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Evaluation of Toxicity and Antibacterial Activity of Sr²⁺-Substituted β -Tricalcium Phosphate Prepared by the Polymeric Precursor Route

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Abstract: The development of bioceramics for biomedical use demands biocompatibility, biodegradability, non-toxicity, and osteoconductivity. Beta-tricalcium phosphate β -Ca₃(PO₄)₂, commonly referred to as β -TCP is widely researched for these properties, with the polymeric precursor method offering improved homogeneity, stoichiometric control, and low-temperature synthesis. This paper investigates the effect of strontium ion (Sr²⁺) doping on β -TCP's structural, toxicological, and antibacterial characteristics. Sr²⁺ was incorporated at concentrations of $x = 0, 5, 10$, and 15 mol%, corresponding to the partial substitution of Ca²⁺ by Sr²⁺ within the crystal lattice. This substitution is represented by the general formula β -Ca_{3-x}Sr_x(PO₄)₂. Characterization techniques included thermogravimetric (TG), differential scanning calorimetry (DSC), X-ray diffraction (XRD) patterns, Rietveld refinement (RR) data, Fourier transform infrared spectroscopy (FT-IR), X-ray fluorescence (XRF), scanning electron microscopy (SEM), toxicity assays with *Artemia salina*, antibacterial test using the direct contact test and cell viability was determined using the MTT assay. TG and DSC curves indicate the mass-loss steps and endothermic events associated with the removal of adsorbed water, combustion of organic residues, and formation of the crystalline β -TCP phase. XRD patterns and RR data confirm a rhombohedral structure without secondary phases, while FT-IR and XRF spectra further confirm Sr²⁺ incorporation and the presence of vibrational modes associated with P–O bonds. Finally, SEM analysis confirmed a flake-like surface exhibiting significant porosity. The zeta-potential results show a progressive shift toward more negative values (from –8 to –12.5 mV) as Sr²⁺ substitution increases from 0% to 15%, indicating enhanced surface negativity with higher Sr content. All samples were non-toxic by *A. salina* and MTT cytotoxicity test. Notably, 5% Sr²⁺ doping achieved 79% antibacterial activity against *S. aureus*, demonstrating enhanced antibacterial performance.

Keywords: Beta-tricalcium phosphate; Strontium; Toxicity; Antibacterial.

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1. Introduction

Calcium phosphates have attracted increasing interest in biomaterials research due to their chemical similarity to the mineral phase of bone and their excellent biocompatibility. As a class of bioactive ceramics, they are known for their ability to bond to host tissue, absence of local and body-wide toxicity, and minimal inflammatory response. Among them, β -tricalcium phosphate [β - $\text{Ca}_3(\text{PO}_4)_2$] in short as β -TCP, stands out because of its resorbable nature and balanced degradation rate, which makes it especially suitable for bone regeneration applications. In addition to its osteoconductivity, β -TCP supports cell adhesion and promotes osteogenic differentiation, often yielding advantages over more stable calcium phosphates, such as hydroxyapatite, in situations when controlled resorption is desirable [1–3].

In recent years, ionic substitution has emerged as an effective strategy to customize the physicochemical and biological properties of calcium phosphates[4–6]. Several metal ions have been incorporated into the β -TCP structure to enhance particular functionalities [7,8]. Notably, ions such as Ag^+ , Cu^{2+} , and Zn^{2+} have been reported extensively for their intrinsic antibacterial activity, acting through mechanisms including membrane disruption, oxidative stress induction, and enzyme inhibition [9–11]. Other ions, including Mg^{2+} and Si^{4+} , primarily enhance bioactivity and osteogenesis rather than exert direct antimicrobial effects. Therefore, when discussing ion substitution in β -TCP, it is necessary to distinguish between ions that confer antibacterial properties and those that mainly modulate bone regeneration.

Within this context, strontium (Sr^{2+}) occupies a distinct and well-established position. Unlike classical antibacterial dopants such as silver or copper, Sr^{2+} is mainly recognized for its dual action on bone remodeling: it stimulates osteoblast proliferation and differentiation while inhibiting osteoclast-mediated bone resorption [12]. Approximately 99% of Sr^{2+} in the human body is localized in bone tissue, showing its strong affinity for calcium-binding sites. Due to its ionic radius and chemical characteristics similar to those of Ca^{2+} , Sr^{2+} can partially substitute for calcium in the β -TCP lattice, leading to modifications in lattice parameters, crystallinity, solubility, and biological response

[13]. Although strontium is not typically considered a potent antibacterial ion, its incorporation may indirectly alter antibacterial performance by altering surface chemistry, ion release behavior, and cellular interactions. Therefore, its role must be clearly understood as predominantly osteogenic, with potential secondary biological effects [14].

The synthesis route is important for structural homogeneity and performance of ion-substituted β -TCP. Standard methods can cause particle agglomeration and limit compositional control. The polymeric precursor (Pechini) method is an attractive alternative. Its sol–gel nature permits precise stoichiometric control, molecular-level homogeneity, and reduced processing temperatures [15]. This method helps achieve controlled particle size, improved dispersion, and lower energy use [16]. Although these benefits, its use in β -TCP synthesis is scarcely explored, especially for ion-substituted compositions [17].

In this context, the incorporation of Sr^{2+} into the β -TCP structure has been widely investigated due to its ability to modify the structural and physicochemical properties of the material, which can directly influence its biological performance [8,18–20]. Such modifications may affect parameters including crystallinity, solubility, and ion release dynamics, potentially leading to changes in toxicity and antibacterial potential in calcium phosphate–based biomaterials [21–24]. Furthermore, strontium has been frequently highlighted among substituent ions used in calcium phosphates because of its ability to promote osteogenic effects and contribute to the development of multifunctional biomaterials for bone regeneration applications [25–27]. In this regard, the polymeric precursor route (Pechini method) has emerged as a promising approach for the synthesis of advanced ceramic materials, enabling high chemical homogeneity and precise compositional control, and has therefore been widely employed in scientific research and in the development of new calcium phosphate–based biomaterials [28,29].

Despite the significance of the Pechini method, very few studies investigate its application in synthesizing various calcium phosphates. Only one study has been identified globally that uses this method to obtain β -TCP [30]. Peña and Vallet-Regí [30] synthesized different phases of calcium

phosphates using the Pechini method, including β -TCP. They produced nine compositions by varying the calcium to phosphorus (Ca/P) molar ratio between 1.5 and 1.667, subjecting them to thermal treatments of up to 1400 °C. The Ca/P ratio of 1.5 yielded two distinct phases depending on the thermal treatment: β -TCP was obtained after firing between 900 and 1100 °C, while the α -tricalcium phosphate (α -TCP) could be isolated after firing at 1400 °C for 72 hours. Omori *et al.*, [25] highlight that calcium phosphate powders produced through variations of the Pechini method demonstrate high dispersibility in liquid media, as well as excellent thermal and chemical stabilities, which instills confidence in their potential applications.

Therefore, this study intends to synthesize and characterize pure and Sr^{2+} -substituted β -TCP powders using this method. It focuses on how Sr incorporation alters structural and electrical characteristics, with possible consequences for toxicity and antibacterial applications. By clearly positioning Sr^{2+} among antibacterial and osteogenic ion substitutions, this research aims to support the rational design of multifunctional calcium phosphate biomaterials.

2. Materials and methods

2.1. Starting reagents

The reagents used for synthesis were deionized water, anhydrous citric acid ($\text{C}_6\text{H}_8\text{O}_7$; Cinética®, 99% purity), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; Cinética®, 99% purity), dibasic ammonium phosphate ($(\text{NH}_4)_2\text{HPO}_4$; Neon®, 99% purity), ethylene glycol ($\text{C}_2\text{H}_6\text{O}_2$; Synth®, 99% purity), and strontium nitrate ($\text{Sr}(\text{NO}_3)_2$; Dinâmica®, 99% purity).

2.2. Synthesis of pure and strontium-doped beta-tricalcium phosphate

Initially, 100 mL of deionized water was kept under constant agitation at temperature of 70 °C for 2 h. Citric acid (AC) was then added to the solution, followed by fractionally adding the precursors, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and $(\text{NH}_4)_2\text{HPO}_4$ for better homogenization. The step involving constant agitation at 70 °C for 2 h plays a crucial role in promoting precursor homogenization and improving reaction kinetics, which contributes to better control over nucleation and phase formation. **Figure 1**

provided a visual representation of the synthesis of Sr^{2+} doped β -TCP via the polymeric precursor method, giving a clear overview of the process and its outcomes.

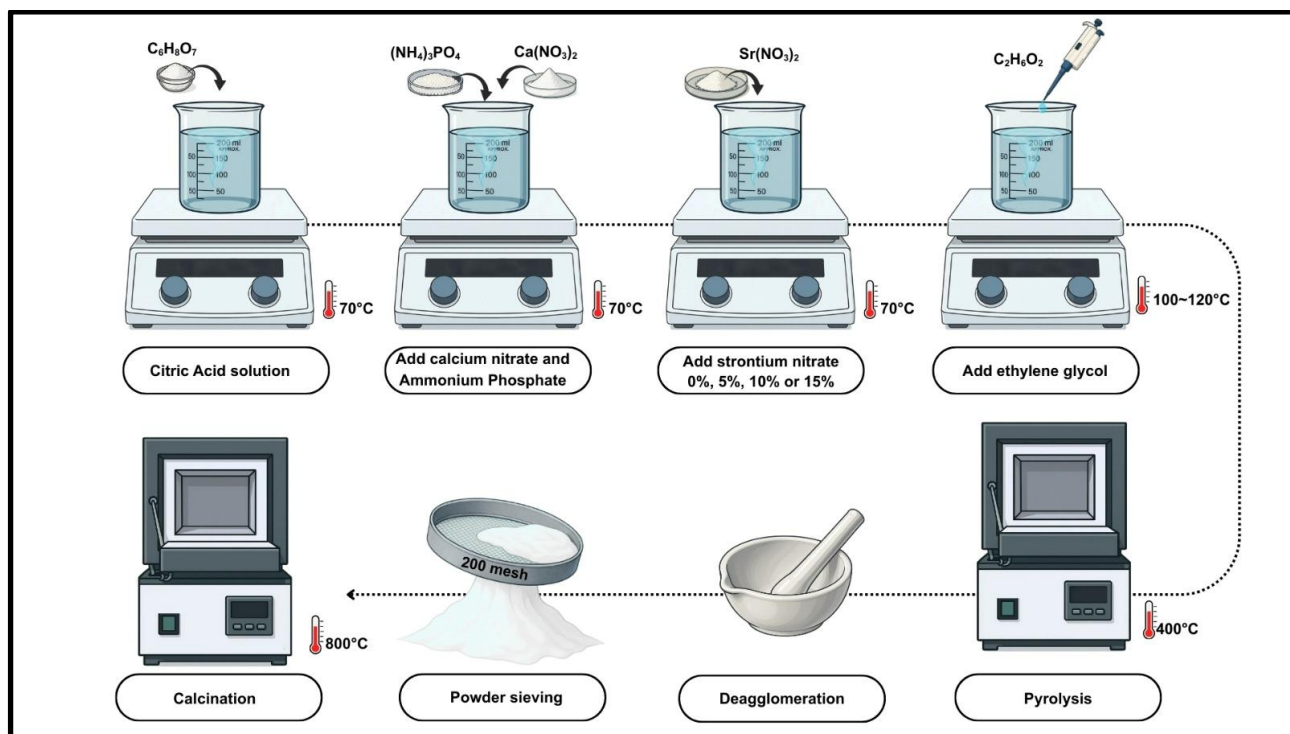


Figure 1: Stages of synthesis via the polymeric precursor method for obtaining pure β -TCP and Sr^{2+} -doped β -TCP powders.

