Rapid-Sweep Polarography of Cystine

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In their brochure *The Principles and Applications of Cathode-Ray Polarography* Southern Analytical Ltd. (Camberley, Surrey, U.K.) refer to a paper in which one of their cathode-ray polarographs is used for the determination of amino acids containing thiol and disulphide groups (Coult, 1958). This work apparently takes no account of the fact that the shape of a rapid-sweep polarogram is more dependent on the direction through which the particular potential range is swept than on the oxidation state of the species in bulk solution.

Consider a rapid-sweep polarogram of cystine (R-S-S-R) when the 'start potential' of the dropping-mercury electrode is too positive for reaction (1) to occur:

If the potential of the dropping-mercury electrode is then rapidly swept in a negative direction, a reduction peak is observed, corresponding to reaction (1), when the potential becomes sufficiently negative. A conventional polarogram (in which a relatively slow potential sweep is applied) gives a diffusion-controlled plateau at a similar negative potential. If the start potential is sufficiently negative for reaction (1) to occur and the potential is swept in a positive direction the conventional polarogram is virtually unchanged. A rapid reverse potential sweep (making the dropping-mercury electrode more positive), however, gives a polarogram corresponding to the oxidation of R-SH. This is due to the formation of R-SH on the mercury-drop surface at the negative start potential before the reversed potential sweep commences. As the complete polarogram is rapidly obtained during the lifetime of this same mercury drop a polarogram of R-SH oxidation results. Thus a solution of R-S-S-R gives the same anodic oxidation peak as a solution of R-SH if the potential is rapidly swept in a positive direction, and it is difficult to understand how Coult (1958) was able to measure one in the presence of the other. An anodic oxidation peak will be observed in one case as the result of the oxidation of R-SH in bulk solution and

in the other case as the result of the oxidation of R-SH produced on the surface of the mercury drop before the potential sweep commences. Coult (1958), however, claimed that a mixture of cystine and cysteine gives two distinct and well-separated peaks when a polarogram is obtained by rapidly sweeping in a positive direction (this paper is further complicated by the use of a mercury-pool reference electrode, which would be affected by the presence of thiol). As shown below, however, it is possible to measure cystine in the presence of cysteine if the rapid sweep is in a negative direction.

Fig. 1(a) shows the double-peaked trace obtained in this laboratory with a Southern Analytical A1670 Davis Polarograph when a potential sweep from 0 to -0.5 V (with respect to an Ag/AgCl electrode) is applied to a de-aerated solution of 0.1 mM-cystine in 0.1 M-perchloric acid. The cathode-ray polarogram clearly indicates two cathodic reduction reactions. Kolthoff & Barnum (1941) observed two plateaus in the conventional polarogram of cystine, but argued that one of these was merely an apparent cathodic reduction. However, their argument is not applicable to a rapid-sweep polarogram.

In Fig. 1(b) a sweep from -0.5 to -1.0 V was applied to the drop on the dropping-mercury electrode immediately before the curves shown, which are for potential sweeps from 0 to -0.5 V, were observed. The first trace shows an increase in size of the first peak and a decrease in that of the second peak. The second and subsequent traces have reverted to that in Fig. 1(a).

The traces in Fig. 1(c) were obtained by applying a potential sweep from -1.0 to -1.5 V and then recording subsequent traces produced by sweeps from 0 to -0.5 V. In these circumstances the polarogram reverted much more slowly, and all these traces apparently intersect at a point corresponding to an isosbestic point in spectroscopy.

The more negative of the two peaks (at -0.43 V) in Fig. 1(a) is due to the cathodic reduction of cystine to cysteine. This agrees well with the conventional polarograms published by Kolthoff & Barnum (1941).

Fig. 1. Rapid-sweep polarograms of de-aerated solutions of 0.1 mM-cystine in 0.1 M-HClO₄ with a Southern Analytical A1670 polarograph at 1/180 full sensitivity. (a) Traces obtained on the oscilloscope when no precautions were taken; (b) traces obtained immediately after the application of a potential sweep from -0.5 to -1.0 V to one mercury drop from the dropping-mercury electrode; (c) traces obtained immediately after the application of a potential sweep from -1.0 to -1.5 V to one mercury drop from the dropping-mercury electrode; (d) first trace obtained when the dropping-mercury electrode was not connected to the sweep generator until immediately before the trace was recorded.

The less negative peak at -0.08 V is due to the reaction (Kolthoff & Barnum, 1940):

The mercurous cysteinate in eqn. (2) is formed by the anodic oxidation of mercury at the dropping-mercury electrode as in eqn. (3):

Normally there would be no cysteine present in the solution, but because the A 1670 polarograph still applies the potential sweep to the cell when the appropriate switch is in the 'cell off' position some cysteine has been electrolytically formed as in eqn. (1) by the time a trace is recorded.

Application of a potential sweep from -0.5 to -1.0 V to the previous mercury drop as in Fig. 1(b) causes reaction (1) to proceed throughout the entire

lifetime of this mercury drop. Some of the cysteine produced forms mercurous cysteinate when the potential of the dropping-mercury electrode is subsequently resting at 0 V before a sweep from 0 to -0.5 V is commenced. The peak at -0.08 V in Fig. 1(b) is larger in the first trace owing to the reduction of an increased amount of mercurous cysteinate to cysteine (it is often assumed that virtually all the product formed is carried away by the previous mercury drop, but this is evidently not so). Subsequent traces then revert to that in Fig. 1(a). In Fig. 1(c) the -0.08 V peak has not been increased so much, but does not revert to the same height as in Fig. 1(a) until about seven sweeps from 0 to -0.5 V have occurred. This is apparently due to the fact that during the preceding sweep from -1.0 to -1.5 V the dropping-mercury electrode becomes sufficiently negative for H₂ gas to be evolved. It is proposed that the H₂ bubbles displace

the cysteine product from the surface of the mercury drop. Consequently when the mercury drop breaks off the fragment of the drop left still attached to the dropping-mercury electrode does not have cysteine on its surface. Thus the mercury drop of the first trace in Fig. 1(c) has no cysteine residue from the previous drop, but has an enhanced peak at -0.08 V due to some of the cysteine displaced by H_2 diffusing back to the dropping-mercury electrode. Thus this peak is lower than that in the first trace of Fig. 1(b) but takes longer to revert to that in Fig. 1(a).

In Fig. 1(b) most of the cysteine residue is carried away by one mercury drop, whereas in Fig. 1(c) the cysteine formed by the sweep from -1.0 to -1.5 V has been displaced into the surrounding solution and a series of decreasing -0.08 V peaks are obtained while this cysteine diffuses into the bulk solution.

The expected single-peaked polarogram of cystine was obtained only if the cell was completely disconnected from the polarograph and not connected until immediately before the first potential

sweep was recorded, as in Fig. 1(d). Subsequent traces then approached those of Fig. 1(a). Supporting evidence for the cathodic peak at -0.08 V being due to the reaction of mercurous cysteinate (eqn. 2) was obtained on adding O_2 -free cysteine to the de-aerated cystine solution. The peak at -0.08 V then increased in proportion to the added cysteine.

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