The Polarographic Determination of Thyroxine and 3:5-Diiodotyrosine

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(Received 26 October 1945)

Although biological methods are used for assessing the value of thyro-active materials, chemical methods are also available to determine the thyroxine content of such substances. These methods are mainly based on the estimation of iodine as 'thyroxine iodine' (Harington & Randall, 1929; Leland & Foster, 1932; Brand & Kassell, 1939). Polarographic analysis suggests itself as a means of simplifying this determination and this note records a preliminary study of the behaviour of thyroxine and diiodotyrosine at the dropping mercury cathode.

EXPERIMENTAL

The apparatus used was a standard Cambridge Polarograph and the samples examined were obtained from British Drug Houses Ltd. The electrolysis cells were maintained at 25° during the experiments and potentials were measured against the saturated calomel electrode. The *m* and *t* values of the dropping electrode were respectively 2.075 mg./sec. and 2.9 sec., with the capillary dipping into distilled water at 25°.

3:5-Diiodotyrosine

3:5-Diiodotyrosine was found to be electro-reducible and to give a polarographic wave. The half-wave potential varied with the base electrolyte used. Thus, 0.004 g. diiodotyrosine in 10 ml. 0.1N-HCl showed a wave in the current-e.m.f. curve at -0.98, rising to a peak at -1.08 V. In 10 ml. 0.5N-Na₂CO₃, 0.004 g. diiodotyrosine gave a wave possessing a point of inflexion at mid-voltage. There is thus a hint of a double step wave, but treating it as a single wave, $E_{\frac{1}{2}} = -1.72$ V. measured against the saturated calomel electrode. Diiodotyrosine acetate gave an identical wave under the same conditions.

These base solutions, however, did not produce very satisfactory waves and better ones were obtained by using 0.5N-Na₂CO₃ containing tetramethylammonium bromide or iodide. In these base solutions, after removal of oxygen, diiodotyrosine gave two polarographic waves having half-wave potentials of -1.50 and -1.70 V. These two waves formed a double wave system and the height of both together was roughly proportional to the concentration of the diiodotyrosine in solution.

Fig. 1a shows the current-e.m.f. curve obtained from 0.02 g. diiodotyrosine dissolved in 20 ml. 0.5N-Na₂CO₃ solution containing 0.2 g. tetramethylammonium iodide. Oxygen was removed by passing nitrogen through the solution for 10 min.

Fig. 1. Polarograms of (a) 0.02 g. diiodotyrosine in 20 ml. 0.5N-Na₂CO₃ containing 0.2 g. tetramethylammonium iodide; (b) 0.02 g. thyroxine in 20 ml. ethanol/0.5N-Na₂CO₃ solution containing 0.2 g. tetramethylammonium iodide.

Fig. 2. Relation between wave height and concentration of diiodotyrosine acetate.

A range of concentrations of diiodotyrosine acetate, between 5×10^{-4} and $2 \times 10^{-3} M$ was measured using 20 ml. portions of $0.5 N-Na_2CO_3$ solution containing 0.2 g. tetramethyl salt. The series of polarograms thus obtained without removal of oxygen did not show any voltage shift and the relationship between polarographic wave height ($4.65 \times 10^{-1} \mu a./$ unit scale division) and concentration is shown in Fig. 2. This suggests the basis of a new analytical method for diiodotyrosine.

Thyroxine

The polarogram of 0.007 g. of thyroxine (monosodium salt) in 10 ml. $0.5 N-Na_2CO_3$ solution consisted of a composite wave. The first discharge occurred at a half-wave potential of $-1.24 V.$ and

Fig. 3. Relation between wave height and concentration of thyroxine without removal of oxygen from test solution.

the second produced a small maximum at $-1.40 V.$, while the third occurred at about $-1.66 V.$

After several experiments with various base electrolytes, the following method was found to yield reproducible polarograms. The thyroxine (0.02 g.) was dissolved in 20 ml. of a mixture of ethanol/ $0.5 N-Na_2CO_3$ solution in the ratio of 2 : 3; 0.2 g. tetramethylammonium iodide was added and the polarogram recorded after elimination of oxygen. The current-e.m.f. curve obtained for thyroxine under these conditions is reproduced in Fig. 1b. Three waves are apparent at half-wave potentials

-1.20 , -1.42 and $-1.70 V.$ respectively. Polarograms of the same solution with other thyroxine concentrations were recorded at identical half-wave potentials. The over-all height of the composite wave structure was measured and the height of this was found to be proportional to the concentration of the thyroxine.

