Preconcentration and Voltammetric Determination of Indomethacin at Carbon Paste Electrodes

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Abstract
The electrochemical oxidation and detection of indomethacin in phosphate buffer (pH 7) at carbon paste electrodes is described. The response is characterized with respect to paste composition, preconcentration time, accumulation potential, indomethacin concentration, stirring effect, reproducibility and other variables. Two mechanisms of accumulation are differentiated: an adsorptive accumulation of the analyte at the graphite particles of the Nujol or silicone oil electrodes and extractive accumulation into the castor-oil based electrode. The preconcentration/medium-exchange approach was exploited for selective determination of indomethacin in urine sample at the castor oil electrode. A detection limit of 2.5×10^{-8} M was obtained for a dilute urine sample after 20 min accumulation and a medium-exchange procedure.

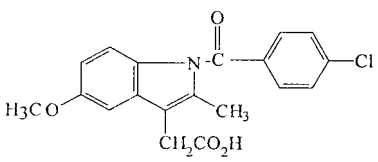
Keywords: Indomethacin, Carbon paste, Castor oil

1. Introduction

Indomethacin (Scheme 1) {1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid} is a nonsteroidal anti-inflammatory analgesic which acts as a therapeutic agent for arthritis [1]. Indomethacin is metabolized in vivo. The unchanged indomethacin, *O*-desmethyl-indomethacin and *N*-deschlorobenzoylindomethacin are the major components in urine [2]. It is possible to determine this compound by direct or differential pulse polarography owing to the presence of reducible group on the molecule [3]. Other methods reported for the determination of indomethacin includes conventional HPLC [4] and HPLC with spectrofluorometric detection [5]. Hampp's group [6] developed an ion-selective electrode based on bis (triphenylphosphoranylidene)ammonium-indomethacin ion-pair complex for the determination of indomethacin.

The application of chemically modified electrodes has received considerable attention in the recent years [7–10]. One promising approach is the accumulation of organic analytes at electrodes displaying hydrophobic properties prior to voltammetric measurement. This preferential accumulation coupled with medium exchange can be used advantageously for the measurement of accumulated analyte in presence of nonaccumulated species with similar redox potential in biological fluids with no sample pretreatment. Hydrophobic CMEs have been used to determine a wide range of organic compounds, including promethazine [11], anti-inflammatory drugs [12], tricyclic antidepressants [13], antihypertensive drugs [14], ergot alkaloids [15], butylated hydroxyanisole [16], methylated indoles [17], phenanthrenequinone and oxoapomorphine [18], adriamycin [19] and antitumour celiptium [20].

As no work dealing with the electrooxidation of indomethacin has been reported, it was of interest to pursue the anodic behavior of the compound due to the presence of electrooxidizable groups, probably the indolic moiety or the amide group. Indomethacin is readily dissolved in castor oil [21] due to its lipophilicity. We exploited this behavior for developing a new carbon paste electrode based on a castor oil pasting liquid for accumulating indomethacin prior to its sensitive and selective voltammetric measurement.



2. Experimental

2.1. Reagents

Stock solution (1×10^{-2} M) of indomethacin was prepared daily in pure methanol and stored at 4 °C in the dark. Phosphate buffer (0.02 M), pH 7 was used as supporting electrolyte. All solutions were prepared from AnalaR-grade reagents in double distilled water.

2.2. Apparatus

Cyclic voltammetry measurements were performed with an Oxford PVSU potentiostat connected to Philips PM 8043 *X–Y* recorder. Differential pulse polarography measurements were made with a Polarecord E506 Metrohm (Herisau, Switzerland). All experiments were performed with a three electrode cell configuration containing the carbon paste working electrode, a Ag/AgCl reference electrode and a platinum wire as auxiliary electrode.

The pH of solution was measured with a Schott Geräte digital pH-meter with glass combination electrode.

2.3. Procedure

Adsorptive voltammetric measurement consisted in accumulation of the analyte at the electrode surface by stirring at ca. 400 rpm, by magnetic stirrer and stirring bar 1 cm long, for a given period of time followed after a delay period of 15 s to settle the solution and decrease the background current and recording of the anodic voltammogram at scan rate of $50\,mVs^{-1}$. The accumulation step was accomplished at a potential range from −0.3 to +0.3 V or at open circuit potential.

For medium exchange experiments, two 20 mL cells were used, a preconcentration cell containing the analyte solution and a measurement cell containing a blank solution. After preconcentration, the electrode was washed with water then transferred to the measurement cell to record the voltammogram between −0.1 and 1.0 V. Quantitative measurement was done by the standard addition method.

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For chronoamperometric experiment, after preconcentration step the medium was exchanged and then the potential was stepped from −0.1 to 0.850 V while recording the *i*–*t* curve.

