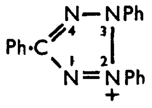
320. *Problems Involved in the Polarography of Triphenyltetrazolium Chloride.*

By B. Jámbor.

Triphenylformazan gives an oxidation wave which is not due to impurities in the solution. Both the micro- and the macro-cathode reductions of triphenyltetrazolium chloride lead to formazan formation to an extent depending on applied potential, pH, and concentration. The more acid the solution the slower is formazan formation because the reduction will involve $N_{(1)}$ and $N_{(2)}$ instead of $N_{(2)}$ and $N_{(3)}$. Further evidence of this is that a colourless compound is produced, instead of formazan, when triphenyltetrazolium chloride disproportionates under the influence of light in acid medium. Its polarographic wave lies between those of the tetrazolium salt and its photo-derivative. When reduced cathodically, in acid medium, the first step of reduction does not lead to this colourless product, only to its precursor intermediate, of as yet unknown structure, which is reduced further at once. In alkaline medium the first step of reduction proceeds in an analogous way: the first two-electron wave does not indicate formazan formation, but that of its precursor intermediate, from which formazan is formed only secondarily. Since the half-wave potentials, and the character of the half-wave potential–pH curves, of polarograms are much altered by the presence of alcohol, it is only with careful reservations that proton uptake can be deduced from alcoholic solutions.

Campbell and Kane[1] have disagreed with our conclusions from earlier work[2] on the polarography of triphenyltetrazolium chloride (as I). They say that (*a*) as the triphenylformazan (II) produced has no oxidation wave, the system triphenyltetrazolium chloride–triphenylformazan cannot be reversible, and hence that Jámbor's oxidation wave must have been due to impurities in the buffer, and (*b*) neither their results nor ours confirm our view that in acid medium the reduction takes place on the nitrogen atoms 1 and 2 (III). Although these disagreements arise from a difference in methods and a deficiency of experimental results in our earlier paper, we now present further results in support of our views and compare them with those of Campbell and Kane. We further disagree with them that the mechanism of reduction can be derived from the tangent of the half-wave potential–pH curves of 50% methanol–water solutions; this would lead to results improbable from the viewpoint of organic chemistry.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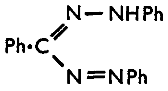
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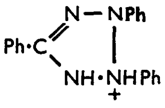
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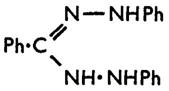
(IV)

Experimental

Polarograms were prepared as previously.[2] For electrolysis with a stationary mercury cathode an unusually large (100 ml.) Heyrovský vessel was used, in which the mercury pool at the bottom was the cathode and a platinum spiral anode replaced the capillary electrode (Fig. 1).

At a dropping mercury cathode, formazan formation could be seen distinctly around the

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[1] Campbell and Kane, *J.*, 1956, 3130.

[2] Jámbor, *Acta Chim. Acad. Sci. Hung.*, 1954, **4**, 55.

drop with a magnifying lens (replacing the horizontal microscope [2]) and a strong light suitably directed. A Methrohm " titriscope " was used in attempts to titrate formazan with alkali.

To prove the reduction involving nitrogen atoms 1 and 2, 10^{-4}M-triphenyltetrazolium chloride at pH 1 under nitrogen was exposed to daylight. Successive samples were polarographed after adjustment to pH 4 (see Fig. 4), and polarograms were also prepared at various pH values from the solution exposed to daylight for 2 hr. to establish the pH-dependence of half-wave potential in the reduction product.

RESULTS AND DISCUSSION

(*a*) To decide whether the oxidation wave attributed [2] to formazan was due to impurities, polarograms were prepared of the formazan in ethanol and methanol, and also of the supporting electrolyte. Fig. 2 shows that the oxidation wave is visible for both media containing formazan, but is absent from polarograms of the supporting electrolyte.

