

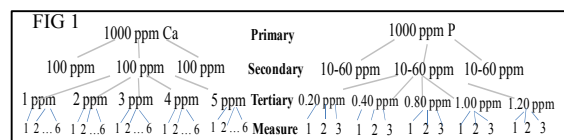
Practical Considerations in Accurate Determination of Ca/P Ratios of Calcium Phosphate Materials

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Statement of Purpose: Wet chemical analysis of calcium (Ca) and phosphorus (P) content by atomic absorbance (AA) and UV-vis spectrophotometry is a generally accepted method for determination of Ca/P molar ratio for synthetic calcium phosphate as well as monitoring compositions of simulated body fluid and solution deposition of biomimetic Ca-P coatings. The latter usually contains electrolytes, including NaCl and KCl. Accurate determination of Ca and P ratios require robust measurements of calcium concentration [Ca] and [P]. Variations of [Ca] or [P] can originate from combinations of repeatability of absorbance measurement by equipment, calibration curve, volumetric tool accuracy, solution compositions and other random errors. Among all variables, robust absorbance measurement and calibration curve play a vital role. For instance, Ca absorbance is related to ground state Ca^0 atoms and can be affected by electrolyte composition (NaCl, KCl). On the other hand, a robust Ca calibration curve is critical to convert absorbance into reproducible [Ca] data. Deviation can be introduced during the process of preparing calibration standards. In fact, a 2-5% deviation in determination of [Ca] and [P] is not uncommon. A 5% deviation of [Ca] and [P] data could mistakenly generate Ca/P ratios ranging from 1.508 to 1.842 for a stoichiometric HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, Ca/P = 1.667). The goal of this study was to investigate the effect of electrolytes on Ca absorbance and use a design of experiment (DOE) study to identify key variables in determination of Ca/P molar ratios.

Methods: Stoichiometric HA (NIST SRM 2910a) was used as a gold standard for variable analysis. 1 mg HA powder was dissolved in 1.1 ml of 6N HCL and finalized in a 50mL volumetric flask with DI water. Ca primary standard (1000±4 ppm, Sigma Aldrich) was diluted to prepare AA calibration standards from 1 to 5 ppm in 0.8 mM LaCl_3 ; 0.02 N HCl in order to determine linear working range of AA. Additional amount of KCl (0 to 160 mM) was also added to standards to investigate the effect on absorbance. P primary standard (1000±4 ppm, Sigma Aldrich) was used to prepare UV-vis calibration standards from 0.2 to 2 ppm with 0.01M ascorbic



acid:0.004M ammonium molybdate matrix solution (0.6M H_2SO_4). All P samples were placed in an oven at 37°C for 3 hours. For AA calibration, three sets of standards from 1 to 5 ppm were generated as illustrated in Fig. 1. Similar calibration standards were prepared for P (Fig. 1). Fig. 2 shows design of experiment (DOE) to analyze Ca/P ratio of NIST HA solution. Ca absorbance was measured by AA (Perkin Elmer Analyst 800) with air-acetylene flame and a 422.7nm wavelength line. P absorbance was measured colorimetrically using UV-vis (Thermo Fisher Scientific Helios β) with an 826nm wavelength line following the Molybdenum Blue Method.

Results: Fig. 3 shows the effect of KCl on absorbance of a 3.2 ppm Ca solution. Ca absorbance varies randomly (18% deviation) when [KCl] ranges between zero and 1 mM. At $[\text{KCl}] > 50$ mM, Ca absorbance is more reproducible. Compared to Ca absorbance without KCl, there is a roughly 10% absorbance increase at $[\text{KCl}] > 50$ mM. The optimum [KCl] was determined to be 110mM, allowing at least ±10% variation without affecting absorbance. Fig. 3 (right) shows Ca absorbance between 0.1 and 60ppm, which is best fitted ($R^2 = 0.9997$) by the third polynomial curve. Linearity is most strictly maintained between 1-5ppm ($R^2 \geq 0.9990$). It is generally erroneous to assume (use) a linear calibration curve to calculate [Ca] outside the linear zone. Similar linear range is confirmed for [P] between 0.2-2.0 ppm ($R^2 \geq 0.9990$).

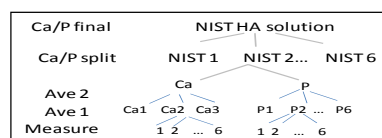


FIG 2

Table 1 shows the minimum (Min) and maximum (Max) Ca/P ratios of NIST HA using the designated Ca calibration curves (Ca-set1, set2, set3) and P calibration curves (P-set1, set2, set3). In each paired calibration curve, range of Ca/P ratios is almost unchanged but the Min drifts from 1.614 to 1.664 and Max from 1.680 to 1.725. Average of all Ca/P ratios in the DOE shows a $\text{Ca/P} = 1.668 \pm 0.033$, which agrees well with NIST data of 1.667 ± 0.037 [1].

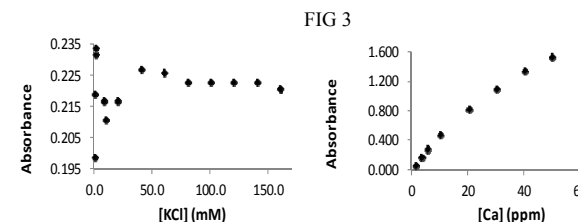


FIG 3

Table 1	P-set1	P-set2	P-set3
Ca-set1	1.615,1.683	1.614, 1.680	1.621,1.688
Ca-set2	1.657,1.728	1.656,1.725	1.664,1.732
Ca-set3	1.640, 1.707	1.633, 1.700	1.640, 1.707

Conclusions: Electrolyte concentration (KCl) can significantly increase Ca absorbance. A minimum of 50 mM KCl is required to reach a stable Ca absorbance reading. The calibration curve is a major contributor of variation in determination of Ca/P ratio. Using three sets of calibration standards greatly reduces this variation and results in ratios that are repeatable and reproducible.

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References: [1] Markovic, M. In: Mineral and Scale Formation, Proc of ACS Symposium, (Ed. Z. Amjad, Plenum, NY, pp. 271-282 (1995).