The Pechini method, also known as the polymeric precursor method, is a widely used chemical route for synthesizing high-purity, multicomponent metal oxides. It was originally patented by Maggio Pechini in 1967 [31] and remains a staple in materials science for creating powders with precise stoichiometry. In this synthesis method, the two primary chemical reactions to produce a stable, homogeneous resin are the chelation of metal cations and the esterification reaction using a polyhydroxy alcohol. $\text{Sr}(\text{NO}_3)_2$ salt was added only in the substituted samples, varying the proportion at 5, 10, and 15 mol %. The AC/metal cations ratio used was 3:1. After complete homogenization of the solution, ethylene glycol (EG) was added according to the 60/40 ratio of AC/EG. The temperature was then increased and maintained between 100 to 120 °C for the polyesterification reactions and consequent gel formation. The gel was pyrolyzed at 400 °C for 1 h, with a heating rate of 10 °C/min, to eliminate organic matter. The obtained material was disaggregated by means of a sieve with a screen with 200-mesh sieve, with openings between the wires of 74 microns.

After the synthesis process, the gel underwent a critical step of calcination at a temperature of 800 °C for 1 h, with a heating rate of 10 °C/min, to obtain the desired phase. The resulting samples were named in a way that clearly indicated the strontium percentage: β -TCP 0% Sr²⁺, β -TCP 5% Sr²⁺, β -TCP 10% Sr²⁺, and β -TCP 15% Sr²⁺, signifying the samples with 0, 5, 10, and 15% Sr²⁺ ions, respectively. The naming represented a critical component of the study, facilitating straightforward identification and comparison of the samples.

2.3. Characterization

The thermogravimetric (TG) curves were obtained using a Shimadzu® DTG-60H thermogravimetric analyzer with TA60 software, under a nitrogen atmosphere of 50 mL/min, at a heating rate of 10°C/min, in the temperature range from 25 to 900 °C. A sample mass of approximately 3 mg ($\pm$ 0.1 g) was used, contained in an alumina crucible. The equipment was verified using a standard sample of calcium oxalate monohydrate to assess temperature and mass variations. Thermal analysis curves were generated using a Shimadzu DSC-60 Plus Differential Scanning Calorimeter under a nitrogen atmosphere at a flow rate of 50 mL per minute. The heating rate was set to 10 °C/min over a temperature range from 30 to 300 °C. Samples, each containing 3 mg ($\pm$ 0.1 g) of dry mass, were sealed in aluminum sample holders. The temperature scale and enthalpy response were calibrated using indium and zinc standards.

The synthesized samples were characterized by X-ray diffraction (XRD) to identify the phases formed, calculate lattice parameters and occupancy factors, quantify the phases, and determine crystallinity percentage and crystallite size. X-ray diffraction patterns were recorded using an X-ray diffractometer from (Shimadzu®, XRD-6000) under experimental conditions using a Cu-K α radiation at 40 kV and 30 mA, scanning from 10° to 110° at a scanning rate of 2° min⁻¹, with intervals of 0.02° and a time of 0.6 seconds per step. The diffractometric aspects of the obtained samples were thoroughly analyzed using the Rietveld refinement method, a comprehensive and detailed technique. The experimental diffractograms of the samples were fitted to a theoretical curve (diffractometer pattern), minimizing the difference between the experimental data points and the calculated data

points, using the least squares method, enabling the comprehensive determination (refinement) of lattice parameters and occupancy factors of the samples. The lattice parameters and occupancy factors of the samples were calculated using the FullProf (PF) software through patterns generated by neutron or X-ray diffraction, collected with a constant counting step in 2θ . The X'pert Panalytical High Score Plus software and the JCPDF (Joint Committee on Powder Diffraction and Standards) database were used to identify and quantify the phases. Using Origin Lab® 2022 software, crystallinity was determined from the ratio between the integrated area of the peak corresponding to the crystalline phase and the area corresponding to the amorphous fraction. Finally, the average crystallite size of the $\square$ -TCP nanoparticles was obtained using the full width at half maximum (FWHM) of the diffractograms peaks, applying the Scherrer equation (1) [32]:

$$D = \frac{k \cdot \lambda}{\beta \cdot \cos \theta} \quad (1)$$

In the equation, D corresponds to the average crystallite size. K is a constant that depends on the experimental setup and sample geometry, which in this case is 0.90. The $\lambda = 0.15418$ nm is the wavelength of the incident X-ray radiation used in the diffraction experiments for Cu-K α , referring to the wavelength of copper anode. β is the FWHM of the diffraction peaks, and θ is the diffraction angle of the crystal plane or Bragg angle. The diffraction peak corresponding to the (211) plane, located at ($2\theta = 31.97^\circ$), was used due to its highest relative intensity (100%). This peak was selected for the determination of crystallite size using the Scherrer equation.

Fourier-transform infrared spectroscopy (FT-IR) was performed to identify functional groups using a VERTEX/70 model spectrophotometer from Bruker® equipped with an Attenuated Total Reflectance (ATR) module, recording wavenumbers in the range from 400 to 4,000 cm^{-1} . The spectra were collected at a resolution of 4 cm^{-1} with 45 scans. The morphological aspects of the obtained samples were analyzed by scanning electron microscopy (SEM) using a Carl Zeiss® Auriga Field Emission Scanning Electron Microscope at 5.00 kV of working acceleration voltage and a secondary electron (SE) detector. The elemental chemical composition was checked on an Epsilon 3X X-ray fluorescence (XRF) spectrometer (PANalytical®, Netherlands) operated at 50 kV and 3 mA.

The zeta potential of the samples was determined using a dynamic light scattering instrument, the Zetasizer Nano ZS (Malvern Instruments), employing the laser Doppler electrophoresis method. Measurements were performed at 25 °C using disposable capillary cells (DTS1070). The samples were dispersed in water, and the refractive index and viscosity of the dispersant were set according to the standard values provided by the instrument software.

2.4. Evaluation of acute toxicity using the *Artemia salina* test

This test was carried out according to the methodology proposed by Meyer et al., [33]. Initially, the material extracts to be tested were prepared according to ISO 10993-5 [34] using distilled water as the extraction vehicle. The extraction conditions were 24 h at 37 °C under light agitation.

At the same time as the extracts were being prepared, *Artemia salina* eggs were laid to hatch in synthetic seawater using 15.153 g of sodium chloride (NaCl; Dinâmica®, 99% purity), 1.398 g of magnesium chloride (MgCl₂; Synth®, 99% purity), 1.888 g of magnesium sulfate (MgSO₄; Dinâmica®, 99% purity), 0.652 g of calcium chloride (CaCl₂; Dinâmica®, 99% purity), 0.414 g of potassium chloride (KCl; Dinâmica®, 99% purity), 0.116 g of sodium hydrogencarbonate (NaHCO₃; Sigma-Aldrich®, 99% purity) were dissolved in 1.0 L of distilled water, under artificial lighting and oxygenation for 48 h.

After the eggs hatched, 10 nauplii were separated for each test tube using the positive phototropism characteristic of the species, directing them toward the illuminated region of an aquarium with partially darkened compartments.

The tested concentrations were 5.0, 1.0, 0.5, and 0.1 mg/mL. Artificial seawater was used as the negative control, while a 0.1% potassium dichromate solution was used as the positive control. The formulations were evaluated at serial concentrations in triplicate, with 10 nauplii added to each test tube. After exposure periods of 24 and 48 h, the surviving nauplii were counted. Organisms that exhibited any spontaneous movement or motor response after gentle agitation of the tube were considered alive when observed under a light source.

2.5. Cell viability

Cell viability was evaluated using the MTT method following the guidelines of the ISO 10993-5 protocol [34]. L-929 murine fibroblasts cell lines were maintained in RPMI 1640 or DMEM supplemented with 10 % fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37 °C in a 5 % CO₂ atmosphere and routinely observed under light inverted microscopy (Olympus).

In vitro cytotoxicity was evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, which is based on the ability of NADPH-dependent cellular oxidoreductase enzymes to reduce MTT, a yellow tetrazolium salt, into insoluble purple formazan crystals. This reaction enables the indirect quantification of cell viability through measurement of formazan formation.

Cells were seeded in 96-well plates at a density of 0.1×10^6 cells/mL and incubated for 24 h to allow adherence. Subsequently, the culture medium was replaced with 200 µL of fresh medium containing varying concentrations of β-TCP. The cells were then exposed to the nanoparticles for 72 h under standard culture conditions (37 °C, 5 % CO₂). After incubation, the supernatant was discarded, and 150 µL of MTT solution (0.5 mg/mL) was added to each well. Plates were incubated for an additional 3 h to allow for formazan crystal formation. Absorbance was measured at 595 nm using a microplate reader (SpectraMax 190, Molecular Devices). Cell viability was determined by normalizing the absorbance values to the mean absorbance of the negative control and analyzed using GraphPad Prism software (version 8.0).

2.6. Antimicrobial activity

Bacterial cultures of *Escherichia coli* (ATCC-25922) and *Staphylococcus aureus* (ATCC 25923) were prepared by transferring the inoculum from the nutrient medium to the BHI medium. Aliquots of 50 µL were transferred to tubes containing 3 mL of saline solution and incubated at 37 °C for 24 h. The inoculums were standardized by MacFarland scale, which is equivalent to a concentration of approximately 1.5×10^8 CFU/mL (colony-forming unit).

The direct contact test in solid medium was performed according to Zheng and Zhu [35]. To carry out these tests, 2000.0 µg of the material to be tested and 2000.0 µL of the bacterial suspension

were transferred to an Eppendorf, followed by homogenization in a vortex mixer. Then, 100.0 μL were transferred to Petri dishes containing Mueller Hinton agar, which were sown with the aid of a Drigalsky loop using the spread plate method, followed by incubation at 37 °C for 24 h. Plates sown only with the bacterial suspension were used as a positive control. The tests were performed in triplicate and the inhibitory effect produced by each test solution was calculated using Eq. (2):

$$\eta = \frac{N_1 - N_2}{N_1} * 100\% \quad (2)$$

where η is the inhibitory effect, N_1 is the arithmetic mean of the colony-forming units of the control plates, and N_2 is the arithmetic mean of the colony-forming units of each of the tested solutions.

2.7. Statistical analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Data are expressed as mean $\pm$ standard deviation (SD) from three independent experiments, each performed in triplicate ($n = 3$ per experiment). Differences among groups were evaluated using one-way ANOVA followed by Tukey's multiple comparisons post hoc test, unless otherwise stated. Differences were considered significant when $p\text{-value} < 0.05$. All assays in the experimental biological section of this paper were performed in triplicate, ensuring reproducibility of results.

3. Results and discussion

3.1. Thermogravimetric analysis

Figures 2(a–d) illustrate the TG curve of precursor $\beta\text{-Ca}_3(\text{PO}_4)_2$ 0%Sr, $\beta\text{-Ca}_3(\text{PO}_4)_2$ 5%Sr, $\beta\text{-Ca}_3(\text{PO}_4)_2$ 10%Sr, and $\beta\text{-Ca}_3(\text{PO}_4)_2$ 15%Sr precursor powders prepared via the polymeric precursor method, showing total mass loss with events correspond to water (H_2O) removal, organic decomposition, and residual mass loss associated with structural rearrangement before thermal stabilization.

