Table V—Rate Constants for the Degradation of V and the Appearance of II (k_3) and VI (k_5) in 52.5% Ethanol at 85°

pH	k, hr^{-1} × 10^3	k_3, hr^{-1} × 10^3	k_5, hr^{-1} × 10^3
1.7	1.37	0.58	0.79
3.8	1.30	0.75	0.55
4.7	1.43	0.70	0.73
6.9	1.27	—	1.27

kcal/mole; for the formation of VI and II, it is 10.3 and 26.7 kcal/mole, respectively. This large difference results because VIIIb is much less favored than VIIIa, as in the degradation of I.

Comparison of the rate constants for the degradation of I and V under the same conditions shows that V is less easily oxidized. This finding is unexpected because the introduction of a methyl group should increase the electron density in the ring system and, therefore, favor the loss of an electron. This explanation can be a steric effect. Phenothiazines are bent around the nitrogen–sulfur axes (17), resulting in two possible conformations. In one conformation, the lone pair on the nitrogen can easily interact with π electrons of the ring system; in the other, the lone pair is forced outside the system. In I itself, the configuration is such that interaction is possible; however, in V, the lone pair is forced outside the system. The methyl group thus decreases the electron density in the aromatic system and increases the stability.

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Mechanism for Phenothiazine Oxidation

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Abstract □ The mechanism of phenothiazine degradation was studied by following the degradation of 3,10'-diphenothiazine in ethanol–water mixtures as well as the electrochemical oxidation of phenothiazine. A mechanism, including the formation of an oxidized dimer and some polymers, is suggested.

Keyphrases □ Phenothiazine—electrochemical oxidation degradation in ethanol–water mixtures, mechanism suggested □ Oxidation—phenothiazine degradation in ethanol–water mixtures, mechanism suggested □ Degradation—phenothiazine in ethanol–water mixtures, mechanism suggested

The isolation and identification of some degradation products of phenothiazine were described previously (1), as were the kinetics of the oxidative degradation in an acidic medium (2). The degradation has been the subject of many investigations (3–9); however, no definite mechanism was established, particularly since 7-(10'-phenothiazinyl)-3H-phenothiazine-3-one was not recognized as a degradation product until recently (1, 2).

In this paper, further experiments to elucidate the mechanism of formation of 7-(10'-phenothiazinyl)-3H-phenothiazine-3-one (XIV) are described, and a mechanism for the overall oxidation is proposed (Scheme I).

EXPERIMENTAL

Materials—All materials were as previously described (2).

Phenothiazine (I) Oxidation in Presence of 3H-Phenothiazine-3-one (VII)—In a mixture of ethanol and water buffered with sodium acetate and hydrochloric acid, 500 µg of I/ml and 50 µg of VII/ml were dissolved. The solutions were saturated with oxygen, placed in screw-capped bottles, and stored in the dark at 75°. The solutions were assayed for I, VII, and XIV as described previously (10).

Oxidation of 3,10'-Diphenothiazine (IXb)—Solutions of 100 µg of IXb/ml were prepared and stored as described. The solution was extracted with carbon tetrachloride; the products were separated by TLC, using silica gel GF$_{254}$ and developing with ether–petroleum ether (1:2). The products were assayed as described previously (10).

Electrochemical Oxidation of I—After dissolving 100 µg of I/ml in the buffered ethanol–water and flushing with nitrogen, a current–voltage curve[1] was recorded at various pH values, using a rotating platinum electrode and a silver–silver chloride reference electrode. Then I was oxidized at a constant potential[2], using a platinum working and auxiliary electrode and a silver–silver chloride reference electrode. The potential of the working electrode was 400 mv throughout this study.

The solution was stirred by bubbling nitrogen continuously through it. After completion of the reaction, the solution was allowed to stand in the dark in a nitrogen atmosphere until a permanent color was obtained.

