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DIFFERENTIAL PULSE POLAROGRAPHY OF CADMIUM- AND LEAD-URATE AND ADSORPTIVE STRIPPING VOLTAMMETRIC DETERMINATION OF URIC ACID

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Summary—The complex formation between uric acid and zinc, cadmium and lead ions has been investigated using differential pulse polarography in 0 01*M* $NaNO_3$ It is found that the complexes formed by Cd(II) and Pb(II) ions with uric acid have the stoichiometry of 1 2 and the logarithmic values of the apparent stability constant are 9 47 and 11 7, respectively On the other hand, zinc(II) ions do not give any indication of complexation with uric acid A sensitive voltammetric method is developed for the quantitative determination of uric acid This method is based on controlled adsorptive preconcentration of uric acid on the hanging mercury drop electrode (HMDE), followed by tracing the voltammogram in the cathodic going potential scan The modes used are direct current stripping voltammetry (DCSV) and differential pulse stripping voltammetry (DPSV) The detection limits found were $8 \times 10^{-9}M$ (quiescent period 15 sec) by DPSV and $1\,6 \times 10^{-8}M$ by DCSV

Most purine derivatives in the diet are converted to uric acid and are excreted as such in man. Gout is characterized by an error in the metabolism of uric acid. It is normally excreted through the glomeruli and reabsorbed by the tubules in the kidney.[1] The metal–uric acid complexes are rarely studied,[2] whereas the electrochemical detection of uric acid have been studied by direct and stripping voltammetry using different electrodes [3–9] To the best of our knowledge the interaction of Cd(II), Pb(II) and Zn(II) ions with uric acid has not yet been studied

EXPERIMENTAL

Apparatus and reagents

The polarographic and voltammetric measurements were recorded with a PAR 264 A polarographic Analyzer coupled with a RE 0089 *x–y* recorder. The working electrode was a PAR 303 (static mercury drop electrode) equipped with a platinum auxiliary electrode and an Ag–AgCl saturated KCl reference electrode. The drop time was 1 sec and 0.5 sec in the DPP and DPSV, respectively.

Reagents and solutions

A stock solution of uric acid (BDH) was prepared by dissolving the appropriate amount in bidistilled water. Sodium hydroxide was added to facilitate the solubility process of uric acid. Supporting electrolytes were analytical grade reagents and were prepared in doubly distilled water. Stock solution (0 01*M*) of the metals as used. All measurements were carried out at 25 ± 1°C.

Procedure

A known volume (10 ml) of the 0 01*M* supporting electrolyte was added to the cell and deaerated by passing nitrogen for 12 min The scan rate was 100 mV/s for DCSV and 5 mV/s for DPSV. Pulse amplitude was 25 mV

RESULTS AND DISCUSSION

Differential pulse polarography of the metal–uric acid interaction at low concentrations

The complexes between uric acid and lead (II) and cadmium (II) ions were investigated using differential pulse polarography in 0.01*M* sodium nitrate ($\mu = 0.01$) pH 7 ± 0.05. The experiments were carried out by keeping the concentration of the metal ions at $1.0 \times 10^{-5}M$ to which different increments of uric acid were added $(0.8–8\,0) \times 10^{-4}M$. It is noticed that on

