

Esters were determined by the standard procedure; 91% of 2,4-D ester and 93% 2,4,5-TP ester were recovered from orange peel (Table III).

Free acid was also determined for each sample to determine whether hydrolysis of ester could have occurred in the procedure.

Initially, no free acid was detectable in the ester standard solutions. Approximately 0.3% of the 2,4-D ester added to the orange peel samples was hydrolyzed during extraction. No evidence for hydrolysis of 2,4,5-TP in the orange peel was found. Neither 2,4-D ester nor 2,4,5-TP ester was hydrolyzed during recovery from water. Consequently, no serious errors are introduced into the analysis through hydrolysis of the esters.

Residues in Citrus Sprayed under Grove Conditions

When citrus trees are sprayed with 20 p.p.m. (free acid basis) of these growth

regulators, usual concentrations of 2,4-D and 2,4,5-TP found in the three fractions extracted from the peel were well below 0.1 p.p.m. During the first 2 weeks following spraying, the total residue in peel appears to be about 0.1 p.p.m. After this period, the residue slowly decreases.

No significant concentration of free acid has been found in the juice and no ester or bound fractions have been detected in this portion of the fruit.

These findings demonstrate the applicability of the method of analysis for determination of these growth regulators in sub-part per million concentrations for investigation of their fate when applied as physiological sprays to citrus.

Acknowledgment

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POLAROGRAPHIC DETERMINATION

Bipyridylum Herbicides. Polarography of 1,1'-Ethylene-2,2'-bipyridylum Dibromide

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Reduction of 1,1'-ethylene-2,2'-bipyridylum dibromide at the D.M.E. in 0.1M KCl gives two diffusion-controlled waves. The currents are linear functions of the concentration in the 1.00×10^{-5} to 9.80×10^{-3} M concentration range, and they vary directly as the square root of the mercury pressure. The $E_{1/2}$'s are independent of the pH within the 2.2 to 12.0 range. The reduction involves two steps of 1-electron transfer, and the reversibility of the first step is established. The lower limit of reproducible detection is approximately 0.5 μ g. per ml. The method can be extended for the polarographic analysis of all N-substituted, 2,2'- and 4,4'-bipyridylum herbicides.

CURRENT interest in bipyridylum salts is the result of the discovery (2) of the herbicidal properties of 1,1'-ethylene-2,2'-bipyridylum dibromide (Diquat). Subsequent developments in this field are summarized by Boon (1).

The need for a sensitive analytical method for the determination of Diquat has arisen in connection with the estimation of residue levels in crops and food products. Some bipyridylum compounds undergo a reversible, 1-electron reduction to form free radicals. The reduction of 1,1'-dimethyl-, 1,1'-diethyl-, 1,1'-betaine-, and 1,1'-dibenzyl-4,4'-bipyridylum cations by CrCl_2 in CH_3COOH , or by alkaline $\text{Na}_2\text{S}_2\text{O}_4$, has been reported (10, 11). Elofson and Edsberg demonstrated (5) that 1,1'-dimethyl- and 1,1'-dibenzyl-4,4'-bipyridylum dichloride are re-

duced at the D.M.E. Calderbank, Morgan, and Yuen (3) have proposed the spectrophotometric determination of the intensely colored free radical derived from Diquat by alkaline $\text{Na}_2\text{S}_2\text{O}_4$ reduction. However, Diquat is not stable in a strongly alkaline medium (2), and the formed free radicals, as other free radicals of bipyridylum origin, are only fleetingly stable under aerobic conditions even in the presence of a large excess of the reducing agent (3, 4, 10, 11).

The suitability of the polarographic method of analysis was investigated then with special emphasis on anaerobic conditions and supporting electrolytes with favorable pH's, and the work was directed at finding the general conditions under which the method could be applied to other bipyridylum herbicides.

