# A Spectrophotometric Study of Phosphomolybdenum Blue Formed by the Reaction of Phosphate with a Mixture of Molybdenum(V) ($Mo_2O_4^{2+}$) and Molybdenum(VI) and Application to the Spectrophotometric Determination of Small Amounts of Phosphates

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The spectrophotometric determination of trace amounts of phosphates, based on the color development of phosphomolybdenum blue formed by the reaction of phosphate with a mixture of molybdenum(V) ($Mo_2O_4^{2+}$) and molybdenum(VI), is described. The rates of formation of phosphomolybdenum blue were measured at 25 °C in a perchloric acid medium. The maximum constant color development was obtained in the perchloric-acid range from 0.20 to 0.41 M under given conditions by warming the solution for 10 min at 80 °C. The method obeys Beer's law in the phosphate-concentration range from 0.08 to 1.16 μg/ml. The molar absorptivity at 840 nm was calculated to be $2.4\times10^4$ l mol$^{-1}$ cm$^{-1}$. The rate is inverse fifth-order with respect to the perchloric-acid concentration in the range from 0.20 to 0.41 M and is first-order with respect to the molybdenum(V) concentration. The formation rates increase with the phosphate and molybdenum(VI) concentrations, although the rate dependence on the concentrations of these reactants is complicated. The ratio of P: Mo(V) for phosphomolybdenum blue was determined to be 1: 1 using the molar-ratio method.

Analytical methods for the determination of phosphates are usually based on the formation of molybdophosphoric acid from the reaction between molybdenum(VI) and the phosphate in an acidic solution,[1] and the subsequent reduction to phosphomolybdenum blue.[2-5] Extensive investigations of the analytical methods and their numerous modifications have been carried out.

Lucena-Conde and Prat[6] developed a spectrophotometric determination method for phosphates based on the blue color development of phosphomolybdenum blue formed by the reaction of the phosphate with a mixture of molybdenum(VI) and molybdenum(V). They examined the effect of the ratio Mo(VI): Mo(V) on the color development.

Hosokawa and Ohshima[7] have modified the method established by Lucena-Code and Prat and applied it to the determination of phosphate in sea water. They also examined the effect of hydrogen-ion concentration on the color development.

The present authors found that the rate of formation of phosphomolybdenum blue formed by the reaction of phosphate with a mixture of molybdenum(VI) and molybdenum(V) is notably dependent on the perchloric-acid concentration. Therefore, the phosphomolybdenum blue formation and the spectrophotometric determination of phosphate, based on the color development of phosphomolybdenum blue, were investigated in perchloric-acid media. The effects of various ions on the color development are described. The composition of molybdenum blue was also studied using the molar-ratio method.

## Experimental

*Materials.* A $1.00\times10^{-1}$ M molybdenum(VI) solution was prepared by dissolving 24.196 g of sodium molybdate dihydrate into 1 litre of redistilled water. Working solutions were prepared by dilution with redistilled water to the desired concentrations. A molybdenum(V) ($Mo_2O_4^{2+}$) perchlorate solution was prepared using a method similar to that reported in Ref. 8. The concentration of this molybdenum(V) solution was determined spectrophotometrically at 384 nm ($\varepsilon=103$).[9] The hydrogen-ion concentration of the molybdenum(V) solution was determined using a cation-exchange resin of hydrogen form. Thus, the hydrogen-ion concentration was established at 2.0 M. The stock solution of molybdenum(V) perchlorate was stored in a refrigerator. A $1.00\times10^{-2}$ M phosphate solution was prepared by dissolving known quantities of sodium dihydrogenphosphate dihydrate or disodium hydrogenphosphate dodecahydrate into redistilled water. All the other chemicals were of analytical grade.

*Apparatus.* All the absorption spectra and absorbance at the given wavelengths were measured using a Hitachi EPS-3 type automatic recording spectrophotometer. The measurements of absorbances at a fixed wavelength were made on a Hitachi 101 manual spectrophotometer. Cells having a light path length of 1 cm were used in all cases. A Yamato BS-44 type water bath was used for warming the solution.

