THE VIOLOGEN INDICATORS

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INTRODUCTION

The quaternary bases derived from γ,γ'-dipyridyl have proven to be useful as oxidation-reduction indicators of properties very desirable for biological purposes, especially because their potential range is very negative, under certain conditions more negative than that of any member of the series of indicators worked out by W. M. Clark and his associates (1) and supplemented by various other authors. The new indicators will be designated as *viologens* according to a proposition made in a preliminary communication (2). They differ from other indicators in several respects; in the first place, the color is exhibited by the reduced form, whereas usually the oxidized form is the colored one.[1] Secondly, the oxidation-reduction potential of these substances is independent of pH. In a neutral solution the normal potential is very close to the potential of a hydrogen electrode at the same pH, therefore in an alkaline solution the potential is more positive, and in an acid solution it is more negative than the hydrogen potential at the same pH. Therefore, in acid solution their normal potential is a hydrogen overvoltage, in other words, rH is negative.

The preparation of one representative of these indicators has been briefly described previously (2, 4). Several improvements and simplifications in the preparation of this compound and the preparation of various homologous compounds, and their properties as regards oxidation-reduction potential, as well as their optical properties, will be described in this paper.

[1] Also the anthraquinone sulfonates, studied by Conant, Kahn, Fieser, and Kurtz (3), have a more intense color in the reduced state than in the oxidized. Their color intensity, however, is rather small even in the reduced state.

Preparations

It has been known for a long time that γ,γ'-dipyridyl can attach two molecules of an alkyl-halide thus forming a bi-quaternary base. The method of preparation consisted in preparing first a quaternary base of pyridine and condensing two molecules of it to a dipyridyl compound by treatment with sodium amalgam and successive oxidation. As early as 1881, A. W. Hofmann (5) prepared dibenzyl-dipyridylium iodide in this way. He considered his preparation as an α,α'-dipyridyl derivative. Much later Emmert (6) proved it to be a γ,γ'-dipyridyl compound. The yields of this method are poor. Since Dimroth and Heene (7) discovered a convenient method of preparing γ,γ'-dipyridyl it seemed more convenient to use this compound as the parent substance for the preparation of the bi-quaternary bases. The following procedure of preparing γ,γ'-dipyridyl is essentially that followed by Dimroth and Heene, and Dimroth and Frister (7, 8).

(*a*) *Preparation of γ,γ'-Dipyridyl.*—50 cc. pyridine, dried over barium oxide, 250 cc. acetic anhydride, and 50 gm. zinc dust are either shaken by machine for 6 hours in a stoppered bottle, or stirred mechanically for 2 hours in a beaker. The yellow precipitate developed is dissolved as well as possible by heating the mixture in a water bath at 90°C. for 1–2 hours. The liquid is filtered off and allowed to crystallize in the ice box. The yellow crystalline precipitate is collected on a Büchner filter, without washing, and exposed to the air in a flat dish at 50°C. It gradually changes in a not easily reproducible way. Sometimes it becomes light brown and within several days the colorless needles of γ,γ'-dipyridyl crystallize out within the brown substance. Sometimes the substance becomes brown altogether. Usually the whole mass becomes liquescent and later dries out again. According to the course of these changes, the final yield of γ,γ'-dipyridyl may vary widely. In any case, after the substance has been exposed in this way to the air for several days it is boiled with 200 cc. water and enough sodium hydroxide added to make the solution strongly alkaline. It is filtered hot and allowed to crystallize in the ice box. Then γ,γ'-dipyridyl crystallizes in long, white needles and may be recrystallized by dissolving in hot, slightly acidified water and precipitated by sodium hydroxide. The crystals contain two molecules of water, which they lose on drying at 50°. The melting point of the anhydrous substance is 111.0–112.0°C.

A test for γ,γ'-dipyridyl is as follows: a small amount is dissolved in 50 per cent acetic acid and some solid chromous chloride is added.

An intense violet color is developed, which fades on shaking in the air. Instead of chromous chloride, zinc dust may be applied. In this case the violet color is only transient and the reduction goes further, to a colorless state. This second step of reduction is irreversible.

