Preparation of Lyophilized Sodium Alginate Fluorapatite Nanocomposites for Biomedical Applications

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**Abstract:** The synthesis of sodium alginate fluorapatite (Alginate-FAP) and the evaluation of its physical and chemical properties were investigated in this study. Fluorapatite, a calcium phosphate mineral found in bones and teeth, offers advantages such as higher elastic modulus and acid resistance compared to hydroxyapatite. Alginate, a polysaccharide derived from marine algae or bacteria, is known for its ability to form hydrogels through ionotropic gelation. The aim of this study was to assess the physical and chemical properties of Alginate-FAP. Various characterization techniques were employed, including FTIR, XRD, SEM, and hemocompatibility tests. FTIR analysis provided insights into the chemical properties of Alginate-FAP, while XRD analysis determined the crystallographic structure. SEM imaging allowed the examination of surface morphology and particle characteristics. Hemocompatibility tests assessed the compatibility of Alginate-FAP with blood components.

**Keywords:** Biomedical, Good Health, Novel Alginate-Fluorapatite composites, Antimicrobial activity, Tissue engineering

# Introduction

Fluorapatite (FAp, Ca₅(PO₄)₃F) is a key component of bones and teeth, known for its role in drug delivery and gene therapy. Compared to hydroxyapatite, it offers higher bulk and elastic modulus and superior acid resistance [1-3]. Fluorapatite, a biocompatible calcium phosphate, resembles hydroxyapatite but contains fluoride ions, enhancing stability, osteogenic properties, and acid resistance for biomedical applications. [(Harsha & Subramanian, 2022)](https://paperpile.com/c/XlLjTy/Rt5TF). Polymers serve as scaffolds in tissue engineering, mimicking the extracellular matrix with tunable mechanics, biocompatibility, and cell encapsulation.[(Deepika et al., 2022)](https://paperpile.com/c/XlLjTy/7MvlK).Common examples include PLGA, PCL, and PEG [4]. Alginate, a polysaccharide from brown algae and bacteria, is widely used in tissue engineering and drug delivery. Sodium alginate, its water-soluble form, undergoes ionotropic gelation with cations, making it valuable in biomedical, food, and pharmaceutical applications [5]. Degradation time can be set to correspond with the pace at which the target tissue is being regenerated. The use of fluorapatite polymer composites in tissue engineering holds promise for various applications, including bone regeneration, cartilage repair, and dental tissue engineering [(Borkowski et al., 2021; Rohani et al., 2022; Taktak et al., 2018)](https://paperpile.com/c/XlLjTy/9IFc4+4S397+8Vgh8)6].

In this study, we have doped fluorapatite with sodium alginate and we aim to assess its physical and chemical properties.

# Materials and methods

## Chemicals

FAp was synthesized by the wet chemical route using calcium nitrate tetrahydrate (Ca(NO3)2.4H2O, Merck), diammonium hydrogen phosphate ((NH4)2.HPO4, Merck), Calcium Fluoride (CaF2), ammonia solution (Merck), and triple distilled water (pH is adjusted using ammonia solution). Sodium alginate purchased from Merck is used to prepare sodium alginate incorporated in FAp.

## Experimental procedure

### Synthesis of Fluorapatite

1.0 M Ca(NO3)2.4H2O, 1.0M of CaF2 and 0.6 M ((NH4)2.HPO4) solutions were prepared separately. The calcium and fluoride sources were slowly added to the phosphate solution drop by drop. The white precipitate of calcium phosphate is formed. The pH of the solution was maintained at 10 throughout the experiment using an ammonia solution. The white precipitate obtained is kept for aging overnight. The precipitate was washed several times and lyophilized at -80 °C. The resultant powder is named FAp.

### Synthesis of Sodium alginate Fluorapatite nanocomposite

1 wt % of sodium alginate solution is added to the phosphate source along with the calcium and fluoride sources as mentioned in the FAp preparation. The resultant lyophilized sample is named Alginate-FAp.

