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Cathodic adsorptive stripping voltammetric determination of the anti-inflammatory drug indomethacin

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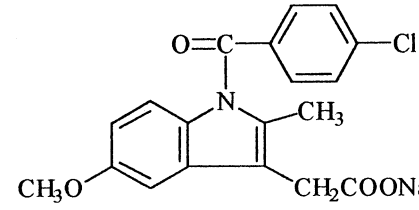
Abstract

Sensitive voltammetric methods have been developed for the determination of the anti-inflammatory, anti-pyretic and analgesic drug indomethacin sodium. The methods are based on the controlled adsorptive preconcentration of the drug on a hanging mercury drop electrode (HMDE), followed by tracing the voltammogram in a cathodic potential scan. The modes used are cyclic voltammetry (CV), cathodic stripping voltammetry (CSV) and differential pulse stripping voltammetry (DPSV). Amounts as low as 10 nM (10 ng ml^{-1}) (60 s preconcentration) by CSV and 0.5 μM (190 ng ml^{-1}) (300 s) by DPSV can be determined accurately. The R.S.D. at the 1×10^{-6} M level is 1.4%. The interference of some metal ions and the application of the method to analysis of urine, plasma and pharmaceutical formulations are described.

Keywords: Indomethacin sodium; Cathodic adsorptive stripping voltammetry; Urine; Serum; Indomethain capsule

1. Introduction

Indomethacin is a non-steroidal, anti-inflammatory agent with anti-pyretic and analgesic properties [1]. It is extensively used because of its excellent pharmaceutical properties. It is used to relieve the symptoms of ankylosing spondylosis, osteoarthritis, rheomatoid arthritis and gout. In addition to its analgesic uses, indomethacin promotes the constriction of patent ductus arteriosus (the opening that allows total blood to bypass the lungs) in newborns. The structural formula of indomethacin sodium is:



Several analytical methods have been used for the quantitative determination of the drug, e.g.

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spectrophotometry [2–4], fluorimetry [4], HPLC [5], UV spectrophotometry [6], colorimetry [7], ion selective electrode [8] and some electrochemical techniques have been studied [9–13]. The polarographic behaviour of the drug were studied using tast polarography and differential pulse polarography [14].

So far, it seems, no report has appeared in the literature describing the analytical utility of stripping voltammetry in the determination of indomethacin. However, no agreement has been found in the interpretation given for the electrode process [14]. Stripping voltammetry is an important technique for trace determination of many inorganic and organic substances [15]. The adsorptive stripping technique has been used successfully for the determination of subnanogram level of several drugs [16–20]. This technique eliminates both time-consuming solvent extraction steps and calculations of recovery common to photometric and chromatographic methods while the resulting accuracy and precision are at least comparable if not better than the above mentioned methods [21].

The present study deals with the quantitative determination of indomethacin using direct current and differential pulse stripping voltammetric methods. This technique is simple, rapid, sensitive, reproducible and easy to apply in routine usage.

2. Experimental

2.1. Instrumentation

An EG&G Princeton Applied Research (PAR, Princeton, NJ) model 264A polarographic analyzer stripping voltammeter was coupled with a PAR 303A static mercury drop electrode (SMDE) (drop size, medium, area of the drop, 0.014 cm^2). The polarographic cell (PAR model K0060) was fitted with an Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode. A PAR 305 stirrer was connected to the PAR 303A SMDE. A PAR RE 0089 X–Y recorder was used for the collection of the experimental data.

2.2. Materials and reagents

A 0.01 M stock standard solution of indomethacin sodium (supplied by E.I.P.I. (Egypt) under licence from Merck, Rahway, NJ) was prepared by dissolving the appropriate weight in doubly distilled water. Solutions of 1 mM copper(II), lead(II), zinc(II) and calcium nitrate (Merck) were prepared and used in the interference studies. Urine samples were taken from healthy persons, while serum samples were of human albumin.

