Calcium phosphate compounds such as Hydroxyapatite (HAp) were prepared by hydrothermal synthesis with phycogenic CaCO₃ as starting material. Material obtained was characterised by usual methods (XRD, FTIR, TG, N₂-adsorption, SEM and EDX) in order to study its physical-chemical characteristics. The prepared HAp showed that it may be suitable for use as a biomaterial.

**Table 1. Intermediate compounds in the formation of HAp²⁸,²⁹.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Chemical Formula</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyapatite</td>
<td>HAp</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>1.67</td>
</tr>
<tr>
<td>Carbonated hydroxyapatite</td>
<td>CHA</td>
<td>Ca₁₀(PO₄)₆CO₃</td>
<td>1.67</td>
</tr>
<tr>
<td>Fluorapatite</td>
<td>FHA</td>
<td>Ca₁₀(PO₄)₆F₂</td>
<td>1.67</td>
</tr>
<tr>
<td>α, β - tricalcium phosphate</td>
<td>α, β - TCP</td>
<td>α₀(Ca₃(PO₄)₂)</td>
<td>1.55</td>
</tr>
<tr>
<td>Amorphous calcium phosphate</td>
<td>ACP</td>
<td>Ca₉(PO₄)₆</td>
<td>1.55</td>
</tr>
<tr>
<td>Octacalcium phosphate</td>
<td>OCP</td>
<td>Ca₉H₂(PO₄)₁₀</td>
<td>1.33</td>
</tr>
</tbody>
</table>
point of the water, reaching the pressure of vapour saturation. One specific method of hydrothermal synthesis consists of submitting an aqueous solution containing Ca\(^{2+}\) and PO\(_4^{3-}\), to high temperatures (200°C-500°C). Thus, a calcium phosphate compound is obtained which is able to maintain the morphological structure of the original material (e.g., CaCO\(_3\) of corals) and which possesses physical-chemical characteristics very similar to human bones, is relatively stable in a physiological medium, and also originates a pure crystalline product when it is used as a synthetic reagent\(^{17,29-33}\). The hydrothermal method has been widely\(^{7,13}\) used for the preparation of materials for prosthetic purposes.

It is believed that the use of natural raw materials for the synthesis of HAp enables it to be accepted better by the organism because of its similar physical-chemical characteristics\(^{27,28}\) to bone. Calcium carbonate for the synthesis of calcium phosphates similar to bone can be obtained from several natural sources. Only the calcium carbonate originating from marine algae and corals shows characteristic porosity and interconnectivity that makes it like human bone. To use these calcium carbonates as a starting material for the synthesis of HAp, they cannot be pulverised. These materials should be used, as taken out, in fragments in the case of algae and in shaped blocks in the case of corals. Thus, the morphological structure of the natural calcium carbonate with macropore and interconnections will be maintained. The way to do this is through hydrothermal synthesis, which allows ionic exchange, without modification of the morphological structure of HAp in order to use it as an auxiliary in bone regeneration\(^7\).

The use of corals as the only source of CaCO\(_3\) for this synthesis has the disadvantage of destroying the banks of corals, which form a delicate and important ecosystem for the maintenance of marine life. The use of marine algae appears then as a less aggressive alternative, since the banks of algae can be managed with relative simplicity without destroying the seabed. The exploitation of banks of algae in Brazil is viable, since they are frequently found on the Brazilian coast, the available stock being of approximately 2 x 10\(^{11}\) tons\(^{36}\). The use of phycogenic calcium carbonate in the synthesis of HAp has already shown promise in relation to human bone substitution and regeneration\(^27,28\).

In recent years, research has been demonstrating that the usefulness of HAp also extends to other areas of great interest. Its use as a support of proteins in column chromatography is already established as a method that unites good performance with high affinity in the separation of several proteins such as γ-globulin, lysozyme and human albumin, among others\(^{37,38}\). In general, awareness of the importance of protecting the environment has grown greatly in recent years, and the products and processes that aid in the removal of substances harmful to nature are more welcome than hitherto: thus, HAp will have a contribution to make in this field. Several papers\(^{39}\) have demonstrated that this material presents very convenient qualities for the removal of toxic metals such as Cd, Pb, As, Mn, Al and Ni. The facility of producing ionic exchanges of hydroxyapatite, allied to its application over a great surface area, makes it a material with great potential in the treatment of industrial effluents. The possibility of HAp being used as catalyst for the treatment of toxic gases before they reach the atmosphere is also being researched\(^{44,45}\).

The present work describes the synthesis and characterisation of a calcium phosphate compound such as HAp, which has calcium carbonate from marine algae as starting material.

