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Short Communication

Polarographic determination of doxorubicin and daunorubicin in pharmaceutical preparations and biological media*

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Introduction

The electrochemical behaviour of doxorubicin, daunorubicin and their corresponding aglycones in aqueous media has already been studied by different authors using various electrochemical methods. These compounds are reducible at the mercury electrode by a two-electron process [1, 2]. Investigation of the electrochemical behaviour of doxorubicin at carbon paste electrode also shows similar results and the reduction process was found to be pH dependent [3]. On the basis of these studies, the methods for the determination of doxorubicin in plasma by differential pulse polarography [4] and in urine by differential pulse voltammetry at glassy carbon or carbon paste electrodes [5, 6] were proposed. In addition to the electrochemical methods, procedures based on spectrofluorimetry [7–9], radioimmunoassay [10–12], gas chromatography–mass spectrometry [13, 14], liquid chromatography [15–17] and high-performance liquid chromatography [18–23] have also been used for the analysis of doxorubicin and other derivatives of the anthracycline family. In this work, the electrochemical behaviour of doxorubicin, daunorubicin and their corresponding aglycones was investigated in chloroform and an ac tast (ac_T) polarographic method is described for the determination of these compounds after extraction from pharmaceutical preparations or biological media (serum, plasma, urine and blood) into chloroform.

Experimental

Reagents and chemicals

The solvent used in this work was chloroform G.R. from E. Merck or Fluka. This solvent was used without further purification. Tetrabutylammonium perchlorate (TBAPC), Purum from Fluka and piperidinium perchlorate (PPC) + piperidine (P) were used as supporting electrolytes. This latter compound was prepared by the procedure described previously [24]. Other chemicals, including doxorubicin HCl and daunorubicin HCl, were of analytical reagent grade from E. Merck or Sigma.

Instrumentation

A Polarecord E 506 + E 505 stand, from Metrohm (Switzerland) was used to plot the dc, ac_T and dp polargrams. A Coulostat E 524 connected to a Metrohm E 525 integrator was used for coulometry at controlled potentials. A function generator from Metrohm (V.A. Scanner E 612) connected to the Polarecord E 506 was used for cyclic voltammetry and the voltammograms were recorded by an X–Y Hewlett–Packard 3310A recorder. The reference electrode was Ag/AgI in 0.05 M tetrabutylammonium iodide + 0.50 M TBAPC solution in chloroform.

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Extraction of doxorubicin and daunorubicin from aqueous solutions

Chloroform was used for extraction of the drugs from buffered pH 9 aqueous solution. The extraction efficiency was pH dependent and approached 100% when the pH of the aqueous phase exceeded 8 (Fig. 1). A 2-ml aliquot of solution containing 3–25 μg of anthracycline was transferred into a separatory funnel, the pH was adjusted to 9 with 0.5 ml of 2 M ammonia buffer and the total volume of the aqueous phase was brought to 5 ml with distilled water. The extraction was carried out with two 5-ml portions of chloroform for 2 min each. The efficiency of extraction was examined by the spectrofluorimetric determination of anthracycline in both aqueous and organic phases and was calculated to be >98%.

Extraction of doxorubicin and daunorubicin from human serum or plasma

A 4:1 mixture of chloroform–isopropanol was used for the extraction of drugs from serum or plasma. Isopropanol acts as a protein denaturing agent [25]. One millilitre of serum or plasma spiked with 5–25 μg of drug was transferred into a 15 × 100 mm test tube. The pH was adjusted to 9 with 0.5 ml of ammonia buffer and the total volume was brought to 5 ml with distilled water. The extraction was carried out by rigorous shaking for 2 min of the aqueous phase with two 5-ml portions of the chloroform–isopropanol mixture. After each step, the phases were separated by centrifugation for 10 min at 5000 rpm. The water layer and protein interface were discarded and the lower organic phase was transferred to a polarography cell. ac_T Polarography was used for recovery determinations and under these conditions, the recoveries were calculated to be >98%.

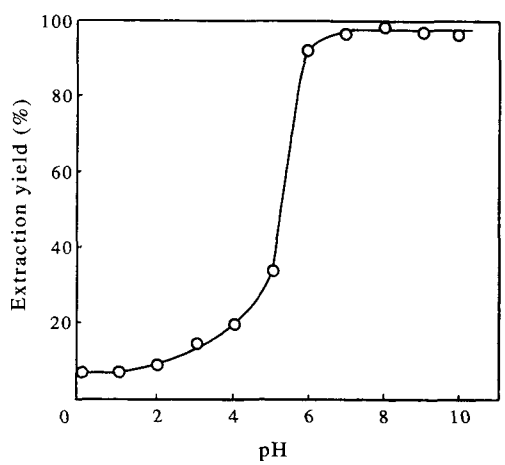

Figure 1
Effect of aqueous phase pH on extraction yield of anthracyclines into chloroform.