2.3. Supporting electrolytes

Solutions of 0.1 M perchloric, phosphoric, Britton Robinson universal buffer and potassium chloride were used as the supporting electrolytes. A standard sodium hydroxide solution (0.1 M) was used to adjust the pH of supporting electrolyte using an Orion 601 A Precision Research ionalyzer digital pH meter.

2.4. Procedure

A universal buffer (1 ml, 10 mM) and 1 ml (10 mM) KCl solution (pH $\approx$ 4.8) as the supporting electrolyte were transferred and diluted to 10 ml in the cell. The cell content was de-aerated by passing pure nitrogen for about 16 min. An accumulation potential of -0.6 V was applied to a fresh mercury drop, the scan rate used for the cyclic voltammetry (CV) and the cathodic stripping voltammetry (CSV) was 100 mV s^{-1} while in differential pulse stripping voltammetry it was 10 mV s^{-1} with a pulse amplitude of -50 mV and a pulse duration of 1 s. After the accumulation step and a further 15 s equilibration time, the voltammogram was recorded (quiescent solution) and the potential was terminated at -1.6 V. The drug sample was added using an automatic pipettor (Volac (0–100 μl)). The solution was stirred while purging with nitrogen, then proceeded through the deposition and stripping step as before. All results were obtained at room temperature (25 ± 1°C) with a nitrogen atmosphere maintained over the solution surface.

2.5. Analysis of capsule

The whole content of one capsule (50 mg) was mixed with ethanol, then any undissolved excipients were removed by filtration. The filtrate and wash were collected quantitatively in a 25 ml measuring flask, then the analysis was done by the recommended procedure.

2.6. Analysis of urine

Polarographic methods are ideally suited for the analysis of urinary metabolites since they are usually very polar compounds. This is in contrast to gas chromatographic methods which often require derivatization to reduce the polarity of the compounds to yield volatile derivatives suitable for analysis [21].

A urine sample (10 μl) taken from a healthy person (female, age 14 years) was added to the polarographic cell containing the supporting electrolyte (0.01 M universal buffer, 0.01 M KCl and pH ≈ 4.8), i.e. the dilution factor of the urine sample in the cell was (1:1000). The voltammogram was recorded, then 10 μl spikes of the standard solution of indomethacin (10^{-5} M) was introduced into the cell and the voltammogram was recorded after each addition.

2.7. Analysis of serum

Analytical procedures of high sensitivity are required for the simultaneous determination of the parent compound and/or its administration. Therefore, differential pulse polarography and fast linear sweep polarography are the voltammetric techniques currently used for the routine determination of drugs in blood or plasma at these low concentration [21].

The voltammogram was recorded for the solution containing the supporting electrolyte and 10 μl serum. The standard solution of the drug was introduced into the cell as 10 μl spikes and the voltammogram was recorded after each addition.

3. Results and discussion

Preliminary investigations for 1 μM indomethacin in the presence of phosphate, nitrate or acetate using different pH values (1–12) and different concentrations of the supporting electrolytes (0.0–0.2 M) give small and unusable peaks. The effects of the concentration of universal buffer as the supporting electrolyte (0.01, 0.02, 0.05 and 0.1 M) and also the influence of pH (2, 4, 6, 8, 10 and 12) were studied. The effect of KCl concentration has been studied in the presence of 0.01 M universal buffer and 1 μM indomethacin. In the absence of KCl, an ill-defined peak was observed at different pH, viz 2.68, 4.8 and 6.18, whereas at pH = 7.08 the peak has completely disappeared. However, on the addition of a low concentration of KCl (1 mM), a small peak appeared and by increasing the concentration of KCl to 0.01 M, pH ≈ 4.8, a sharp cathodic peak was observed. This peak is due to the reduction of the carbonyl group (C=O) [14] and the presence of KCl may increase the ability of the analyte to adsorb onto the electrode surface. Therefore, the optimal conditions for studying the adsorptive stripping voltammetry of indomethacin is a mixture of 0.01 M universal buffer and 0.01 M KCl at pH ≈ 4.8.

