Color Development Based on the Ion Association of Molybdophosphate with Cationic Dye Chloro-Malachite Green

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Color development based on the ion association of molybdophosphate with a cationic dye, a chloro derivative of Malachite Green (Cl-MG), was investigated in an aqueous acidic solution. Cl-MG is very stable as its quinoid form in an acidic medium, and can easily react with molybdophosphate to form a green ion associate, which shows maximum absorption at 685 nm. The experimental conditions for optimum color development have been examined. This color development reaction could be applied to the determination of trace amounts of orthophosphate. The molar absorptivity was 1.3×10^5 dm^3 mol^{-1} cm^{-1} at 685 nm.

Keywords Color development, ion association, molybdophosphate, chloro-Malachite Green

Most of the color developments for the spectrophotometric determination of orthophosphate have been based on the formation of heteropolyacids, such as molybdophosphate and vanadomolybdophosphate, in acidic media. The formed heteropolyacid can be used as a light-absorbing species either as it is, after reduction to its heteropoly blue species, or after extraction into an organic solvent. The molar absorptivities of molybdophosphate are on the order of 10^3 (in dm^3 mol^{-1} cm^{-1}) near 400 nm. A sensitivity enhancement for the determination of phosphate has been achieved by using ion associates of molybdophosphate with cationic dyes. Several cationic dyes have been used as a pairing ion of an ion associate in solvent extraction[1-6], ion association in an aqueous medium[7-9], filtration on a membrane[10,11] and flotation between an aqueous and organic phase.[12] Of these procedures, the methods using color development based on ion association in aqueous media are very simple and more sensitive than that using the absorption of the molybdophosphates. Malachite Green (MG) has been favorably used in color development in aqueous media[9,13-16], because it is the most stable of the cationic triphenylmethane dyes commercially available today. MG, however, is gradually converted into its colorless carbinol, even in an acidic medium. According to the data reported by Cigén *et al.*[17], halogeno derivatives of MG are converted into their colorless carbinols much slower than MG itself.

In this work, the authors aim was to investigate the ion association of a chloro derivative of MG (Cl-MG) with molybdophosphate, and to develop a simple, sensitive spectrophotometric method for the determination of phosphate in an aqueous medium.

Experimental

Apparatus

Absorption measurements were carried out on a Shimadzu (Model 140-02) spectrophotometer and a Shimadzu (UV-300) scanning spectrophotometer with 10-mm pathlength cells. A high-rate stirring apparatus was assembled with a 10-mm quartz cell, a small stirrer bar and a small magnetic stirrer (Iuchi, CC-301), which was placed just under the quartz cell in the cell holder of the 140-02 spectrophotometer.

Reagents

Molybdate solution, 0.4 M as molybdate: ammonium molybdate, $(NH_4)_6(Mo_7O_{24})\cdot4H_2O$ (7.1 g), was dissolved in distilled water to give a 100 ml solution.

Sulfuric acid: commercially available concentrated sulfuric acid (97%, 18.2 M) was used after dilution with distilled water.

Poly(vinyl alcohol) (PVA) solution (0.5w/v%): commercially available PVA (number-averaged degree of polymerization, 500) (1 g) was dissolved in distilled water to give 200 ml of solution.

Standard phosphate solution (2.00×10^{-3} M): potassium dihydrogen orthophosphate was dried at reduced pressure (about 400 Pa) and 50°C to constant mass. The dried compound (0.2722 g) was dissolved in distilled water to give a 1 dm^3 of solution. For calibration purposes, the solution was accurately diluted with distilled water before use.

Cl-MG: this cationic dye was synthesized with reference to the literature[18] with several procedures were changed; the final product differed from that obtained by

Cigén *et al.*, in the counter anion.

The synthetic procedure was as follows.

After dissolving 12.4 g of 2-chlorobenzaldehyde in 10 ml of ethanol, we added 19 ml of concentrated sulfuric acid under cooling with ice. We then added 27.8 g of dimethylaniline dropwise and refluxed the mixture for 1 d. After refluxing, the mixture was added into 150 ml of 2.5%(w/v) ammonia water. Then, after removing residual dimethylaniline by steam distillation, the precipitate of the leuco base was separated by filtering the mixture. We then recrystallized the precipitate twice from an acetone–water mixture. After dissolving the recrystallized product into ethanol, it was refluxed with 3-fold moles of chloranil for 2 h. Cl-MG as a salt of zinc chloride or perchlorate was then precipitated, and the salts from a diluted acetic acid solution recrystallized. The purity of Cl-MG (ClO_4 salt) was examined by a molar-ratio method based on the solvent extraction of the ion associate of lauryl sulfate into benzene. The purity was more than 96%; the result of an elemental analysis was as follows. Found: C, 58.79%; H, 5.37%; N, 5.68%. Calcd: C, 59.62%; H, 5.22%; N, 6.05%.

