Polarographic Behaviour of DL-nor-leucine at the dropping mercury electrode

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Summary: The polarographic behaviour of DL-nor-leucine in 1.0 percent DMSO, 0.1M tetraethyl ammonium perchlorate and 0.002 percent Triton-X-100 has been studied at the d.m.e. The effects of changes in pH, depolariser concentration, Hg-pressure and temperature on the polarographic waves have been investigated. The electrode process is found to be irreversible and diffusion controlled involving one electron transfer under experimental conditions and hence kinetic parameters viz. transfer coefficient (αn) and formal rate constant ($K^{\circ}_{f,h}$) for the electrode reaction have been determined. The mechanism of electrode reaction has also been explained.

Introduction

Very few references are available in the literature on the polarography of amino acids and on the mechanism of their electrode reduction. Okazaki and Otsuki [1] have reported half wave potentials of few amino acids in 80 percent dioxane. The work of Thomas R. Koch and W.C.Purdy [2] on the polarographic behaviour of fourteen amino acid appears to be the main but brief relevant reference. In the absence of detailed polarographic investigations on individual amino acids, it was considered worthwhile to make comprehensive polarographic studies on amino acids under different conditions. Consequently Saxena and co-workers have earlier reported the polarographic behaviour of N-glycyl-glycine [3], L-pyrrolidine-2-carboxylic acid [4] and L-2-amino-3 hydroxy butanoic acid [5].

This communication reports the polarographic behaviour of DL-nor-leucine in 1.0 percent DMSO at the d.m.e. using 0.002 percent Triton-X-100 as maximum suppressor and 0.1M tetraethyl ammonium perchlorate as supporting electrolyte.

Experimental

DL-non-leucine [$CH_3(CH_2)_3.CH(NH_2).COOH$] was a guaranteed biochemical reagent and its solution was prepared in 1.0 percent DMSO by warming on a water bath. Tetraethyl ammonium perchlorate (TEAP) (Fluka, 99 percent pure) was used as supporting electrolyte. TEAP is polarographically inert in 1.0 percent DMSO in the potential range of +0.4 to -2.7V vs S.C.E. Triton-X-100 was used as maximum suppressor.

A Cambridge general purpose polarograph with scalamp galvanometer as current recorder and S.C.E. as reference electrode in conjunction with a thermostated H-cell was used for recording the polarograms at 25° ± 0.1°C. The capillary used had the

following characteristics in 0.1M TEAP, 0.002 percent Triton-X-100 and 1mM DL-nor-leucine in 1.0 percent DMSO at -2.0V vs S.C.E. with h_{Hg}=50cms.

m=2.953 mg/sec, t=3.5 sec/drop

$m^{2/3}t^{1/6} = 2.536\ mg^{2/3}S^{-1/2}$

The polarograms of solutions containing 0.1M TEAP, 0.002 percent Triton-X-100 and different concentrations of DL-nor-leucine in 1.0 percent DMSO were recorded in unbuffered media (pH - 6.5) and also at higher pH range upto 10 in an inert atmosphere of nitrogen.

The pH of the test solution was measured by 'EC' digital pH meter equipped with combined glass-calomel electrode assembly.

Results and Discussion

The polarograms of DL-nor-leucine in 1 percent DMSO yielded a single well defined, cathodic wave which was found to be diffusion controlled involving one electron reduction. The conventional log plots log i/i_d-i vs $-E_{d.e.}$ were linear but their slope values were not in agreement with the theoretical value for the reversible nature of the electrode reaction. The half wave potentials were also found to be slightly dependent on concentration - a characteristics feature of the irreversible waves. The number of electrons consumed in the reaction was also determined by millicoulometric method of DeVries and Kroon [6] and found to be one.

Effect of pH:

The polarograms of solutions containing 1mM DL-nor-leucine, 0.1M TEAP and 0.002 percent Triton-X-100 were recorded in unbuffered media, pH = 6.5 and also at higher pH range upto 10. It was observed that polarographic waves beyond pH 7 were highly irregular and hence the analysis is difficult; however a well defined cathodic wave was obtained in unbuffered media pH 6.5 at which subsequent polarographic investigations were made.

Effect of [Leucine], Hg-pressure and temperature:

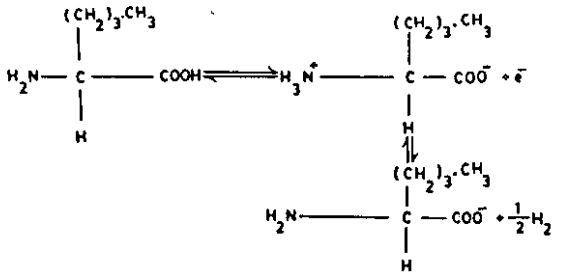
DL-nor-leucine in DMSO gave a well defined cathodic wave and exhibited rounding on the plateau which was due to the rapid increase in drop time at the rising part of the wave over the range of potentials.

The diffusion current i_d changes linearly with amino acid concentration in the range 0.5 to 2.0 mM and the mean value of diffusion current (I) is found to be 1.02. The value of $i_d/h_{eff}^{1/2}$ was found to be constant with the height of mercury column varying from 35 to 60 CMS and its mean was found to be 0.495 $\mu Acm^{-1/2}$; the temperature coefficient of i_d in the range 25° - 40°C was found to be 2.276 percent per degree as calculated by Nejedly's method [7] within experimental conditions, which indicates the diffusion controlled nature of the cathodic wave.