Experimental

Apparatus. A capillary (E. H. Sargent and Co.) was cut to 15.0-cm. length. Later, in order to renew the orifice, it was shortened to 13.7 cm. Estimation of the radius of the capillary orifice by the critical pressure and drop weight methods (12) yielded 32.8 and 32.2 microns, respectively. Direct microscopic measurement in polarized light gave 31.0 microns. A batch of mercury (Fisher, reagent) used throughout the experiments was vacuum-distilled three times in a nitrogen atmosphere to give a satisfactory foam test (13). The head of mercury was variable between 25.0 and 100.0 cm. An H-cell (9), maintained at $25.0^\circ \pm 0.1^\circ \text{C}$., was used with a S.C.E. For iR drop corrections the cell resistance was obtained from the shift of the apparent $E_{1/2}$ of Tl^+ with concentration. The nitrogen gas used for deoxygenating the test solutions was freed from

traces of oxygen (less than 10 p.p.m.) by copper flakes heated to approximately 450° C. The gas was cooled to ambient temperature before it was led through a portion of the supporting electrolyte that was kept in a gas-washing bottle at 25.0° ± 0.1° C.

Most of the polarograms were obtained with a semimanual polarograph that was built from readily available parts according to the circuit of Lingane (8). The potential difference of 2.70 volts from a bank of mercury cells (Mallory) was manually applied to the D.M.E. by means of a 150-ohm, 15-turn Beckman Helipot (BSP, linear tolerance ± 0.5%, ESS), coupled through a 2 to 1 stepup gear to a 7A27R Metron driver (Instrument Co., Denver 9, Colo.) to give an over-all drive reduction of 1 to 13.5. The *iR* drop from the measuring resistance of 26,000 ± 25 ohms was fed to the Y axis of an X-Y recorder (F. L. Moseley Co., Pasadena, Calif.) and the potential difference to the X axis. Recorder speed was found to be approximately 1 second per full scale travel along both axes. During trial tests the $E_{1/2}$ for Zn⁺² in 0.1M KCl at 25° C. was found to be -1.01 volts *vs.* S.C.E. Thereafter, circuit performance was checked by the linearity and slope of the current *vs.* potential relationship having a known resistance in place of the cell. A few measurements were repeated using a Leeds & Northrup Electro-Chemograph, Type E, in order to ascertain the over-all behavior of the X-Y recorder in a polarographic circuit. (The semimanual polarograph proved to be more precise but less sensitive.)

The pH of test solutions was obtained with a Metrohm E 336 potentiograph fitted with a UX combination glass electrode, and the absorption spectra by a Cary M 11 recording spectrophotometer using 1-cm. Ultrasil cells (Pyrocell Co., New York, N. Y.).

Reagents. Samples were prepared by using distilled water that was deionized over a column of Dowex 1-X8 and Dowex 50W-X2 resins in their OH⁻ and H⁺ forms, respectively. Final resistance was 1.0 × 10⁶ ohm-cm. at 25° C. Diquat (Plant Protection, Ltd., Bracknell, Berks., England), KCl (British Drug Houses), and tetra-*n*-butylammonium iodide were recrystallized from the water above, dried at 105° C., and stored in a desiccator over anhydrous CaSO₄. Other reagents were of analytical grade, and were used as supplied. Buffer solutions were made by mixing 0.1M citric acid with 0.2M Na₂HPO₄ (McIlvaine) and 0.2M H₃BO₃ with 0.15M NaOH (Sørensen). The free radical of Diquat, having an intense green color, was prepared under anaerobic conditions *in situ* by the use of Zn dust.

Polarographic Procedure. Solutes were weighed out to ±0.1 mg. and made up to appropriate volumes. Some solutions were made by diluting stocks. Test solutions to be polarographed were pipetted into the H-cell and freed from oxygen by passing through purified and preconditioned nitrogen gas for 15 minutes. A nitrogen atmosphere was then maintained over the test solution. The capillary, the tip of which was

tapped free of gas bubbles, was visually set into vertical position, and the mercury head was adjusted. After obtaining the open circuit drop time, the polarogram was recorded. It was found advantageous to drive the voltage divider of the semimanual instrument in equal increments. Each step commenced after the breakoff of a mercury drop, and terminated when half a drop time had elapsed. The pH of the test solutions, where applicable, was measured at 25.0° ± 0.1° C. The pH scale was calibrated with 0.05M potassium hydrogen phthalate and 0.01M borax buffers (National Bureau of Standards).

