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The Analysis of Phosphate, Arsenate, and Silicate by Reverse Phase Liquid Chromatography

I-Zenq Lin^a; William K. Nonidez^a; Li-Fen Hsieh^a

^a Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama

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THE ANALYSIS OF PHOSPHATE, ARSENATE, AND SILICATE BY REVERSE PHASE LIQUID CHROMATOGRAPHY

I-ZENQ LIN, WILLIAM K. NONIDEZ,

AND LI-FEN HSIEH

Department of Chemistry

University of Alabama at Birmingham

Birmingham, Alabama 35294-1240

Abstract

The separation of 12-Molybdoarsenic Acid (12-MAA), 12-molybdosilicic Acid (12-MSA) and 12-molybdophosphoric Acid (12-MPA) by a disposable commercially available SEP-PAK™ C18 reverse phase liquid chromatography column is described with UV-visible detection. The mobile phase is an acetonitrile-water solution which is 0.050 F in HClO_4 and 0.016 F in MoO_4^{2-} . In these solutions lowering the acetonitrile concentration to 10.0% (v/v) inhibits the formation of 12-MAA while allowing the formation of 12-MPA and 12-MSA resulting in the retention of 12-MPA and 12-MSA upon infusion through a SEP-PAK C18 column. The arsenate anion remains in the column effluent. 12-MSA is eluted from the column by 3.0 ml of a solution which is 25.0% in acetonitrile, 0.060 F in HClO_4 and 0.016 F in MoO_4^{2-} and the 12-MPA is eluted from the column by 3.0 ml of a solution which is 55.0% (v/v) in acetonitrile, 0.060 F in HClO_4 and 0.016 F in MoO_4^{2-} . The column effluent which contains AsO_4^{3-} is then brought up to 35.0% acetonitrile to allow the 12-MAA to form. This solution is injected into a SEP-PAK column and the 12-MAA eluted by 3.0 ml of a solution which is 55.0% (v/v) in acetonitrile, 0.060 F HClO_4 and 0.016 F in MoO_4^{2-} . The absorbance of each of the separated 12-MAA, 12-MPA and 12-

MSA solution are measured by a UV-visible spectrophotometer (Perkin Elmer Lambda 6) at 440 nm, and related to PO_4^{3-} , AsO_4^{3-} or SiO_3^{2-} concentration by standard methods. The detection limit is 1.25×10^{-6} M for PO_4^{3-} , 8.68×10^{-6} M for AsO_4^{3-} and 2.86×10^{-6} M for SiO_3^{2-} . The anions may be analyzed at 10 times their detection limits with relative percent standard deviations of 0.40% for PO_4^{3-} , 1.66% for SiO_3^{2-} , and 2.27% for AsO_4^{3-} . An additional method is described for phosphate analysis in solutions with $[\text{PO}_4^{3-}] < 10^{-6}$ M.

Introduction

In 1989 Nonidez and Samanifar (1) reported the separation of 12-molybdophosphoric acid (12-MPA) from 12-molybdosilicic acid (12-MSA) by reverse phase liquid chromatography utilizing a commercially available styrene-divinylbenzene column and a acetonitrile-water mobile phase. Although that system was capable of determining small amounts of phosphate in the presence of large amounts of silicate the authors were unable to separate arsenate in the form of 12-molybdoarsenic acid (12-MAA) from 12-MPA making it impossible to quantitate arsenate, if present in the sample, or to prevent it from acting as a positive interference upon phosphate.

Additionally that year a reversed-phase HPLC separation of highly charged polyoxometalates using ion-interaction reagents and competing ions was published (2). Since this separation required the use of a C18 bonded column no attempts were made to study or quantitate heteropolyacids such as 12-MPA, 12-MSA and 12-MAA which form in highly acid solutions due to the pH = 2-8 limitations of those columns.

