[FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Potentiometric Studies on Semiquinones

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I. General and Theoretical Part

There are numerous aromatic compounds on an oxidation level intermediary between a quinone and hydroquinone, or between a diamine and a diimine, or between phenazine and dihydrophenazine, and comparable instances. The cases of α-oxymethylphenazine (pyocyanine),[1a] of α-oxyphenazine[1b,1c,1d] and of Wurster's dyes[1e] have been discussed in previous papers. A potentiometric study showed that the intermediary compounds are radicals with an odd number of electrons and of the same molecular size as the fully oxidized or the fully reduced form, and not bimolecular meriquinones such as suggested by Willstätter and Piccard.[2] Our assumption is in agreement with Weitz,[3] who, in special cases, succeeded in determining the molecular weight by the boiling point method. In contrast herewith, our method was a potentiometric one.

Certain difficulties in the application and interpretation of the potentiometric method had to be overcome to arrive at these results. In the first place, the substances concerned may be, in one form or other of the three levels of oxidation, difficult to dissolve. This prevents the establishment of a homogeneous system during the oxidative or reductive titration and makes the interpretation of the titration curve difficult. An instance of this kind is phenazine, of which a green intermediary state of reduction has been known for a very long time. This difficulty can be overcome by applying an organic solvent, especially acetic acid. Another difficulty may arise from the fact that sometimes one of the three forms is an unstable molecule undergoing a rapid and irreversible rearrangement or destruction. In some cases, the intermediary form is the most unstable one, *e. g.*, in the benzidine compounds. Sometimes the completely oxidized form is the most unstable, *e. g.*, in Wurster's dyes. In some cases the completely reduced form is the unstable one, namely, in γ,γ'-dipyridyl.

None of the cases to be described in this paper shows any evidence that the radical might undergo a partial polymerization, in the way triphenylmethyl partially associates to hexaphenylethane. The explanation may be found in the fact that all these semiquinones have the character of cations and very likely do not exist at all or have only a very short lifetime in an un-ionized condition. It is, however, unlikely that two molecules of a

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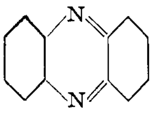
[1] (a) Friedheim and Michaelis, *J. Biol. Chem.*, **91**, 355 (1931); see also Elema, *Rec. trav. chim. Pays-Bas*, **50**, 807 (1931). (b) Michaelis, *J. Biol. Chem.*, **92**, 211 (1931). (c) Michaelis, *ibid.*, **96**, 703 (1931). (d) Michaelis, Hill and Schubert, *Biochem. Z.*, **255**, 66 (1932). (e) Michaelis, THIS JOURNAL, **53**, 2953 (1931).

[2] Willstätter and Piccard, *Ber.*, **41**, 1458 (1908).

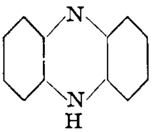
[3] (a) Weitz, *Z. Elektrochemie*, **34**, 538 (1928); (b) Weitz, König and Wistinghausen, *Ber.*, **57**, 153 (1924); (c) Weitz and Fischer, *ibid.*, **59**, 432 (1926).

univalent cation should combine to any appreciable extent to form one molecule of a bivalent cation, for electrostatic reasons. In contrast herewith, in the case of triphenylmethyl or diphenyl nitride, two electroneutral molecules associate to form a double molecule.

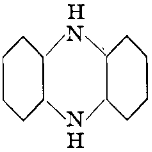
To illustrate the matter, let us consider what takes place with phenazine. Disregarding all ionizations, we may write the three forms in this way



I

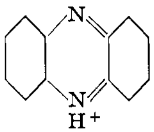


II

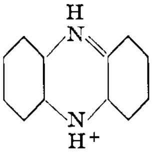


III

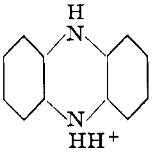
I is the oxidized form of phenazine, II the intermediary form, which in the language of these formulas contains a "bivalent" nitrogen atom, and III is the completely reduced form. Experience, however, shows that II is obtainable only in acid solutions. On writing these formulas as they occur in moderately acid solutions, as univalent cations, we obtain



Ia



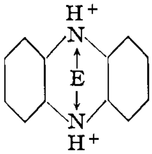
IIa



IIIa

In order to explain the existence of a molecule such as IIa we rewrite it in the following way

IIb



Here E means an electron. We assume that this electron has no definite place in one N-atom but is shared by both N-atoms. The outer electron shell of each nitrogen atom is a septet, and one odd electron alternatively fills up the one or the other septet to an octet. Each N-atom with a septet may be very unstable and liable to secondary, possibly irreversible, reactions. Nevertheless, its life-time will have some short duration (there are even cases known in which the septet is a fairly stable arrangement, as in triphenylmethyl and biphenyl nitride). Before any irreversible disruption happens to the whole structure, the septet is filled up again to form an octet. Thus the oscillation of the odd electron between the two septets imparts to the whole structure sufficient stability, even where the septet structure, without the support of the oscillating eighth electron, might be a more labile structure.