## Characterization

The obtained FAp and Alginate-FAp were analyzed using Bruker, D8 Advance XRD CuKα radiation (0.154nm) with step size 0.021 in the2θ range from 20° to 70° in a continuous scan mode. [(Solanki et al., 2022)](https://paperpile.com/c/XlLjTy/EMqLv) The functional groups of the samples were characterized using the Bruker-ALPHA II compact FT-IR spectrometer. The morphology and elemental mapping of the samples were examined using JEOL JSM –IT800 scanning electron microscope. [(Chidambaram et al., 2022)](https://paperpile.com/c/XlLjTy/OUJi).

## Hemocompatibility assay

The hemocompatibility assay was conducted using fresh human blood from healthy volunteers. Blood (5 mL) was collected in a heparin-coated tube, centrifuged at 750 rpm at 4°C for 5 min, and washed with PBS three times. RBC lysis was quantified in the presence of FAp and Alginate-FAp at 5 mg/mL and 10 mg/mL. Samples were incubated in 2.5 mL RBC-suspended PBS at 37°C for 1 hour. Distilled water and PBS served as controls. Absorbance was measured at 545 nm, and hemolysis was calculated using a standard formula.

% hemolysis = (1)

The calculated percentages of hemolysis for all the samples were compared with the ASTM standard [(Ramya et al., 2019)](https://paperpile.com/c/XlLjTy/o9ziz).

## Antimicrobial activity

The antimicrobial activity of the samples was assessed using the plate diffusion technique. Mueller Hinton agar (38 g/L) was prepared, autoclaved at 15 lbs pressure for 15 min, and poured (30 mL) into petri plates for gelation. A 300 µL suspension of *E. coli* and *S. aureus* was spread separately using a cotton swab. Wells were bored, and the samples were placed inside. After incubation at 37°C overnight, the zone of inhibition was measured and photographed. Gentamicin served as the positive control, while distilled water acted as the negative control [(Ramya et al., 2022)](https://paperpile.com/c/XlLjTy/hCBng).

# Results

## XRD analysis

XRD analysis determines the crystallographic structure and properties of materials. The XRD pattern of FAp showed similar peak distribution like HAp and the peaks at 2θ angles of approximately 25.9°, 31.8°, 32.2°, 32.9°, 39.6°, 46.8°, 49.5°, and 53.0° correspond to the crystallographic planes of the FAp crystal structure. The most intense peak is usually found at around 31.8°. The XRD patterns coincide with the standard JCPDS No. 15-876. The crystallinity of the HAp was affected on the incorporation of polymer and peak broadening was noticed in the characteristic peaks of HAp.The main characteristic XRD peaks of alginate are present at 2θ values of 32° [(Bhagyaraj & Krupa, 2020; Zhang et al., 2022)](https://paperpile.com/c/XlLjTy/pc5lt+PCG7L).

