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# A Method of Preparation and Characterization of Carbonato-apatites

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*Isomorphous carbonate anion can be introduced into the lattice of calcium hydroxyapatite at two different sites: either at the hydroxyl position or by replacement of the phosphate anion. In the present study, a successful attempt with the method of preparation of carbonato-apatites in aqueous media is reported in which the samples have been characterized by chemical analyses, X-ray diffraction, infrared and scanning electron microscopic techniques. It has been proven that the substitution is taking place at the phosphate anion site.*

## Introduction

Calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, (HA) is an important inorganic crystalline constituent of human calcified tissues (bones and teeth). It undergoes a series of cationic and anionic isomorphous replacement reactions [1], resulting in the formation of solid solutions with substituted ions. The replacement of phosphate by carbonate in HA is of biological significance as it explains the mechanism of incorporation of carbonate in teeth and bones [2]. The exchange process results in the formation of a series of solid solutions of carbonato-apatites and, when completed, leads to the formation of pure carbonato-apatite according to the following scheme:

$$Ca_{10}(PO_4)_6(OH)_2 + nCO_3^{2-} + nNH_4OH \rightarrow$$

$$Ca_{10}(PO_4)_{6-n}(CO_3)_n(OH)_2(OH)_n + nPO_4^{3-} + nNH_4^+$$

In biological apatites, carbonate ions are present in two different environments:

(i) an environment involving substitution of $CO_3^{2-}$ for $OH^-$ ions representing about 5–10%.

(ii) an environment involving substitution of $CO_3^{2-}$ for $PO_4^{3-}$ ions representing 90–95%. This shows that the preparation of synthetic apatites of the second type is necessary to study the influence of incorporation of carbonate ions in biological apatites.

All the methods used earlier for the preparation of carbonato-apatites have been solid state reactions using either high temperature or pressure and sometimes both [3]. The samples prepared by those methods were, however, found to be discontinuous and non-homogeneous. Hence the present work has been a successful attempt to elaborate a new method for the preparation of homogeneous solid solutions over the entire compositional range by the novel method of co-precipitation in an aqueous medium and to confirm the homogeneity of the samples obtained by X-ray, infrared and scanning electron microscopic techniques. By this method of preparation, carbonato-apatites have been obtained having substitution of carbonate at the phosphate position only in the apatite lattice structure which is in accordance with the earlier observations [4].

## Material and Methods

All the chemicals used in this work were of analytical grade Merck or Fluka. Deionised water was used in order to take special precautions to eliminate carbonate content of water, as the samples were synthesized in aqueous media. To a two necked 2L Pyrex flask, separating funnels A and B were fitted through one holed rubber stoppers. Stoichiometric quantities of a mixture of ammonium carbonate and ammonium dihydrogen phosphate solutions were added to funnel A and stoichiometric quantities of calcium nitrate solution were placed into funnel B. The pH of the solution was scrupulously maintained above 9 to avoid the formation of calcium-deficient apatites [5]. Proper care was taken to prevent the absorption of atmospheric carbon dioxide by fixing guard tubes to the funnels filled with lime. Then the solutions were carefully and slowly added to the flask containing a dilute solution of ammonium hydroxide in order to maintain the pH of the media above 9 during precipitation. The solution was stirred by means of a magnetic stirrer. After precipita-

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TABLE 1. Chemical Analysis and X-ray Data of the Synthetic Apatite and Its Solid Solutions with Varied Quantity of Carbonate.

| Sample No. | Wt.% Ca | P | C | OH | Molecular formula | Ca/P + C g atom ratio | Molecular volumes in Å$^3$ | X-ray data in Å *a* | *c* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 39.95 | 18.61 | – | 3.4 | $Ca_{10}(PO_4)_6(OH)_2$ | 1.67 | 338.6 | 9.492 | 6.886 |
| 2 | 42.66 | 13.55 | 2.43 | 6.9 | $Ca_{10}(PO_4)_{\underset{4}{4.1}}(CO_3)_{1.9}(OH)_2(OH)_{1.9}$ | 1.68 | 331.0 | 9.491 | 6.888 |
| 3 | 46.65 | 6.51 | 5.88 | 11.3 | $Ca_{10}(PO_4)_{1.8}(CO_3)_{4.2}(OH)_2(OH)_{4.2}$ | 1.67 | 326.0 | 9.446 | 6.886 |
| 4 | 50.39 | – | 9.08 | 15.1 | $Ca_{10}(CO_3)_6(OH)_2(OH)_6$ | 1.67 | 322.7 | 9.325 | 6.893 |