One way of picturing the status of the odd electron is to assume that this electron has a path around both N-atoms jointly. In this way, the situation was tentatively pictured in the previous paper.[1e] It seems advisable not to take this picture too literally. Mechanical models for atomic structures involving definite circuits of electrons have been abandoned by atom physicists. Suffice it to express the situation by saying that the probability of the odd electron being a constituent of the one nitrogen atom or the other is the same. At any rate this configuration is equivalent to an electric oscillator and can be considered responsible for the very intense coloring of these compounds.

The molecule of formula IIa or IIb is a univalent cation. In no other state of dissociation can the regular periodic oscillation be imagined. If this oscillation be the principle maintaining the structure of the otherwise labile molecule, the monovalent cation should be more stable than all the other forms of dissociation. In fact, all these semiquinones are stable only in moderately acid solutions; and in the case of the aromatic diamines it will be shown in the special part that the lability is very considerably increased when, by changes in *P*H, the state of ionization is altered.

The idea suggests itself to correlate the hypothesis of the alternating odd electron of the semiquinone-like molecules with the very distinct and beautiful band spectrum exhibited by the majority of these compounds (namely, all alkylated aromatic diamines, and the derivatives of γ,γ'-dipyridyl) in their semiquinoid form. This is even more so as another group of radicals with an odd electron exhibits the same type of band spectrum, such as triphenylmethyl and related compounds.[4] It fits the hypothesis presented very well, that the very distinct band spectrum is shown, in the case of triphenylmethyl, by the electroneutral radical, whereas in the case of Wurster's dyes or the half-reduced dipyridyl compounds, the band spectrum is encountered in the form of the univalent cations.

II. Special and Experimental Part

1. **Materials Used.**—Dimethyl-, tetramethyl-, diethyl- and tetraethyl-*p*-phenylenediamine were used in the form of the commercial hydrochlorides (Eastman), the two first mentioned after recrystallization according to previous directions[1e] without changing the results.

Diphenyl-*p*-phenylenediamine was prepared according to v. Bandrowski[5] and Calm,[6] by what the first of these authors calls his third method.

γ,γ-Dipyridyl was prepared in the following way. Diacetyl-[tetrahydro-γ,γ'-dipyridyl] was prepared according to Dimroth and Heene.[7] This substance on suitable oxidation directly yields γ,γ'-dipyridyl. This oxidation is a somewhat variable reaction, the yields differing widely. Dimroth and Heene recommend the bubbling of oxygen

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[4] Meyer and Wieland, *Ber.*, **44**, 2557 (1911).

[5] V. Bandrowski, *Monatsh.*, **8**, 475 (1887).

[6] Calm, *Ber.*, **16**, 2786 (1883); *cf.* p. 2805.

[7] Dimroth and Heene, *ibid.*, **54**, 2934 (1921).

through the suspension of the above substance. We found the following method most convenient. The freshly prepared substance was exposed to the air at 48° in an open dish for two or three days until it had been converted into dark brown lumps with a strong smell of acetic acid. Ten grams of these lumps was suspended in 300 cc. of water and ten grams of manganese dioxide added and the mixture shaken at room temperature for half an hour. Then enough saturated solution of disodium phosphate is added to bring the acid reaction almost to neutrality. The liquid is now filtered off. It is yellow-brown in color. By adding a suitable amount of sodium hydroxide the γ,γ'-dipyridyl directly crystallizes in white, shiny needles. The yield is far from the theoretical but satisfactory for the preparation.

N,N'-Dimethyl-γ,γ'-dipyridylium chloride was prepared in the following way. Five-tenths gram of γ,γ'-dipyridyl is heated in a test-tube with 3 cc. of dimethyl sulfate for a short time until the crystals are completely dissolved. This solution is poured into 30 cc. of water, the excess of dimethyl sulfate removed by washing with ether, enough aqueous solution of picric acid is added to bring about a complete precipitation of the picrate. The crystals are collected on a Buchner filter, washed with picric acid solution and shortly with water, then dried. After grinding, they are suspended in a rather strong hydrochloric acid solution. For each gram of the picrate 40 cc. of concentrated hydrochloric acid plus 160 cc. of water is an appropriate amount. This suspension is heated to complete solution, and while still warm, before recrystallization starts, is extracted with toluene. This extraction is repeated later on with ether, to the complete removal of the picric acid. The colorless solution is evaporated to dryness, either on a steam-bath, or *in vacuo* at 50°, the dry residue dissolved in methyl alcohol. Then benzene is added. This solution is heated on the steam-bath until enough methyl alcohol has been removed to allow a separation of the chloride of the base. The crystals are filtered off, washed with benzene, dried and redissolved in ethyl alcohol. On addition of a suitable amount of benzene, crystallization begins. In the ice box beautiful, long, shiny, colorless needles are formed and can be collected on a filter. Five-tenths gram of γ,γ'-dipyridyl gave 0.53 g. of the dry chloride of the ammonium base, or 65% of the theoretical yield.

The analysis gave the following results (Dr. Elek): C, 55.11; H, 5.70; N, 11.07; Cl, 26.59. Calcd. for $C_{12}H_{14}N_2Cl_2$: C, 56.25; H, 5.47; N, 10.94; Cl, 27.34.

