Characterization of α- and β-Dodecamolybdophosphate Complexes Formed in Aqueous Solutions Including Acetonitrile, Acetone, or Dioxane

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On the basis of a spontaneous conversion reaction of molybdophosphate (formed in an aqueous medium including acetonitrile, acetone, or dioxane), the existence of β-modification of dodecamolybdophosphate has been substantiated. The conversion reaction was highly affected by the presence of the organic solvents. In the absence and/or the presence of the organic solvent of less than 20% by volume, the reaction proceeded instantaneously and, in consequence, β-modification practically did not exist, but α-modification existed entirely. Whereas, in the presence of the organic solvent ranging from 30 to 60%, β-modification was so stable, it could be observed for a fairly long duration. However, it was still thermodynamically unstable and finally converted to α-modification at a certain rate depending on the concentration of the organic solvent. With more of the organic solvent than 65%, the formation of β-modification and thence α-modification, were prohibited. As a result of these facts, α- and β-modifications were individually prepared in solutions consisting of 0.05 M Mo(VI)/1.2 M HCl/40% acetonitrile. On each modification thus formed, a chemical reduction (with such metals as nickel, iron, or lead) and solvent-extraction isolation (using chloroform as an auxiliary solvent) were performed. The inherent absorption spectra of the two modifications were clearly distinguished in both oxidized yellow and reduced blue forms. The utility of β-modification in a sensitive spectrophotometric determination of phosphate ion was also discussed.

It has been well known that the yellow color of molybdophosphate in aqueous solutions is markedly enhanced by the presence of certain water-miscible organic solvents such as acetone,[1,2,3] dioxane,[4] ethanol,[5] tetrahydrofuran,[6] and acetonitrile.[7] The enhanced yellow color has been widely utilized for spectrophotometric determinations of the orthophosphate ion.

Chalmers and Sinclair investigated[2] in detail the color enhancement in an aqueous acetone medium and ascribed it to the formation of β-modification of dodecamolybdophosphate (abbreviated as β-molybdophosphate, hereafter). This modification might exhibit higher absorbances in the near-ultraviolet region than the ordinary α-modification of dodecamolybdophosphate (abbreviated α-molybdophosphate). They explained that β-molybdophosphate might be stabilized to exist in an aqueous acetone medium since acetone molecules preferably replace with water molecules in the coordination sphere of the molybdate complex and suppress the hydrolytic dissociation of the complex (which dissociation would be the initial step for the spontaneous conversion of β to α). On the basis of these concepts, Chalmers and Sinclair[2] postulated a possible structure for β-molybdophosphate. The similar structure was found, in fact, by Matsumoto *et al.*[8] in β-modification of dodecatungstosilicate. Nevertheless, as for molybdophosphate, the spontaneous conversion reaction itself, which must be the most inherent property to β-molybdophosphate and is most likely to occur from the analogy with the β to α conversion in molybdosilicate,[9] has not been substantiated yet. It is only known that the two modifications of α and β would also exist in molybdophosphate and would be distinguishable from their different intensities in the yellow color.

Employing acetonitrile as an organic solvent, we[10] previously investigated the color enhancement of molybdophosphate as a function of the concentration of the solvent. As a result, it was found that the solvent had two different effects on the yellow color.

The first effect was observable at acetonitrile concentrations of around 5% by volume. It became evident with an increase of the solvent up to 20%, and finally saturated with more of the solvent. The most notable point regarding this effect was that it was a reversible reaction, that is, the yellow color of molybdophosphate was intensified up to the same extent in either case when the organic solvent was present before or was added after the formation of the molybdate complex. This effect might be attributable to the reversible formation equilibrium of α-molybdophosphate, which is favoured by the presence of the organic solvent.

The second effect of the organic solvent was only appreciated when molybdophosphate was formed in the presence of acetonitrile more than 30%. Most strikingly, it involved the irreversible reaction. Actually, the yellow color had an intensity approximately twice as strong as that due to the first effect, but faded gradually on standing and reached the same intensity as that attained by the first effect. This extraordinary intense yellow color was then estimated to be due to β-molybdophosphate and the fading of the yellow color was due to the conversion of β to α-molybdophosphate.