**MATERIAL AND METHODS**

Starting material
The biogenic material was obtained from algae of the Rodophycophyta division collected in the coastal area of Santa Catarina Island. These algae are characterised by a high content of CaCO\(_3\) in their vegetative structure. The collected algae were selected in the laboratory for removal of greater impurities such as mollusk shells or small marine organisms. This material was washed with tap water and then dried in an oven at 80°C for 48 hours. After drying a new selection was made and the dry weight was measured. The organic matter present was digested through treatment with a dilute aqueous solution (10%) of sodium hypochlorite. The material was then washed until its pH was close to that of physiological pH. This white material formed by particles of 3mm length on average, was dried in the oven at 80°C for 30 hours and then it was stocked for later analyses and utilisation. The obtained material was considered as being entirely free of organic matter\(^{46}\). The use of CaCO\(_3\) from the algae was evaluated as 80% of the dry weight, a remarkable value considering the natural origin of the material.

The calcium carbonate from the algae was characterised as calcitic according to X-Ray powder diffractometry and FTIR spectroscopy\(^{47}\). The porous microstructure of the material was analysed by gas adsorption, showing that the material was mesoporous. Photomicrographs by scanning electron microscopy also confirmed the presence of macropores and interconnections. The porous structure of this phycogenic calcium carbonate, allied to its surface morphology without edges, makes it a promising material as a precursor for biomedical use.

**Hydrothermal synthesis**

Two different methodologies were used in the synthesis, both involving hydrothermal reactions:

1. \((\text{NH}_4)_2\text{HPO}_4\) was dissolved in distilled water in the desired proportions. This solution was placed in the pressure vessel on top of the phycogenic CaCO\(_3\).
2. \((\text{NH}_4)_2\text{PO}_4\) was dissolved in an NH\(_4\)F (20ppm) aqueous solution, and this solution alone was later run onto the phycogenic CaCO\(_3\) for the synthesis.

The pH was controlled prior to the reactions in the range of 8.5 - 9.0 with use of NH\(_4\)OH when necessary.

Next, the solutions were placed in the pressure vessels, which were then closed and put into the oven. The temperature was controlled at 200°C. The time of permanence in the oven varied between 24 and 48 hours. After the withdrawal of the samples from the vessel, they were measured for pH and washed until all excess of HPO\(_4^{2-}\) had been removed and the pH was close to that of the physiological pH. The samples as prepared were fully dried in an oven at 80°C for 30 hours. The prepared material with particles not bigger than 3 mm was stocked for analysis.

From the many proportions of the main reagents that were tested, two are presented in this paper because they showed the closest similarity with non-stoichiometric HAp, like bone. The reaction conditions for the hydrothermal synthesis are presented in Table 2.

**X-ray diffraction analysis (XRD)**

Samples of the synthesised material were pulsed and examined in a Phillips diffractometer model APD15 and Seiffert model 300TT. The XRD patterns being recorded in the range of 5° ≤ 2θ ≤ 50° using CuKα radiation (λ=1.54 nm), with 0.05° 2θ step size and step scanning 1 s per 2θ value. To determine the peaks from HAp or other phases that might occur, the diffractograms were compared with the ICDD cards - for HAp (9-432); α– TCP, 9 – 348; β – TCP ( 9-169). The x-ray diffraction patterns from commercial calcium phosphate prepared by Aldrich (Milwaukee, USA), catalogue number 12167-74-7, were also recorded as reference material.
Fourier transform-infrared spectrometry (FTIR)

After crushing, the samples were prepared in KBr discs and analysed in Mattson Galaxy 2030 and Nicolet DXB Fourier transform IR spectrometers in the range of 4000 cm\(^{-1}\) and 400 cm\(^{-1}\). The obtained spectra were compared with that of the commercial material from Aldrich, used as reference, and with the literature.\(^{19-20}\)

Thermogravimetry (TG)

Thermogravimetric analysis in air up to 1000°C was performed at a heating rate of 10°C min\(^{-1}\) using a Linseis L81 thermobalance operated by the Lynseis Acquisition Program. For this analysis, the samples were not crushed. A platinum crucible was used as reference.

Scanning Electron Microscopy (SEM)

Fragments of the synthesised material were analysed employing scanning electron microscopy (SEM - ZEISS DSM962). For SEM analyses, the fragments were not crushed so that the material was very similar to the starting raw material for the synthesis.

Gas adsorption analysis (BET)

For a verification of the morphology of the microstructure of the obtained material, gas adsorption analysis was made, using N\(_2\) at boiling point (Micrometrics ASAP 2110 and Quantachrome Autosorbt MGS). The synthesised material was not crushed so results could be obtained from the material without morphological alteration.

Chemical analysis

The phosphorus content was analysed colorimetrically. The complexation titration method was used for the calcium determination. In order to verify the presence of other elements contaminating the material, energy-dispersive electron probe X-ray analysis (EDX-PHILIPS) was used. Some trace elements, which might have been connected with the biocompatibility, were detected by spectrochemical analysis.