Extraction of doxorubicin and daunorubicin from whole blood

The comparison of extraction efficiencies of drugs from serum and whole blood under similar conditions suggested that a considerable quantity of drug was bound to blood cells to cause a significant decrease in the extraction efficiency from whole blood. Haemolysing the blood cells by the addition of distilled water (1:1), increased the extraction efficiency to 95%. The procedure used for the extraction of the drugs from a (1:1) mixture of blood–distilled water is the same as for serum and plasma.

Extraction of doxorubicin and daunorubicin from urine

The use of pure chloroform or a (4:1) mixture of chloroform–isopropanol for the extraction of the drugs from buffered urine at pH 9 gave the same results. The recovery from urine was >98% in both cases. The procedure for the extraction of the drugs from urine was the same as from aqueous solution.

Extraction of adriamycinone and daunomycinone

Acid or alkaline hydrolysis of the anthracyclines causes the release of the glycoside moiety and the formation of an aglycone. The aglycone is less polar than the corresponding anthracycline and therefore can be extracted from acid solutions. An acid hydrolysis procedure was used to prepare the aglycones of doxorubicin and daunorubicin [5]. A 0.5–1 ml aliquot of 2 M hydrochloric acid was added to each 1-ml sample and the volume was adjusted to 5 ml with distilled water. The samples were placed in a boiling water bath for 10 min to complete the transformation of anthracycline to aglycone. The extraction of the aglycone followed the same procedure as that for anthracyclines from aqueous solution. In serum, plasma and blood, the addition of acid caused protein denaturation and thus the extractions were carried out with pure chloroform. Separation of two phases was accomplished by centrifugation for 10 min at 5000 rpm.

Results and Discussion

Electrochemical behaviour of doxorubicin, daunorubicin and their aglycones in chloroform

The electroactivity of anthracyclines in chloroform is due to the presence of the quinone ring in their structure. Reduction of these compounds, in the absence of proton donors, should be similar to that of the simple quinones, which occurs in two one-electron steps [26]. In the presence of proton donors, the electrode reaction changes to a two-electron reduction process [24, 27].

dc Polarography of doxorubicin, daunorubicin and their corresponding aglycones in chloroform and in the presence of 0.5 M TBAPC gives two reduction waves, with the second wave nearly coalescing with the reduction wall of the solvent. In ac polarography, two separate peaks with peak potentials very close to the $E_{1/2}$ of the dc waves appear (Fig. 2).

A comparison of the $E_{1/2}$ of the first reduction wave of doxorubicin and its aglycone (adriamycinone) in neutral medium showed that the aglycone was reduced at a potential ~25 mV less negative. The plots of $E_{e1} = f(\log[(i_d - i)/i])$ for the first reduction waves of both compounds gave straight lines with slopes of 58 mV. Coulometry at the level of the first cathodic wave of doxorubicin and adriamycinone gives a value of 1 for n. These results confirm the reversibility of the electrode process.

ac_T Polarography of doxorubicin, daunorubicin and their correspondence aglycones in the above mentioned conditions produces two separate peaks due to the two-step reduction of these compounds at dme. A comparison of the peak potentials showed that the adriamycinone was reduced at less negative potentials than the doxorubicin (Table 1). The plots of $\log\{(i_p/i)^{1/2} + [(i_p - i)/i]^{1/2}\}$ vs the electrode potential for the first peak gave straight lines with slopes of 118.6 mV and an intercept of −0.354 and −0.342 V for doxorubicin and adriamycinone, respectively. These results confirm that the electroreductions are reversible one-electron processes.

