The polarographic behavior of α-lipoic acid

α-Lipoic acid (6,8-dimercapto-octanoic acid) is widely distributed in animal, plant and microbial cells. It is found to be essential in a number of biochemical reactions involving hydrogen transfer and acyl transfer[1], *e.g.*, in the oxidative decarboxylation of α-keto-acids and in the formation of energy-rich S-acyl derivatives. Available evidence indicates that only the naturally occurring dextrorotatory isomer is biologically active. α-Lipoic acid has also been suggested by CALVIN AND BARLTROP[2] to play a crucial role as the primary quantum conversion agent in photosynthesis.

REED *et al.*[3] have shown that the disulfide bond of α-lipoic acid is reducible at the dropping mercury electrode. GUNSALUS[1] suggested that the standard potential of lipoic acid lies near or below that of DPN ($E'_0 = -0.32$ V at pH 7 and 30° C) — considerably more negative than values reported for linear disulfides of the glutathione type. In view of the unique oxidation–reduction behavior of the intramolecular SH-groups in the α-lipoic acid, further polarographic studies have been made and are reported here. This note is one of a series of studies on hydrogen-transferring coenzymes[4,5].

The experimental methods were described in previous reports[4,5]. The characteristics of the dropping mercury electrode used in the present study are: $m = 1.306$ mg·sec^{-1}; $t = 3.805$ sec (open circuit); $m^{2/3}t^{1/6} = 1.493$ mg $^{2/3}$sec$^{-1/2}$. The synthetic DL-α-lipoic acid was obtained from California Foundation for Biochemical Research (Los Angeles). A molar extinction value[6] of 154 cm^2·mole^{-1} at 332 mμ was used for determining its concentration. The dithiol form was prepared according to GUNSALUS *et al.*[7,*].

All current-voltage curves were measured in standard aqueous buffers and at a lipoic acid concentration of $2 \cdot 10^{-4}$ M. Over the entire pH range studied, all polarograms of α-lipoic acid contain a main wave which is a well-defined single step reduction wave, and a pre- or post-adsorption wave depending on the pH of the medium (*vide infra*). It was also found that the presence of $2 \cdot 10^{-4}$% Triton X-100 (Rohm and Haas) was necessary to eliminate the maximum and other slight irregularities in the curve. The single step two electron reduction of lipoic acid may be represented by the following equation:

$$\mathrm{LipS_2} + 2\mathrm{H}^+ + 2e \longrightarrow \mathrm{Lip(SH)_2}$$

where $\mathrm{LipS_2}$ and $\mathrm{Lip(SH)_2}$ represent the oxidized and reduced lipoic acid respectively. α-Lipoic acid gives a double wave in unbuffered supporting electrolytes such as 0.1 M KCl, KNO_3, $N(CH_3)_4Cl$, etc., where the second wave corresponds to a local pH increase at the electrode surface.

In the acid pH region post-adsorption waves appear. However, below pH 3.5 the post-wave is masked by the hydrogen wave. The first post-wave was observed at pH 4.15. at pH's greater than 8, the post-wave disappears and only the pre-wave remains. Typical polarograms containing the main reduction wave and a post- or a pre-wave respectively are shown in Fig. 1. At acid pH's, the adsorption of the oxidized form is facilitated by both the free carboxylate group as well as the disulfide group, whereas at alkaline pH's the reduction product apparently becomes more strongly adsorbed, probably due to the presence of the anion form of the dithiol. The electrocapillary curves of both the oxidized and reduced forms of lipoic acid are constructed by plotting the drop time *versus* the potential of the dropping mercury electrode. Fig. 2 shows a set of typical electrocapillary curves at various lipoic acid concentrations. When lipoic acid is present, the positive branch of the electrocapillary curve is lowered appreciably. The electrocapillary zero is shifted to more negative values with increasing lipoic acid concentrations. In contrast to the electrocapillary curves of most simple capillary active substances, the curves of lipoic acid shows a slower initial increase, and then a more abrupt increase starting at −0.5 V. All curves coincide with the normal electrocapillary curve at about −0.85 V.

The $\log(i/i_d - i)$ *versus* E plot for the lipoic acid polarograms gives a value of 0.054, indicating semi-quinone formation. At a lipoic acid concentration of 10^{-3} M in a pH 7 supporting medium, the index potential E_i is 0.031 V corresponding to a semi-quinone formation constant $K = 6.03$[8].