*General Procedure for the Spectrophotometric Determination of Phosphate Content.* In each experiment, 5 ml of $1.60\times10^{-2}$ M molybdenum(VI), 2 ml of $2.20\times10^{-2}$ M molybdenum (V), and a given quantity of a phosphate at $4.00\times10^{-4}$ M were pipetted into a 25-ml measuring flask. 3 ml of 1.0 M perchloric acid was added to adjust the final hydrogen-ion concentration to 0.28 M. The final volume was brought to 25 ml by the addition of redistilled water. The solution was warmed for at least 10 min at 80 °C in a thermostatically-controlled water bath. A portion of the solution was then transferred into a cell. The measurements of absorbances were made at 840 nm.

*Measurements of the Formation Rate of Phosphomolybdenum Blue.* The solutions of molybdenum(VI), molybdenum(V), and sodium dihydrogen phosphate were maintained at 25 °C. In each experiment, portions of these solutions were pipetted into a 25-ml measuring flask, the molybdenum(V) perchlorate solution was added last to the solution containing molybdenum(VI) and phosphate, and the time was measured from its addition. A portion of the solution was transferred into a cell, which was placed in a thermostatically-controlled cell compartment. The kinetic measurements were carried out by recording the change in absorbance at 840 nm as a function of the time. The plots of the absorbance at 840 nm *vs.* time

were linear for at least 15% of the entire reaction. Therefore, the zeroth-order rate constants of the formation of phosphomolybdenum blue were determined from the initial slope of the absorbance *vs.* time plots.

## Results and Discussion

*Absorption Spectra of Phosphomolybdenum Blue Formed by the Reaction of Molybdenum(V) with Molybdenum(VI).* The absorption spectra of a product formed by the reaction of a given concentration of phosphate with a mixture of $1.76\times10^{-3}$ M molybdenum(V) and $3.20\times10^{-3}$ M molybdenum(VI) in 0.28 M perchloric acid are shown in Fig. 1. The absorption spectra coincide with that of phosphomolybdenum blue formed by the reduction of molybdenum(VI) by hydrazinium sulfate in the presence of a phosphate.

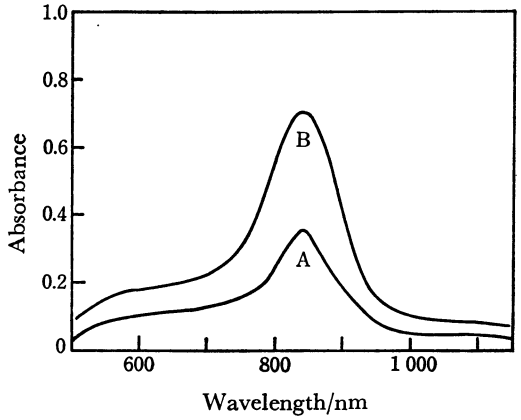


Fig. 1. The absorption spectra of phosphomolybdenum blue formed from the reaction of $1.76\times10^{-3}$ M molybdenum(V) with $3.20\times10^{-3}$ M molybdenum(VI) in the presence of phosphate at 0.28 M perchloric acid. (A): $[NaH_2PO_4]=1.45\times10^{-5}$ M, (B): $[NaH_2PO_4]=2.90\times10^{-5}$ M.

*Effects of the Reaction Time and Temperature on the Color Development.* The effects of the reaction time at 25 and 80 °C on the color development were examined with $1.76\times10^{-3}$ M Mo(V), $3.20\times10^{-3}$ M Mo(VI), $2.90\times10^{-5}$ M $NaH_2PO_4$, and 0.28 M $HClO_4$. The results are shown in Fig. 2. The rate at 25 °C is very small, but the formation rate at 80 °C is relatively large. It requires at least 3 min for the color development to proceed completely at 80 °C. The color is quite stable even after standing for 6 h at 80 °C.

*Effect of Perchloric-acid Concentration on the Color Development and the Formation Rate of Phosphomolybdenum Blue.* The effect of the perchloric-acid concentration on the color development was studied with $2.90\times10^{-5}$ M phosphate, $1.76\times10^{-3}$ M Mo(V), and $3.20\times10^{-3}$ M Mo(VI). The absorbances were measured after warming the solution for 10 min at 80 °C. The results are shown in Fig. 3. The maximum constant absorbance was obtained in the range for perchloric-acid concentration from 0.20 to 0.41 M. Above 0.43 M perchloric acid, the absorbances decrease with increasing perchloric-acid concentration. Below 0.20 M perchloric acid, the reaction of molybdenum(V) with molybdenum(VI) gives a blue precipitation.

