1   **Substitution of sulfate in apatite**

2   **REVISION**

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12   **Abstract**

13   The substitution of sulfate in apatite is of potential importance in

14   synthetic biomaterials used in bone repair and reconstruction. The

15   counter ion (e.g., Na^+, K^+, Mg^{2+}, Sr^{2+}) in the sulfate reagent may also be

16   used as a source of medically beneficial ions. An understanding of the

17   structural parameters controlling sulfate substitution is also important in

18   expanding our knowledge beyond the substitution of carbonate in apatites.

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20   The incorporation of sulfate in calcium and strontium hydroxylapatites,

21   prepared in aqueous solution at pH 9, was verified by combustion

22   analysis of sulfate, infrared and Raman spectroscopy, and by

23   determination of unit cell parameters. Sulfate could not be incorporated

24   into barium hydroxylapatite because of the preferential formation of

25      $BaSO_4$.

26

27   The amount of sulfate substituted in the apatite was affected by the mole

28   ratio of sulfate to phosphate in the reaction mixture and by the nature of

29   the counter ion in the sulfate reagent. When sodium is the counter ion in

30   the sulfate reagent, the molar amounts of both sodium and sulfate in the

31   product apatite can be explained by assuming charge compensation by

32   sodium ions and sulfate displacement of phosphate and calcium. With

33   lithium as the counter ion, a greater molar amount of lithium than sulfate

34   is incorporated into the apatite, an observation that requires an additional

35   charge-compensation mechanism. With potassium and rubidium as

36   counter ions, less of the counter ion is incorporated than sulfate, probably

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37   a result of less favorable accommodation of the larger cation in the

38   apatite structure.

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40   The maximum molar amount of sulfate incorporated in hydroxylapatite

41   (prepared in the presence of Na^+) is more than three times lower than the

42   maximum molar amount of carbonate that can be incorporated, a

43   difference that can be explained by the relative solubilities of the

44   substituted apatites. The unit cell parameters determined for both

45   sulfated calcium and strontium hydroxylapatites synthesized with the

46   sodium counter ion show a slight increase in the a-axis length and a

47   nearly constant c-axis length with increasing sulfate content. The

48   difference in the variation of unit cell parameters with anion content can

49   be rationalized by the difference in size of the anion.

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51   The results indicate that sulfate can be incorporated into biomaterials

52   such as apatite or in composites with calcium sulfate and that the design

53   of new apatites and composites could include the use of medically

54   desirable counter cations.

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56 **Keywords:** Apatite, sulfate, incorporation of sulfate, unit cell, IR,

57 strontium apatite, sulfated apatite, calcium sulfate-apatite composite

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59 **Introduction**

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61 Calcium sulfate has been used as a biomaterial since the later part of the

62 19th century. It has been used to repair bone defects, augment sinuses,

63 and in a variety of dental applications (Thomas and Puleo 2008; Ricci et

64 al. 2000). Discussions of the mechanism by which this extraordinary

65 compound operates usually focus on the efficacy of its resorption and its

66 ability to initiate production of a calcium-phosphate lattice; that is, to

67 stimulate new bone growth (Ricci 2000). Increased calcium ion

68 concentrations may act as a stimulus to osteoblasts (bone-producing

69 cells) and inhibitor of osteoclast (bone-dissolving cells) activity (Thomas

70 and Puleo 2008). Calcium sulfate has been combined with organic

71 compounds such as gelatin, poly(lactic) acid, and carboxymethylcellulose,

72 as well as calcium phosphate and hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$, in

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73   this work designated as CaApOH). Parsons et al. (1988) report on

74   mixtures that are "osteoconductive composite grouts for orthopedic use."

75   Mixtures of calcium sulfate with either apatite or calcium phosphate have

76   been reported to resorb more slowly, which can have clinical advantages

77   (Parsons et al. 1988; Urban et al. 2007; Fillingham et al. 2012; Yang et al.

78   2012; Kuo et al. 2015). Because apatite has a singular ability to

79   accommodate ions by substitution, it is possible that the efficacy in bone

80   healing/renewal of calcium sulfate alone and in composites together with

81   hydroxylapatite may involve substitution of sulfate in the newly formed

82   biological apatite. Moreover, the use of sulfated apatite (SCaApOH),

83   rather than CaApOH, in mixtures with calcium sulfate may be

84   advantageous.

85

86   Among the many anions that can function as substituents in apatite,

87   carbonate has received the most attention primarily due to its presence in

88   bones and teeth. The substitution of sulfate ion, on the other hand, is

89   poorly understood. One might anticipate that on the basis of its charge,

90   sulfate would behave like carbonate and replace either phosphate (B-type

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91   substitution) or the monovalent ion (A-type substitution). On the other

92   hand, its structural and electronic similarity to phosphate—sulfate and

93   phosphate are isoelectronic-- suggest that sulfate might only replace

94   phosphate, with appropriate accommodations to maintain local charge

95   neutrality in the apatite structure. The larger size of sulfate mitigates

96   against its ability to substitute for monovalent anions in the apatite

97   channels. The smaller carbonate ion, which has a volume of 28 $Å^3$ (as

98   opposed to 43 $Å^3$ for sulfate, both calculated from thermodynamic radii

99   (Roobottom 1999)), can replace channel hydroxide to a limited extent in

100   apatites that have estimated channel volumes of about 30 $Å^3$ or less

101   (Goldenberg et al. 2015).