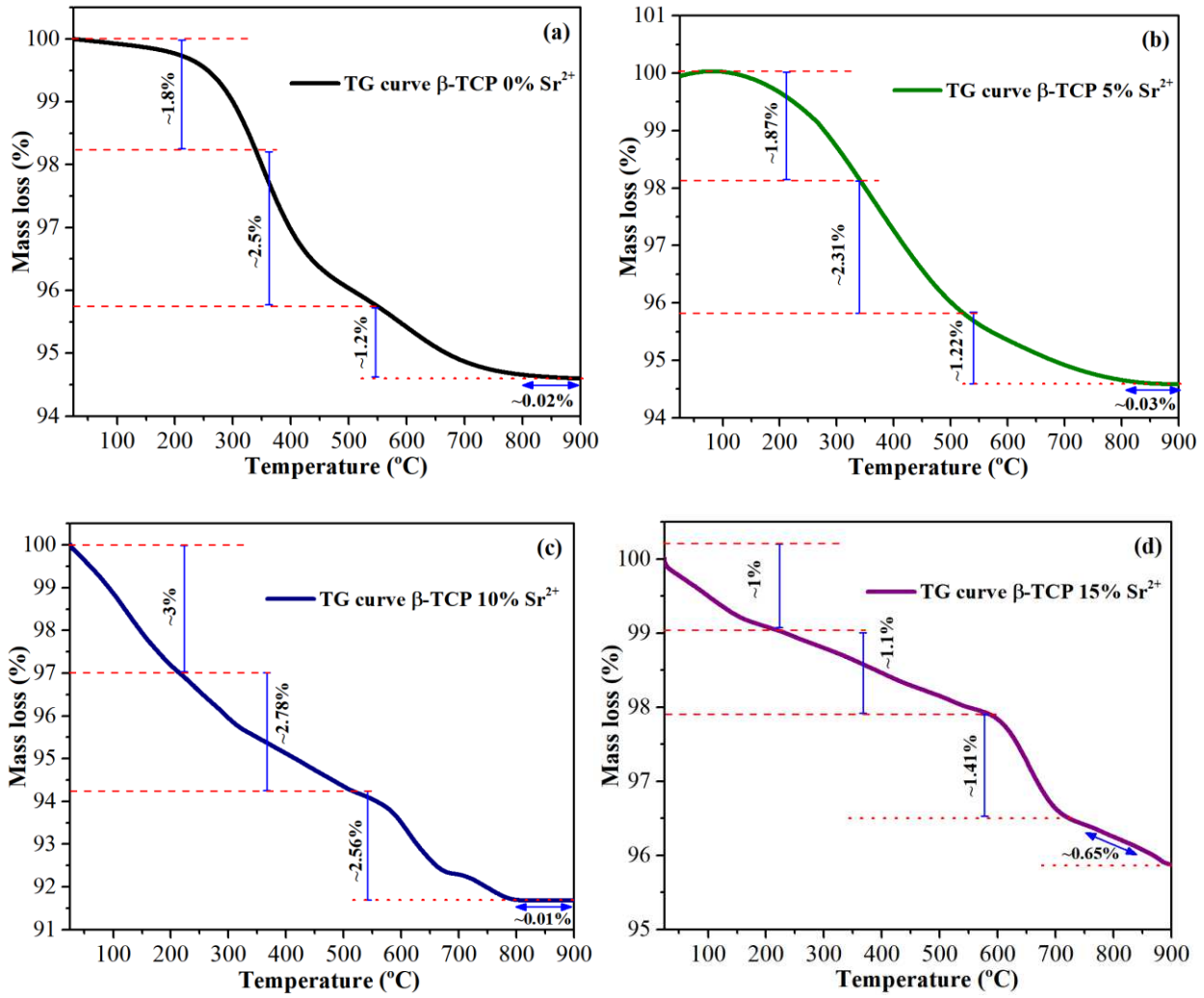


Figure 2: TG curves of the synthesized samples: (a) β -TCP 0% Sr^{2+} (total mass loss $\approx 5.5\%$), (b) β -TCP 5% Sr^{2+} ($\approx 5.5\%$), (c) β -TCP 10% Sr^{2+} ($\approx 5.5\%$), and (d) β -TCP 15% Sr^{2+} ($\approx 5.5\%$) in the range 25–900 °C. Thermal events are assigned as follows: 25–200 °C (removal of adsorbed water), 200–550 °C (decomposition of organic residues such as citric acid and ethylene glycol), 550–800 °C (oxidation of residual carbon and partial decarbonation), and >800 °C (phase stabilization and improvement of crystallinity).

As displayed in **Figures 2(a–d)**, it is noted the thermogravimetric (TG) curves of $\beta\text{-Ca}_3(\text{PO}_4)_2\text{:Sr}^{2+}$ precursor powders synthesized via the polymeric precursor method with different Sr^{2+} contents: (a) 0%, (b) 5%, (c) 10%, and (d) 15%, respectively. All compositions exhibit multistep mass losses (%) from 25 °C to 200 °C associated with removal of adsorbed water, or powder precursor dehydration. This weight loss is attributed primarily to the removal of physically adsorbed water and the dehydration of weak hydroxyl groups or solvated molecules on the surface of the nanocrystals.

Moreover, the weight loss from 200 °C to 550 °C ascribed to decomposition of organic residues (citric acid, ethylene glycol), while the process from 550 °C to 800 °C related to oxidation of residual carbon and partial decarbonation, and structural rearrangement processes up to 900 °C [36]. The total mass loss (%) gradually increases with Sr²⁺ incorporation, indicating modifications in the decomposition profile and thermal stability of the precursors. Specifically, the 0% Sr²⁺ sample shows a total mass loss of ~5.5%, while the 15% Sr²⁺ sample presents a more continuous and extended degradation behavior, suggesting that Sr²⁺ ions influence the polymeric network and delay the complete thermal stabilization of β -TCP powders. These processes are fast and have low enthalpy; they are typically observed as a gentle drop at the beginning of the curve. Overall, the TG evolution indicates that Sr²⁺ incorporation does not simply act as an inert dopant but actively modifies precursor chemistry and the energetics of phase formation, influencing both the kinetics and thermodynamic stability of the β -TCP phase [37].

In addition, DSC curves of polymeric β -TCP precursors containing 0%, 5%, 10%, and 15% Sr²⁺ show the progressive influence of Sr²⁺ substitution on thermal behavior. For pure β -TCP, two clear endo/exothermic events correspond to water removal and organic decomposition. As Sr²⁺ content increases, the curves become smoother with lower exothermic intensity, indicating enhanced thermal stability [38]. The progressive substitution of larger Sr²⁺ ions ($r = 1.14 \text{ \AA}$) for Ca²⁺ ions ($r = 0.99 \text{ \AA}$) leads to lattice relaxation and alters the bonding environment. At 10–15% Sr²⁺ content, the heat flow profile approaches linearity, indicating uniform decomposition of polymeric species. These findings demonstrate that Sr incorporation decreases the energy released during combustion and stabilizes the precursor network, thereby promoting controlled β -TCP crystallization at elevated temperatures, as illustrated in **Figs. S11(a–d)** in the *Supporting Information*.

3.2. X-ray diffraction and Rietveld refinement analysis

Figures 3(a,b) illustrates the XRD patterns in range from 20° to 60° for samples β -TCP 0% Sr²⁺, β -TCP 5% Sr²⁺, β -TCP 10% Sr²⁺, and β -TCP 15% Sr²⁺, and XRD patterns in from 25° to 40° which were doped with 0%, 5%, 10%, and 15% of Sr²⁺, respectively.

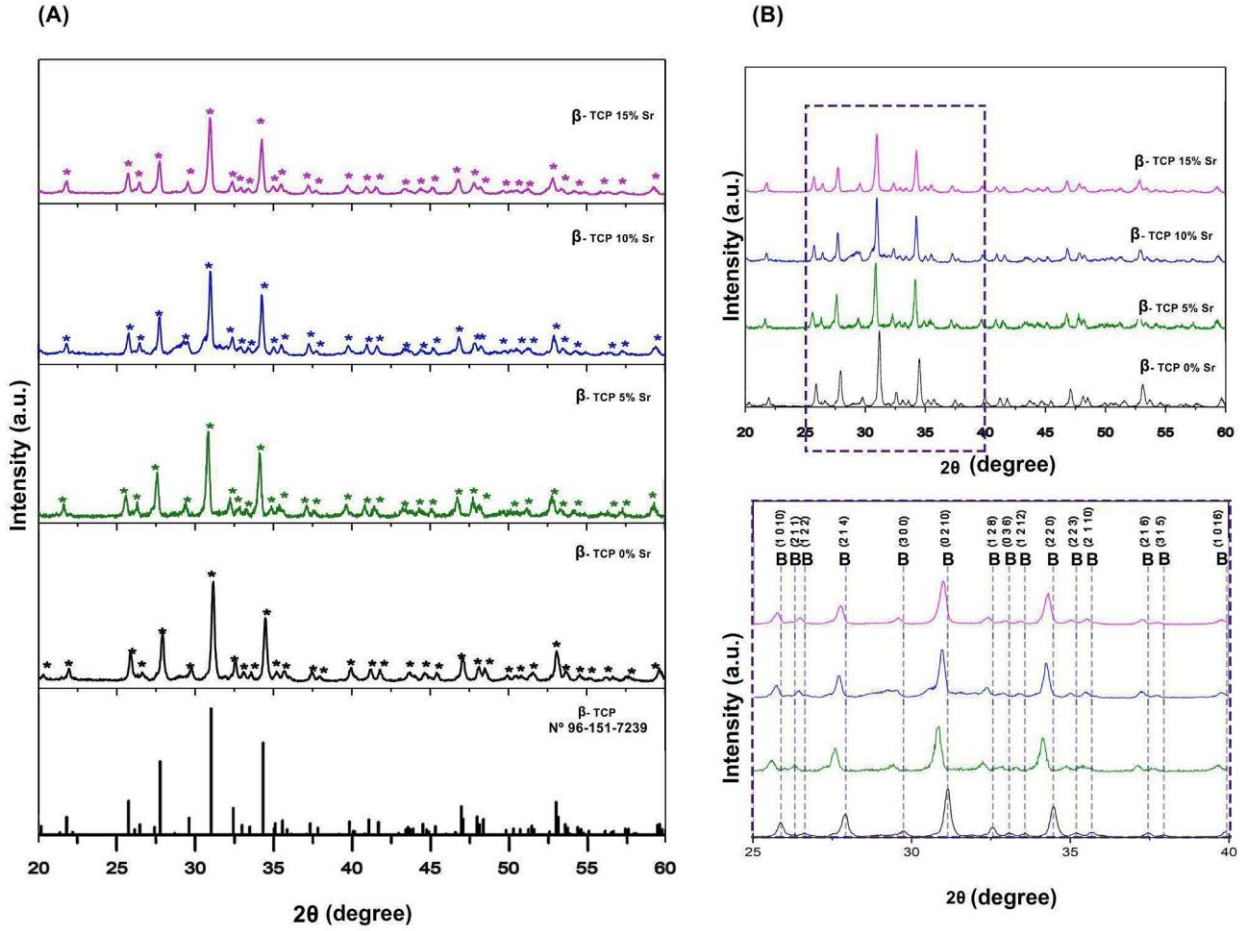


Figure 3: X-ray diffraction patterns of (a) β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} samples, and (b) corresponding peak shifts indicating lattice modifications induced by Sr^{2+} incorporation.

Figure 3(a) shows that the pattern is similar for all samples, with well-defined peaks of beta-tricalcium phosphate phase observed in all samples, according to the ICDD card N°. 96-151-7239. It is noticeable that with the addition of strontium (Sr), there is a slight shift of the prominent peaks towards smaller angles since Sr^{2+} ions with a same coordination has an ionic radius ($r_{\text{Sr}^{2+}} = 1.18 \text{ \AA}$), more than Ca^{2+} ionic radius ($r_{\text{Ca}^{2+}} = 0.99 \text{ \AA}$), promotes a slight increase in unit cell volume. This shift can be considered evidence of the successful incorporation of Sr^{2+} ions into the β -TCP structure, as shown in **Figure 3(b)**. Additionally, there was no formation of secondary phases, confirming the effectiveness of the polymeric precursor method.