Mechanism of electrode reaction:

The reduction process of DL-nor-leucine is probably the catalytic reduction of a proton on the carboxyl site of the amino acid molecule in aqueous solution as well as 1.0 percent DMSO. Amino acid is supposed to exist as 'Zwitterion' and an equilibrium exists between two neutral forms (i.e.

the molecular forms and Zwitterion form) and the reduction proceeds by the following mechanism:



or

$$AH \rightleftharpoons (A^- H^+) + e^- \rightleftharpoons A^- + \tfrac{1}{2}H_2$$

(where AH represents the amino acid molecule)

$$H^+ + e^- \rightleftharpoons \tfrac{1}{2}H_2$$

The above mechanism suggests that DL-nor-leucine is a monofunctional acid and one electron is involved in the reduction process.

The reduction of hydrogen envisaged in the process was established by studying the effect of acidity on the limiting current. This was carried out by titrating the amino acid first with HCl and then with NaOH. The limiting current was found to be linearly related to the acidity of the solution. These observations suggest that the reducible proton probably resides on the carboxyl group.

Kinetic parameters:

Since the reduction of 1mM DL-nor-leucine in 1 percent DMSO is irreversible, kinetic parameters viz. transfer coefficient (αn) and formal rate constant ($K^\circ_{f,h}$) for the electrode reaction have been determined at 25° and 35°C by applying Koutecky's treatment [8] as extended by Meites and Israel [9].

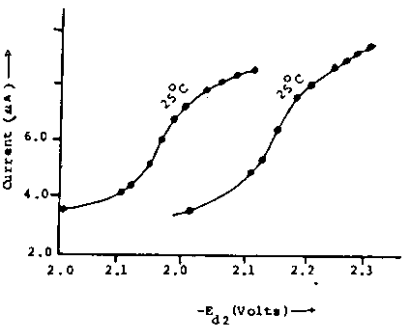


Fig.1: Polarograms of 1mM Leucine in 10% DMSO + 0.1% Temp.+0.002% Triton X-100 at 25°C and 35°C.

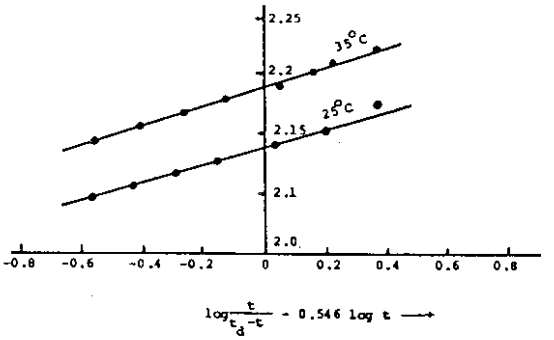


Fig.2: Plot of $\log \frac{i}{i_d - i} - 0.546 \log t$ VS-E_{d-e} (Voldts) at 25°C and 30°C.

As 't' varies appreciably on the rising part of the wave over the range of potentials, it was considered necessary to apply the correction for drop time [10]. Values of 't' are measured at different potentials on the rising portion of the wave so that 't' lies between 10 and 95 percent of i_d which are approximate limits of validity of the equation:

$$E_{d.e.} = -0.2412 - \frac{0.05915}{\alpha n} = \frac{\log 1.349 k^\circ_{f.h}}{Do^{\frac{1}{2}}} - \frac{0.0542}{\alpha n} [\log i/id - i - 0.546 \log t] \quad (1)$$

A plot of $-E_{d.e.}$ vs$[\log(i/i_d - i) - 0.546 \log t]$

(Fig. 1 & 2 and Table 1) produces a straight line whose slope is equated to $\frac{-0.0542}{\alpha n}$ from which the value of αn is calculated and is recorded in

Table-1

Values of $\log \frac{i}{i_d - i}$ - 0.546 log t at various values of $-E_{d.e.}$ at different temperatures

(A) At 25°C		i_d = 3.5 μA	
$-E_{d.e.}$ (Volts)	t (seconds)	i (μA)	$\frac{i}{\log i_d - i}$ − 0.546 log t
2.14	3.3	0.89	-0.75
2.15	3.23	1.19	-0.567
2.16	3.2	1.45	-0.421
2.17	3.13	1.7	-0.296
2.18	3.1	2.01	-0.134
2.19	3.0	2.29	+0.021
2.20	2.9	2.53	0.163
2.22	2.7	2.71	0.305

(B) At 35°C		i_d = 4.4 μA	
$-E_{d.e.}$ (Volts)	t (seconds)	i (μA)	$\frac{i}{\log i_d - i}$ − 0.546 log t
2.14	3.23	1.32	-0.652
2.15	3.2	1.65	-0.498
2.16	3.3	1.99	-0.351
2.17	3.1	2.30	-0.226
2.18	3.0	2.61	-0.096
2.19	2.9	2.91	+0.04
2.20	2.8	3.1	+0.142
2.21	2.6	3.33	0.212
2.22	2.4	3.47	0.356

Table-2: Values of i_d, $-E_{1/2}$ and kinetic parameters at 25° and 35°C

Quantity	Temperature	
	25°C	35°C
i_d (μA)	3.5	4.4
$-E_{1/2}$	2.19	2.18
$D_o^{1/2}$	2.17×10^{-3}	2.85×10^{-3}
αn	0.774	0.637
$K^\circ_{f,h}$	5.3×10^{-8}	2.3×10^{-7}

Table 2. The intercept of the same plot is equal to $E_{1/2}$ defined by the equation:

$$E_{\frac{1}{2}} = 0.2412 + \frac{0.05915}{\alpha n} \log \frac{1.349 k^\circ_{f.h.}}{D_o^{\frac{1}{2}}} \qquad (2)$$

The value of $E_{1/2}$ obtained from the above plot is substituted in equation (2) to obtain $K^\circ_{f,h}$ at different temperatures (Table 2).

It was observed that with the increase in temperature, $E_{1/2}$ shifts slightly towards more positive value showing the easier reduction which also supports the irreversible nature of the electrode process.

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