Analysis of phosphatic bivalves

This method was designed for the analysis of phosphatic shells. All measurements are best done by weight, or at the very least using weight-calibrated pipettes. It is wise to prepare a set of blanks in parallel with the unknowns. Blank subtraction will be necessary in case of contamination from the acid or elsewhere.

Sample preparation

1. Thoroughly rinse the samples in deionized water and dry at 110°C for one hour.
2. Mix up 100 ml of 1:5 HCl (~2.4 molar).
3. Weigh 5 to 50 mg of shell sample into a 13 ml test tube.
4. Add 1 ml of the 1:5 HCl and let sit overnight to dissolve.
5. Add 9 g of water to bring the total volume to 10 g.
6. To rinsed ion chromatograph autosampler vials add 4.5 g of water, and then 0.5 g of the sample solution, to yield an additional dilution factor of ~10. In this solution, 5 to 50 mg of shell is effectively diluted by factors of between 20,000 to 2000, respectively.
7. You may wish to rinse, dry, and weigh the organic residue left after shell dissolution. This will allow you to calculate more accurately the composition of the dissolvable part.

Prepare the anion standard

To a 100 ml volumetric flask, add:

Ion	Stock concentration, ppm	g used	Standard/1, ppm
F^-	1000	1	10
Acetate	1000	0.1	1
Formate	1000	0.1	1
SO_4^{2-}	1000	1	10
Oxalate	1000	0.5	5
PO_4^{3-}	1000*	4	123.2

*Check label, may be 1000 ppm as P, 3080 ppm as PO_4^{3-}.

Dilute to volume and transfer into a clean 125 ml plastic bottle. This is Standard/1, which is not diluted further. Transfer the following numbers of 5.5 ml aliquots of DI water and Standard/1 to three other bottles for calibration standards. The numbers of 5.5 ml aliquots are as follows:

	Standard/1	Standard/2	Standard/5	Standard/10
5.5 ml Aliquots of DI water	-	5	8	9
5.5 ml Aliquots of Standard/1	-	5	2	1
Dilution factors	1	2	5	10

Ion	Resulting concentrations, ppm			
F^-	10	5	2	1
$Acetate^-$	1	0.5	0.2	0.1
$Formate^-$	1	0.5	0.2	0.1
SO_4^{2-}	10	5	2	1
$Oxalate^-$	5	2.5	1	0.5
PO_4^{3-}	40	20	8	4

The result will be 56 ml of Standard/1, 49.5 ml of Standard/2, and 55 ml of the others.

Prepare the cation standard

In general, standards should be similar in composition to the samples being analyzed. Since samples vary enormously, you may want to start with a generally useful standard. For this, dilute the following stock solutions in a 100 ml volumetric flask.

Ion	Stock concentration, ppm	ml used	Standard/1, ppm
Li^+	1000	0.05	0.5
Na^+	1000	0.5	5
NH_4^+	1000	0.1	1
K^+	1000	0.1	1
Mg^{2+}	1000	0.3	3
Ca^{2+}	1000	10	100
Sr^{2+}	1000	0.2	2

Dilute to volume and transfer into a clean 125 ml plastic bottle. This is Standard/1, which is not diluted further. Transfer the following numbers of 5.5 ml aliquots of DI water and Standard/1 to three other bottles for calibration standards. The numbers of 5.5 ml aliquots are as follows:

	Standard/1	Standard/2	Standard/5	Standard/10
5.5 ml Aliquots of DI water	-	5	8	9
5.5 ml Aliquots of Standard/1	-	5	2	1
Dilution factors	1	2	5	10

Ion	Resulting concentrations, ppm			
Li+	0.5	0.25	0.1	0.05
Na+	5	2.5	1	0.5
NH4+	1	0.5	0.2	0.1
K+	1	0.5	0.2	0.1
Mg2+	3	1.5	0.6	0.3
Ca2+	100	50	20	10
Sr2+	2	1	0.4	0.2

The result will be 56 ml of Standard/1, 49.5 ml of Standard/2, and 55 ml of the others.

Analysis setup

Run DI water washes before the unknowns and the blanks. Monitor analytical quality with one or more standards run as unknowns within or at the end of the run. Set all dilution factors to 1. Off-line subtract the blanks and then apply the dilution factors.

Note on the shell dissolution reaction

The shell dissolution reaction is approximately:

$Ca_5(PO_4)_3F + 10HCl = 5CaCl_2 + 3H_3PO_4 + HF$, with OH^- substituting for F^- to variable degrees.

Therefore, on a molar basis 10 times as much HCl is needed as there is shell phosphate. The 1 ml of HCl used to dissolve the sample is ~2.4 M, and contains $2.4*10^{-3}$ moles HCl. 5 to 50 mg of fluorapatite shell material has $1*10^{-5}$ to $1*10^{-4}$ moles of $Ca_5(PO_4)_3F$. So, remembering that 10 times as much HCl is needed as there is fluorapatite, there is an excess of HCl ranging from 24 (5 mg shell) to 2.4 (50 mg shell). The shell material, however, is not pure apatite and contains considerable organic material, so the actual excess of HCl is larger. This organic material will remain after dissolution and may resemble a transparent version of the original shell.

Note on acid purity

It is important that the acids be reasonably pure. The table below shows several runs made with 1:1000 HCl and HNO_3, both un-distilled reagent grade and distilled (in-house two-bottle Teflon still), and reagent grade acetic acid. As can be seen, all of the acids are very clean, with most components near or below detection limits. HCl is recommended since the chloride peak will only interfere with Cl- and NO2-, which are unimportant in these samples. HNO_3, on the other hand, elutes later and will potentially interfere with phosphate.

	HCl ACS 1	HCl ACS 2	HCl distilled	HNO_3 ACS	HNO3 distilled	HAc ACS	Blank	Blank
Glycolate		0.04	0.00	0.01	0.01		0.00	0.01
Acetate				0.01		Added		
Formate			0.01					
Propionate			0.05		0.02			0.02
Methyl-sulfonate	0.01	0.01						
Oxalate	0.00			Interference				0.02
F		0.002	0.002	0.002	0.001	0.001	0.001	
Cl	Added				0.00			0.01
NO2		0.00	0.00		0.08	0.00		0.00
SO4								
Br					0.01			
NO3	0.00	0.06		Added		0.09	0.03	0.01
PO4	0.01	0.02	0.04	0.01	0.01	0.01	0.01	0.02
Li								
Na	0.01	0.00	0.02		0.00	0.00	0.01	0.02
NH4								
K								
Mg	0.00						0.01	0.02
Ca	0.01	0.00	0.00	0.00	0.00		0.05	0.06

ACS = American Chemical Society, reagent grade acid, not distilled.

Blank cells indicate that no peak was detected.

Gray cells indicate that acids either contained the anion of interest, or caused a large interference on another ion.