102

103   That sulfate can take the place of phosphate to produce compounds with

104   an apatitic stoichiometry and structure is supported by the existence of

105   "sulfate" apatites, such as $Na_6Ca_4(SO_4)_6F_2$, and $Na_6Pb_4(SO_4)_6F_2$ (Klement

106   et al. 1939; Kreidler and Hummel 1970), and compounds containing

107   both orthosilicate and sulfate, such as $M_{10}(SiO_4)_3(SO_4)_3F_2$, where M =

108   Ca, Sr, and Pb (Kreidler and Hummel 1970) occur naturally (ellestadite is

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109   the F/OH end member for M = Ca, McConnell 1937; Khorari et al. 1994)

110   and also have been synthesized. A recent study of sulfate substitution in

111   hydroxylapatite, using microwave heating of the aqueous reaction

112   mixture, produced a compound whose formula was given as $Ca_{10}(PO_4)_{6-}$

113    $_x(SO_4)_x(OH)_{2-x}$, with x = 0.05-0.5 (Alshemary et al. 2013). Evidence for

114   the incorporation of sulfate included X-ray diffraction and IR peak

115   broadening, as well as an increase in both the a- and c-axis lengths with

116   increased sulfate concentration in the product. Hydrothermal processing

117   of a mixture of sulfate and amorphous calcium phosphate produced

118   sulfated hydroxylapatites that showed no change in lattice parameters

119   (Toyama et al. 2013).

120

121   In order to explore the possible role of sulfate substitution in apatite as

122   part of the biocompatibility and resorbability of calcium sulfate and

123   sulfated hydroxylapatite, we report here our studies of sulfate

124   incorporation into hydroxylapatite. Because biomaterials can be

125   designed with ancillary ions that can provide additional nutrients

126   (Pasteris 2016), we have also studied the effect of the counter ion in the

127   sulfate reagent on the incorporation process. The use of selected counter

128   ions (e.g., Li^+, Na^+, K^+, Zn^{2+}, Mg^{2+}) in the sulfate reagent may produce

129   apatites deliberately doped with medically desirable ions that could be

130   released during dissolution/resorption of the biomaterial. To provide

131   greater depth of understanding about substitution processes in apatite, we

132   also include our work on sulfate substitution in strontium and barium

133   hydroxylapatites.

134

135   **Experimental Methods**

136   Milli-Q deionized water and ACS reagent grade reagents with purities

137   above 98% were used for the preparation of all samples.

138   **Syntheses**

139   Sulfated calcium and strontium apatites were synthesized in a round

140   bottom flask using the following solutions: 0.33 M $Ca(NO_3)_2 \cdot 4H_2O$ or

141   0.33 M $Sr(NO_3)_2$, 0.20 M A_2SO_4, and 0.20 M $NH_4H_2PO_4$ with A = Li,

142   Na, K, Rb. The amounts of each solution used were based on the 5:3:1

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143   stoichiometry of $M_5(PO_4)_3OH$ rather than the formula (vide infra) of the

144   desired apatite. The amount of the sulfate solution was varied to provide

145   sulfate to phosphate mole ratios of 0.5 to 1 up to 4 to 1 (sulfate to

146   phosphate). In separate dropping funnels, the solutions of $NH_4H_2PO_4$ and

147    $M(NO_3)_2$ were added dropwise at 0.5 drop per second to the A_2SO_4

148   solution, magnetically stirred in a round bottom flask at 80 ˚C. The pH of

149   the mixture was adjusted to 9 with 3 M NH_3 after 10 drops of each

150   reagent were added. The pH of the mixture was maintained at 9 during

151   the addition and digestion periods using 3 M NH_3. After the digestion

152   time of 24 hours, during which the reaction mixture was stirred and

153   heated, the white precipitate was suction filtered and washed 6 times

154   using Milli-Q water. The product was dried in a vacuum oven for 24

155   hours at 120 ˚C and 5 torr. The yields were 85-95%.

156   **Identification and Analysis**

157   All products were identified by X-ray diffraction using a PANalytical

158   X'Pert PRO Multi purpose diffractometer Theta-Theta System with Cu-

159   Ka radiation (λ=1.54060 Å) and analyzed using the PANalytical program

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160   X'Pert Highscore Plus. Samples were prepared on a 32 mm glass slide

161   for scanning (30 minutes) with a range from 5 to 70° 2θ.