The effect of the deposition potential on the peak current using the CSV technique was studied. The peak height increased on applying more negative potential, i.e. (from 0 to −0.6 V) and became a maximum when the voltammogram was taken between −0.6 and −1.4 V. Therefore, a potential of −0.6 V was used as the accumulation potential for all the experimental measurements.

When the cyclic voltammogram was taken for 1 μM indomethacin in the presence of 0.01 M universal buffer, 0.01 M KCl and pH ≈ 4.8, a single peak was obtained at a potential of −1.2 V as shown in Fig. 1 and no peak was observed on scanning in the positive direction. This means that either the concentration of the reduction product at the electrode surface is so small that it is not sufficient to bring about an oxidation peak or the electrode process is irreversible.

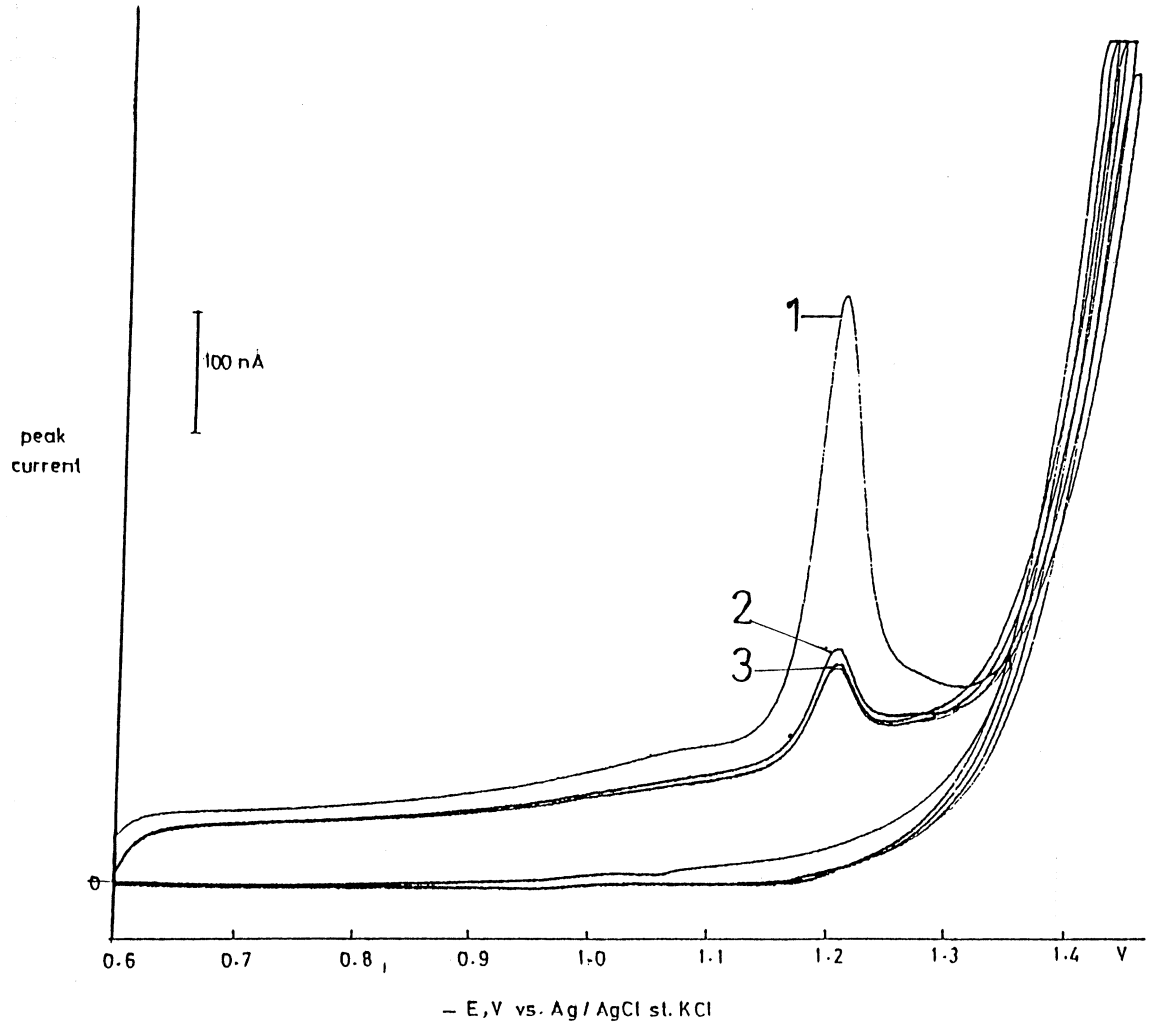

Fig. 1. Repetitive cyclic voltammograms for 1 μM indomethacin sodium in the presence of 0.01 M universal buffer, 0.01 M KCl, pH ≈ 4.8, accumulation potential −0.6 V and scan rate 100 mV s^{-1}. (1) First cycle, (2) second cycle and (3) third cycle.

3.1. Effect of potential scan rate

The effect of the potential scan rate v on the peak current or the peak potential was studied. The plot of log i_p (peak current) versus log v (scan rate) is given in line a, Fig. 2, where a straight line with a slope of 0.81 over the range 10–200 mV s^{-1} was obtained. This slope is in close agreement with a slope of 1.0 that is to be expected for the ideal reaction of the surface species [22]. A 15 mV negative shift in the peak potential was observed when the scan was increased in the given range. This is further evidence for the adsorption of the drug onto the electrode surface [23]. The plot of E_p versus log v as shown in line b, Fig. 2, is also linear (correlation coefficient = 0.995).

The current versus preconcentration time was studied for 0.1 μM indomethacin, 0.01 M KCl solution, two different concentrations of universal buffer (0.01 and 0.02 M) and pH ≈ 4.8, as shown in Fig. 3. It was noticed that the surface coverage occurs at 240 s at 0.01 M universal buffer while at the higher concentration (0.02 M) no surface coverage was observed up to 360 s. However, in the