Benzidine, *o*-tolidine, tetramethylbenzidine were commercial preparations, recrystallized. Tetrabromobenzidine and dibromotolidine were prepared according to Schlenk.[8]

Phenazine was prepared in the following way, which is rather obvious, though we could find no reference to just this modification probably because of the small yield procurable by the method.

Two grams of pyrocatechol is dissolved in 250 cc. of dry ether. Twenty grams of dry lead peroxide is added. After shaking for thirty minutes the lead peroxide is removed by filtering and washed out with some more ether. The red-brown filtrate is mixed with a solution of 2 g. of *o*-phenylenediamine in a mixture of glacial acetic acid and ether. After an hour this ether solution is extracted successively once with 2% hydrochloric acid, twice with 5% sodium hydroxide and with water. The remaining ether is amber-yellow. The ether is evaporated and the residue sublimed *in vacuo*. After one sublimation the sample melted at 171°. Nitrogen, 15.46; calcd., 15.55%.

Methoxyphenazine was prepared according to Wrede and Strack[9] with a slight modification especially in the preparation of the asymmetrical pyrogallol monomethyl ether, which is requisite for the synthesis, as described in a previous paper.[1e]

A series of various naphthazonium compounds was placed at our disposal by Dr. W. A. Jacobs. Thanks are due to him.

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(8) Schlenk, *Ann.*, **363**, 313 (1908).

(9) Wrede and Strack, *Z. physiol. Chem.*, **181**, 58 (1929); *Ber.*, **62**, 2051 (1929).

The methods of titration are the same as described in previous papers.[10] All titrations were performed in a stream of nitrogen purified by heated copper.[11]

Special Part

1. The Alkylated Aromatic Diamines.—In *p*-phenylenediamine the first step of oxidation is too labile a compound to be worthy of a careful study. On oxidation with bromine or any other strong oxidant it turns successively to light yellow, green, red, colorless. The first yellow product is probably the semiquinone, but the potentials drift too much for any reliable information. Piccard[12] considers the green stage as what he called the meriquinone. It is possible that he did not give attention to the first yellow stage which is obtained by adding a very small amount of oxidant to a large excess of the diamine in the P_{H} range of the acetate buffer.

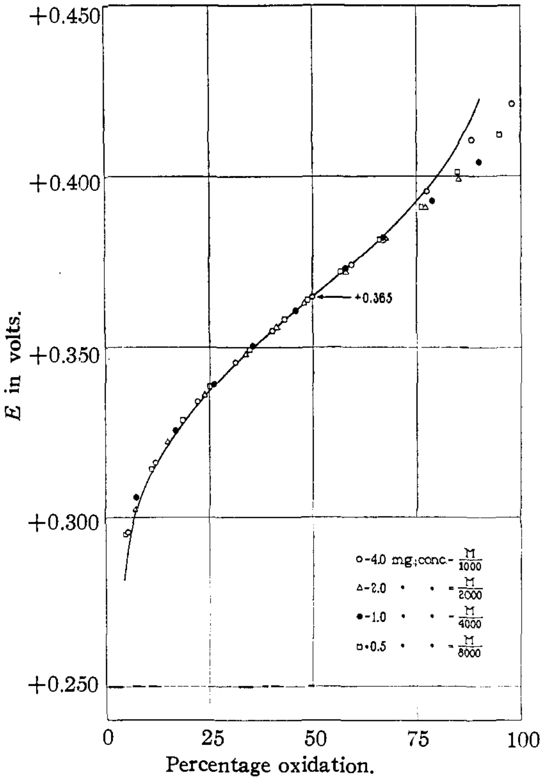

Fig. 1.—Independence of the normal potentials of tetramethyl-*p*-phenylenediamine on the initial concentration: potentials are plotted against percentage of oxidation; ordinate is potential in volts; abscissa is percentage of oxidation; potentials referred to the normal hydrogen electrode; P_{H} 4.620, varying no more than ±0.01 in the individual titration experiments.

In asymmetrical dimethyl-*p*-phenylenediamine, the substance produced in the first stage of the oxidation, Wurster's red, is much more stable, and in tetramethyl-*p*-phenylenediamine it is a perfectly stable substance (Wurster's blue). In presence of an excess of the unoxidized diamine, the potentials are perfectly stable. Otherwise even with the tetramethyl compound, a drift of the potential occurs due to the fact that the second step of oxidation, the tetramethyl-diimonium compound, is extremely labile. Stable and interpretable potential readings can be obtained, therefore, only up to about 60–70% of

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(10) Michaelis and Eagle, *J. Biol. Chem.*, **87**, 713 (1930).

(11) Michaelis and Flexner, *ibid.*, **79**, 689 (1928).

(12) Piccard, *Ber.*, **46**, 1843 (1913).

the theoretical end-point of the titration of the first step. Therefore, in the previous paper only the first half of the curves was used to calculate the results. It is desirable, however, to prove that the neglect of the second half of the curve, with drifting potentials, is justified. Such proof can be given by showing that a number of consequences ensuing from our interpretation can be corroborated by independent methods. There are three corollaries of our interpretation: (a) if our hypothesis is correct, the potential, as plotted against percentage of oxidation, should be independent of the initial concentration of the substances. In part this has been shown in the previous paper.[1e] It can, however, be shown to a much greater extent by the following experiments (see Fig. 1).