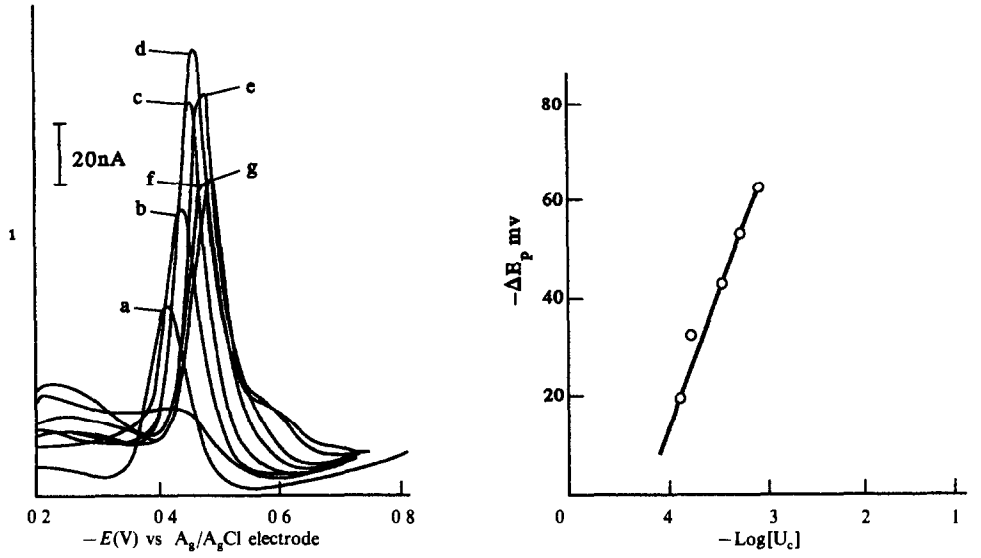

Fig 1 Differential pulse polarograms for $1 \times 10^{-5}M$ Pb(II), in presence of 0 01M $NaNO_3$ and at different concentrations of uric acid (a) Pb(II) alone, (b) $0\,8 \times 10^{-4}M$, (c) $1\,6 \times 10^{-4}M$, (d) $3\,2 \times 10^{-4}M$, (e) $4\,8 \times 10^{-4}M$, (f) $6\,4 \times 10^{-4}M$, (g) $8 \times 10^{-4}M$ UC

increasing the amount of uric acid, the shift in peak potential occurs as shown in Fig. 1 (for Pb) and Table 1.

The linear relation between ΔE_p and log [UC] does not cover the whole range of uric acid concentrations. At $6.4 \times 10^{-4}M$ concentration of UC the surface coverage is attained during the drop life (1 sec). At a higher concentration of uric acid, it is assumed that Pb(II)–UC and Cd(II)–UC complexes and uric acid are accumulated at the electrode surface. The current of simple metal ion (Pb and Cd) is lower than that observed in the presence of ligand. On addition of uric acid, enhancement in the current was noticed indicating the adsorption of lead and cadmium complexes. The shift in peak potentials may, therefore, be due to complex formation and adsorption.

The half-peak width ($\omega_{1/2}$) values are 50–60 mV indicating that two electrons are consumed in the reduction process and the reaction is reversible (pulse amplitude = 25 mV) in close agreement with theoretical value calculated by Parry and Osteryoung [10]

Table 1 Variation of differential pulse peak potential, of the peak current as a function of uric acid, for $1 \times 10^{-5}M$ Pb(II) and Cd(II) uric acid systems

[UC] ($\times 10^{-4}M$)	Pb(II)		Cd(II)	
	$-\Delta E_p$ (mv)	i_p (nA)	$-\Delta E_p$ (mv)	i_p (nA)
0 0	—	24	—	64
0 8	20	78	8	67
1 6	33	112	15	93
3 2	43	126	25	152
4 8	53	84	35	210
6 4	63	84	45	190
8 0	63	84	45	225

The basic relationship between potential shift and the ligand concentration can be expressed by Linganes equation[11]

$$E^c_{1/2} - E^s_{1/2} = \frac{0.059}{n} \log K_F - \frac{0.059}{n} p \log[X]$$

The measured peak potential with differential pulse polarography is correlated to the ordinary half-wave potential (direct current polarography) by the following equation

$$E_p = E_{1/2} - \frac{\Delta E}{2}$$

where ΔE is the pulse amplitude. Hence, the shift in the peak potential in complex formation can be rewritten in the form

$$E^c_p - E^s_p = \frac{0.059}{n} \log k_F - \frac{0.059}{n} P \log[X],$$

where E^c_p is the peak potential of the complexed ion in the presence of ligand with concentration X, E^s_p is the peak potential in the absence of ligand (aquo-metal ion), n is the number of the electrons gained in the reduction, K_F is the apparent formation constant and p is the complex coordination number.

