

## INDIRECT DETERMINATION OF MALACHITE GREEN WITH CERIUM(IV) SULPHATE

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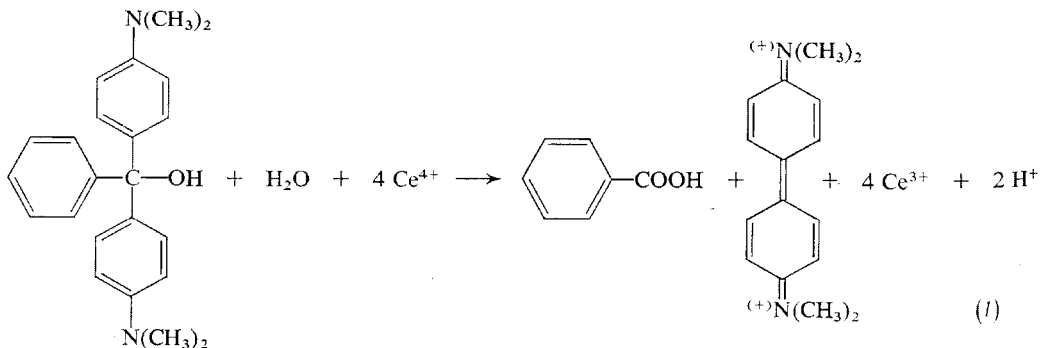
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An indirect determination of malachite green based on its oxidation with cerium(IV) sulphate and on the ascorbinometric or spectrophotometric determination of the formed diphenoquinone-(4,4')-bis-dimethylimine has been developed.

So far malachite green has been determined usually by reductometric titration<sup>1-3</sup>, and a number of precipitation methods<sup>4-6</sup> and a bromination method<sup>7</sup> have been described, too. All the methods mentioned are relatively complex and little selective. Considerably simpler, nevertheless also little selective, is the method based on oxidation of malachite green with cerium(IV) sulphate<sup>8</sup> according to Eq. (1).

In this method malachite green is first isolated in the form of the carbinol base, which is then dissolved in acid and determined by potentiometric titration with a standard cerium(IV) sulphate solution. The reprecipitation of the carbinol base serves for removing of oxalic acid which is usually contained in commercial samples, and besides that it secures that the dyestuff is present in the titrated solution in its hydrated form, which is necessary for quantitative course of the reaction<sup>8,9</sup>. The reprecipitation of the dyestuff, however, cannot remove number of the aromatic amines which also react with cerium(IV) sulphate and thus interfere with the cerimetric determination of malachite green.



In this work we tried to increase the selectivity of the determination by evaluating the amount of the formed oxidation product instead of that of the consumed oxidation agent. Therefore, first we studied the oxidation of N,N,N',N'-tetramethylbenzidine (which is accessible sufficiently pure) – the intermediate of the malachite green oxidation, and we developed a titration and a spectrophotometric method of determination of the formed diphenoquinone-(4,4')-bis-dimethylimine. Then the applicability of these newly developed methods was proved also for the indirect determination of malachite green based on its oxidation with cerium(IV) sulphate and on spectrophotometric or titration determination of the formed diphenoquinone(4,4')-bis-dimethylimine.

## EXPERIMENTAL

### Reagents and Apparatus

0.01N and 0.001N cerium(IV) sulphate solutions in 0.25M sulphuric acid and 0.01N ferrous sulphate in 0.25M sulphuric acid were prepared in usual way. The ferrous sulphate solution was standardized with dichromate every day, the same being applied for 0.01N ascorbic acid solution<sup>10</sup>. 0.005M solution of N,N,N',N'-tetramethylbenzidine in 0.02M hydrochloric acid was prepared by dissolution of the precisely weighed sample prepared according to Ullmann and Dieterle<sup>11</sup>. 0.00025M solution was prepared by dilution of the 0.005M solution with distilled water. 0.025M malachite green solution in 0.25M sulphuric acid was prepared by dissolving the weighed sample of the malachite green carbinol base which was prepared by alkalization of the solution of the dyestuff and purification of the precipitated product by crystallization from ethanol and benzene<sup>12</sup>. The solution was standardized with titanium(III) chloride<sup>3</sup>. The respective 0.0025M and 0.00025M solutions were obtained by dilution of the 0.025M solution with distilled water.