In this **Table 1** are presented the lattice parameters, crystallinity, and average crystallite size for samples β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} , respectively.

Table 1: Lattice parameters, unit cell volume, average crystallite size, and degree of crystallinity of β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} samples.

<i>Samples code</i>	<i>Lattice Parameters</i>		<i>Unit cell volume ($\text{\AA}^3$)</i>	<i>Average crystallite size (nm)</i>	<i>Degree of crystallinity (%)</i>
	<i>a = b ($\text{\AA}$)</i>	<i>c ($\text{\AA}$)</i>			
β -TCP 0% Sr	10.440	37.403	3530.4	43.55	94.66
β -TCP 5% Sr	10.447	37.404	3534.2	30.32	76.44
β -TCP 10% Sr	10.470	37.475	3557.6	32.40	69.26
β -TCP 15% Sr	10.497	37.628	3590.5	34.41	63.26
Refinement reliability factors: [β -TCP 0% Sr $\rightarrow R_p = 13.30\%$; $R_{wp} = 17.58\%$; $R_{exp} = 11.83\%$ and $\chi^2 = 2.21$], [β -TCP 5% Sr $\rightarrow R_p = 14.20\%$; $R_{wp} = 18.30\%$; $R_{exp} = 11.00\%$ and $\chi^2 = 2.75$], [β -TCP 10% Sr $\rightarrow R_p = 13.13\%$; $R_{wp} = 16.56\%$; $R_{exp} = 10.85\%$ and $\chi^2 = 2.33$], and [β -TCP 15% Sr $\rightarrow R_p = 12.05\%$; $R_{wp} = 15.26\%$; $R_{exp} = 10.48\%$ and $\chi^2 = 2.12$].					

After analyzing the data from **Table 1**, it is evident that incorporating Sr^{2+} ions tends to decrease the crystallinity of the samples. Due to the expansion of the material's crystal lattice, the Sr^{2+} ions at same coordination with an ionic radius ($r_{\text{Sr}^{2+}} = 1.18 \text{ \AA}$), causing distortions in the crystal lattice and creating new crystal defects such as vacancies and interstices, leading to a reduction in the crystallinity percentage of the samples. The increase at unit cell volume from to $3530.4 \text{ \AA}^3$ to $3590.5 \text{ \AA}^3$ is again indicative of the successful incorporation of Sr^{2+} ions into the lattice of β -TCP crystal structure [39,40]. The polymeric precursor method enabled the attainment of nanometer-scale crystallites, ranging from 30.32 to 43.55 nm, which potentially exerts a significant impact on the interaction of cell implants within the body environment. The nanomaterials possess adjustable surface properties and a bioactive nature; such materials offer enhanced surface area, adequate rigidity, roughness, and altered physicochemical properties to promote adhesion, proliferation, synthesis of bone-related proteins, and deposition of calcium-containing minerals. Moreover, the incorporation of Sr^{2+} ions has been found to decrease the crystallite size, possibly due to microstrains

in the lattice, as well as changes in the crystal nucleation and growth process during synthesis. This intriguing finding adds to the potential of nanomaterials for orthopedic implants [41–43]. The incorporation of Sr^{2+} into the β -TCP lattice and its preferential occupation of Ca sites has been widely reported and confirmed by Rietveld refinement, with Sr substitution leading to lattice expansion due to its larger ionic radius compared to Ca^{2+} in the in the literature [RSI] at Reference for *Support Information*.

A slight increase in lattice parameters, as well as in the volume of unit cells of the samples doped with Sr^{2+} ions, compared to the pure sample, is noticeable, indicating that the substitution of Ca^{2+} ions by Sr^{2+} ions into the rhombohedral β -TCP lattice was adequate, consistent with the diffraction patterns displayed in **Figure 3(b)**. The increase occurs because of the occupation of interstitial spaces by Sr^{2+} ions causing changes in interplanar distances and distortions in the crystal lattice of the material, resulting in variations in lattice parameters and unit cell volume [39]. Moreover, the unit-cell expansion remains consistent with the substitution of Ca^{2+} ions by larger Sr^{2+} ions, while slight deviations in individual reflections may arise from local structural distortions and anisotropic lattice effects. After Rietveld refinement analysis, as displayed in **Figs. SI2(a–d)** in the *Supporting Information*, it was found that β -TCP crystallized in the rhombohedral or trigonal crystal structure, with $R3c$ space group, N°. 161 at International Table for Crystallography and twenty-two molecular formula units per unit cell ($Z = 22$). We have employed some the CIF number 36966 [41] to modeling unit cell, and the samples showed lattice parameters consistent as previously works [42,43].

3.3. Schematic representation of unit cells and clusters coordination for $\beta\text{-Ca}_3(\text{PO}_4)_2$ crystals

Figure 4 illustrates the schematic representation for the unit cell of β -TCP crystal with a rhombohedral structure.

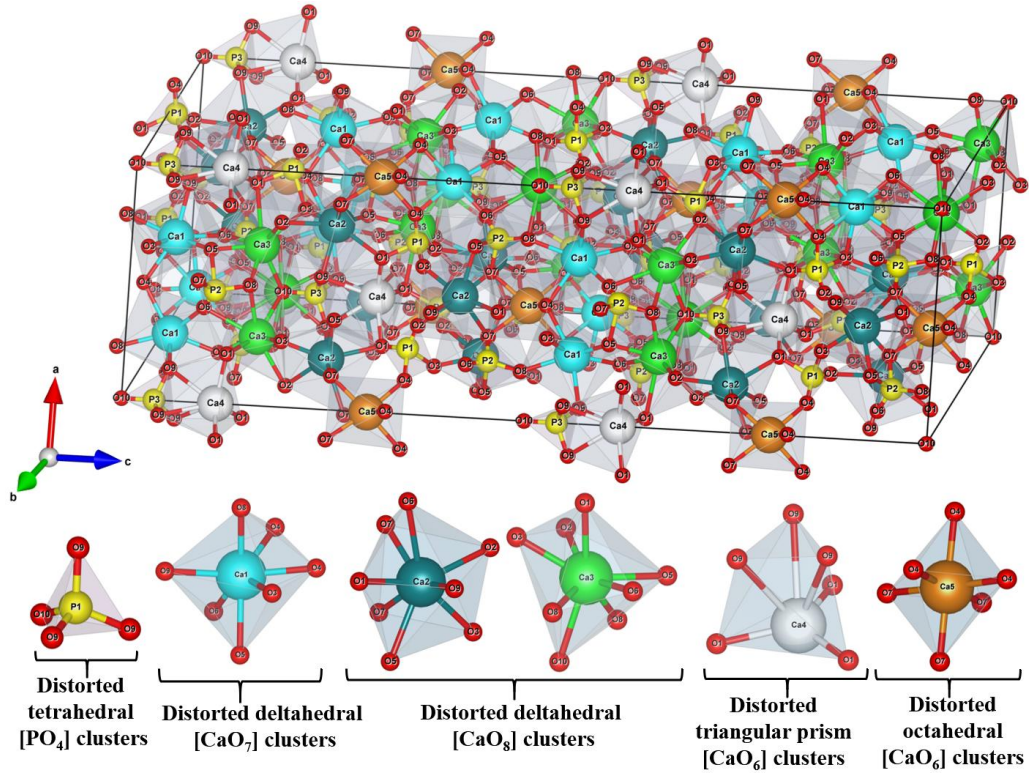


Figure 4: Schematic representation of the rhombohedral unit cell of pristine β - $\text{Ca}_3(\text{PO}_4)_2$, modeled using ICSD data (CIF No. 36966) with adjusted bond distances, as detailed in **Table 1**.

The structural data obtained from CIF number 36966, as illustrated in **Figure 4**, and presented in **Table 1**, were utilized to model the rhombohedral unit cell and the clusters coordination of five calcium (Ca) atoms (Ca1, Ca2, Ca3, Ca4, and Ca5), and three phosphorus (P) atoms (P1, P2, and P3). This rhombohedral unit cell was drawn using the program 3D Visualization for Electronic and Structural Analysis (*VESTA*), version 3.90.9a for Windows 11®-64 bits [44,45]. In addition, we have observed in the literature [46–49] that there is no consensus for modeling and correct description for the coordination of all calcium (Ca) atoms in this rhombohedral structure of β -TCP crystal at N° 161 according to the international table of crystallography, due to its high structural complexity with 22 molecular formula units per unit cell. **Figure 4** shows that there is only one type of cluster for the three types of P atoms (P1, P2, and P3), while there are four types of clusters for the five Ca atoms, which have coordination numbers of eight, seven, and six. The P atoms are associated with a single type of coordination related to distorted tetrahedral $[\text{PO}_4]$ clusters, which have a point symmetry

group of T_d . In contrast, the Ca atoms exhibit different types of clusters coordination. The Ca1 atoms form distorted deltahedral $[\text{CaO}_7]$ clusters, characteristic of polyhedron known as an irregular pentagonal bipyramid, and have a point symmetry group of D_{5h} . The Ca2 and Ca3 atoms form distorted deltahedral $[\text{CaO}_8]$ clusters, exhibiting a polyhedron of type biaugmented triangular prisms and have a point symmetry group of C_{2v} . The Ca4 atoms demonstrate greater symmetry breaking, forming distorted triangular prism $[\text{CaO}_6]$ clusters with a point symmetry group of D_{3h} . Lastly, the Ca5 atoms are typically characterized by distorted octahedral $[\text{CaO}_6]$ clusters that belong to the point symmetry group O_h . Moreover, according to the Dickens *et al.* [45], the Ca1 atom is coordinated strongly to seven oxygen's (2 $[\text{PO}_4]$ clusters edges, and 3 apexes) with a thermal ellipsoid approximately isotropic. The Ca2 and Ca3 atoms are coordinated strongly to six oxygen's and less strongly to two others due to longer chemical bond distances. The thermal parameters of Ca2 and Ca3 atoms are less isotropic than those of Ca1 atom. The Ca4 atom is coordinated with three oxygen atoms with very close bond distances, present a slight disordered along c -axis and/or weakly bound to others three oxygen atoms. However, the large bond distance, can be leads to formation a possible vacant clusters $[\text{CaO}_3.3V_{\text{O}}^{\bullet\bullet}]$. Finally, the Ca5 is coordinated to six oxygen atoms and may be ascribed to normal thermal motion with small thermal parameter. Typically, the work reported in the literature [47] has prepared this material at high temperatures $> 1,100$ °C. Yashima *et al.*, [48], performed investigations by means of neutron powder diffraction to $\beta\text{-Ca}_3(\text{PO}_4)_2$ and explained the following coordination numbers (CN) clusters for the atoms of Ca1 (CN = 7), Ca2 (CN = 8), Ca3 (CN = 8), Ca4 (CN = 3), and Ca5 (CN = 6), respectively. Liu *et al.*, [49] have prepared a $\beta\text{-Ca}_3(\text{PO}_4)_2\text{-Ca}_9\text{La}(\text{PO}_4)_7\text{:Eu}^{2+}/\text{Mn}^{2+}$ solid solutions phosphors and has explained the same about the cluster coordination of Ca atoms as the paper reported by Dickens *et al.*, [46]. However, with a modification in the NC for Ca atoms when a percentage (%) increase in substitution of $x.\text{Ca}_9\text{La}(\text{PO}_4)_7\text{:Eu}^{2+}$ is introduced into the crystal lattice of $1-x.\beta\text{-Ca}_3(\text{PO}_4)_2$ we have a change at CN clusters from 7 and 8 at Ca1 and Ca2 atoms, and a change to 8 and 9 to at Ca3 atoms. Moreover, theoretical studies using density functional theory (DFT) methods [41] have been reported to pure $\beta\text{-Ca}_3(\text{PO}_4)_2$ and doped with

Nd^{3+} ions, which can be added impurity centers over five Ca^{2+} positions. The results indicated the discovery of different pair $\text{Nd}^{3+} - \text{Nd}^{3+}$ centers, the formation of which is energetically favorable for charge compensation according to the $3\text{Ca}^{2+} \rightarrow 2\text{Nd}^{3+} + \text{vacancies}$ scheme, when one Ca position remains vacante. In this **Table 2** are presented the occupancy factors for samples $\beta\text{-TCP 0\% Sr}^{2+}$, $\beta\text{-TCP 5\% Sr}^{2+}$, $\beta\text{-TCP 10\% Sr}^{2+}$, and $\beta\text{-TCP 15\% Sr}^{2+}$, respectively.