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[1] Polarograph E 44 S, Bruker, Brussels, Belgium.
[2] Potentiostat PRT 10-05, Tacussel, Villeurfanne, France.
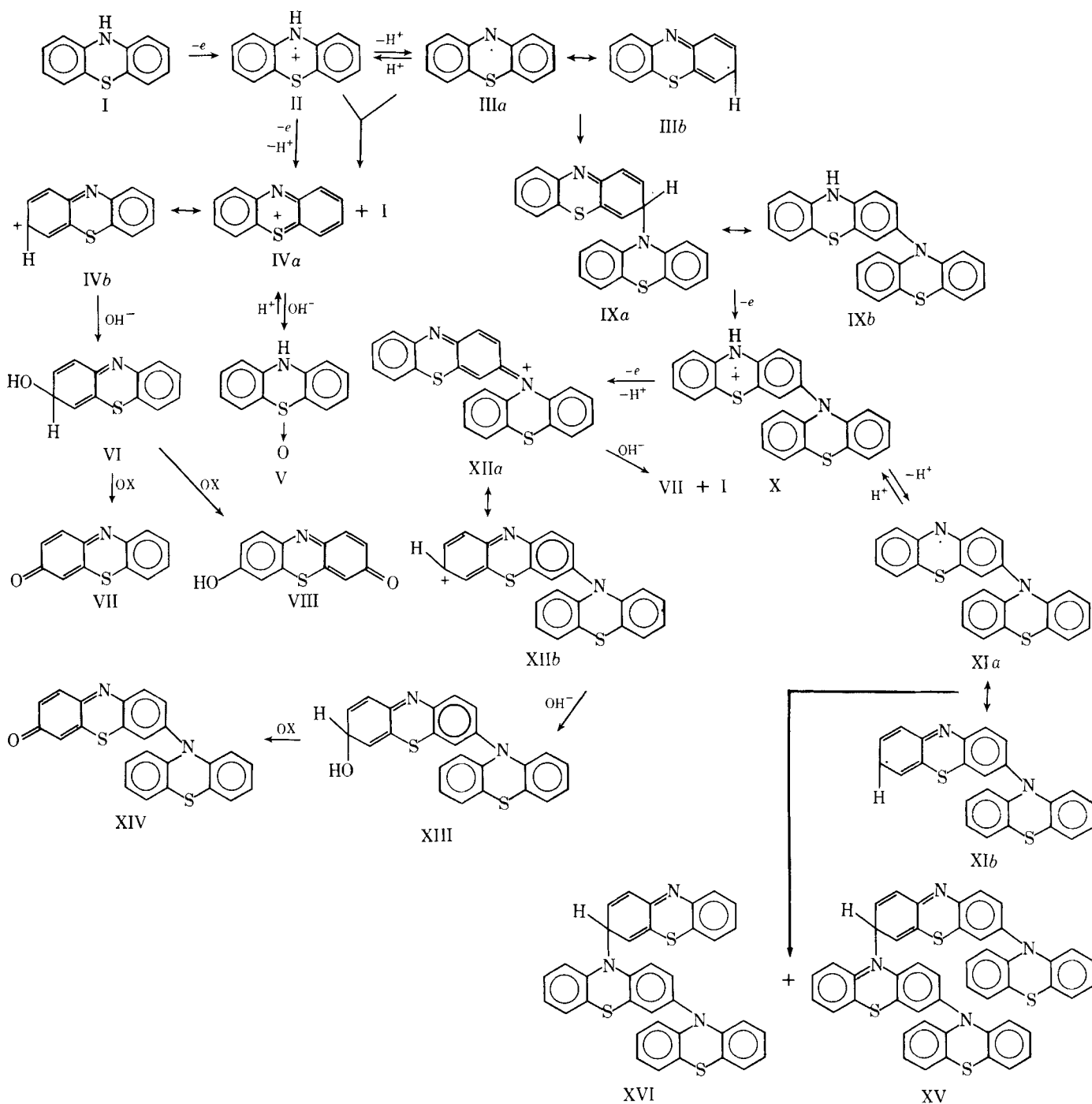
Scheme I

Then the solution was extracted, and the components were separated and analyzed for VII and XIV as described.