The potentiometric titrations were carried out with a valve millivoltmeter Multoscop V (Laboratorní přístroje, Prague) using a glossy platinum indication electrode and a saturated calomel reference electrode. A 10 ml burette divided by 0.02 ml was used whose outlet was modified in such a way that one drop corresponded to 0.02 ml. The titrated solutions were stirred with an electromagnetic stirrer. The spectrophotometric measurements were carried out with a Unicam SP 800 apparatus (Unicam Instruments, Ltd., England) using 1 cm quartz cells. The infrared spectra were measured with a UR 20 spectrophotometer (Carl Zeiss, Jena) using 0.1 mm cells. Commercial thin layers Silufof UV 254 (Kavalier, Votice) were used for the chromatography.

## PROCEDURE

### Oxidation of N,N,N',N'-Tetramethylbenzidine

*Time dependence of the exchanged electrons number.* 5.00 ml 0.005M-N,N,N',N'-tetramethylbenzidine solution was added to 10.00 ml 0.01N cerium(IV) sulphate solution, the solution was mixed and left to stand at the room temperature. After a time  $t$  the non-reacted excess of the oxidation reagent was determined by potentiometric titration with 0.01N ferrous sulphate. At the same time a blank test was carried out, and the reading of the oxidation reagent (in equivalents per 1 mol of N,N,N',N'-tetramethylbenzidine) were obtained from the difference in the readings of ferrous sulphate in the blank test and that in the proper determination.

*Spectrophotometric evidence of the oxidation product.* The obtained solution ( $t = 2$  min) was transferred quantitatively to a 1 l calibrated flask, filled to the mark with distilled water and its spectrum was measured in the region 325–700 nm.

#### Proof of Oxidation Reversibility

*Thin layer chromatographical evidence of reversibility of the oxidation.* 5 ml 0.005M-N,N,N',N'-tetramethylbenzidine was added to 10 ml 0.01N cerium(IV) sulphate, and after 2 minutes 15 ml 0.01M ascorbic acid was added thereto. Then pH of the solution was adjusted at the value 7 by addition of solid  $\text{NaHCO}_3$  (checked by universal pH-paper test), and the solution was extracted by  $3 \times 10$  ml benzene. Combined extracts were evaporated under reduced pressure, the evaporation residue was dissolved in 0.5 ml acetone, and about 0.5  $\mu\text{l}$  solution was spotted on the chromatogram. At the same time the standard solution prepared by dissolving 10 mg of the original diamine in 10 ml acetone was spotted. The thin layer chromatogram was developed in the ascending arrangement with the mixture benzene–ethanol 4 : 1, the atmosphere of the chromatographic box being saturated with the vapours of the eluent system. The spots were made visible by spraying with 0.0025N cerium(IV) sulphate.

*Infrared evidence of the oxidation reversibility.* 100 ml 0.005M N,N,N',N'-tetramethylbenzidine was added to 150 ml 0.01N cerium(IV) sulphate, the solution was mixed, and after 2 minutes 0.5 g ascorbic acid was added. Then pH of the solution was adjusted at the value 7 (checked by universal pH-test paper) by addition of solid  $\text{NaHCO}_3$  and the solution was extracted by  $4 \times 25$  ml benzene. The combined extracts were evaporated under reduced pressure, the evaporation residue was recrystallized from ethanol and dissolved in tetrachloromethane; IR spectrum of the solution was measured in the range 800 to 1700  $\text{cm}^{-1}$ .

#### Determination of the Oxidation Product

*Ascorbinometric determination after removal of excess cerium(IV) sulphate by potentiometric titration with ferrous sulphate.* 5.00 ml 0.005M N,N,N',N'-tetramethylbenzidine was added to 10.00 ml 0.01N cerium(IV) sulphate, the solution was mixed, and after 2 minutes the excess of cerium(IV) sulphate was accurately reduced with 0.01N ferrous sulphate with potentiometric indication. Then the present quinonediimine was titrated with 0.01N ascorbic acid, the equivalence point being indicated by disappearance of the orange colour corresponding to the titrated diphenoquinone-(4,4')-bis-dimethylimine.

*Ascorbinometric determination after removal of excess cerium(IV) sulphate with excess hydrazine sulphate.* 5.00 ml 0.005M N,N,N',N'-tetramethylbenzidine was added to 10.00 ml 0.01N cerium(IV) sulphate, the solution was mixed, and after 2 minutes 5.00 ml 0.01M hydrazine sulphate was added thereto. After further 2 minutes diphenoquinone-(4,4')-bis-dimethylimine was determined by titration with ascorbic acid with visual indication.