Fig. 3 shows a plot of polarographic wave height ($4.65 \times 10^{-1} \mu a./$ unit scale division) against thyroxine concentration, without elimination of oxygen. The concentration ranges from about 3×10^{-4} to $1.25 \times 10^{-3} M$. In this range, it may be noted that the proportionality exists whether or not oxygen has been removed from the electrolysis solution.

For a more dilute range of thyroxine, 3×10^{-5} to $1.25 \times 10^{-4} M$, it was necessary to remove oxygen

Fig. 4. Relation between wave height and concentration of thyroxine. More dilute range: oxygen removed from test solution.

from the test solution by means of nitrogen. Fig. 4 shows the linear relationship between polarographic wave height ($6.975 \times 10^{-2} \mu a./$ unit scale division) and concentration of thyroxine over this range.

This does suggest that polarography may provide a new method of thyroxine determination.

Thyroxine in the presence of diiodotyrosine

Preliminary work indicated that within certain concentration limits it may be possible to determine thyroxine in presence of diiodotyrosine by means

of the first polarographic thyroxine wave at -1.20 V. Diiodotyrosine under the same conditions does not commence to discharge until about -1.30 V. (see Fig. 1). In a typical experiment the following data were obtained.

Diiodotyrosine (0.05 g.) was dissolved in 50 ml. $0.5N$ - Na_2CO_3 /ethanol (3 : 2) and thyroxine (0.048 g.) was dissolved in 20 ml. of the same ethanolic carbonate solution. Two polarographic test solutions were prepared from the above solutions thus:

Thyroxine		Diiodotyrosine		0.5N- Na ₂ CO ₃ / ethanol (3 : 2)	Tetra- methyl- ammonium iodide	
ml.	g.	ml.	g.	(ml.)	(g.)	
(a)	4	0.0096	10	0.005	6	0.2
(b)	6	0.0144	10	0.005	4	0.2

Observing the usual precautions, nitrogen was bubbled through each solution for 10 min. The resultant polarograms are reproduced in Fig. 5. It

Fig. 5. Thyroxine in presence of diiodotyrosine. Polarograms of solutions containing 20 ml. $0.5N$ - Na_2CO_3 /ethanol (3 : 2), 0.005 g. diiodotyrosine and 0.2 g. tetramethylammonium iodide plus (a) 0.0096 g. thyroxine, (b) 0.0144 g. thyroxine. Potentials against mercury pool anode.

may be seen that the heights of the first thyroxine wave at -0.90 V. (measured against a mercury

anode) in both (a) and (b) are approximately in the ratio of 2 : 3, which is the ratio of the thyroxine concentrations.

DISCUSSION

In this work it has been found that tyrosine does not undergo polarographic reduction in sodium carbonate solution (cf. Roberts, 1941). Thus it would appear that the formation of the waves for the electro-reduction of thyroxine and diiodotyrosine at the dropping mercury cathode is due to the presence of the iodine atoms. It is evident that the iodine atoms in diiodotyrosine possess two separate reduction potentials, producing as they do, two waves of approximately equal height. The polarogram of thyroxine is not so simple; the first fairly well-defined wave, constituting one-fourth of the entire wave, presumably indicates the reduction of one iodine atom while the remainder of the current-e.m.f. curve represents the reduction of the remaining three iodine atoms.

It has been demonstrated that the composite wave height for thyroxine is proportional to the concentration. As the clearly defined first wave of thyroxine appears to bear a constant ratio (1 : 4) to the composite wave, then in the determination of thyroxine in presence of diiodotyrosine, the height of the wave at -1.2 V. with respect to the saturated calomel electrode may be taken as being proportional to the thyroxine concentration.

SUMMARY

1. Thyroxine has been determined polarographically using a mixture of ethanol and sodium carbonate solution containing either tetramethylammonium iodide or bromide. A similar technique may be used for the determination of diiodotyrosine, using a base solution without ethanol.

2. Preliminary indications are given that thyroxine may be determined polarographically in the presence of diiodotyrosine.

Thanks are due to Mr J. P. Cavanagh for assistance rendered during the course of this work.

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