162   IR spectra were obtained on a Bruker Tensor 37 IR Spectrometer with a

163   Ge ATR mount using 256 scans and a resolution of 2 cm^{-1}.

164   In addition to combustion analysis of sulfur (Galbraith Laboratories,

165   Knoxville TN and in-house use of a Costech ECS 4010 CHNS-O

166   system), the presence of sulfate in each apatite sample was confirmed by

167   precipitation of $BaSO_4$ from an acid solution (3M HNO_3) of the sulfated

168   apatite.

169   The weight percent of metal ions, phosphorus, and sulfur were obtained

170   using a SPECTRO Analytical ICP-AES SPECTROBLUE spectrometer

171   (Wavelengths (nm): Ca=422.673, P=177.495, Na=589.592, Li=670.780,

172   K=769.896, S=182.034). Calibration curves were prepared by dilution of

173   commercial standards of Ca, K, Na, Li, S, and P (1000 ppm) with 8 M

174   HNO_3. Apatites were dissolved in 8 M HNO_3 solution before analysis.

175   The errors in all elemental data are expected to be no greater than 5%.

176   Elemental composition was also obtained using X-ray fluorescence

177   spectroscopy with a Panalytical PW 2404 Vacuum Spectrometer

178   equipped with a 4kW Rh X-ray tube. Sample preparation involved

179   ignition to 1200 °C to create an anhydrous powder, followed by

180   preparation of glass discs using nine parts lithium tetraborate and one part

181   anhydrous sample material.

182   Unit cell parameters were determined from 2.5-hour XRD scans of

183   samples. Peaks were indexed following a star-quality PDF card that

184   matched the experimental pattern with the highest score. The program

185   UnitCell for OS X (Holland et al. 1997) provided an initial set of a- and

186   c-axis values that were refined by eliminating potentially deleterious

187   peaks. Unit cell parameters were also determined with the Panalytical

188   X'pert Highscore Plus (version 2.3e) program. Based on repeated

189   determinations of unit cell parameters on one compound the error is

190   estimated to be ±0.001 Å. The two methods produced cell parameters

191   generally within ±0.001 Å.

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193   **Results and Discussion**

194

195   **Synthesis**

196   Sulfated calcium and strontium hydroxylapatites were prepared by

197   aqueous addition of reagents containing lithium, sodium, potassium, and

198   rubidium as the sulfate counter ion. The reaction of sodium sulfate with

199   calcium nitrate and ammonium dihydrogen phosphate produced sulfated

200   calcium hydroxylapatites (SCaApOH) containing 2 to 8 wt% sulfate. The

201   relationship between the weight percent sulfate in the apatite and the

202   mole ratio of sulfate to phosphate used in the reaction mixture is shown

203   in Figure 1, which indicates saturation of the apatite structure with sulfate

204   at about 8 wt% (with sodium as the sulfate counter ion). $CaSO_4$

205   (gypsum) was detected by Raman spectroscopy in reactions that utilized a

206   4 to 1 mole ratio of sodium sulfate to phosphate: at this mole ratio

207   formation of the slightly insoluble $CaSO_4$ becomes competitive with

208   continued incorporation of sulfate.

209   **Figure 1**.

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210   By contrast, the maximum weight percent carbonate incorporated in

211   calcium hydroxylapatites is about 17% (Pasteris et al. 2014) (22.5% was

212   reported by LeGeros et al. 1967), indicating that a significantly greater

213   number of moles of carbonate can be incorporated in the structure. This

214   difference in the amount of anion incorporated can be attributed to the

215   greater insolubility of carbonated apatite relative to sulfated apatite, as

216   rationalized using the simple salt approximation for the solubility of

217   double salts (Yoder and Rowand 2006).

218

219   Sulfated strontium hydroxylapatites were prepared analogously, but the

220   formation of $SrSO_4$ was observed at mole ratios (sulfate to phosphate) of

221   0.4 to 1 and higher. The use of mole ratios of 0.1 to 1 up to 0.4 to 1

222   produced sulfated strontium hydroxylapatite with sulfate weight

223   percentages ranging from 1.2 to 2.2 (and no $SrSO_4$ detectable by XRD).

224   Attempts to prepare sulfated barium hydroxylapatites (SBaApOH) lead to

225   the formation of $BaSO_4$ even at a mole ratio of 0.1 to 1. The change in

226   the amount of sulfate incorporated in the alkaline earth apatites is likely

227   due primarily to the decrease in solubility of the sulfate salts (K_{sp} $CaSO_4$,

13

228   10^{-5}; $SrSO_4$, 10^{-7}; $BaSO_4$, 10^{-10}), which are formed in the reaction

229   mixtures.

230

231   **Composition**

232   Powder X-ray diffraction patterns of the sulfated apatites (SCaApOH)

233   were in good agreement with the pattern for CaApOH (PDF 98-002-

234   2060) and showed no appreciable change in line widths with increased

235   incorporation of sulfate. The wt% Na and SO_4 of selected calcium

236   hydroxylapatites prepared with sodium sulfate present in the reaction

237   mixture are shown in Table 1. The samples are arranged in order of

238   increasing wt% SO_4, and it is clear that the wt% Na increases in the same

239   order. Indeed, the number of moles of sodium is roughly the same as that

240   of sulfate. Thus, the incorporation of sulfate is accompanied by the

241   incorporation of sodium.