This paper describes a method for the separation of 12-MPA, 12-MAA and 12-MSA and quantitative analysis of arsenate ion, silicate ion and phosphate ion

using inexpensive disposable C18 bonded SEP-PAK columns. The effect of solution conditions on the formation of 12-MPA, 12-MSA and 12-MAA were studied as well as chromatographic studies to determine SEP-PAK capacity, and necessary elution volumes. Finally an analytical procedure is specified for the serial analysis of PO_4^{3-} , SiO_3^{2-} and AsO_4^{3-} in the same sample. The linear range, detection limits and reproducibility of the technique is determined.

MATERIALS AND METHODS

Reagents

All solutions were prepared from high purity (greater than 99.7%) reagent grade chemicals. Deionized water used for all solution preparation was prepared with a HYDRO Model 1000 Reagent Water System (Hydro Service & Supplies, Inc., 3200 Sandy Creek Drive, Durham, N.C. 27705). All organic solvents used were HPLC grade. Glassware used in solution preparation was soaked in 6 M HCl followed by multiple rinsings with deionized water and was isolated from other laboratory glassware to prevent contamination from laboratory detergents.

SEP-PAK C₁₈TM bonded C₁₈ cartridges were obtained from the Waters Division of Millipore Corporation Milford, Massachusetts and were used after wetting with pure acetonitrile (ACN). Cartridges were discarded after one use.

All absorbance measurements were made on a Perkin Elmer Lambda 6 Spectrophotometer.

Solution Studies

A) ACN Concentration vs. Absorbance. Three series of solutions were prepared each of which was 0.050 F in HClO_4 , 0.016 F in molybdate ion and 10.0,

15.0, 20.0, 25.0, 30.0, 35.0, and 40.0% (v/v) ACN. One series was made 2.00×10^{-3} M in PO_4^{-3} , a second 2.00×10^{-3} M in AsO_4^{-3} and the third 2.00×10^{-3} M in SiO_3^{-2} . The absorbance of each solution was measured at 440 nm vs. a reference solution identical to the sample except for the absence of the heteroanion.

B) Molybdate Ion Concentration vs. Absorbance. Three series of solutions 0.050 F in HClO_4 , 10.0% ACN, and 0.0080, 0.012, 0.016, 0.020, 0.024, 0.028, 0.032, and 0.036 F in molybdate ion were prepared. Each series was made 1.00×10^{-4} F in either PO_4^{-3} , AsO_4^{-3} , or SiO_3^{-2} . The absorbance of each solution was measured at 440 nm vs. a reference solution identical to the sample but without heteropolyanion.

C) Perchloric Acid Concentration vs. Absorbance. Three series of solutions 0.016 F in molybdate, 10.0% ACN and 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, and 0.10 F in HClO_4 were prepared. Each series was made 1.00×10^{-4} M in either PO_4^{-3} , AsO_4^{-3} , or SiO_3^{-2} . The absorbance of each solution was measured at 440 nm vs. a reference solution identical to the sample but without heteropolyanion.

D) Sep-Pak Capacity Study. Solutions 0.050 F in HClO_4 , and 1.00×10^{-4} M in either phosphate or silicate were made 10.0% (v/v) in ACN. An additional solution 0.050 F in HClO_4 and 1.00×10^{-4} M in arsenate was made 35.0% (v/v) in ACN. After pre-conditioning a Sep-Pak column with 100% ACN a solution was injected into the column in 2.0 or 3.0 mL increments. After each injection the column effluent was captured and its absorbance measured vs. a reference solution identical to the above without the heteropoly oxylon present.

E) Elution Volume Study. Columns which were fully loaded in D) were eluted in 1.0 or 2.0 mL increments with solutions which were 0.016 F in molybdate ion, 0.06

F in HClO_4 and either 25.0% ACN (for 12-MSA) or 55.0% ACN (for 12-MAA or 12-MPA). After each injection the column effluent was captured and its absorbance measured vs. a reference solution identical to the eluting solution except for the absence of heteropoly anion.

F) Calibration Curves. Solutions of various concentrations between 1.00×10^{-4} M and 1.00×10^{-5} were analyzed by the procedure specified in steps 1-5 found under Analytical Procedure.

