

The concept of pseudodiffusion-controlled reactions stimulates new approaches to a substituent and solvent effect on reaction rate. In certain cases an increase of a substituent volume or an adhering of a solvent molecule to a reagent molecule may result only in a more severe steric constraint to a reaction controlled by diffusion. Concrete examples of such a behaviour have been found /3/.

Many pseudodiffusion-controlled reactions have been discovered recently. They are certain electron and proton transfer reactions, enzyme reactions, energy transfer, DNA recognition and binding, fluorescence quenching /2,9/. It has been shown recently that chemical anisotropy strongly affects reactivity of photogenerated radical pairs /10/.

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#### POLAROGRAPHIC STUDIES ON THE COPPER-ADENINE SYSTEM

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#### SUMMARY

The polarographic behaviour of adenine and of the copper-adenine system was studied in a medium of sulphuric acid.

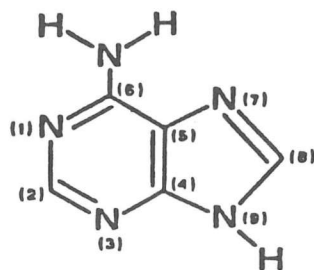
It was found that adenine is irreversibly reduced at dropping mercury electrode (DME) and that its differential pulse polarography (DPP) peaks are suitable for the analytical determination of adenine concentrations.

In the presence of adenine, copper(II) ions are reduced in two consecutive steps indicating that adenine may complex with copper(I) ions, forming a species sufficiently stable in solution.

The influence of pH and of the concentrations either of copper(II) ions or adenine on the electrochemical reduction of the system were also studied.

#### 1 - Introduction

Adenine (6-aminopurine) is one of the three most important components of nucleic acids, and is composed of a pyrimidinic nucleus attached to an imidazole nucleus.



Given the great biological interest of adenine and some of its derivatives, either for its catalytical action in biochemical reactions in the cell, as constituents of nucleic acids or because compounds with an imidazole nucleus are of great clinical importance in the treatment of cancer, these substances have been the subject of study by many investigators whose work has been the subject of a series of review articles [1, 2, 3].

Equally interesting, is the study of the influence of metallic cations on the reactivity of imidazole compounds [4-9], amongst which adenine is included.

The Cu(II)/adenine system stands out as particularly important, due to the biological action not only of adenine but of the Cu(II)/Cu(I) system as well. That is the reason for our interest in studying these systems by electrochemical means.

The electrochemical behaviour of adenine and of the Cu(II)/Cu(I) system in the presence of adenine was studied in a sulphuric acid medium.

The techniques used were DC and DP Polarography and Cyclic Voltammetry. Preference was given to DPP due to the clarity of results obtained.

## 2 - Instrumentation and Reagents

For the DC and DP polarography an EG & G / PAR model 174-A polarographic analyser was used connected to an Omnigraphic Houston 2000 recorder. For cyclic voltametry a EG & G/PAR model 175 Universal Programmer was coupled to the model 174-A. A three electrode, thermostatted cell was used to carry out experiments at  $25.0 \pm 0.1$  C.

The working electrode was the dropping mercury, the auxiliary electrode of platinum and a standard calomel electrode was used as reference.

Adenine, sulphuric acid, copper sulphate and other chemicals were of "analytical" quality. Solutions were prepared with de-ionised and doubly-distilled water.

Solutions of adenine and of copper sulphate were made up in 0.25 M sulphuric acid, this acid also serving as supporting electrolyte.

The influence of pH was investigated by varying the concentration of the acid or by the addition of aqueous sodium hydroxide.

Mesurements of pH were made with a Beckman 4500 digital pH meter.

Solutions were de-oxygenated by the passage of oxygen-free nitrogen.

## 3 - Results and discussion

### 3.1 - Voltammetric Measurements of Adenine

Voltammetric techniques show that, in a sulphuric acid medium, adenine is irreversibly reduced at the mercury electrode.

In cyclic voltammetry a reduction peak was observed at potentials between -1.0 and 1.1 volt (vs. SCE) that was not followed by a corresponding oxidation peak. This is indicative of the irreversibility of the reduction of adenine under these experimental conditions.