(*b*) *N-N'-Dimethyl-* γ,γ'*-Dipyridylium-Bichloride (or Methyl Viologen).*—10 cc. dimethyl sulfate and 1 gm. γ,γ'-dipyridyl are mixed and heated over a free flame just to the beginning of full boiling. After spontaneous cooling the heating is repeated once in the same way. The mixture is poured into a separatory funnel and 50 cc. water added. The excess of methyl sulfate is removed by repeated extraction with ether. The aqueous solution is poured into a beaker and an excess of a concentrated picric acid solution is added. The crystals of the picrate are collected on a Büchner filter, washed with a little acetone, sucked nearly dry, and suspended in 100 cc. acetone. A small amount of concentrated hydrochloric acid is added (1.0 cc.). Gradually the yellow picrate is converted into the colorless chloride which is insoluble in acetone. The crystals are collected and may be recrystallized by dissolving in a small amount of methanol and precipitating with a large excess of acetone. The substance forms perfectly colorless needles, which, on drying at 50°C., lose the water of crystallization. The yield is almost the theoretical one. The substance, dissolved in water, on adding sodium hydrosulfite and ammonia, turns deep violet; in higher dilution it is a pure blue, in higher concentration a little more violet. (γ,γ'-Dipyridyl itself does not give this test but develops color only when reduced in an acid medium according to the above directions.)

(*c*) *N-N'-Diethyl-*γ,γ'*-Dipyridylium-Bichloride (or Ethyl Viologen).*—This compound is prepared analogously by using diethyl sulfate.

(*d*) *N-N'-Dibenzyl-*γ,γ'*-Dipyridylium-Bichloride (or Benzyl Viologen).*—The benzyl compound is prepared analogously, using benzyl chloride instead of methyl sulfate. γ,γ'-Dipyridyl, dissolved in an excess of benzyl chloride, directly yields a precipitate on heating. The boiling is extended to 2 or 3 seconds, the mixture then poured into a large volume of acetone, and a few drops of concentrated hydrochloric acid are added. The precipitate is filtered off and is recrystallized by dissolving in a small amount of methyl alcohol and precipitating with acetone. The preliminary preparation of a picrate is not necessary. If a picrate be prepared it can be converted into the chloride in the same way as with the other picrates. This picrate does not crystallize so easily as the others. Usually it arises first in an amorphous state which on gentle heating gradually becomes crystalline.

(*e*) *N-N'-Dibetaine-*γ,γ'*-Dipyridylium-Dichloride (or Betaine Viologen).*—The nitrogen of pyridine can be attached to monochloracetic acid. Then, the chlorine of this compound is present in ionic form and the compound is analogous to the chlorides of a betaine (9). So it could be expected that γ,γ'-dipyridyl might

give a double betaine compound. This compound was prepared as follows: 10 gm. monochloracetic acid are melted in a test-tube and 1 gm. γ,γ'-dipyridyl is added. The solution is heated quickly to the boiling point and kept boiling for 2 to 3 seconds. Due to the formation of by-products it turns amber-yellow. The betaine was recovered from this solution in the form of the chloride as the free betaines are very hygroscopic substances. The solution is mixed with a large excess of acetone and several drops of concentrated hydrochloric acid are added. The chloride of the betaine crystallizes from this solution and can be recrystallized by dissolving the dry crystals in a small amount of methanol, adding a large volume of acetone, and, if necessary, reducing the volume on a steam bath until crystallization begins. Further recrystallization may be performed by dissolving with methanol and precipitation with acetone, or benzene.[2]

Analytical data have been presented for the methyl compound in a previous paper (2). The analysis of the benzyl compound, dried at 60° *in vacuo*, is:

3.786 mg. substance gave 9.590 mg. CO_2 and 2.020 mg. water.
3.174 mg. substance gave 8.185 mg. CO_2 and 1.550 mg. water.
7.244 mg. substance gave 0.430 cc. N_2 (20°, 759 mm.).
5.555 mg. substance gave 3.885 mg. AgCl.
Found: C, 69.05, 70.32 per cent; H, 5.97, 5.46 per cent; N, 6.90 per cent; Cl, 17.30 per cent.
Calculated for $C_{24}H_{22}N_2Cl_2$:C, 70.57 per cent; H, 5.44 per cent; N, 6.86 per cent; Cl, 17.16 per cent.

We are indebted to Dr. H. Elek for the analyses.

Peculiarities with Respect to the Technique of the Potentiometric Titration Experiments

Particular difficulties are involved in the potentiometric titration of these substances, due to a tendency of the potentials to drift. The cause of this drift and its influence upon the interpretation of the results obtained shall first be discussed.