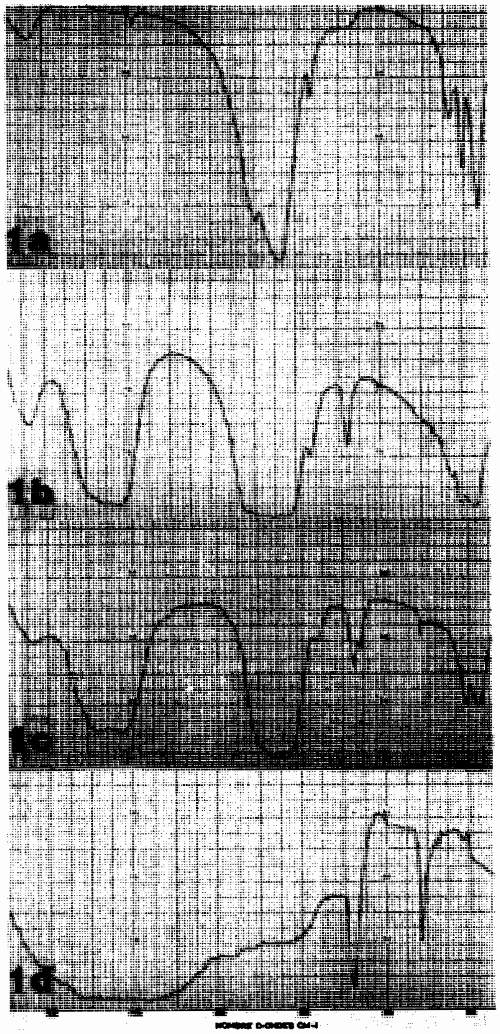

Fig. 1. Infrared spectra for the synthesized compounds. a) $Ca_{10}(PO_4)_6(OH)_2$; b) $Ca_{10}(PO_4)_{4.1}(CO_3)_{1.9}(OH)_2(OH)_{1.9}$; c) $Ca_{10}(PO_4)_{1.8}(CO_3)_{4.2}(OH)_2(OH)_{4.2}$; d) $Ca_{10}(CO_3)_6(OH)_2(OH)_6$.

tion, the contents of the flask were refluxed for about 1 h in order to improve the homogeneity and crystallization of the samples. These were filtered. The precipitate was washed until it was free from adsorbed ions, dried at 105 °C for 6 h, powdered to 200 mesh (BSS) and analysed for chemical composition. The density of the samples was determined with a specific gravity bottle using toluene as a solvent.

Infrared spectra of the samples were recorded using the KBr pellet technique with the help of a Perkin-Elmer No. 1.c 396 double beam spectrophotometer. Scanning electron micrographs were obtained on selected areas of the samples using the Jeol 35C SEM operating at 12 KV. X-ray diffraction spectra were obtained with Philips PW1130 equip-

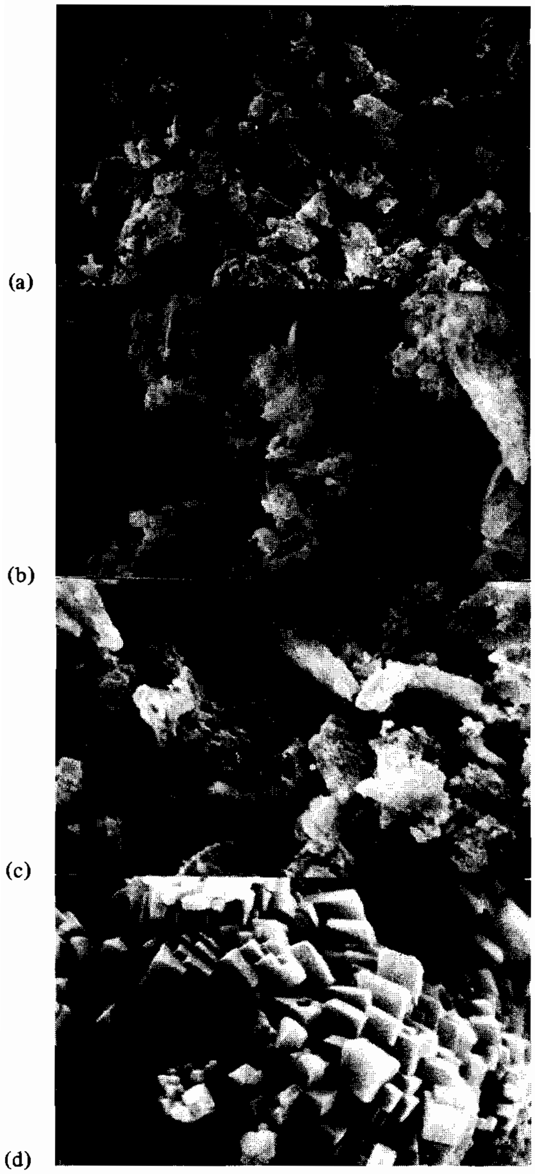

Fig. 2. Scanning electron micrographs of the different samples. a) Presence of tiny crystals for $Ca_{10}(PO_4)_6(OH)_2$ (×3300). b, c) Presence of needle shaped crystals for compounds with 2.4% and 5.9% of carbon (×2150 for b and ×8000 for c). d) Presence of large crystallites for d (×10 000).

ment using Ni filtered $CuK_\alpha$ radiation generated at 10 KV and 25 mA, with a scanning speed of $1/2°$ per minute.