#### Spectrophotometric Determination

The calibration curve was constructed in the following way: 1.00 to 5.00 ml 0.00025M N,N,N',N'-tetramethylbenzidine was added to 10.00 ml 0.001N cerium(IV) sulphate, the solutions were made up to 50 ml each, and after 2 minutes their absorbances were measured at 462 nm.

The proper determination was carried out in the following way: 5.00 ml solution containing  $25 \cdot 10^{-8}$  to  $125 \cdot 10^{-8}$  mol N,N,N',N'-tetramethylbenzidine was added to 10.00 ml 0.001N cerium(IV) sulphate, the solution was made up to 50 ml with distilled water, and after 2 minutes

its absorbance was measured at 462 nm. Concentration of the diphenquinone-(4,4')-bis-dimethylimine formed by oxidation was then read from the calibration curve constructed in the abovementioned way.

#### Determination of Malachite Green

*The determination based on ascorbinometric titration of the diphenquinone-(4,4')-bis-dimethylimine formed by oxidation.* 10.00 ml Malachite green solution is added to 20.00 ml 0.01N cerium(IV) sulphate, the dyestuff content in the former being  $12 \cdot 10^{-7}$  to  $38 \cdot 10^{-7}$  mol; the solution obtained is mixed, and after 2 minutes 20.00 ml 0.01M hydrazinium sulphate is added. After further 2 minutes the present diphenquinone-(4,4')-bis-dimethylimine is determined by visual titration with 0.01N ascorbic acid.

*The determination based on spectrophotometric determination of diphenquinone-(4,4')-bis-dimethylimine amount formed by oxidation.* 5.00 ml Malachite green solution containing  $25 \cdot 10^{-8}$  to  $125 \cdot 10^{-8}$  mol of the dyestuff is added to 10.00 ml 0.001N cerium(IV) sulphate, the solution is made up to 50 ml with distilled water, and after 2 minutes its absorbance is measured at 462 nm. The malachite green content is read from the calibration curve constructed with the use of pure N,N,N',N'-tetramethylbenzidine (see the paragraph describing the spectrophotometric determination of diphenquinone-(4,4')-bis-dimethylimine formed by oxidation of N,N,N',N'-tetramethylbenzidine with cerium(IV) sulphate).

## RESULTS AND DISCUSSION

The time dependence of the number of exchanged electrons is as it follows:

Time, min	2	5	10	20	30	60
Number of exchanged electrons	2.00	2.02	2.02	2.04	2.06	2.10

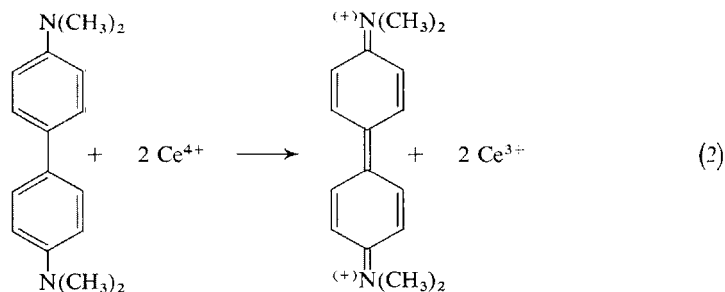
The given values represent mean values from three measurements whose results did not differ more than by  $\pm 0.2\%$ ; it can be seen that N,N,N',N'-tetramethylbenzidine is immediately oxidized by excess cerium(IV) sulphate, 2 electrons being exchanged, and subsequent deeper oxidation is only very slow.

#### *Spectrophotometric Evidence of the Oxidation Product*

The spectrum measured shows a sharp absorption maximum at 462 nm, its position and intensity being in accordance with the known<sup>13</sup> absorption spectrum of diphenquinone-(4,4')-bis-dimethylimine. This fact along with the proved exchange of 2 electrons indicate that oxidation of N,N,N',N'-tetramethylbenzidine with cerium(IV) sulphate proceeds quantitatively according to Eq. (2).

#### *Evidence for the Oxidation Reversibility*

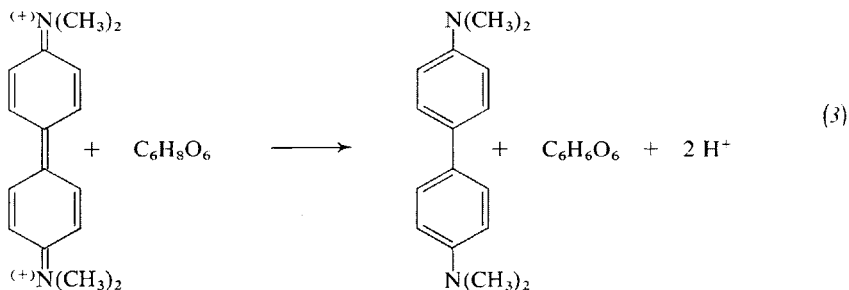
Position of the spots obtained after development of the chromatograms is the same in the case of N,N,N',N'-tetramethylbenzidine and in the case of the compound