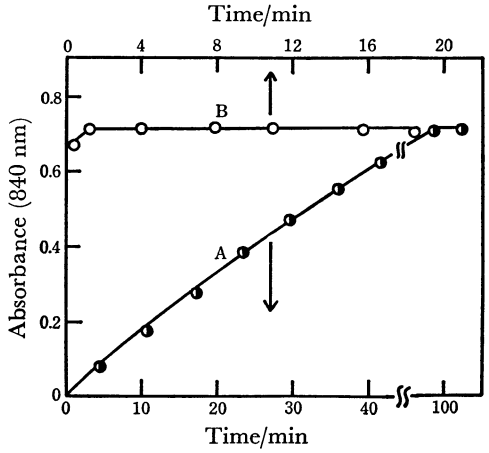


Fig. 2. The Effect of the heating time on the color development. $[Mo(VI)]=3.20\times10^{-3}$ M, $[Mo(V)]=1.76\times10^{-3}$ M, $[NaH_2PO_4]=2.90\times10^{-5}$ M, $[HClO_4]=0.28$ M. (A): 20 °C, (B): 80 °C.

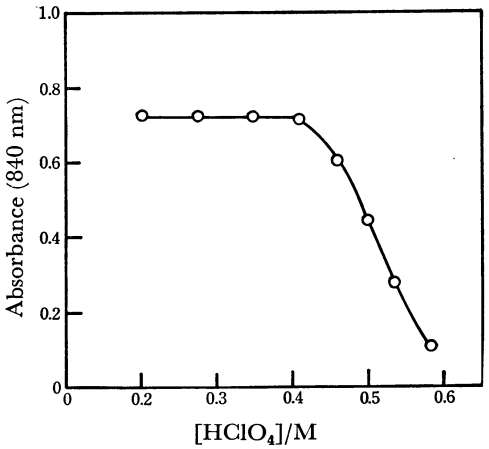


Fig. 3. The effect of the concentration of perchloric acid on the color development. $[Mo(VI)]=3.20\times10^{-3}$ M, $[Mo(V)]=1.76\times10^{-3}$ M, $[NaH_2PO_4]=2.90\times10^{-5}$ M, 10 min, 80 °C.

The effect of the perchloric-acid concentration on the rate of formation of phosphomolybdenum blue was examined in the range from 0.20 to 0.55 M for $1.01\times10^{-3}$ M Mo(V), $1.60\times10^{-3}$ M Mo(VI), and $5.80\times10^{-5}$ M $NaH_2PO_4$ (Fig. 4). The result that the slope of the logarithm of the rate *vs.* pH plot is −5 indicates that the rate of the formation is inversely fifth order with respect to the perchloric-acid concentration in the range from 0.20 to 0.40 M.

*Effects of Molybdenum(VI) and Molybdenum(V) Concentrations on the Color Development and the Formation Rate of Phosphomolybdenum Blue.* The effect of molybdenum(VI) concentration on the formation of phosphomolybdenum blue was examined under given conditions. The results are shown in Fig. 5. Constant maximum color development was obtained in the range for molybdenum(VI) from $2.30\times10^{-3}$ to $6.40\times10^{-3}$ M with $1.76\times10^{-3}$ M Mo(V), $2.90\times10^{-5}$ M phosphate, and 0.28 M $HClO_4$. In the case of $1.00\times10^{-3}$ M Mo(V), any concentration of molybdenum(VI) greater than $1.50\times10^{-3}$ M results in constant maximum color

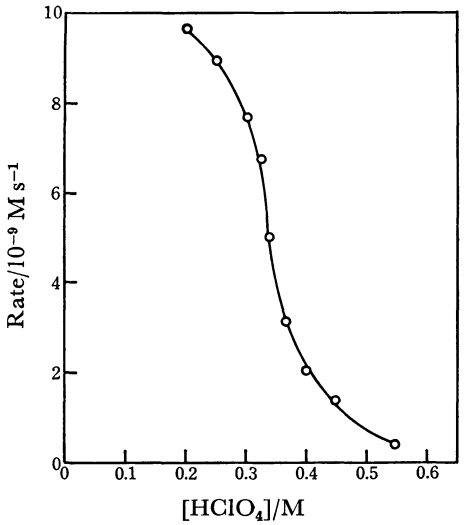


Fig. 4. The relation between the formation rate of phosphomolybdenum blue and the concentration of perchloric acid.
[Mo(VI)]=$1.60\times10^{-3}$ M, [Mo(V)]=$1.01\times10^{-3}$ M, [$NaH_2PO_4$]=$5.80\times10^{-5}$ M, $I$=0.70 M ($NaClO_4$), 25 °C.

