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Distinguishing between organic and inorganic phosphorus in hydroxyapatite by elemental analysis

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Abstract

A method is developed to determine the amount of organic and inorganic phosphorus in an inorganic polymer (hydroxyapatite (HAP)) modified with an organic phosphorus – containing complexant. The simplicity and precision of the vanadate method has made it useful for measuring the total phosphorus content in phosphorus – containing organic polymers that are first digested in concentrated sulfuric acid. However, it can be important to quantify the organic and inorganic phosphorus capacities in modified (hybrid) polymers and this method does not distinguish between the two. In the current report, HAP was modified with 1-hydroxyethane-1,1-diphosphonic acid (HEDP) and a method developed to give the respective phosphorus capacities. HAP was contacted with HEDP for 17 h, 28 h, 56 h, 84 h, 112 h, and 140 h. By combining results from the sulfuric acid digestion of the modified polymer with those from a separate digestion in HCl, it was determined that there was a monotonic increase in the organic phosphorus capacity from 0.04 to 2.44 mmol / g, and a decrease in the inorganic phosphorus capacity from 4.68 to 2.54 mmol / g.

Introduction

Determination of the phosphorus content in soil,¹ glass² and phosphorus – containing compounds has been extensively evaluated by spectrophotometric methods.^{3,4} The phosphorus capacities of orthophosphates, condensed phosphates, and phosphorus ligands bound to organic polymers can be measured using the vanadate-molybdate reagent.^{5,6,7} The method requires strong acid (HClO₄ or H₂SO₄) digestion and peroxodisulfate oxidation to convert all phosphorus species into orthophosphate. The method also cleaves stable P-C bonds in organophosphonates to produce orthophosphates and organic fragments (Fig. 1).

Orthophosphates react with the ammonium salts of vanadate (H₂VO₄⁻) and molybdate (MoO₄²⁻) to produce a yellow phosphovanadomolybdate complex. The intensity of this yellow color is proportional to the concentration of phosphate and is analyzed at 470 nm.⁸

While the vanadate method is useful for determining the total phosphorus content of pure organic and inorganic phosphorus compounds, it is incapable of distinguishing between

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organic and inorganic phosphorus in materials containing both types of phosphorus since all phosphorus-containing species become orthophosphate. Distinguishing organic from inorganic phosphorus is important in the growing area of hybrid polymers^{9,10,11,12} and thus a method to distinguish between the two was developed.¹³

Organic phosphorus capacity of organic polymers

Precise measurements of the phosphorus capacity of polystyrene-bound phosphinic and phosphonic acids were obtained using the vanadate-molybdate method:⁶ digestion of the modified polymer (20 mg) in concentrated sulfuric acid (10 mL) was accelerated by the addition of 3.19 mg of copper sulfate catalyst. Mixing the digested sample with 10 mL of the ammonium vanadate-molybdate reagent resulted in formation of an intense yellow complex that was measured at 470 nm on a Milton Roy Spectronic 21D. The accuracy of the phosphorus capacities was supported by their agreement with the acid capacities. Therefore the total phosphorus capacity can be accurately determined using this method to analyze phosphorus-containing polymers.

Inorganic phosphorus capacity of inorganic polymers

Unlike organophosphates, inorganic polymers such as HAP, can dissociate into their respective ions and orthophosphates in the presence of dilute acid. Oxidizing agents, catalysts and heat are not required. The dissociation of hydroxyapatite in aqueous HCl is shown eq. 1 to produce calcium chloride, water and protonated orthophosphates.

To determine the inorganic phosphorus capacity, 20 mg of the polymer was added to 10 mL of 1.0 M HCl and stirred for 3 h. Five drops of 0.1% phenolphthalein indicator solution were added and the pH was adjusted with dilute NaOH until the solution turned slightly pink. At the equivalence point, a few drops of 0.10 M HCl were added to turn it back to clear. The dilute HCl was added drop-wise to remove any cloudiness while keeping the solution neutral. The final solution was diluted to 100 mL with deionized water and a 25 mL aliquot was transferred to a 50 mL volumetric flask along with 10 mL of the ammonium vanadate-molybdate reagent. It was filled to the mark with deionized water and allowed at least 15 min to develop. The yellow color intensity was measured at 470 nm on a Milton Roy Spectronic 21D. Calibration standards were generated with K₂HPO₄ solutions and the absorbance was plotted against mg P. The concentration of P in the unknown solutions was calculated using the Beer-Lambert equation.

Organic phosphorus capacity in hybrid polymers

The total phosphorus capacity in a phosphorus-containing hybrid polymer can be determined by the procedure noted above with organic polymers and the inorganic phosphorus capacity can be determined as noted above by dissolution in HCl. The organic phosphorus (%) in hybrid polymers is thus the difference of the total and inorganic phosphorus capacities (eq. 2).

$$\% P_{org} = (P_T - P_{inorg}) / P_T \times 100 \quad (2)$$

P_T is the total phosphorus capacity, P_{inorg} is the inorganic phosphorus capacity and P_{org} is the organic phosphorus capacity.

Phosphorus capacity of HEDP-modified HAP

HAP was modified by stirring 2.0 g of HAP with 100 mL of 0.50 M HEDP at 20°C. Six contact times were studied (17 h, 28 h, 56 h, 84 h, 112 h and 140 h) to determine the effect of reaction time on the extent of modification. The phosphorus capacities are reported in Table 1. Multiple runs show the capacities to have a standard deviation of ± 0.2 . Results indicate that the amount of organic phosphorus increases monotonically from 0 to 2.5 mmol /g over a 140 h period.

Discussion

Digestion of modified HAP with H_2SO_4 / $CuSO_4$ gave a higher (total) phosphorus capacity than digestion with HCl. As the reaction time increased, HEDP-modified HAP had a larger organic phosphorus capacity which was indicative of a greater degree of modification. Results show that the organic phosphorus capacity of HEDP-modified HAP can be determined by a combination of the P_T and P_{inorg} vanadate-molybdate procedures. The procedure will be applicable to any inorganic polymer soluble in HCl modified with organic compounds (e.g., lead phosphate as the inorganic support¹⁴ or calcium phosphate modified with poly(vinyl phosphonic acid)).¹⁵

Conclusion

An experimental procedure has been developed for hybrid hydroxyapatite but will be applicable to any inorganic polymer soluble in HCl that is modified with phosphorus-containing organic compounds. In this research, the effect of modification of HAP with HEDP (an ion – selective complexant) on the removal of metal ions from water in the environment is being investigated and the selectivity correlated with the ratio of organic to inorganic phosphate.

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Figure 1.
Oxidation of organophosphates (A) and organophosphonates (B)

Table 1

Phosphorus capacity of HEDP-modified HAP

Sample	Reaction Time	P_T (mmol/g)	P_{inorg} (mmol/g)	P_{org} (%)
A	17 h	4.72	4.68	0.7
B	28 h	4.78	4.41	7.8
C	56 h	4.92	3.84	22.0
D	84 h	5.85	3.80	35.0
E	112 h	5.15	2.64	48.7
F	140 h	4.98	2.54	49.0