## Results

The samples were analysed for calcium, phosphate, hydroxide and carbonate content by means of complexometric [6], spectrophotometric [7], and titrimetric [8] methods, respectively, and the experimental Wt% errors of the determination of calcium, phosphate, and carbonate when present together were 0.1, 0.05 and 0.1, respectively. Based on the fact that one molecule of each sample has a total of 6 g atoms of P and/or C, the molecular formulae of the samples were determined and from them the atomic ratio of Ca/P + C in one mol of each sample was calculated. The results are given in Table I. The atomic volumes were determined from the density of the samples. The infrared spectra in the range 4000–400 $cm^{-1}$ were recorded and the spectra are given in Figs. 1a–d. The scanning electron micrographs are included in Figs. 2a–d always indicating the presence of crystallites. In the absence of $CO_3^{2-}$, the inorganic compound was found as tiny agglomerated crystals (Fig. 2a), whereas in the compounds with 2.4 to 5.9% of carbon, the crystals were distinctly needle shaped (Figs. 2b and 2c). In the case of complete substitution of $PO_4^{3-}$ by $CO_3^{2-}$ the flat surface areas of large crystals were clearly seen (Fig. 2d). To obtain lattice parameters and the unit cell volumes of the crystals from the 2θ values of the X-ray diffraction spectra, the equidistances were determined and the corresponding $d_{hkl}$ values were obtained by comparison with the values given for hydroxyapatite [9]. The least squares method of refinement for *a* and *c* parameters was employed using a UNIVAC 1100 electronic computer with the program (W9214). The statistical analysis was performed by adjusting the statistical weights. For each value, a weighting factor of 10 was given to the prominent peaks and a factor of 1 to all the other peaks.

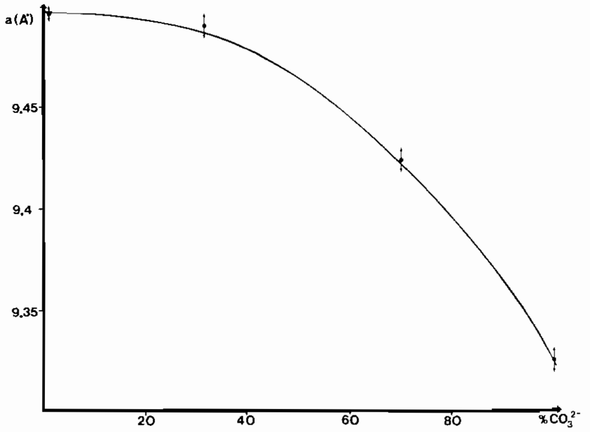

Fig. 3. *a* Parameter as a function of the $CO_3^{2-}$ content.

The observed values of the molar atomic ratio (theoretical 1.66) of Ca/P and Ca/C for the end member and Ca/P + C for the intermediates, and the closeness of the values of the molar volumes of the end members and those of intermediate samples which lie within the range of end members, indicated the formation of homogeneous solid solutions [10]. This interpretation can further be substantiated with the infrared spectral data for which shifts of the $\nu_3$ and $\nu_4$ frequencies due to $PO_4^{3-}$ were clearly observed. This is in accordance with the Barnes equation [11] which gives a relationship between frequency, atomic mass, and force constant. The vibration frequency was dependent on the reduced mass of the participating atoms and the restoring forces between atoms. The $\nu_3$ and $\nu_4$ frequencies of $PO_4^{3-}$ (approximately 560 and 1050 $cm^{-1}$, respectively) were not observed in the spectrum of Fig. 1d. Also the appearance of the frequency due to carbonate ion (1410 $cm^{-1}$) in Fig. 1b, 1c revealed that substitution is taking place at the $PO_4^{3-}$ site only instead of $OH^-$ in the crystalline lattice. Hence, it was considered that the desired compound had been synthesized.

This observation is in agreement with those observed earlier [4]. The scanning electron micrographs indicated clearly crystallites characteristic of apatites. The absence of extraneous phases in the micrographs indicated the presence of a single phase of apatite in the samples.

In Fig. 3 the different values of the *a* parameter *versus* mol percent carbonate content are represented.

## Discussion

The novel method of preparation of the carbonate substituted apatites has been used since the earlier methods of solid state reaction were found not to be useful because of the formation of discontinuous non homogeneous samples. For the substitution of one phosphate group by one carbonate group, there should be a balance of negative charge of units and this should be achieved by the incorporation of $OH^-$ from the alkalinity of the medium of precipitation. The actual determination of the total hydroxide content of the samples gives more hydroxide content than is normally present in synthetic hydroxyapatite, hence the general formula that has been proposed for the carbonate substituted apatite is $Ca_{10}(PO_4)_{6-n}$-$(CO_3)_n(OH)_2(OH)_n$.

The synthetic apatite will be of the general formula $Ca_{10}(PO_4)_6(OH)_2$; the extra OH units that are present in the above carbonate substituted apatites will probably be accommodated outside the hydroxyapatite lattice as may be concluded from the rapid decrease of the *a* parameter.

Nevertheless, the decrease of the *a* parameter with increasing amount of incorporated carbonate can also be related to the smaller ionic radius of $CO_3^{2-}$ ions as well as to the plane symmetry of $CO_3^{2-}$ substituting the tetrahedral $PO_4^{3-}$ group.

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