The recrystallized Cl-MG was dissolved in distilled water to give a 8×10^{-4} M solution.

Standard procedure for the spectrophotometric method

Transfer 5 ml of a sample solution containing phosphorus up to 3 μg to a 10-ml volumetric flask. Add each 1 ml of 5.4 M sulfuric acid, 0.4 M molybdenum and a 8×10^{-4} M Cl-MG solution; then mix. To it, add 0.2 ml of a 0.5%(w/v) PVA solution and dilute the mixture to the mark with distilled water. Measure the absorbance of the solution at 685 nm after standing for at least 3 min.

Results and Discussion

Absorption spectra and stability of color development

Figure 1 shows the absorption spectra of an ion associate of molybdophosphate with Cl-MG and a reagent blank. In the presence of molybdate, the absorbances at longer wavelengths near to 500 – 700 nm increased. This colored product was assumed to be the ion associate of isopolymolybdate and Cl-MG, which showed maximum absorption at 632 nm. When orthophosphate was added to the solution in the presence of molybdate, a large absorbance increase was found at wavelengths near to 685 nm. This strong absorption is attributed to the formation of the ion associate of molybdophosphate with Cl-MG$^+$ (C$^+$). The absorption spectra of the ion associates are broadened compared to that of Cl-MG$^+$, itself. This phenomenon is the same as that with Malachite Green (MG$^+$). The ion-association reaction in an acidic medium can be represented as follows:

$$H_3PMo_{12}O_{40} + \text{Cl-MGH}^{2+}(CH^{2+}) \rightleftharpoons C^+\cdot H_2PMo_{12}O_{40}^- + 2H^+, \quad (1)$$

$$C^+\cdot H_2PMo_{12}O_{40}^- + CH^{2+} \rightleftharpoons (C^+)_2\cdot HPMo_{12}O_{40}^{2-} + 2H^+, \quad (2)$$

$$(C^+)_2\cdot HPMo_{12}O_{40}^{2-} + CH^{2+} \rightleftharpoons (C^+)_3\cdot PMo_{12}O_{40}^{3-} + 2H^+. \quad (3)$$

Figure 2 shows the absorbance change under continuous mixing after mixing cationic dye solutions and a molybdophosphate solution using a rapid-mixing apparatus. It can be seen that Cl-MG reacts rapidly with molybdophosphate to form a colored ion associate, and the absorbance of the ion associate formed according to Eq. (1) and/or Eq. (2) is almost constant over a long

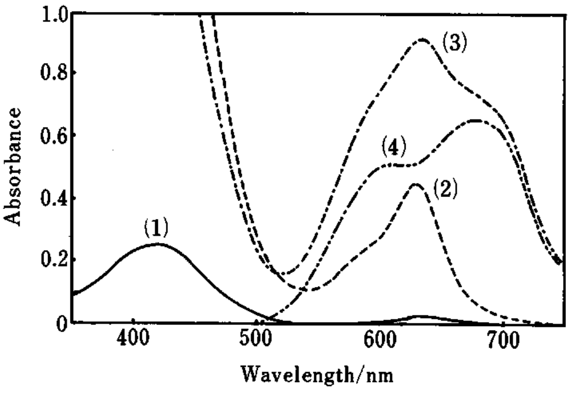

Fig. 1 Absorption spectra of the ion associate of molybdophosphate with Cl-MG. (1) 10^{-5} M Cl-MG (pH 0.3); (2) without phosphate; (3) 4×10^{-6} M phosphate, ref., water; (4) 4×10^{-6} M phosphate, ref., reagent blank. (2), (3) and (4): molybdate, 0.04 M; H_2SO_4, 0.54 M; Cl-MG, 8×10^{-5} M.

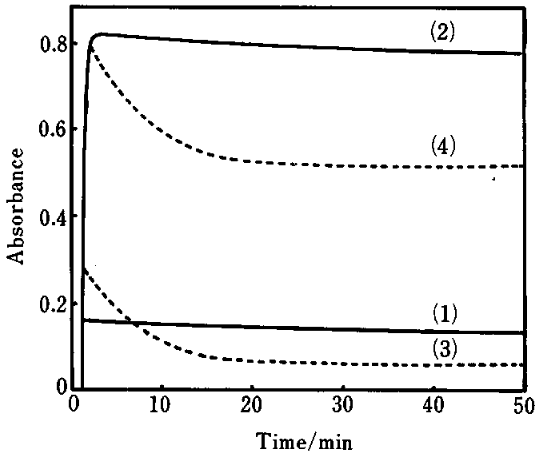

Fig. 2 Absorbance change obtained with MG or with Cl-MG under continuous mixing. (1) and (2): 1×10^{-4} M Cl-MG; (3) and (4): 1×10^{-4} M MG; (1) and (3): reagent blank; (2) and (4): 4×10^{-6} M phosphate. Solution A: 0.08 M molybdate and 0.8 M sulfuric acid with and without 8×10^{-6} M phosphate; solution B: dye solution (pH 5). Solution A and B were mixed rapidly, and absorbances were measured at 650 nm under continuous mixing.

period.