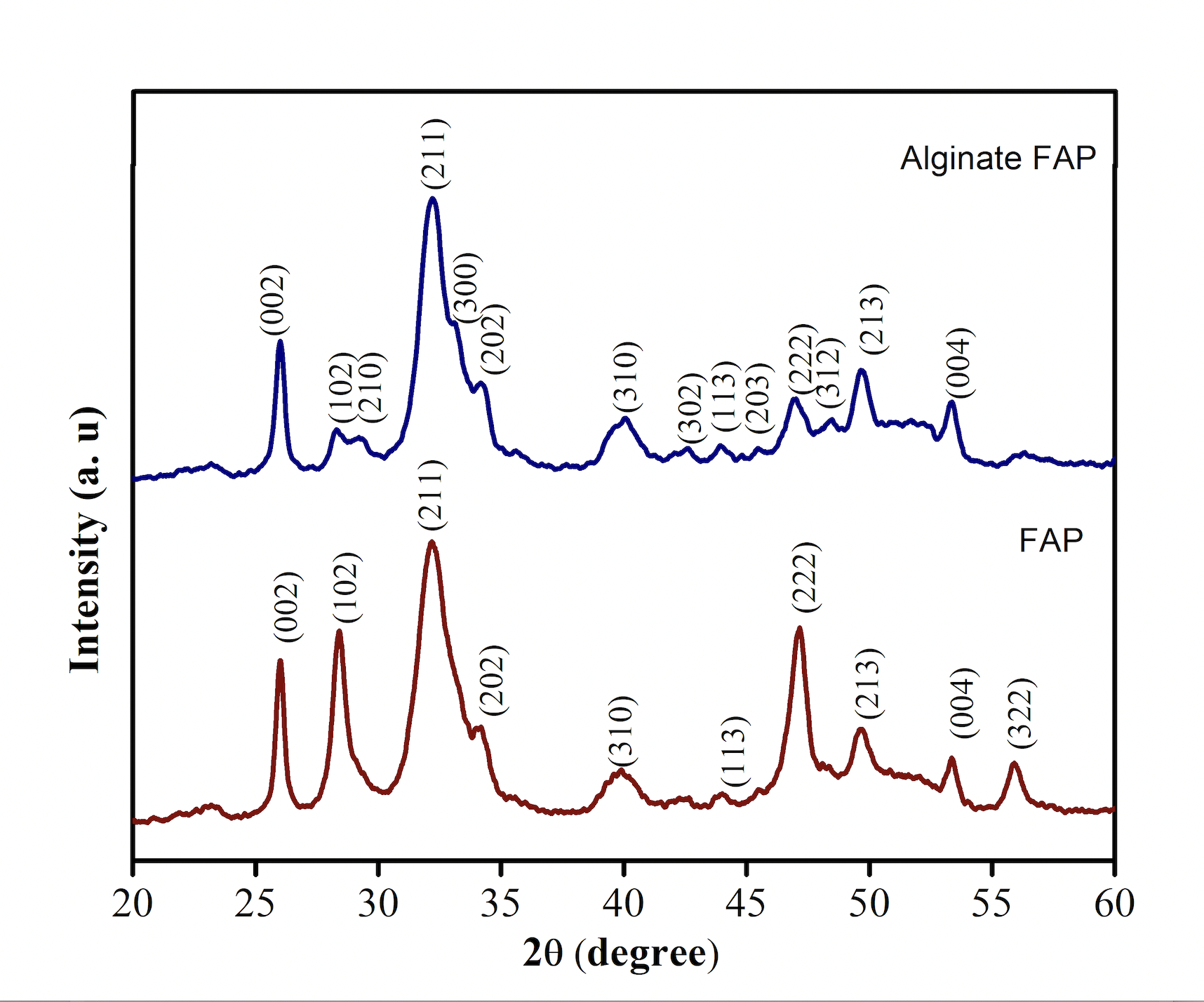
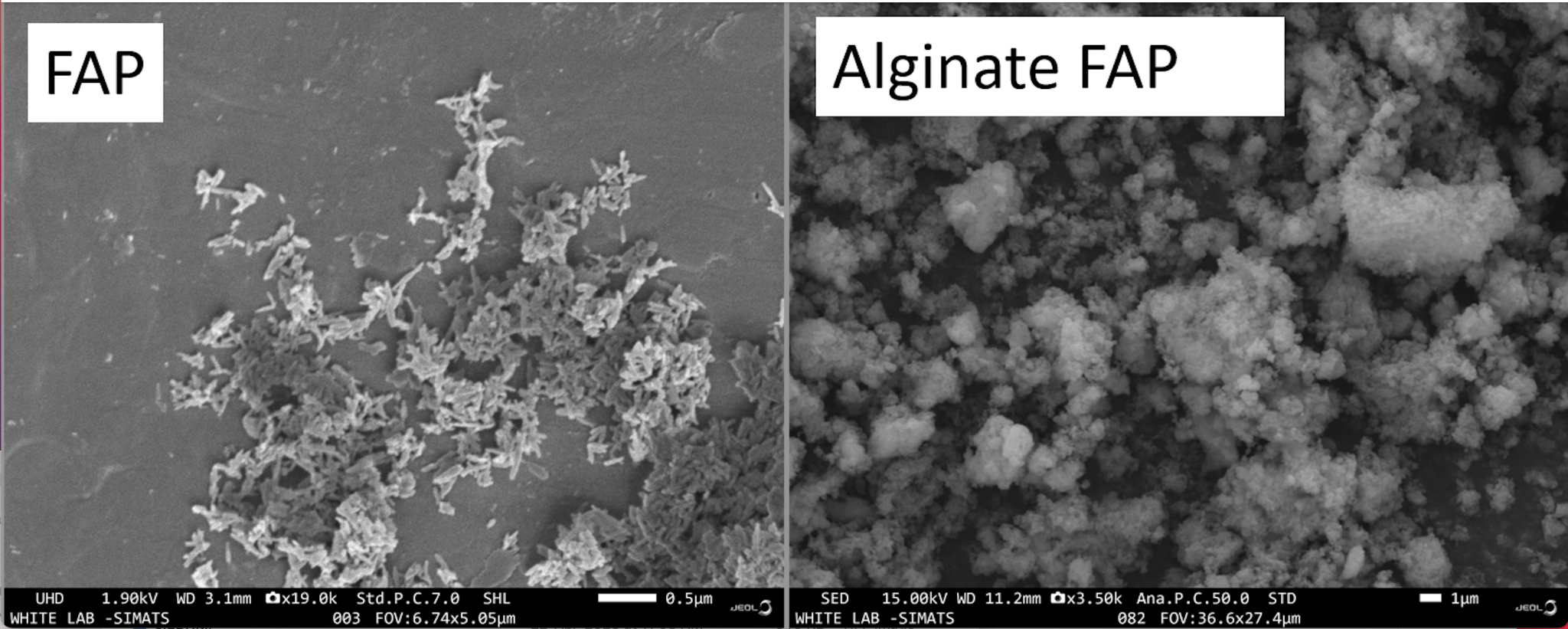
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Fig. 1. XRD patterns of (a) FAp and (b) Alginate FAp