In DC polarography, a well defined reduction wave is obtained, the half wave potential of which,  $E_{1/2}$ , appears at -1.2 v (vs. SCE) (Fig.1).

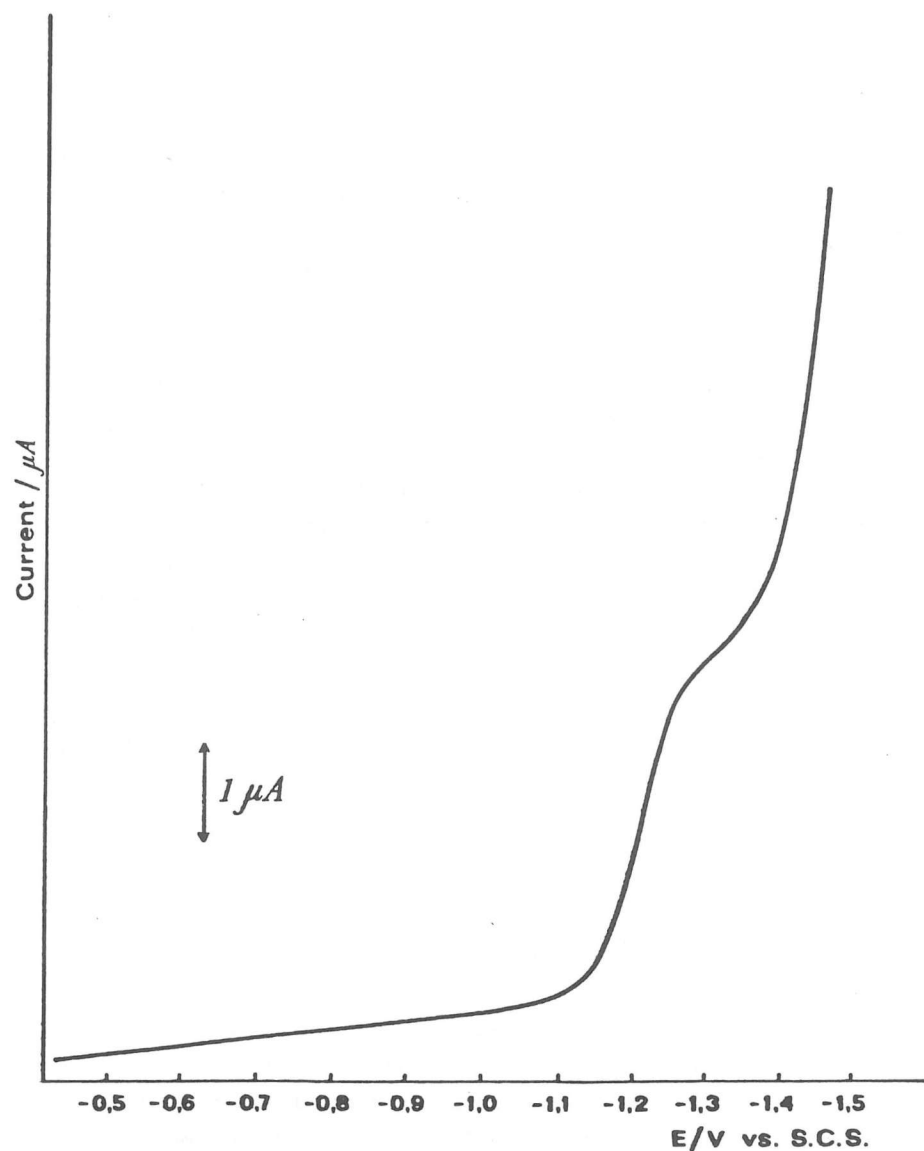


Fig. 1 - DC polarogram of  $10^{-4}$  M adenine in 0.25M  $H_2SO_4$   
 Drop Time (DT)=0.5 s;  
 Scan Rate=10 mV/s; T=25°C; Height of Mercury Reservoir ( $h_{Hg}$ )=  
 =75 cm.

Data obtained from DC polarograms for the relationship  $\log i / (i_d - i)$  as a function of applied potential, allow determination of  $\alpha n$  by means of the following equation available in the literature (10):

$$E = E_{1/2} - \frac{0.0542}{\alpha n} \log \frac{i}{i_d - i}$$

the value of 0.954 obtained for  $\alpha n$  comparing favorably with the value of 1.09 quoted for adenine by Janik and Elving (11).

