Effects of acidity and molybdate concentration on the kinetics of the formation of the phosphoantimonylmolybdenum blue complex

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SUMMARY

The effects of variation of acidity and molybdate concentration on the spectrophotometric determination of phosphate by the method of Murphy and Riley were studied. The $[H^+]$: [Mo] ratio was found to be a crucial parameter which not only influences the form of the final reduced complex, but also plays a key role in controlling the reaction kinetics. Normal colour formation of the reduced phosphoantimonylmolybdenum blue complex was only observed with a ratio between 60 and 80; the reaction was faster at low acidities. Silicate is a possible source of interference at high temperatures, but the formation of the silicoantimonylmolybdenum blue complex was found to be much slower than that of the corresponding phosphorus species.

The most frequently used method for the determination of low concentrations of phosphate is that developed by Murphy and Riley [1], which is based on the formation of a phosphoantimonylmolybdenum blue species using ascorbic acid as reductant. The reaction is complicated and probably initially involves the formation of a mixture of two isomeric heteropoly acids (the α - and β -forms) in proportions which depend on the acidity in the final solution. For this reason, the effects on the reaction rate of changes in the hydrogen ion and molybdate concentrations have been studied by several investigators.

In the original Murphy and Riley procedure, the H^+ and molybdenum concentrations in the final solution were 0.4 M and 5.438 mM, respectively, corresponding to an $[H^+]$: [Mo] ratio of ca. 74. Although the reagent concentrations recommended by other workers [2–5] vary considerably,

the final [H⁺]: [Mo] ratios used are similar. Indeed, those employed in all recent oceanographic publications are within the range 70-78 (see Fig. 1). Going and Eisenreich [6] studied the influence of the [H⁺]: [Mo] ratio on the absorbance of the phosphoantimonylmolybdenum blue species. They found that the optimum range for the ratio lies between 60 and 80. Above this level, colour formation is incomplete, whereas below it the molybdate ion undergoes self-reduction even in the absence of phosphate. Unfortunately, they did not study the effect of variations in the $[H^+]$: [Mo] ratio on the reaction rate. A knowledge of the kinetics of the reaction is of particular value when adapting the process to flow-injection analysis (FIA) for phosphate since the time spent by the sample in the manifold is so short, and heat is frequently applied to enhance the rate of colour development. Although Janse et al. [7] optimized



Fig 1 H⁺ vs Mo concentration matrix diagram in which final acidities and concentrations of molybdate are presented as percentages of those used in the Murphy and Riley method [1] Solid lines indicate the [H⁺] [Mo] ratio contours, closed circles show the concentration ratios of several published procedures MR, Murphy and Riley [1], [H⁺]: [Mo] = 74, APHA, American Public Health Association [5], [H] \cdot [Mo] = 73, SP, Strickland and Parsons [2], [H] [Mo] = 71, K, Koroleff [4], [H] [Mo] = 76

the FIA conditions for phosphate, they did not study the effect of changes in the $[H^+]$: [Mo] ratio on the response. As a preliminary to the development of such a procedure, the effects of variation of the $[H^+]$: [Mo] ratio on the kinetics of the spectrophotometric determination were examined using a matrix scheme at various temperatures. The possibility of interference from silicate at elevated temperatures was also studied in a similar fashion.

EXPERIMENTAL

All standards and reagents were prepared in deionized, distilled water. Chemicals were of analytical-reagent grade.

Standards

A stock standard solution of phosphate (2.5 \times 10⁻³ mol P 1⁻¹) was prepared by dissolving 0.3402

g of potassium dihydrogenphosphate in and diluting to 1 l with water. A phosphate working standard solution $(1.25 \times 10^{-4} \text{ mol P l}^{-1})$ was prepared fresh daily by diluting a 25-ml aliquot of the the stock standard solution to 500 ml with water.

A silicate stock standard solution (1000 mg Si 1^{-1}) was prepared by diluting the contents of a tube of Merck Titrisol silica standard to 1 l with water. A working standard solution $(5.0 \times 10^{-3} \text{ mol Si } 1^{-1})$ was prepared by diluting 70 ml of the stock standard solution to 500 ml with water.

Reagents

Concentrated sulphuric acid (95-97%, w/w) (112 ml) was diluted to 2 l with water. The exact acidity of the solution was determined by titration with standard alkali solution.

Ammonium molybdate reagent was prepared by dissolving ammonium molybdate tetrahydrate (4.8 g) and diluting to 1 l with water in a plastic beaker.

Antimony reagent was prepared by dissolving antimony potassium tartrate (0.5486 g) in 1 l of water.