RESULTS AND DISCUSSION

The XRD recorded patterns of the samples are indexed based on the ICDD Card N.9-432, in the recorded diffractogram of the commercial material and in the available literature.\(^{17,19-20}\) They are showed in Figure 1a-c.

It was assumed that the unit cell of all probes was hexagonal, and the dimensions \(a\) and \(c\) for all samples were calculated using a least squares refinement program. The lattice parameters from ICDD Card N.9-432 were used as reference to the refinements, commercial HAp and synthesised samples 1 (without the presence of NH\(_4\)F) and 2 (in the presence of NH\(_4\)F) are given in Table 3.

The peaks on the pattern from sample 2 (Figure 1c), where NH\(_4\)F was used, seem sharp and well resolved, and can all be attributed to the hexagonal crystal form of HAp in good agreement with the patterns on the ICDD Card N.9-432 and the recorded diffractogram from commercial HAp (Figure 1a). The calculated lattice parameters tend to be different from that in the reference (ICDD Card N.9-432). It was shown that the increasing of CO\(_3\)\(^{2-}\) content in the HAp lattice can cause a decrease of \(a\) and increase of \(c\)\(^{51,52}\), as observed in sample 2. This can be attributed to a substitution of PO\(_4\)\(^{3-}\) ions for CO\(_3\)\(^{2-}\)\(^{19,20}\), typical for the so called type B-HAp. For sample 1 (Figure 1b), the occurrence of atypical XRD peaks was verified. They are attributed to other phases that can be usually formed in the system (e.g. octacalcium phosphate or tertiary calcium phosphate (TCP))\(^{17}\). Although the lattice parameters were close to that of the HAp, the peaks were not so well resolved as in sample 2.

Figure 2a-c depicts the FTIR spectra of the analysed samples. Spectra of synthesised samples are shown in Figure 2b and Figure 2c. A spectrum of commercial HAp is also shown in Figure 2a.

### Table 2. Reaction conditions for the hydrothermal synthesis

<table>
<thead>
<tr>
<th>Sample N.</th>
<th>Reagents (mass / mass)</th>
<th>pH initial</th>
<th>pH final</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1CaCO(_3) : 1(NH(_4))(_2)HPO(_4) : 4 H(_2)O</td>
<td>8.5</td>
<td>10</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>1CaCO(_3) : 1(NH(_4))(_2)HPO(_4) : 4 SF(^{a})</td>
<td>9.5</td>
<td>9.5</td>
<td>200</td>
<td>48</td>
</tr>
</tbody>
</table>

\(^{a}\) NH\(_4\)F (20 ppm) aqueous solution

### Table 3. Reference and calculated lattice parameters.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(a) (nm)</th>
<th>(c) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICDDN. 9-432</td>
<td>0.9418</td>
<td>0.6884</td>
</tr>
<tr>
<td>Commercial HAp</td>
<td>0.9432</td>
<td>0.6893</td>
</tr>
<tr>
<td>1</td>
<td>0.9357</td>
<td>0.6827</td>
</tr>
<tr>
<td>2</td>
<td>0.9377</td>
<td>0.6890</td>
</tr>
</tbody>
</table>

\(\theta\) (degrees)

**Figure 1.** XRD patterns of the material (a) commercial, (b) synthesised without the presence of NH\(_4\)F and (c) in presence of NH\(_4\)F.
the spectra of the synthesised samples (Figures 2b and 2c) can be explained by the substitution of the groups OH for CO$_3^{2-}$ in the lattice of HAp, which is characteristic of carbonated type A-HAp$^{49}$. The band at 3572cm$^{-1}$ is usually masked in the spectra by a wide band of H$_2$O between 3500 and 3700cm$^{-1}$ $^{48}$. The extraction of this water included in the lattice is only possible with treatment of the samples at 650°C, bringing the risk of deterioration of the samples for liberation of the CO$_3^{2-}$ ions together with the water, as discussed later. But the absence of the band located at 635cm$^{-1}$ in the two spectra is an indication that this type of substitution really occurs.

In sample 2, where NH$_4$F was used (Figure 2c), one can see the appearance of bands located at 875cm$^{-1}$ derived from HPO$_4^{2-}$-ions$^{55}$. A remarkable increase in the band intensities at 1456 and 1430cm$^{-1}$ was also observed, besides the appearance of a band at 864cm$^{-1}$. These bands are characteristic of carbonated type B-HAp, in which occurs the substitution of groups PO$_4^{3-}$ for CO$_3^{2-}$ in the lattice of HAp$^{57-60}$. These results allied to the absence of the band at 635cm$^{-1}$ lead to the conclusion that the synthesised material is HAp of the type AB. Moreover this agrees with the results of XRD. For this HAp, probably existing together with a fluorapatite phase, due to the high content of F$^{-}$ added, the appearance of the bands derived from this ion was expected, but this did not occur. These bands were probably masked by more intense bands of PO$_4^{3-}$ $^{65}$.