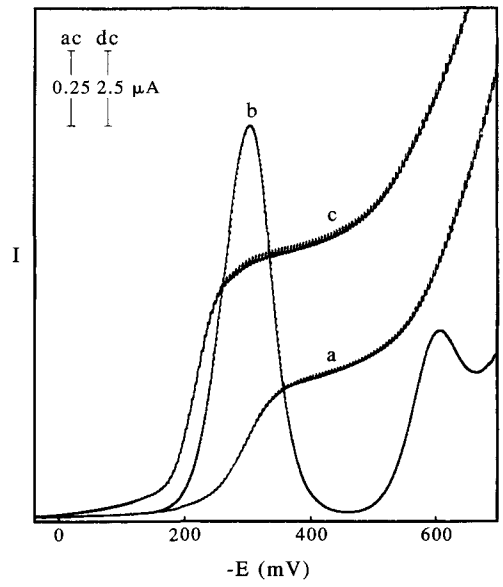

Figure 2
Polarograms of 5×10^{-4} M doxorubicin after extraction into chloroform. (a) dc; (b) ac_T polarogram ($E\sim$ = 15 mV). Supporting electrolyte, 0.5 M TBAPC; (c) a + 10^{-2} M PPC.

In dc polarography of doxorubicin and adriamycinone, with a mixture of 0.75 M PPC + 0.25 M P used as the supporting electrolyte, a single reducation wave appeared at less negative potentials due to the protogenic property of piperidinium ions. The height of this new wave was twice that of the first wave obtained in the presence of proton donors. The plots of $\log[(i_d - i)/i]$ vs E_{e1} gave a straight line with a slope of approximately 30 mV. This

Table 1

Compounds	dc Polarography TBAPC $E_{1/2}$*	dc Polarography PPC + P $E_{1/2}$‡	ac Polarography TBAPC $E_{p.1}$	ac Polarography TBAPC $E_{p.2}$	ac Polarography PPC + P E_p	Cyclic voltammetry† PPC + P $E_{p\,a}$	Cyclic voltammetry† PPC + P $E_{p.c}$
Doxorubicin	−0.354	−0.250	−0.354	−0.742	−0.240	−0.195	−0.240
Adriamycinone	−0.325	−0.250	−0.342	−0.684	−0.239	−0.195	−0.240
Daunorubicin	−0.360	−0.255	−0.360	−0.755	−0.240	−0.205	−0.258
Daunomycinone	−0.335	−0.255	−0.355	−0.695	−0.240	−0.215	−0.255

* $E_{1/2}$ of the second wave is not measurable because of its proximity to the reduction wall of the solvent.
† Scan rate was 100 mV s^{-1}.
‡ All potentials are in V vs ref.

corresponds to a reversible 2e process. The $E_{1/2}$ of this new wave was −0.240 V vs ref.

ac_T Polarography at the above mentioned conditions shows a peak with E_p = −0.240 V vs ref. The plot of $\log\{(i_p/i)^{1/2} + [(i_p - i)/i]^{1/2}\}$ vs E_{e1} gave a straight line with a slope of 64 mV. This can be taken as a measure of the reversibility of the electrode process [28].

The cyclic voltammetry of doxorubicin, daunorubicin and their corresponding aglycones at hmde and in the presence of 0.75 M PPC + 0.25 M P produced a cathodic and an anodic peak (Fig. 3). At a scan rate of 100 mV s^{-1}, ΔE_p was 40 mV and decreased with decreasing scan rate. The peak current ratio ($i_{p.a}/i_{p.c}$), however, remained constant and equal to 1 at all scan rates. This indicates the reversibility of the electrode reaction, as well as the contribution of 2e in the reduction process by an $E_rE_rC_rC_r$ mechanism [24]. Table 1 gives the characteristic potentials of the compounds studied.

Recommended method for the determination of anthracyclines and its aglycones after extraction into chloroform

Both dp and ac_T polarography were used for the determination of the anthracycline drugs. On the basis of the results obtained for a

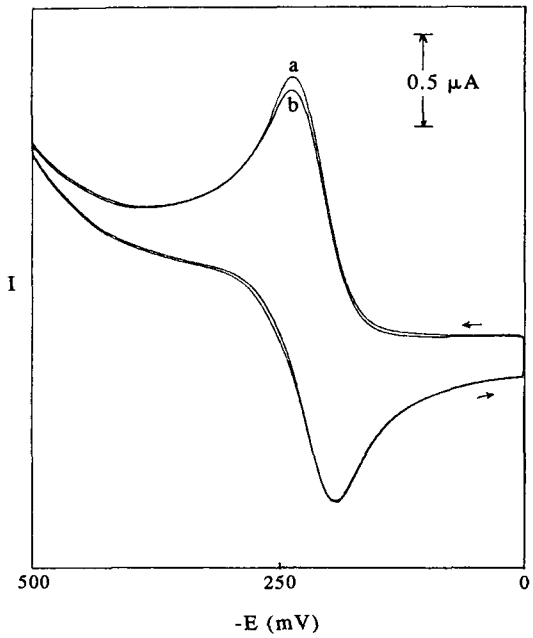

Figure 3
Cyclic voltammogram of 5 × 10^{-4} M doxorubicin after extraction into chloroform. (a) First scan; (b) second scan. Supporting electrolyte: 0.75 M PPC + 0.25 M P. Scan rate: 200 mV s^{-1}.