According to these previous observations,[10] we report in the present paper on preparation of individual α- and β-molybdophosphate using an aqueous medium containing acetonitrile, acetone, or dioxane. Specifically, regarding β-molybdophosphate, its inherent properties were carefully investigated and distinguished from those of the well-established α-molybdophosphate. The so-called solvent effects on the color development of molybdophosphate can be thoroughly explained in terms of the charactersistic conversion reaction between α and β. Some confusions which have been hitherto experienced regarding the different intensities of the yellow color of molybdophosphate, are considered consistent.

Experimental

Reagents. A standard 0.100 M (1 M=1 mol dm^{-3}) phosphate solution and 0.5 M molybdate(VI) solution were prepared from KH_2PO_4 and $Na_2MoO_4 \cdot 2H_2O$, respectively.

A mixed molybdate reagent, with which phosphate ion was reacted to form molybdophosphate, was prepared by mixing 20 cm^3 of a 0.5 M molybdate(VI) solution, 20 cm^3 of concentrated hydrochloric acid and the desired amounts (usually 80 cm^3) of acetonitrile, and diluting to 200 cm^3 with water. The acetonitrile (Wako Pure Chemicals, GR grade) was used without further purification. The mixed reagent, otherwise indicated, was a 40%(v/v) acetonitrile solution. It was stored in a polyethylene bottle without appreciable deterioration for at least one month. Also, a mixed molybdate reagent in acetone or dioxane was similarly prepared using acetone and dioxane (Wako Pure Chemicals, GR grade) in place of acetonitrile.

Nickel metal with 99.95% purity (in a sponge type and pulverized to a size to pass through a 300-mesh filter) was used for the reduction of molybdophosphate. The metal could be used as supplied (Wako Pure Chemicals), but, if necessary, further purified by washing with 40% aqueous acetonitrile including 1.2 M hydrochloric acid. It was subsequently washed with 40% aqueous acetonitrile and dried in the open air for 2 d. Iron with 99.99% purity (in a sponge type and pulverized to pass through a 100-mesh filter), cobalt with 99% purity (in a powder form), copper with 99.85% purity (in a powder form), and lead with 95% purity (in a powder form) were also commercially available products and were used in the comparative studies with the nickel reduction. These metals were usually weighed into a 60-mg dose and wrapped in glazed paper.

As an auxiliary solvent, chloroform was used after the removal of a stabilizer ethanol, just before use, by washing three times with water and drying over anhydrous sodium sulfate.

Apparatus. A Shimadzu UV 240 automatic recording spectrophotometer equipped with a constant-temperature housing (kept at 25±0.2 °C) for the cuvettes, was used. Quartz cuvettes with path-lengths of 1 (with a glass-stopper), 0.2, and 0.1 cm were properly used according to the magnitude of the absorbances of the samples.

Results and Discussion

Formation and Conversion of β-Molybdophosphate in Aqueous Media Including Acetonitrile, Acetone, or Dioxane. At various concentrations of acetonitrile in the reaction medium, the formation of molybdophosphate was monitored by measuring the changes in absorbance at 420 nm according to the following techniques.

Into a quartz cuvette having a path-length of 1 cm, take a 4-cm^3 aliquot of mixed molybdate reagent composed of 0.05 M Mo(VI)/1.2 M HCl/ acetonitrile ranging from nil to 70% (v/v), and equilibrate it at 25 °C. Into the reagent solution, inject 40 mm^3 of 0.1 M phosphate standard solution using a calibrated micropipette. Shake it quickly. Then, set the reaction time to zero and record the absorbance change with regard to time.

The typical absorbance-time curve obtained in the 40% acetonitrile medium is shown in Fig. 1. It can be seen from the curve that the absorbance increases rapidly and reaches a higher stational level (1.600 in absorbance unit) within 2 s, and then decreases quite slowly, until it goes down to a lower constant level (0.970 in abs. unit). The higher absorbances temporarily observed at the initial period seem to be attributable to the formation of an extraordinary molybdophosphate other than the ordinary α-molybdophosphate. The extraordinary one was expected to be β-molybdophosphate. In a later paragraph, this will be further confirmed by discussion involving both the extraction isolation and the chemical reduction of the complex.

