Synthesis of calcium phosphate nanoparticles in a droplets-based microfluidic device

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Abstract. Calcium phosphates (CaP) biomaterials are one of the commonly used synthetic bone graft alternatives, due to their chemical resemblance to the mineral bone component. Nanoparticles of CaP and especially hydroxyapatite (HAp) ,which represents one of the most thermodynamically stable calcium phosphate materials, were synthesized using a custom-designed 3D-printed microfluidic device, enabling the continuous-flow synthesis with precise control over size and uniformity. HAp were synthesized using calcium chloride and disodium phosphate with a starting molar ratio (Ca/P) of 1.67. By optimizing the $CaCl_2$ concentrations, particle sizes were effectively reduced, achieving uniform and dispersible nanoparticles with sizes as small as 41.5 nm for a concentration of 0.01 M. Characterization included FT-IR analysis, confirming the presence of phosphate and carbonate groups, SEM imaging revealing spherical morphologies, and X-ray diffraction highlighting good crystallinity. DLS and Zeta potential measurements demonstrated homogeneous size distribution. The synthesized nanoparticles demonstrated effective fluoride adsorption, with an adsorption capacity of 63 mg/g, and efficiency of 83%. Adsorption isotherms fitted the Langmuir model, and thermodynamic study indicated a slightly endothermic and non-spontaneous process.

1 Introduction

Nanoparticles (NPs) are nanometer-sized particles that, due to their small size, exhibit fascinating physicochemical properties, making them highly sought after in various sectors such as electronics, catalysis, the food industry, and especially medicine and the biomedical field [1]. In fact, nanomedicine is known as the use of nanotechnology to promote innovation in healthcare. It entails applying a substance's nanoscale properties, which may be different from the same material's properties at large scale [2]. These NPs can be used to develop or enhance preventive solutions, enable earlier disease detection, optimize the efficiency of existing therapies, or accelerate research in regenerative medicine. For instance, iron oxide nanoparticles have already been used to increase the sensitivity of magnetic resonance imaging (MRI) because they aggregate around tumor cells, making them easier to detect at the earliest stages of growth.

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In batch mode, a variety of synthetic techniques have been developed such as electrochemical processes, chemical vapor deposition, spray pyrolysis and sputtering [3]. Nevertheless, most of these techniques have drawbacks, including long synthesis time, poor repeatability, and limited control over particle size, distribution and form.

To address this issue, microfluidic devices or microreactors, which are designed for continuous flow systems with channel sizes ranging from 10 to 1000 μm, offer a promising alternative. The development of microreactors in the field of process engineering mainly concerns fine chemistry and the pharmaceutical industry, and it continues to expand into other application areas. Miniaturization provides numerous advantages, the most significant being an important surface-to-volume ratio, enhanced safety due to the small quantities of reactants used, and efficient, precise, localized, and nearly instantaneous control of operating conditions during reactions [4]. These advantages make the application of this technology for nanoparticle production an innovative and promising approach, as it imparts highly desirable intrinsic properties to the nanoparticles, particularly uniform nanometric size, which is crucial for sensitive applications.

In this study, a 3D printed microreactor was developed and used to produce calcium phosphate material called Hydroxyapatite nanoparticles (NHAp) which are the primary inorganic component of human teeth and bones [5-6]. Hydroxyapatite is a crystalline calcium phosphate phase with a Ca/P ratio of 1.67 and represents one of the most thermodynamically stable calcium phosphate materials. Due to its exceptional biocompatibility and bio-activity, HAp is widely used in dental clinics and bone substitute material [7-8]. NHAp were prepared by simple co-precipitation method occurs in the 3D-printed microreactor as a precipitate from solution of calcium chloride and disodium hydrogen phosphate, fed at a steady flow rate, under atmospheric pressure and ambient temperature. Characteristics of the prepared powders such as element composition, particles size distribution, crystal structure and morphology were evaluated in the same operating conditions. The obtained nanoparticles were tested for the fluoride adsorption in batch system.

2 Materials and methods

2.1 Microfluidic device design

The 3D printed microreactor device used to perform the production of the nanoparticles was designed using computer-aided design (CAD) software. The CAD script was exported as stereolithographic (STL) file which is converted to G-code using ideaMaker software. The prototype of microreactor is Y-shaped, the reagents are supplied through two inlet channels of length 20 mm at an angle of 60°, connected to a serpentine-shaped outlet channel through a series of five U-tube of constant section with a total length of 80 cm. The designed microreactor is a cylindrical canal section with diameter of 1 mm. This miniaturized reactor has a volume of 8 cm^3.