The drift may vary, according to the circumstances, from 0.1 to 2.0 millivolts per minute, and is always in the direction of the potential becoming more positive in time. Such a drift may be due to two causes: either the reduced form of the substance is a labile molecule

[2] It is worth while mentioning that the picrate of the betaine compound cannot be converted to the chloride by the simple method described for the other representatives of this group.

which gradually disappears by an irreversible process, or the nitrogen, bubbling through the electrode vessel, contains oxygen. To begin with the first possibility, it can be shown that the reduced form of any of these substances, in absence of oxygen, is perfectly stable. When a small amount of the oxidized form is dissolved in a suitable buffer, a suitable reductant added, and the tube then sealed, the color developed by reduction does not fade but lasts for weeks. This can be shown as follows: A test-tube with a drawn-out neck is filled with a solution of M/10 sodium carbonate, some glucose, and 0.5 mg., or less, of methyl viologen, and the neck of the tube sealed. The test-tube is kept at 50°C. Gradually the blue color of the reduced form will appear. On shaking, the color will disappear, due to the oxygen of the air bubble remaining in the tube. By repeated shaking and re-reduction at rest, the oxygen will be exhausted, and when this point is reached the color will remain permanently for weeks. Thus it can be proven that the colored substance is not liable to an irreversible destruction during any reasonable period of time, even at pH 10 or 11.

There remains the second explanation that the nitrogen used for the titration experiments contains a trace of oxygen. In all earlier experiments, however, the nitrogen appeared to be free of oxygen. The gas was purified over copper at 450°C. as described previously (10), and the titration vessel was entirely sealed by mercury as described in the same paper. It could be shown also that this nitrogen is satisfactorily purified, by the fact that other dyestuffs showed no drift of the potentials during titration. In order to prove this statement, a series of dyestuffs was selected, which were known to be perfectly stable substances, both in the oxidized and the reduced forms, and yet very sensitive to oxygen in the reduced form. The latter condition will, in general, coincide with the condition that their potential range is very negative. The nitrogen was re-tested with thionine, gallocyanine (11), and rosinduline G G (12). The last dye seemed to be especially suitable on account of its very negative potential range. Sodium hydrosulfite was used as reductant. One experiment will be described in detail. 35 cc. phosphate buffer (pH 7.0), containing 2.94×10^{-6} mol of rosinduline G G, were titrated with hydrosulfite to approximately 50 per cent reduction. The

nitrogen was bubbled continuously at 74.2 cc./min. (4.45 liter/hour). After allowing several minutes for the establishment of the potential, the potential was read at regular intervals. During an observation of 60 minutes there was no drift, not even of 1/10 of a millivolt. Considering the exceptionally strict conditions, *i.e.* the very high dilution of the dye and its very high sensitivity for oxygen, this experiment seems to show a perfect condition of the nitrogen. When, however, the same experiment was performed at pH 11.0, a drift was observed, amounting to 0.2–0.5 mv. per minute. The drift became negligible when the flow of gas was stopped. The interpretation is as follows:

Reid (13) has shown that methylene white is oxidized by molecular oxygen only in a neutral or alkaline medium, whereas at pH 4.0 it is virtually stable in an oxygen atmosphere, unless there is a catalyst, such as a copper salt, present. One may infer from this that the spontaneous oxidizability of the reduced dyes increases with increasing pH. Our nitrogen contained so little oxygen that the rate of oxidation of the reduced rosinduline was practically zero at pH 7.0, but was detectable at pH 11.0. It is understandable that using a gas with a very low oxygen content, the rate of oxidation will be somewhat proportional to the partial pressure of the oxygen. The oxygen pressure being constant, the oxidation may be negligible at pH 7.0, but not so at pH 11.0.