In DPP a reduction peak was observed for potentials about -1.2V, the current intensity of which,  $i_p$ , being practically independent of pH for pH < 4. In this range of pH, the current intensity depends on the adenine concentration, showing a linear relationship for the range of concentrations from  $2.73 \times 10^{-5}$  M up to  $6.62 \times 10^{-5}$  M. For adenine concentrations less than  $2.73 \times 10^{-5}$  M the ratio  $i_p / [\text{adenine}]$  is somewhat greater which could be explained assuming that adenine is adsorbed on the electrode surface before being reduced (Fig. 2).

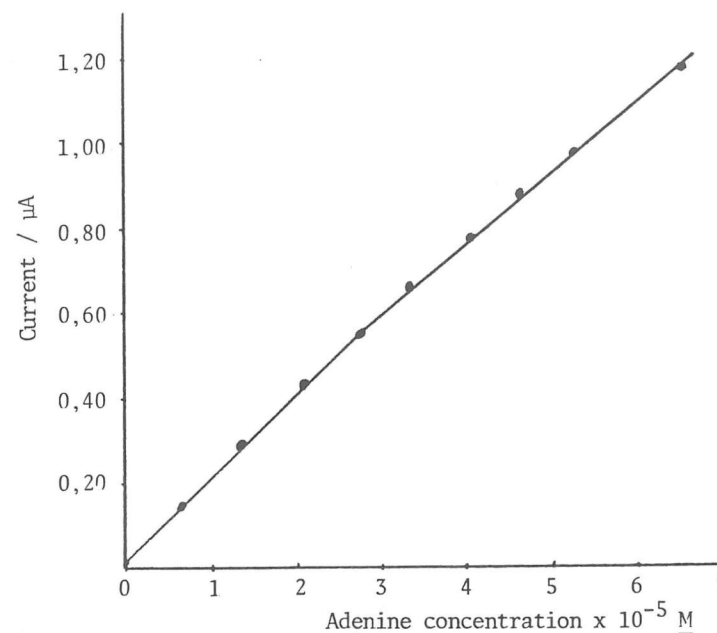


Fig. 2 - Peak current of adenine reduction as a function of adenine concentration.

### 3.2 - pH influence on adenine reduction

We have observed in DPP a small variation of the current intensity of the adenine reduction peak as the pH changes in the interval  $1 < \text{pH} < 4$ .

By increasing pH beyond 4, the peak current gradually diminishes and disappears at pH values greater than 6. This is in agreement with similar results of several authors (Ref. 2 and references there in).

Considering that the  $\text{pK}_a$  of protonated adenine equals 4.1 (2), such behaviour suggests that the disappearance of the peak for  $\text{pH} > 4.8$  is related to the disappearance of the protonated form of adenine. These results confirm the information, available in the literature, that the electroactive species is the protonated adenine. This protonation of adenine seems to occur at the N-1 position (2, 11, 12, 13, 14).

To extend the study of pH effects experiments on reduction of adenine in more acidic media ( $\text{H}_2\text{SO}_4$  up to 2 M) have been undertaken. At these pH values the adenine reduction peak separates into two close peaks. Such behaviour could be explained by assuming that the adenine molecule accepts a second proton in much more acidic media, this doubly protonated species being electroactive (Fig. 3).

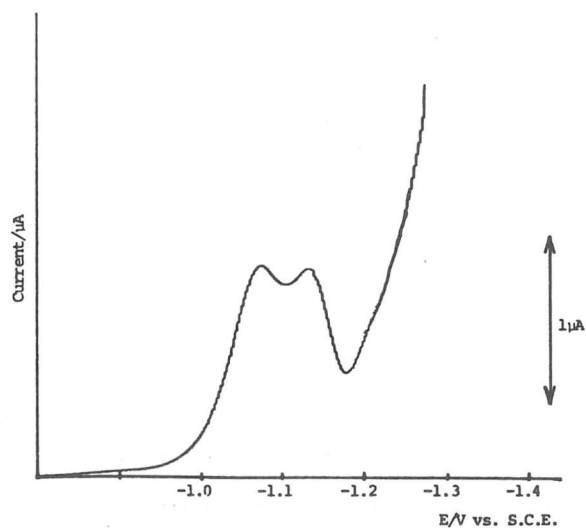


Fig. 3 - DP polarogram of adenine  $10^{-4}$  M in 2 M  $\text{H}_2\text{SO}_4$ .  
 $\text{DT}=0.5\text{s}$ ;  $A=25\text{mV}$ ; Scan Rate= $5\text{mV/s}$ ;  $T=25^\circ\text{C}$ ;  $h_{\text{Hg}}=75\text{cm}$ .