2.2 HAp Synthesis

A total volume of 50 mL of first reagent calcium chloride solution ($CaCl_2$) were prepared to an equal volume of second reagent disodium hydrogen phosphate solution (Na_2HPO_4), and maintains the solution having a molar ratio Ca/P equal to 1.67, equivalent to the stoichiometric HAp. Substrates were supplied to the microreactor by a peristaltic micro-pump. After reaction, the resulting suspension of HAp nanoparticles was collected in the collection beaker. To maintain the solution's pH at 10, NaOH is added under continuous

stirring. The obtained solution was filtered, and the precipitate was cleaned with ultrapure water. After that, it was dried in the oven at 50°C for 24 h to obtain powder sample. The hydroxyapatite powders obtained were subjected to numerous analyses, chemical composition, size distribution, zeta potential and morphology.

2.3 HAp application:

As application, the obtained HAp particles were tested for the fluoride batch adsorption. To determine the fluoride adsorption isotherm, a series of experiments was conducted. Fluoride solutions with different concentrations ranging from 50 to 500 ppm were prepared in separate flasks, each containing a fixed amount of HAp (0.4 g). The samples were subjected to continuous agitation for 3 hours at temperatures of 25, 50, and 60 °C. After this period, the samples were filtered, and the potential difference was measured to determine the residual fluoride concentration, thereby allowing the calculation of the amount of fluoride adsorbed by the HAP.

3 Results and discussion

3.1 Hap Characterization

3.1.1 Fourier Transform Infrared Spectrometry

The powders' functional groups were identified using an FT-IR spectrometer operating in the 400-4000 cm^{-1} wavelength range. Figure 1 displays The Fourier Transform Infrared spectrum (FTIR) of the hydroxyapatite sample prepared in 3D-printed microreactor. The presence of stretching and bending vibrations of the phosphate, PO_4^{3-}, carbonate ion and hydroxyl ion approves the production of a typical apatite structure.

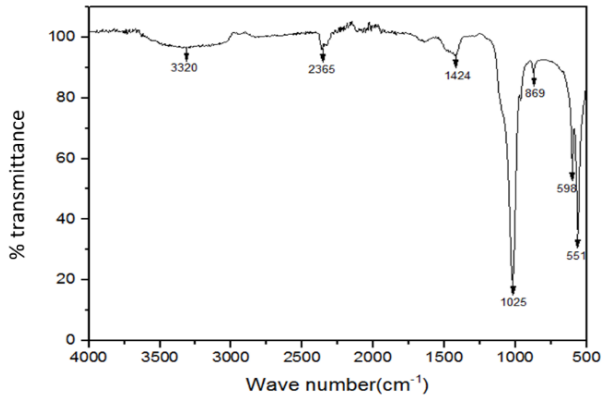

Fig. 1. FTIR spectrum of HAp particles.

3.1.2 XRD analysis

The particles' crystalline nature was identified by X-Ray Diffractometer (XRD). The XRD diffractograms of the synthesized HAp powder is depicted in Figure 2. The data were gathered for the 2θ between 20˚C and 70˚C. The XRD pattern displays a strong reflection peak in 32 of 2θ value, which is similar to the apatite phase's distinctive peak. The peaks' sharpness and excellent resolution confirm the HAp's crystalline structure. Furthermore, by showing the distinctive peak at 2θ:25.8°, the diffraction pattern is consistent with HAp

crystalline material and the synthesis of single-phase stoichiometric HAp with a Ca/P molar ratio of 1.67 has been verified.

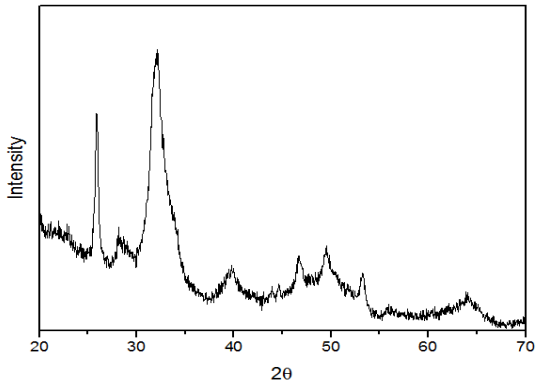

Fig. 2: XRD pattern of hydroxyapatite nanoparticle.

3.1.3 Particle size distribution

The Dynamic Light Scattering data show clusters with symmetric size distributions, and the average particle size is 99.48 nm for HAp synthesized. The intensity weighted DLS measurement of the sample exhibits one peak as depicted in figure 3.

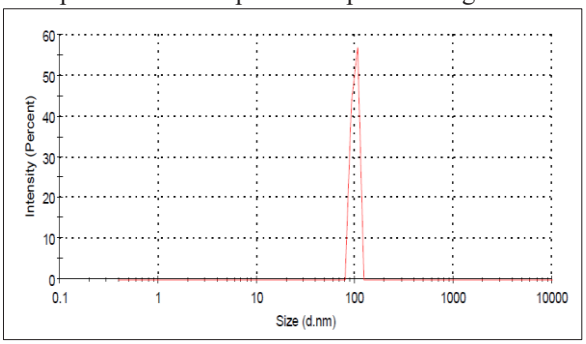

Fig. 3: Dynamic light scattering measurements of hydroxyapatite size particle (T = 25°C, C_{CaCl2}=0.1M, Q = 1 ml/min)

The effect of $CaCl_2$ concentration on the particle size has been investigated. Indeed, by decreasing this reagent concentration from 0.06 to 0.01 M, the size decreases from 626.3 to 41.5 nm respectively as depicted by figure 4.

