A.C. Processes at the Dropping Mercury Anode

by

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(Received Nov. 18, 1967)

I should like to dedicate this paper to the Polarographic Society of Japan and to the Japan Society for Analytical Chemistry in gratitude for their assistance which enabled me to attend the International Polarographic Congress in Kyoto in September 1966.

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Summary

A new type of a.c. wave that occurs in some supporting electrolytes (e.g., sulfate, nitrate, perchlorate) at high positive polarizations of the dropping mercury electrode is described. It has been named “predissolution wave” because it occurs at potentials of the beginning mercury dissolution. The wave is due to the exceeding of the solubility product of the mercurous salts of the respective anions.

On the addition of salts whose anions form complexes with mercurous ions (e.g., thiosulfate, thiocyanate, sulfite), the peak of the wave shifted to more positive potentials and increased in height because of the solubilization of the film at the electrode surface. It was also found that the indistinct a.c. wave obtained in thiosulfate solutions at $-0.19V$ (S.C.E.) was transformed into a sharp peak on addition of chlorides; the peak at first increased with increasing chloride concentration, to decline again at still higher chloride concentrations.

The addition of serum albumin had the same effect on the predissolution wave as had complexing ions: the wave increased in height and its peak shifted to more positive potentials. Based on this finding, an analytical procedure was worked out permitting the estimation of serum protein in a concentration range from 2 to 200 µg/ml.

Anodic a.c. polarography is a most sensitive method for the early detection of growing microorganisms, especially moulds, contaminating distilled water.

In a number of previous publications (1–6) it has been pointed out that the region of anodic polarizations of the dropping mercury electrode (d.m.e.) seems to hold great promise for future developments in the field of a.c. polarography. The present communication deals with such developments. It will be shown that anodic a.c. polarography lends itself to a number of most varied investigations, such as the study of electrode reactions when different anions simultaneously interact with the dropping mercury anode, the study and sensitive analysis of proteins, the investigation of anion interaction with proteins; finally, we wish to outline the usefulness of the method for the early detection of products of cellular growth in supposedly pure distilled water.

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The Predissolution Wave

A new type of a.c. wave has been observed in a number of electrolytes at high positive polarizations of the d.m.e. (Fig.1). This wave will be called the predissolution wave, because it appears at potentials corresponding to the anodic dissolution of mercury, it occurs in supporting electrolytes whose anions form mercurous salts of intermediate solubility, such as sulfate, nitrate, and perchlorate. In the corresponding d.c. polarogram there appeared but a very indistinct step which obviously does not lend itself to accurate investigations.

As is well known, the potential at which the anion discharge takes place at the d.m.e. is connected with the solubility product of the mercurous salt formed (7). Now, in the case of sulfate, nitrate, and perchlorate the solubilities of the corresponding mercurous salts are relatively high; consequently, the solubility product will be exceeded only at rather high positive polarizations. Thus, in the case of a supporting electrolyte of 1 M sodium sulfate, given a solubility of mercurous sulfate of 0.06% at 25°C (8), one calculates that the peak of the wave should occur at about +0.380 V, in good agreement with the value found in our experiments (+0.374 V)*. Further support for the hypothesis that the predissolution wave is due to the exceeding of the solubility product of the mercurous salt formed at the electrode surface comes from the experimental result that the peak of the wave shifted about 30 mV to more negative values with a tenfold increase in electrolyte concentration (cf. Fig. 2). Finally, the fact that the wave was not

* All potentials are given relative to the saturated calomel electrode.
seen in supporting electrolytes containing anions that form rather well soluble mercurous salts, such as in acetate solutions, further supports our assumption; the solubility of mercurous acetate being 0.75% at 13°C (8) there is no possibility of exceeding the solubility product under polarographic conditions.

**Influence of Anions on the Predissolution Wave**

In view of what has been said, it seemed of interest to investigate the influence on the predissolution wave of the addition of salts whose anions react with mercurous ions.

We found that the addition of, for instance, thiosulfate \((2 \times 10^{-5} M)\) caused a shift of the predissolution peak towards more positive potentials; at the same time the height of the wave increased (Fig.3). Keeping in mind that the predissolution wave owes its origin to the equilibrium process

\[
\text{Hg}_2\text{SO}_4(s) \rightleftharpoons \text{Hg}_2^{2+} + \text{SO}_4^{2-}
\]

these findings are at once understandable: the mercurous ions react with the complexing anions, the equilibrium moves to the right with the consequent shift of the peak potential to more positive values. At the same time the surface concentration of mercurous ions also increases causing a rise in both the faradaic current and the differential capacitance; hence the increased waveheight. Or, to express it differently, the effect of the complexing anion is to solubilize the film of the sparsely soluble mercurous salt at the electrode surface.