242

14

243   **Table 1**. Weight percent of sodium and sulfate in selected sulfated

244   calcium hydroxylapatites prepared using sodium sulfate.

Sample	Wt% Na	Wt% SO$_4$
1	0.02	0.04
2	0.07	1.69
3	0.93	2.79
4	1.13	3.90
5	1.22	4.19
6	1.68	5.51
7	1.95	6.47

245

246

247   A formula for the sulfated hydroxylapatites may be determined from the

248   relationship between the number of moles of calcium and sulfate and the

249   number of moles of alkali metal counter ions in each of the

250   hydroxylapatites. These relationships appear in Figures 2 and 3. Figure 2

251   shows an inverse 1 to 1 relationship between calcium and sulfate ions:

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252   substitution of sulfate is accompanied by a loss of calcium ions. With the

253   number of moles of calcium normalized to 10 for the compound with

254   zero moles of sulfate (where the formula of the compound should be

255    $Ca_{10}(PO_4)_6(OH)_2$) the intersection of the extrapolated line in Figure 2

256   with the x-axis suggests that a limiting sulfated hydroxylapatite prepared

257   with sodium as the counter ion may contain three moles of sulfate and

258   seven moles of calcium.

259   **Figure 2**.

260   Figure 3 shows the relationship between moles of sulfate and the moles

261   of alkali metal counter ion (present in the sulfate reactant) incorporated.

262   This reactant in most cases was Na_2SO_4, but in five reactions Li_2SO_4 was

263   used, and in five K_2SO_4 was one of the reactants (Rb_2SO_4 was also

264   employed to confirm observations, vide infra). It is well known that Na^+

265   can be incorporated into the apatite lattice. Limited substitution of Li, Na,

266   and K for calcium has been reported (Simpson 1968; Mayer et al. 1983;

267   Fleet and Liu 2007; Whyte et al. 2008; Zyman et al. 2013; Yoder et al.

268   2016).

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16

270   **Charge-compensation**

271   The presence of the counter ion in the apatite structure, presumably as

272   replacements for some of the Ca(2) cations in the apatite channel (Pan

273   and Fleet,, 2002), is to be expected given the charge compensation

274   necessary for substitution of a -2 anion (SO_4^{2-}) for a -3 anion (PO_4^{3-}).

275   De Maeyer et al. (1996) provide six fundamental substitution schemes for

276   carbonate substitution, of which five are pertinent to B-type substitution.

277   These are rewritten below for sulfate substitution and without designation

278   of vacancies (M represents the counter ion):

279

280    $\quad\quad$ (1) SO_4^{2-} → PO_4^{3-} + Ca^{2+} + OH^-

281    $\quad\quad$ (2) $2\,SO_4^{2-}$ → $2\,PO_4^{3-}$ + Ca^{2+}

282    $\quad\quad$ (3) SO_4^{2-} + M^+ → PO_4^{3-} + Ca^{2+}

283    $\quad\quad$ (4) M^+ → Ca^{2+} + OH^-

284    $\quad\quad$ (5) SO_4^{2-} + OH^- → PO_4^{3-}
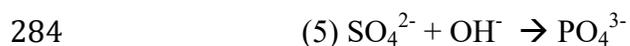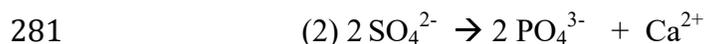
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286   Although our reactions were carried out at pH 9, scheme (5) is probably

287   not likely and was not observed in the extensive study of De Maeyer et al.

17

288   (1996). De Maeyer et al. (1996) found that schemes (1) and (2) were

289   predominantly employed in their synthetic methods using sodium and

290   potassium salts to synthesize carbonated apatites. It is important to realize

291   that the charge compensation scheme used during the synthesis is

292   dependent on the reagents and conditions. The stoichiometry of the

293   product apatite also may be best explained by a combination of charge

294   compensation schemes.

295

296   Interestingly, each of the alkali metal cations has its own relationship (Fig.

297   3) to sulfate content, with the lines for all three counter ions extrapolating

298   to close to zero at 0 moles of sulfate. The slopes of the lines are very

299   different, presumably a result of a change in charge compensation

300   mechanism and/or stereo-structural constraints. The relationship between

301   moles of counter ion and moles of SO_4^{2-} incorporated is approximately 1

302   to 1 for Na^+, less than 1 for K^+, and greater than 1 for Li^+. In other words,

303   the incorporation of, say, 0.05 mole of sulfate is accompanied by the

304   incorporation of roughly the same amount of Na^+, about 0.01 mole of K^+

305   and almost 0.1 mole of Li^+.