The occurrence of a doubly protonated adenine molecule in strong acidic media was also admitted by other investigators (16,17,18,19) on the bases of spectroscopic measurements.

### 3.3 - Interaction of adenine with copper cations

In the presence of adenine, the copper(II) ions are reduced to copper(0) at the mercury electrode in two distinct steps (Fig.4; Curve 1)

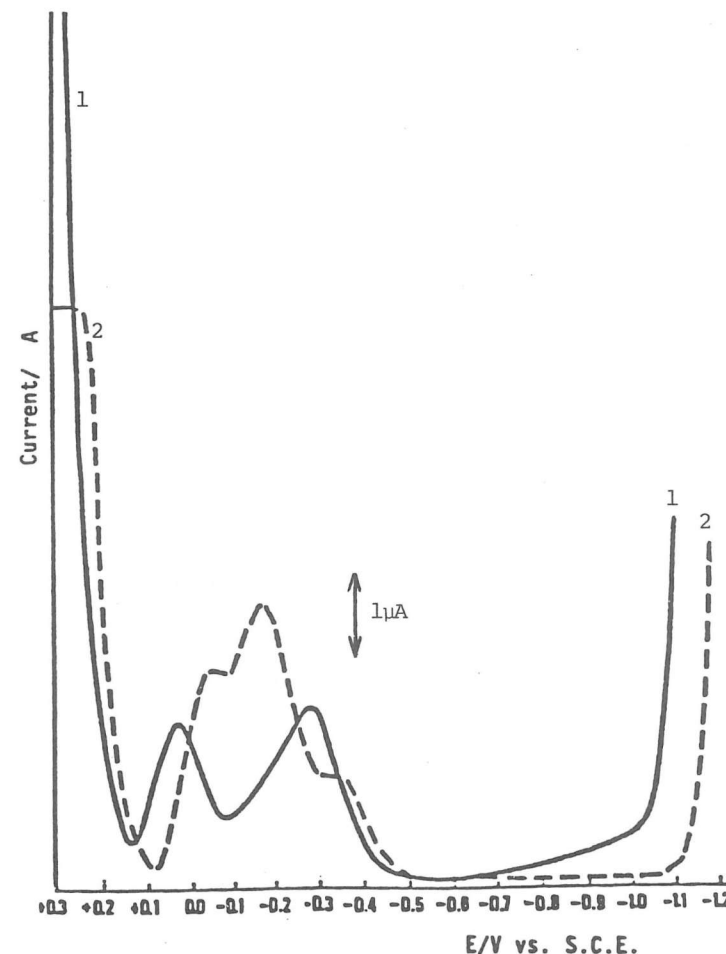


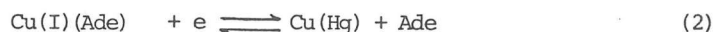
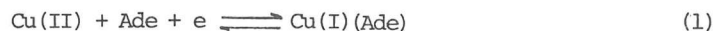
Fig. 4 - DP polarograms of  $10^{-4}$  M  $\text{CuSO}_4$  in the presence of adenine for different ratios copper(II): adenine. Supporting electrolyte  $0.25\text{M}$   $\text{H}_2\text{SO}_4$ . Ratio Copper(II): adenine = 1:1 (curve 1) and  $> 1:1$  (curve 2).  
 $\text{DT}=0.5\text{s}$ ;  $A=50\text{mV}$ ; Scan Rate= $10\text{mV/s}$ ;  $h_{\text{Hg}}=75\text{cm}$ ;  $T=25^\circ\text{C}$ .