Although the polarographic wave does not obey the Nernst equation as a result of semi-quinone formation, the half-wave potential of the main reduction wave still shifts in the regular fashion with respect to pH. The $E_{1/2}$ values of the pre- and post-waves remain fairly constant. At pH 7 the main wave has an $E_{1/2}$ of −0.567 V with respect to the standard calomel electrode (or −0.325 V with respect to the normal hydrogen electrode). When $E_{1/2}$ is plotted against pH for the normal reduction wave the curve is bent into two sections over the pH range studied, with slopes of 0.058 and 0.028 respectively. The inflection occurs at 9.85, which presumably corresponds to the pK_a value of one of the sulfhydryl groups. The pK_a of the carboxyl group of α-lipoic acid has been determined by REED *et al.*[3] using electrometric titration and found to be 4.76.

It is worthy of note that in a recent paper on the polarographic study of some α,α^1-dimercaptocyclic disulfides, NYGÅRD AND SCHOTTE[9] have measured the $E_{1/2}$ for the five-membered

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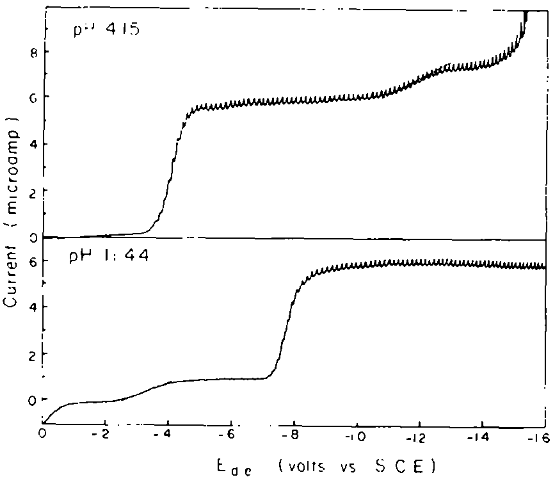
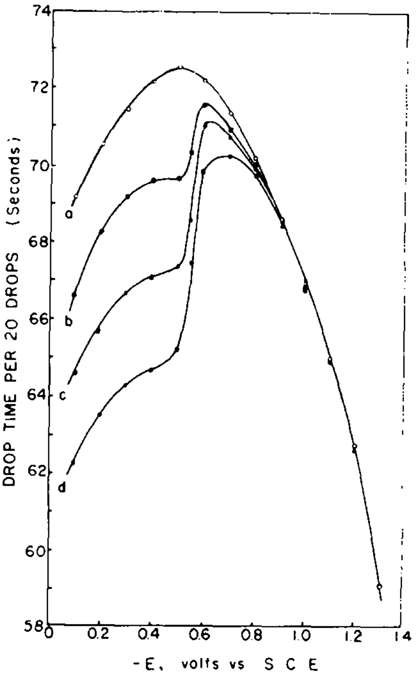


Fig. 1. Polarograms of α-lipoic acid containing a main reduction wave and a post- or a pre-wave obtained in different pH media. Lipoic acid, 10^{-3} M; Triton X-100, $2 \cdot 10^{-4}$%.

Fig. 2. Typical electrocapillary curves of α-lipoic acid: a, pH 7 phosphate buffer alone; b,c,d, $2.29 \cdot 10^{-4}$, $4.58 \cdot 10^{-4}$ and $9.15 \cdot 10^{-4}$ M lipoic acid respectively.



ring 1,2-dithiane-3,5-dicarboxylic acid at pH 2.2 and found it to be —0.330 V with respect to the standard calomel electrode. This value is almost identical with that of α-lipoic acid measured at the same pH. The six- and seven-membered cyclic disulfides have successively more negative half-wave potentials, indicating a greater ring stability for the six- and seven-membered cyclic disulfides.

Dihydrolipoic acid gives a double anodic wave, which apparently results from steric hindrance that the two-electron transfer cannot be made in a single step. The $E_{1/2}$ of the first anodic wave coincides with that of the cathodic wave, while the second anodic wave occurs at a potential 0.175 V more positive. At higher pH values, less well-defined anodic waves were observed.

Like many other compounds containing disulfide and sulfhydryl groups, the oxidized and reduced forms of α-lipoic acid also produce characteristic catalytic hydrogen waves. Experiments were performed in 0.1 N NH_4Cl-NH_4OH buffers 2×10^{-3} M in $CoCl_2$. The concentration range of α-lipoic acid used was from 10^{-5} to 10^{-4} M. In the absence of α-lipoic acid, the cobaltous ion gives a wave with $E_{1/2} = -1.12$ V with respect to the standard calomel electrode. Upon the addition of α-lipoic acid, the cobaltous wave is shifted about 0.1 V toward the positive side. The height of the catalytic hydrogen wave increases with increasing concentration of lipoic acid, but not in a linear manner. The height of the catalytic wave is 102 times greater than that of the corresponding reduction wave at 10^{-4} M lipoic acid concentration. The catalytic waves obtained with dihydrolipoic acid are identical with those of the oxidized form at equal concentrations.

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