# SEM

The SEM micrographs showed rod-like morphology in the FAp sample. The particle size was reduced due to the lyophilization of the sample. the Sodium alginate mediated FAp nanocomposite showed agglomerated nanobeads.[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/XlLjTy/p6Ogs) Alginate is responsible for the formation of nanobeads [(Bhagyaraj & Krupa, 2020; Liu et al., 2021)](https://paperpile.com/c/XlLjTy/c6G82+PCG7L). Jerome et al. reported the formation of [alginate](https://www.sciencedirect.com/topics/physics-and-astronomy/alginate) nano-aggregates, [nanocapsules](https://www.sciencedirect.com/topics/chemistry/nanocapsule), and [nanospheres](https://www.sciencedirect.com/topics/chemistry/nanosphere) by varying the preparation techniques [(van der Linden Cees J. M. van Rijn Leonard M. C. Sagis, 2014)](https://paperpile.com/c/XlLjTy/aS963).

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1. (b)

Fig. 2. SEM micrographs of (a) FAp and (b) Alginate FAp

# EDX

The presence of fluoride is confirmed from the EDAX spectrum of FAp. The calcium and phosphorous were found to be high in the spectrum. The replacement of fluoride ions on the calcium site was evidenced from the spectrum(Saadh et al., 2024). The Alginate-FAp sample showed the presence of carbon which eventually implies the presence of polymer chains of the alginate. The calcium percentage was high in Alginate-HAp sample which was due to the binding affinity of the polymer with the calcium ions. Daemi et al.reported that as the concentration of sodium alginate decreases and the concentration of calcium cations increases, the distribution of calcium alginate nanoparticles is affected This is due to the ionic interactions of calcium croslinkers white polymeric chains of alginate [(H. Daemi, 2012)](https://paperpile.com/c/XlLjTy/Cmf3g).