The TG curves for the synthesised material as well as the commercial HAp from Aldrich are shown in Figure 3a-c. They can be generically described as a gradual weight loss starting with the detachment of adsorbed water on the surface of the sample up to 250°C $^{64}$, and continuing with losses of hydroxyls from the crystalline lattice until the complete conversion to β-TCP, which begins at approximately 650°C. Above 900°C, the complete decomposition of the material begins. A loss of weight was observed between 200°C and 500°C, probably originating from the firing of residual organic matter, which is common in view of the biological origin of the material$^{61}$. These weight losses might also originate from the detachment of HPO$_4^{2-}$, but this cannot be observed even when submitting the material to temperatures of 450°C and conducting an FTIR analysis in an attempt to verify the presence or absence of the band at 875cm$^{-1}$, typical of this ion. It is accepted$^{49,50}$ that carbonated HAp with low crystallinity already loses OH and CO$_3^{2-}$ in considerable amounts at temperatures below 700°C, as may be observed by comparing the three diagrams. For commercial HAp (Figure 3b), called stoichiometric HAp, the weight loss is gradual and smooth. The total percentage of weight loss was 5.12%. For non-stoichiometric HAp synthesised without NH$_4$F (Figure 3a), a sudden weight loss between 880°C and 931°C occurs. This weight loss corresponds to 0.45% of the mass in an interval of temperature of only 50°C and is due to the phase change non-stoichiometric HAp → α-TCP that is determined by the gradual loss of OH and probably CO$_2$ from non-stoichiometric HAp and possible decomposition of the carbonates linked to the chain$^{56-60}$. This same curve already occurs at 650°C for HAp synthesised in presence of NH$_4$F (Figure 3c), rising to 782°C. The relative loss in weight (2.08%) is substantially larger than in the previous case. Also, the process occurs in a temperature range which is twice as that of the previous case. This is attributed to the higher content of carbonate in HAp synthesised without NH$_4$F, reducing the thermal stability of the HAp lattice$^{64}$.

**Figure 2.** FTIR spectra of the material (a) commercial, (b) synthesised without the presence of NH$_4$F and (c) presence of NH$_4$F.

**Figure 3.** TG diagrams of the material (a) synthesised without the presence of NH$_4$F, (b) commercial and (c) synthesised in the presence of NH$_4$F.

Figure 4 shows the result of the EDX analysis. The presence of a peak of Mg$^{2+}$ is observed at 1.2keV, and in general the peaks belong to expected elements - calcium (Kα) at 3.69keV, calcium (Kβ) at 4.01keV, phosphorus at 2.01keV, oxygen at 0.5keV and carbon at 0.2keV. The presence of carbon and oxygen provides further evidence of the ionic substitution in these samples. In non-stoichiometric HAp synthesised in presence of NH$_4$F (Figure 4a), the peaks of C and O are more intense probably due to substitution of CO$_3^{2-}$ at the sites of PO$_4^{3-}$ and OH in the lattice, as discussed before. The presence of contamination in this material of natural origin is expected, since HAp presents relative facility for ionic exchanges, especially with charged elements and ionic radii similar to Ca$^{2+}$ as in the case of Mg$^{2+}$ and Na$^+$, respectively$^{51}$. The fact that the EDX analysis has shown such expressive relative amounts of Mg$^{2+}$, indicates the incorporation of the Mg$^{2+}$ into the formation of the crystalline lattice of hydroxyapatite, since this ion usually links in small amounts to the hydroxyapatite molecule during its formation$^{52}$. This ion also occurs in HAp of the human bone.

The photomicrographs obtained by SEM (Figure 5a-b) revealed a porous interconnected structure similar to the phycogenic CaCO$_3$. The maximum distance between two walls of the pores was an average of 20µm, that is smaller than is generally accepted as the size required for a porous HAp implant. The surface seems to be homogeneous and regular, without ridges (Figure 5a). The interconnected pore structure is similar to the trabecular bone structure$^{53}$.
The adsorption isotherms of nitrogen of the synthesised material are of type IV, presenting a very defined and reproducible hysteresis loop of the type H3, typical of mesoporous materials with slit-like pores (\(r_m\approx 250\) Å). The BET specific surface area calculated using 5 points, varied between 30-50 m\(^2\)g\(^{-1}\) in the synthesised samples.

These results, associated to that SEM analysis, indicate the presence of macro and mesopores in these samples. They are similar to those found for the phycogenic CaCO\(_3\) (BET specific surface area of 20-30 m\(^2\)g\(^{-1}\); \(r_m\approx 250\) Å). The BET specific surface area calculated using 5 points, varied between 30-50 m\(^2\)g\(^{-1}\) in the synthesised samples.

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