(b) If the hypothesis is correct, the amount of a mono-equivalent oxidant used up at the supposedly 50% point of the titration should be half the molar amount of the initial diamine. This is proved by the data shown in Table I.

TABLE I[a]

5.17×10^{-5} MOLE OF DIMETHYL-*p*-PHENYLENEDIAMINE HYDROCHLORIDE DISSOLVED IN 20 CC. OF ACETATE BUFFER, *P*H 4.62, AND TITRATED WITH BROMINE, THE WHOLE TITRATION LASTING ABOUT THREE MINUTES

E calculated according to the formula $E = 0.447 + 0.0601 \log \%\text{ oxidation}/100 - \%\text{ oxidation}$.

Br_2 soln., cc.	% Oxidation, to the first step	E in volt Obs.	E in volt Calcd.
0.0	0.0		
.250	10.8	+0.394	+0.392
.641	27.6	.424	.422
.988	42.5	.440	.439
1.398	60.2	.456	.457
1.780	76.7	.470	.478
2.082	89.7	.478	.503

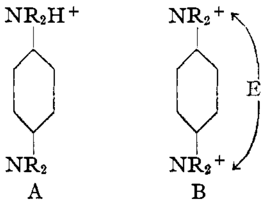
By titration, 10 cc. of the bromine solution after adding an excess of potassium iodide, used up 2.255 cc. of $N/10$ sodium thiosulfate. At the midpoint (50% oxidation), 1.16 cc. bromine had been used up according to graphic interpolation.

$1.16 \times 2.255 \times 10^{-5} = 2.61 \times 10^{-5}$ gram atom of bromine used for one-half of the first step of oxidation and = one-half the initial concentration of the diamine (2.59×10^{-5}).

[a] All potentials in this paper are referred to the normal hydrogen electrode. They were measured against a saturated calomel half cell the potential of which was frequently checked by comparison with the hydrogen electrode in standard acetate buffer the *P*H of which was taken as 4.620. The temperature was always 30 ± 0.1° and maintained by working in a constant temperature room.

(c) If the hypothesis is correct, a series of titration curves over a wide range of *P*H would enable us to draw a plot showing the normal potential as a function of *P*H. The slope of this curve should be constant as long as no change of the dissociation interferes. Considering the state of ionization in moderately acid solution, we have to assume the primary cation (formula A) for the diamine, where R is alkyl or hydrogen, and the formula

B for the oxidized form. These two molecules differ by one hydrogen atom, hence the slope of our curves should be 0.06 volt per *P*H unit in moderately acid solutions. This is true, as Fig. 2 shows. It is obvious that this slope cannot be maintained throughout all ranges of *P*H. In fact, the diagram (Fig. 2) shows bendings of the slope at either end of the curves. Where these bendings begin, the potentials become so strongly drifting that accurate determinations of the normal potentials become difficult or even impossible. The change of the slope indicates that the substances change their state of dissociation. It fits very well the theory presented, that simultaneously with the change of dissociation the stability of the substances is lost. The arrows in Fig. 2 show the dissocia-



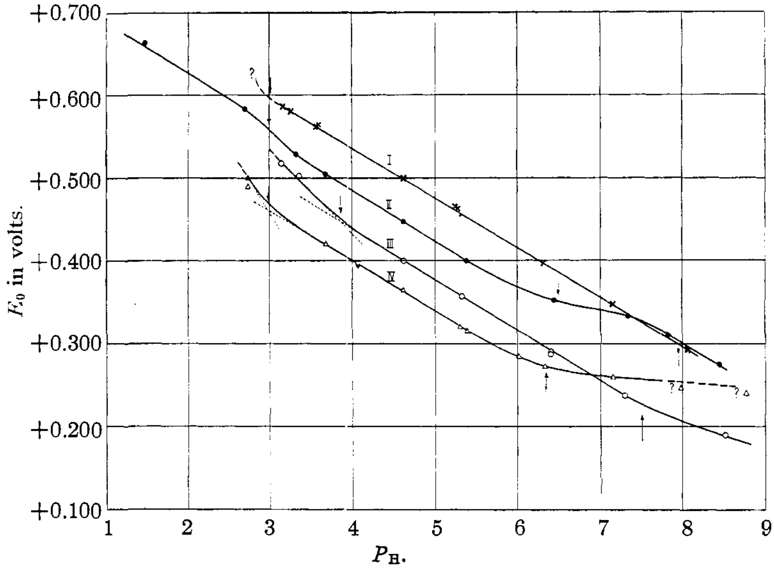


Fig. 2.—Normal potentials are plotted against *P*H for the four alkylated diamines as follows: x, diethyl-*p*-phenylenediamine; ●, dimethylphenylenediamine; ○, tetraethylphenylenediamine; △, tetramethylphenylenediamine; ordinates, normal potential in volts; abscissas, *P*H; potentials referred to the normal hydrogen electrode.

tion exponents of the diamines (in their original, reduced form) as directly determined by acidimetric titration with the hydrogen gas electrode. They agree fairly well with the bends of the curves considering the limits of error in those *P*H ranges where the potential drift becomes disturbing. Tables II, III, and IV are records of representative individual titration experiments, included to show the agreement between our observed potential values and those calculated according to the theory. The agreement is in general much better for the tetramethyl compound because there is practically no drift of the potential up to about 60% of the oxidation. This statement is in an unexplainable contradiction to the one of Clark, Cohen and Gibbs,[13] who report stronger drifts for the tetramethyl than for the dimethyl compound.