Spectrophotometric Procedure. After obtaining the pH, a portion of the test solution was transferred into the ultraviolet light-transparent cell. The absorption spectra of the electroreducible species were recorded against the supporting electrolyte between 350 and 200 mμ.

Mathematical Procedure. Maximum currents were read off between the residual and diffusion current regions (no damping was used). The voltage

values were corrected for *iR* drop. The $E_{1/2}$'s and *n*'s were obtained for each polarogram by the method of linear regression, fitting points of the diffusion current region to the potential *vs.* current equation (73).

For each statistic calculated the number of variates is at least 12, and the boundary values are to signify the standard error of the mean.

Results and Discussion

pH. The effect of pH is shown in Figure 1. Statistical analysis reveals that the $E_{1/2}$ *vs.* pH values for both the first and second waves are not significantly different, except at pH 12. Omitting these values, the results are shown in Table I. The wave form is distorted from neutrality toward both ends of the pH scale. There is a rise in diffusion currents of both steps of reduction shifting from the citric acid-Na₂HPO₄ to the H₃BO₃-NaOH buffer. However, the ratio of the diffusion current of the second step to that of the first (I_{d2}/I_{d1})

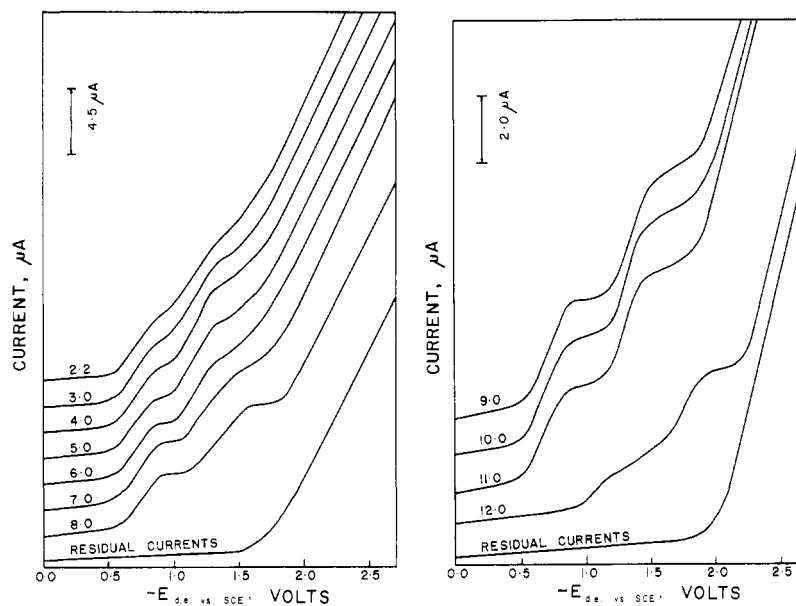


Figure 1. Polarograms of 1.00 × 10⁻³M solution of 1,1'-ethylene-2,2'-bipyridylum dibromide at various pH's

Left. Citric acid-disodium phosphate buffer
Right. Boric acid-sodium hydroxide buffer

Table I. Polarographic Data for Various Treatments of Diquat

	pH, 2.2-11.0	Supporting Electrolyte		
		0.02M, [CH ₃ (CH ₂) ₃] ₄ Ni	0.1M, KCl	Reversibility, 0.1M, KCl
Diquat concn., M	1.00 × 10 ⁻³	1.00 × 10 ⁻³	5.00 × 10 ⁻⁴ to 5.00 × 10 ⁻³	5.00 × 10 ⁻⁴
D.M.E.				
<i>m</i> , mg./sec.	1.98	1.92	2.52	2.16
<i>t</i> , sec.	4.13 ± 0.02	3.60 ± 0.08	3.74 ± 0.01	3.69 ± 0.08
- $E_{1/2}$, volts <i>vs.</i> S.C.E.				
First wave	0.64 ± 0.01	0.53 ± 0.01	0.56 ± 0.01	0.52 ± 0.04
Second wave	1.16 ± 0.02	1.20 ± 0.02	1.06 ± 0.02	N.A. ^a
Current, I_{d1} , μa.	2.1 ± 0.1	2.95 ± 0.05	N.A. ^a	1.02 ± 0.05
I_{d2}/I_{d1}	1.25	0.83	1.03	N.A. ^a
Electron transfer, <i>n</i>				
First wave	N.A. ^a	0.88	0.98	1.00
Second wave	N.A. ^a	1.06	0.88	N.A. ^a