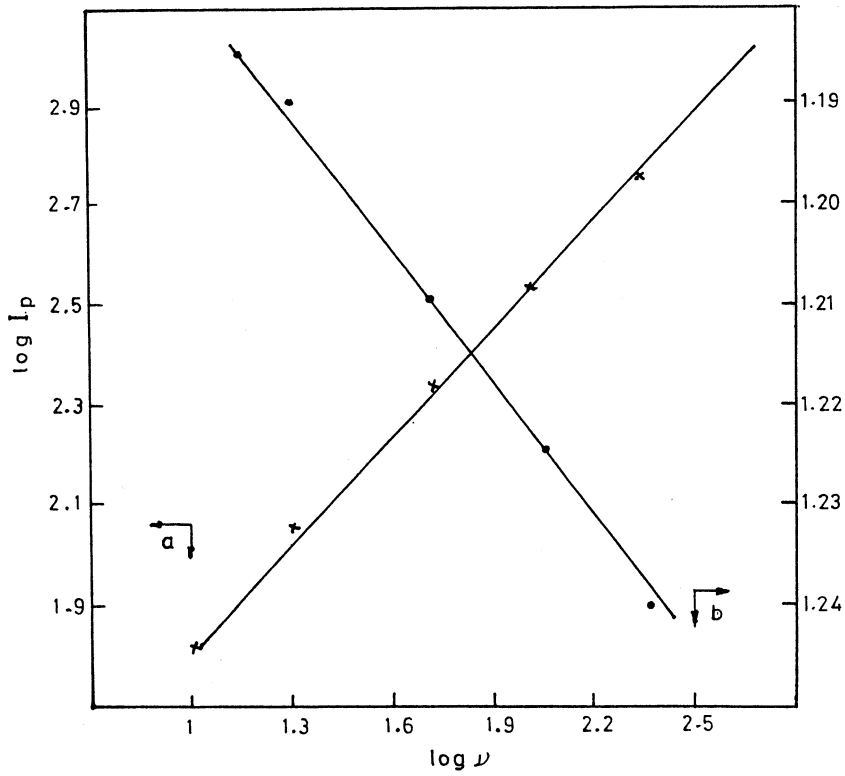

Fig. 2. Log v (scan rate) vs. log i_p (peak current a) and log v vs. E_p (peak current b) for 1 μM indomethacin in the presence of 0.01 M universal buffer, 0.01 M KCl and pH ≈ 4.8.

latter case the current is greatly diminished. Therefore, it is recommended to carry out the experiment at 0.01 M universal buffer to bring about a better lower detection limit.

Fig. 4 shows the relationship plot between the current and the preconcentration time for 1, 2, 5 and 10×10^{-8} M indomethacin in the presence of 0.01 M universal buffer, 0.01 M KCl at pH ≈ 4.8. Straight lines were obtained and the magnitude of the peak current increased with increasing concentration of the drug, the intersection of these lines with the peak current axis may be attributed to the fact that adsorption takes place at the equilibrium time [18] which was fixed at 15 s. The break at certain stirring times means that the surface coverage was attained at 0.1 μM indomethacin after (240 s) deposition time.

Table 1 summarizes the characteristics of the calibration plots established with different deposition times. The extension of the limit of linearity up to 1×10^{-7} M drug, indicates the strong adsorption behaviour of the drug for all deposition times applied.