RESULTS AND DISCUSSION

Solution Studies

Spectroscopic studies were first performed to determine the effects of ACN HClO_4 , and molybdate concentration on 12-MPA, 12-MAA and 12-MSA formation in ACN-water solutions. Even small amounts of ACN in water promote the formation of a yellow isopolymolybdate from the excess molybdate present which has a λ_{max} of 325 nm which is identical to the λ_{max} given for $\text{Mo}_6\text{O}_{19}^{2-}$ (3)⁴. This species absorbs in the visible region at wavelengths almost identical to the heteropolyacids under study in this paper, and hence must be compensated for in any spectral study of heteropolyacids.

Figure 1 is a plot of ACN concentration vs. heteropolyacid absorbance (440 nm) for the three heteropolyacids discussed above. The MoO_4^{2-} and HClO_4 were held at 0.016 F and 0.050 F respectively. The absorbance of the isopolymolybdate was compensated for by placing a solution identical to the sample but containing no PO_4^{3-} , AsO_4^{3-} or SiO_3^{2-} in the reference cell. At ACN concentrations between 10.0% and 25.0% no 12-MAA formation is detected while the 12-MPA and 12-MSA

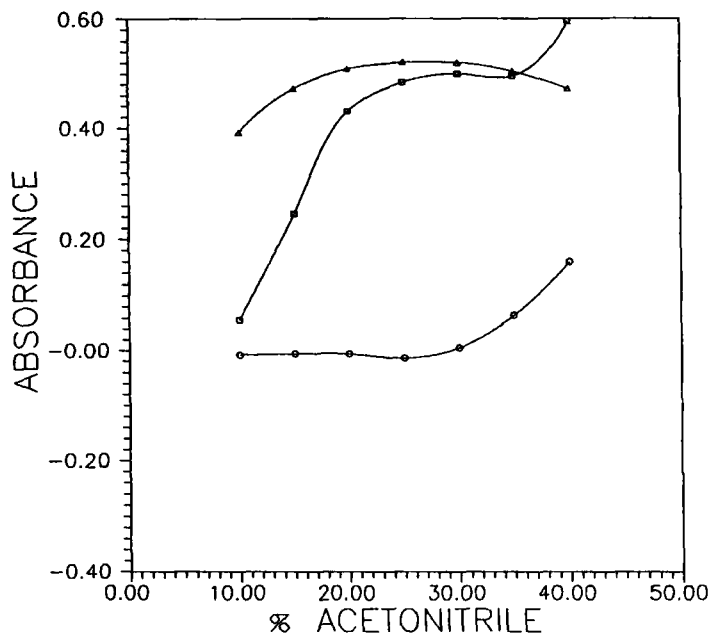


Figure 1. Plot of acetonitrile concentration vs. heteropolyacid absorbance for 12-MAA (O), 12-MPA (□) and 12-MSA (Δ). Acetonitrile concentration varied between 10.0% and 40.0% (v/v). Solutions for 12-MAA, 12-MPA and 12-MSA were 2.00×10^{-2} M in AsO_4^{3-} , PO_4^{3-} and SiO_3^{2-} . The MoO_4^{2-} and HClO_4 were held at 0.016 F and 0.05 F respectively.

absorbances are almost at their maxima at 25.0%. 12-MAA absorbance is seen to increase substantially at ACN concentrations greater than 30.0%.

The effect of MoO_4^{2-} concentration on this system was studied by holding the ACN concentration at 10.00% and varying the MoO_4^{2-} concentration between 0.0080 F and 0.036 F. As shown in Figure 2, no 12-MAA formation was detected between these limits and an actual decrease in absorbance of 12-MPA and 12-MSA were observed at higher MoO_4^{2-} concentrations.