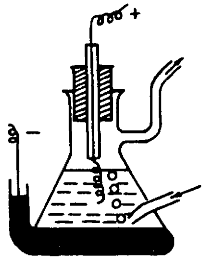

FIG. 1. *Cell for macro-electrolysis.*

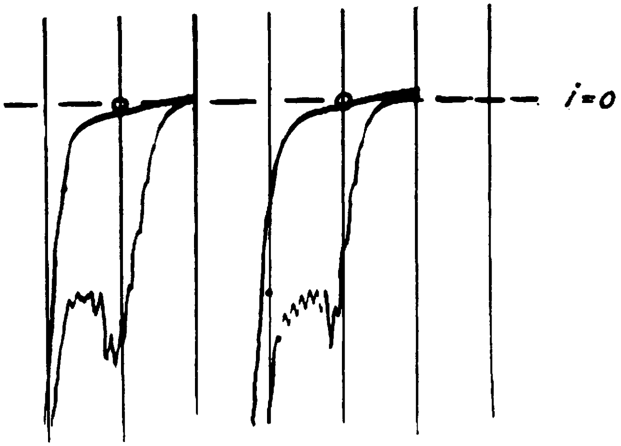

FIG. 2. *Polarograms of triphenylformazan (lower curves) in ethanol (left-hand side) and methanol (right-hand side). The upper curves are of the supporting electrolyte alone. Each horizontal division is* 0·2 v. *Sensitivity* 1/15.

Since the lower horizontal parts of these oxidation waves are obscured by a maximum, particularly in non-automatic polarograms, Campbell and Kane [1] may easily have overlooked the wave. But their Fig. 3 shows that the downward-pointing parts of the polarograms of the tetrazolium chloride and the formazan do not coincide, the curve for the chloride beginning to bend downward at a more positive potential than that of the formazan, so showing that the formazan did produce the wave in question. We agree that, under the conditions of the polarograms in their Fig. 3, the formazan does not produce a wave at the same potential as the tetrazolium chloride does, so that the chloride–formazan system is not *fully* reversible. The formazan produces an anodic wave at a more positive potential. We studied the question of reversibility in alcohol containing concentrated potassium hydroxide, whereas Campbell and Kane used alcohol containing lithium hydroxide, a solution much less alkaline, and we have shown [3,4] that the reversibility of the system increases with pH—at 20° it is complete only above pH 14 and at 70° at pH *ca.* 13. We were also able, by potentiometry of the chloride and the formazan in various proportions, to obtain the sigmoid curve described by the Nernst formula.

In Cambell and Kane's Fig. 3 the β_1 wave of the triphenyltetrazolium salt is separated

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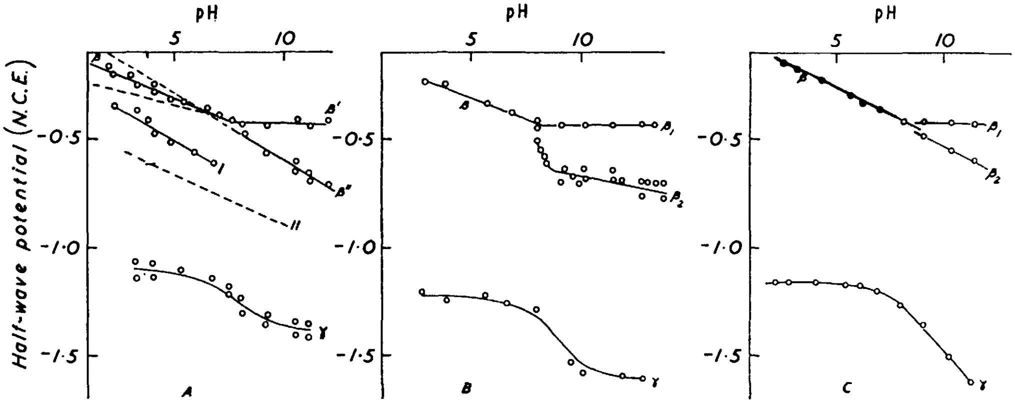
[3] Jámbor, *Nature*, 1955, **176**, 603.

[4] Jámbor, *Acta Chim. Acad. Sci. Hung.*, 1957, **13**, 293.