RESULTS

Oxidation of I in Presence of VII—The rate constants were determined as described previously (2) and were not significantly different from those obtained when no VII was added. The amount of XIV formed was not significantly different from the amount formed from I without the addition of VII. This result indicates that XIV is not formed from a coupling of I and VII.

Oxidation of IX*b*—The thin-layer chromatograms of partially oxidized IX*b* showed four spots, three of which were IX*b*, VII, and XIV. The fourth spot, with R_f 0.00, was colorless but turned green in daylight; the color was less intense after oxidation at low pH values. Figures 1 and 2

give the amounts of XIV and VII, respectively, formed as a function of time at different pH levels.

These figures show that IX*b* was completely oxidized in 80 hr. They also show the instability of VII and XIV at low pH values. Figure 3 shows the ultimate amount of VII and XIV formed from IX*b* as a function of pH. There was a maximum in the formation of XIV at pH 3.8 and of VII at pH 4.7. Table I shows the amounts of VII and XIV formed from IX*b*

Table I—Amounts of VII and XIV Formed on Oxidation of IX*b* at pH 4.7 and 75° at Various Ethanol Concentrations

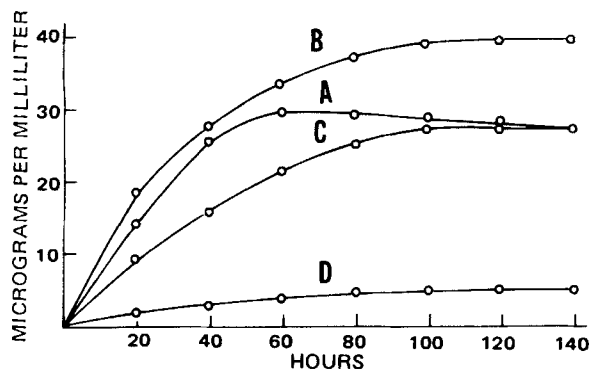| Ethanol, % (w/w)	VII, μg/ml	XIV, μg/ml
47.5	24.2	25.5
52.5	22.1	24.4
62.0	24.5	25.5
72.5	25.5	23.6

Figure 1—*Amount of XIV formed on oxidation of IXb in 52.5% ethanol at 75° at different pH levels as a function of time. Key: A, pH 1.7; B, pH 3.8; C, pH 4.7; and D, pH 6.9.*

Figure 3—*Ultimate amount of XIV (○) and VII (△) formed from IXb in 52.5% ethanol at 75° as a function of pH.*

at different ethanol concentrations at pH 4.7 and 75°; ethanol had no significant influence on the amounts of products formed.

Electrochemical Oxidation of I—Figure 4 shows the current–voltage curves of I in 52.5% ethanol at various pH values. The half-wave potential of the first oxidation step was pH independent; the second half-wave potential was lowered at higher pH levels. Thin-layer chromatograms showed that, in all cases, V, VII, and XIV were formed. Two other spots were formed, both colorless but turning green in daylight; one had the chromatographic characteristics of IXb and the other had R_f 0.00.

Figure 5 shows the amounts of VII and XIV formed from the electrolysis of I as a function of pH, Fig. 6 gives the amounts as a function of the ethanol concentration in the solvent mixture, and Fig. 7 gives the amounts as a function of the working potential. The amount of VII produced increased with increasing pH, and there was a maximum in the formation of XIV at pH 4.7. The amounts of both products increased with increasing ethanol concentration, but this increase was less at higher concentrations. The amounts formed increased with increasing working potential but reached a plateau at 450 mv.