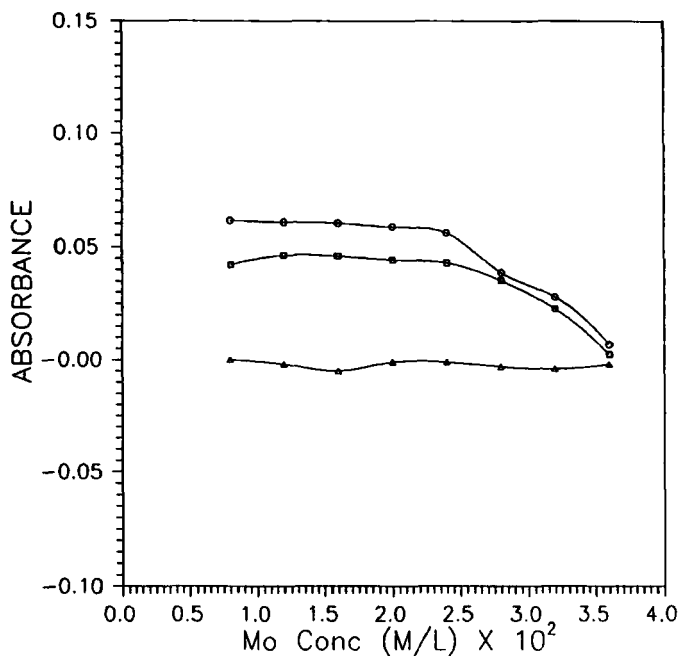


Figure 2. Plot of MoO_4^{2-} concentration vs. heteropolyacid absorbance for 12-MAA (\blacktriangle), 12-MPA (\square) and 12-MSA (\circ). MoO_4^{2-} concentration was varied between 0.008 F and 0.036 F. Solutions for 12-MAA, 12-MPA and 12-MSA were 1.00×10^{-4} M in AsO_4^{3-} , PO_4^{3-} and SiO_3^{2-} . The HClO_4 and acetonitrile were held at 0.05 F and 10.0% (v/v) respectively.

Finally the effect of HClO_4 on the system was studied by varying its concentration between 0.03 F and 0.10 F as seen in Figure 3. Again varying the acid concentration through the above range did not promote the formation of 12-MAA while allowing the formation of 12-MPA and 12-MSA at concentrations above 0.030 F.

The above solution studies indicate that 12-MPA and 12-MSA can be produced in the presence of AsO_4^{3-} without 12-MAA formation in a wide range of

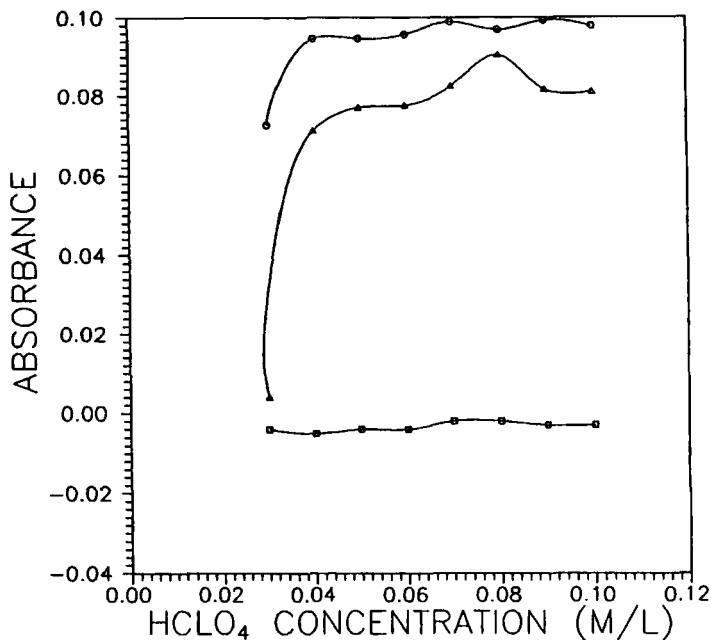


Figure 3. Plot of HClO_4 concentration vs. heteropolyacid absorbance for 12-MAA (\square), 12-MPA (Δ) and 12-MSA (\circ). HClO_4 concentration was varied between 0.03 F and 0.10 F. Solutions for 12-MAA, 12-MPA and 12-MSA were 1.00×10^{-4} M in AsO_4^{3-} , PO_4^{3-} and SiO_3^{2-} . The MoO_4^{2-} and acetonitrile were held at 0.016 F and 10.0% (v/v) respectively.

