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Short Communication

A simple spectrophotometric determination of submicromolar quantities of vanadium oxyions

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Introduction

Widespread interest has developed in the study of vanadium as an essential nutrient [1] and as a possible regulator of several ATP hydrolysing enzymes [2–4] in mammalian species. Available methods for the determination of vanadium are costly [1, 5, 6], complex [7–12], and of very low sensitivity [13, 14].

Oxyvanadium species are known to react with molybdic acid in the presence of phosphate to form a heteropolymolybdate complex [15, 16]. The complex, molybdivanadophosphoric acid, is characterized by a yellow colour that maximally absorbs light at 385 nm. The intensity of colour resulting from the formation of this heteropoly complex has been shown to vary with changes in the concentration of inorganic phosphate. The dependence of the reaction on the concentration of vanadium oxyions has not been evaluated.

In the present study we examined the possibility that the intensity of yellow colour development resulting from the reaction of oxyvanadium with molybdic acid and phosphate may vary as a function of oxyvanadium concentration. The effect of phosphate ions and the pH of the medium on the colour intensity were also investigated. The study included the stoichiometric determination of the composition of the complex species.

Experimental

Materials

Sodium orthovanadate (Fisher Scientific, purified grade); ammonium molybdate and potassium phosphate dibasic (certified ACS, Fisher Scientific) were utilized.

Reagents

Vanadium oxyion standard solutions ranging in concentration from 5×10^{-7} to 5×10^{-4} M were freshly prepared by dissolving sodium orthovanadate in deionized distilled water.

Potassium phosphate 0.3 mM solution in water; and potassium phosphate 3.0 mM solution in water were used.

Ammonium molybdate solutions ranging in concentration from 0.25 to 2.5% in water and in 5 N sulphuric acid.

Apparatus

A DU-40 Beckman UV-Visible Spectrophotometer with 1-cm silica cells was used for recording spectra and absorbance or per cent transmittance measurements.

Procedure

One millilitre standard solutions of sodium orthovanadate in water were mixed with an equal volume of water or 0.3 mM or 3.0 mM potassium phosphate, and 0.1 ml of 0.5%

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ammonium molybdate in water or in 5 N sulphuric acid was then added. After 5 min the percentage transmittance was measured at the absorption maximum against a reagent blank.

Samples containing phosphate were also measured against blanks having water in place of phosphate solution. Percentage transmittance was plotted against oxyvanadium concentration on semilogarithmic charts. A total of six vanadium oxyion standard plots were obtained under these conditions. The slope of the resulting regression line of pooled data was determined by regression analysis. Comparison of slopes was done using analysis of covariance.

Varying molar concentrations of vanadium and ammonium molybdate solutions were used to measure the molar ratio composition of the complex.

Results and Discussion

The formation of a heteropolymolybdate when the hetero moieties are phosphate and oxyvanadium is known to vary with changes in phosphate concentration [15]. When acid molybdate was added to oxyvanadium standards in the absence of phosphate the reaction mixtures did not absorb light of 385 nm wavelength. In the presence of the low phosphate concentration (0.3 mM), however, oxyvanadium standards developed a yellow colour upon the addition of acid molybdate. Colour intensity varied proportionately with the oxyvanadium concentration (Fig. 1). The presence of a higher phosphate concentration (3.0 mM) further increased the slope of the oxyvanadium standard curve (Fig. 1) indicating that the reaction was dependent on the concentration of both phosphate and oxyvanadium for formation of a coloured complex in highly acid medium (pH < 1.0) such as that provided by molybdate in 5 N sulphuric acid. Consequently, a colorimetric determination of oxyvanadium in solution under these conditions would be limited by a phosphate requirement as well as possible phosphate interference. At this pH the minimal concentration of oxyvanadium detected by the reaction with acid molybdate was 10 μM.

In a less acidic environment; achieved through preparation of ammonium molybdate in deionized distilled water, we found that oxyvanadium alone reacted with ammonium molybdate to yield a yellow species. Since phosphate was not a requirement at this pH (4.0–5.0) the complex was not molybdivanadophosphoric acid. Job's method of continuous variation and the molar-ratio method [17] were used to determine the stoichiometry of the complex. A 1:1 stoichiometric ratio of molybdate to the vanadate ion was found (Fig. 2). Under these conditions to slope of oxyvanadium standard curves was not altered by the presence of phosphate. The *y*-intercept of oxyvanadium standard curves, however, was increased by the presence of phosphate (Fig. 3). The magnitude of this effect was not dependent upon phosphate concentration, since the phosphate is not part of the reaction. When readings for standard solutions were taken against blanks containing the same con-

