated as described above. Two hundred microlitres of serum were added to 5 ml of an aqueous phosphate buffer (1 M) solution of pH 6 in the polarographic cell, and the concentration of paracetamol was determined.

In all the experiments the temperature in the cell was kept at 25 ± 0.1°C, and nitrogen gas was used for deaeration.

Results and Discussion

Influence of pH

The electrooxidation of paracetamol has been studied previously by cyclic voltammetry [19, 26]. It has been established that it is a pH-dependent two-electron process that yields the *N*-acetyl-*p*-quinoneimine. The occurrence of a secondary chemical reaction,

also pH-dependent, was inferred from the influence of scan rate in the cyclic voltammetry experiments. Therefore, the influence of pH on the DPV curves is essential in order to fix the optimum pH values for the analytical determination.

Only one peak is observed in the pH range studied, which becomes broader and smaller in basic media (Fig. 1). The peak potential, *Ep*, shifts towards less positive values when the pH is increased, indicating the participation of protons in the process.

There may be several reasons for this behaviour: first, the decrease of the heterogeneous rate constant (affected by proton transfer) when pH is increased can produce a reduction of the peak intensity, ΔIp, and an increase in peak half-width. This has been proved to occur in differential pulse polarography [27–29]. The increase in homogeneous rate constant when the pH is increased, observed in the cyclic voltammetric experiments is also expected to affect the pulse curves, although this effect has not been studied so extensively. Finally, the acid–base catalysed hydrolysis of the drug [30, 31] could result in the distorted peak observed at pH 8.1, which could contain the peak of 4-aminophenol. Hydrolysis of paracetamol can also be the cause for the lower value of ΔIp observed at pH 1 (in comparison with the value obtained at pH 3) although at this pH value the 4-aminophenol is not stable and does not interfere in the curve.

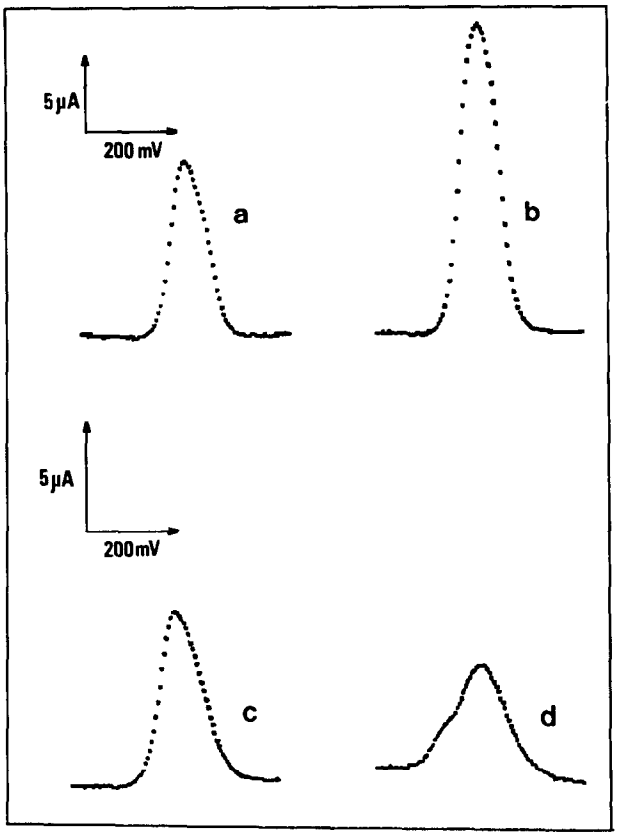

Figure 1
DPV curves for paracetamol at different pH values with the same electrode. (a) pH 0.9, *Ep* = 560 mV; (b) pH 3.1, *Ep* = 500 mV; (c) pH 6.0, *Ep* = 345 mV; (d) pH 8.1, *Ep*= 285 mV.

In view of these observations, it seems that a pH value between 4 and 7 is the most satisfactory for the analytical determination. Somewhat lower values produce better sensitivity, but the data may be more affected by the hydrolysis of the drug.

Influence of concentration

The range 10^{-6}–10^{-4} M in aqueous buffered solutions of different pH was studied. The *Ep* values remain constant within the experimental error indicating the absence of adsorption phenomena or second-order reactions. Linear correlations between ΔIp and concentration were observed (Table 1) allowing the analytical determination of the drug.

Interferences

A characteristic which makes the use of the DPV technique attractive in this case is the fact that many of the substances which may be co-administered with paracetamol are not electroactive. For instance, barbiturates which interfere in colorimetric and in some chromatographic methods are not oxidised on a carbon paste electrode. The benzodiazepinic tranquilisers undergo only reduction reactions [32]. Unfortunately, that is not the case with some paracetamol metabolites, such as the 4-conjugated sulphates or glucuronides [19]. It has been proved, however, that the 4-hydroxy derivatives are more easily oxidised than paracetamol. Thus, 4-aminophenol, the main hydrolysis product, gives a peak at less positive potentials, clearly separated from the paracetamol peak (Fig. 2). The simultaneous determination of both compounds is then possible with this technique.