The final absorbance, on the other hand, was due to α-molybdophosphate. This was confirmed from the fact that the same absorbance could be attained with a solution which was prepared by dissolving into 40% aqueous acetonitrile a corresponding amount of synthesized crystal of molybdophosphate, *i.e.*, $24MoO_3 \cdot P_2O_5 \cdot 3H_2O$,[11] which had been well-

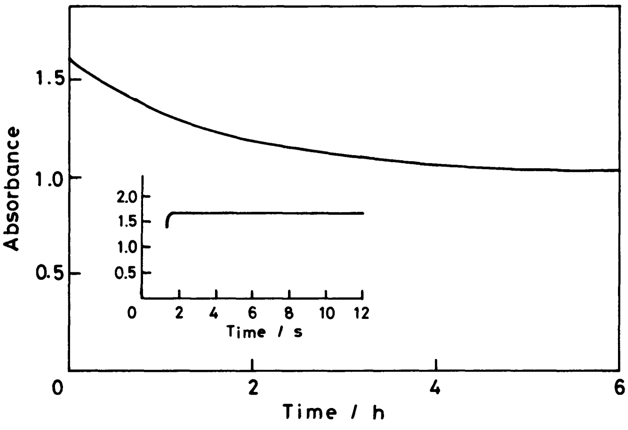

Fig. 1. Formation and conversion of β-molybdophosphate in the 40% aqueous acetonitrile medium at 25 °C.
A 40 mm^3 of 0.1 M phosphate standard solution was injected into a 4 cm^3 aliquot of the mixed molybdate reagent consisting of 0.05 M Mo(VI)/1.2 M HCl/40 % acetonitrile, and the absorbance change at 420 nm of the mixture was recorded against time.

established as Keggin structure.[12]

In consequence, it can be said that the decreasing absorbance recorded in Fig. 1 corresponds to the spontaneous conversion of β to α-molybdophosphate.

By the kinetic analysis for the absorbance-time curve, it was assured that the conversion proceeded according to first-order reaction kinetics with respect to the concentration of β-molybdophosphate, and the rate constant in the 40% acetonitrile medium was observed as $1.79\times10^{-4}\ \mathrm{s}^{-1}$.[†]

Although the formation and the conversion of β-molybdophosphate were similarly observed in an aqueous medium including more than 20% of acetonitrile, the reaction rate depended on the acetonitrile concentration. This dependence is featured in Fig. 2. As shown in the right-hand part of curve a in the Figure, extraordinarily higher absorbances were recorded at an early period around 2 min, indicating the formation of β-molybdophosphate. Upon standing, however, these absorbances decreased gradually through curves b—e and finally reached curve f (which was the ordinary absorbance level attributable to the formation of α-molybdophosphate).

In a 60% acetonitrile medium, for instance, it can be seen from the Figure that β-molybdophosphate takes more than 30 h to convert completely to α-molybdophosphate.

With the presence of more acetonitrile than 65% in the medium, the formation of β-molybdophosphate, thence that of α-molybdophosphate as well, was fairly prohibited. The reason, however, has not been explained yet.

In the absence and/or the presence of acetonitrile less than 20% (see the left-hand part of Fig. 2), the absorbances of molybdophosphate solutions increased rapidly and reached directly to those indicated by curve a, neither the extraordinarily higher absorbances nor the gradual decrease of the absorbance being

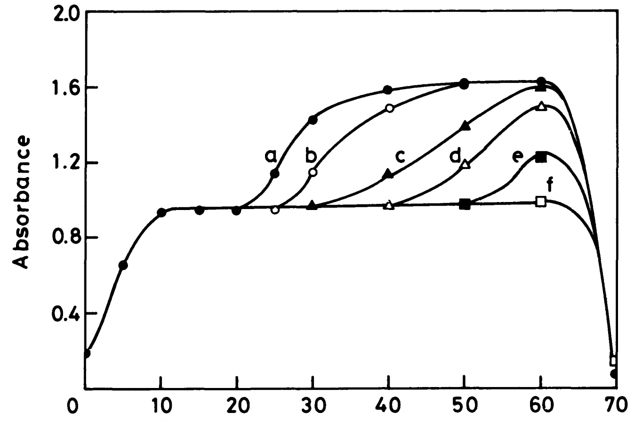

Fig. 2. Profile for the formation and survival of β-molybdophosphate in aquoeus acetonitirle media at the time: (a) 2 min, (b) 20 min, (c) 2 h, (d) 5 h, (e) 14 h, and (f) 38 h, elasped since the injection was made of a 40 mm^3 of 0.1 M phosphate solution into a 4 cm^3 of the mixed molybdate reagent consisting of 0.05 M Mo(VI)/1.2 M HCl/varing concentrations of acetonitrile.