DISCUSSION

Scheme I represents the most probable mechanism for the oxidation of I. The first step is the loss of an electron from I to give the cationic free radical II. The radical can lose a proton to give the neutral radical III, lose a proton and an electron to give the phenazothionium ion IVa, and disproportionate together with III to give I and IVa, as shown by Hanson and Norman (8).

The phenazothionium ion exists in two mesomeric forms, IVa and IVb, which can hydrolyze to the 5-oxide (V) and to 3-hydroxyphenothiazine (VI). Compound VI is rapidly oxidized to 3H-phenothiazine-3-one (VII) and 7-hydroxy-3H-phenothiazine-3-one (VIII). The existence of the neutral radical III was first confirmed by Gilbert et al. (9), whose calculations of spin densities showed the largest densities to be located at the nitrogen atom and at C-3 and C-7. This situation is represented by the equilibrium between IIIa and IIIb.
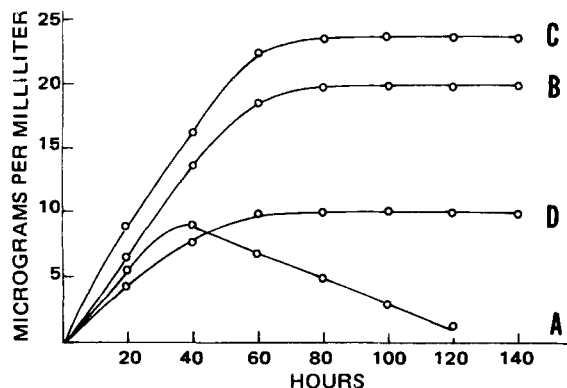

Figure 2—*Amount of VII formed on oxidation of IXb in 52.5% ethanol at 75° at different pH levels as a function of time. Key: A, pH 1.7; B, pH 3.8; C, pH 4.7; and D, pH 6.9.*
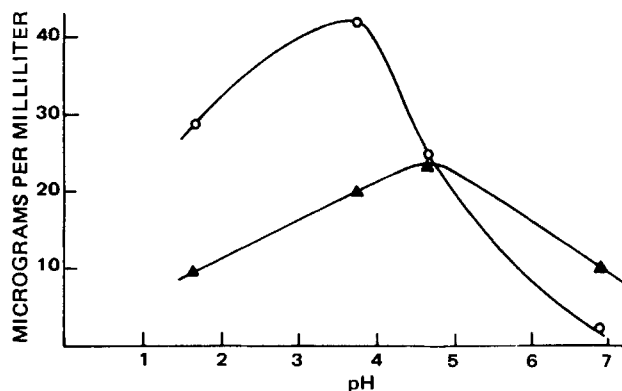
The formation of the dimer IXb has been reported to proceed via IXa (6, 8). This dimer can lose an electron to give the radical X, which can then behave like radical II, forming the ion XIIa and the neutral radical XIa. The disproportionation of X and XI to give XIIa and IXb is not shown for reasons of simplicity. The existence of XIIa has been confirmed (8). Compound XIIa hydrolyzes to I and VII; its mesomeric form XIIb hydrolyzes to a 7-hydroxy dimer (XIII), which is rapidly oxidized to XIV.

The equilibrium between XIa and XIb is in principle the same as that between IIIa and IIIb. These radicals can give several products; two possibilities, shown in Scheme I, are the trimer XVI and the tetramer XV. Although these products were not isolated, their formation is analogous to that of the dimer IX; on oxidation of IXb, at least one product is formed that turns green in air, as do I and IXb. Also, the amounts of VII and XIV produced on oxidation of IXb do not account for all of the starting material, especially at higher pH values (Fig. 2). Raising the pH causes a shift in the equilibrium between X and XI, so that less of the ion XIIa is formed and subsequently less VII and XIV. Compound VII is formed via two pathways, from the phenazothionium ion IV and from the ionic dimer XII. That the latter process occurs is proven by the appearance of VII from the oxidation of IXb.