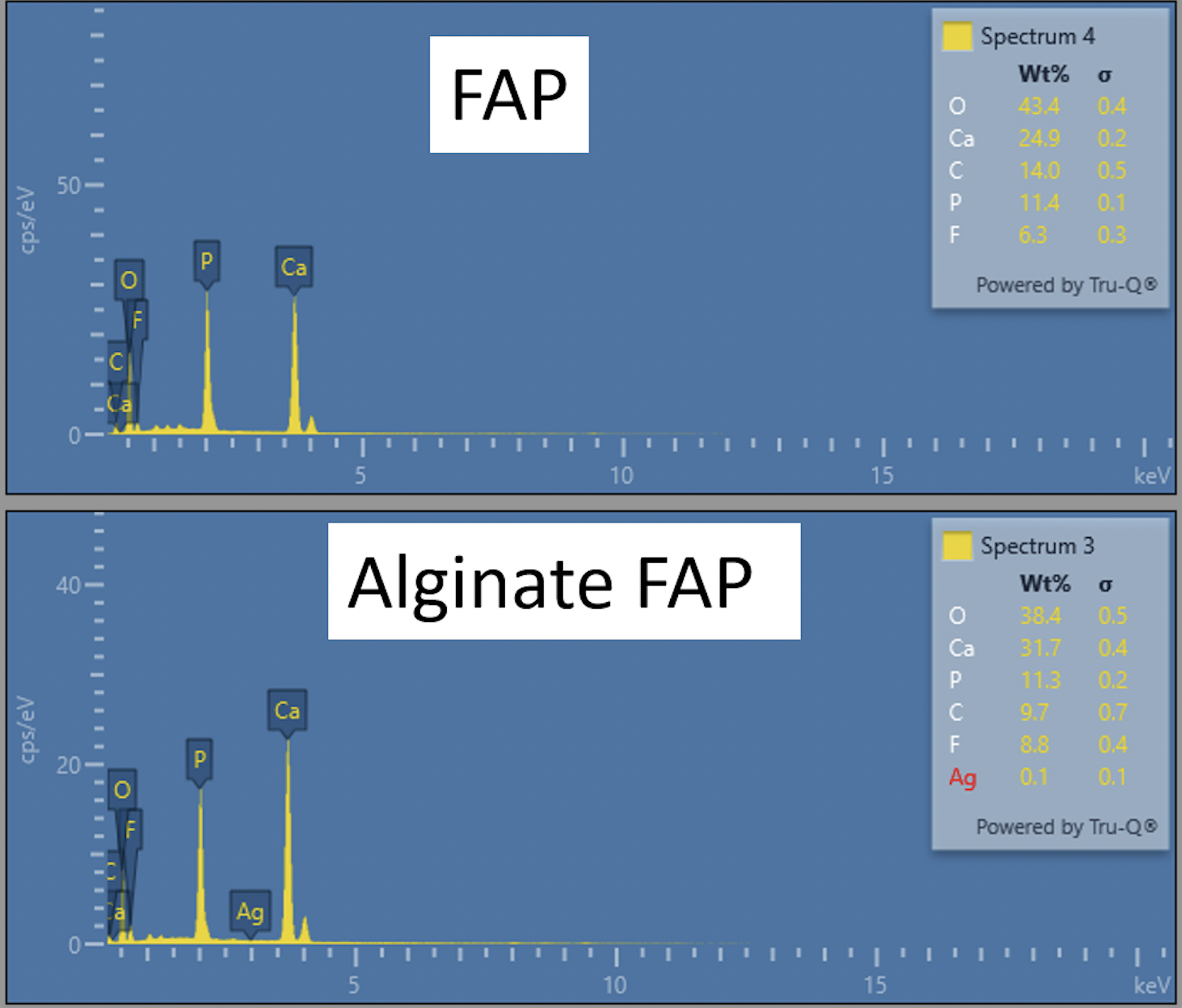
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Fig. 3. EDAX spectrum of FAp and Alginate FAp

## Hemocompatibility assay

The results revealed that both FAp and Alginate-FAp exhibited favorable hemocompatibility characteristics, the hemolysis percentage was well below 5% indicating that they are highly hemocompatible.[(Katyal et al., 2021)](https://paperpile.com/c/XlLjTy/yHl0G). Hemocompatibility tests were done to evaluate the effects on blood and/or blood components by Alginated-FAp and FAp Saadh, M. J., Rasulova, I., Almoyad, M. A. A., Kiasari, B. A., Ali, R. T., Rasheed, T. & Ciongradi, C. I. (2024). Recent progress and the emerging role of lncRNAs in cancer drug resistance; focusing on signaling pathways. Pathology-Research and Practice, 253, 154999.