TABLE II

TETRAMETHYL-*p*-PHENYLENEDIAMINE

Oxidant, bromine. *P*H, 3.657 (acetate buffer). Concn. = 2 mg./20 cc. = *M*/2000. $E = 0.420 + 0.0601 \log$ % oxidation/ (100 − % oxidation)

% oxidation	Observed potential	Calculated potential
4.09	+0.3420	+0.3381
10.0	.3639	.3632
15.3	.3766	.3760
23.1	.3894	.3889
30.4	.3995	.3991
37.6	.4075	.4072
44.5	.4151	.4149
53.06	.4229	.4231
60.2	.4293	.4317
70.1	.4379	.4422
78.2	.4442	.4532

The agreement of the observed and calculated values is satisfactory up to 53% of oxidation.

TABLE III

TETRAMETHYL-*p*-PHENYLENEDIAMINE

Oxidant, bromine. *P*H, 4.628 (acetate buffer). Concn. = 0.5 mg./20 cc. = *M*/8000. $E = 0.365 + 0.0601 \log$ % oxidation/(100 − % oxidation)

% oxidation	Observed potential	Calculated potential
11.2	+0.3143	+0.3130
18.2	.3283	.3261
25.7	.3387	.3380
34.5	.3492	.3483
43.2	.3580	.3579
48.8	.3639	.3638
57.7	.3725	.3731
66.7	.3812	.3831
76.3	.3909	.3954
85.2	.4010	.4100
94.6	.4120	.4390

Agreement between observed and calculated values is satisfactory up to about 60% of oxidation.

TABLE IV

DIMETHYL-*p*-PHENYLENEDIAMINE

Oxidant, bromine. *P*H, 4.620 (acetate buffer). Concn. = 2 mg./20 cc. = *M*/1725. $E = 0.450 + 0.0601 \log$ % oxidation/(100 − % oxidation)

% oxidation	Observed potential	Calcd. potential
8.26	+0.3886	+0.3873
19.2	.4140	.4126
40.3	.4409	.4402
52.3	.4520	.4523
71.5	.4710	.4739
88.8	.4877	.5039

Note to Tables II to IV.—Duration of the whole titration experiment for the dimethyl compound two to three minutes. For the tetramethyl compound the duration of the experiment is irrelevant up to 60% of oxidation.

2. Phenylated Diamines.—The diimines of the phenylated aromatic diamines, in contrast to those of the alkylated ones, are stable substances. Symmetrical diphenyl-*p*-phenylenediimine was described under the name of azophenylene by v. Bandrowski[14] as a red dyestuff. The quinhydrone-like intermediary form has been described by Piccard.[12] It is a fortu-

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(13) Clark, Cohen and Gibbs, Public Health Reports, Suppl. No. 54 (1926).

(14) Von Bandrowski, *Monatsh.*, **8**, 475 (1887).

nate circumstance that in this case the substances produced at each stage of oxidation are both stable compounds. The difficulty in obtaining good titration curves consists only in the insufficient solubility of the diamine itself in aqueous media. Yet the solubility in acetic acid is satisfactory. It is not necessary to work in water-free acetic acid. A mixture of 90% glacial acetic acid and 10% water is sufficient and offers the advantage of a higher electric conductivity. Diphenyl-*p*-phenylenediamine was dissolved in the medium and titrated with a solution of bromine in the same medium. The potentials showed scarcely any drift except toward the end of a titration, and an easily reproducible curve could be obtained.

TABLE V

DIPHENYL-*p*-PHENYLENEDIAMINE–HCl

Solvent, 90% acetic acid. Oxidant, bromine, in the same solvent. *P*H, −0.49 (90% HAc).[a] Concn. = 1 mg./20 cc.

1st step

$$E = 0.592 + 0.0601 \log \frac{\%\ \text{oxid.}}{100 - \%\ \text{oxid.}}$$

2nd step

$$E = 0.795 + 0.601 \log \frac{\%\ \text{oxid.}}{100 - \%\ \text{oxid.}}$$

1st step % oxid.	Observed potential	Calcd. potential	2nd step % oxid.	Observed potential	Calcd. potential
10.2	+0.5405	+0.5386	12.2	+0.7375	+0.7436
31.1	.5735	.5727	37.6	.7820	.7819
40.0	.5830	.5825	62.6	.8080	.8083
54.5	.5980	.5977	81.4	.8230♀	.8330
70.0	.6155	.6147	98.0	.8355♀	.8660
86.5	.6395	.6404			

♀ signifies drift of the potential

[a] The *P*H has been calculated from the potential of the hydrogen electrode as though it were an aqueous solution.