2.4. Electrode Preparation and Regeneration

Pastes were made by hand mixing 70/30 or 60/40% (w./w.) spectroscopic graphite powder (Aldrich 1-2 micron) and pasting liquid in mortar and pestle. The pasting liquids were Nujol oil (Sigma), silicone oil (Edward, Sussex, England) and castor oil (ADWIC). The paste was pressed into the glass barrel of a Metrohm electrode (8 mm diameter) and smoothed on a clean paper.

After each electrochemical measurement, the working electrode was transferred to a blank solution. The electrode was held at 0.850 V and the solution was stirred for 2 min at ca. 400 rpm. The removal of the analyte was indicated from the subsequent voltammogram recorded in a blank solution. The electrode was then ready for use in the next measurement cycle.

3. Results and Discussion

The cyclic voltammograms for the oxidation of indomethacin in phosphate buffer (pH 7.0) at the three electrodes are shown in Figure 1. In all instances, the first forward scan exhibited a single anodic peak owing to the oxidation of indomethacin, probably at the indolic moiety or the amide group. In the reverse sweep only a small cathodic peak was observed. The cyclic voltammogram at castor oil electrode exhibits a relatively higher background current compared to the CPE and this may be related to the presence of molecules with unsaturated functionality in the castor oil. However, the enhanced peak current due to the high solubility of indomethacin in castor oil compensates for the increased background current.

Figure 2 shows the dependence of the peak heights on accumulation time for 2.5×10^{-5} M indomethacin in phosphate buffer (pH 7.0) at carbon paste electrodes containing different pasting liquids with different composition ratios. For the castor oil electrode the relation between the peak current and the accumulation time was linear for short accumulation periods. After 10 min, the rate of extraction decreased, but the extraction process went on for 30 min. The Nujol electrode shows a current increase within the first 2 min and a leveling-off with longer preconcentration times. It is evident that the response is strongly dependent on the pasting liquid and the composition ratios. Pastes containing castor oil yielded substantially larger peak current upon increasing the oil content. For the Nujol oil electrode, the peak current, decreases with increased Nujol content. The current enhancement at the Nujol oil electrode was attributed to preconcentration by mainly adsorption mechanism, whereas, at the castor oil electrode, the extraction mechanism was the dominant one. Hence, the increased coverage of Nujol oil on the surface resulted in increased blocking of the adsorption sites at graphite particles [22] and hindered penetration of the molecules through the oily layer to the graphite redox centers [23]. The increased coverage of the surface by castor oil means increased capacity of the electrode to extract the analyte. Since indomethacin is soluble in castor oil, they form a separate layer of homogeneous solution which permitted unrestricted diffusion of the molecules through the oily layer. Among the other pasting liquid examined, the silicone oil yielded a larger response than that containing Nujol oil. This might be attributed to the differences in viscosity and the graphite-wetting properties of pasting liquids. The wetting of graphite particles by the less viscous silicone oil ($\eta = 180$ cP, at 25 °C) occurred to be lesser than by Nujol ($\eta = 400$ cP) and resulted in an increased degree of exposure of graphite particles to the aqueous solution.

All the measurements were made at neutral pH of 7.0 since indomethacin is only stable in neutral or slightly acidic media [21]. The efficiency of the preconcentration of 1×10^{-6} indomethacin solution at castor oil electrode was studied at different ionic strength of solution from 0.001to 0.04 M phosphate buffer. The degree of accumulation was found to be of comparable magnitude.

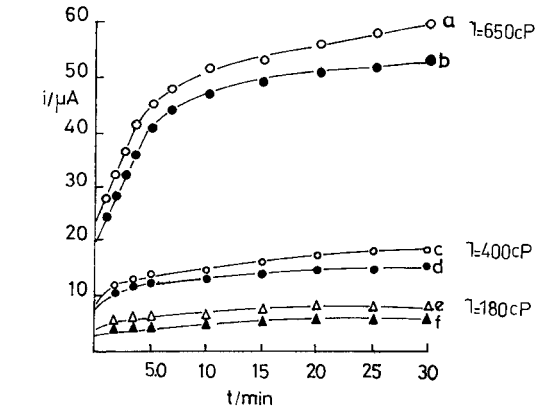

Fig. 2. Effect of accumulation time on peak current for 2.5×10^{-5} M indomethacin; a,b) castor oil electrode, c,d) silicone oil electrode, and e,f) Nujol electrode. a,c,e) graphite-to-oil ratio: 70/30 % (w./w.); b,d,f) graphite-to-oil ratio: 60/40 % (w./w.); Other conditions as in Figure 1.