If this contention is correct then the action of complexing ions, different from thiosulfate, on surface films other than mercurous sulfate, should produce the same effect. Experimental results completely confirmed this prediction. Table 1 shows that the effect of thiocyanate addition on the a.c. wave of chloride was entirely analogous to that which thiosulfate addition had on the predissolution wave: the peak was shifted

![Fig. 3. Effect of thiosulfate on the predissolution wave in 0.5M and 0.25M sodium sulfate.](image)
Table 1. Effect of thiocyanate on the peakpotential and height of the chloride wave (2 × 10⁻⁴M KCl in 0.5M sodium sulfate.

<table>
<thead>
<tr>
<th>Thiocyanate concn. (×10⁴M)</th>
<th>$E_p$ (V vs. S.C.E.)</th>
<th>$i_p$ (µA r.m.s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>+0.324</td>
<td>4.39</td>
</tr>
<tr>
<td>1.0</td>
<td>+0.325</td>
<td>5.10</td>
</tr>
<tr>
<td>2.0</td>
<td>+0.327</td>
<td>5.84</td>
</tr>
<tr>
<td>4.0</td>
<td>+0.331</td>
<td>7.45</td>
</tr>
<tr>
<td>8.0</td>
<td>+0.343</td>
<td>11.4</td>
</tr>
</tbody>
</table>

to more positive potentials and the waveheight increased.

Similar results were obtained with sulfite ions also.

It is interesting to note that the presence of halogen ions in concentrations of about 2 × 10⁻⁴M diminished (Cl⁻) or suppressed (Br⁻ and I⁻) the predissolution wave, most probably because of the formation of an insoluble mercurous halogenide film at the electrode surface.

A peculiar effect was observed when the solution contained both chloride and thiosulfate ions. In 10⁻⁴⁴M solutions of thiosulfate two d.c. steps appeared (Fig.4), one at −0.13 V, corresponding to the complexing reaction between mercurous and thiosulfate ions (9), and a second one at about +0.19 V. The latter is most probably identical with the step observed by Kolthoff and Miller (9) at about +0.15 V in 10⁻³M thiosulfate solution, a step which they attributed to the irreversible oxidation of thiosulfate to sulfate ion.

Fig. 4. Effect of chloride addition on A.C. and D.C. polarograms of thiosulfate; (A) 0.5M sodium sulfate; (B) same + 10⁻⁴M sodium thiosulfate; (C) as in (B) + 8 × 10⁻⁴M potassium chloride.
Fig. 4 shows that in the potential regions corresponding to the two d.c. steps there appeared two very flat, little-pronounced a.c. waves. If, however, chloride was added to the thiosulfate solution, the flat hump at +0.19 V was transformed into a sharp peak whose height at first increased with increasing chloride concentration, to decline again at still higher chloride concentrations. Completely analogous results were obtained when the procedure was reversed; a sharp peak appeared at +0.19 V when the chloride concentration was kept constant and thiosulfate added to the solution (cf. Fig. 5). Fig. 5 also shows that very small additions of thiosulfate enhance the height of the chloride a.c. wave, at about +0.3 V), whilst higher concentrations diminish it. Fig. 6 summarizes the results in form of a waveheight vs. thiosulfate-concentration diagram. It can be seen that the ionic ratio at which the maximum peakheight was obtained depended on the initial chloride concentration.

We hoped to obtain some elucidation of the underlying electrode process by applying d.c. polarography. Unfortunately, however, the corresponding d.c. steps were so ill defined that they were hardly usable. Obviously more work is required to clear up the mechanism of this phenomenon.

Proteins

The a.c. polarography of proteins has first been studied by Berg (10). He found that a number of proteins containing disulfide bonds suppress the base current, especially on the positive side of the electrocapillary maximum, and that at rather high negative potentials there appears a tensammetric wave.
We ourselves have so far limited our investigations to crystalline bovine serum albumin (fraction V). The potential region we studied was that extending from about the electro-capillary maximum to the extreme positive regions, limited by mercury dissolution. A typical polarogram obtained with a 0.02% solution of serum albumin in a supporting electrolyte of 1 M sodium sulfate is shown in Fig. 7. Two facts are at once apparent: the suppression of the base current at potentials more negative than +0.1 V and the steep increase in height of the predissolution wave. At the same time the peak potential was shifted slightly towards more positive values. The obvious reason for this behaviour is the complexing of the mercurous ions by the protein.