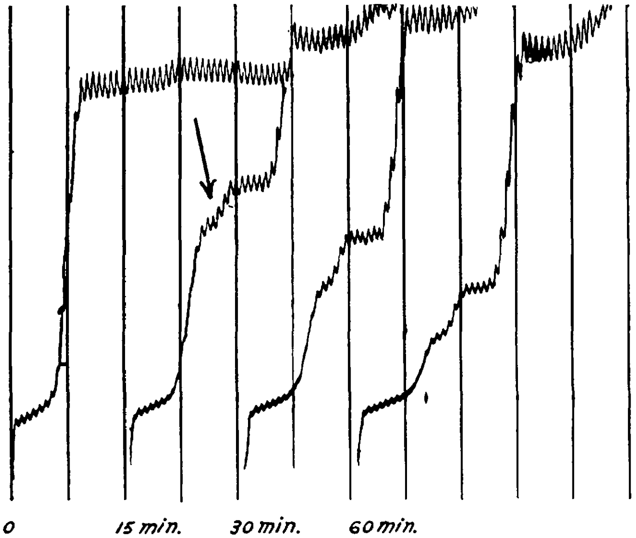
from that of the formazan by *ca.* 0·2 v. We encountered[3] this potential difference in solution of about pH 12·5, whereas our earlier, contested polarograms[2] were prepared at much higher pH's where the distance between the reduction and the oxidation waves was negligible, which means that the mixture of triphenylterazolium salt and the formazan gave a single confluent wave.

FIG. 3. *Plots of half-wave potential (relative to the normal calomel electrode) against* pH *for the* β *and* γ *waves of triphenyltetrazolium chloride, of triphenyltetrazolium chloride reduced at* $N_{(1)}$ *and* $N_{(2)}$ *(I), and of " photo-tetrazolium chloride " (II), obtained (A) in aqueous solution, (B) in 50% ethanol, and (C) the values of Campbell and Kane, obtained in 50% methanol.*



(*b*) Campbell and Kane do not regard our or their results as evidence for our assumption that in acid medium reduction involves the nitrogen atoms 1 and 2, instead of 2 and 3, but think that the tetrazolium salt is transformed into the benzhydrazyl (IV) by simultaneous uptake of 4 electrons; only at pH > 8 does reduction proceed in two waves *via* formazan. We admit that little evidence was given for the temporary formation of the compound (III) reduced at $N_{(1)}$ and $N_{(2)}$, but neither is the simultaneous uptake of 4 electrons proved.

FIG. 4. *Polarograms of oxygen-free solutions of triphenyltetrazolium chloride exposed to light for various periods. The arrow shows the first production of the colourless compound. Each horizontal division is* 0·2 v. *Sensitivity* 1/5.



The essence of our argument is as follows. Formazan has definitely been detected in reductions at pH's less than 7. Furthermore, the formazan concentration increases with pH, which means that the importance of the " formazan reduction route " is a function of pH. Now, because the importance of the formazan reduction route can vary, it follows that there must be some alternative reduction route, which involves an intermediate other than formazan. Experiments with triphenyltetrazolium chloride, which has been exposed to light, suggest that this other intermediate is triphenyltetrazolium chloride, in which reduction has occurred at $N_{(1)}$ and $N_{(2)}$. We had assumed the formation of this colourless compound because the unequal slopes of the half-wave potential–pH curves of the β' and β'' waves, obtained in aqueous solution, indicated that the curves intersect at a lower pH where the order of the two waves is then reversed (Fig. 3*A*). This agrees with the non-formation of formazan in reduction in acid media. The deficiency of this idea lies in that the β wave in the polarogram of triphenyltetrazolium chloride ought to be steepest at the pH of intersection (*i.e.*, about 6) since the potentials of its component β' and β'' waves only coincide at this point and should diverge above and below it. In the alkaline direction the two components do in fact diverge owing to the change in the slope of the half-wave potential–pH curve of the β' wave. In the acid direction the two components remain fused, as the β' and β'' waves do not attain sufficient difference in half-wave potential to separate.

FIG. 5. *Plot of minimum applied voltage at which formazan is detectable for formazan formation from* 10^{-2}M-*triphenyltetrazolium chloride against* pH.