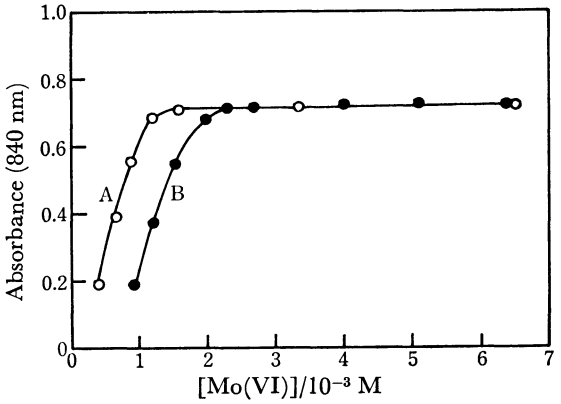


Fig. 5. The effect of the concentrations of molybdenum-(VI) on the color development.
[$NaH_2PO_4$]=$2.90\times10^{-5}$ M, [$HClO_4$]=0.28 M.
(A): [Mo(V)]=$1.00\times10^{-3}$ M, (B): [Mo(V)]=$1.76\times10^{-3}$ M.

development. These results indicate that the color development is significantly affected by the Mo(VI): Mo(V) ratio.

The effect of the molybdenum(VI) concentration on the formation rate of phosphomolybdenum blue was examined for 0.36 M $HClO_4$, $6.76\times10^{-4}$ M Mo(V), and $4.36\times10^{-5}$ M $NaH_2PO_4$. The results are shown in Fig. 6. At low concentrations of molybdenum(VI), the formation rate is greatly affected by the molybdenum(VI) concentration, but at high concentrations the effect is small.

The effect of molybdenum(V) concentration on the formation of phosphomolybdenum blue was also examined. The rate has a first-order dependence on the molybdenum(V) concentration in the range from $2.00\times10^{-4}$ to $1.76\times10^{-3}$ M for $2.40\times10^{-3}$ M Mo(VI), $5.80\times10^{-5}$ M $NaH_2PO_4$, and 0.28 M $HClO_4$.

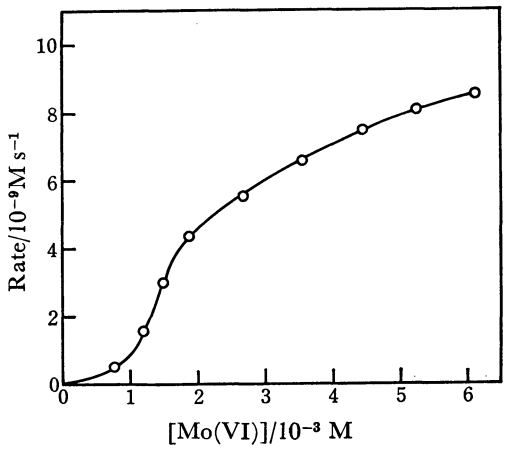


Fig. 6. The relation between the formation rate of phosphomolybdenum blue and the concentration of molybdenum (VI).
[Mo(V)]=$6.76\times10^{-4}$ M, [$NaH_2PO_4$]=$4.36\times10^{-5}$ M, [$HClO_4$]=0.36 M, $I$=0.70 M ($NaClO_4$), 25 °C.

*Determination of Phosphate Concentration.* The calibration curve was obtained by the general procedure for the determination of phosphate concentrations. The phosphomolybdenum blue color system obeys Beer's law in the phosphate range from 0.08 to 1.16 μg/ml. Based on the phosphate concentration, the molar absorptivity at 840 nm can be calculated to be $2.4\times10^4$ l $mol^{-1}$ $cm^{-1}$. The sensitivity is very high. Standard solutions prepared from $Na_2HPO_4$ and $NaH_2PO_4$ give the same result.

*Precision and Accuracy.* The accuracy of the results is represented in Table 1. An estimate of the

Table 1. Accuracy of the results

| Measured absorbance[a] (840 nm) | Amount of phosphate in 25 ml (μg) | | |
|---|---|---|---|
| | Taken | Found | Error (%) |
| 0.780 | 21.7 | 21.3 | −1.84 |
| 0.610 | 15.9 | 16.4 | +3.14 |
| 0.453 | 12.6 | 12.4 | −1.59 |
| 0.309 | 8.0 | 8.3 | +3.75 |
| 0.150 | 4.4 | 4.3 | −2.27 |

a) [Mo(VI)]=$3.20\times10^{-3}$ M, [Mo(V)]=$1.76\times10^{-3}$ M, [$HClO_4$]=0.28 M.