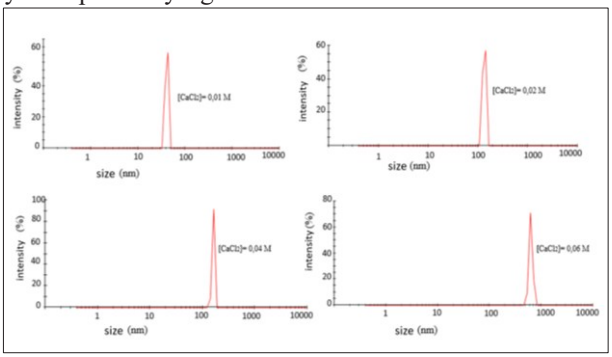

Fig.4 Effect of reagent concentration (T = 25°C, Q = 0.8 ml/min)

3.2 HAp Application

The synthesized nanoparticles were applied as adsorbent for fluoride removal in batch system. They demonstrated effective fluoride adsorption, with an adsorption capacity of 63 mg/g, and efficiency of 83%. This can be explained by its high chemical stability and the substitution of OH^- groups by F^- ions. The obtained isotherms are depicted in figure 5.

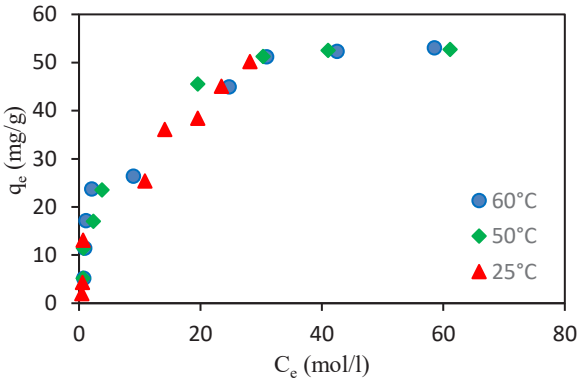

Fig. 5. Fluoride adsorption isotherms (pH = 5.79, V = 50 mL, m_HAp = 0.4 g, and time = 3 h).

Table 1: Adsorption isotherm parameters of fluoride on HAp nanoparticles

	Temperature (°C)	K_L	Q_{max} (mg/g)	R^2
Langmuir	25	0.083	67.56	0.94
	50	0.1506	63.29	0.99
	60	0.184	57.803	0.98
	Temperature (°C)	K_F	N	R^2
Freundlich	25	2,312	1,795	0.86
	50	2,76	2,26	0.84
	60	2,878	2,405	0.92
	Temperature (°C)	K_T	B_T	R^2
Temkin	25	1.104	9.9	0.91
	50	2.364	9.66	0.95
	60	0.954	10.32	0.95

Several adsorption models were evaluated to describe the experimental isotherms as Freundlich, Langmuir and Temkin. Among them, the Langmuir model offered the greatest fit to the experimental data as mentioned in table 1 with a correlation coefficient near to one. In addition, a thermodynamic analysis was performed to determine the relevant thermodynamic parameters. The calculated enthalpy and Gibbs energy values indicates a slight endothermic and non-spontaneous process.

4 Conclusion

This study was conducted in two main parts. The first part was devoted to the design and manufacture of microreator device using a 3D printer. A new conception of the prototype of microreactor is Y-shaped in the inlet channels, connected to a serpentine-shaped outlet channel were created to boost the nanoparticles' production rate without degrading their characteristics. The second part was intended for the preparation of hydroxyapatite powders by the precipitation chemical method. The results of the analyses of HAp obtained in 3D-printed reactor were presented. The infrared spectra of synthetic HAp shows the P-O and O-H bands of the phosphate and hydroxyl groups that make up hydroxyapatite. The nanometric crystal size of the synthesized HAp was corroborated by X-ray diffraction showed the apatitic phase of the analyzed powders. The produced HAp sample presents a uniform distribution size particle remained in the nanoscale range obtained by DLS. The created microreactor allows the production of HAp, useful in biomedical field, especially in medical implant applications.
As application, the obtained nanoparticles were used to assess the fluoride adsorption in a batch system. The adsorption isotherms fitted the Langmuir model with a maximum adsorption capacity of 67 mg/g at 25°C and the estimated thermodynamic parameters show a slight endothermic and non-spontaneous process.

Acknowledgements

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