(Chehelgerdi et al., 2023). The hemocompatibility of the samples was analyzed by measuring the hemolytic activity (percentage of hemolysis). The values are in good agreement with the standard limits of hemocompatibility values for the biomaterials [(Amri et al., 2016; Han et al., 2013; Notara et al., 2009; Pal et al., 2008)](https://paperpile.com/c/XlLjTy/YTl5Z+aOcHl+ntMaC+hljCC).

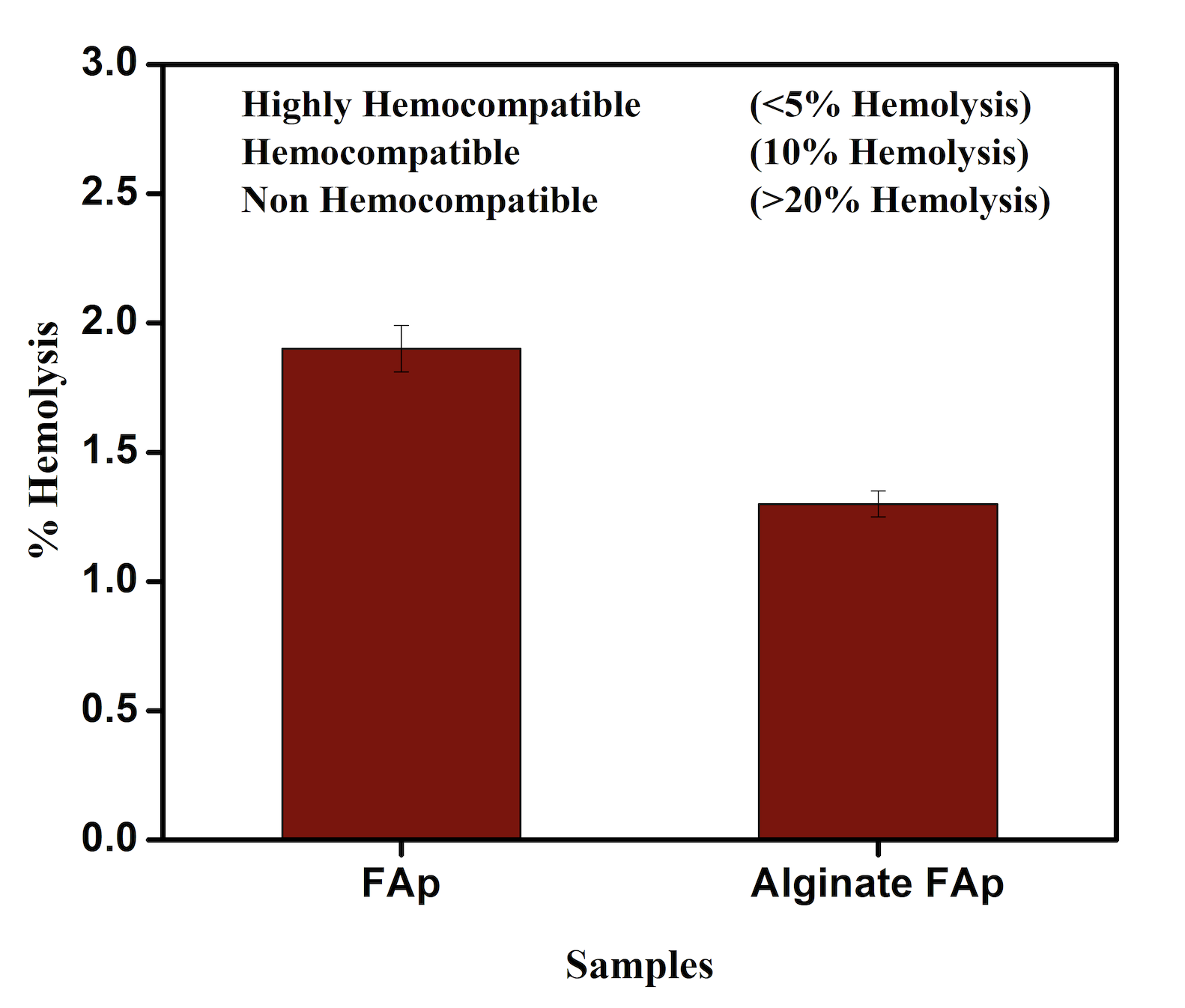
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Fig. 4. Percentage of Hemolysis of the FAp and Alginate-FAp