Table 2: Atomic occupancy factors for $\beta\text{-TCP 0\% Sr}^{2+}$, $\beta\text{-TCP 5\% Sr}^{2+}$, $\beta\text{-TCP 10\% Sr}^{2+}$, and $\beta\text{-TCP 15\% Sr}^{2+}$ samples.

<i>Samples code</i>	Ca1	Ca2	Ca3	Ca4	Ca5
$\beta\text{-TCP 0\% Sr}$	100.00	100.00	100.00	43.00	100.00
$\beta\text{-TCP 5\% Sr}$	100.00	100.00	90.00	25.00	96.00
$\beta\text{-TCP 10\% Sr}$	94.43	98.02	80.79	25.30	100.00
$\beta\text{-TCP 15\% Sr}$	81.60	81.60	81.60	40.80	100.00

As shown in **Table 2**, the relationship between Sr^{2+} ion concentration and occupancy factors is a key aspect of our research. We found that as the concentration of Sr^{2+} ions increases, the occupancy factors tend to decrease due to the enlargement of the unit cell. This leads to an increase in the total number of occupancy sites within the unit cell, while the quantity of atoms of a specific element remains constant. Consequently, the ratio between the number of atoms of that element and the total number of available sites decreases, resulting in a decrease in the occupancy factor of that element [38]. We also observed that the Ca^{2+} (5) site obtained higher values, possibly due to the low defect formation energy for small cations that occupy this site, as reported in the literature [40]. The variations in the atomic positions related to oxygen (O) atoms were noted, while the calcium (Ca), strontium (Sr), and phosphorus (P) atoms had fixed positions. The substitution positions of Ca atoms by Sr were not fixed under the applied constraints and without inducing any site-preference claims. Instead, Sr atoms were allowed to remain mobile to identify the Ca1, Ca2, Ca3, C4, and C5 sites that best satisfied the statistical parameters of the Rietveld Refinement, yielding values consistent with the most relaxed electronic structure. In this way, the Sr^{2+} substitutes isovalently for Ca^{2+} and

therefore does not require charge-compensation mechanisms involving vacancies. Hence, there is a disturbance in the respective positions of O atoms into the lattice, provoked by the replacement of Ca^{2+} by Sr^{2+} , yielding of distortions in (O–Ca–O)/(O–Sr–O) and (O–P–O) bonds of their clusters, respectively.

These findings have significant implications for the crystal structure, as illustrated in **Figures 5(a–c)**, which shows the crystal structure of β -TCP incorporated with (a) 5% Sr, (b) 10% Sr, and (c) 15% Sr, respectively.

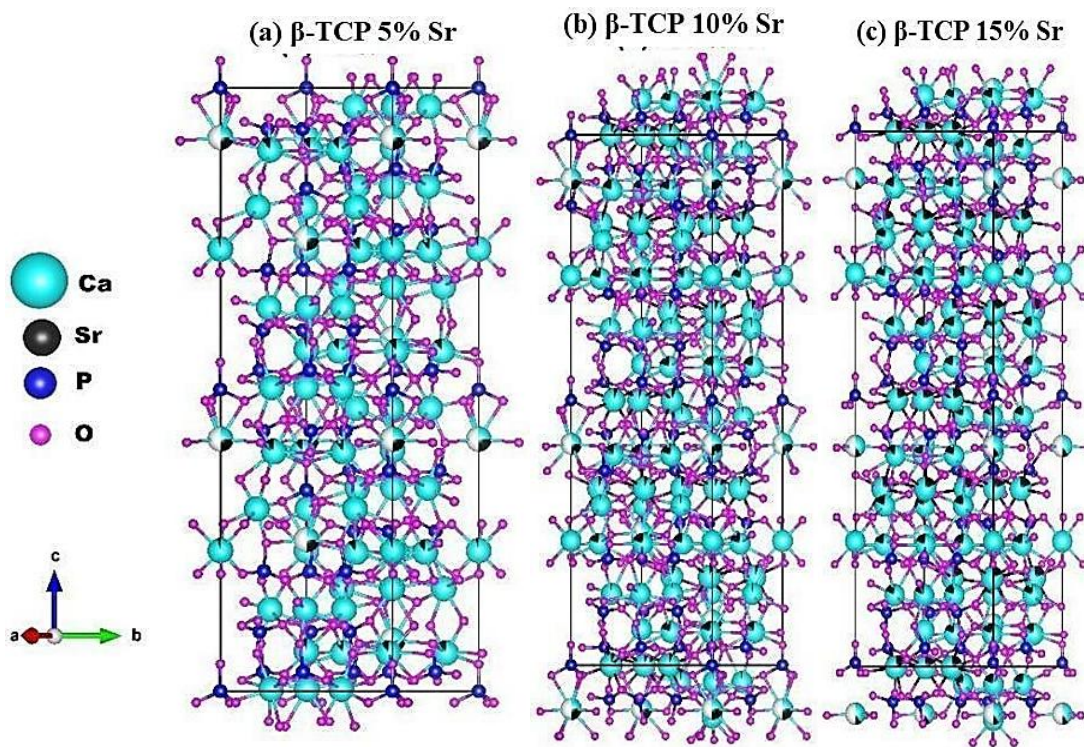


Figure 5: Refined crystal structures of (a) β -TCP 5% Sr^{2+} , (b) β -TCP 10% Sr^{2+} , and (c) β -TCP 15% Sr^{2+} , illustrating structural evolution with increasing Sr^{2+} substitution.

As displayed in **Figures 5(a–c)**, the unit cell models of β -tricalcium phosphate (β -TCP) incorporate different Sr^{2+} concentrations: (a) 5% Sr, (b) 10% Sr, and (c) 15% Sr. The substitution of Ca^{2+} by Sr^{2+} ions within the β -TCP lattice is clearly evidenced, maintaining the overall β -TCP framework while inducing subtle lattice distortions associated with Sr^{2+} larger ionic radius Sr^{2+} . These structural variations highlight the progressive incorporation of Sr^{2+} and its influence on the crystal symmetry and local coordination environment.

The chemical composition for the elements present in the samples was obtained by X-ray fluorescence (XRF). The calcium-to-phosphorus molar ratio, calculated through molar ratio (mol %), is described in **Table 3**.

Table 3: Semi-quantitative phase analysis of pure and Sr-substituted β -TCP samples (0–15% Sr^{2+}).

<i>Samples code</i>	Ca (%)	P (%)	Sr (%)	Calculated (Ca+Sr)/P (at%)
β -TCP 0% Sr	60.22	39.78	0.00	1.51
β -TCP 5% Sr	58.88	36.40	4.72	1.75
β -TCP 10% Sr	57.48	34.84	7.68	1.87
β -TCP 15% Sr	51.97	34.01	14.02	1.94

Elemental analysis by X-ray fluorescence (XRF) enabled the semiquantitative evaluation of the Ca/P atomic ratio and confirmed the presence of Sr^{2+} ions in the modified samples. According to Table 3, the pure sample (β -TCP 0% Sr) exhibited a Ca/P ratio of 1.51, which is in good agreement with the theoretical value of 1.50 reported in the literature [50,51]. However, with increasing Sr concentration, the (Ca + Sr)/P ratios showed values higher than that of the pure sample. This behavior is attributed to the partial substitution of Ca^{2+} ions by Sr^{2+} within the β -TCP crystal structure, as strontium contributes to the total amount of divalent cations present in the material [39,52]. Furthermore, the $\text{Sr}/(\text{Ca}+\text{Sr})$ ratios obtained by XRF confirm the progressive incorporation of strontium into the synthesized powders, indicating that increasing amounts of Sr were effectively incorporated into the material composition [53,54]. However, XRF analysis has intrinsic limitations when quantifying light elements, particularly phosphorus, and therefore may introduce uncertainties in calculated stoichiometric ratios, as shown in **Table 3**.

3.4. *Fourier-transform infrared analysis*

Figure 6 shows the FT-IR spectra of the β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} .

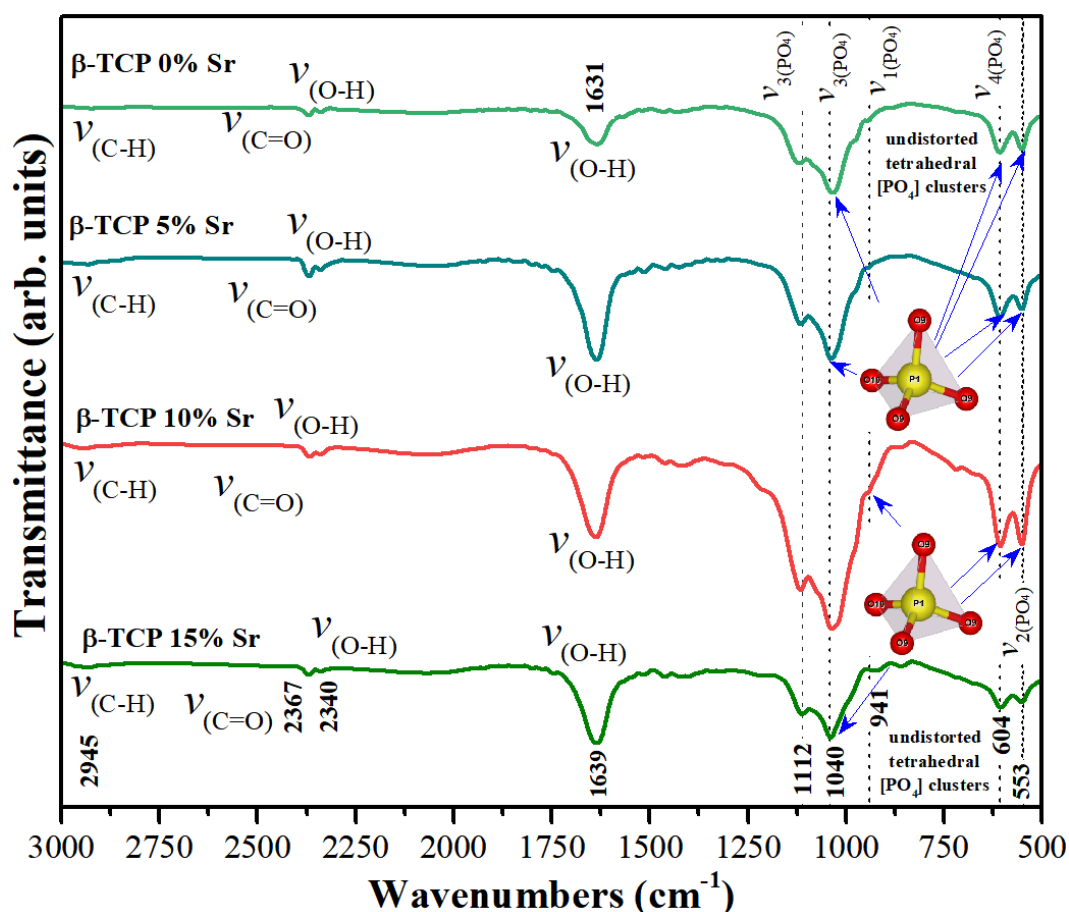


Figure 6: FT-IR spectra of β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} samples, highlighting characteristic phosphate vibrational bands and possible structural modifications.