Ascorbic acid solution (0.12 M) was prepared by dissolving DL-ascorbic acid (10.56 g) and diluting to 500 ml with water. The solution was stable for several days in a refrigerator.

Apparatus

A Hitachi Model 100-60 double-beam spectrophotometer fitted with 1-cm cuvettes was used for absorbance measurements.

Measurements of the kinetics of formation of the molybdenum blue complexes were made in a magnetically stirred glass reaction cell mounted in a thermostatically controlled bath. The lid of the cell was fitted with a funnel for the introduction of ascorbic acid. Absorbance was measured using a Brinkman PC-800 dipping probe colorimeter equipped with a plastic probe giving a light path of 1 cm, and with an optical filter having its maximum transmission at ca. 880 nm.

Investigation of effect of variation of $[H^+]$: [Mo] ratio

The measurement matrix was set up by varying the relative proportions of sulphuric acid and molybdate reagents around those used in the Murphy and Riley method [1]. Aliquots of x ml (up to 5 ml) of sulphuric acid were pipetted into a series of dry 30-ml tubes. To these were added sequentially, with mixing, aliquots of y ml of the molybdate reagent (up to 5 ml), 1 ml of antimony reagent, (23 - x - y) ml of water, 1 ml of working standard phosphate $(1.25 \times 10^{-4} \text{ mol } P \text{ } l^{-1})$ or silicate $(5.0 \times 10^{-3} \text{ mol Si } 1^{-1})$ solution and 1 ml of ascorbic acid solution, giving a total volume of 25 ml. The tubes were then placed in a thermostatically controlled bath at the desired temperature for 30 min. After cooling, the absorbances of the solutions were measured at 880 nm in a 1-cm cuvette. Blanks were run in the same manner but substituting 1 ml of water for the 1 ml of standard phosphate or silicate solution.

For convenience, the final hydrogen and molybdate concentrations are shown in the figures as percentages of those used in the Murphy and Riley method.

Kinetic measurements

To the reaction vessel were transferred the appropriate volumes of sulphuric acid (x ml) and

molybdate reagent (y ml), together with antimony reagent (1 ml), water (23 - x - y ml) and 1 ml of working standard phosphate or silicate solution. The cell was closed and placed into the constanttemperature bath at the desired temperature. Sturring was commenced and when the temperature had stabilized the colorimeter and its associated recorder were set to zero. Ascorbic acid (1 ml) was pipetted into the cell through the funnel and the recorder was switched on immediately. The absorbance was monitored for the desired period.

RESULTS AND DISCUSSION

Self-reduction of reagent blank

As a preliminary, a matrix of tests was carried out in the absence of phosphate and silicate to determine the $[H^+]$: [Mo] range under which selfreduction of molybdate ion occurs. This process was found to be slow and the reaction was therefore allowed to proceed for 0.5 h at 20 or 70 °C. It was found (Fig. 2) that under these conditions significant absorbance ($\lambda_{max} = 790$ nm) developed when the $[H^+]$: [Mo] ratio was low (< 60). When



Fig 2 Absorbance ($\times 10^3$) of the reagent blank at various H⁺ and Mo concentrations Measurements were made at 880 nm in 1-cm cuvettes, (a) at 20 °C for 30 min and (b) at 70 °C for 30 min



Fig. 3 Absorbance ($\times 10^3$) of a 5 μ mol P 1⁻¹ phosphate solution at various H⁺ and Mo concentrations. Measurements were made at 880 nm in 1-cm cuvettes. Zone A, self-reduction occurs; zone B, complete reaction, zone C, incomplete reaction, zone D, no reaction detected. (a) At 20 °C for 30 min; (b) at 70 °C for 30 min.

the ratio was very low, precipitation of molybdic acid tended to occur.

 $([H^+]:[Mo] \text{ ratio } \geq 200)$ negligible molybdenum blue formation occurred.

Optimization of $[H^+]$: [Mo] ratio for phosphate determination

A matrix of determinations at various [H⁺]: [Mo] ratios was carried out in the same fashion as for the blank, but in the presence of $0.125 \,\mu$ mol P per 25 ml final volume. The matrices obtained at both 20 and 70°C can both be divided into four zones (Fig. 3). In zone A colour formation was complete, but self-reduction of the molybdate 10n had also occurred, causing the absorbance to be high. In zone B no self-reduction had occurred but the formation of molybdenum blue from phosphate was complete, as evidenced by absorbance readings of 0.112 ± 0.001 , which corresponds closely with the accepted molar absorptivity of $22\,700\,1\,\text{mol}^{-1}\,\text{cm}^{-1}$. This zone corresponds approximately with that suggested for the optimum ratio by Going and Eisenreich [6], but the exact limits cannot be specified without taking reaction kinetics into account. In zone C the reaction was very slow and incomplete and in zone D

