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Spectrophotometric determination of bisazo dye malachite green in water sample

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ABSTRACT

In this paper, a new spectrophotometric method was established for the determination of malachite green. The optimal pH for the determination of malachite green for the method is 4.0 with a suitable temperature of 35°C. In a pH 4.0 acetic acid-sodium acetate buffer solution medium, the maximum absorption wavelength of malachite green was located at 614 nm. A good linearity is presented over the concentration range of 0–6.0 μg/mL of malachite green and absorbance at this wavelength. The apparent molar absorption coefficient of the method was 7.43×10^4 L/(cm·mol) at 614 nm with a detection limit of 0.13 μg/mL and a quantification limit of 0.43 μg/mL. Then influences of thirty-three co-existing substances on the determination of malachite green were determined. The inter-day and intra-day relative standard deviation for polluted water sample is 0.60% and 0.83%, respectively. The present method was used for the determination of malachite green in a few kinds of water samples with good precision and accuracy. A rapid and accurate method has been established for the determination of malachite green in water samples.

Keywords: Malachite green; Spectrophotometry; Water sample; Determination

1. Introduction

Malachite green (MG, molecular formula: $C_{23}H_{25}ClN_2$, as shown in Fig. 1). The scientific name is tetramethyl diaminotriphenylmethane, which is also called alkaligreen, alkaline green, or Chinese green. As a dye used in leather, textile, pottery manufacture industry, food and biological dyeing, it is a synthetic triphenylmethane kind compound [1]. At the same time, malachite green can also be used as fungicide and insect repellent to prevent and treat the infection of bacteria, fungi and parasites in aquatic animals. Because of its low cost and remarkable effect, malachite green is widely used in aquaculture. However, since 1990, researchers at home and abroad have successively found that the carbon linked to the three phenyls by malachite green contains a double bond, which is unstable in chemical properties and generally exists in the form of chlorides or oxalate. It has been found by scientific research that when malachite green enters the water and after it was dissolved by water body, it enters aquatic animal body inner. Malachite green changes in organisms, 80% of which react to create colorless malachite green (Leuco Malachite Green, LMG, molecular formula: $C_{23}H_{26}N_2$, as shown in Fig. 1). The carbon linked to the three phenyl groups is a single bond, so it is more stable. Colorless malachite green is also called the recessive malachite green. In the structural formula, the carbon linked to the three phenyl groups is a single bond, which is more stable than the malachite green, but more toxic and has a longer residual time in the organism. The damage produced to biological production is greater. They both have high toxicity,

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high residual property, high carcinogenicity, high teratogenicity and other risks [2–4]. With the rapid development of industrial production, a large number of toxic and harmful environmental pollutant malachite green is discharged into the environment, malachite green residues in aquatic products and the environment for a long time, leading to a serious increasing pollution phenomenon. In view of the harm of malachite green and its metabolite, colorless malachite green, malachite green has been listed as a banned drug in aquaculture in China [5,6], the United States, the European Union and many countries [7].

At present, the main detection methods of malachite green are physicochemical method and immunological method. Physicochemical assays include thin layer chromatography [8], spectrophotometry, gas chromatography, high-performance liquid chromatography [9], electrochemical sensor [10], tandem liquid chromatography-mass spectrometry [11,12], gas chromatography-mass spectrometry [13], and immunoassay mainly uses enzyme linked immune sorbent assay [3]. Although high-performance liquid chromatography [14,15] and liquid chromatography-mass spectrometry tandem detection [16,17] have the advantages of high speed, high efficiency, high sensitivity and high automation, these methods have the disadvantages of expensive instrument and complicated operation, etc. Spectrophotometry has the advantages of simple and convenient operation, low cost of instrument and equipment, high sensitivity and so on [18]. At present, the determination of some dye Congo red by this method has attracted much attention [19–23]. Mo et al. [24] determined the malachite green of the lake water, but the method required dichloromethane extraction and the operation was inconvenient.

In this paper, a quantitative method for the determination of malachite green was established by spectrophotometry. The optimum conditions were studied, the influence of co-existing substances was investigated, and the working curve for the determination of malachite green was established. The method has been applied to the determination of malachite green in water samples with satisfactory results. This method can provide a referential content detection method for the cleaning treatment of the waste water containing malachite green dye.

2. Experimental section

2.1. Chemicals

Malachite green standard working solution: 1.0000 mg/mL of stock solution was prepared by dissolving 0.1000 g of malachite green (Xilong Chemical Co., Ltd., China) in 100 mL water. Appropriate amount of malachite green standard reserve solution was appropriately diluted to get working solution. pH = 4.0 acetic acid (HOAc)-sodium acetate (NaOAc) buffer solution: 4.00 g of $NaOAc{\cdot}3H_2O$ (Beijing Chemical Plant, China) were dissolved in proper amount of water. 9.46 mL glacial acetic acid were added (Beijing Chemical Plant, China). It was diluted to 100 mL with water. The reagents used were analytical pure and water was deionized water.