The slopes of the straight lines obtained in plotting ΔE_p *vs.* log[UC] are 0.055 and 0.049 for lead and cadmium, respectively. The corresponding coordination number is 1.63 and 1.8 for Cd(II) and Pb(II), respectively, which can be

approximated to 2. This gives the stoichiometry of 1:2 metal to ligand ratio and the logarithmic values of the apparent stability constant are 9.47 and 11.7 for lead and cadmium, respectively

Effect of metal concentration

Differential pulse polarograms of Pb(II)–UC and Cd(II)–UC systems in the range of 5×10^{-6}–$1 \times 10^{-4}M$ of lead or cadmium ions and in a constant concentration of uric acid ($6.4 \times 10^{-4}M$) in $0.01M$ sodium nitrate as supporting electrolyte are represented graphically in Fig. 2 (for Pb only). A new, less electronegative peak at concentration $\geqslant 5 \times 10^{-6}M$ of lead (−0.64 V) and cadmium (−0.615 V) ions is observed which may be attributed to the reduction of the aquo-metal ions (easily reduced) which is in equilibrium with the metal urate complex. On plotting the peak current *vs.* concentration of metal ions, straight lines passing through the origin are obtained.

The complex formation between uric acid and Zn(II) ion was also investigated by differential pulse polarography in the presence of $0.01M$ $NaNO_3$ as the supporting electrolyte ($\mu = 0.01$), and different concentrations of uric acid. It is found that, on increasing the concentration of UC, no shift in peak potential occurs. The peak potential of Zn(II) ions either in nitrate solution or nitrate–urate mixture is at −1.03 V (Ag/AgCl saturated). This indicates that Zn(II) ion does not form complexes with urate solution under the present set of conditions.

Linear sweep adsorptive stripping analysis of uric acid

From the above results, it is assumed that the metal(II)–uric acid systems exhibit adsorption behavior at the mercury surface and uric acid displays surface-active characters too. Uric acid is not reducible at the mercury electrode and becomes reducible after prolonged exposure to air.[12] Zongpeng *et al.*[9] reported that the polarographic currents and cathodic stripping peaks of uric acid are due to the formation of slightly soluble compounds with mercury.

In the present work, the cathodic stripping analysis of these Hg compounds has been reinvestigated in a trial to verify the optimal condition for uric acid analysis. The modes used are linear sweep (100 mV/sec) and differential pulse stripping voltammetry (5 mV/sec, 25 mV pulse amplitude.

Adsorptive linear sweep stripping voltammetric determination of uric acid

The effect of the supporting electrolyte composition such as sodium nitrate, sodium perchlorate, potassium chloride, disodium hydrogen phosphate (pH = 9.8), Borax solution (pH = 9.1) and sodium acetate–acetic acid buffer (pH = 5.65) on the direct current adsorptive cathodic