These experiments show both steps of the oxidation, and these two steps do not overlap as in the case of Wurster's dyes, but are separated by a distinct jump (Fig. 3 and Table V). So, the normal potentials of both steps of the oxidation can be determined and they were found also to be independent of the initial concentration of the substance. The slope of the curve for each step was precisely the one expected for a one-electron system.

3. The Benzidine Compounds.—Much time was devoted, without success, to a search for some benzidine compound which might give a non-drifting potential curve at any *P*H in which the green intermediary oxidation form is developed in any solvent. We tried benzidine, *o*-tolidine, tetramethylbenzidine, dibromotolidine and tetrabromobenzidine. It may be mentioned that in very acid solution with tolidine a regular titration curve could be obtained as in any ordinary dyestuff without any intermediary green form. This is a confirmation of the statement made by Clark, Cohen and Gibbs.[13]

4. γ,γ'-Dipyridyl and Dimethyl-dipyridylium Chloride.—When γ,γ'-dipyridyl, dissolved in diluted acetic acid, is reduced by chromous chloride, a deep violet color is developed. The substance so produced has been studied by Dimroth and Heene[15] and found to be on the oxidation level of a quinhydrone, intermediary between dipyridyl and a hypothetical dihydrodipyridyl. It is easily reoxidized to dipyridyl. When the reduction is performed with a still stronger reductant, zinc dust, the reduction goes through this violet stage to a second colorless stage, and this second reduction is irreversible. It is obvious from these remarks that the potential range even of the first step of reduction lies in a hydrogen overvoltage range. Thus it is not surprising that we could not obtain trustworthy potential curves by reducing γ,γ'-dipyridyl with chromous chloride.

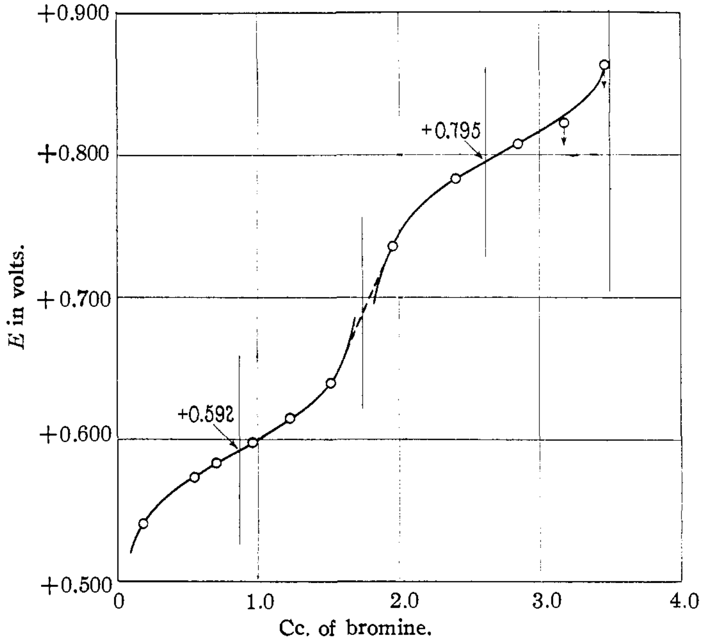


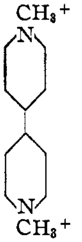
Fig. 3.—Titration curve for diphenyl-*p*-phenylenediamine in 90% acetic acid: ordinates, potentials referred to the normal hydrogen electrode; abscissas, cc. of bromine solution.

The violet dye can be produced only in moderately acid solutions. According to the reasoning presented in the general part of this paper, the radical should be stable only in the form of a univalent cation. It thus appeared probable that an increase in the basic character of the radical would increase its stability. Since the quaternary ammonium bases derived from pyridine are more strongly basic than the original substance, we examined an alkylated dipyridyl. When N,N'-dimethyl-γ,γ'-dipyridylium chloride was reduced a deep blue color was developed, with one distinct absorption band, the maximum of absorption being 602 mμ,

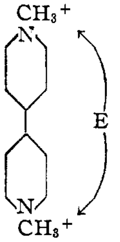
(15) Dimroth and Heene, *Ber.*, **54**, 2984 (1921); Dimroth and Frister, *ibid.*, **55**, 3693 (1922).

whereas that of the unsubstituted γ,γ'-dipyridyl dye is 582 mμ. According to expectation this dye was developed also in alkaline solution, and in this case sodium hydrosulfite could be used as a reductant. The reductive titration of this quaternary ammonium compound in alkaline solution with sodium hydrosulfite gave very distinct potential curves. The potentials were instantaneously established and showed only a slow drift even in strongly alkaline solutions. Titration experiments, when performed not too slowly, gave reproducible titration curves which leave no doubt about the interpretation. The colorless solution turns blue on adding the reductant and the end of the first step of titration is recognizable by a jump of the potential to a more negative level. The second titration curve cannot be completed because it lies in the range of hydrogen overvoltage even in alkaline solution.