This indicates that adenine complexes with copper(I), stabilizing this form in solution. We confirmed that the formation of this complex, and its electrochemical reduction, is influenced not only by the pH values but also by the concentrations of copper(II) and of adenine, and by the relative proportion of these species in solution.

a) Influence of copper and adenine concentrations

As mentioned previously, copper exhibits two one-electron reduction waves when complexed with adenine for copper concentrations up to  $10^{-4}$  M and as long as the adenine/copper(II) ratio is such that there is no excess of copper(II) over adenine (Fig. 4; Curve 1).

The reactions giving rise to the first and second reduction waves may be represented as follows:



Changes in half-wave potentials with increasing adenine concentrations are exemplified in Table 1.

Table 1 - Half-wave potentials of the reduction waves of  $2 \times 10^{-4}$  M copper(II) in the presence of adenine in a 0.25 M  $\text{H}_2\text{SO}_4$  medium

Concentration of adenine M	$-\log[\text{adenine}]$	First wave $E_{1/2} / \text{V}^*$	Second wave $E_{1/2} / \text{V}^*$
$3.1 \times 10^{-3}$	2.62	-0.033	-0.365
$4.0 \times 10^{-3}$	2.40	+0.045	-0.375
$6.0 \times 10^{-3}$	2.22	+0.105	-0.375
$7.0 \times 10^{-3}$	2.15	+0.125	-0.375
$8.0 \times 10^{-3}$	2.10	+0.135	-0.375
$8.8 \times 10^{-3}$	2.06	+0.145	-0.375
$9.8 \times 10^{-3}$	2.01	+0.165	-0.375

\* - Half-wave potentials measured by DPP were corrected for Pulse Amplitude using the relation  $E_{1/2} = E_{\text{peak}} - (\text{Pulse Amplitude})/2$ .

From this table it is possible to deduce, for the two reduction waves, the relationship between the half-wave potential and the concentration of adenine over the whole range of concentrations studied:

$$E_{1/2} [\text{Cu(II)/Cu(I)}] = +0.817 + 0.325 \log [\text{Adenine}] \quad (3)$$

$$E_{1/2} [\text{Cu(I)/Cu(0)}] = -0.375 \quad (4)$$

In a series of experiments, in which the adenine concentration was kept equal to  $1 \times 10^{-4}$  M and the copper(II) concentration was varied, it was observed that the two above mentioned peaks appeared as long as Cu(II)/Ade proportion did not exceed the 1:1 ratio. For relative concentrations of copper(II)/adenine greater than 1:1, there appears between these two peaks a third reduction peak, the  $E_p$  potential of which remains between -0.10 to -0.17 V (vs. SCE) (Fig. 4; Curve 2), which is the reduction potential of copper(II) in this medium without the presence of adenine.

Furthermore, the current of this peak increases with increasing Cu(II) concentration such that this peak may correspond to the reduction of non-complexed copper(II) to copper(0):



b) Influence of pH on the copper-adenine interaction

Even though the  $i_p$  values are not altered when the pH of the solution is varied, the  $E_p$  values are sensitive to those variations.

In fact, the potentials of the peaks concerned with the reduction of the copper/adenine complexes vary differently as pH increases: the peak corresponding to the first reduction moves to more positive potentials, where as the peak corresponding to the second reduction moves to more negative potentials (Fig. 5).

