Synthesis and Comparison of Chemical Changes Using FTIR Spectroscopy for Copper Substituted Hydroxyapatite

S. Mounika¹, Praveen Ramakrishnan²

¹²Department of BioMedical Engineering, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, pincode: 602105

Abstract: The study aims to scrutinise and compare the chemical modifications present in copper-substituted hydroxyapatite utilising FT-IR spectroscopy. The synthesis of both HAp and Cu-modified HAp was conducted via the chemical precipitation method, involving specific chemicals like ammonium hydroxide (HN4OH) solution, diammonium hydrogen phosphate ((NH4)₂HPO₄), copper chloride hexahydrate (CuCl₂.6H₂O), and calcium nitrate tetrahydrate (Ca(NO₃)₂.4H₂O). The overall sample size was determined through clinlac.com considering the copper-substituted dataset (N=4), an alpha error of 0.05, G power at 80%, an enrolment ratio of 0.1, and a confidence interval of 95%. The acquired FT-IR spectra denote minimal differences between the CuHAp and HAp samples. Each peak bears a resemblance to the distinctive features of HAp, indicating structural similarity between pure hydroxyapatite and the copper-substituted variant. The investigation utilised SPSS 27.0.1 software to compute the copper-doped hydroxyapatite percentages at 0%, 1%, 5%, and 10%. A One Sample T-test revealed a statistically significant difference with a p-value of 0.001 (p<0.05), particularly highlighting the statistical significance of copper hydroxyapatite weight percentage (p=0.006). Significant alterations in peak values were observed in the novel copper-substituted hydroxyapatite concentrations of 1%, 5%, and 10%. Notably, the study identifies the 10% copper and hydroxyapatite combination as yielding the most noteworthy chemical modifications via spectroscopy. This finding suggests the potential utility of these compounds in medicinal applications.

Keywords: Hydroxyapatite, Novel Copper Substituted Hydroxyapatite, Fourier Transform Infrared (FT-IR) Spectroscopy, Biomedical, Biocompatible, Healthy Lives.

1 Introduction

The chemical composition of hydroxyapatite is typically denoted as Ca₅(PO₄)₃(OH), although it's more commonly expressed to signify the presence of two entities within its crystal unit cell. The mineral form of calcium apatite that occurs naturally is called hydroxyapatite (HAp). Hydroxyapatite-based bone regeneration therapy holds promise in
fostering robust health. Hydroxyapatite, the hydroxyl endmember within the intricate apatite group, is commonly employed by healthcare practitioners in bone restoration procedures. Both synthetic and naturally occurring hydroxyapatite are utilised, despite its natural presence in bone [1]. The addition of copper or copper oxide can increase the stability of the hydroxyapatite layer. The copper-doped hydroxyapatite (CuHAp) materials may present fresh possibilities for the range of biomedical uses. As a bone substitute in medicine, synthetic hydroxyapatite material assumes a crucial role due to its biocompatibility and bioactivity [2]. Hydroxyapatite-based biomaterials can serve as both a bone substitute and an osteoconductive scaffold, owing to their exceptional biocompatibility and close resemblance in chemical composition to the mineral phase found in bone. Incorporating hydroxyapatite on their surfaces enhances the stability and biocompatibility of copper oxide nanoparticles [3]. Research findings indicate that the incorporation of copper into the structure of HAp enhances its bioactivity [4]. Additional exploration into the magnetic HAp scaffolds within living organisms demonstrated no observable impact of magnetism on the development of bone or its biocompatibility. Fourier Transform Infrared (FT-IR) Spectroscopy analyses how the material absorbs a beam of light. Unlike using a single light frequency, this technique emits a beam encompassing various frequencies, measuring the absorption by the sample. A second data point is obtained by modifying the beam’s frequencies. Operating within the infrared spectrum, which has longer wavelengths and lower frequencies than visible light, FT-IR presents a less convenient alternative for obtaining equivalent data. The study focuses on evaluating various proportions of copper-doped hydroxyapatite. Over the last five years (2018-2022), extensive research has been conducted on this subject, amounting to approximately 17,200 articles as per Google Scholar. Notably, PubMed has released 39 articles, while ScienceDirect has published 423 articles in the current year (2022) alone. The synthesis of Cu-HAp involved varying levels of copper as a substitutive element. The examination for antibacterial properties carried out in a liquid medium indicates a decline in viable microorganism cells across various species upon exposure to metal-doped hydroxyapatite samples. The effectiveness of these materials against bacteria is contingent upon the specific metal ions, their quantities, and the bacterial strain. These observations draw inference from the outcomes of the antimicrobial test [8]. Antimicrobial agents serve multiple functions, such as addressing skin infections and bone irregularities [5]. They find application in coating orthopaedic implants, purifying water contaminated by microorganisms, and in general applications [6]. Across the entirety of the research, the quantity of hydroxyapatite employed to supplant copper in the investigation involving characterisation, synthesis, and assessment of antibacterial efficacy in copper-doped hydroxyapatite displays variability.[7] The study highlights the most promising outcome through the substitution of 10% in the creation of novel copper-substituted hydroxyapatite. This research aims to encompass a comprehensive exploration of diverse copper substitution ratios within hydroxyapatite. The study's objective is to discern the optimal proportion of new copper-substituted hydroxyapatite (CuHAp) by examining the chemical alterations through Fourier Transform Infrared (FT-IR) Spectroscopy. It entails a comparison between hydroxyapatite formulations with 1%, 5%, and 10% copper substitution to determine the most favourable ratio for CuHAp.