obtained after reduction of the oxidation product of *N,N,N',N'*-tetramethylbenzidine with ascorbic acid. Also the infrared spectra of the compound obtained by reduction of the oxidation product of *N,N,N',N'*-tetramethylbenzidine and of *N,N,N',N'*-tetramethylbenzidine itself are identical. These facts indicate that ascorbic acid reduces the diphenylquinone-(4,4')-bis-dimethylimine formed by oxidation back to the original *N,N,N',N'*-tetramethylbenzidine.

#### Determination of the Oxidation Product

*Ascorbinometric determination after removal of excess cerium(IV) sulphate by potentiometric titration with ferrous sulphate.* In this context it had to be proved that the reduction of diphenylquinone-(4,4')-bis-dimethylimine with ascorbic acid proceeds quantitatively. Therefore, the excess of cerium(IV) sulphate was removed with the accurately corresponding amount of ferrous sulphate with potentiometric indication, and the remaining quinonediimine was titrated with 0.01N ascorbic acid. In this experiment use was made of the fact that the titrated diphenylquinone-(4,4')-bis-dimethylimine has extraordinary intensive colour, so that the point of equivalence can reliably be indicated by disappearance of the orange colour



corresponding to the quinonediimine being titrated. The transition in the point of equivalence is very sharp and the found readings of ascorbic acid do not differ by more than  $\pm 0.2\%$  from the theoretical ones. This fact confirms that diphenoquinone-(4,4')-bis-dimethylimine is reduced with ascorbic acid quantitatively according to Eq. (3).

*Ascorbinometric determination after removal of excess cerium(IV) sulphate with excess hydrazine sulphate.* In order to avoid the potentiometric titration of excess cerium(IV) sulphate with ferrous sulphate, such a reagent was looked for which would remove excess cerium(IV) sulphate without reducing markedly the present diphenoquinone-(4,4')-bis-dimethylimine. Out of a large number of the investigated reagents hydrazine sulphate met these requirements best. It was found, that its use can remove the excess cerium(IV) sulphate within less than 2 minutes, the amount of the present quinonediimine being not markedly decreased by reduction with hydrazine sulphate even after 10 minutes.

Table I gives the accuracy and reproducibility of the determinations carried out according to the procedure given in Experimental. The values found (Tables I–III) represent mean values from 10 determinations each, the standard deviation being calculated according to the relation

$$s = \left[ \frac{\sum_i \Delta_i^2}{(n - 1)} \right]^{1/2},$$

where  $\Delta_i$  and  $n$  stand for the deviation of the  $i$ -th determination from the mean and number of the determinations, respectively.

#### *Spectrophotometric Determination*

The fact that diphenoquinone-(4,4')-bis-dimethylimine formed on oxidation has an extremely high value of molar absorption coefficient can be used for its spectro-

TABLE I

Accuracy and Reproducibility of Indirect Titration Determination of N,N,N',N'-Tetramethylbenzidine

N,N,N',N'-Tetramethylbenzidine, mg		Relative error, %	Standard deviation mg
given	found		
3.004	2.994	-0.33	0.005
6.008	5.978	-0.50	0.008
9.012	8.990	-0.25	0.010

photometric determination. Excess of oxidation reagent need not be removed, as the molar absorption coefficient value of cerium(IV) sulphate at 462 nm is negligible as compared with that of the quinonediimine being determined ( $\lambda_{\max} = 462 \text{ nm}$ ). It was found<sup>14</sup> that intensity of the colour is constant within 2 to 20 minutes after preparation of the solution, provided the procedure described in Experimental is observed. The calibration line constructed according to the given procedure is linear in the concentration range 0 to  $20 \cdot 10^{-6} \text{ M}$  N,N,N',N'-tetramethylbenzidine, and the molar absorptivity value obtained from its slope is  $7 \cdot 24 \cdot 10^4 \text{ mol}^{-1} \text{ l cm}^{-1}$ . It was proved that N,N,N',N'-tetramethylbenzidine can be determined down to the concentrations of  $2 \cdot 10^{-6}$  to  $2 \cdot 10^{-5} \text{ M}$  with relative error less than  $\pm 2\%$ .