^a Not applicable.

is constant throughout. No meaningful polarograms can be obtained either below pH 2.2 because of hydrogen discharge or above pH 12 where the rapid decomposition of Diquat takes place.

The ultraviolet absorption spectra (Figure 2) indicate that the decrease of the absorption of the coplanar (*l*) 1,1'-ethylene-2,2'-bipyridylium rings at 303 and 218 m μ , *K*-band, is discernible at pH 7 and marked at pH 12. At the same time, the appearance of an absorption peak at 210 m μ shows the emergence of R—CH=CH—CHO groups (*l*).

The buffering of the Diquat solution during polarography is not necessary. A supporting electrolyte of near neutrality, if otherwise satisfactory, is acceptable.

Supporting Electrolytes. Tetra-*n*-butylammonium iodide was evaluated as a potential supporting electrolyte because of its additional utility as an efficient eluent in the ion exchange type separation of Diquat from extraneous materials. The resulting polarogram is shown in Figure 3, and the corresponding data are given in Table I.

The nature of the prewave, at -0.1 volt *vs.* S.C.E., and the maximum, at -2.3 volts *vs.* S.C.E., were not investigated, as these do not interfere with the analysis. On the other hand, the existence of a third, irregular wave having $E_{1/2} = -1.67$ volts *vs.* S.C.E. and $n = 7.9$ requires the use of the first current plateau only at Diquat concentrations of approximately $10^{-5}M$ or lower.

Though less efficient as an eluent in ion exchange separations, $0.1M$ KCl as the supporting electrolyte permits the development of more satisfactory polarograms (Figure 4, left, and Table I).

The reduction is reversible at least for the first step, as shown in Figure 4 and Table I (75). Characteristic values could not be obtained to test the reversibility of the second step of reduction because the free radical is not reduced further by Zn dust in KCl solution. Therefore, the second step of the polarogram in Figure 4(right), corrected for the overlapping Zn⁻² wave, corresponds to the cathodic reduction of the preformed free radical and not to the oxidation of the 2-electron reduced form of Diquat.

The above findings are in agreement with the potentiometric studies of Homer and Tomlinson (7) and Homer, Mees, and Tomlinson (6).

The relationship between the polarographic current and the back pressure-corrected mercury head was also evaluated at five pressure points between 48.5 and 88.5 cm. of mercury at seven different concentrations of Diquat ranging from 1.0×10^{-4} to $9.8 \times 10^{-5}M$ solutions, all $0.1M$ with respect to KCl. Coefficients of correlation were obtained to test the goodness of fit of the I_d *vs.* h^x relationships, where $x = 0, 1/2, \text{ and } 1$.