2 Materials and Methods
The synthesis investigation was conducted within the Nanomaterials Department laboratory situated at Saveetha University. As human involvement was absent in this research endeavour, ethical approval was deemed unnecessary. The study involved the
categorisation of four groups where copper substituted hydroxyapatite at varying proportions of 0%, 1%, 5%, and 10%.

The determination of sample size utilised clinicalc.com [9] for Fourier Transform Infrared (FT-IR) spectroscopy analysis of novel copper-substituted hydroxyapatite alongside pure hydroxyapatite data, resulting in an N=4. Parameters considered included an 80% G power, an error level set at 0.05, and a 95% confidence interval. Each category comprised one sample, allocated as follows: Group 1 represented pure hydroxyapatite with 0% copper substitution, Group 2 included 1% copper substitution, Group 3 entailed 5% copper substitution, and Group 4 encompassed 10% copper substitution.

In the laboratory procedure, a synthesis method was employed. Groups 2, 3, and 4 underwent testing and control employing the FT-IR method, involving novel copper-substituted hydroxyapatite concentrations of 1%, 5%, and 10%. Meanwhile, Group 1 consisted of pure hydroxyapatite, which underwent processing and subsequent testing.

HAp and Cu-substituted HAp synthesis was conducted through the chemical precipitation method. The elements utilised in this process encompassed calcium nitrate tetrahydrate (Ca(NO3)2.4H2O), copper chloride hexahydrate (CuCl2.6H2O), diammonium hydrogen phosphate ((NH4)2HPO2), and ammonium hydroxide (HN4OH) solution.

1.0 mole of calcium nitrate tetrahydrate was combined with deionised water alongside specified percentages of Cu salts (0%, 1%, 5%, 10 wt%). The addition of ammonium hydroxide solution, drop by drop, facilitated the formation of the precipitation mixture. This resulting solution underwent continuous stirring at 600 rpm for a duration of 4 hours, maintained at a temperature of 60°C.

Sonication was utilised during the precipitation process to facilitate settling at room temperature. Similarly, the base HAp underwent a comparable preparation method, incorporating the addition of Cu salts. The resulting precipitation underwent filtration and subsequent drying in a hot air oven for a duration of 24 hours. The dried powder underwent compression via a hydraulic press to form tablets, subsequently subjected to heat treatment at 1100°C for a duration of 2 hours. The heat-treated HAp and Cu-substituted HAp were then subjected to FT-IR characterisation.

Fourier Transform Infrared (FT-IR) Spectroscopy employs infrared radiation to scrutinise the chemical composition of a substance. It operates by observing atomic vibrations when exposed to infrared light. Different atoms possess distinct vibrational wavelengths, which, along with the varied energy levels of infrared, are utilised to identify the functional groups at a molecular level, enabling the identification of the sample's chemical composition.

All four samples underwent FT-IR analysis as part of this study. Hydroxyapatite-based bone regeneration therapy holds potential for fostering healthier lives. To prepare the synthesized samples, a grinding process using a pestle and mortar yielded a powdered form. These powders were then combined with potassium bromide (KBr) at a 1:2 ratio to capture spectra across the 400 to 4000 cm⁻¹ range. A total of 100 scans were conducted at a resolution of 4 cm⁻¹, with additional background scans performed to mitigate instrumental errors.

Statistical Analysis

For this research, SPSS 27.0.1 served as the tool to precisely compute the mean, variance, and standard deviation of copper-substituted hydroxyapatite across 1%, 5%, and 10% concentrations [10]. This examination specifically considered the concentration of copper in hydroxyapatite as the independent variable, without involving any dependent variables.[11]
3 Results

The study shows that 10% of copper-substituted hydroxyapatite has superior qualities than 0%, 1%, 5%, and 10%. Table 1 presents the highest FT-IR (Fourier Transform Infrared Radiation) values for the stretching mode of the hydroxyl group, reaching a maximum value of 3430.