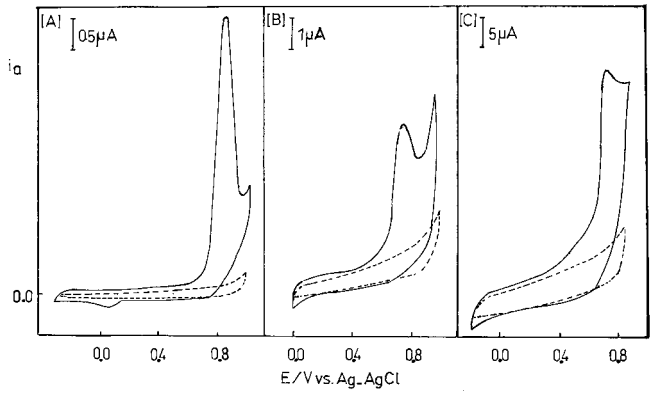

Fig. 1. Cyclic voltammetry for 2.5×10^{-5} M indomethacin in 0.02 M phosphate buffer at a castor oil electrode (A), silicone oil electrode (B), and Nujol electrode (C). Graphite-to-oil ratio: 70/30 % (w./w.) ; scan rate: 50 mV s^{-1}.

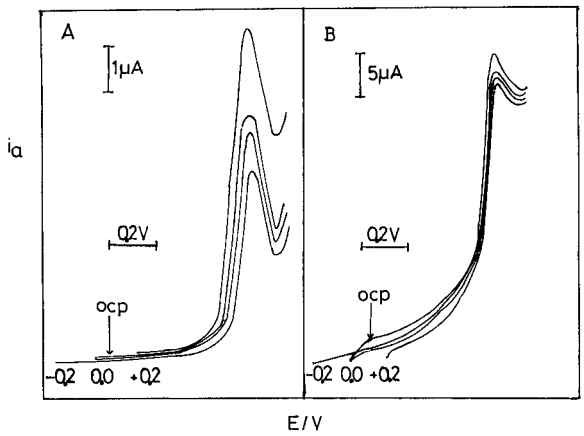

Fig. 3. Voltammograms obtained for 2.5×10^{-5} M at castor oil and Nujol electrodes at different accumulation potentials.

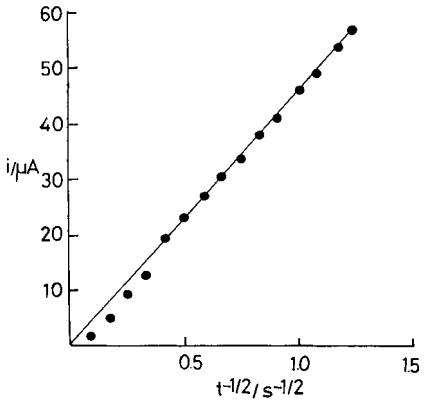

Fig. 4. i vs. $t^{-1/2}$ plot for chronoamperometric oxidation for 2.5×10^{-5} M at 70/30 % (w./w.) graphite-castor oil paste electrode (potential step from −0.1 to 0.850 V).

The effect of the accumulation potential was also investigated at a potential range from −0.3 to +0.3 V or at open circuit potential. As shown in Figure 3 the extraction efficiency at the castor oil electrode is essentially independent of the accumulation potential. This may be due to the nonelectrochemical nature of the extraction process. On the other hand, significant difference in the response at negative or positive accumulation potential was observed at Nujol or silicon oil electrodes. Considering these data, open circuit condition was selected for further study at castor oil electrode.

The stirring of solution causes more drug to be partitioned into the oily phase of the paste. Convection also assists in maintaining an interfacial concentration of indomethacin as close as possible to the bulk concentration. Accumulation from solution stirred at 400 rpm gave 4.5 fold and 2.0 fold larger peak currents compared with those obtained in unstirred solution at castor oil and Nujol oil electrodes, respectively.

The effect of the scan rate on the peak current response of 2.5×10^{-5} M indomethacin was studied using CV. At the Nujol carbon paste electrode, a direct proportionality was observed between the peak current i_p and the scan rate (ν) in the range from 5 to 200 mV s^{-1}, indicating an oxidation process which is governed by adsorption of the molecules at graphite particles exposed to the solution. At the castor oil electrode the dependence shows that the peak currents are nearly proportional to the square root of sweep rate ($\nu^{1/2}$), which is expected for diffusion-controlled process. This may be the diffusion of dissolved molecules from the castor oil layer to the graphite particle surface. Potential-step

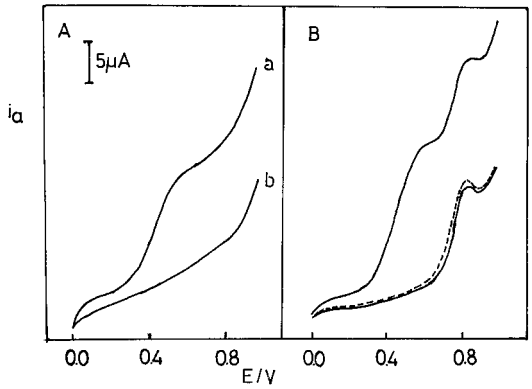

Fig. 5. A) Linear sweep voltammograms for a) diluted (1:10) urine with phosphate buffer. b) Medium exchange after preconcentration step at $t_{acc}=3$ min and open circuit condition. B) Conditions as in (A) + 2.5×10^{-5} M indomethacin. Dotted lines: medium exchange response after accumulation from aqueous buffer solution of 2.5×10^{-5} M indomethacin.