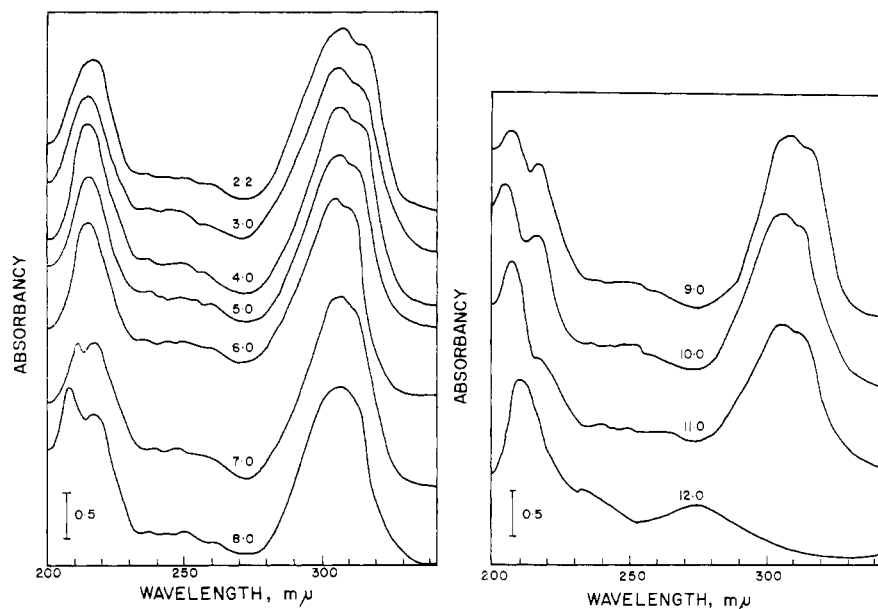


Figure 2. Absorption spectra of $1.00 \times 10^{-3}M$ solution of 1,1'-ethylene-2,2'-bipyridylium dibromide at various pH's

Left. Citric acid-sodium phosphate buffer
Right. Boric acid-sodium hydroxide buffer

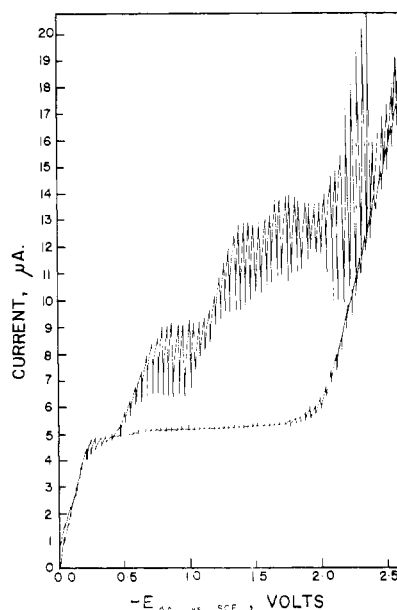


Figure 3. Polarogram of $1.00 \times 10^{-3}M$ solution of 1,1'-ethylene-2,2'-bipyridylium dibromide in $0.02M$ tetrabutylammonium iodide

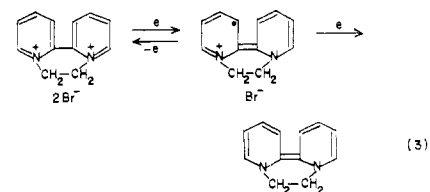
$$C_{(\text{moles/liter})} = 1.28 \times 10^{-6} + 2.18 \times 10^{-4} \times I_{d1} \quad (1)$$

$$C_{(\text{moles/liter})} = 4.26 \times 10^{-5} + 2.26 \times 10^{-4} \times I_{d2} \quad (2)$$

The diffusion currents are to be obtained in microamperes for both the first, I_{d1} , and the second, I_{d2} , wave. The existence of a small, positive intercept in both equations represents random variation in the diffusion currents. The use of the first wave is more satisfactory.

Reaction Mechanism

On the basis of the potentiometric studies (6, 7) and the above results the following reaction is proposed.



Only one of the 18 possible canonical forms of the free radical (6) is shown.

Applications of Analysis

The polarographic analysis of Diquat is feasible either in $0.02M$ tetra-*n*-butylammonium iodide or, especially, in $0.1M$ KCl for the determination of the purity of manufactured products or commercial concentrates.

With adequate separation of high molecular weight and some electroreducible compounds the method should be applicable for the determination of Diquat residues in a wide range of crops