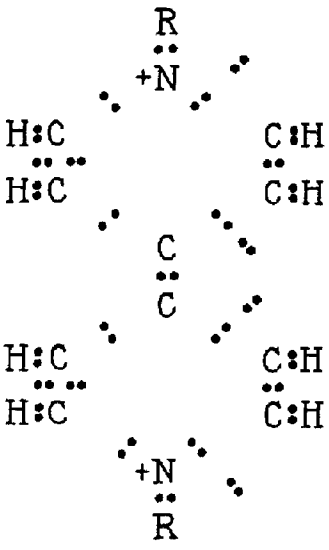
There can be no doubt that any method of purification of nitrogen has its limits. An estimation of the efficiency of the method used for these experiments may be obtained as follows: According to von Wartenberg (14), the cloudy halo that surrounds a piece of yellow phosphorus is still visible in an atmosphere containing 2 parts of oxygen in 100,000 parts of an indifferent gas but not visible in an atmosphere containing 1 part in 100,000. Our nitrogen caused the halo around phosphorus to disappear, so we may estimate the oxygen content of our nitrogen to be 1 in 100,000, or less. If it were only half this amount, it would be sufficient to cause a slight drift in the potential, provided the rate of the effect of this oxygen is appreciable. Experience suggests that this effect is unappreciable at pH 7.0 but is quite detectable at pH 11.0.

On titrating the viologens, we are faced with a situation where we not only have to deal with exceptionally oxygen-sensitive substances, but also are compelled to titrate at, or very near to, pH 11.0. The potential range of these substances, even at pH 11.0 or 10.0, is so close to the hydrogen potential at the same pH that full titration curves cannot be obtained in less alkaline solutions.

Therefore, we are justified in attributing the drifts to traces of oxygen and must be satisfied with a smaller degree of accuracy than might be desirable, but one has to bear in mind that on performing the titration not too slowly, the whole drift during the course of the titration will only amount to 2 or 3 mv. This will cause only a slight distortion of the titration curve and an uncertainty of the normal potentials of only a few millivolts.

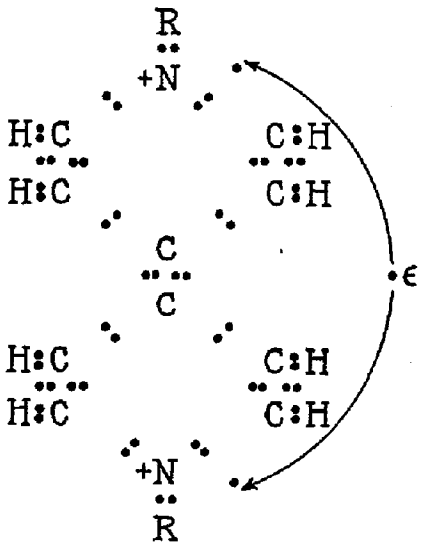
Interpretation of the Potentiometric Curves and Suggestions as to the Chemical Structure of the Compounds

On taking ±2–3 mv. as limits of error, and applying the principles developed previously (15), we may state that the shape of the titration curve is that of a one-electron system and that the normal potential is independent of pH throughout that range in which a full titration curve can be obtained; *i.e.*, pH 10.0–13.0. Both the oxidized and the reduced forms have the character of a quaternary base and exist in a strongly, if not completely, ionized state at any pH, without changing the state of dissociation with pH. So the two forms differ from each other only by an electron and never by a full hydrogen atom (or a hydroxyl group) and the normal potential must be considered as independent of pH, not only for that range of pH in which titration experiments can be executed, but also in acid solution, in which, on account of the overvoltage, such experiments cannot be performed. The following formula will give a picture of the structure.



I

The bivalent cation of the (colorless) bi-quaternary ammonium base. *R* means a univalent radical such as CH_3. The pyridine rings have the benzenoid structure.



II

The univalent cation of the (violet) reduced form. One electron (designated as ϵ) has been added to formula I and the double bonds have been rearranged. The odd electron ϵ is shared by both N atoms to supplement the septet to an octet, alternately, in rapid succession; this electron belongs to the one and the other N atom. The pyridine rings have the quinoid structure.

The molecule II can be reduced farther. The next step of reduction is the attachment of another electron to formula II. Then the structure of the right hand formula is in general maintained, except for the fact that the octet of either N atom is permanently complete without any sharing of an odd electron being necessary. These compounds are almost colorless, slightly yellow. One representative, namely the benzyl derivative, can be easily produced by reducing benzyl viologen in a very alkaline solution (pH >12) by an excess of hydrosulfite or by glucose beyond the violet stage. On exposure to the air, oxidation to the violet form and subsequent oxidation to the colorless viologen takes place. This, however, is true only for the freshly prepared solution; an irreversible destruction of this molecule gradually takes place. This substance is on the reduction level of a dihydrodipyridyl. Still further reduction leads to the level of the tetrahydrodipyridyl, of which the substance produced by reduction of pyridine by zinc in the presence of acetic anhydride is an example. No substance on the trihydrodipyridyl level has been known.