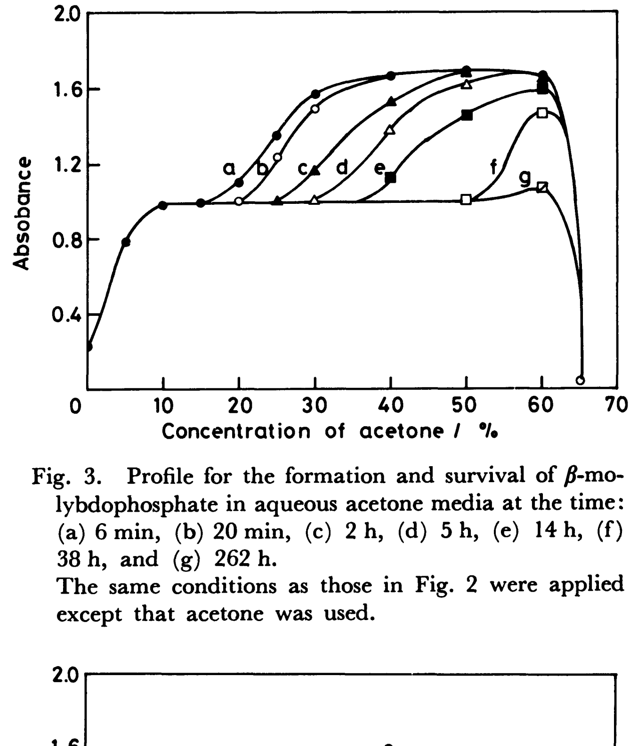

Fig. 3. Profile for the formation and survival of β-molybdophosphate in aqueous acetone media at the time: (a) 6 min, (b) 20 min, (c) 2 h, (d) 5 h, (e) 14 h, (f) 38 h, and (g) 262 h.

The same conditions as those in Fig. 2 were applied except that acetone was used.

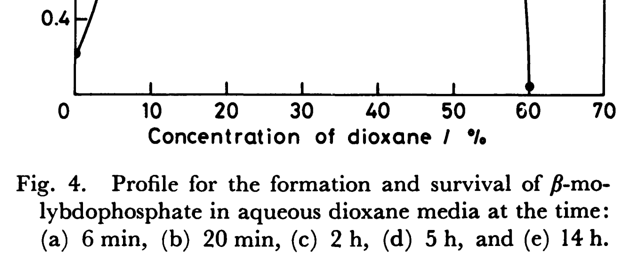

Fig. 4. Profile for the formation and survival of β-molybdophosphate in aqueous dioxane media at the time: (a) 6 min, (b) 20 min, (c) 2 h, (d) 5 h, and (e) 14 h.

[†] Let $C_{\alpha,t}$ and $C_{\beta,t}$ be the respective concentrations of α and β-molybdophosphate at time t during the conversion. The values of $C_{\alpha,t}$ (or those of $C_{\beta,t}$) were calculated by solving the following equations: $E_t=\varepsilon_\alpha\cdot C_{\alpha,t}+\varepsilon_\beta\cdot C_{\beta,t}$ and $C=C_{\alpha,t}+C_{\beta,t}$, where ε_α and ε_β denote the molar absorption coefficients of α- and β-molybdophosphate at 420 nm (being calculated from the final and the initial absorbances in Fig. 1 as 1.60×10^3 and 9.70×10^2 $\mathrm{l\ mol^{-1}\ cm^{-1}}$, respectively), C is the total concentration of molybdophosphate and is known as 1×10^{-3} M in this experiment. The plot of log $C_{\beta,t}$ against time gave a straight line and the first-order rate constant for the conversion reaction was evaluated from the slope of the line.

observed at all. Only at elevated temperatures above 30 °C, extraordinary absorbances could be observed,[7] although they decreased to the ordinary values within several seconds. In consequence, it can be said that at these lower acetonitrile concentrations, β-molybdophosphate scarecely exists because of a rapid conversion to α-molybdophosphate.

β-Molybdophosphate can similarly be stabilized by virtue of acetone or dioxane in the reaction medium. The formation and the conversion of the complex in these media are illustrated in Figs. 3 and 4. By comparing Figs. 2—4, it is shown that any of the organic solvents employed here have essentially the same effect on the formation of β-molybdophosphate but the largest effect is attained by acetone, the complex being stabilized to exist even after 5 h in 60% acetone medium (curve d in Fig. 3, for example).

