



Spectrophotometric Determination of Dissolved Oxygen in Water Sample with the System of I_3^- -Malachite Green-Polyvinyl Alcohol

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A new spectrophotometric method with the system of I_3^- -malachite green (MG)-poly(vinyl alcohol) (PVA) was first proposed for determination of dissolved oxygen in water sample. The I_3^- which reacted with malachite green to form an ion-associated complex has a characteristic absorbance at 699 nm. KIO_3 which adopted as the primary standard substance was used to determine the dissolved oxygen directly. The calibration graph was linear over the range of 0.05-1.70 $\mu\text{g/mL}$ IO_3^- (0.0137-0.466 $\text{mg O}_2/\text{L}$). The relative standard deviation ($n = 6$) was below 0.3 %. A complete analysis could be performed in 0.5 h including pretreatment and detection, giving a high throughput of 20 samples in about 40 min. Compared with the Winkler methods, this method is simple and sensitive and consumes limited and cheap reagents. It was successfully applied for the determination of dissolved oxygen in fresh or seawater samples, sewage, *etc.* It is suggested as a suitable alternative to titration in most applications of the Winkler determination of dissolved oxygen.

Key Words: Dissolved oxygen, Spectrophotometry, Malachite green.

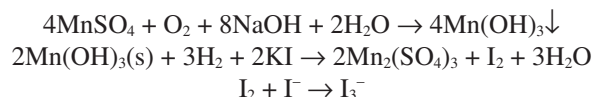
INTRODUCTION

Dissolved oxygen (DO) is one of the most important substances present in water and it involves in a variety of chemical, biological and biochemical processes in environmental waters. Generally, the dissolved oxygen level equilibrated with atmosphere is in a range of 5-15 $\text{mg O}_2/\text{L}$, which depend on water temperature, pollution, salinity and altitude¹.

Many methods have been proposed for the determination of dissolved oxygen in water since the original work of Winkler². Recently a lot of researchers put forward other methods³⁻¹⁶ for the measurement of dissolved oxygen, such as electrochemical methods, spectrophotometric method, the optical sensor method and the flow injection method, *etc.* In these methods the Winkler method and electrode method were the mostly used for the dissolved oxygen measurement. The Winkler method¹⁷ (it has been developed as the standard iodometric method (ISO 5813 Guide) for the determination of dissolved oxygen) has been proven precise and robust for determination of dissolved oxygen regardless of the actual matrix components of the sample, *e.g.*, in contrast, the contents of dissolved salts or organic matter can severely influence the diffusive membrane passage of oxygen when resorting to amperometric detection by the Clark electrode. The electrode further requires a constant approach flow of the sample to the membrane. Due to a considerable delay time and the consumption of dissolved

oxygen by the sensor itself, its application to small changes in the concentration or low contents of dissolved oxygen is troublesome.

In this paper, a new spectrophotometric method for determination of dissolved oxygen in water sample with the I_3^- -malachite green-poly(vinyl alcohol) system was first proposed to determine the content of the dissolved oxygen. Two solutions are added to the oxygen containing water sample: one containing KI and KOH and the other containing $MnSO_4$. Oxygen reacts with Mn^{2+} ions under alkaline conditions and forms manganese(III) hydroxide. Then this solution is acidified. Under acidic conditions Mn^{3+} ions oxidize iodide to iodine and in the presence of excess KI, the iodine eventually convert into triiodide. Related chemical reactions were as follows:



Then approximate water sample with the formed I_3^- ions reacted with malachite green to form the ion-associated complex which has a characteristic absorption at 699 nm. KIO_3 adopted as the primary standard substance was used to determine the dissolved oxygen directly and the standardization relied on the preparation of iodate solution. IO_3^- could react with excessive iodide to form I_3^- in the acidic medium. The reaction equation is:



From the reaction above, it could be easily deduced that one mole of iodate equivalent to 3/2 mol of dissolved oxygen in samples (that is to say, 10 mg/L IO_3^- is equivalent to 2.74 mg/L O_2), so the content of dissolved oxygen could be calculated directly from the work curve prepared by using KIO_3 as standard solution in the I_3^- -MG-PVA system.

$$\rho_{\text{O}_2} = \frac{(A-a)}{b} \times \frac{1.5 \times M_{\text{O}_2}}{M_{\text{IO}_3^-}} \times \frac{V_1}{V_2}$$

where ρ = dissolved oxygen concentration (mg/l LH_2O); A = absorbance for the water sample (against the reagent blank); V_1 = the volume of the colour comparison tube; V_2 = sample volume shift from the dissolved oxygen bottle. a and b are slope and intercept of the equation $A = a\rho_{\text{O}_2} (\text{mg/L}) + b$.