MoO_4^{2-} and HClO_4 concentrations at ACN concentrations less than 30%. 12-MAA formation can be induced by increasing the ACN concentration to values greater than 30.0%

Chromatographic Studies

The capacity of the C-18 Sep-Paks was studied by injecting various volumes of IPY, 12-MPA, 12-MSA, and 12-MAA into C-18 Sep-Paks. The solutions were 1.00×10^{-4} M in either SiO_4^{2-} , PO_4^{3-} or AsO_4^{3-} , 0.016 F in MoO_4^{2-} , 0.050 F in

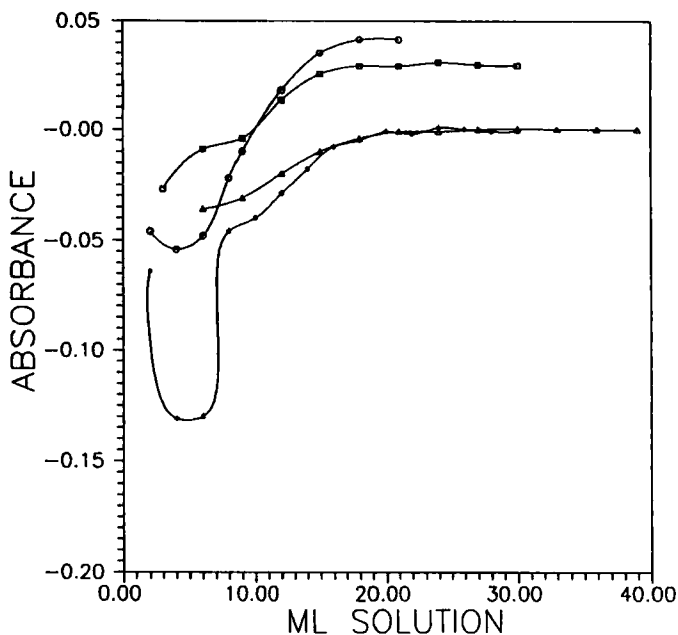


Figure 4. Plot of total accumulated injected volume vs. absorbance for IPY (*), 12-MAA (□), 12-MPA (Δ) and 12-MSA (O). Solutions were 1.00×10^{-4} M in either AsO_4^{3-} , PO_4^{3-} or SiO_3^{2-} , 0.016 F in MoO_4^{2-} , 0.05 F in HClO_4 and either 10.0% (v/v) acetonitrile for 12-MPA and 12-MSA studies or 35.0% (v/v) acetonitrile for 12-MAA studies.

HClO_4 and either 10.00% ACN for 12-MPA or 12-MSA studies or 35.0% ACN for 12-MAA studies. Each solution was injected into a Sep-Pak column in 2.0 or 3.0 mL increments which had been pre-injected with a solution of 100% ACN. The column effluent was collected after each injection and its absorbance measured at 440 nm against a reference solution identical to the above except for the absence of heteropoly anion. A plot of absorbance vs. total accumulated injected volume appears in Figure 4. The drop in absorbance of the 12-MSA and the IPY at low