When Malachite Green (MG) was used as a pairing ion of molybdophosphate instead of Cl-MG, the absorbances of both the molybdophosphate and the reagent blank solution gradually decreased until 20 min after mixing. In the case of MG, the ion associate of molybdophosphate with MG^+ is considered to have been formed instantaneously after mixing the solutions. The gradual decreases in the absorbances are attributed to the formation of colorless carbinol of MG^+, according to the following reaction:

$$MG^+ + H^+ \xrightarrow{\text{very fast}} HMG^{2+}, \tag{4}$$

$$HMG^{2+} + H_2O \xrightarrow[k]{\text{slow}} H_2MGOH^{2+}. \tag{5}$$

The protonation reaction (4) is very fast, whereas the hydration reaction (5) is slow. According to Cigén *et al.*, the rate constants of the carbinol formation (k in Eq. (5)) are 31.7 and 1.34 min^{-1} for MG and Cl-MG, respectively.[17]

Figure 3 shows the absorbance change with Cl-MG after preparing solutions according to the standard procedure. In the absence of PVA, the absorbance of a phosphate solution gradually decreased with time. This was because the ion associate of molybdophosphate with Cl-MG gradually precipitates. To prevent the formation of a precipitate, the addition of PVA was examined by varying its amount; 0.01%(w/v) PVA was found to be sufficient for preventing precipitation formation.

In the presence of 0.01%(w/v) PVA, the absorbance of the phosphate solution was constant over a long time, though the absorbance of the ion associate was smaller than the initial absorbance obtained in the absence of PVA. This result shows that PVA acts as a stop agent for the formation of the ion associate and as a solubilizing agent of the ion associate.

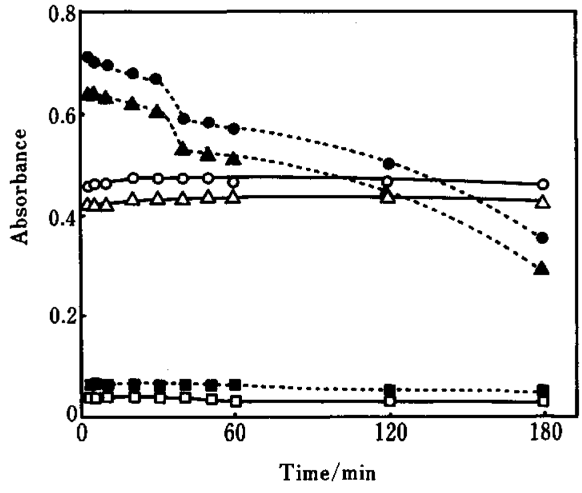

Fig. 3 Effect of PVA on absorbance. ○, □ and △: with 0.01%(w/v) PVA; ●, ■ and ▲: without PVA; ○ and ●: 4×10^{-6} M phosphate; □ and ■: reagent blank; △ and ▲: ref., reagent blank; molybdate, 0.04 M; H_2SO_4, 0.54 M; Cl-MG, 8×10^{-5} M.

Considering the composition of the ion associate based on the apparent molar absorptivities, the ion associates of (1 : 1) and (2 : 1) composition (represented by Eqs. (1) and (2)) seem to be present in the absence of PVA, whereas the ion associate of (1 : 1) composition seems to be mainly present in the presence of PVA.

Effect of the sulfuric acid concentration

Figure 4 shows the effect of sulfuric acid concentrations on the formation of the ion associate, which was examined according to the standard procedure, except for the sulfuric acid concentration.