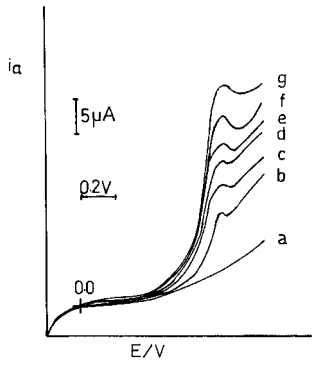

Fig. 6. Linear sweep voltammograms obtained after increasing the indomethacin concentration in 5×10^{-5} steps (b–g) and urine blank (a).

chronoamperometric electrolysis for a short time also showed a current decay with the square root of electrolysis time ($t^{-1/2}$) as expected from the Cottrel equation for a diffusion-controlled process (Fig. 4). At larger times the drop of the current from the Cottrel line may be due to the depletion of the indomethacin molecules from the oily layer due to the back diffusion from the oil phase of the paste to the solution or to the onset of finite diffusion. The latter effect is established when the layer containing electrode reactant becomes comparable to the depth of the diffusion layer. Analogous effect was encountered in thin layer electrochemistry [24] and in electrochemistry of thin electroactive polymer film [25, 26].

The peak current of indomethacin as measured by LSV was found to increase with concentration. The peak current of indomethacin increase linearly with concentration from 3.5×10^{-6} up to 7.5×10^{-5} M {C [mM] = 0.0008 i_{pa}[μA] − 0.0001, r = 0.987}. At concentration of 1.0×10^{4} M, a curvature of the calibration plot was observed. The curvature presumably indicates the saturation of the electrode surface [14]. A detection limit of 2.5×10^{-7} M for indomethacin was obtained after a 3 min accumulation period. Improved sensitivity and lowered detection limit were achieved by applying the differential pulse mode. The detection limit calculated from the signal-to-noise characteristic ($S/N=3$) was 1.0×10^{-8} M . This was lowered down to 1.5×10^{-9} M if the preconcentration time was extended to 20 min. The reproducibility of the peak current at new surfaces as measured by the relative standard deviation (RSD) was 5.0 % and at same electrode after consecutive accumulation and cleaning step, was 3.5 % (n = 5).

3.1. Determination of Indomethacin in Urine

As shown in Figure 5 the direct determination of indomethacin in a diluted urine sample was not feasible due to the large oxidation peak of the blank urine. In contrast, after medium exchange, none of the urine component contribute to the response and the indomethacin can easily be measured. A similar elimination of interference from ascorbic acid and acetaminophen was obtained for analogous measurements of doped urine samples.

Assays of doped urine samples diluted 10-fold with phosphate buffer were performed at the castor oil electrode. Figure 6 illustrates the voltammetric response to successive standard additions of indomethacin after 3 min accumulation at open circuit condition and applying a medium-exchange procedure. The electrode response was linearly related to the indomethacin concentration within the range 1×10^{-5} M–3.0×10^{-4} M with a detection limit of 1.5×10^{-6} M by LSV. A linear range from 8.5×10^{-8} M to 1.5×10^{-7} M with a detection limit of 5×10^{-8} M was obtained in the differential pulse mode. Addition of 1×10^{-3} M ascorbic acid

or acetaminophen to the same urine samples did not affect the determination of indomethacin after medium exchange. The reproducibility of the total analytical process was determined from multiple measurement at each of the urine samples ($n = 5$). An average deviation of 3.0 % was obtained. Urine samples collected from volunteers yielded response characteristics similar to that of doped urine samples. Thus, It may be possible to determine indomethacin without interference from its metabolites.

4. Conclusion

The present study demonstrates that indomethacin is effectively extracted from aqueous solution into the castor oil electrode and that this process imparts high selectivity and sensitivity. Rapid and convenient recovery of the electrode allows a single electrode for multiple determination. The detection limit found for indomethacin was ca. $0.1\,\mu g\ mL^{-1}$ in urine sample after medium exchange. This is comparable to the detection limits of conventional high performance liquid chromatography. Moreover, the proposed method is faster and less expensive than chromatographic procedures where time-consuming steps for sample preparation are required.

5. References

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