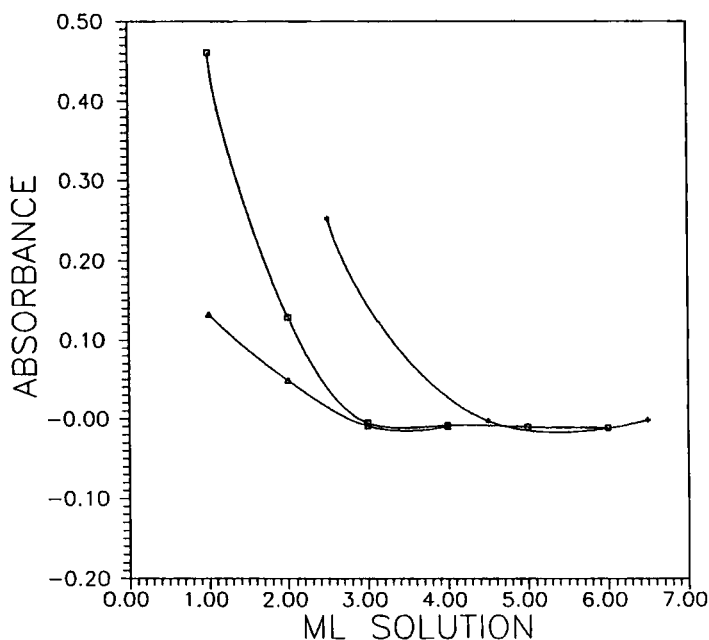


Figure 5. Plot of total accumulated eluting volume vs. absorbance for 12-MAA (Δ), 12-MPA (\square) and 12-MSA (*). Solutions were 0.016 F in MoO_4^{2-} , 0.016 F in HClO_4 and either 55.0% (v/v) acetonitrile for 12-MPA and 12-MAA studies or 25.0% (v/v) for 12-MSA studies.

injection volumes is due to the initial removal of both IPY and heteropolyacid by the column. When the absorbance of the 12-MAA and 12-MSA curves reach values greater than the absorbance of the IPY curve, elution of the 12-MAA and 12-MSA is indicated. 12-MAA and 12-MSA elution begins at a total elution volume of 10.0 mL thus defining the maximum volume of 12-MSA and 12-MAA analyte which can be injected on a column at the above analyte concentrations and solvent conditions. The absorbance of the 12-MPA never increases past IPY absorbance

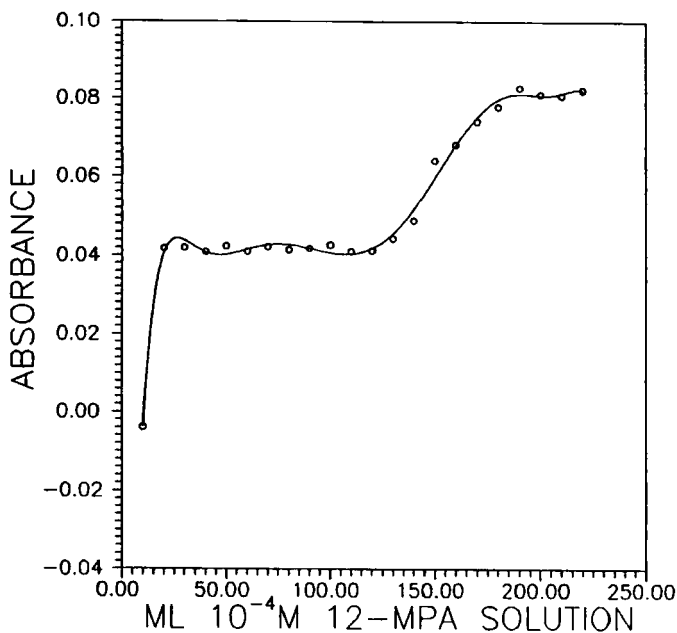


Figure 6. Plot of total accumulated 12-MPA injected volume vs. absorbance. The solution of 12-MPA was 1.00×10^{-4} M in PO_4^{3-} , 0.05 F in HClO_4 , 0.016 F in MoO_4^{2-} and 10.0% (v/v) in acetonitrile.

because it is not eluted by the 10.0% ACN solution. After an elution volume of 15.0 mL is reached a steady state absorbance is obtained which is evidence that the column is saturated with IPY.

An additional capacity study for 12-MPA was performed by injecting 220.00 mL of a solution which was 1.00×10^{-4} F in PO_4^{3-} , 10.0% (v/v) ACN, 0.050 F in HClO_4 and 0.016 F in MoO_4^{2-} through a SEP-PAK in 10.0 mL increments. The last three mL of each increment was captured and its absorbance measured vs. water.