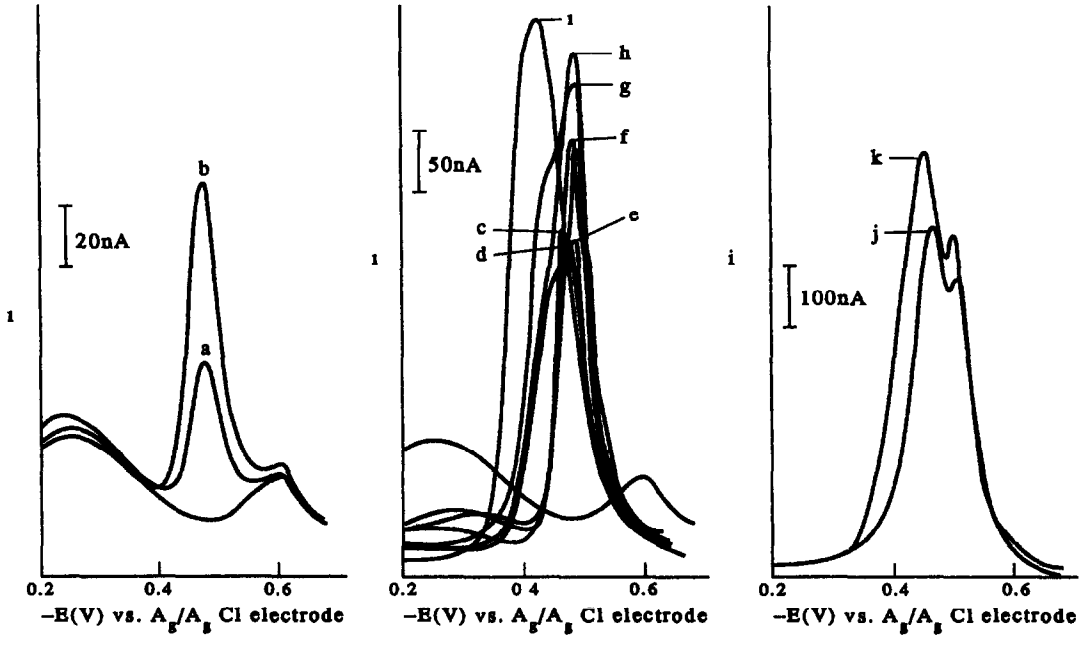

Fig 2 Dp polarograms for $6.4 \times 10^{-4}M$ UC, in the presence of $0.01M$ $NaNO_3$, and different concentrations of Pb(II) ion (a) $5 \times 10^{-6}M$, (b) $1 \times 10^{-5}M$, (c) $2 \times 10^{-5}M$, (d) $3 \times 10^{-5}M$, (e) $4 \times 10^{-5}M$, (f) $5 \times 10^{-5}M$, (g) $6 \times 10^{-5}M$, (h) $7 \times 10^{-5}M$, (i) $8 \times 10^{-5}M$, (j) $9 \times 10^{-5}M$, (k) $1 \times 10^{-4}M$, of Pb(II) ion

stripping voltammetry at $1.6 \times 10^{-7}M$ of UC at constant ionic strength ($\mu = 0.01$) was studied.

Preliminary investigation showed that the preconcentration potential had a drastic effect on the voltammograms. For experiments being done in KCl, $NaNO_3$ and Na_2HPO_4 it is concluded that the preconcentration potential should be held at +0.25 V against Ag–AgCl electrode. More positive potentials may give a large peak due to the oxidation of mercury itself and the interferences from chloride and hydroxyl ions Application of a more negative potential may produce ill-defined peaks or no oxidation of uric acid occurs prior to the negative going scan.

In the case of borax, potassium chloride and disodium hydrogen phosphate solutions, large peaks in the same position as that of the uric acid peak were observed due to interference, therefore these media are not recommended in the analysis of uric acid by cathodic stripping voltammetry.

It has also been shown from our experiments that these peaks are produced even in the absence of the analyte. This may be due to the interferences of OH^- ions.

However, Zonbeng *et al.*[9] reported that alkaline borax and Britton–Robinson buffer can be used. The erroneous conclusion may come from the fact that the supporting electrolytes should be tested alone (*i.e.* without the addition of the analyte) by cathodic stripping voltammetry under the same set of conditions to clarify the interferences that may be inherent with the analysis.

In the presence of $NaNO_3$ and $NaClO_4$ (pH = 6.4), a well-defined peak was observed with a small increase in height as the preconcentration time increases as shown in [Figs. 3(a) and 3(b)]. However, a more sensitive peak was observed at +0.05 V for $1.6 \times 10^{-7}M$ uric acid in $0.01M$ acetic acid–acetate buffer (pH 3.76–6 75) using different preconcentration times. The plot of the peak current versus pH values shows that current decreases on increasing the pH of the solution and the current attains a constant value at pH values of 5.65 and 6.75. Above these ranges of pH, the OH^- ion obscures the reduction peak of uric acid.