All the FT-IR spectra displayed in **Figure 6** revealed similar vibrational modes, with the leading adsorption bands being one $\nu_1(\text{PO}_4)$ vibration at 941 cm^{-1} , two $\nu_3(\text{PO}_4)$ vibrations at 1040 cm^{-1} and $1,112\text{ cm}^{-1}$, one $\nu_4(\text{PO}_4)$ vibrations at 604 cm^{-1} , and one $\nu_2(\text{PO}_4)$ at 553 cm^{-1} [31,54,55]. The observed variations in band intensities are described as relative changes that may result from structural modifications, differences in local bonding environments, or variations in sample homogeneity. It is important to note that intensity comparisons in FT-IR spectra require careful interpretation, as they may also be affected by factors including particle size, packing effects, and scattering. The bands at $1,040$ and $1,112\text{ cm}^{-1}$ demonstrate the asymmetric stretching of distorted tetrahedral $[\text{PO}_4]$ clusters in β -TCP. Meanwhile, the bands at 604 and 553 cm^{-1} are attributed to the O–P–O bending vibrations and symmetric bending vibration of distorted tetrahedral $[\text{PO}_4]$ clusters

[27,39]. Overall, it is noticeable that the band intensities increase as the concentration of Sr^{2+} ions increases. It occurs due to alterations in the chemical nature of the phosphate bonds present in the material's structure due to Sr^{2+} ions occupying Ca sites. This leads to decreased Ca sites available to interact with the distorted tetrahedral $[\text{PO}_4]$ clusters. Consequently, the phosphate group exhibits more vital interaction and, thus, higher band intensities [39,56]. In our FT-IR spectra at midregion from 1,500 to 3,000 cm^{-1} is due to the presence of water (H_2O) adsorbed molecules, adsorbed organics and carbon dioxide (CO_2) at crystals surface and arising from the room atmosphere and humidity. A IR-bands at 2,945 cm^{-1} is related to symmetric stretching of CH groups from $\nu(\text{C-H})$ due to possibly from adsorbed organics or synthesis residues. A IR-bands at 236 and 2,340 cm^{-1} is related to $\nu(\text{O-H})/\nu(\text{C=O})$ assigned to stretching vibration of surface hydroxyl or atmospheric CO_2 weak, while a médium IR-bands from 1631 and 1639 cm^{-1} are assigned to bending vibration ($\delta\text{H-O-H}$) of adsorbed bound water (H_2O) molecules adsorbed at crystals surface. A IR-bands located at 2,269 cm^{-1} is due to adsorbed CO_2 or or atmospheric CO_2 . In general, the dominant phosphate bands between 1,200–500 cm^{-1} confirm the β -TCP crystalline phase. The slight shift and intensity reduction with increasing Sr^{2+} concentration (5–15%) indicate local lattice distortion and possible changes in P–O bond strength, as shown in **Figure 6**. The broad O–H absorption bands observed in the FT-IR spectra are attributed primarily to adsorbed atmospheric moisture and surface hydroxyl groups commonly present in calcium phosphate materials due to their hygroscopic nature and high specific surface area. The preservation of bands near 1,112 cm^{-1} , 1,040 cm^{-1} , and 960 cm^{-1} suggests undistorted tetrahedral $[\text{PO}_4]$ clusters even at higher Sr substitution, aligning with structural stability.

3.5. Scanning electron microscopy analysis

Figure 7 presents the SEM images for β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} , respectively.

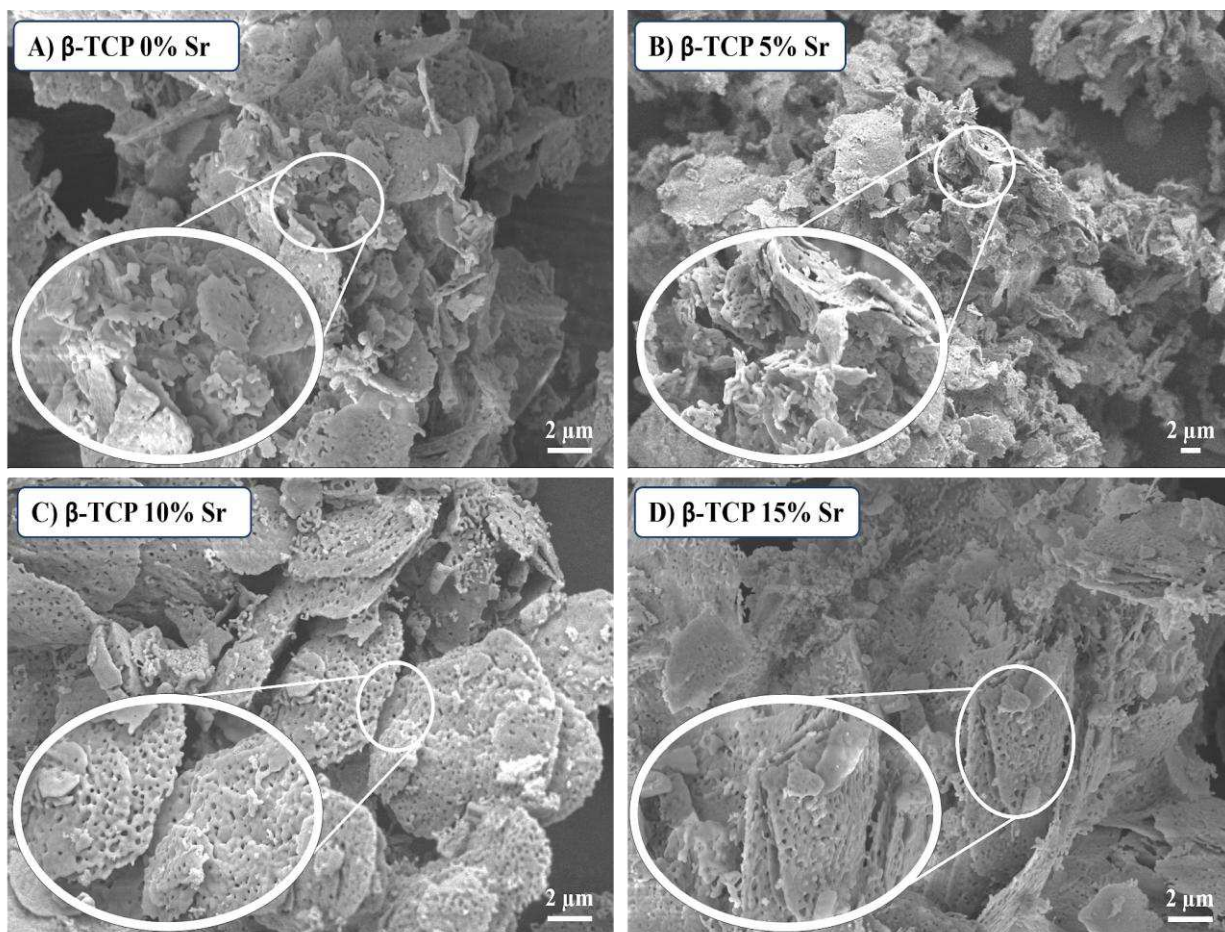


Figure 7: SEM micrographs of β -TCP 0% Sr^{2+} , β -TCP 5% Sr^{2+} , β -TCP 10% Sr^{2+} , and β -TCP 15% Sr^{2+} powders, showing morphological features and particle distribution.

As displayed in **Figures 7(a–d)**, the SEM images reveal as a calcium phosphate material formed by aggregated flakes of β -TCP, showing a tendency for pore size to increase as Ca^{2+} ions are replaced by Sr^{2+} ions, as noted in the literature [56]. The presence of several pores in all samples confirms that the polymeric precursor method, composed of citric acid and ethylene glycol, promotes the formation of multiple agglomerates after pyrolysis. Further structural details are evident in higher-resolution SEM images, as presented in **Figs. SI3(a–d)** in the *Supporting Information*. Corresponding histograms of average pore size distribution are provided in **Figs. SI4(a–d)** in the *Supporting Information*. This results in the formation of microparticles with a higher degree of porosity, which is crucial for practical applications such as mineralization, nutrient diffusion in bone, and cell

proliferation. These findings highlight the significance of β -TCP ceramics in these fields, where these characteristics notably enhance their functionality [57,58].

3.6. Zeta potential analysis

Figures 8(a–d) display the Zeta potential (ZP) plots for β -TCP (0% Sr), β -TCP:5% Sr, β -TCP:10% Sr, and β -TCP:15% Sr, respectively.

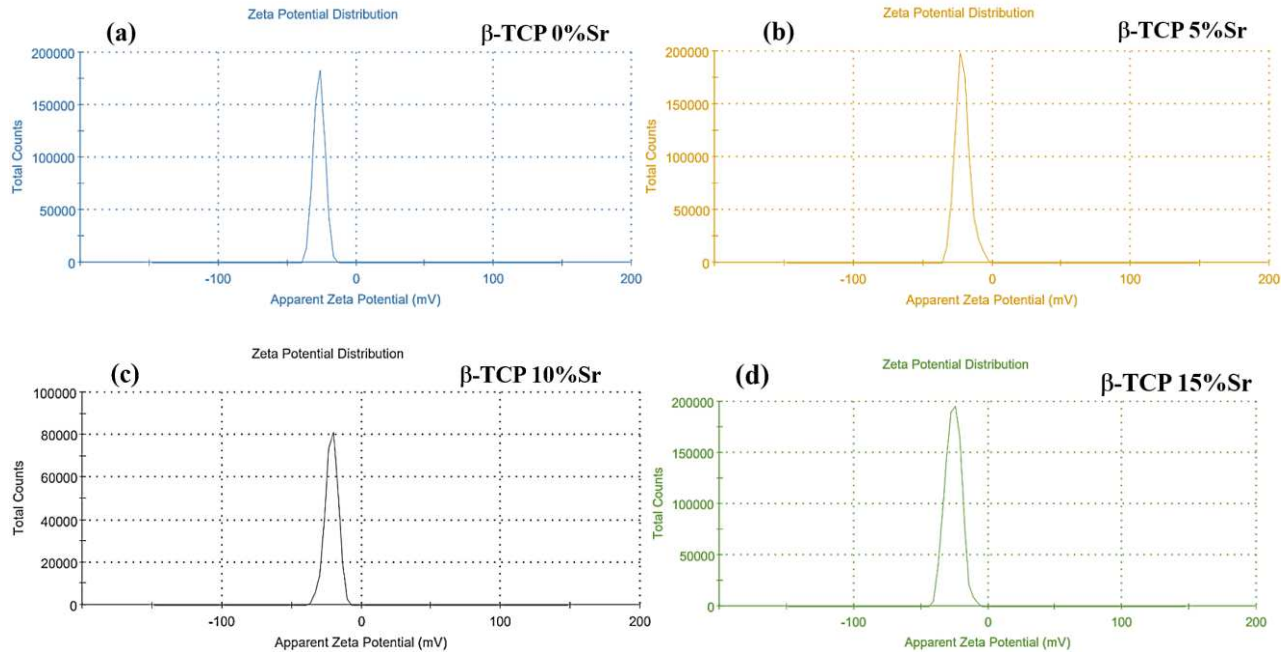


Figure 8: Zeta potential distribution curves of β -tricalcium phosphate (β -TCP) powders with increasing Sr^{2+} substitution: (a) 0% Sr, (b) 5% Sr, (c) 10% Sr, and (d) 15% Sr.