## Antimicrobial activity

The antimicrobial activity was detected by measuring the zone of inhibition (including the diameter of the well) that appeared after the incubation period using a direct contact test. [(Tiwari & Jain, 2023)](https://paperpile.com/c/XlLjTy/nd281). The antimicrobial activity of Escherichia coli, Staphylococcus aureus was assessed and both exhibited dose-dependent antimicrobial activity. The zone of inhibition increased marginally with increased alginate dosage. The antimicrobial activity of Alginate-FAp and Fap was evaluated by measuring the diameter of the zone of inhibition observed after the incubation period [(Asadpoor et al., 2021; Notara et al., 2009)](https://paperpile.com/c/XlLjTy/ntMaC+xsmjA).

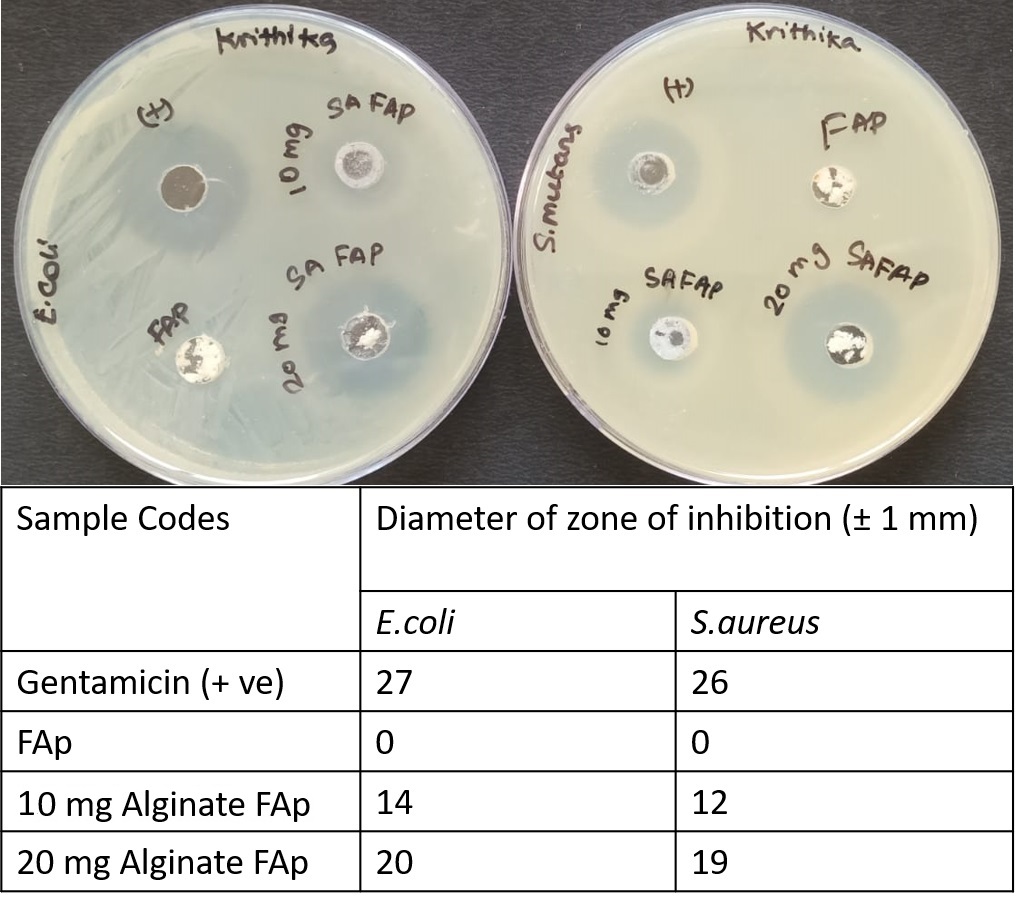
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Fig. 5. Antimicrobial activity of FAp and Alginate-FAp against (a) E.coli and (b) S.aureus