The detection limit of 10 nM (3.8 ng ml^{-1}) indomethacin was determined using a 120 s preconcentration time by CSV, whilst for 0.5 μM (190 ng ml^{-1}) a 300 s deposition time was used for differential pulse stripping voltammetry. Generally, differential pulse stripping voltammetry is more sensitive than linear sweep stripping voltammetry due to the low charging current in the former [24], however, in the present situation this statement is not true. According to our results, the detection limit observed in the differential pulse mode is about 50 times greater than that required in the direct current mode. This can be explained by the low scan rate of the differential pulse technique. At this low scan rate, more and more buffer constituent may be adsorbed onto the electrode surface. Therefore, a lesser amount of the analyte is adsorbed in this case.

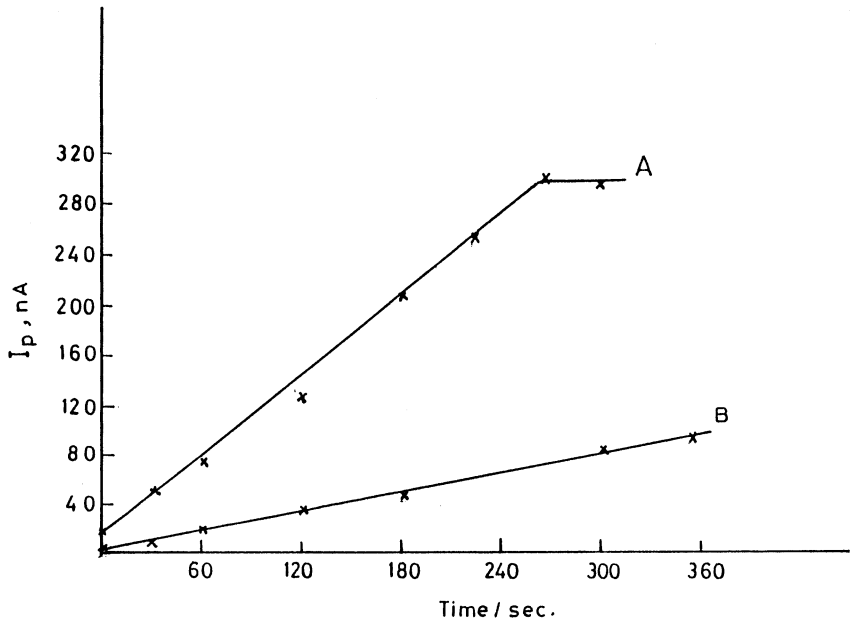

Fig. 3. Peak current vs. accumulation time in the presence of 100 nM indomethacin, 0.01 M KCl and pH ≈ 4.8 for (A) 0.01 and (B) 0.02 M universal buffer.