Preparation of α- and β-Molybdophosphate. As the most convenient method for preparing α-and β-molybdophosphate individually, the following procedures and conditions are recommended.

To obtain a 10-cm^3 solution of either α- or β-molybdophosphate add a desired amount (should be less than 200 mm^3, however) of 0.1 M phosphate standard solution into a 10-cm^3 aliquot of the mixed molybdate reagent composed of 0.05 M Mo(VI)/1.2 M HCl/40% acetonitrile (being equilibrated at 25 °C) and shake the mixture well.. Thus, β-molybdophosphate will be formed instantaneously and will survive almost entirely through the following 20 min (during which, the degree of the conversion can be practically neglected). To obtain α-molybdophosphate, stand the same solution for more than 6 h. It is quite possible, of course, to prepare the complexes in an aqueous acetone medium. But, note that when acetone is used instead of acetonitrile β-molybdophosphate takes several minutes to form and takes a few days to convert to α completely. Also note, when dioxane is used, the complex forms in 2 min and converts to the other complex in *ca.* 6 h.

From the foregoing examinations, it is obvious that β-molybdophosphate should be prepared freshly for every use and exercised out within 20 min after preparation. However, α-molybdophosphate is stable enough to be stored for at least one month.

Chemical Reduction of α- and β-Molybdophosphate to the Respective Blue Species. In order to obtain a pure blue species of β-molybdophosphate by retaining a critical concentration of acetonitrile in the medium, several kinds of metals were tested as the dry reductants.

For the test, a series of 10-cm^3 solutions of 5×10^{-5} M β-molybdophosphate were prepared in the 40% acetonitrile medium and shaken vigorously with a 60-mg dose of each metal such as nickel, iron, cobalt, lead, and copper. The formation of the blue species was monitored by measuring the absorbance at 788 nm. The results obtained with nickel are represented in Fig. 5A as a function of the shaking time with the metal and the standing time thenafter. As indicated by curve a in the Figure, when the reduction was initiated by shaking for several seconds, the solution slowly turned to blue and the absorbance reached a constant value on standing. Curves b and c show that the longer the shaking time is prolonged, the more rapidly the reduction proceeds. It is also shown by d and e, however, that the longer-shaking exceeding over 1 min is not profitable for the reduction, since other unstable blue species are produced (which give more deeply blue color but decay slowly on standing). The absorption spectrum of the most stable blue species of β-molybdophosphate is shown in Fig. 6 as curve a.

Quite similarly, α-molybdophosphate was reduced

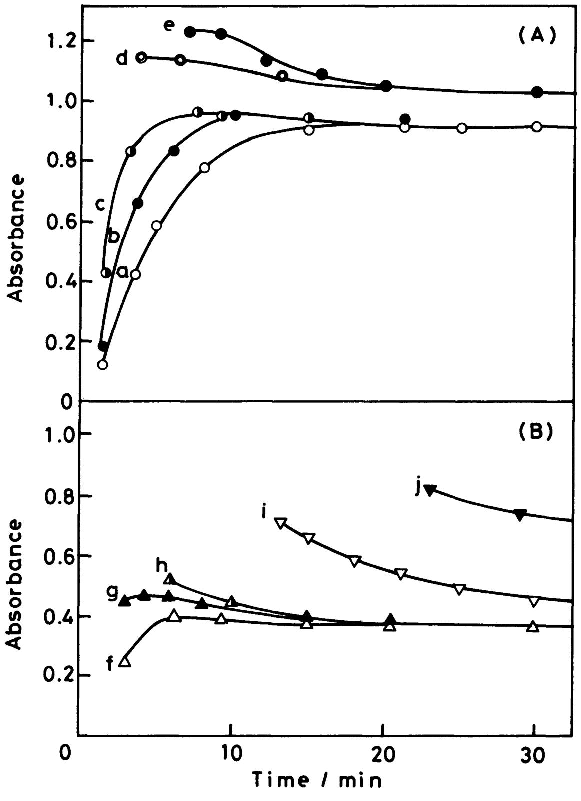

Fig. 5. The shaking time with nickel reductant and the standing time needed for the reduction of α- or/and β-molybdophosphate to the respective blue species. A 10 cm^3 of 5×10^{-5} M solution of either α- or/and β-molybdophosphate prepared in the 40 % aqueous acetonitrile medium, was shaken with a 60 mg dose of nickel. For β (in the upper part of the Figure), the shaking time was varied as: (a) 6 s, (b) 10 s, (c) 30 s, (d) 1 min, and (e) 15 min and the formation of the blue species was monitored as a function of the standing time thenafter. For α (in the lower part of the Figure), the shaking time was as: (f) 30 s, (g) 1 min, (h) 4 min, (i) 10 min, and (j) 20 min.