2.2. Instruments

A 722S spectrophotometer (Shanghai Lengguang Technology Co., Ltd., China) equipped with 1 cm cells, was used for the determination of absorbance.

2.3. Experimental procedure

In 25 mL volumetric flask, 0–150 μg malachite green standard solutions were added. Then, 2 mL of pH 4.0 HOAc-NaOAc buffer solution were added. Water was used to fix the volume to the mark. The solution was set for 40 min. The corresponding reagent blank was used as reference and the absorbance was determined by using 614 nm as determination wavelength on spectrophotometer.

2.4. Method for determination of water sample within a day and between days

The method for determination within a day took 13 parallel measurements to obtain the relative standard deviation. For the determination method between days, by using 6-d parallel measurements, results obtained were calculated to obtain their relative standard deviation.

3. Results and discussion

3.1. Absorption spectra

Determination of the absorption spectrum of the malachite green standard working solution for 1.0–5.0 μg/mL different concentration vs. the reagent blank was carried out in the wavelength range of 400–800 nm according to the experimental method, and the absorbance-wavelength curve was plotted. The determined result is shown as Fig. 2. The results show that the maximum absorption wavelength is 614 nm. The reagent blank has not the absorption in the range of 400–800 nm. In this paper, for the experimental wavelength of determination 614 nm was adopted.

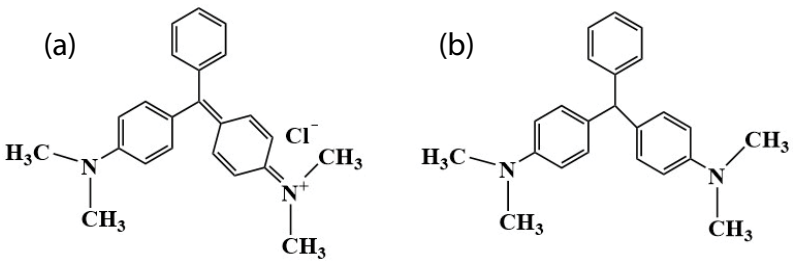

Fig. 1. Molecular structure of (a) malachite green and (b) Leuco Malachite Green.

3.2. Effect of pH on absorbance

The results of absorbance variation situation with pH show (Fig. 3) that the absorbance of malachite green increases with the increase of pH. At pH 4.0, the absorbance was maximum. The absorbance decreased with the increase of pH over pH 4.0–10.0. The optimum pH for the determination of malachite green was 4.0, and the pH value was controlled by pH 4.0 HOAc-NaOAc buffer solution for later experiments. Over the range of the dosage 0.5–2.0 mL of acetic acid-sodium acetate buffer solution with pH = 4.0, the absorbance was increased with the increase of the dosage of the buffer solution. The absorbance was larger and stable in the range of 1.8–4.0 mL. In the experiment, 2.0 mL was selected.

3.3. Influence of temperature

The temperature effect experiments were carried out in the range of 25°C, 35°C, 45°C, and 55°C, in the range of 25°C–35°C the absorbance was increased and the sensitivity of the determination was increased with the increase of temperature. In the range of 35°C–55°C, the absorbance was decreased and the sensitivity of determination was decreased with the increase of temperature. The experiment was carried out at 25°C ± 1°C at room temperature.

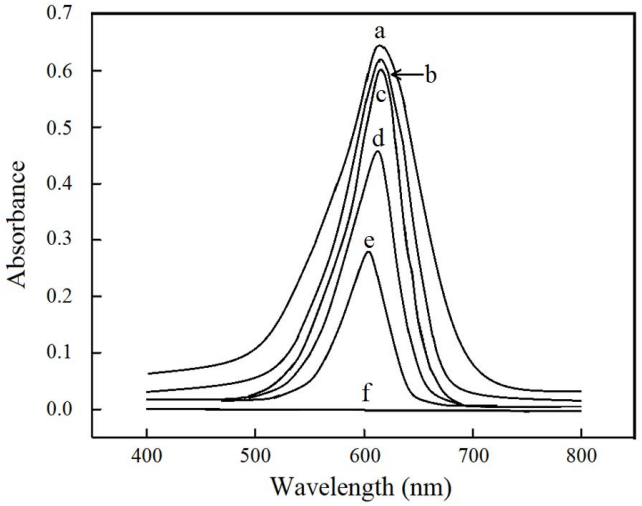

Fig. 2. Absorption spectra: (a–e) malachite green vs. reagent blank, (f) reagent blank vs. water, [malachite green] = (a) 5.0 μg/mL, (b) 4.0 μg/mL, (c) 3.0 μg/mL, (d) 2.0 μg/mL, and (e) 1.0 μg/mL, pH = 4.0.