Potentiometric Titrations

Potentiometric titrations were performed for the aqueous solution with sodium hydrosulfite at 30°C. The most convenient buffer is made from disodium phosphate and sodium hydroxide, but the titrations can be performed at any pH between 13.0 and 10.0, or even 9.0. At lower pH the titration curves are incomplete because of the overlapping with the hydrogen potential. The completeness of the titration curve is indicated by a jump of the potential into a more negative level at the end.

The following tables are examples of the titrations.

Methyl Viologen

pH = 11.0 (Na_2HPO_4 + NaOH).
Titrated with sodium hydrosulfite at 30°C. ±0.05.
Potentials calculated according to the formula

$$E = -0.446 - 0.0601 \log \frac{\text{Per cent reduction}}{100 - \text{per cent reduction}}$$

All potentials referred to the normal hydrogen electrode at 30°C.

Reduction	Observed potential	Calculated potential
per cent		
0.0		
13.4	0.3977	0.3986
19.6	0.4102	0.4107
30.5	0.4252	0.4254
39.8	0.4355	0.4360
49.0	0.4449	0.4450
57.5	0.4531	0.4538
67.7	0.4634	0.4652
77.0	0.4740 drifting	0.4774
89.5	0.4940	0.5075

Similar results were obtained in many other experiments, in which the initial amount of the substance was varied from 4×10^{-7} mols up to 4×10^{-6} mols, dissolved in 30 cc. of the buffer; furthermore in pH range varying from 9 to 13.

Ethyl Viologen

pH = 11.0 (Na_2HPO_4 + NaOH).

$$E = -0.449 - 0.0601 \log \frac{\text{Per cent reduction}}{100 - \text{per cent reduction}}$$

Reduction	Observed potential	Calculated potential
per cent		
0.0		
7.73	−0.3830	−0.3844
15.4	−0.4050	−0.4053
22.6	−0.4165	−0.4170
35.1	−0.4326	−0.4330
44.6	−0.4431	−0.4434
60.1	−0.4595	−0.4596
74.4	−0.4750	−0.4767
92.8	−0.5120	−0.5122

Betaine Viologen

pH = 11.0 (Na_2HPO_4 + NaOH).

$$E = -0.444 - 0.0601 \log \frac{\text{Per cent reduction}}{100 - \text{per cent reduction}}$$

Reduction	Observed potential	Calculated potential
per cent		
0.0		
7.10	−0.3770	−0.3770
14.4	−0.3980	−0.3984
21.0	−0.4103	−0.4105
30.6	−0.4225	−0.4233
39.4	−0.4331	−0.4332
53.4	−0.4472	−0.4475
71.5	−0.4672	−0.4679
96.8	−0.5235	−0.5320

Benzyl Viologen

pH = 8.0 (Na_2HPO_4 + KH_2PO_4).

$$E = -0.359 - 0.0601 \log \frac{\text{Per cent reduction}}{100 - \text{per cent reduction}}$$

Reduction	Observed potential	Calculated potential
per cent		
13.1	−0.3087	−0.3097
18.0	−0.3203	−0.3205
22.5	−0.3279	−0.3272
28.0	−0.3353	−0.3356
33.6	−0.3420	−0.3427
39.6	−0.3487	−0.3480
46.0	−0.3555	−0.3554
54.1	−0.3645	−0.3648
63.3	−0.3773	−0.3772
71.1	−0.3924	−0.3924
80.6	−0.3961 drifting	−0.4145
91.6	−0.4070 drifting	−0.4215

Absorption Spectra of the Dyes

The absorption spectra of all these indicators, in their reduced form, consist of a very distinct band. Within the band one can distinguish