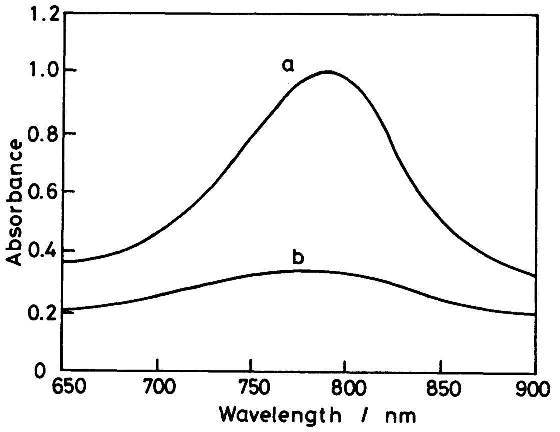

Fig. 6. Absorption spectra of the blue species of α- or/and β-molybdophosphate in the 40 % aqueous acetonitrile medium.
Curve a is for 5×10^{-5} M β-molybdophosphate, reduced with a 60 mg dose of nickel by shaking for several seconds and standing it for 15 min, and curve b for 5×10^{-5} M α-molybdophosphate reduced by shaking for 30 s and standing for 15 min.

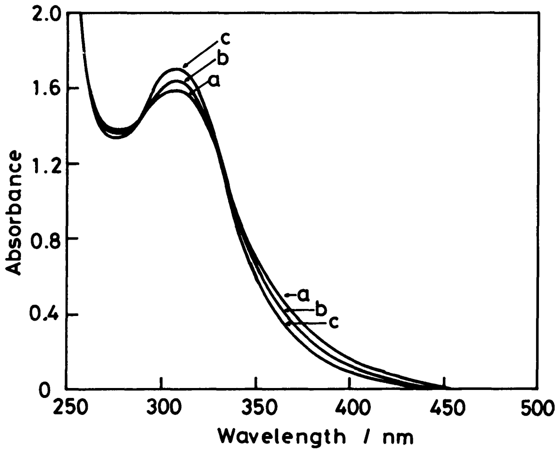

Fig. 7. Absorption spectra inherent to the respective α- or/and β-molybdophosphate.
Curve a is for β extracted at $t=10$ min and curve c is for α at $t=8$ h, during the conversion of β to α(see the text for the detailed extraction procedure). Thence, curve b for the mixture of α and β extracted at $t=1$ h. The initial concentration of the complexes in the 40 % aqueous acetonitrile medium, 4.5×10^{-5} M, was finally concentrated up to 7.5×10^{-5} M through this extraction.

to the corresponding blue species by using nickel. The shaking time and the standing time required for the reduction are shown in Fig. 5B. As shown by curve f, one of the most stable blue species was attained by shaking for 30 s and standing for 15 min. The absorption spectrum is given in Fig. 6 as curve b and compared with that of the reduced β-molybdophosphate (curve a). α- and β-molybdophosphate can be characterized even in their reduced forms; the former showing an absorption maximum at 770 nm with $\varepsilon=(6.43\pm0.15)\times10^3$ and the latter at 788 nm with $\varepsilon=(1.99\pm0.06)\times10^4$ l mol^{-1} cm^{-1}, respectively.

Similar to nickel, iron and lead also reduced the complexes although, the reduction with these metals proceeded rather faster than with nickel. Moreover, with these metals it was seen by visual observation that the solutions of the molybdophosphate complexes turned to a greenish blue. This was solely because the background color (reddish yellow) due to the reduction of the reagent molybdate covered the blue color of the reduced complexes. In fact, in the absorption spectrum being measured using a spectrophotometer, the inherent absorption maxima of the blue species were clearly revealed since the background color only occurred in a spectral region shorter than 500 nm.

Copper was less reactive compared with the three metals mentioned above and the reduction remained incomplete. Cobalt was even less reactive, the molybdophosphate complexes being slightly blue.