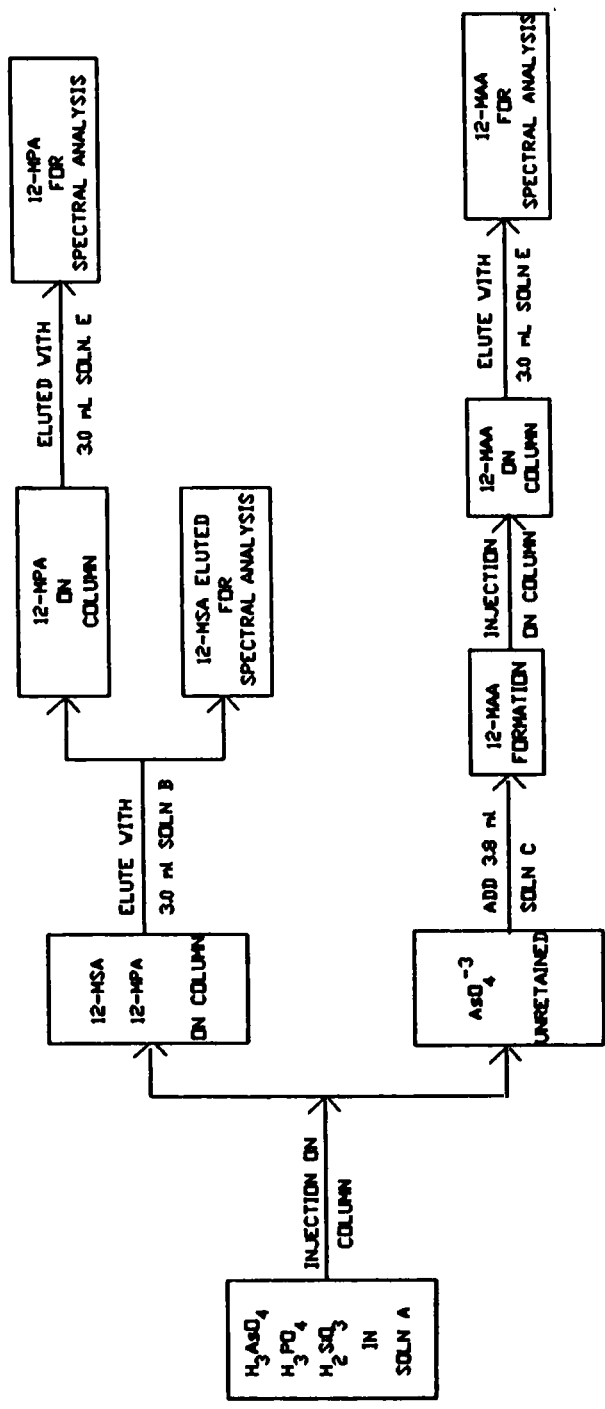


Figure 7. Flow chart for analysis of PO_4^{3-} , AsO_4^{3-} , and SiO_3^{2-} .

The results of this experiment appear in Figure 6.

The first absorbance plateau is due to the elution of IPY until an elution volume of 120.00 mL is reached. After this the increase in absorbance is due to the elution of 12-MPA indicating that 12-MPA may be concentrated on a column from large volumes of sample. This 12-MPA may then be eluted with as little as 2.0 mL of eluant allowing a sample concentration of 60:1.

Solvent volumes necessary to completely elute 12-MPA, 12-MSA and 12-MAA were determined by injecting various volumes of eluting solvent into columns already saturated with heteropolyacid. The absorbance of column effluent after each injection was measured and plotted vs. total injected volume in Figure 5. It is evident from this Figure that 12-MPA and 12-MAA may be totally removed from a column at a total elution volume of 2.00 mL when using a solvent 55% in ACN, 0.016 M in MoO_4^{2-} and 0.06 M in HClO_4 . 12-MSA may be removed with the same solvent with a lower ACN concentration (25.0%) with a total elution volume of 2.5 mL.

From the above experiments an analytical scheme was proposed and tested for the systematic determination of arsenate, silicate and phosphate in aqueous solutions. A flow chart for this scheme appears in Figure 7.

Analytical Procedure

Step 1. The sample solution is made 0.05 F in HClO_4 , 0.016 F in Na_2MoO_4 and 10.0%(v/v) in ACN resulting in Solution A.

Step 2. Eight mL of Solution A is then injected onto a SEP-PAK column which has been previously wet with 100% ACN. 12-MPA and 12-MSA are retained on the

column. Any residual amount of mobile phase is then forced from the column by a small injection of air into the previously collected effluent which is saved for arsenate analysis.