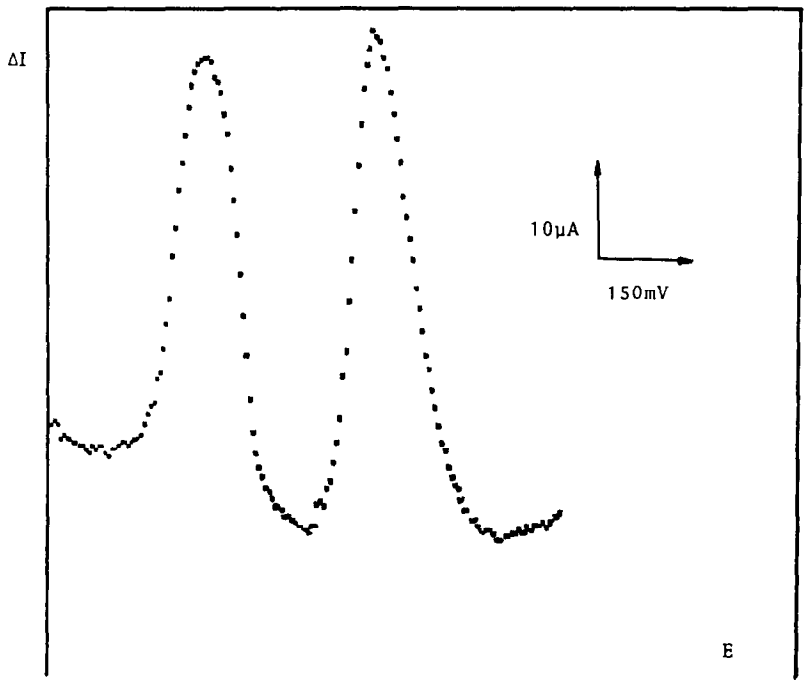

Figure 2
DPV curves for a solution of paracetamol (1.5×10^{-5} M) and *p*-aminophenol (5×10^{-5} M) at pH 6. *Ep* paracetamol = 315 mV; *Ep p*-aminophenol = 45 mV.

The major sulphonate or glucuronide metabolites give peaks at more positive potentials [19], so that they do not interfere in the high resolution technique of DPV. This is true also of phenacetin, which may be co-formulated with paracetamol in pharmaceutical preparations.

Simultaneous determination of vitamin C

Oxidation of vitamin C can take place at potentials very close to that of paracetamol oxidation depending on pH [33]. A more detailed analysis of this interference was carried out at different pH values in the range 2–8. In Fig. 3, some of the DPV voltammograms are shown. It can be observed that the separation between both peaks is better at lower pH values. At pH >7 the vitamin peak is almost completely masked by the paracetamol peak. The simultaneous determination of both drugs is optimum around pH 4. This value can be increased in order to prevent the hydrolysis of paracetamol, but in that case, deconvolution of the peaks is necessary.

To check this possibility, deconvolution was performed assigning Gaussian forms to both peaks, determined by the number of electrons transferred (two in both cases) and the irreversibility of the process (represented by the Tomes slope, which can be obtained in experiments with each drug separately). A program then searches for the ΔIp values giving the minimum standard deviation of the complex curve. In Fig. 4, the theoretical and experimental curves are shown for a solution of vitamin C (3.8×10^{-5} M) and of paracetamol (7×10^{-5} M). The deconvolution afforded in this case were 7.6 and 72.5 μA, respectively. When the vitamin concentration was doubled, the intensity of the first peak was also doubled, while that of the other remained constant.

Determination in tablets

An aliquot of the sample prepared as described in the experimental section was added to the buffered solution in the cell and the DPV curve was recorded. No interference was observed and the shape and characteristics of the pulse voltammograms were the same as those obtained with the aqueous buffered solutions of pure paracetamol. The standard addition method was adopted for the quantitative determination. Successive aliquots of a standard solution were added to the solution in the cell containing the sample, and the pulse voltammogram was recorded after each addition. Plots of ΔIp versus the amounts added were straight lines (Table 1) from which the concentration of paracetamol was calculated.

The precision of the method was calculated by analysing 10 aliquots of a sample. The relative standard deviation was 2.5% and the average recovery 107%.

Determination in serum

The possibility of analysing serum without pretreatment was discounted because significant changes in the *Ep* values and shape of the curve were observed when compared with the results obtained with standard aqueous solutions. The reason for this may be the influence of proteins on the double-layer structure.