#### Determination of Malachite Green

The abovegiven methods for determination of diphenoquinone-(4,4')bis-dimethylimine were used for indirect determination of malachite green based on its oxidation with cerium(IV) sulphate and ascorbinometric or spectrophotometric determination of the diphenoquinone-(4,4')-bis-dimethylimine formed by the oxidation.

TABLE II

Accuracy and Reproducibility of Indirect Titration Determination of Malachite Green

Malachite Green, mg (Cl <sup>-</sup> )		Relative error, %	Standard deviation mg
given	found		
4.2834	4.2241	-1.38	0.009
8.5667	8.4467	-1.40	0.014

TABLE III

Accuracy and Reproducibility of Indirect Spectrophotometric Determination of Malachite Green

Malachite Green, mg (Cl <sup>-</sup> )		Relative error, %	Standard deviation mg
given	found		
0.0858	0.0817	-4.78	0.0014
0.1715	0.1631	-4.90	0.0028
0.2569	0.2449	-4.67	0.0040
0.3427	0.3255	-5.28	0.0056

*Determination based on ascorbinometric titration of diphenoquinone-(4,4')-bis-dimethylimine formed by oxidation.* The recommended procedure of this determination was suggested on the basis of measurements of the time dependence of the quinonediimine amount found by ascorbinometry, from which it followed that it is sufficient to use excess of the reagent for 2 minutes. Accuracy and reproducibility of malachite green determination in a commercial sample (Malachite Green B, Chemapol, Prague) carried out according to the abovementioned procedure is given in Table II.

*Determination based on the spectrophotometrically found amount of the diphenoquinone-(4,4')-bis-dimethylimine formed by oxidation.* The recommended procedure for the determination was suggested on the basis of measurements of the time dependence of absorbance, wherefrom it followed that the absorbance can be measured as early as 2 minutes after preparation of the solution. Practical results of the analysis are given in Table III.

## CONCLUSION

The investigations carried out proved that N,N,N',N'-tetramethylbenzidine is oxidized by cerium(IV) sulphate with exchange of 2 electrons to give diphenoquinone-(4,4')-bis-dimethylimine which can be quantitatively reduced by ascorbic acid back to the original N,N,N',N'-tetramethylbenzidine. Further it was proved that oxidation of N,N,N',N'-tetramethylbenzidine can be used for determination of this substance on the basis of ascorbinometric or spectrophotometric determination of the quinonediimine formed. The submitted new methods of determination of diphenoquinone-(4,4')-bis-dimethylimine were then used for indirect determination of malachite green based on its oxidation by excess cerium(IV) sulphate and spectrophotometric or ascorbinometric determination of the formed diphenoquinone-(4,4')-bis-dimethylimine.

Compared with the reductometric methods used so far the new titration method is simpler, not requiring the work under inert atmosphere, and substantially more sensitive, too. Compared with the direct titration with cerium(IV) sulphate the new method is more selective. The direct titration is complicated by the fact that all the substances reacting with cerium(IV) sulphate interfere therewith, whereas only coloured substances or those reacting with ascorbic acid interfere with the new method. The new method with the spectrophotometric variant is also considerably more selective and, in addition to it, substantially more sensitive than all the methods used so far. It can be anticipated that triphenylmethane dyestuffs having structures analogous to that of malachite green will be determinable in analogous way.



## REFERENCES

1. Matrka M., Ságner Z.: Chem. Prům. 8, 22 (1958).
2. English F. L.: Ind. Eng. Chem. 12, 994 (1920).
3. Calcott W. S., English F. L.: Ind. Eng. Chem. 15, 1042 (1923).
4. Kozlov V.: Zh. Prikl. Chim. 9, 558 (1936).
5. Knecht E.: Chem.-Ztg. 12, 817 (1888).
6. Rawson R.: J. Soc. Dyers Colourists 4, 82 (1888).
7. Vaubel W.: J. Prakt. Chem. 50, 347 (1894).
8. Hanousek V., Matrka M.: Chem. Listy 52, 755 (1958).
9. Hanousek V., Matrka M.: Chem. Listy 52, 1774 (1958).
10. Barek J., Berka A.: Anal. Lett. 8, 57 (1975).
11. Ullmann F., Dieterle P.: Ber. Deut. Chem. Ges. 37, 29 (1904).
12. Swein C., Herberg K.: J. Amer. Chem. Soc. 72, 3373 (1950).
13. Matrka M.: Chem. Listy 57, 163 (1963).
14. Nováková L.: *Thesis*. Charles University, Prag 1975.

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