The effect of ionic strength ($1\ 6 \times 10^{-7}M$ UC) was studied using different ionic strengths *viz.* 0.01, 0.03, 0.05 and $0.07M$ sodium acetate–acetic acid buffer (pH = 5.65). A single peak was observed in each case. The enhancement of the peak current is largely decreased by increasing the ionic strength The plot of current *vs* preconcentration time (Fig. 4) gives straight lines with slopes which equal 3 7, 3.0, 2.3 and

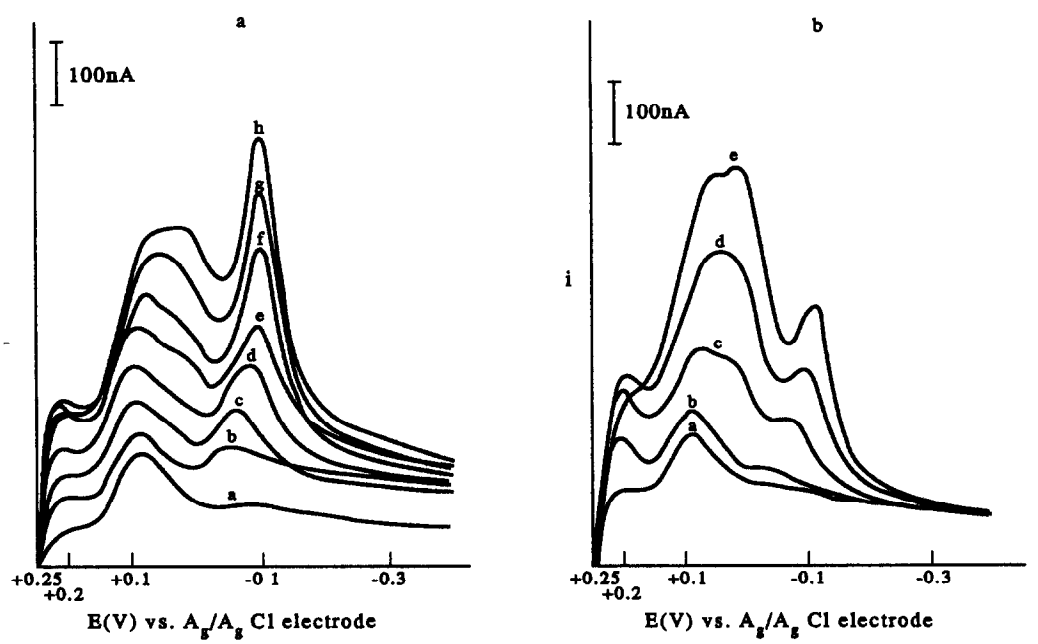

Fig 3 (a) DC stripping voltammograms for $1\ 6 \times 10^{-7}M$ uric acid, in the presence of $0\ 01M$ sodium nitrate, scan rate = 100 mV/sec and equilibrium time = 15 sec (a) Residual, (b) 0 0, (c) 30, (d) 60, (e) 90, (f) 120, (g) 150, (h) 180 sec (b) DC stripping voltammograms for $1\ 6 \times 10^{-7}M$ uric acid, in the presence of $0\ 01M$ $NaClO_4$, scan rate = 100 mV/sec and equilibrium time = 15 sec (a) Residual, (b) 0 0, (c) 30, (d) 60, (e) 90 sec