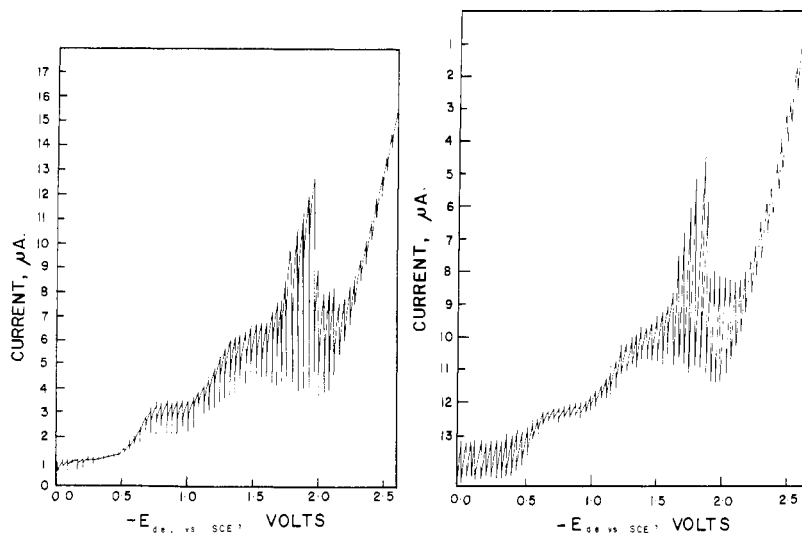


Figure 4. Polarogram

Left. $5.00 \times 10^{-4}M$ 1,1'-ethylene-2,2'-bipyridylum dibromide in 0.1M KCl

Right. Free radical of $5.00 \times 10^{-4}M$ 1,1'-ethylene-2,2'-bipyridylum dibromide in 0.1M KCl

and food products. Ion exchange type elimination of interfering materials is readily achieved according to the method of Calderbank *et al.* (3) and Calderbank and Yuen (4) using KCl or tetra-*n*-butylammonium iodide for eluents. In this manner samples of potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), rutabagas (*Brassica napobrassica*), turnips (*Brassica rapa*), and radishes (*Raphanus sativus*) were analyzed. Recoveries, standards added right after the weighing of the samples, range from 84 to 97% against appropriate blanks. The limit of residue detection

with respect to the raw sample is 0.01 to 0.1 p.p.m.

The given procedure is expected to be reliable for the determination of all bipyridylum-type herbicides because the reduction of 2,2'- and 4,4'-bipyridylum ions at the D.M.E. is only slightly influenced by the nature of the *N*-substituents. However, the $E_{1/2}$'s of these herbicides will be modified somewhat by the composition of the supporting electrolytes.

Acknowledgment

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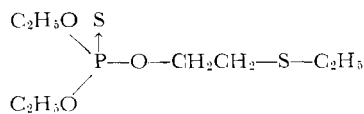
RESIDUE ESTIMATION

Determination of Demeton Residues by Infrared Spectrophotometry

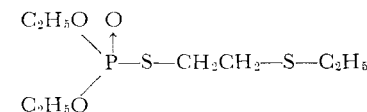
COMMERCIAL demeton is a mixture of about 40% of thiol and about 60% of thiono isomers [*O,O*-diethyl *S*- (and *O*)-2-(ethylthio)ethyl phosphorothioate; Systox]. It was first synthesized by Schrader in 1948 (17). Ever since the publication of its insecticidal properties in 1952 (19), it has attracted wide interest, and it is now recognized as one of the most valuable systemic insecticides (6, 11, 13, 16, 18), although it also has contact activity (13, 16, 18).

In studies of the metabolism of demeton in plants, Metcalf, Fukuto, March, and their coworkers (8-10) found four important oxidation products which are insecticidally active and which have high mammalian toxicity—thiono demeton sulfoxide, thiono demeton sulfone,

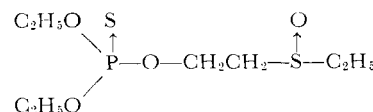
thiol demeton sulfoxide, and thiol demeton sulfone—the phosphate-sulfoxide and phosphate-sulfone being of lesser significance. Hydrolysis or further oxidation results only in decomposition products having little toxicity to insects or mammals. These authors also found from *in vitro* enzymatic studies that the sulfones are ten times more potent as inhibitors of cholinesterase than the respective sulfoxides and that pure thiono demeton itself is a weak inhibitor.



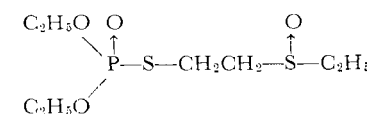
Thiono-demeton



Thiol demeton



Thiono demeton sulfoxide



Thiol demeton sulfoxide

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