Table 2. Precision of the results

| 0.8 μg/ml phosphate Measured absorbance (840 nm)[a] | 0.4 μg/ml phosphate |
|---|---|
| 0.696 | 0.345 |
| 0.710 | 0.342 |
| 0.693 | 0.361 |
| 0.701 | 0.357 |
| 0.717 | 0.359 |
| 0.720 | 0.363 |
| Av. 0.706 | 0.355 |
| Std. dev. 0.011 | 0.0029 |

a) [Mo(VI)]=$3.30\times10^{-3}$ M, [Mo(V)]=$1.76\times10^{-3}$ M, [$HClO_4$]=0.28 M.

precision was obtained for the results of replicate samples at two different phosphate concentrations. These results are listed in Table 2.

*Effect of Various Ions.* The effect of many different ions was examined by the proposed procedure for the determination of 0.8 μg/ml of phosphate. The metal ions, such as $Mg^{2+}$, $Al^{3+}$, $Ca^{2+}$, $Cr^{3+}$, $Mn^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $UO_2^{2+}$, and $SiO_3^{2-}$ in moderate amounts (4 μg/ml) do not interfere with this determination.

*Determination of the P: Mo(V) Molar Ratio in Phosphomolybdenum Blue.* There are a few investigations of the formation mechanism and composition of phosphomolybdenum blue. Arnold and Wacker,[10] Baman *et al.*,[11] and Hahn and Schmidt[12] have investigated the composition of phosphomolybdenum blue formed by the reduction of 12-molybdophosphoric acid by reducing agents. Assuming that phosphomolybdenum blue contains the same number of molybdenum atoms as the unreduced 12-molybdophosphoric acid, they proposed that the Mo(VI): Mo(V) ratios in phosphomolybdenum blue are 2: 1 or 5: 1, depending on the type of reducing agents and reduction time.

Recently, Meiklejohn *et al.*[13] isolated $(Bu_4N)_4PMo^{V}Mo^{VI}_{11}O_{40}$, which was prepared by the interaction of $PMo_{11}O_{39}^{7-}$ and $Bu_4N[MoOCl_4]$ in acetonitrile or propylenecarbonate, where $Bu_4N$ represents the tetrabutyl ammonium ion.

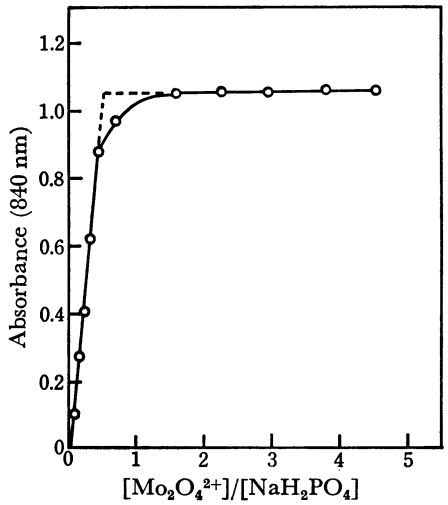


Fig. 7. The determination of molar ratio of P: Mo(V) in phosphomolybdenum blue.
$[Mo(VI)]=2.40\times10^{-3}$M, $[NaH_2PO_4]=4.36\times10^{-5}$M, $[HClO_4]=0.28$ M,

In this work, the P: Mo(V) molar ratio in phosphomolybdenum blue, which was formed by the reaction of molybdenum(V) with molybdenum(VI), was determined by means of the molar-ratio method, under the condition of the presence of amounts of molybdenum(V) small with respect to that of molybdenum(VI). The results are shown in Fig. 7. Since the formation rate of phosphomolybdenum blue is markedly small for these experimental conditions, it requires at least 300 min at 80 °C for the color to completely develop. The results shown in Fig. 7 illustrate that the P: Mo(V) molar ratio (as a monomer) is 1: 1 for 0.28 M $HClO_4$. The same result was obtained with 0.36 M $HClO_4$. A determination of the P: Mo(VI) molar ratio based on the molar-ratio method was unsuccessful.

The formation mechanism of phosphomolybdenum blue determined from this work has not yet been ascertained. The present authors are planning a detailed kinetic treatment of the formation of phosphomolybdenum blue.

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