EXPERIMENTAL

Absorption spectra were recorded on a model UV-2501 spectrophotometer (Shimadzu, Japan). Conventional 1.0 cm quartz cells were used in this instrument.

All chemicals were of analytical reagent grade. Distilled water was used throughout. Iodate standard stock solution of 1.00 g/L was prepared by dissolving 0.3057 g KIO_3 in 1000 mL distilled water and stored in the dark. This solution was diluted to 10 mg/L before using. Alkaline iodide solution was prepared by dissolving 250 g sodium hydroxide and 75 g potassium iodide in 500 mL ultra pure water.

KI: 0.2 mol/L; malachite green (MG): 2.0×10^{-4} mol/L; poly(vinyl alcohol): 10 g/L; MnSO_4 : 0.05 mol/L and 2 mol/L; Clark-Lubs buffer solutions over the pH range 1.0-4.4 were prepared according to Dean¹⁸. In this case 0.2 mol/L KCl solution, 0.2 mol/L HCl and 0.2 mol/L potassium phthalate were the buffer components.

General procedure: Collect water samples with a flask bottle sampler (narrow mouth ground glass stopper bottles, 250 ± 1 mL). 1 mL of 6 mol/L MnSO_4 solution and 1 mL of the alkali-KI solution were added to the water sample. The solution is then acidified by 1 mL of 18 mol/L H_2SO_4 . Let the water sample stand for 5 min in the dark. Appropriate volume of the I_3^- solution was sampled for the measurement of the dissolved oxygen with the proposed I_3^- -malachite green-PVA spectrophotometric method.

Detection method: Appropriate volumes of the iodate standard solution (or the water sample after preparation) in the iodate concentration range were placed in a series of 10.0 mL colour comparison tube equipped with plug and treated with 1.00 mL 0.2 mol/L KI, 1.00 mL pH = 1.0 clack-lubs solution, 1.00 mL 0.05 mol/L MnSO_4 (during preparation of the water samples, 2 mol/L MnSO_4 was added to react with oxygen forming manganese(III) hydroxide, so Mn(II) must be considered firstly

in the spectrophotometric method). 1.00 mL 10.0 g/L PVA solution, 2.00 mL 2×10^{-4} mol/L malachite green. The solution allowed to stand for 20 min at room temperature then completed to volume with distilled water. The absorbance was measured at 669 nm against a reagent blank. The working curve of iodate or dissolved oxygen could be obtained on the base of the absorbance corresponding to iodate concentration.

RESULTS AND DISCUSSION

Absorption spectra: Under the experimental conditions, the absorption spectra of I_3^- -MG-PVA system and I_3^- -MG system were scanned against the reagent blank, respectively in the wavelength range 630-750 nm (Fig. 1). As it could be seen that the $A_{\lambda_{\text{max}}}$ of I_3^- -MG-PVA was larger than that of the I_3^- -MG and the absorbance was increased with the concentration of the IO_3^- .