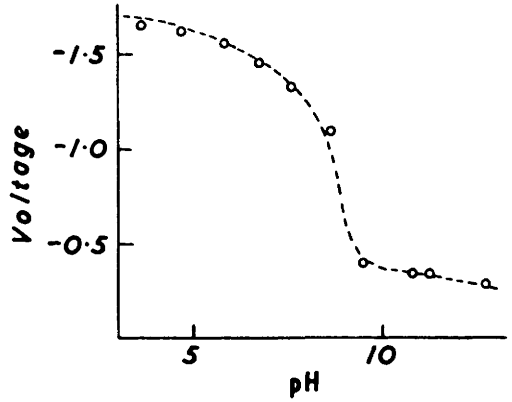
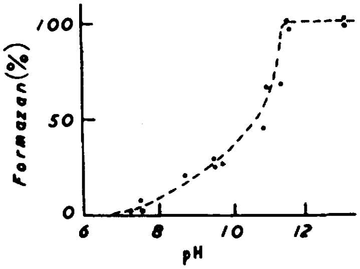


FIG. 6. *Formazan formation* (%) *as a function of* pH *in the macro-electrolytic reduction of triphenyltetrazolium chloride* (10^{-7}M).



That the two half-wave potential–pH curves intersect does not mean that at higher pH's the reduction can only occur *via* formazan and at lower ones by the colourless compound; it means that at this critical point the two routes have equal probability, so that we can assume that a change in pH involves only a change in the probability of route.

In Campbell and Kane's Fig. 4, based on results in 50% methanol, the β wave does not appear to be the resultant of β_1 and β_2, nor does it appear that they intersect as indicated for β' and β'' by our Fig. 3*A* (for aqueous solution). Comparison of Campbell and Kane's curves and ours for alcoholic solution with ours for aqueous solution shows that the alcohol profoundly changes both the half-wave potential and the character of the curve. Nevertheless it seems very probable that the waves β' and β'' (for aqueous solution) can be regarded as identical with β_1 and β_2 respectively (for 50% alcohol). We consider results obtained in aqueous solution to be more reliable as a basis for the mechanism.

As has been shown elsewhere,[5] in acid medium formazan fails to form not only in the course of chemical and electrolytic reduction but also during disproportionation caused

[5] Jámbor, *Agrokémia ésTalajtan*, 1956, **5**, 89.

by light. In the latter reaction one each of two tetrazolium chloride molecules is oxidised to " photo-tetrazolium chloride ", while the other is mostly reduced to formazan in alkaline medium, and to a water-soluble colourless product in an acid one.

If the latter colourless product is to be prevented from being photo-oxidised *via* triphenyltetrazolium chloride into the photo-compound, the reaction with light must be performed in absence of oxygen. In the polarogram of such a solution a new wave does appear but this cannot be due to the tetrazolium salt or the photo-compound (Fig. 4). Its half-wave potential–pH curve is compared with that of the photo-compound in Fig. 3*A*, and evidence is thus given that in acid medium a colourless reduction product of triphenyltetrazolium chloride exists which is not identical with the formazan. It is oxidised rapidly to the tetrazolium salt in air.

Finally, if triphenyltetrazolium chloride were reduced in acid media by simultaneous uptake of four electrons, no formazan should be formed; benzhydrazyl would have to arise in one step. But precipitation of formazan is observed whenever the light-induced disproportionation or the cathodic reaction is carried out with a sufficiently high concentration of triphenyltetrazolium chloride. This can be understood by remembering that pH changes only alter the probability of the route: if large amounts of the tetrazolium salts are reduced sufficient formazan may be produced to be precipitated and thus not be further reduced.

Jámbor's results [4] and Figs. 5 and 6 bear this out. Fig. 5 shows that a large enough potential difference causes perceptible formation of formazan at the dropping-mercury cathode even at as low a pH as 3, and Fig. 6 shows that application of the same potential causes the amount of formazan of cathodic origin to increase exponentially with pH.

Figs. 5 and 6 do not show the full extent of formazan formation because their course is affected by continued reduction to benzhydrazyl. For example, Fig. 6 indicates that no formazan is formed at pH 6 although half of the total triphenyltetrazolium chloride must have been converted into it. Since at this low pH the half-wave potential of the β'' wave, representing continued reduction, coincides with that of the β' wave, the formazan produced can immediately continue to be reduced to the colourless benzhydrazyl.

All this indicates that in acid medium reduction proceeds through some intermediate other than the formazan.