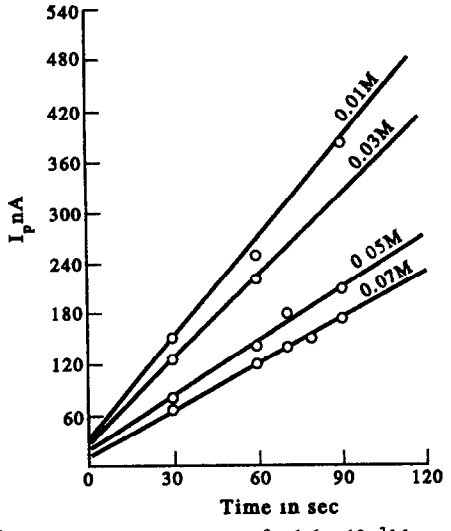

Fig 4 t_p *vs* preconcentration time for $1\,6 \times 10^{-7}M$ uric acid

1.7 nA/sec for 0.01, 0 03, 0.05 and 0.07*M*, respectively This indicates that the best ionic strength is $0\,01M$ sodium acetate–acetic acid (pH = 5.65).

The effect of potential scan rate (v) on both current and potential of the peak of $8 \times 10^{-7}M$ uric acid as evaluated for the adsorbed UC. A plot of log i_p *vs.* log is linear over the 10–200 mV/sec range with a slope of 0.88, which is in close proximity to a slope of 1.0 that is expected for an ideal reaction of surface species.[13] A 20 mV negative shift in the peak potential p was observed upon increasing the scan rate in the range given The plot of E_p *vs.* log v is also linear ($r = 0.98$).

Figure 5 shows the peak current *vs.* preconcentration time plots for $1\,6 \times 10^{-7}$ and $8 \times 10^{-7}M$ uric acid. The slopes of the linear sections are 3.6 and 5.9 nA/sec for $1.6 \times 10^{-7}M$ and 9.5 nA/sec for $8 \times 10^{-7}M$ UC, with intersects at current axis and breaks at certain preconcentration times. The differences in the slopes are due to the differences in the concentration of the analyte, (slopes being greater for higher concentration). The breaks at certain preconcentration times means that full surface coverage is attained.

To reveal the adsorption of the analyte without convection (stirring), some experiments were carried out with $8 \times 10^{-7}M$ UC in quiescent solution, a linear dependence of the peak current on the square root of the preconcentration time was observed for a quiescent solution in good agreement with the prediction of Delahay and Fike[14] for the case of semi-infinite linear diffusion.

With $2.6 \times 10^{-7}M$ UC solution, full surface coverage was achieved after 30 sec of stirring. Under these conditions, the charge transferred in the reduction step corresponds to 1.86×10^{-6}C as calculated by integration of the peak. A monolayer surface coverage of 6.00×10^{-10} mole/cm^2 can be estimated by division of the charge by nFA ($n = 2$ electrons, $F = 96500$ C and $A = 0.016$ cm^2). Consequently, each adsorbed uric acid occupies an area of 0.276 nm^2.

Adsorptive differential pulse stripping voltammetric determination of uric acid

Adsorptive stripping of uric acid can also be performed by changing the working mode.