The reproducibility of the adsorption process was tested by repeating five experiments using CSV on 1 μM indomethacin in the presence of 0.01 M universal buffer and 0.01 M KCl (pH ≈ 4.8) with a 60 s deposition time. The R.S.D. is 1.42 and the coefficient of variation is 0.997.

The major sources of interferences in adsorptive stripping measurements are likely to be organic surfactants that compete with the drug for space on the mercury surface and other metal ions some of whose complexes may form adsorbable electroactive species. Interference by several metal ions were tested for 1 μM indomethacin in the presence of 0.01 M universal buffer, 0.01 M KCl (pH ≈ 4.8) and a 60 s deposition time. On the addition of 100 μM of Zn^{2+}, Pb^{2+}, Cu^{2+} and Ca^{2+} ions, individually or in an admixture, no change in the current signal was observed. On the other hand, on addition of 10 μM Fe^{3+} to 1 μM indomethacin the peak height of the drug decreased to one sixth of the original iron-free analyte. The large interference is mainly due to the formation of a phosphate and/or acetate complex of iron which adsorbed onto the electrode surface.

3.2. Application to a pharmaceutical formulation capsule

The adsorption of indomethacin can be used as an effective preconcentration step prior to the voltammetric measurements. In this way the amount of any pharmaceutical formulation capsule can be quantified by means of the method described above. The stripping voltammogram (60 s preconcentration time) was recorded. The content in the cell was determined by the standard addition method C.F. 5 from which 6.46 ppm or 6.5 ng ml^{-1} from the original sample can be analyzed and the recovery is 98.5% of the amount claimed.

3.3. Application to biological samples

3.3.1. Urine

The CSV was used for the determination of indomethacin in a urine sample. A well defined peak ($E_p = -1.2$ V) was observed. The peak current increased with increasing drug concentration from 10 to 100 nM as shown in Fig. 5. However