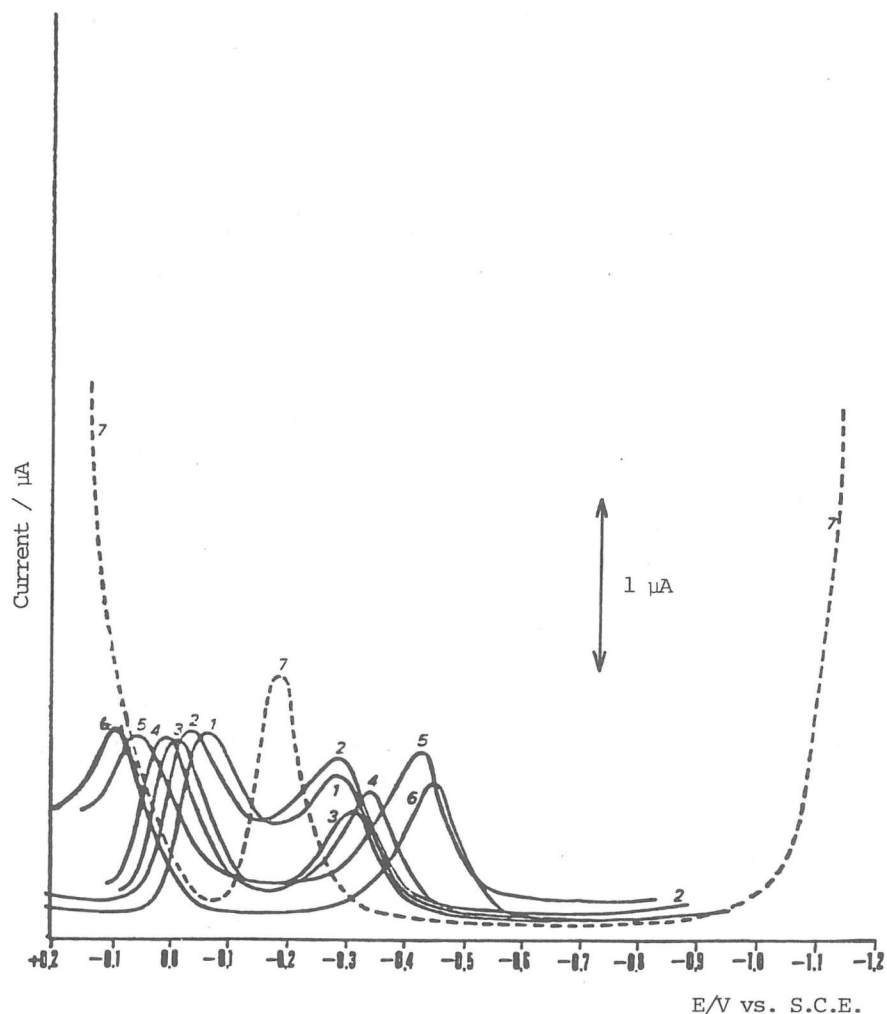


Fig. 5 - DP polarograms of  $10^{-4}$  M  $\text{CuSO}_4$  and  $10^{-4}$  M adenine in 0.25 M  $\text{H}_2\text{SO}_4$

1 - pH=1.54; 2 - pH=1.62; 3 - pH=2.00; 4 - pH=2.63; 5 - pH=3.68;  
6 - pH=4.32; 7 - pH=0

DT=0.5s; A=50mV; Scan Rate=10mV/s;  $h_{\text{Hg}}=75\text{cm}$ ; T=25°C

These facts suggest that, when pH increases the reaction



is favoured, whereas the reaction



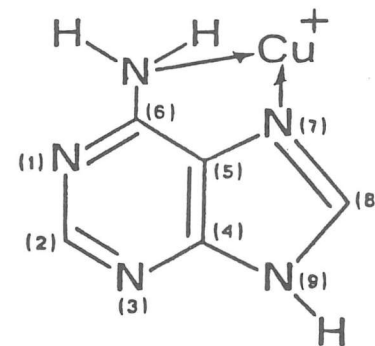
is unfavoured, showing a greater stabilization of Cu(I)(Ade) complex as pH increases.

If we assume that a second protonation takes place at higher concentrations of  $\text{H}^+$  ion, the instability of the complex for lower pH values may be explained assuming that the second protonation occurs at one of the coordination positions. In fact, for very low pH values, near to zero, where adenine shows two reduction peaks, meaning that it is doubly protonated, only the peak corresponding to the  $\text{Cu(II)} \rightarrow \text{Cu(0)}$  reduction is to be seen.

The fact that a peak corresponding to the  $\text{Cu(II)} + (\text{Ade}) + e \rightarrow \text{Cu(I)(Ade)}$  is not obtained, suggests that copper does not complex with adenine for very low pH values.

Having in mind that, as was referred previously, it is generally accepted that adenine is protonated at the N-1 position for  $\text{pH} < 4$ , then the coordination point should be any N other than N-1. If copper ions do not complex with adenine when this molecule is doubly protonated then the coordination point should be the one of the second protonation namely N-7. In fact, as soon as this position is blocked by the proton, complexation cannot take place.

Consequently, we suggest that copper complexes with adenine in N-7 and in the  $\text{NH}_2$  group at C-6, forming a stable five membered ring.



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