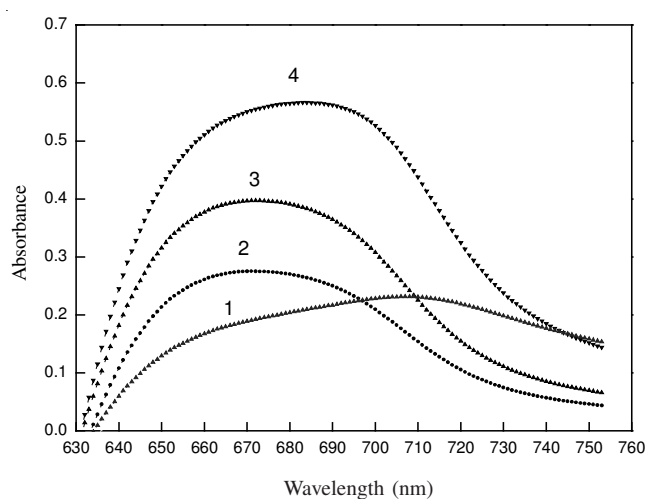


Fig. 1. Absorption spectra of ion-associated complex (against reagent blank). (1) $0.5 \mu\text{g IO}_3^- + \text{MG}$; (2) $0.5 \mu\text{g IO}_3^- + \text{MG} + \text{PVA}$; (3) $3.07 \mu\text{g IO}_3^- + \text{MG} + \text{PVA}$; (4) $1 \mu\text{g IO}_3^- + \text{MG} + \text{PVA}$

In addition, surfactants may shift the absorption peak of associated complexes and the shift is usually accompanied by increase in molar absorptivity. Effect of type of surfactant on spectra and sensitivity were examined and results displayed in Table-1. The results showed higher sensitivity in the PVA solution than in other surfactant solutions when the same concentration of the iodate standard solution was added. So poly(vinyl alcohol) was selected for further studies.

Effect of pH: The reaction among the I_3^- -KI-malachite green could be carried out in the acidic medium. So the effect of pH on the determination of $1.0 \mu\text{g IO}_3^-$ was studied by measuring the absorbance of associated-complex in the range of pH 1.0-6.0. The results (Fig. 2), displayed that the complex has the constant and maximum absorbance at pH 1.0-3.8. In higher pH, the absorbance of I_3^- -MG complex was

TABLE-1
EFFECT ON SELECTIVITY AND THE SENSITIVITY OF THE SURFACTANTS

Surfactant	0	PVA	OP	AG	Tween 20	TBAB	SDS
Wavelength (nm)	669	669	642	669	670	Precipitation	Precipitation
Absorbance	0.469	0.566	0.0576	0.301	0.134	—	—

TBAB: Tetrabutyl ammonium bromide; SDS: sodium dodecylsulfate; PVA: poly(vinyl alcohol); AG: gum arabic.

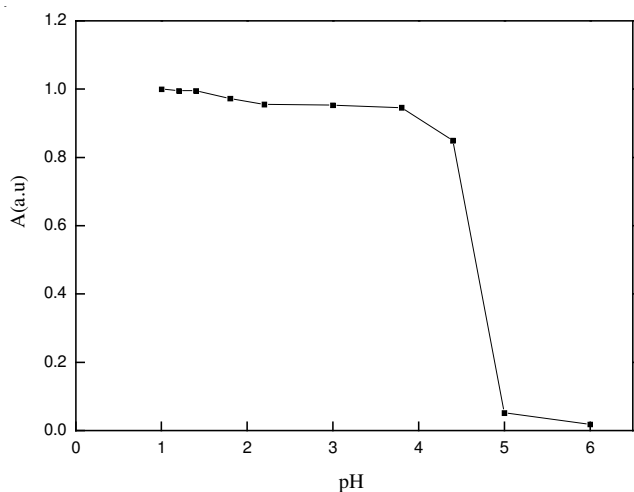


Fig. 2. Effect of pH on A (1.00 mL, 10 mg/L IO₃⁻ + 2.00 mL 2.0 × 10⁻⁴ mol/L malachite green)

decreased due to the less I₃⁻ generated by IO₃⁻ and I⁻ in the weak acid solution. In subsequent work, pH 1.0 has been selected.

The influence of different amounts of buffer solution (pH 1.0) on the system was also studied. the presence of 0.50-3.00 mL of Clark-Lubs solution (pH 1.0) gave a maximum and constant absorbance. Thus, in subsequent experiments the solution volume of the buffer solution (pH 1.0) was fixed at 1.00 mL.

Effect of the amounts of poly(vinyl alcohol) (PVA): Effect of amounts of 10 g/L PVA solution on the complex formation reaction was tested (Fig. 3). It was found that the absorbance leveled off with a maximum when the volume amount of PVA solution was tested from 0.50-3.0 mL so 1.00 mL was selected for further experiment.