The slope of the titration curve for the first step of reduction is the one for a one-electron system. If it is true that the original substance has the molecular size of a dipyridyl compound, then the blue, reduced form must have the same molecular size and hereby it is proved that this blue dye is also a semiquinone-like radical. The normal potential was -0.446 volt according to the best experiments performed by rapid titration and this result varied, between *P*H 8.4 and 13, no more than might be expected in systems of a not entirely stable nature, namely, only by a few millivolts. This observation suggests that the normal potential is independent of *P*H and, according to the *P*H, therefore, is in the hydrogen overvoltage range in acid solution, but is in the well measurable potential range in alkaline solution. The assumption of the independence of *P*H can be theoretically justified by the following argument. This compound, being a quaternary ammonium base, will be ionized even in alkaline solution and may be formulated both in acid and in alkaline solution as in Formula I, which represents a bivalent cation. The reduction consists in the acceptance of one electron, which will be shared by the two ammonium groups as in Formula II. This is a univalent cation. It is also of the character of a very strong base and will remain ionized even in alkaline solution. As I and II differ only by an electron and not by a hydrogen atom, the normal potential will be independent of *P*H. This behavior enables us to use this ammonium compound as an oxidation-reduction indicator for a potential range in the very neighborhood of the hydrogen potential.



I



II

The above interpretation of the structure of the violet or blue dyes originating from the reduction of γ,γ'-dipyridyl and related compounds is at variance with that of Emmert,[16] who considers that the compounds of the

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[16] Emmert, *Ber.*, **53**, 370 (1920).

type of dipyridyl dissociate into two radical-like molecules, just as hexaphenylethane splits into two molecules of triphenylmethyl. Our interpretation also deals with radicals but without splitting of the dipyridyl nucleus into two halves. Dimroth assumes for the violet dye, generated by reduction of γ,γ'-dipyridyl, a bimolecular formula such as was customary for Wurster's dyes. The potentiometric titration curves are at variance with this interpretation, not only for Wurster's dyes but also for that particular dipyridyl dye which was accessible to a potentiometric study. Weitz[3] was the first to advocate the radical formula.

5. **Phenazine and its Derivatives.**—It was a phenazine derivative which, due to its very obvious two step oxidation and its great stability in all of its three stages of oxidation–reduction, gave the impetus for this whole investigation, namely, pyocyanine, the blue dyestuff of *Bacillus pyocyaneus*.[1a] It was recognized by Wrede and Strack[17] as a phenazine derivative and was considered by these authors as a polymerization product of two molecules of α-oxy-N-methylphenazine. It was however shown by Friedheim and Michaelis[1a] that it is simply α-oxy-N-methylphenazine itself. It was shown by Michaelis[1b] that α-oxyphenazine which also was first synthetized by Wrede and Strack[17] showed the same behavior. The particular suitability of these dyes other than their stability in all forms of oxidation–reduction, consists in the fact that they are sufficiently soluble in water and accessible for titration curves in all P_H ranges. This matter has been exhaustively examined in previous publications.[1a,b,c,d]

On further investigation it became manifest that many other phenazine compounds behave in the same way, with the difference that most of them are not water soluble enough, especially in the completely reduced state, to allow the use of the potentiometric method in aqueous solution, and many of those soluble in water did not show this phenomenon but behaved like ordinary dyestuffs. No phenazine dye containing an amino group showed two steps of reduction in any solvent or at any P_H. The following showed no intermediary form of reduction: symmetrical and asymmetrical diaminophenazine, triaminophenazine, *m*-methylrosinduline, N-dimethylisorosinduline, asymmetrical anilinoaminophenazine, aminoacridine, asymmetrical aminohydroxyphenazine, rosinduline,[18] isorosinduline, phenylacridine. In contrast herewith phenazine itself and those phenazonium compounds containing no amino groups showed two steps of reduction: phenazine, α-oxyphenazine, α-methoxyphenazine, phenylnaphthazonium nitrate, phenylisonaphthazonium nitrate, methylnaphthazonium chloride, rosinduline GG (Rosindon-monosulfonate). The semiquinoid forms are green in all phenazine derivatives, and red to violet in the naphthaphenazine compounds. Many of the semiquinoid forms have been known for a

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(17) Wrede and Strack, *Z. physiol. Chem.*, **181**, 58 (1929); *Ber.*, **62**, 2051 (1929).

(18) Not to be confused with Rosinduline GG of the paper quoted (1b).

long time and have been described by Claus[19] for phenazine, by Kehrmann[20] for methylphenazonium compounds, for which, after a discussion between Kehrmann and Hantzsch,[21] the meriquinoid character of the intermediary form was accepted.

The best specimen for a potentiometric study is phenazine itself. In 80% acetic acid, the reduction by hydrogen in the presence of colloidal palladium does not lead to a complete reduction because the potential of the dye system overlaps with the potential of hydrogen of one atmosphere pressure. Phenazine, dissolved in 80% acetic acid, practically colorless, first turns intensely green on reduction with hydrogen, then the green becomes paler but never disappears completely. An oxidative titration curve, therefore, shows only the end of the first step of oxidation and then jumps into the potential range of the second step, which is obtained in its full extent. When dissolved, however, in 50% acetic acid plus 50% water, the dye is reduced by palladium plus hydrogen, through the green intermediary stage to the colorless stage. This reduced solution, on oxidative titration with quinone, shows in its full extent the potential curve for both steps, as shown in the experiment (Table VI). These potentials are perfectly stable. The slope for each step of the titration is precisely the one calculated for a one-electron system.