**Table 1.** The table exhibits the highest values for the stretching mode of the hydroxyl group's peak FT-IR (Fourier Transform Infrared) readings, reaching a maximum of 3430.[12]

<table>
<thead>
<tr>
<th>PEAK (cm(^{-1}))</th>
<th>ASSIGNMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>3430</td>
<td>Stretching mode, vs, of hydroxyl groups OH(^{-})</td>
</tr>
<tr>
<td>1643</td>
<td>Adsorbed water</td>
</tr>
<tr>
<td>1390</td>
<td>Stretching mode, v3, of B-type CO(_3)^{2-}</td>
</tr>
<tr>
<td>1034</td>
<td>Triply degenerate asymmetric stretching mode, v3c, of the P–O bond of phosphate groups PO(_4)^{3-}</td>
</tr>
<tr>
<td>962</td>
<td>Non degenerate symmetric stretching mode, v1, of the P–O bonds in phosphate groups PO(_4)^{3-}</td>
</tr>
<tr>
<td>602</td>
<td>Triply degenerate bending mode, v4a, of the O–P–O bonds in phosphate groups PO(_4)^{3-}</td>
</tr>
<tr>
<td>565</td>
<td>Triply degenerate bending mode, v4c, of the O–P–O bonds of the phosphate group</td>
</tr>
</tbody>
</table>

Table 2 illustrates the quantities of reactants utilised in the chemical precipitation technique for producing innovative copper-substituted hydroxyapatite.

**Table 2.** Weights of reactants in chemical precipitation method to make copper substituted Hydroxyapatite (units).

<table>
<thead>
<tr>
<th>Cu(%)</th>
<th>Ca(NO(_3))(_2).4H(_2)O</th>
<th>Cu(NO(_3))(_2)</th>
<th>(NH(_4))(_2)HPO(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.20</td>
<td>0</td>
<td>14.66</td>
</tr>
<tr>
<td>1</td>
<td>46.73</td>
<td>0.33</td>
<td>14.66</td>
</tr>
<tr>
<td>5</td>
<td>44.84</td>
<td>4.56</td>
<td>14.66</td>
</tr>
<tr>
<td>10</td>
<td>42.48</td>
<td>5.92</td>
<td>14.66</td>
</tr>
</tbody>
</table>

Table 3 represents the comparison of mean, standard deviation of novel copper substituted hydroxyapatite and pure hydroxyapatite using the FT-IR Spectroscopy method.

**Table 3.** Comparison of Mean, Standard Deviation for FT-IR of copper substituted hydroxyapatite and pure hydroxyapatite (N=4).

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>N</th>
<th>MEAN</th>
<th>STD. DEVIATION</th>
<th>STD. ERROR MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT% OF COPPER IN HAp</td>
<td>4</td>
<td>4.000</td>
<td>4.546</td>
<td>1.187</td>
</tr>
<tr>
<td>MEAN OF COPPER IN HAp</td>
<td>4</td>
<td>4.620</td>
<td>0.848</td>
<td>0.245</td>
</tr>
</tbody>
</table>
Table 4 represents One sample T-test comprising the novel copper substituted hydroxyapatite and the pure hydroxyapatite. 

Table 4. One Sample T-test comprising the copper substituted hydroxyapatite and the pure hydroxyapatite \( p=0.001 \), \((p<0.05)\). The wt\% of copper hydroxyapatite is statistically significant \( p=0.006 \). There exists a statistical significance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Levene's Test For Equality of Variance</th>
<th>T-Test for Equality of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT-IR spectroscopy of copper in HAp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>df</td>
<td>Significance (one-sided ( p ))</td>
</tr>
<tr>
<td>WT% of copper in HAp</td>
<td>3.37</td>
<td>0</td>
</tr>
<tr>
<td>MEAN OF COPPER IN HAp</td>
<td>18.8</td>
<td>53</td>
</tr>
</tbody>
</table>

Figure 1 shows the FT-IR spectroscopy of HAp and copper substituted HAp.