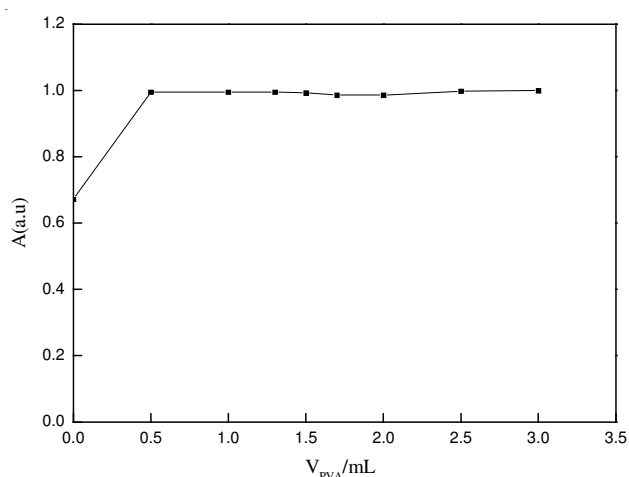


Fig. 3. Effect of volume of poly(vinyl alcohol) on A (1.00 mL 10 mg/L IO₃⁻ + 2.00 mL 2.0 × 10⁻⁴ mol/L malachite green)

Effect of the amounts of malachite green: With the increase of the volume of 2 × 10⁻⁴ mol/L malachite green solution, the absorbance increased rapidly almost at a maximum and unchanged (Fig. 4). In 10 mL of solution, the addition of 1.75-3.00 mL of 2 × 10⁻⁴ mol/L malachite green solution gave maximum and constant absorbance for 1 µg IO₃⁻, thus an addition of 2.00 mL malachite green solution was recommended.

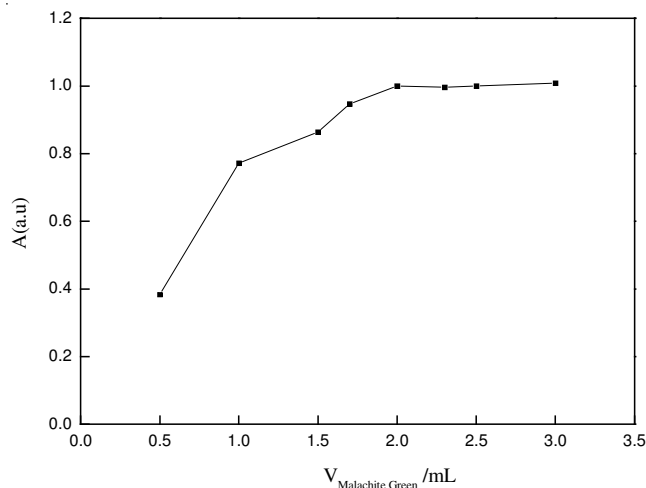


Fig. 4. Effect of volume of malachite green on A (1.00 mL 10 mg/L IO₃⁻ + 2.00 mL, 2.0 × 10⁻⁴ mol/L malachite green)

Effect of reaction time: The optimum reaction time was determined in 2 h. The absorbance of the complex was increased in the initial 10 min and then remained stable for 40 min after which the absorbance gradually decreased. So 20 min were recommended for the further study.

Effect of interfering ions: The interference of foreign substrates were discussed with a relative error of less than ± 5 %, the tolerance limits for various foreign ions were listed in Table-2. It was showed that most of metal ions did not influence the determination of dissolved oxygen.

Analytical parameters: Under the optimum experimental conditions, analytical characteristics were shown in Table-3.

Water sample analysis: Collect the water samples from the river, distilled water, seawater and the sewage treatment plants, then the dissolved oxygen in the water sample was converted to iodine as described earlier. The amount of dissolved oxygen (mg/L) in the water sample was determined by the proposed spectrophotometric method. The Winkler's method was also used to determine the dissolved oxygen in the same sample at 20 °C. The results were shown in Table-4. Averages and variances of the differences between the two methods were homogenous (T-test and Student's test at the 95 % confidence level). Furthermore, 20 samples were analyzed in 40 min by spectrophotometry instead of 1-2 h by titration.