The question still arises, why the wave of this colourless product of disproportionation by light does not appear on polarograms of triphenyltetrazolium chloride within proper pH limits. If this product were an intermediate product of cathodic reduction of the salt in acid medium, the appearance of this wave could be expected. We did not investigate this question further. It seems probable that the intermediate reduction product of triphenyltetrazolium chloride is not identical with the product of the photo-reaction. It can be assumed that in both cases the reduction occurs *via* the same unknown intermediate, which in cathodic reduction is reduced further at once to benzhydrazyl, and in the case of photo-reduction a secondary rearrangement of this intermediate results in the colourless product, the polarogram of which is shown on Fig. 4.

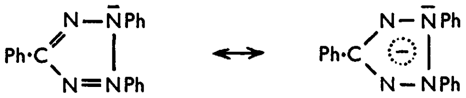
It is striking that on the polarogram of the tetrazolium salt, in strongly acid medium, the 4-electron wave is followed by another 2-electron wave, which disappears on increase in pH. It is not unlikely that this wave is in some way related to the above colourless reduction product.

Essential to us at the moment is only the fact, for which our data appear to furnish support, that in acid medium the reduction of the tetrazolium salt proceeds not *via* formazan but by way of a colourless reduction product, involving not $N_{(2)}$ and $N_{(3)}$ but $N_{(1)}$ and $N_{(2)}$.

(*c*) We are opposed in principle to Campbell and Kane's basing the mechanism of the reduction on $dE/d(pH)$ values taken from polarograms of methanolic solutions. The Nernst formula is strictly valid only for reversible systems, but reduction of triphenyltetrazolium chloride is only reversible in very alkaline medium, even in respect of its first wave. As Campbell and Kane say, the formula can sometimes be applied to irreversible processes, as Jámbor did [2] for aqueous solutions in finding that dE/d(pH) for the β, β'', and β' wave was 45, 60, and 0 mv respectively, in harmony with the number of protons involved. But for alcoholic solutions the curves are much altered (cf. Figs. 3*A*, *B*, and *C*), so that it is not possible to deduce the number of protons from them. For instance, between pH 8·0 and 8·5 the β_2 curve of Fig. 3*B* breaks upwards sharply, and for the steeply ascending portion the Nernst formula is obviously not valid. Campbell and Kane appear not to have observed this break, perhaps because their pH intervals were too wide, or because they polarographed a solution of higher concentration or, probably, because they used methyl and not ethyl alcohol.

Complications also arise in connection with dE/d(pH) for the β' wave measured in aqueous medium. Above pH 9 the reduction product should be completely dissociated, and if, as we maintain, it is the formazan, it should be an acid of pK' *ca.* 8, *i.e.*, titratable with alkali. Attempts at such titration failed: the pH of the aqueous alcoholic solution of the formazan was already above 7 before alkali was added and additions of alkali increased the pH of the solution by the same amounts as that of the formazan-free blank.

Accordingly, we maintain that the formazan is not the primary product of the polarographic reduction in alkaline solution, but that it arises from the primary product by a secondary reaction. Jámbor [3,4] showed that the half-wave potential–pH curve of the oxidation wave of the formazan was quite different from that of the corresponding reduction wave: above pH 9 it is not horizontal but has a slope of *ca.* 75 mv/pH unit. Combined with other results, this shows that in the reduction triphenyltetrazolium chloride, using only a small activation energy, first takes up two electrons only, giving the following unstable product:



This intermediate, with liberation of much energy, is converted into the formazan which is more stable than triphenyltetrazolium chloride. The polarogram of triphenyltetrazolium chloride indicates only the primary reaction, the following ring-fission and proton uptake not affecting the β' wave of the polarogram. Therefore the pK' value indicated by this wave refers to this intermediate, and not to the formazan.

A similar situation may obtain, as we suggested, with the colourless reduction product, and explain why the half-wave potential of the product arising on the action of light differs from that of the intermediate product on the dropping electrode. Further experiments are needed to settle the details of the mechanism of the reduction.

I thank Dr. E. Sándi for carrying out the formazan titrations, and Miss Clara Kisbán for technical assistance.

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[*Received, March* 18*th*, 1957.]