18

306   **Figure 3.**

307

308   The charge balance mechanism for the sulfated hydroxylapatites made

309   with sodium as the counter ion is therefore almost surely the replacement

310   of one calcium ion by one sodium ion (scheme 3):

311

312                         $SO_4^{2-} + Na^+ = PO_4^{3-} + Ca^{2+}$
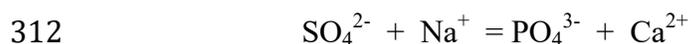
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314   This relationship is similar to that found by Montel et al. (1981) for

315   carbonated hydroxylapatites. Thus, the formula of the sulfated

316   hydroxylapatites precipitated in the presence of sodium ion can be written

317   as $Ca_{10-x}(Na)_x(PO_4)_{6-x}(SO_4)_x(OH)_2$, where x = moles sulfate,. For the

318   limiting apatite containing 3 moles of sulfate (vide supra) the formula is

319     $Ca_7Na_3(PO_4)_3(SO_4)_3(OH)_2$, which contains 30% sulfate and cannot be

320   prepared using our experimental procedure.

321

322   Because the sulfated hydroxylapatites prepared using lithium sulfate

323   contain more than twice as much lithium as sulfate, the charge balance

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324   mechanism cannot be expressed using only schemes (1), (2), or (3). The

325   addition of scheme (4), the independent incorporation of Li^+, is necessary

326   and also requires removal of calcium and hydroxide ions.

327   (4) $M^+ \rightarrow Ca^{2+} + OH^-$

328 Enhanced uptake of lithium in the synthesis of carbonated apatites was also

329 reported by Mayer et al. (1986). In the IR spectra (Figure 4) of the lithium

330 sulfate incorporated apatites, the OH peak at 3570 cm^{-1} decreases as the

331 amount of sulfate increases, consistent with the operation of schemes (1) or

332 (4).

333   **Figure 4**.

334

335   For those apatites sulfated using K_2SO_4, considerably less K^+ is present

336   than required by scheme (3). For these compounds most of the sulfate

337   incorporation occurs via scheme (1) or (2). The small amount of

338   potassium in the sulfated apatites is contrary to the composition of

339   carbonated apatites containing potassium prepared at high temperatures

340   where the mole ratio of potassium and carbonate is close to 1 to 1

341   (Verbeeck et al. 1995).   It is also clear from Figure 3 that the amount of

20

342   sulfate incorporated when the counter ion is potassium is two to three

343   times smaller than the amount incorporated with Na as counter ion. Thus,

344   some sulfate may be incorporated with simultaneous incorporation of

345   potassium (scheme (3)) but most of the sulfate enters through the

346   operation of schemes (1) or (2). Because the total amount of sulfate is

347   low, this observation indicates that the use of scheme (1) or (2)

348   contributes a smaller amount to charge-compensation than does scheme

349   (3) when lithium and sodium are the counter ions.

350

351   The relatively minor incorporation of K^+ suggests that larger cations may

352   not easily be incorporated into the apatite structure. This observation is

353   supported by the even smaller molar amounts of rubidium found in

354   SCaApOH prepared using Rb_2SO_4. For the apatite prepared using the

355   largest mole ratio of 3 to 1 (sulfate to phosphate) only 0.04 wt% Rb was

356   found compared to 0.2 wt% K in an apatite prepared using a similar mole

357   ratio (this corresponds to the incorporation of about a tenth as many

358   moles of Rb as K). It is worth noting that the molar amount of alkali

359   metal ions incorporated follows the trend $Li^+ > Na^+ > K^+ > Rb^+$, which is

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360   the inverse order of their 6-coordinate ionic radii. Both K^+ (1.38 Å) and

361   Rb^+ (1.52 Å) are significantly larger than Ca^{2+} (1.00 Å).

362

363   **IR and Raman spectra**

364   The presence of incorporated sulfate in both CaApOH and SrApOH

365   samples was confirmed by IR and Raman spectra. The Raman spectrum

366   of SSrApOH containing 4 wt% sulfate contains a peak at 1004 cm^{-1},

367   consistent with the sulfate symmetric stretching (v_1) peak at 1008 cm^{-1} in

368   gypsum, as well as a peak at 3570 cm^{-1} for the OH stretch (Fig. 5). These

369   assignments are consistent with those of Pasteris et al. (2014) for the OH

370   stretch and Liu et al. (2009) for the symmetric sulfate (v_1) stretching

371   mode. IR spectra for four SCaApOH samples prepared using various

372   mole ratios of sulfate to phosphate show an increase in the asymmetric

373   sulfate stretching (v_3) peak intensity at ca. 1100 cm^{-1} consistent with an

374   increase in sulfate content (Fig. 6).