Tested ions	Tolerance ration (w ₁ /w)	Tested ions	Tolerance ration (w ₁ /w)	Tested ions	Tolerance ration (w ₁ /w)
Na ⁺	1800	Mg ²⁺	1400	Fe ²⁺	500
Zn ²⁺	1800	NH ₄ ⁺	1400	H ₂ PO ₄ ²⁻	700
Cu ²⁺	700	Cr ⁶⁺	10	Ni ²⁺	350
K ⁺	1400	Fe ³⁺	20	Hg ²⁺	200

TABLE-3
PARAMETERS OF ANALYSIS

Parameters of analysis	Proposed method
LR of DO (mg/L)	0.0137-0.466
Regression equation	$A = 0.021 + 1.8228\rho_{O_2}$ (mg/L)
Correlation coefficient	0.9977
LOD of DO (mg/L, S/N = 3)	6.14×10^{-4}
SD (%)	0.02

LR: Lineal range; LOD: detection limit (defined on the basis of three times the standard deviation of the blank).

TABLE-4
CONCENTRATION OF DISSOLVED OXYGEN
OF DIFFERENT SAMPLE (95 % CONFIDENCE)

Sample	Proposed method (n = 10)/(mg/L)	Winkler method (n = 6) (mg/L)
Distilled water (20 °C)	11.24 ± 0.03	10.92 ± 0.03
Seawater (20 °C)	8.85 ± 0.04	8.64 ± 0.02
Water of Slender West Lake sampled at 7:00 am (10 °C)	11.60 ± 0.05	11.25 ± 0.02
Water of Slender West Lake sampled at 5:00 pm (10 °C)	12.05 ± 0.04	11.87 ± 0.02
Sewage (10 °C)	11.04 ± 0.03	11.10 ± 0.04

Relationship between dissolved oxygen concentration and temperature: The content of dissolved oxygen in the water has a close relation with the partial pressure of oxygen in the air and the temperature of the water. The dissolved oxygen in the natural water at different temperature was described in the Fig. 5. It indicated that the dissolved oxygen diminished with increasing temperature. Warm water holds less dissolved oxygen than cold water. Our test result also showed that the lowest levels of dissolved oxygen usually occur in the morning, because photosynthesis stops at night while respiration continues.

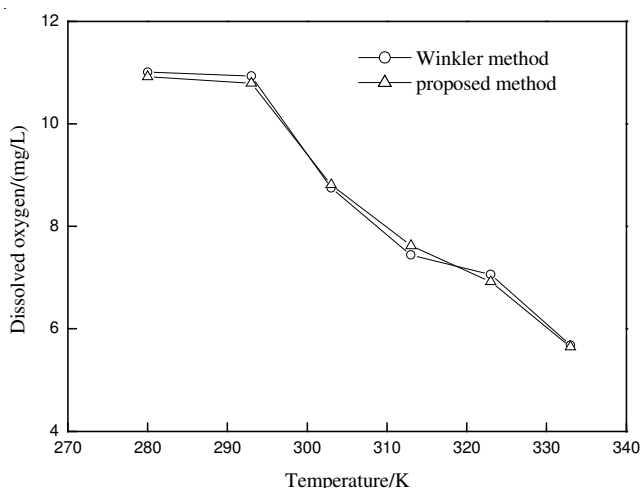


Fig. 5. Relationship between dissolved oxygen concentration and temperature

Conclusion

A new I_3^- -malachite green-poly(vinyl alcohol) spectrophotometric method for the determination of dissolved oxygen in water sample has been proposed. This method can be applied to the determination of dissolved oxygen in fresh water, seawater samples or sewage water. Compared with the Winkler method the IO_3^- instead of $Na_2S_2O_3$ or starch solution in present research work were used and less frequent calibration was required in the proposed spectrophotometric method. All the solutions of the reagents used could be kept stable for longer time. The water samples after pretreatment were only taken less than 2 mL. Because of using spectrophotometric method instead of traditional redox titration method, the changes in oxygen concentrations are less than 1 mg/L and the volatilization of iodine and the oxygen dissolved in the reagents used could also be ignored in the spectrophotometric method. Furthermore, a complete analysis could be performed in 0.5 h including pretreatment and detection, giving a high throughput of 20 samples in about 40 min. It is suggested as a suitable alternative to titration in most applications of the Winkler determination of dissolved oxygen.

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