The kinetic measurements of Ref. 2 support Scheme I. More VII and less V were formed at higher temperatures, and the relative amount of XIV formed was independent of temperature. The degradation of I was independent of pH, and there were a minimum in the formation of V at pH 4.7 and maxima in the formation of VII and XIV at pH's 4.7 and 3.8, respectively.

Increasing the percentage of ethanol in the solvent mixture decreases the degradation rate of I and increases the relative amounts of VII and XIV, up to a given ethanol concentration. The relative increase in the formation of VII at higher temperatures suggests that the mesomer IVa is energetically favored over mesomer IVb, so the formation of VII has a higher energy of activation than does the formation of V. The temperature independence of the amount of XIV formed is probably due to a shift of the acid–base equilibrium of II and III being compensated for by a comparable shift in the equilibria between X and XI, resulting in a temperature independence of the formation of the ion XIIa and, therefore, of XIV.
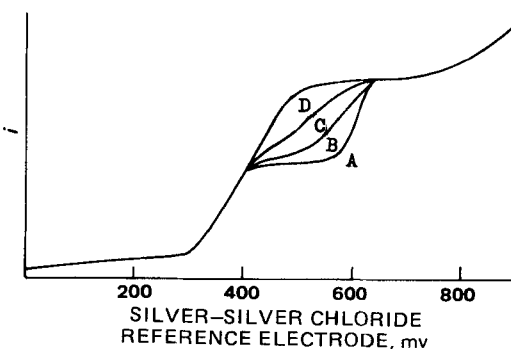

Figure 4—*Current (i)–voltage curves of I in 52.5% ethanol at various pH levels. Key: A, pH 1.7; B, pH 3.8; C, pH 4.7; and D, pH 6.9.*
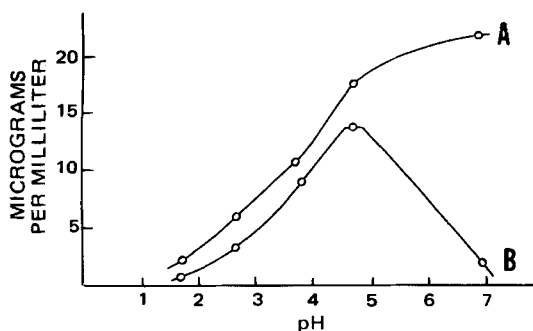
Figure 5—*Amounts of VII (A) and XIV (B) formed on electrolysis of I in 52.5% ethanol as a function of pH.*

The first step of the oxidation of I does not involve hydrogen ions, so the half-wave potential of the first step of the electrochemical oxidation (Fig. 4) and the degradation of I is pH independent. As a result of the acid–base equilibrium between II and III, there is more dimerization, so less IV and V are formed at higher pH values. At still higher pH values, the half-wave potential of the second oxidation step (II → IV) is lowered to such an extent that there are a large increase in the velocity of this step and a resultant increase in the formation of V. The net result is a minimum in the formation of V at pH 4.7 (2).

Because VII is produced *via* two mechanisms, the influence of pH is more difficult to predict; however, some estimate of the amounts produced by both pathways can be made from the data on the oxidation of IXb. The amount of VII produced *via* XIIa is calculated from the ratio of the amounts of VII to XIV produced on the oxidation of IXb; this ratio is multiplied by the amount of XIV formed on oxidation of I. This value, subtracted from the total amount of VII, gives the amount produced *via* IV. The instability of the products at low pH values makes these calculations only approximate. Figure 8 shows the decrease in the amount of VII formed in one half-life of phenothiazine as the pH is raised, in spite of the fact that there is an increase in the formation of IV at pH levels above 4.7. This result is due to the pH dependence of the reaction IV → V (4, 5), favoring the formation of V at higher pH's.