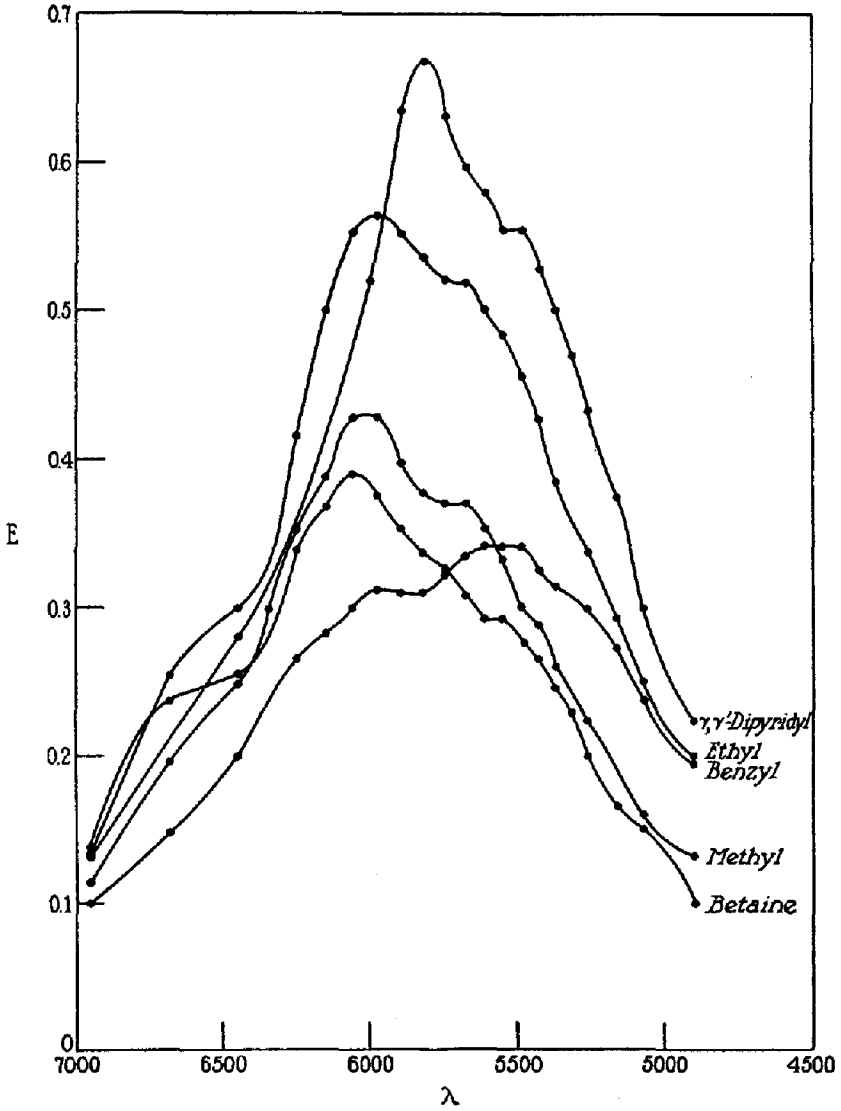


FIG. 1. Extinction, E, plotted against wave length, λ. E is the logarithm of the ratio of the intensity of the incident light to the transmitted light.

one distinct maximum of absorption and a noticeable secondary maximum. The primary peak of absorption is the one towards the longer wave length side, except for the benzyl compound, where the converse is true. Beer's law holds for these dyes within wide limits. The absorption for a highly concentrated solution in a very thin layer matches that of a solution more than 20-fold weaker in a correspondingly thicker layer. The spectrophotometric measurements were made with the spectrophotometer according to König and Martens in a solution of the substances kept in the reduced state by adding glucose in a phosphate buffer (pH 10.0–11.0). The curves in Fig. 1 show the extinction plotted against wave lengths. The concentration of the dye is different for each curve in order to allow the plotting on one graph. Therefore the absolute value of the ordinate of one curve must not be compared with that of any other. But the general shape and especially the location of the peaks are comparable. For comparison, the spectrum of the reduction product of γ,γ'-dipyridyl as produced by reducing this substance in dilute acetic acid with chromous chloride has been plotted. Because of the instability of this color over a longer period of time the accuracy of this particular curve as to details may not be very high, but good enough to determine the maximum and the secondary peak of the absorption. The location of the various maxima is as follows:

	First peak	Second peak	
	mμ	mμ	
γ,γ'-Dipyridyl	582	550	Smaller than the first peak
Methyl viologen	601	570	
Ethyl viologen	598	570	
Betaine viologen	605	558	
Benzyl viologen	598	555	Higher than the first

The color of the reduced methyl, ethyl, and betaine viologen is indigo blue in very high dilution, somewhat more violet in more concentrated solution; that of the benzyl compound is violet in very high dilution and more blue in higher concentration. The reduced form of γ,γ'-dipyridyl itself is more red-violet.