375   **Figure 5**

376   **Figure 6**

377

22

378   **Unit cell parameters**

379   Additional evidence for the incorporation of sulfate into the apatite

380   structure comes from the relationship between wt% sulfate and the unit

381   cell axial lengths (Fig. 7). The increase in the a-axis length over a range

382   of 2 wt% sulfate is about 0.015 Å. The increase in a-axis length is not

383   consistent with the thermodynamic radius of sulfate (2.18 Å, Roobottom

384   et al., 1999) relative to that of phosphate (2.47 Å, derived from the

385   apatite-appropriate volume of 0.063 nm^3, Flora et al. 2004) but is

386   consistent with Dasent's values (Dasent 1982) of 2.58 Å for sulfate and

387   2.38 Å for phosphate as well as the ion volumes of 0.091 nm^3 for sulfate

388   (Marcus et al. 2002) and 0.063 nm^3 for phosphate (Flora et al. 2004). An

389   increase in a-axis length (as well as the c-axis length) was also observed

390   by Alshemary et al. (2013), whereas Toyama et al. (2013) found no

391   change in axial lengths.

392

393   The variation in axial lengths was also determined for a series of sulfated

394   strontium hydroxylapatites using four samples prepared with Na_2SO_4 at

395   low mole ratios (0.1 to 1 up to 0.4 to 1) and two obtained at higher mole

23

396   ratios where $SrSO_4$ was also present as a separate phase. These axial

397   lengths (Fig. 8) show the same increase in a-axis with percent sulfate

398   observed for the calcium analogs (Fig. 7).

399   **Figure 8**.

400

401   **Implications**

402   Our study reveals that sulfate can be incorporated into CaApOH, that the

403   sulfated apatites can be identified by IR spectroscopy, and that the

404   crystallite size, at least as indicated by XRD line widths, does not vary

405   significantly with increased incorporation of sulfate. The lattice

406   parameters for the sulfated apatites are similar to those of CaApOH, but

407   the a-axis length increases with an increase in wt% sulfate.

408

409   Because on the recognition of the therapeutic properties of calcium

410   sulfate in bone healing and reconstruction, it is reasonable to assume that

411   the incorporation of sulfate in apatite should be explored in creating new

412   biomaterials using sulfated calcium apatite alone or in a composite with

413   calcium sulfate. Sulfation of apatite may also be involved in creating

24

414   new bone material using calcium sulfate and calcium sulfate-

415   phosphate/apatite composites.

416

417   Because the counter ion of the sulfate reagent is incorporated into the

418   apatite, to an extent dependent on the size of the ion and the ratio of the

419   reagent to phosphate, the ions present in the sulfate reactant can be

420   tailored to produce beneficial effects on the bone mineral (LeGeros et al.

421   2009; Shepherd and Best 2011; Shepherd, et al. 2012). Substituted

422   apatites generally have a greater solubility than the parent apatite,

423   potentially increasing their resorbability and biocompatibility.

424

425   Although the solubility of sulfated apatite has not yet been determined, it

426   is likely that sulfated apatite is more soluble than carbonated apatite

427   (Yoder and Rowand 2006), but it contains a substituent (the sulfate ion)

428   that is the conjugate base of a strong acid, whereas carbonate is the

429   conjugate base of a weak acid. This difference in basicity of the

430   substituent ion may affect the solubility of the substituted apatite as a

25

431   function of pH, which is likely to be related to bioresorption of the apatite

432   (Arnett 2008).

433

434   More generally, our work shows that the extent of sulfation (and, perhaps,

435   most substitutions in the apatite structure) depends on the nature of the

436   counter ion in the sulfate reactant.   We also suggest that the size of the

437   substituent determines the type of substitution (A- vs B-type) and the

438   relative solubilities of the substituted apatite (SCaApOH vs CCaApOH).

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440

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26

449   analyses by Karen Mertzman and XRF analyses by Stanley Mertzman at

450   Franklin & Marshall College.

451

452

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608

609   **Tables**:

610

611   **Table 1**. Weight percent of sodium and sulfate in selected sulfated

612   calcium hydroxylapatites prepared using sodium sulfate.

Sample	Na^+	SO_4^{2-}
1	0.02	0.04
2	0.07	1.69
3	0.93	2.79
4	1.13	3.90
5	1.22	4.19
6	1.68	5.51
7	1.95	6.47

613

614   **List of Figure captions**:

615

36

616   **Figure 1**. The wt% sulfate incorporated into CaApOH as a function of

617   the mole ratio of sulfate to phosphate in the reaction mixture with sodium

618   as counter ion.

619

620   **Figure 2**. Relationship between calcium content and amount of sulfate

621   incorporated in calcium hydroxylapatites with sodium as counter ion.

622

623   **Figure 3**. Relationship between sulfate and counter cation content.

624   Squares = Li, diamonds = Na, triangles = K.

625

626   **Figure 4**. The OH region of the IR spectra of several SCaApOH apatites

627   prepared using lithium as the counter ion. Spectra normalized to the ν_3

628   phosphate band.

629

630   **Figure 5.** The Raman spectra of the phosphate and sulfate stretching

631   regions of top, SSrApOH showing the ν_1 phosphate stretch at 948 cm^{-1}

632   and the ν_1 sulfate stretch at 1000 cm^{-1}, middle, gypsum (1008 cm^{-1}) and,

633   bottom, non-sulfated CaApOH (Sigma-Aldrich).