Extraction Isolation of α- and β-Molybdophosphate. For either α- or/and β-molybdophosphate formed in the 40% acetonitrile medium, an addition of the proper amount of chloroform was quite effective to extract the complex. Such an extra addition of chloroform served to extract a sufficient amount of acetonitrile from the medium into an organic phase, to which molybdophosphate was quantitatively extracted, the reagent molybdate being left in the aqueous phase. As to our examination performed here, chloroform was one of the unique solvents and the most excellent for such extraction isolation.

In practice, at appropriate intervals during the conversion of β to α, 3.0-cm^3 of chloroform was added to a 10-cm^3 solution of 4.5×10^{-5} M molybdophosphate, and either α- or/and β-molybdophosphate or their mixture was extracted. At every extraction an approximate 6 cm^3 of organic phase was recovered at the lower layer. The absorption spectra of these organic extracts were measured as superimposed in Fig. 7: Curve a was for β-molybdophosphate (which was attained by the extraction at $t=10$ min), curve b for the mixture of α and β (at $t=1$ h), and curve c for α (at $t=8$ h). According to the conversion reaction, the absorption spectrum changed from that of β to that of α, keeping isosbestic points at around 285 nm and 335 nm.

In paralell to these spectral measurements, the organic extracts were analyzed with respect to the contents of molybdenum and phosphorus, and the Mo/P ratios were determined. As the result, it was confirmed that, irrespective of the differences among the absorption spectra of a, b, and c in Fig. 7, the Mo/P ratios in the organic extracts remained unchanged and equally fell into a value of 12.1 ± 0.3. That is, α- and β-molybdophosphate are quite identical in their compositions but possibly different in their structures. The detailed structure of β-molybdophosphate has not been analyzed yet because of the difficulty in crystallization, but it would be successfully deduced by Chalmers[2] from the Keggin structure.

It was also possible to extract the blue species of α- and β-molybdophosphate by the similar addition of chloroform. It is recommended to add 0.3 cm^3 of the solvent into a 10-cm^3 solution where the molybdophosphate complex is in a reduced form. Usually, an approximate 1.56 cm^3 of the organic phase is recovered at the upper layer, and the reduced complexes are effectively concentrated into the phase.[††] Although the blue color was highly increased in the organic phase, the characteristic absorption maxima of α- and β-molybdophosphate were retained unchanged even after the extraction.

Extraction isolation of the molybdophosphate complexes was unsuccessful in aqueous acetone and aqueous dioxane media because suitable solvents were not yet found for the phase separations of the mixed solvents.

Analytical Consideration. A variety of sensitive methods for phosphate determination have been reported by using water-miscible organic solvents such as acetone. Among these methods, however, the recommended procedures or the optimum conditions are widely different or even contradictory to each other. Now, it can be obviously said that in these methods two different effects by the organic solvent are involved, and either one of the effects has been overlooked. As confirmed in this paper, one of the solvent effect is due to the stabilization of α-molybdophosphate, where the organic solvent serves to shift the *reversible* formation equilibrium of the complex to the completion, and the other is due to the kinetic stabilization of β-molybdophosphate, where the *irreversible* conversion of the complex is retarded by the solvent.

For the same reason as above, one could not obtain β-molybdophosphate even by treating with organic solvents the crystallized molybdophosphate, *e.g.*, $24MoO_3 \cdot P_2O_5 \cdot 3H_2O$, or by extracting molybdophosphate from its aqueous solutions into various organic solvents. In either case, the conversion of β to α had already been completed. In consequence, an aqueous medium (including certain organic solvents) is indispensably needed for preparing β-molybdophosphate.

It was made evident that β-molybdophosphate shows higher absorbances than α-molybdophosphate in both forms of the reduced blue (Fig. 6) and the oxidized yellow (Fig. 7). This spectroscopic nature is capable of more sensitive determination of phosphate ion by utilizing the formation of β-molybdophosphate rather than that of α-molybdophosphate, especially in its reduced blue form. In addition to this, the extraction of the blue complex may further sensitize the phosphate determination by concentrating it into a small volume of the organic phase by the aid of auxiliary solvent of chloroform.

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[††] At this extraction using a relatively small amount of chloroform (0.3 cm^3), approximately 1.4% of reagent molybdate is extracted along with the molybdophosphate complexes. However, the reagent molybdate is colorless and causes no practical interference with the spectral measurements in the visible region of the reduced blue complexes.