The above table does not account for the smaller secondary maxima

which are especially obvious in the betaine compound. A remarkable peculiarity of benzyl viologen is the dependence of its absorption spectrum on the temperature. The color of the reduced form varies, in a reversible way, from reddish-violet at room temperature, to a pure blue at high temperatures. In the other compounds a small trace of this phenomenon is just detectable: the color changes reversibly with increasing temperature from violet-blue to a purer blue. The spectroscopic observation shows that the change with increasing temperature in the benzyl compound is due to a decrease of the first maximum and an increase of the second maximum of absorption. At 80° the first maximum is very high, and the second becomes inconspicuous so that the type of the spectrum becomes the same as for the other compounds, whereas at low temperature it differs greatly. For the reduced form of γ,γ'-dipyridyl itself no change with temperature is noticeable.

The Application of the Substances as Indicators

The determination of the oxidation-reduction potentials, within suitable ranges, by means of these indicators can be made by comparing the color intensity produced by the solution in question with the color intensity which could be produced by the complete reduction of the same amount of indicator. The problem is therefore only to establish a standard solution in which the indicator may be considered as completely reduced. The difficulty in preparing such a standard solution lies in the fact that by applying too strong a reductant the reduction may go beyond the first step and partially destroy the color. A complete reduction without the risk of overreduction can be brought about by means of a solution of 2–5 per cent glucose in M/10 Na_2CO_3, except for benzyl viologen. Here, at pH $\geqq$10.0, gradually an overreduction would take place. The reduction, however, will be complete without overreduction at pH about 9.8; *i.e.*, a mixture of 6 parts of M/5 sodium carbonate plus 4 parts of M/5 sodium bicarbonate.

The Problem of Hydrogen Overvoltage

The problem in question can be best explained by an example. Supposing we wish to reduce methyl viologen in an acid solution. We

can do so by using chromous chloride as a reductant. The success of the reduction becomes evident by the appearance of the deep blue color. The chromous-chromic-ion system is supposed to have a normal potential of about −0.4 volt, which at least in acid ranges of pH will not depend appreciably on pH. So it is understandable that chromous chloride in an acid solution (pH 2 to 4) containing no, or only little, chromic chloride, will reduce to a certain extent viologen, the normal potential of which is about −0.4 volt. The striking fact is that such an overvoltage potential can be maintained in an aqueous medium. One might expect that under the conditions of such an experiment water would be reduced and hydrogen gas be developed rather than that viologen would be reduced. When colloidal palladium is added to the system viologen + chromous chloride, the blue color of the reduced viologen will not arise and the expected development of hydrogen gas within the solution will take place instead. The only interpretation imaginable is the hypothesis that the primary reduction product of water is not H_2, but monomolecular hydrogen. In the absence of a catalyst the hydrogen atoms will be accumulated until equilibrium is attained. When, however, a catalyst is present which brings about the reaction $2H \rightleftharpoons H_2$ with appreciable speed, the overvoltage potential will break down.

In the absence of a catalyst these indicators can be used even for the measurements of potentials in the hydrogen overvoltage ranges. In this respect the potential measurement by means of such an indicator is far superior to a measurement by metal electrodes. Platinum or gold electrodes, even when blank, will allow only a very small overvoltage. Only mercury electrodes will stand an appreciable overvoltage, and for this reason mercury electrodes were used, *e.g.* by Forbes and Richter (16), for measurement of the potential of chromous-chromic systems. The application of this mercury electrode measurement in an overvoltage range meets with great technical difficulties. There is scarcely any difficulty in applying the indicator method.

SUMMARY

The tabulation gives the normal potentials of the various indicators at 30°C.; referred to the normal hydrogen electrode, the accuracy is estimated to be ±0.002 volt.

Normal potentials of the viologens at 30°C.:

Methyl viologen	−0.446 volts
Ethyl viologen	−0.449 volts
Betaine viologen	−0.444 volts
Benzyl viologen	−0.359 volts

Supposing some solution brings about a coloration of one of these indicators to the extent of A per cent of the maximum color, the oxidation-reduction potential of this solution is $E = E_o - 0.06 \log \frac{A}{100 - A}$ where E_o is the normal potential according to the above tabulation. This normal potential is independent of pH.

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