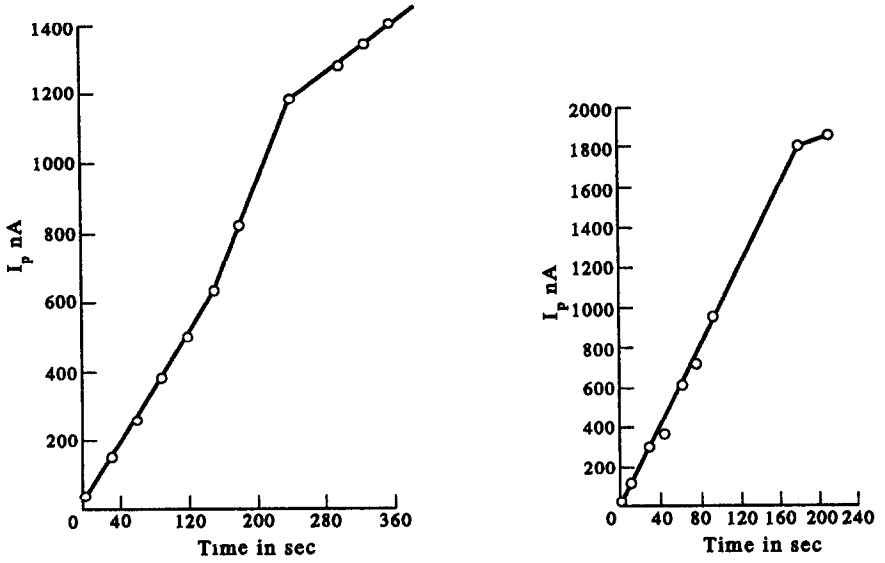

Fig 5 (a) t_p *vs* preconcentration time for $1\,6 \times 10^{-7}M$ uric acid (b) t_p *vs* preconcentration time for $8 \times 10^{-7}M$ uric acid

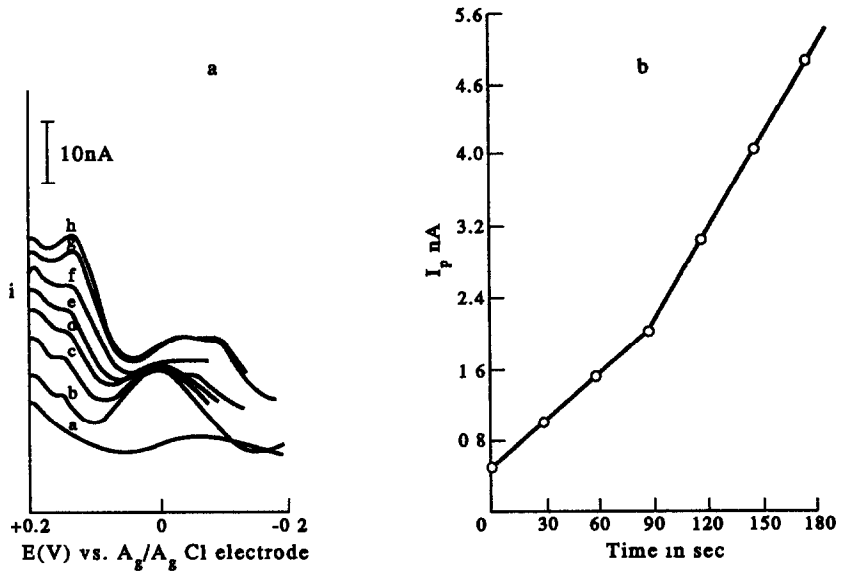

Fig 6 (a) Differential pulse stripping voltammograms for $8 \times 10^{-9}M$ uric acid, in the presence of 0 01M acetate-buffer (pH = 5 65), scan rate = 100 mV/sec and equilibrium time 15 sec (a) Residual, (b) 30, (c) 60, (d) 90, (e) 120, (f) 150, (g) 180, (h) 210 sec (b) i_p *vs* preconcentration time for $8 \times 10^{-9}M$ of uric acid

Differential pulse voltammetry is more sensitive than the linear sweep mode though it takes a longer time due to the slow rate scanning. The differential pulse stripping voltammetry of $8 \times 10p^{-9}M$ UC was carried out in acetate buffer pH = 5.65 at constant ionic strength $\mu = 0.01$. The accumulation of uric acid on the electrode surface may be given by studying the effect of preconcentration time on the peak current [Fig 6(a)]. On plotting the peak current *vs.* preconcentration time, a straight line is observed [Fig 6(b)] with a break at 90 sec and intersect at 0 5 nA, indicating that the full surface coverage is attained at 90 sec

Using DPSV, a linear relation was observed in the range of 1.6×10^{-8}–$3.2 \times 10^{-7}M$ UC with a break at $2\,6 \times 10^{-7}M$ UC which indicates that the surface coverage is attained at this concentration

Linear relations between peak current and preconcentration time of $8 \times 10^{-9}M$ uric acid were observed This indicates that this concentration may be considered as the lower limit of detection, *i e.*, 1.3 ppb using 25 mV pulse height.

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