In the region of low concentrations of sulfuric acid, the absorbances of the reagent blank increase abruptly. This has been attributed to the formation of ion associates of isopolymolybdate with Cl-MG. The net absorbances for phosphate were almost identical at the region from 0.5 M to 0.7 M of sulfuric acid. In the

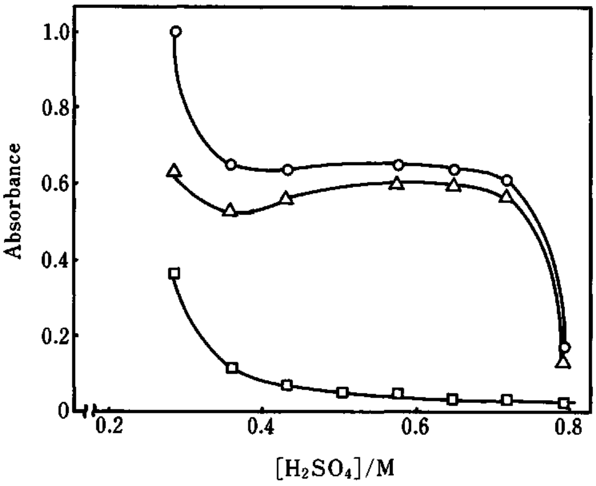

Fig. 4 Effect of the sulfuric acid concentration. ○: 4×10^{-6} M phosphate; □: reagent blank; △: ref., reagent blank. Molybdate, 0.04 M; Cl-MG, 8×10^{-5} M; PVA, 0.01% (w/v).

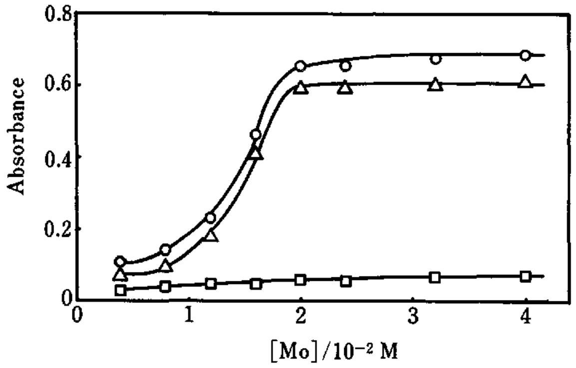

Fig. 5 Effect of the molybdate concentration. ○: 4×10^{-6} M phosphate; □: reagent blank; △: ref., reagent blank. H_2SO_4, 0.54 M; Cl-MG, 8×10^{-5} M; PVA, 0.01% (w/v).

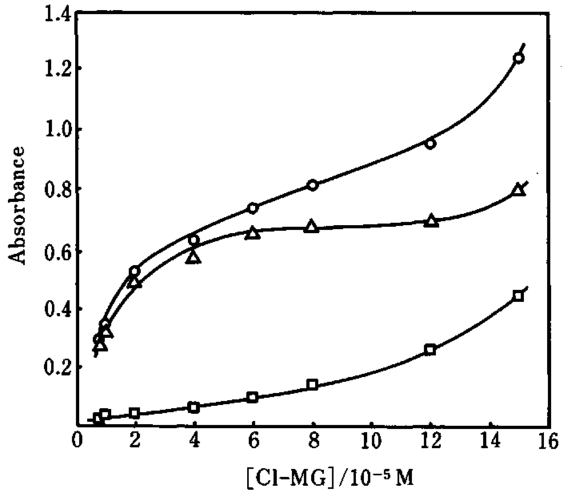

Fig. 6 Effect of the chloro-Malachite Green concentration. ○: 4×10^{-6} M phosphate; □: reagent blank; △: ref., reagent blank. Molybdate, 0.04 M; H_2SO_4, 0.54 M; PVA, 0.01% (w/v).

standard procedure, 0.54 M of the final concentration of sulfuric acid was adopted.

Effect of the molybdate concentration

Figure 5 shows the effect of the molybdate concentration on the formation of the ion associate. The absorbances were almost identical at concentrations above 2×10^{-2} M. In the standard procedure, 4×10^{-2} M (final concentration) was adopted.

Effect of chloro-Malachite Green (Cl-MG)

Figure 6 shows the effect of the Cl-MG concentrations on the formation of the ion associate. The absorbances of both the reagent blank and the phosphate increased with increasing the concentration of Cl-MG. Ion associates of higher order are probably formed along with an increase in Cl-MG. In the standard procedure, 8×10^{-5} M of Cl-MG was adopted as a compromise, considering the absorbance of the reagent blank.

Calibration graph and molar absorptivity

According to the standard procedure, the calibration graph was linear over the range up to 1×10^{-5} M of orthophosphate, and the molar absorptivity at 685 nm was 1.3×10^5 dm^3 mol^{-1} cm^{-1}. The absorbance of the reagent blank was about 0.07.

Color development based on the formation of an ion associate was newly examined for the spectrophotometric determination of phosphate by using a chloro derivative of Malachite Green (Cl-MG). Since the cationic dye adopted in this work is chemically stable and is difficult to be converted to its colorless carbinol, color development can rapidly reach completion. The molar absorptivity of the proposed method is larger than that with Malachite Green. This method will be applicable to trace amounts of orthophosphate.

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