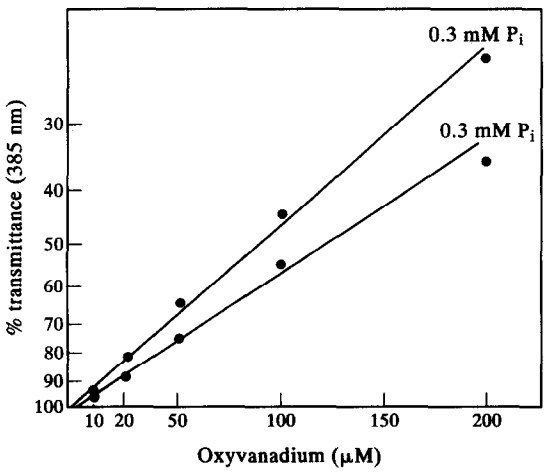

Figure 1
Effect of phosphate ion level on the reaction of oxyvanadium with molybdate in 5 N sulphuric acid.

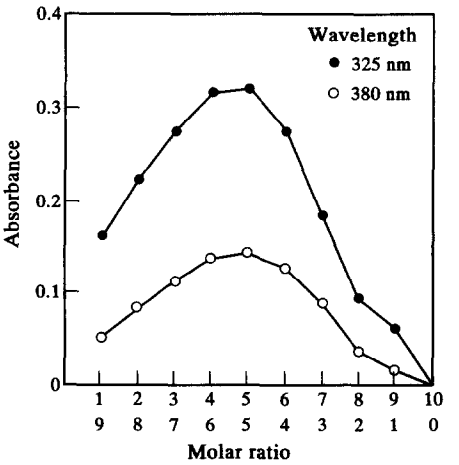

Figure 2
Molar variation curves oxyvanadium with molybdate ions at pH 4.5.

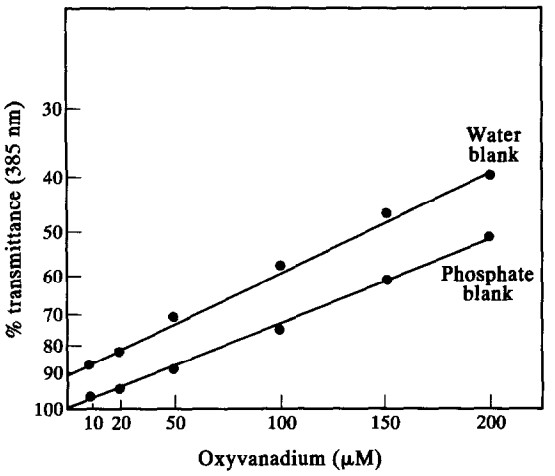

Figure 3
Effect of phosphate ion level on the reaction of oxyvanadium with molybdate at pH 4.5.

centration of phosphate, the oxyvanadium standard curve was similar in the presence and absence of phosphate (Fig. 3). At this pH the effects of phosphate on oxyvanadium standard curves was not dependent on phosphate levels even when a physiological concentration was used. The spectra of the complex, obtained from oxyvanadium solution and ammonium molybdate in water, absorb at a wavelength of 325 nm beside the 385 nm. At 325 nm, the wavelength of the absorption peak, the system obeys Beer's law from 0.5×10^{-7} to 5×10^{-4} M of vanadium oxyion. The system is also characterized by molar absorptivity, $\epsilon = 3.52 \times 10^3$ and 1.64×10^3 l mol^{-1} cm^{-1} at 325 nm and 380 nm, respectively.

Although the logarithm of percentage transmittance was a linear function of oxyvanadium concentration up to 5×10^{-4} M, at vanadium oxyion concentration greater than 3×10^{-3} M, the intensity of the colour developed in the presence of molybdate varied inversely with oxyvanadium concentration. This deviation from linearity as well as the inverse relationship between oxyvanadium concentration and the colour intensity at levels greater than 3×10^{-3} M may have been the result of oxyvanadium polymerization [18]. Our results suggest that polymeric oxyvanadium does not react with molybdate in the same manner as monomeric species. Since the process of polymerization is reversible [18], dilution of concentrated samples will permit measurement using the reaction with molybdate.

Conclusion

The proposed method provided a one-step spectrophotometric determination of submicromolar (5×10^{-7}) quantities of oxyvanadium. At pH 4.0–5.0 oxyvanadium alone reacted with molybdate to yield a yellow coloured species, independent on phosphate levels even when a physiological concentration was used.

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