Practically, all **Figures 8(a–d)** display narrow peaks for ZP curves, indicating relatively monodisperse surface charges for four samples. In **Figure 8(a)**, the β -TCP sample exhibits a peak ζ potential centered around -8 mV, which indicates a slightly negative surface charge due to exposed phosphate groups (PO_4^{3-}) on the particle surface. In **Figure 8(b)**, a small Sr^{2+} substitution slightly increases the surface negative charge, likely due to partial replacement of 95% Ca^{2+} by 5% Sr^{2+} , which may alter the local electronic environment and hydration layer. The behavior is due to substitution of Ca^{2+} ions ($r_{\text{Ca}^{2+}} \approx 0.99 \text{ \AA}$) by larger Sr^{2+} ions ($r_{\text{Sr}^{2+}} = 1.14 \text{ \AA}$), which distorts the lattice and changes surface charge effects and hydration. As Sr^{2+} replaces Ca^{2+} in the lattice, with 5% the peak ζ potential

is around -10 mV, and with 10% it is around -11 mV. Zeta potential measurements are now presented solely as indicators of changes in surface physicochemical properties following Sr incorporation. Moreover, other factors such as the lattice distortions and crystalline defects of β -TCP crystals doped-Sr% increasing free energy at the crystal surface. β -TCP crystals specific surfaces with high energy and structural defects are biologically more reactive. Mesopore size and surface roughness also play roles, while the Sr^{2+} competes with Ca^{2+} for essential enzymatic processes, impairing metabolic pathways and intracellular signaling in bacteria, thereby leading to oxidative stress. The presence of Sr^{2+} during synthesis by polymeric precursors method inhibits excessive grain growth during calcination. Finally, the Sr^{2+} ions also alters the speed of the ionic microenvironment, disrupting the osmotic gradient vital for bacterial survival, where the replacing from Ca^{2+} to Sr^{2+} promotes changes at thermodynamic stability of the β -TCP crystal. In **Figures 10(b,c)**, we have slightly enhanced the negative potential, probably because substitution increases the proportion of exposed phosphate or modifies the interfacial double layer. Finally, in **Figure 8(d)**, it's noted that a peak ζ potential shifts slightly to more negative (~ 12.5 mV) and broadens a little at 15% due to these factors such as high surface energy, structural defects, pore size, surface roughness, grain growth, speed of the Sr^{2+} ionic microenvironment. In addition, a stronger lattice distortion, an increase in unit cell volume, and greater charge heterogeneity of particles with a more negative surface, giving a broader distribution and a more negative mean potential [59].

3.7. Evaluation of acute toxicity using the *Artemia salina* test

Figure 9 displays the results of toxicity assay using *Artemia salina*, showing the percentage (%) mortality of the organisms as a function of the concentration of the tested samples.

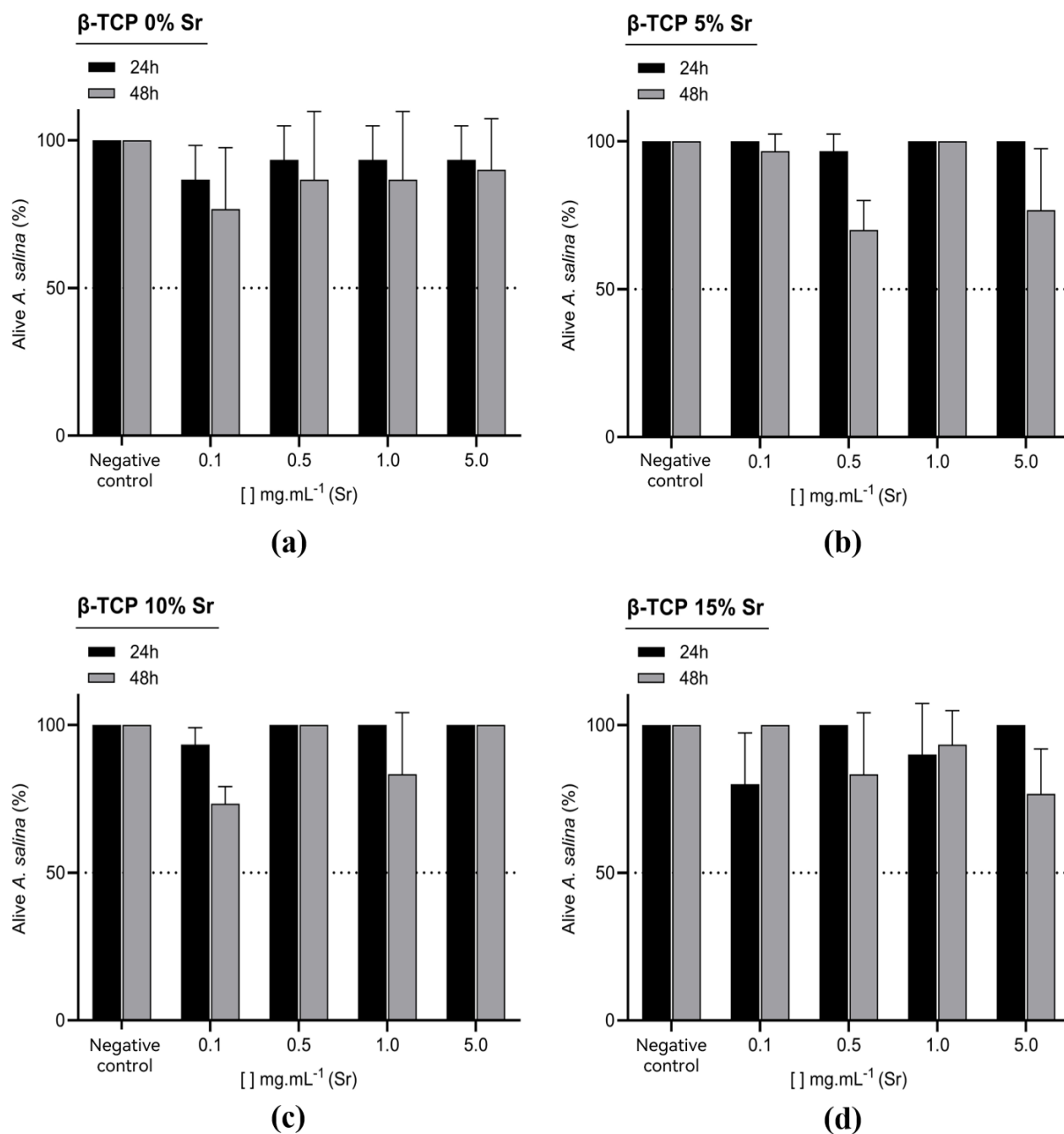


Figure 9: Mortality of *Artemia salina* exposed to (A) β -TCP 0% Sr²⁺, (B) β -TCP 5% Sr²⁺, (C) β -TCP 10% Sr²⁺, and (D) β -TCP 15% Sr²⁺ samples.

The toxicity of the samples was evaluated using the lethality assay with *Artemia salina*, a model widely employed for preliminary toxicity screening due to its high sensitivity to different

chemical compounds and its good correlation with more complex biological systems. As shown in Figure 9, all samples exhibited similar behavior, presenting low mortality rates, with an average of approximately three dead nauplii regardless of the concentration evaluated. The samples were therefore considered non-toxic to *Artemia salina*, since the percentage of dead nauplii remained below 10% at concentrations equal to or lower than 1.0 mg/mL, a threshold considered by the World Health Organization as a necessary condition for a natural-derived material to be classified as non-toxic to *Artemia salina* [60, 61].

This behavior may be related to the bioactive and biocompatible nature of calcium phosphates. During interaction with the aqueous medium, β -tricalcium phosphate (β -TCP) tends to gradually release Ca^{2+} and PO_4^{3-} ions, species naturally present in physiological systems and generally well tolerated by living organisms. The partial substitution of Ca^{2+} by Sr^{2+} also does not result in significant toxicity, since strontium exhibits chemical properties similar to those of calcium and has a recognized affinity for mineralized tissues. Therefore, the ionic release resulting from the partial dissolution of the material likely occurs at levels compatible with biological systems, minimizing potential cytotoxic effects [62]. Additional details are observable in percentage of mortality (%) at different concentrations tested of β -TCP ceramics, as shown in **Figs. SI5(a–d)** in the *Supporting Information*.

3.8. Antimicrobial activity

Figure 10 shows the inhibitory effect for the bacteria *S. aureus* ATCC 25.923 and *E. coli* ATCC 25.922.

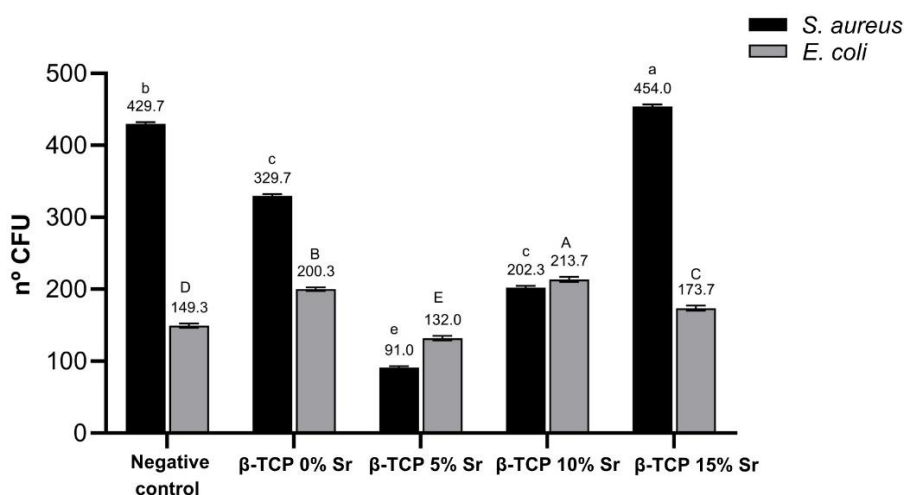


Figure 10: Antibacterial activity of the samples against *Staphylococcus aureus* and *Escherichia coli*, expressed as the number of colony-forming units (CFU). a,b,c,d Lowercase letters above the bars indicate statistically significant differences among the groups for *S. aureus*, determined by ANOVA followed by Tukey's multiple comparison test ($p < 0.05$). A, B, C, D Uppercase letters above the bars indicate statistically significant differences among the groups for *E. coli*, determined by ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

The antibacterial activity of the materials was evaluated using the direct contact method, as shown in **Figure 10**. The results indicate that the incorporation of strontium significantly influenced the number of colony-forming units (CFU), particularly for *Staphylococcus aureus*. The β -TCP 5% Sr sample exhibited the highest antibacterial activity, with a marked reduction in CFU compared to the control group. The superior antibacterial performance observed for the 5 mol% Sr-substituted β -TCP sample represents an experimental observation rather than definitive evidence of a specific mechanism. In addition, the antibacterial activity likely results from a combination of factors, including local ionic release, surface chemistry, crystallographic modifications induced by Sr incorporation, and particle–bacteria interactions. Since ion-release kinetics and molecular-level interaction studies were not performed in the present work, definitive structure–property correlations cannot be established for this paper. However, samples with higher strontium concentrations (10% and 15%) showed low or no antibacterial activity [63,64].

This behavior can be explained by the existence of an optimal concentration at which a favorable balance between ionic release and surface properties of the material occurs [65,66]. At moderate substitution levels, the incorporation of Sr^{2+} promotes slight structural distortions that increase surface reactivity and favor partial material dissolution [67]. This process results in the controlled release of Ca^{2+} , PO_4^{3-} , and Sr^{2+} ions into the surrounding medium, altering the microenvironment around bacterial cells [68,69]. In contrast, at higher concentrations, excessive incorporation leads to more pronounced structural modifications in the crystalline lattice of β -TCP, which may reduce the available surface area for interaction with bacterial cells and decrease the

dissolution rate of the material, thereby limiting the release of active ions into the medium [70,71]. Consequently, the antibacterial effect becomes less pronounced in these samples.