Step 3. The 12-MSA is then eluted from the SEP-PAK with 3.0 mL of a solution 0.06 F in HClO_4 , 0.016 F in Na_2MoO_4 and 25.0% (v/v) ACN (Solution B). The absorbance of the effluent is then measured and related to 12-MSA concentration by a standard calibration curve.

Step 4. 12-MPA remaining on the SEP-PAK is eluted with 3.0 mL of a solution which is 0.060 F in HClO_4 , 0.016 F in Na_2MoO_4 and 55.0% (v/v) in ACN (Solution E). The absorbance of the effluent is then measured and related to 12-MPA concentration by a standard calibration curve.

Step 5. The effluent from Step 2 containing H_3AsO_4 is then treated with a solution which is 0.050 F HClO_4 , 0.016 F in Na_2MoO_4 and 93.4% ACN (Solution C) in order to increase its total ACN concentration to 35.0% thus allowing the formation of 12-MAA whose absorbance is then measured and related to AsO_4^{3-} concentration by a standard calibration curve.

Step 6. If low concentrations of AsO_4^{3-} are to be measured (less than 1×10^{-4}) 10.0 mL of the above solution may be injected into a fresh SEP-PAK and eluted with 3.00 mL of Solution E above resulting in a concentration increase of 3.33.

Alternate Procedure for Phosphate

In cases where phosphate concentrations are very low, the 12-MPA phosphate derivative may be preconcentrated from a starting sample volume of 120.0 mL. In this case 120.0 mL of sample is allowed to pass by gravity through

a SEP-PAK at an approximate rate of 1 mL per minute. An aliquot of 2.5 mL of a solution 25% in ACN, 0.016 F in MoO_4^{2-} and 0.050 F HClO_4 is then passed through the column to remove any SiO_3^{2-} present. The 12-MPA remaining on the column is then eluted with 2.0 mL of a solution which is 55.0% in ACN, 0.016 F in MoO_4^{2-} and 0.06 F in HClO_4 . The absorbance of the phosphate may then be measured and quantitated by standard methods. The detection limit for PO_4^{3-} is 3.60×10^{-8} M in the original sample by this procedure and the method linear to 1.00×10^{-6} M.

Calibration Curves

Plots of absorbance as a function of PO_4^{3-} , AsO_4^{3-} and SiO_3^{2-} concentration were found to be linear up to a concentration of 1.00×10^{-4} M in the original sample. At higher concentrations negative deviations from linearity are to be expected due to decreasing molybdate to phosphate ratios.

Linear regression analysis of calibration curves produced correlation coefficients typically of at least 0.99 for all species. A detection limit of 1.25×10^{-6} M was calculated for phosphate, 8.68×10^{-6} M for arsenate and 2.86×10^{-6} M for silicate. These were calculated by the method reviewed by Miller and Miller (4) which estimates the standard deviation of the blank and the actual value of the blank from statistical deviation of the blank and the actual value of the blank from statistical values calculated from the calibration curve. The limit of detection is defined as the concentration corresponding to:

$$y_B + 3s_B$$

where y_B is the best fit absorbance value of the blank calculated by regression analysis and s_B is the calculated standard deviation of the absorbance of the blank.

Reproducibility

The reproducibility of the technique was estimated by analyzing 5 replicates of a sample which was 1.25×10^{-5} M in phosphate, 8.68×10^{-5} M in AsO_4^{-3} and 2.86×10^{-5} F in SiO_3^{-2} . These values represent concentrations 10 times the detection limit of each oxyanion. The relative standard deviation for PO_4^{-3} was 0.40%, 1.66% for SiO_3^{-2} and 2.77% for AsO_4^{-3} .

REFERENCES

1. Nonidez, W.K.; Samanifar, M. J. *Liq. Chrom.* **12**(3): 397-418 (1989)
2. Kirk, A.D. *et al.*, *Inorg. Chem.*, **28**: 792-797 (1989)
3. M.T. Pope, Heteropoly and Isopolyoxometalates, Springer-Verlag, Berlin, 1983, pp. 45
4. Miller, J.C.; Miller, J.N. "Statistics for Analytical Chemistry"; Wiley, New York, 1986.