37

634

635   **Figure 6**. The 960 to 1200 cm^{-1} region of the IR spectra in SCaApOH

636   samples containing varying amounts of sulfate. The peak at 1030 cm^{-1} is

637   the ν_3 P-O stretching band; the peak at ca. 1100 cm^{-1} is the ν_3 S-O

638   stretching band.

639

640   **Figure 7**. The effect of incorporated sulfate on the unit cell a- and c-

641   axial lengths for SCaApOH synthesized using sodium as the counter ion.

642   The a-axis is represented by triangles and the c-axis by circles.

643

644   **Figure 8**. Variation of unit cell axial lengths with sulfate percentage for

645   SSrApOH. The a-axis is represented by triangles and the c-axis by

646   circles.

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648

649   **Figures**:

650

651   **Figure 1**

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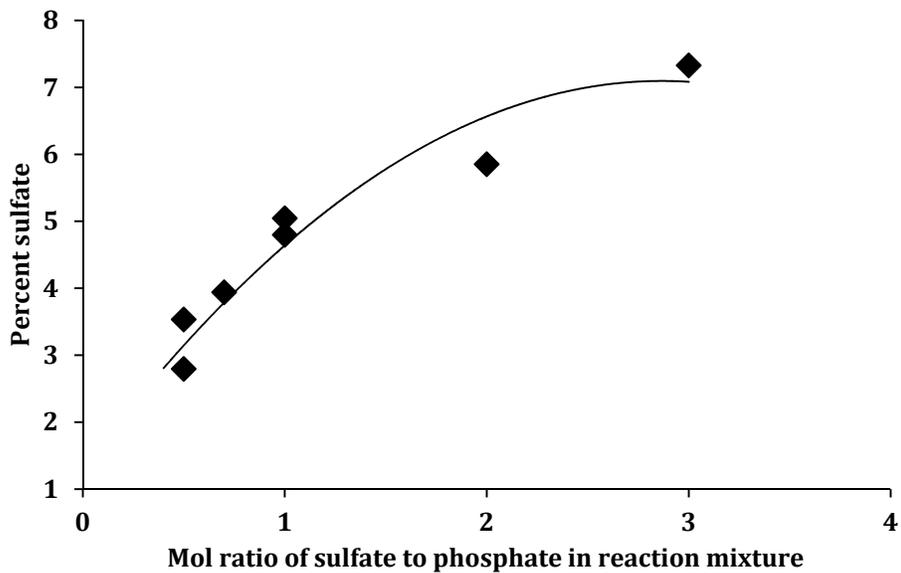
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655   **Figure 2**

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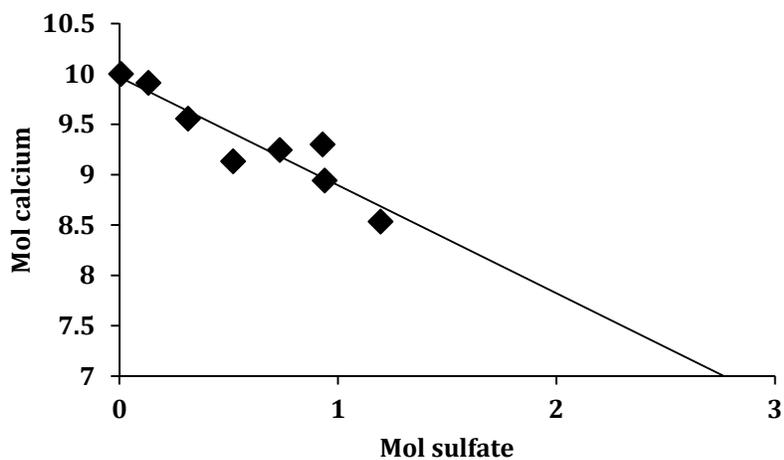
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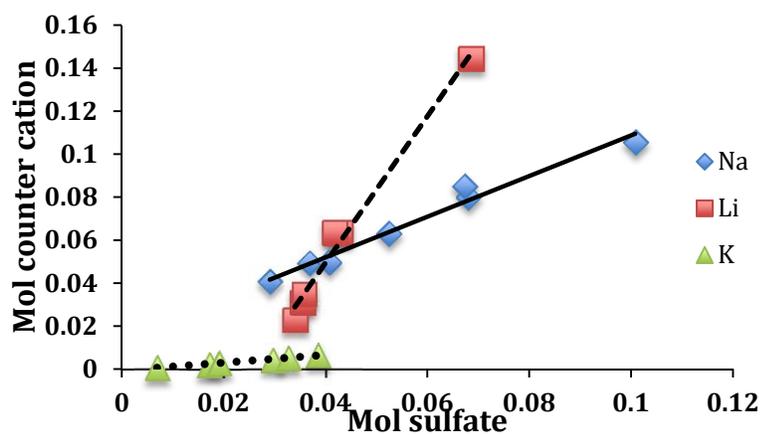
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661   **Figure 4**