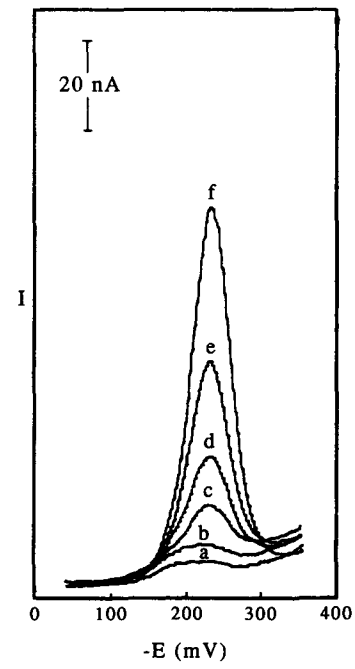

Figure 4
ac_T Polarograms of doxorubicin after extraction into a 4:1 mixture of chloroform–isopropanol from spiked human serum: (a) 6 × 10^{-7} M, (b) 1 × 10^{-6} M, (c) 2.2 × 10^{-6} M, (d) 4.4 × 10^{-6} M, (e) 8.8 × 10^{-6} M, (f) 1.8 × 10^{-5} M. E~ = 15 mV.

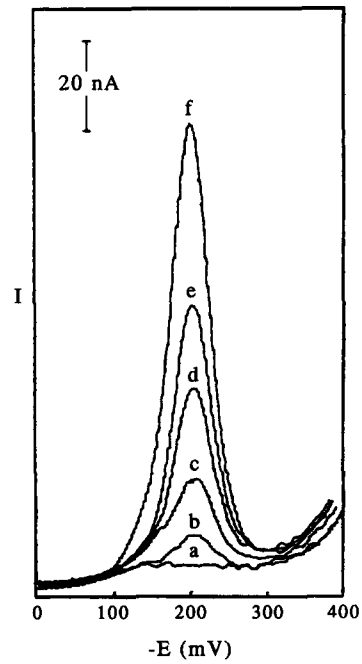

Figure 5
ac_T Polarograms of doxorubicin after extraction into chloroform from spiked human urine: (a) blank; (b) 1.87 × 10^{-6} M; (c) 3.74 × 10^{-6} M; (d) 7.49 × 10^{-6} M; (e) 1.12 × 10^{-5} M; (f) 1.87 × 10^{-5} M. $E\sim$ = 15 mV.

2×10^{-5} M solution of doxorubicin in chloroform and in the presence of 0.75 M PPC + 0.25 M P as the supporting electrolyte, dp polarography with a pulse amplitude of 50 mV and ac_T polarography with an alternating voltage amplitude of 15 mV were found to be suitable methods for deterination. However, for a given drug concentration, the height of the ac_T peak was about twice that of the dp peak. Therefore, the recommended method for analysis of anthracyclines and their aglycones in chloroform is the ac_T procedure. Figures 4 and 5 show the ac_T polarograms obtained from serum and urine, spiked with various quantities of adriamycin. The extraction solvent used was a 4:1 mixture of chloroform–isopropanol for serum; since isopropanol caused a decrease in the peak height, only chloroform was used for extraction from urine. Plots of $i_p = f$(concentration) for a concentration range of $5 \times 10^{-7} - 5 \times 10^{-5}$ M of both drugs gave calibration curves with a correlation coefficient of 0.9998. The proposed method has also been applied to the determination of drugs in whole blood after extraction into chloroform and the results of ac_T polarography were used to make a calibration curve. The correlation coefficient of this curve was 0.9982. The determination of anthracycline concentration in various pharmaceutical and biological samples were carried out using both standard addition and calibration curve methods.

Limit of detection (lod) and precision of method

The general definition given in the literature was used for calculation of the lod [29–31]. The calculated lod concentrations of the anthracyclines were 4.3×10^{-7} M for serum or plasma, 4.2×10^{-7} M for urine and 4.1×10^{-7} M for whole blood. The precision (RSD) of the ac_T method for the determination of 1.8×10^{-5} M doxorubicin in five serum samples was 1.4%.

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