At pH 4.7, there are a minimum in the formation of IV and a maximum in the formation of the dimer IX. On oxidation of the dimer, the cationic radical X is formed, which converts to the neutral radical XI and the ion XIIa. At higher pH values, the formation of XIIa is decreased, so the maximum in the formation of XIIa from I is at a lower pH than the maximum in the formation of IX. From the oxidation of IXb, it is seen that at higher pH values the ratio of VII to XIV increases. Therefore, the maximum in the formation of XIV is at a lower pH than the maxima in the formation of XII and of VII. The maximum in the formation of XIV is at pH 3.8; for VII and IXb, it is at pH 4.7.

Increased ethanol concentration in the solvent mixture favors the formation of the nonionic III, resulting in more dimerization and more VII and XIV. However, at still higher ethanol concentrations, XI is favored over X, resulting in no further increase in the amounts of VII and XIV.
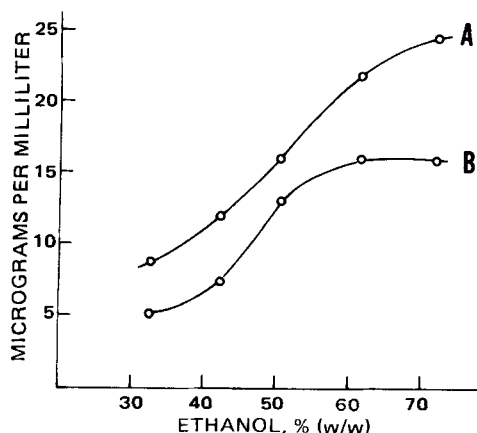

Figure 6—*Amounts of VII (A) and XIV (B) formed on electrolysis of I at pH 4.7 as a function of the ethanol concentration.*
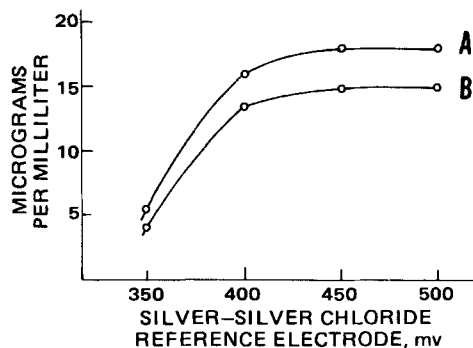

Figure 7—*Amounts of VII (A) and XIV (B) formed on electrolysis of I in 52.5% ethanol at pH 4.7 as a function of the potential of the working electrode.*
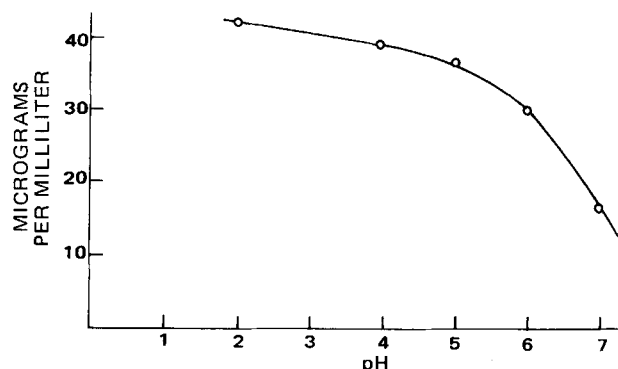

Figure 8—*Amount of VII formed* via *the ion IV in one half-life of I as a function of pH, calculated as described in the text.*

In the electrochemical oxidation of I, the rate-determining step is no longer the formation of the radical II. When the oxidation potential is high enough to oxidize I but not II, there is buildup of II. At high pH values, the radical is deprotonated, allowing considerable dimerization. The rapid oxidation of IXb suggests that it, as well as X, has a low oxidation potential and that the formation of XIIa increases with pH. As the pH is raised, there is an increase of VII at the expense of XIV on electrochemical oxidation, resulting in a maximum in the formation of XIV (Fig. 5).

If it is assumed that increasing the oxidation potential increases the concentration of II, then dimerization and the formation of VII and XIV increase up to a maximum (Fig. 7).

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