The differences observed between the two bacterial strains can also be explained by the structural characteristics of their cell walls. Gram-positive bacteria, such as *S. aureus*, possess a thick peptidoglycan layer associated with a single cytoplasmic membrane and cell walls rich in teichoic and lipoteichoic acids, which are polysaccharides linked to peptidoglycan or inserted into the cytoplasmic membrane [72]. In contrast, Gram-negative bacteria, such as *E. coli*, exhibit a more complex cell structure consisting of an inner cytoplasmic membrane and an outer membrane rich in lipopolysaccharides (LPS), with a thin peptidoglycan layer located between them [73,74]. This outer membrane acts as an additional barrier to the diffusion of antimicrobial agents, reducing the susceptibility of these bacteria to the tested materials [75]. The reduction in antibacterial activity observed at 15% Sr can be attributed to several plausible factors: (i) alterations in ion release behavior and dissolution kinetics, (ii) changes in local pH and ionic strength, and (iii) aggregation of nanoparticles, which decreases the effective surface area available for interaction with bacterial cells. However, our experimental results do not demonstrate a clear Gram-dependent susceptibility pattern.

Therefore, the results suggest that the antibacterial activity of Sr²⁺-doped β -TCP strongly depends on the level of ionic substitution within the crystalline structure of the material, with an optimal doping concentration that maximizes antimicrobial effects. Moderate levels of strontium favor ionic release and interaction with bacterial cells, whereas higher doping levels may alter the structural and surface properties of the material, reducing the observed antibacterial effectiveness. Finally, it is important to note that parameters such as the kinetics of Sr²⁺ ion release, local pH variations, and quantitative measurements of specific surface area were not evaluated in this study. Future investigations considering these factors may contribute to a deeper understanding of the mechanisms responsible for antibacterial activity in Sr-doped β -TCP systems [76].

3.9. Cell Viability

The cytocompatibility of the synthesized materials was evaluated using the MTT assay with murine fibroblasts, as presented in **Figures 11(a-d)**.

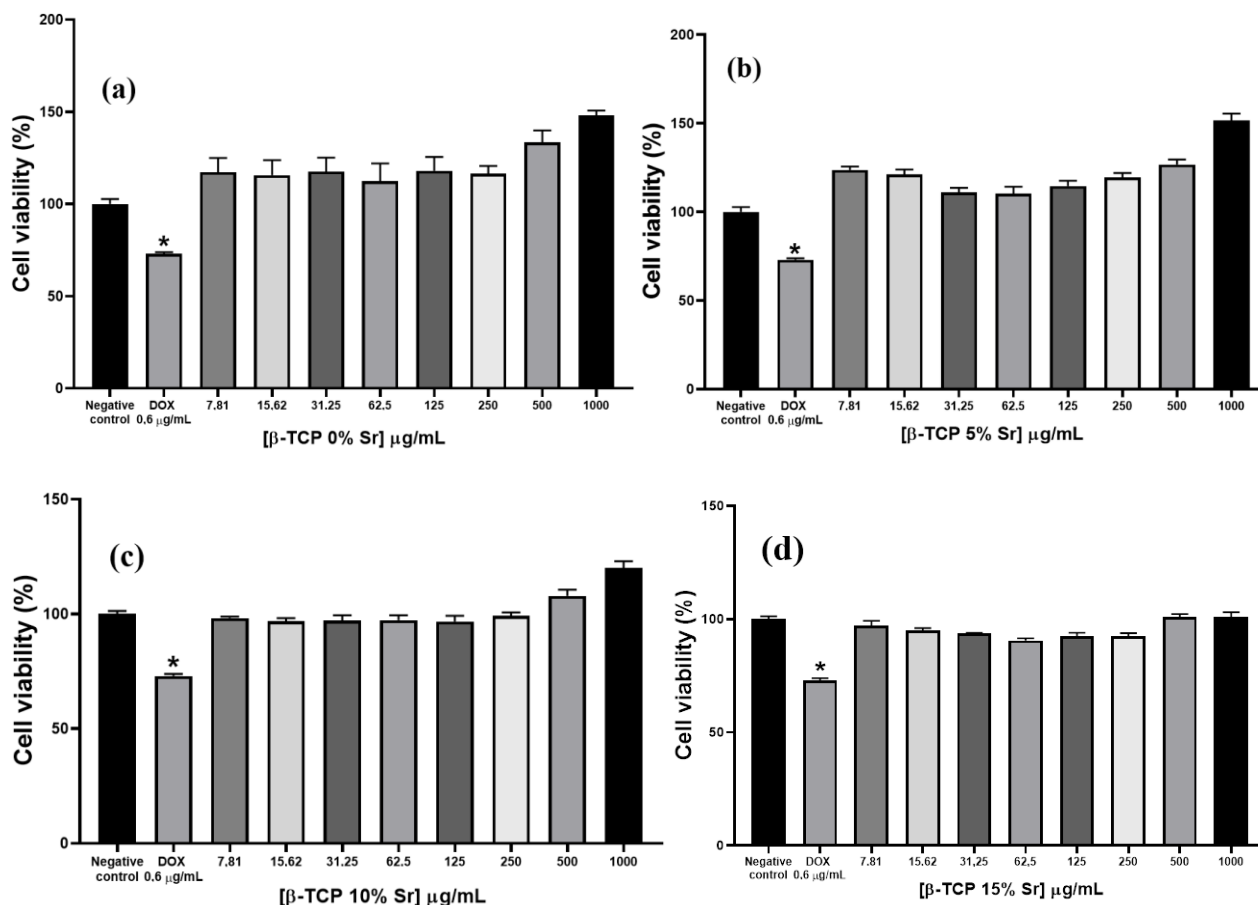


Figure 11(a-d): Percentage of mortality at different β -TCP concentrations containing (a) 0%, (b) 5%, (c) 10%, and (d) 15% Sr^{2+} , evaluated after 24 h and 48 h. All samples exhibited low mortality (<30%) across the tested concentrations, demonstrating non-toxic behavior. The calculated LC_{50} values were higher than the maximum concentration tested ($> 5.0 \text{ mg.mL}^{-1}$), indicating that all β -TCP compositions are atoxic to the bioindicator *Artemia salina* L. All samples

As shown in **Figure 11(a-d)**, none of the tested samples exhibited cytotoxic effects within the evaluated concentration range (7.81–1000 $\mu\text{g mL}^{-1}$). In all cases, cell viability remained above 90%, indicating that the β -TCP powders, both pure and Sr^{2+} -substituted, are biocompatible toward fibroblast cells. In contrast, the positive control (doxorubicin) showed a significant reduction in cell viability, confirming the sensitivity and reliability of the assay [56,72].

The absence of cytotoxicity can be primarily attributed to the intrinsic biocompatibility of calcium phosphate materials. β -Tricalcium phosphate (β -TCP) is chemically similar to the mineral

phase of bone and is widely recognized for its excellent biological performance in contact with mammalian cells. When dispersed in aqueous biological environments, β -TCP undergoes partial dissolution, releasing Ca^{2+} and PO_4^{3-} ions, which are naturally present in physiological systems and play essential roles in cellular metabolism and signaling pathways. These ions contribute to maintaining cellular homeostasis and may even stimulate cellular proliferation and metabolic activity, which explains the cell viability values slightly above 100% observed for some concentrations [72-74].

The incorporation of Sr^{2+} into the β -TCP structure did not induce any detectable cytotoxic effects, even at higher substitution levels, as shown in **Figures 11(a-d)**. This behavior is consistent with the known biological role of strontium in bone metabolism. Due to its similar ionic radius and chemical properties to Ca^{2+} , Sr^{2+} can be incorporated into calcium phosphate lattices without significantly disrupting their biological compatibility. Furthermore, strontium ions are known to exert beneficial biological effects by stimulating osteoblastic activity and inhibiting osteoclastic bone resorption. Although fibroblasts are not directly involved in bone mineralization, they represent a robust model for evaluating the general cytocompatibility of biomaterials, and the observed high cell viability suggests that Sr incorporation does not introduce harmful interactions with mammalian cells [73-75].

Another important aspect relates to the surface chemistry and dissolution behavior of the materials. Moderate ionic substitution in calcium phosphates can induce subtle structural distortions that influence solubility and ion exchange processes without generating harmful byproducts. In the present study, the absence of significant reductions in cell viability suggests that the ionic release from the Sr-doped β -TCP samples occurs within biologically tolerable limits. Additionally, the relatively stable viability profiles across the tested concentrations indicate that the materials do not trigger dose-dependent cytotoxic responses within the investigated range [76-78].

From a biomaterials perspective, these findings are particularly relevant because the development of multifunctional calcium phosphate systems requires a careful balance between

biological activity and cellular safety. The combination of antibacterial potential and high cytocompatibility observed for the synthesized powders indicates that Sr-doped β -TCP may represent a promising candidate for bone-related biomedical applications, such as bone graft substitutes, bioactive coatings, or composite scaffolds for tissue engineering.

Overall, the MTT results confirm that the synthesized materials exhibit excellent cytocompatibility toward murine fibroblasts, even at relatively high concentrations. These findings reinforce the suitability of Sr-substituted β -TCP as a bioactive ceramic with potential for safe interaction with mammalian cells, supporting its continued investigation for applications in bone tissue regeneration and related biomedical fields.

4. Conclusion

In summary, pure and Sr^{2+} -substituted β -tricalcium phosphate (β -TCP) powders containing 0, 5, 10, and 15 mol% Sr were successfully synthesized by the polymeric precursor method. Thermal analyses (TGA/DSC) confirmed the progressive removal of organic precursors and the formation of the crystalline β -TCP phase after heat treatment. XRD patterns and Rietveld refinement confirmed the formation of a single rhombohedral β -TCP phase without detectable secondary phases and revealed a gradual expansion of the unit-cell volume with increasing Sr content, consistent with the incorporation of larger Sr^{2+} ions into the β -TCP lattice. FT-IR spectra showed the characteristic phosphate vibrational modes of β -TCP and confirmed that the crystal structure was preserved after Sr substitution. SEM and XRF analyses revealed porous flake-like agglomerates and verified the presence of Sr in the substituted compositions, indicating successful incorporation of the dopant. Zeta-potential measurements demonstrated a progressive shift toward more negative surface potentials as the Sr content increased, indicating modifications in the surface physicochemical properties of the powders. Artemia salina toxicity assays showed low mortality rates for all compositions, classifying the materials as non-toxic under the investigated conditions. Furthermore, MTT assays using L-929 fibroblasts demonstrated cell viability above 90% for all samples, confirming their cytocompatibility. Antibacterial assays revealed that the β -TCP sample containing

5 mol% Sr exhibited the highest antibacterial activity against *Staphylococcus aureus*, whereas only limited inhibition was observed against *E. coli*. Overall, the combined structural, physicochemical, toxicological, and biological results demonstrate that moderate Sr incorporation can enhance the antibacterial performance of β -TCP without compromising its structural integrity or biocompatibility, highlighting the potential of Sr-substituted β -TCP as a multifunctional biomaterial for bone regeneration and related biomedical applications.

Notes

The authors declare no competing financial interest.

CRediT authorship contribution statement

This manuscript was written through the contributions of all authors who agreed with this submission.

S.L.C. Matias: Conceptualization, methodology, data curation, formal analysis, investigation, and writing – original draft. **J.R.S. Farias, R.P. Feitosa, J.N. Silva, P.M.P. Ferreira,** Data curation, formal analysis, investigation, validation, visualization, and Writing – review & editing, **G.A. Neves:** Data curation, formal analysis, investigation, and methodology. **J.A. Osajima, E.C. da Silva Filho** Writing – review & editing, **L.S. Cavalcante:** Writing - review & editing, supervision, and, investigation, project administration and visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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