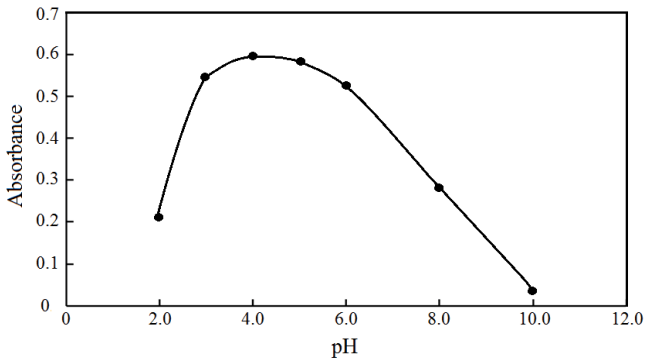

Fig. 3. Determination of optimum pH: [malachite green] = 3.0 μg/mL, determination wavelength = 614 nm.

3.4. Co-existing substance effects

When 3.0 μg/mL malachite green solution is determined, the relative error of the determination does not exceed ±5%. The permitted mass multiples of 33 kinds of coexisting substances (the relative error does not exceed ±5%) are as follows: Ag^{+} (1,500), Li^{+} (400), Be^{2+} (30), Ca^{2+} (800), Cd^{2+} (1,200), Co^{2+} (20), Cu^{2+} (300), Hg^{2+} (150), Mg^{2+} (700), Mn^{2+} (200), Ni^{2+} (1,100), Pb^{2+} (100), Sr^{2+} (20), Zn^{2+} (800), Al^{3+} (60), Bi^{3+} (20), Cr^{3+} (30), Fe^{3+} (50), La^{3+} (1,500), Ce^{4+} (1), Mo(VI) (0.2), MnO_4^- (0.002), NO_2^- (2), $B_4O_7^{2-}$ (2,000), SO_4^{2-} (50), WO_4^{2-} (0.1), PO_4^{3-} (1,000), EDTA (ethylenediaminetetraacetic acid, 4,000), acetone (1.1×10^4), ethanol (1.1×10^4), citric acid (300), tartaric acid (300), salicylic acid (150). The malachite green can be oxidized by Ce^{4+}, Mo(VI), MnO_4^-, WO_4^{2-} in the acid medium. The malachite green can be reduced by NO_2^- in the acid medium. The malachite green in the solution is consumed. So the interference was produced.

The results of the influence on different ionic strength systems show that in the determination of 3.0 μg/mL malachite green solution, 0.1 mol/L of NaCl, $CaCl_2$, and $AlCl_3$ reduced the sensitivity of the determination by 0.3%, 0.7% and 1.1%, respectively.

3.5. System stability

When 3.2 μg/mL malachite green solution was determined and the relative error of determination did not exceeded ±5%, the absorbance of the chromogenic system remained stable at 35 min and the system remained stable within 8 h.

3.6. Drawing of working curve

A certain amount of malachite green standard solution was added to a series of 25 mL volumetric flasks according to the experimental method and the concentration of malachite green was made to be 0, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 μg/mL, respectively. Then the absorbance was measured according to the experimental method. The working curve was drawn with the absorbance as the vertical coordinate and the malachite green concentration as the transverse coordinate. Malachite green concentration was used as the horizontal coordinate to draw the working curve. The results showed that Beer's law is obeyed in the range of mass concentration 0–6.0 μg/mL for malachite green. Its linear regression equation was $A = 0.1988C + 0.0030$ (C: μg/mL), with the correlation coefficient $R^2 = 0.9980$, $R = 0.9990$, and the apparent molar absorption coefficient of the method was $\varepsilon_{616\ nm} = 7.43 \times 10^4$ L/(cm·mol). The detection limit [25] is based on the formula $C_L = 3S_B/m$, and the quantitative limit [26] is according to the formula $C_L = 10S_B/m$, where C_L, S_B and m are the detection limit, the blank signal standard deviation and the calibration curve slope. That is, the slope of the working curve is divided by three times of the standard deviation of the blank solution. The detection

limit and quantitative limits of malachite green were calculated to be 0.13 and 0.43 μg/mL, respectively. The relative standard deviation was determined by 11 times parallel determinations of 3.2 μg/mL malachite green solutions and the relative standard deviation found was 0.79%.