TABLE VI

PHENAZINE

*P*H, 1.26 (50% acetic acid). Oxidant, quinone in 50% acetic acid

1st step			2nd step		
$E = -0.086 + 0.0601 \log \frac{\% \text{ oxid.}}{100 - \% \text{ oxid.}}$			$E = 0.254 + 0.0601 \log \frac{\% \text{ oxid.}}{100 - \% \text{ oxid.}}$		
% oxid.	Observed potential	Calcd. potential	% oxid.	Observed potential	Calcd. potential
3.1	−0.0000	−0.0036	6.8	+0.1905	+0.1860
7.5	+ .0225	+ .0206	20.0	.2190	.2179
15.3	+ .0420	+ .0415	31.5	.2345	.2340
24.0	.0565	.0560	42.6	.2465	.2463
33.5	.0680	.0682	58.8	.2630	.2632
44.0	.0800	.0798	75.1	.2810	.2827
55.3	.0910	.0915	84.06	.2955	.2970
67.1	.1040	.1045	97.7	.3340	.3410
80.2	.1210	.1224			
92.8	.1465	.1514			

Summary

The analysis of the potentiometric oxidation or reduction curves for various classes of semiquinoid dyestuffs shows that always the semiquinoid form differs from the holoquinoid by one electron without any change of

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[19] Claus, *Ann. Chem.*, **168**, 13 (1873); *cf.* also Hinsberg and Garfunkel, *ibid.*, **292**, 260 (1896); Eckert and Steiner, *Monatsh.*, **35**, 1153.

[20] Kehrmann and Danecki, *Ber.*, **47**, 279 (1914); Kehrmann, *ibid.*, **46**, 344 (1913); *ibid.*, **46**, 3003 (1913).

[21] (a) Hantzch, *Ber.*, **49**, 511 (1916) (in which the radical structure of the quinhydrones is advocated for the first time); (b) *ibid.*, **46**, 682 and 1925 (1913).

the molecular size. Semiquinoids are radicals in which one electron is shared by two atoms which possess each a septet of electrons, alternately supplemented to an octet by the odd electron. Several cases are made accessible to a potentiometric study by the application of an organic solvent, acetic acid. Stable and titrable semiquinoids could be demonstrated in the following classes of organic substances: the alkylated diamines, the phenylated diamines, phenazine and many of its derivatives except those containing an amino group, and γ,γ'-dipyridyl and the quaternary ammonium base derived from it.

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The Ternary System: Palmitic, Margaric and Stearic Acids

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The occurrence of palmitic and stearic acids as constituents of many oils, fats and waxes is well established. Margaric acid, the intermediate C_{17} acid, has been reported in numerous natural products[1] but many investigators have denied that this odd carbon acid exists in nature.[2]

The difficulty in establishing the presence of margaric acid in mixtures of fatty acids arises from the fact that the freezing point curves for palmitic and stearic acids indicate compound formation.[3] This compound of approximately equimolecular amounts of palmitic and stearic acids melts at nearly the same temperature as margaric acid. Moreover, the equimolecular mixture of palmitic and stearic acids is stated not to be separable by fractional crystallization.[4] Even the status of margaric acid prepared by some synthetic methods has been questioned. This is of importance because of the proposed use of the synthetic glyceryl trimargarate (Intarvin) as a food for diabetics.

The purpose of the present investigation was to make a complete study of the ternary system composed of mixtures of palmitic, margaric and stearic acids. Such a study would not only indicate the possible compounds formed between these acids but also show the effect of the addition of margaric acid to the compound of palmitic and stearic acids. Com-

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(1) A review to 1912 is given by Bömer and Limprich, *Z. Untersuch. Nahr. Genus.*, **23**, 641 (1912); Hébert, *Bull. soc. chim.*, [IV] **11**, 612 (1912); [IV] **13**, 1039 (1913); Klimont, Meisl and Mayer, *Monatsh.*, **35**, 1115 (1914); **36**, 281 (1915); Jacobson and Holmes, THIS JOURNAL, **38**, 485 (1916); Lipp and Kovács, *J. prakt. Chem.*, **99**, 243 (1919); Lipp and Casimer, *ibid.*, **99**, 256 (1919); Dunbar and Brunneweis, THIS JOURNAL, **42**, 658 (1920); Trillet, *Ann. Physik*, **6**, 1 (1925); Sekito, *Z. physiol. Chem.*, **199**, 225 (1931).

(2) Heintz, *Pogg. Ann.*, **84**, 238 (1851); **87**, 21 (1852); **87**, 553 (1852); **102**, 257 (1857); Heiduschka and Felser, *Z. Untersuch. Nahr. Genus.*, **38**, 241 (1919); Heiduschka and Luft, *Arch. Pharm.*, **257**, 33 (1919); Holde, *Ber.*, **34**, 2402 (1901); **35**, 4306 (1902); **38**, 1247 (1905).

(3) De Visser, *Rec. trav. chim.*, **17**, 182 (1898).

(4) De Visser, *ibid.*, **17**, 346 (1898); Heintz, *Pogg. Ann.*, **102**, 257 (1857)