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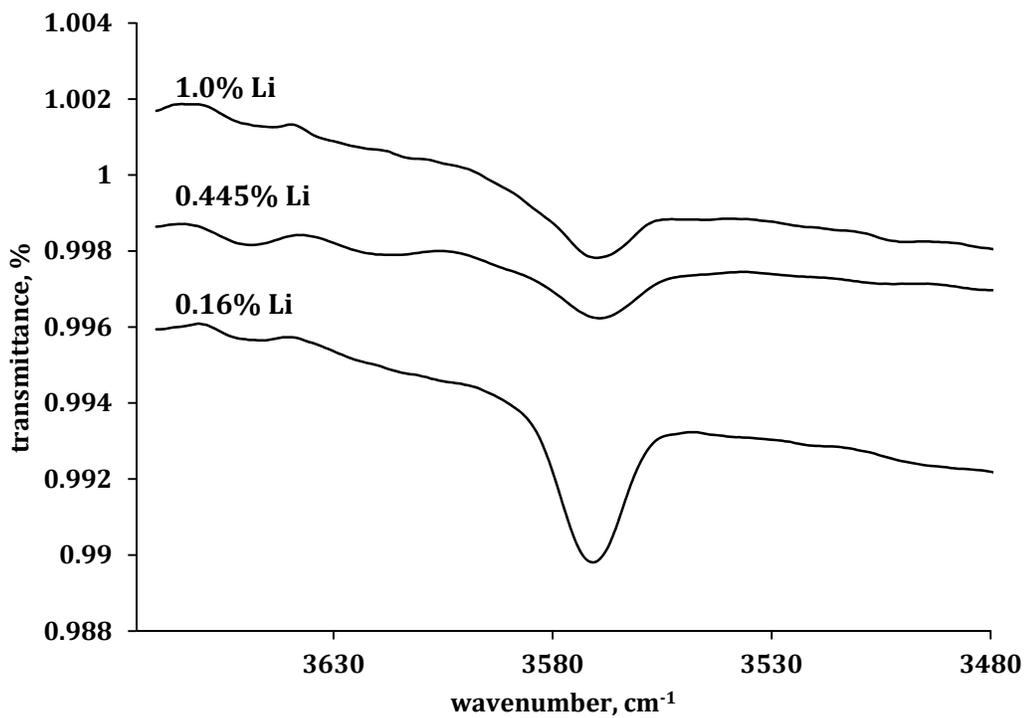
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665   **Figure 5**

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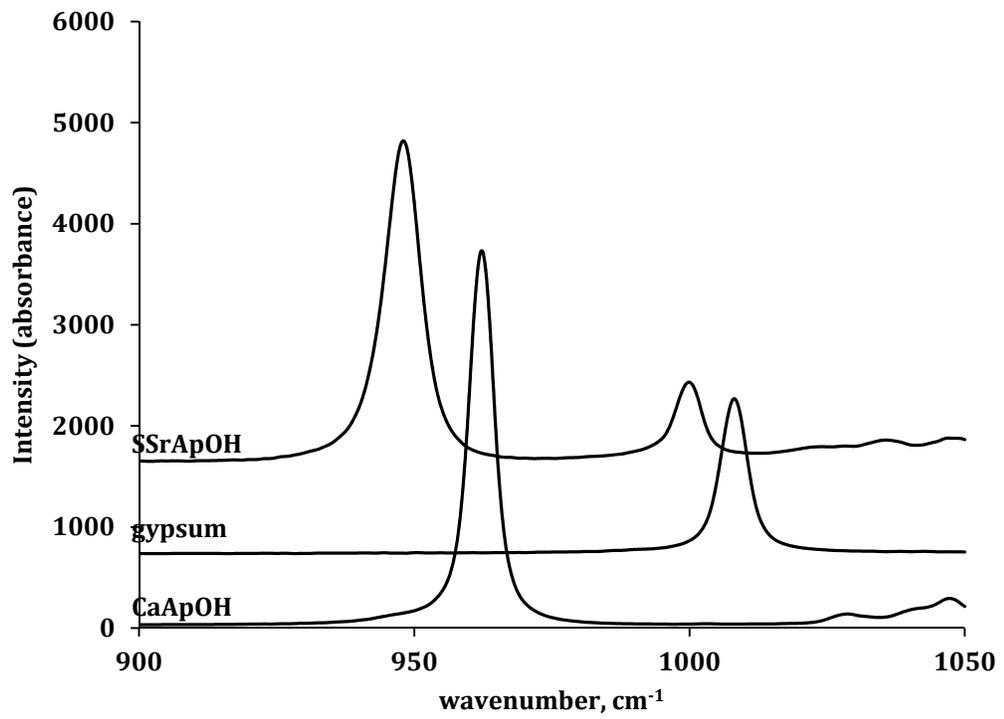
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671   **Figure 6**

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674   **Figure 7**

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678   **Figure 8**

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