000 - - 1618-1

CITE INDEXES

MASTER

H C \$ 3.10; MN .65

AUG 22 1957

Gradient Elution Anion Exchange Chromatography in Hot-Atom Studies

Vincent C. Anselmo
Department of Chemistry
New Mexico Highlands University
Las Vegas, New Mexico 87701

Summary

Gradient elution anion exchange chromatography was applied to the separation of the various oxidation states of phosphorus oxy-anions produced in thermal neutron irradiated orthophosphates, orthophosphites, or hypophosphites. Production of a logarithmic gradient is discussed and the results using several different gradients are illustrated.

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Gradient elution ion-exchange chromatography has been used successfully in both organic and inorganic separations. For organic systems, it is usually used to produce a change in pH, or for a continuous change in solvents. While for inorganics it may be used to produce a change in ionic strength in the eluant solution in order to facilitate separation, principally by reducing separation times. There are several reports in the literature dealing with the subject (1,2). Three types of gradient may be produced: convex, linear, or concave, depending on experimental conditions. One, convex, is the easiest to produce and is the one of choice in this report. It results in a logarithmic increase in eluant concentration. Such gradients have been successfully applied to the phosphate system by Pollard, and coworkers (2) and in general, theirs is the experimental procedure used here. However, there has been little application to radiochemical, or more specifically, hot-atom systems.

In the case of hot-atom systems, particularly neutron irradiated phosphorus oxy-anions, the usual methods of analysis involved chemical separation of one oxidation state (3), or electrophoresis (4). The former suffers from the uncertainty of physical adsorption of tracer quantities in bulk precipitates as well as the difficult chemistry of phosphorus. Electrophoresis just cannot produce the resolution and convenience of gradient elution anion exchange chromatography.

The parameters of importance are flow-rate and chloride gradient. A flow-rate of 5 to 7 ml/min. allows a reasonable time for separation (about 6 hours for separation including tripolyphosphate). The different gradients are discussed here with the major objective being best resolution at the desired flow-rate. All in all, the proper balance must be achieved.

For the separation of the various oxidation states of phosphorus the general procedure of Pollard, and coworkers (2), was followed with two exceptions. The eluant used was sodium chloride instead of potassium chloride and the flow-rate was increased significantly. The gradient was produced by continuously mixing a concentrated solution of sodium chloride from a reservoir into a more dilute one contained in a mixing vessel. A mechanical pump provided the force for movement of the eluant through the column. In Figure 1 there is a semi-schematic representation of the gradient producing system and the column.

The column was 43 cm long, 4.91 cm² in cross sectional area, and contained 38 ml. of Dowex 1-X8, 100 to 200 mesh, resin.

In order to find the best conditions for separating the simple, catenated, and complex orthophosphates, orthophosphites and hypophosphites, a sample known to contain radioactive P⁵, P³, and P¹ (5), as well as several complex phosphates, was separated on the column under differing conditions. The sample was thermal neutron irradiated ammonium hypophosphite to which was added ammonium orthophosphite and ammonium hypophosphite carriers. The sample material, with carriers, was added to a 50 ml volumetric flask, dissolved in and brought up to volume with the eluant solution and then 10 ml aliquots taken for the five experiments. The different gradient producing conditions were: the mixing vessel contained 0.025 M, 0.050 M, or 0.10 M sodium chloride solution while the reservoir contained 0.50 M or 0.30 M sodium chloride solution. At a constant flow-rate of about 6 ml/min., the different results can be analyzed for most efficient separation taking into account time and resolution of activities.

All eluant solutions were buffered by the addition of 25 ml of a 2M solution of ammonium acetate per liter of solution. This results in a solution with a pH of 6.8. Proper buffering is important in order to have the various oxyanions in one predominant ionic species. Failure to do so may result in double peaking for some species as has been shown (6).

In Figures 2 and 3, the results of the several experiments are outlined so that the proper conditions may be chosen depending on the desired results.

A deviation from the usual procedure in handling the column was followed. After conversion of the column to the chloride form, it is then washed with the eluant concentration found in the mixing vessel instead of distilled water as in the usual case. A problem arose in knowing when the column was equilibrated with this eluant. To that end a flow conductivity cell was introduced in the effluent stream immediately below the column. It was constructed of two lengths (2.5 cm) of silver tubing (0.3 cm id) separated by 2.5 cm of the same tygon tubing used to carry the effluent to the detector. The conductivity was measured on a conductivity meter using the conductivity of the eluant solution as a reference.

Radioactivity was measured by passing the effluent through 126 cm of Saran tubing of 0.1 cm id that was wrapped around a 30 mg/cm² metal thin walled GM tube. The usual counting equipment included a count rate meter and then a strip chart recorder completed the assembly.

The formal procedure was as follows:

The resin was put in the chloride form by passing about 200 ml of 6M hydrochloric acid through the resin, which was then rinsed with the eluant solution of the same concentration as is found in the mixing vessel. Washing is continued until the conductivity meter registers no change in conductance. After washing, the upper portion is drained of excess eluant, the solution of the sample in about 10 - 20 ml of eluant solution is then added to the resin. A few ml of eluant is used to wash the remaining sample solution onto the column and then the upper part of the column filled with eluant solution. The column is connected to the pump, the latter started and the system is ready to operate and record. The fraction as each species may be determined by measuring the area under each peak or by cutting each peak out of the recording, weighing the paper, and finding the ratio of this weight to the total weight of all the peaks.

Calibration was achieved by subjecting a compound to thermal neutron irradiation and separating the sample on the column. Since the irradiated material showed the presence of several different oxidation states, each peak must be collected and tested qualitative for mass as follows. In the case of the phosphate system, each fraction must be oxidized or hydrolyzed to orthophosphate before testing. This may be accomplished by boiling with hydrochloric acid or oxidation with bromine water or hot nitric acid. Then, to about 10 ml of the sample is added 3 ml of concentrated nitric acid and 10 ml of a 10% (w/v) solution of sodium molybdate. A yellow color indicates the presence of orthophosphate in the treated sample.

Gradient elution anion exchange chromatography has been shown to be very efficient for the separation of the several oxidation states of phosphorus oxyacids following thermal neutron irradiation of any particular one. The system is flexible enough to be adapted to other chemical systems. Its major advantages are convenience, flexibility, and the ease of automation.

The research was supported, in part, by Contract AT(11-)-1618 with the United States Atomic Energy Commission. Acknowledgement is also made of the cooperation of the Los Alamos Scientific Laboratory at whose facilities the irradiations were performed.

FIG. 1. GRADIENT PRODUCING ARRANGEMENT

Fig. 2. Separation of the Oxidation States of Phosphorus Oxy-Acids Under Different Gradient Producing Conditions.

- (a) 0.5 M NaCl into 2.0 l of 0.025 M NaCl
Flow rate = 5.9 ml/min
- (b) 0.3 M NaCl into 2.0 l of 0.025 M NaCl
Flow rate = 6.0 ml/min
- (c) 0.3 M NaCl into 2.0 l of 0.1 M NaCl
Flow rate = 6.8 ml/min

Fig. 3. Separation of the Oxidation States of Phosphorus Oxy-Acids Under Different Gradient Producing Conditions.

(a) 0.5 M NaCl into 2.0 l of 0.1 M NaCl

Flow rate = 5.8 ml/min

(b) 0.5 M NaCl into 2.0 l of 0.050 M NaCl

Flow rate = 6.2 ml/min

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