3.7. Water sample analysis

3.7.1. Synthetic sample

The synthetic samples were prepared by the following method. 0.5 mL solution containing 10 μg/mL of Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+} were taken out and placed in a 100 mL volumetric flask. 0.0100 g malachite green were weighed, placed in a small beaker. After it was dissolved in a small amount of water, it was also added into this volumetric flask. Deionized water was diluted to the mark and shaken well. The composition of the sample is: 100 μg/mL malachite green was contained. The concentration of these ions Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, all were 0.05 μg/mL. 1.0 mL of this solution was then taken and placed in a 25 mL volumetric flask. Then 2 mL of the acetic acid-sodium acetate buffer solution (pH 4.0) was added, water was used to make the volume to the mark, and then the solution was at rest for 40 min. With the corresponding reagent blank as the reference, the absorbance value was measured at 614 nm and the content of malachite green was calculated by regression equation. The results of determination are shown in Table 1.

3.7.2. Lake water

5.0 mL of water sample were taken and put into a 25 mL volumetric flask. Malachite green was determined according to the experimental method. At the same time, the standard-adding recovery test was carried out. The above results are shown as Table 1.

3.7.3. Simulated water sample

A simulated fish farming pond water was taken and the analysis was carried out for determination. According to the ratio of Ca^{2+}, Al^{3+}, Fe^{3+}, Cl^- as 200–800 mg/L, the pH is 6.5–8.5. The malachite green concentration was in 0.015–0.04 mg/L [27] to constitute a simulated fish farming pond water. After the water sample was extracted by dichloromethane solution [28], the salt and other water-soluble substances were removed. In accordance with the experimental procedure, the determination was made and the determined results are shown as Table 1.

3.7.4. Sewage

2 L of a polluted water sample was taken. After it was extracted by dichloromethane solution [28], the water-soluble substances such as salt were removed. As the malachite green was determined by the experimental method, the determined results are shown as Table 1. The inter-day and intra-day relative standard deviation for polluted water sample is 0.60% and 0.83%, respectively.

From the results determined, this method has the advantages of high precision and good accuracy. The analytical results were accurate and satisfactory.

4. Conclusion

In this paper, the optimum experimental conditions for the determination of malachite green by spectrophotometry

Table 1
Results of water sample analysis

Sample	Found (n = 13, μg/mL)		Average (μg/mL)	Relative standard deviation (%)	Added (μg/mL)	Recovered (μg/mL)	Relative recovery (%)	Reference or control value (μg/mL)	Relative error (%)
Synthetic sample	101.21, 99.97, 101.19, 100.96, 99.71, 99.97, 99.59	99.59, 101.11, 102.17, 101.94, 99.71, 101.11	100.63	0.84	–	–	–	100.00	0.63
Lake water	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0	0	4.00	3.98	99.50	–	0.50
Simulated water[a]	0.021, 0.023, 0.025, 0.023, 0.025, 0.023, 0.021	0.023, 0.025, 0.023, 0.021, 0.023, 0.025	0.023	0.66	1.00	0.999	99.9	0.023	0
Polluted water[b]	0.030, 0.035, 0.033, 0.030, 0.035, 0.033, 0.035	0.033, 0.035, 0.033, 0.035, 0.033, 0.030	0.033	0.60	2.00	1.998	99.9	0.032	–

Notes: (1) The control method of a, b sample determination was high-performance liquid chromatography.
(2) The other major components of the (a) sample were: Ca^{2+}, Al^{3+}, Fe^{3+}, Cl^-, and the composition was 400, 200, 300, and 2,300 mg/L.
(3) The other major components of the (b) sample were: Ca^{2+}, Al^{3+}, Fe^{3+}, and the composition was 50, 22, and 35 mg/L. Their results were obtained by atomic absorption spectrometry.

and the properties of the system were studied. The effects of thirty three co-existing substances on the determination of malachite green were studied. The results showed that, the optimal pH for the determination of malachite green for the method is 4.0 with a suitable temperature of 35°C. In the system of acetic acid-sodium acetate buffer solution with pH = 4.0, the maximum absorption peak of malachite green was found to be 614 nm. In the range of 0–6.0 μg/mL for malachite green, the concentration showed a good linear relationship with the absorbance and obeyed Beer's law. Its linear regression equation was: A = 0.1988C (μg/mL) + 0.0030, and the correlation coefficient was 0.9990. The apparent molar absorptivity of malachite green for the determination of malachite green was $\varepsilon_{614\ nm} = 7.43 \times 10^4$ L/(cm·mol), and the detection limit was 0.13 μg/mL. The absorbance of the system was stable at 35 min for 8 h. The method has good precision and accuracy. A rapid and accurate method for the detection of malachite green in water samples has been established. The operation of the present method is simple and rapid.

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Conflict of interest

The authors declare that they have no conflict of interest.

Statement

Data is available in this manuscript.

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