Extraction of the drug with ethyl acetate from a buffered solution at pH 6.6 has been proposed to eliminate interferences from vitamin C and other drugs because they are not extracted at this pH value [20]. However, when assaying samples containing paracetamol and vitamin C, it was observed that the vitamin C interference was not completely eliminated. Therefore, the method adopted involved the deproteinisation of the serum

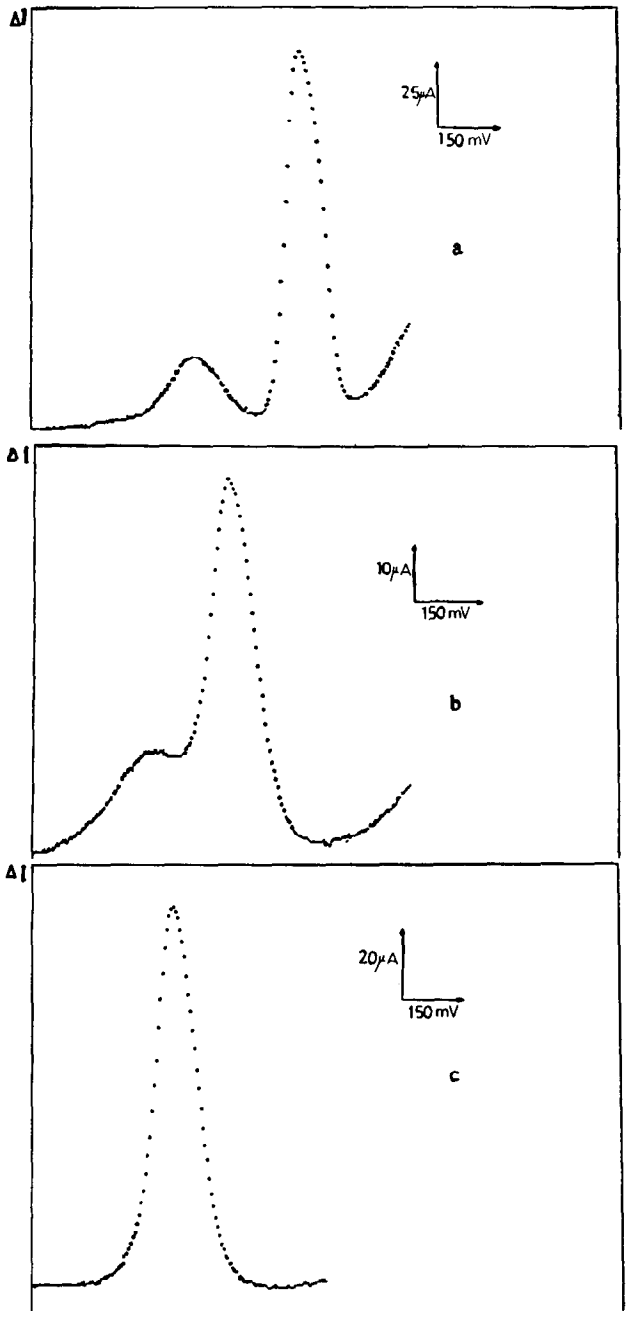

Figure 3
DPV curves for solutions of paracetamol (7×10^{-5} M) and vitamin C (7×10^{-5} M) at different pH values (a) pH 1.9; (b) pH 5.9; (c) pH 8.0. Initial potential = −100 mV.

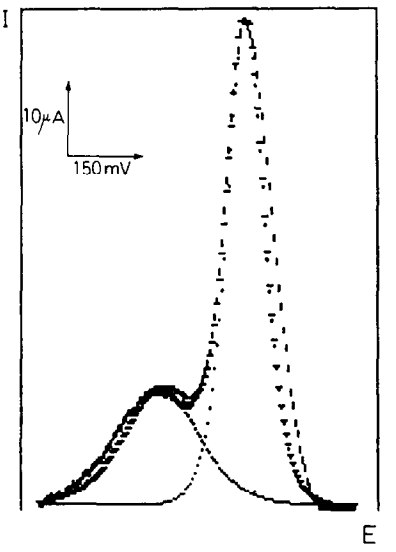

Figure 4
Deconvolution for the solution in Fig. 3b. (. . .) deconvoluted curves; (– – –) sum of the deconvoluted curves; (///) original complex curve.

Table 1
Characteristic of the linear ΔIp-c plots

Aqueous buffered solution (Student's t-test) (with different electrode for each concentration)	r (correlation coefficient)	Student's t-test
pH 2	0.997	34.80
pH 4.8	0.996	31.31
pH 6.6	0.9990	58.65
Tablets (standard addition method)	0.998	96.93
Serum (After administration to rats standard addition method)	0.998	30.70

as described in the experimental section. The standard addition procedure yields linear ΔIp-c correlations (Table 1) at pH 4 and 6.

Monitoring of the concentration of paracetamol in rat blood after administration was carried out in the same way, following the procedure described in the experimental section. The following values were obtained: 24, 68 and 39 $\mu g\ ml^{-1}$ for the concentration in blood at 45, 90 and 135 min after administration. These values are in agreement with the biological half-life previously reported [34, 35]. The determination of serum levels at intervals after the ingestion of the drug offers the possibility of further pharmacokinetic studies with this technique.

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