Interference from sulcate

Silicate causes negligible interference in the determination of phosphate at room temperature as the formation of silicomolybdenum blue is inhibited by the high $[H^+]$: [Mo] ratio when this is used. Thus, Koroleff [4] found that a 200 μ mol Si 1^{-1} silicate solution gave an absorbance of only 0.003 at 880 nm in a 10-cm cuvette after 30 min. It seemed possible that silicate interference might be significant when colour development is carried out at higher temperatures in flow-injection analysis. Using 5 µmol Si (per 25 ml final volume) a series of measurements were made to observe the colour development of silicomolybdenum blue at 70°C for both 0.5 and 2.5 h. The absorbances found (1-cm cell) are shown in Fig. 4. The matrix indicates that significant silicate interference occurs over the whole of the area of the matrix corresponding to the optimum range for the determination for phosphate (i.e. zone B in Fig. 3). For example, if the determination is carried out at



Fig 4 Absorbance ($\times 10^3$) of a 200 μ mol Si 1^{-1} silicate solution using the procedure for phosphate determination (a) At 70 °C for 30 min, (b) at 70 °C for 2.5 h.

70 °C with the same silicate concentration and the ratio employed by Murphy and Riley, absorbances of 0.005, 0.009 and 0.055 would be found after 3, 5 and 30 min, respectively. This shows that moderate concentrations of silicate can cause significant interference in the determination of phosphate.



Fig 5 Colour formation reaction of a 5 μ mol P l⁻¹ standard phosphate solution monitored by the dipping probe colorimeter Both [H⁺] and [Mo] were 100% (a) 70 °C, (b) 55 °C; (c) 35 °C, (d) 20 °C, (e) 200 μ mol Si l⁻¹ solution at 70 °C



Fig. 6 Half-reaction time, $t_{1/2}$ (min), for phosphate determination at various H⁺ and Mo concentrations at 20 °C, $t_{1/2}$ is defined as the time required for a 5 mol P l⁻¹ sample to reach half of the complete absorbance



Fig 7 The effects of H^+ and Mo concentration on the formation kinetics of the phosphomolybdenum blue colour at 880 nm and 20 °C The H^+ -Mo combinations were (a) 20%-100%, (b) 60%-200%, (c) 60%-60%, (d) 100%-100%, (e) 140%-140% and (f) 200%-200%

Kinetics of formation of phosphoantimonylmolybdenum blue

Variations in the [H⁺]: [Mo] ratio exert a considerable effect on the rate of formation of the phosphoantimonylmolybdenum blue species at 20°C (Fig. 5). When the acidity is low (curves a and b), appreciable self-reduction of the molybdate occurs. In contrast, the rate of colour formation decreases rapidly when the ratio exceeds 80. The optimum reaction rate was found within a narrow range centred on a ratio of ca. 74. This is clearly demonstrated by the matrix (Fig. 6) for the half-reaction time $(t_{1/2})$, the time required for the absorbance to attain half its final value, a parameter which provides a useful guide in selecting the optimum conditions for a flow-injection system). Thus, at 20 °C the $t_{1/2}$ for the methods of Murphy and Riley [1] and Strickland and Parsons [2] are ca. 1.6 and ca. 1.0 min, respectively.

These times would be inconveniently long for flow-injection analysis and would mean that much potential sensitivity might be sacrificed. The effect of temperature on the reaction was therefore investigated using the reagent concentrations recommended by Murphy and Riley. It was found (Fig. 7) that colour development was complete within ca. 40 s at 70 °C, about 60 s before any significant absorbance was produced by silicate. The results of matrix measurements (Fig. 3) showed that at 70 °C the optimum range of the [H⁺]: [Mo] ratio is displaced to a higher value centred on ca. 100. If the concentration of the molybdate reagent in the Murphy and Riley technique is halved, no interference results at this temperature from 200 μ mol Si 1⁻¹ silicate even after 30 min (Fig. 4b).

Conclusions

The optimum conditions for the determination of phosphate by the phosphoantimonylmolybdenum blue method were investigated with a view to adapting it for flow-injection analysis. The optimum [H⁺]:[Mo] ratio for colour development was evaluated and found to have a narrow range centred on ca. 74 at 20 °C. Kinetic studies showed that the half-reaction time using the conditions recommended by Murphy and Riley [1] fell from ca. 95 s at 20 °C to ca. 6 s at 70 °C. Colour formation from silicate was much slower and only became detectable after 100 s at 70 °C.

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