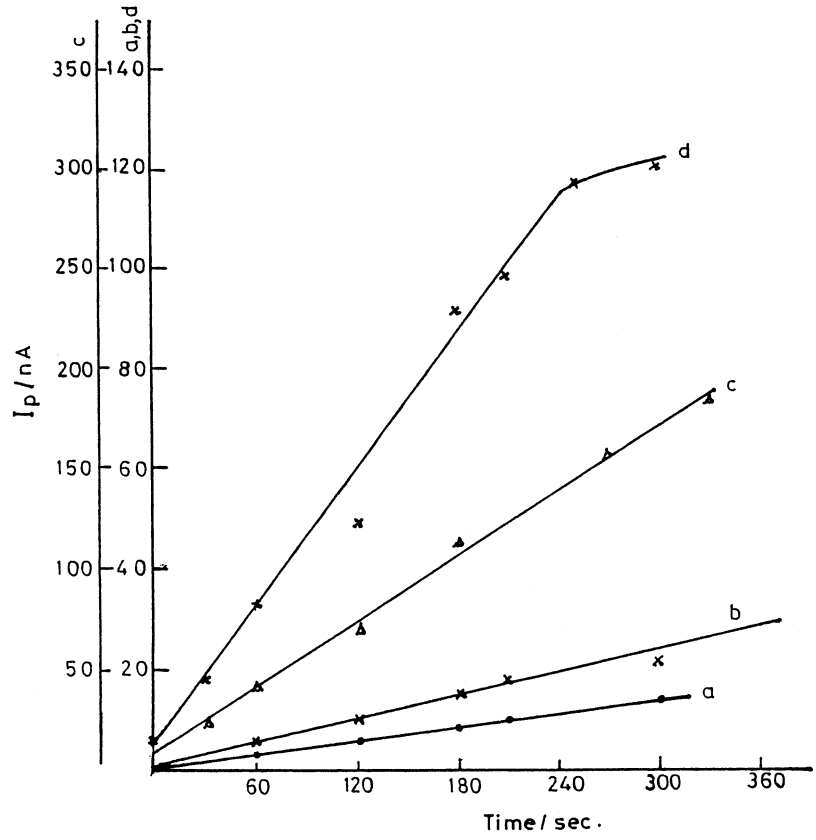

Fig. 4. Peak current vs. accumulation time in the presence of 0.01 M universal buffer, 0.01 M KCl, pH ≈ 4.8 and scan rate 100 mV s^{-1} for (a) 10, (b) 20, (c) 50 and (d) 100 nM indomethacin.

if another 10 μl urine sample is added to the final 100 nM indomethacin in the polarographic cell, the reduction peak completely disappears (dilution 1:500).

3.3.2. Serum

The analysis of the drug has also been studied in more complicated medium, viz serum. Indomethacin could be determined successfully with

Table 1
Characteristics of the indomethacin calibration plots

Deposition time (s)	Equation[a]	R.S.D. slope	R.S.D intercept	Correlation coefficient
30	$Y = 1.48X + 5$	0.19	0.15	0.996
120	$Y = 5.53X + 0$	0.15	0.14	0.994
210	$Y = 21.25X - 2.5$	0.12	0.17	0.992
300	$Y = 26.6X - 15$	0.25	0.17	0.998

In 0.01 M universal buffer, 0.01 M KCl and pH ≈ 4.8.
[a] Peak height (Y) in nA, concentration (X) in 10 nM.

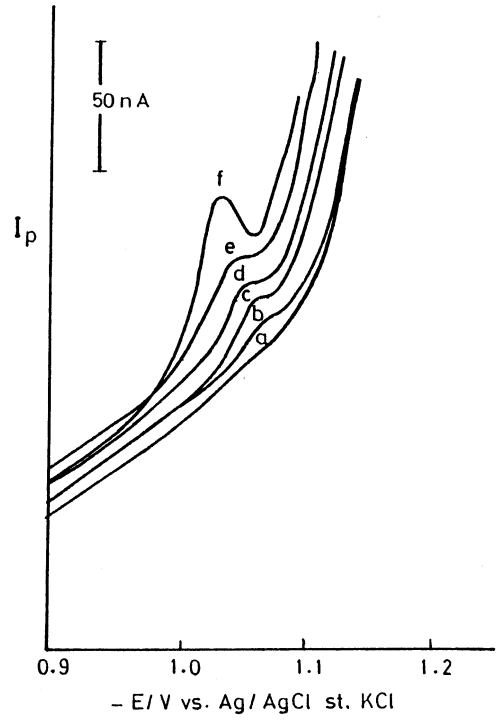

Fig. 5. Differential pulse stripping voltammetry for dilute urine (1:1000) containing different concentrations of indomethacin. (a) Urine sample and 0.01 M universal buffer and 0.01 M KCl; (b) 50; (c) 70; (d) 80; (e) 100 and (f) 200 nM indomethacin.

a deposition time of 60 s and using the standard addition method where 9 nM indomethacin can be detected. This means that 3.4 ng ml^{-1} of serum is detectable.

4. Conclusion

It is concluded that the present voltammetric method described in this paper is more rapid and highly sensitive compared with previous methods. Amounts as low as 3.8, 6.5 and 3.4 ng ml^{-1} could be detected in the analysis of the pure drug, a capsule and the drug in serum, respectively, compared to other methods, i.e. ion selective electrode [18], normal pulse polarography [14] and high performance liquid chromatography [25] where the limits of detection are 6.4, 24.8 and 0.01 ug l^{-1}, respectively.

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