Fig. 1. Plot of different percentages of copper-substituted hydroxyapatite: [a] pure hydroxyapatite, [b] 1\% copper substitution, [c] 5\% copper substitution, and [d] 10\% copper substitution.
Fig. 2. Bar chart comparing the average FT-IR of copper-substituted hydroxyapatite against pure hydroxyapatite.[18]

The Mean FT-IR data illustrates comparisons between novel copper-substituted hydroxyapatite and pure hydroxyapatite. Statistical analysis indicates significant differences with p=0.001 (p<0.05). Furthermore, the weight percentage (wt%) of copper in hydroxyapatite displays statistical significance at p=0.006. The investigation involved samples of hydroxyapatite with varying copper substitutions (ranging from 0 to 10%) along with their corresponding FT-IR spectra. FT-IR spectra for both HAp and CuHAp coatings were recorded within the wave number range of 4000 to 400 cm\(^{-1}\). The outcomes reveal comparable shapes and peaks in HAp across all samples. The absorption peaks at 1034, 962, 602, and 565 cm\(^{-1}\) are attributed to PO\(_4^{3-}\) ions. Specifically, the asymmetric stretching vibrations of the P-O bond were identified at 1034 cm\(^{-1}\) and 962 cm\(^{-1}\). The presence of the asymmetric O-P-O bending mode was evident at 602 cm\(^{-1}\) and 565 cm\(^{-1}\). The characteristic peaks of the OH group were observed at 3430 and 1643 cm\(^{-1}\), while CO\(_3^{2-}\) ions were detected at 1390 cm\(^{-1}\). The graphical representation displays slight alterations in peak values for copper percentages of 1%, 5%, and 10% when employing the FTIR method during analysis.

4 Discussion

This research explores diverse quantities of hydroxyapatite substituting copper, aiming to discern their impact. The findings emphasise the superiority of 10% novel copper-substituted hydroxyapatite, manifesting notably enhanced chemical alterations, substantiated by statistical significance (p=0.001, p<0.05). Statistical significance of wt% in copper hydroxyapatite registers at p=0.006. The study presents averages (4.620), standard deviations (0.848), and mean standard errors for 1% copper HAp (0.462). A similar investigation in characterisation, synthesis, and antibacterial activity of copper-doped hydroxyapatite highlights variations in hydroxyapatite substituting copper. Comparative analyses delve into the structural characteristics of unique porous copper-substituted nano hydroxyapatite powders, juxtaposing 0.5% and 1% copper substitution levels. Optimal hydroxyapatite methods are revealed through 4% doping, examined in the structural and
biological assessment of copper-doped hydroxyapatite nanoparticles. Higher concentrations, however, potentially diminish the total cell count per sample [13]. Pulsed laser deposition research into copper and zinc-doped hydroxyapatite coatings for biomedical usage exhibits successful incorporation of these elements into HAp coatings. Contrarily, opposing studies employing 20% hydroxyapatite doping for Cu-doped hydroxyapatite synthesis, characterisation, scaffold application, antibacterial, and bioactivity studies present conflicting conclusions [15]. The absence of visible OH stretching at 20% doping is noteworthy. For biomedical utilisation, Cu-doped hydroxyapatite was optimally doped at a maximum of 15% [16]. Evaluating textural, structural, and biological attributes reveals a rise in water content with increased copper concentrations in hydroxyapatite through FT-IR analysis [14]. However, drawbacks in CuHAp, including brittleness, poor tensile strength, and fracture toughness, hinder its mechanical robustness[17]. The rapid degradation rate, while contributing to HAp's bioactivity, raises concerns such as macrophage presence, particle formation, and potential implant clinical failures. This highlights avenues for future research. Hydroxyapatite-based bone regeneration therapies promise improved lives. Future applications may utilise novel copper-substituted hydroxyapatite (CuHAp), sourced biologically or synthetically, for bone repair in various forms, including granules, blocks, scaffolds, or in composite structures with polymers or ceramics, and coatings on dental or orthopedic implants.

5 Conclusion

The investigation centred on comparing the chemical changes in copper-substituted hydroxyapatite using FT-IR spectroscopy. Through chemical precipitation, HAp and Cu-modified HAp were synthesised using specific compounds. To ensure statistical reliability, the sample size was determined considering the copper-substituted dataset (N=4). FT-IR spectra revealed minimal disparities between CuHAp and HAp, indicating structural similarity. Statistical analysis confirmed significant differences, notably at 10% copper substitution. This suggests potential medicinal applications for these compounds. The chemical composition of hydroxyapatite, Ca$_5$(PO$_4$)$_3$(OH), holds promise in bone restoration therapies. The incorporation of copper or copper oxide enhances hydroxyapatite's stability, potentially expanding its biomedical uses. Synthetic hydroxyapatite is vital due to its biocompatibility in bone substitutes. The addition of metals like copper may augment bioactivity, although concerns over mechanical strength and degradation rate persist. Nonetheless, such research opens avenues for using novel copper-substituted hydroxyapatite in various biomedical applications, including bone repair and coatings on implants.

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References