# Discussion

The XRD shows that the characteristic peaks of FAp and the presence of alginate affect the crystallinity and peak broadening was experienced due to the cross-linking interactions of calcium ions and alginate. The fluoride ions in the FAp also have the ability to interact with the alginate and form strong covalent bonds as similar to Ca ions. These interactions made the material more stable. The characteristic peak of FAp at 2q=32°, was broadened on the presence of alginate. The SEM-micrograph showed the nano-rod-like morphology in the FAp sample and the formation of nanobeads decorated with calcium and fluoride ions on the surface. The elemental distribution spectrum showed the percentages of Ca, P,F, C, and O revealing the incorporation of alginate. The material is highly hemocompatible. The metal ions like Ca and F didn’t affect the hemolysis percentage. A hemolysis study indicates hemolysis is < 5% for both FAP and Alginate-FAp making them highly hemocompatible. Hemolysis of Alginate-FAp is less than FAP making it more hemocompatible, as indicated in Fig. 4. One crucial aspect of hemocompatibility is the material's ability to prevent excessive blood clotting, which can lead to thrombosis. If FAP can promote the formation of a stable fibrin network without excessive platelet activation, it is considered to have good hemocompatibility [(Weber et al., 2018)](https://paperpile.com/c/XlLjTy/VlSJS). The antimicrobial activity of FAp showed no diameter of zone of inhibition whereas the incorporation of Alginate inherited the antimicrobial properties. On increasing the concentration of Alginate-FAp, the diameter of the zone of inhibition was improved as shown in Fig. 5. Alginate, upon exposure to moisture or specific ions, undergoes gelation, forming a hydrogel or gel-like structure. This hydrogel acts as a physical barrier that hinders microbial movement, thereby preventing their spread and colonization on surfaces. Additionally, the gel's structure restricts the diffusion of essential nutrients required for microbial growth, further impeding their proliferation. The strong calcium-alginate bonds could be responsible for cell rupture and cell death [(Lee & Mooney, 2012)](https://paperpile.com/c/XlLjTy/ZYZmq). Alginate is composed of carboxyl groups that confer negative charges on its molecular structure. These negatively charged groups can interact with the positively charged surfaces of bacterial cells, resulting in an electrostatic interaction. This interaction has the potential to disturb the integrity of the bacterial cell membrane, leading to the release of cellular contents and eventual cell death. Moreover, alginate has the capability to chelate metal ions, including calcium and magnesium. Since certain bacteria rely on these ions for vital cellular processes, alginate's capacity to sequester them can disrupt these processes, thereby impeding bacterial growth. Further, *in-vivo* studies and clinical studies are required to access the material to be a potential candidate for tissue engineering application.

# Conclusion

The XRD revealed that the incorporation of sodium alginate on the FAp matrix resulted in a decrease in the peak intensities and some peaks were masked by the sodium alginate. The SEM micrographs showed nanorods in FAp and agglomerated nanobeads were seen in alginate-FAp. The EDAX confirmed the presence of fluoride and carbon peaks were seen in alginate FAp. The samples are highly hemocompatible and possess antimicrobial activity in Alginate-FAp samples suggesting the material to be a potential candidate for biomedical application.

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