It is a matter for regret that we had to do most of our work in unbuffered solutions, since the majority of anions react with mercury at positive polarizations. One exception is the acetate ion; hence, the only buffer we have used up to the present was acetate buffer, restricting severely the available pH range. We are, however, hopeful that we shall be able to find buffer systems giving a predissolution wave suitable to our purpose.

Applying the same reasoning as that expounded in the previous section, we proceeded to examine the influence of protein addition on the a.c. waves of halogen ions. As is evident from Fig. 8, the effect was completely analogous to that obtained with other complexing anions: the wave was shifted to more positive potentials and increased in height.

Fig. 7. Effect of serum albumin addition on A.C. and D.C. polarograms of sodium sulfate; (A) 1 M sodium sulfate; (B) same +0.02% serum albumin.

Fig. 8. Effect of serum albumin on A.C. and D.C. polarograms of chloride; (A) $4 \times 10^{-4}$ M potassium chloride in 1 M sodium sulfate; (B) same +0.02% serum albumin.
It is interesting to note that in contradistinction to chloride, the a.c. waves of both bromide and iodide ion were completely eliminated by the addition of protein. The reason is immediately apparent from inspection of the corresponding d.c. polarograms (Figs. 8 and 9). It is evident that the addition of protein left the d.c. polarogram of chloride ion almost unchanged, whilst that of bromide ion became completely distorted, indicating a high irreversibility of the electrode process, causing the disappearance of the corresponding a.c. wave. The d.c. step of iodide ion showed the slope due to an irreversible electrode process even in the absence of protein and was little affected when protein was added to the electrolyte (Fig. 10); the a.c. polarogram showed a depression of the hump corresponding to the discharge of iodide ion at about $-0.12\,\text{V}$ and complete suppression of the tensammetric peak at about $+0.3\,\text{V}$.

Finally, we should like to report on the potentialities of anodic a.c. polarography for the analysis of proteins. Our investigations are as yet in the very early stages of development; hence, even some of the most obvious work has not been carried out yet, such as a study of the qualitative and quantitative differences to be expected with various types of proteins, with lipoproteins, lipids, etc.; all our results refer to crystalline serum albumin.

We have carried out the estimations in two different ways:

1. measuring the depression of the a.c. base-current hump seen in a supporting electrolyte of either sodium or magnesium sulfate at about $-0.1\,\text{V}$ (Fig. 11): this method is suitable for estimations of serum albumin at very low concentrations ($2-20\,\mu\text{g/ml}$) (cf. Fig. 12B)
Fig. 11. Suppression of the A.C. hump in half-saturated magnesium sulfate solution by serum albumin; serum albumin concentration in $\mu$g/ml; (A) zero; (B) 2.5; (C) 10; (D) 15; (E) 20

Fig. 12. Calibration curves of serum albumin; (A) in 0.1M sodium sulfate; (B) in half-saturated magnesium sulfate.

(2) at higher concentrations (20–200 $\mu$g/ml) we used the increase in height of the predissolution wave, measuring either the changes in peakheight (against the zero-current line), or else by measuring the alternating current always at the same direct potential. Fig. 12A shows the calibration curves so obtained. It is evident that these methods can be usefully employed for protein analysis.

Appendix

Recently we have found that the growth of micro-organisms, in particular moulds, in either distilled water or in salt solutions, produced typical changes in the anodic a.c. polarogram. As seen in Fig. 13, a hump appeared on the curve at about +0.2 V, first discernible about 16 hours after infection of the solution with a few mould cells. With the passing of time the hump gradually increase. As expected no change was seen in
a sterile control solution.

The changes in the a.c. polarogram became visible about 1–2 weeks before any growth could be detected macroscopically, a useful fact if one remembers how profoundly experimental results can be affected by infection of the distilled water used. Although we make it a rule to use as far as possible freshly distilled water in all our experiments, some of the solutions (especially standards) had to be kept for some time. It gave us an added feeling of security to be able to test them for suitability before use. The problem is a particularly acute one in biological institutions. Not only is the risk of infection of the distilled water in storage heightened because animals are kept in the vicinity, but it also seems likely that infected water can be toxic to biological materials such as microbial cultures and isolated organs.

We propose to study this problem in more detail and shall report the results in due course.

Acknowledgements

The authors wish to thank Prof. E. Trabucchi, Head of the Department of Pharmacology of the University of Milan, for his constant interest and generous support of this work.

References