ISSN: ۲۶۷۶-۵۷۶٤
www.jocrisar.ir

مجله پژوهش های معاصر در علوم و تحقیقات
سال پنجم، شماره ۵۱، مهر ۱۴۰۲

Investigating the effect of temperature and time, and pH parameters on the particle size of hydroxyapatite using the hydrothermal method

[۱]Shamsi Ebrahimi ، [۲]C.S.Sipauta

Chemical Engineering, Faculty of Engineering Universiti Malaysia Sabah [۱]
Chemical Engineering, Faculty of Engineering Universiti Malaysia Sabah [۲]

Abstract

Biomaterials are getting popular because of their numerous uses, including compatibility at the graft site. There are several forms of biomaterials, one of which is hydroxyapatite (HAP), which is frequently employed in medical sciences and scientific research. Hydrothermal synthesis of hydroxyapatite nanoparticles was used in this study. The chemical structure and shape of the produced hydroxyapatite powder were investigated using Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). To identify the ideal conditions for the size of the synthesised hydroxyapatite nanopowder, the response surface method (RSM) with central composite design (CCD) is utilised. Three independent variables, such as pH, temperature, and time of hydrothermal treatment, were investigated in this design. The size of hydroxyapatite nanoparticles was shown to decrease under alkaline conditions. Furthermore, the pH factor has the significant influence on the size of hydroxyapatite powder nanoparticles, according to the analysis of variance (ANOVA).

Keywords: Hydroxyapatite; Response surface methodology; central composite design

Introduction

Technological advancements result in the creation of implant materials that are not corrosive in nature yet are biocompatible. Due to the increased biocompatibility and biological activity of calcium phosphate, nanomaterial structures are now the most highly desired (Mehta and George 2013). Hydroxyapatite is one of these biological materials that can be utilised as a replacement for bone grafting (HAP). Because of its chemical and structural similarities to bone mineral, synthetic hydroxyapatite is quickly being used as an alternative biomaterial. Hydroxyapatite [Ca_{10} $(PO_4)_6$ $(OH)_2$] is a porous and granular bioceramic that it widely has been utilised in the medical sciences as one of the most essential and useful bioceramics, similar to the mineral phase of human hard tissue. Similarity of hydroxyapatite's chemical composition to bone is the determining element for its bioactivity, capacity to stimulate bone formation, and therapeutic actions (Sandukas, Yamamoto et al. 2011, Singh, Kim et al. 2014). Nanoscale hydroxyapatite has been extensively produced. Hydroxyapatite ceramics with nanoscale particle size will perform better and have more strength. Nanoparticles absorb more protein, resulting in increased adhesion and cell proliferation. Because of its capacity to move inside cells, hydroxyapatite nanoparticles may be utilised to deliver a wide range of therapies, including proteins, antibodies, oligonuclides, imaging agents, and liposomes, under a variety of circumstances and biological systems (Cai, Liu et al. 2007). This study used the statistical technique of Response Surface Methodology (RMS) to determine which variables have the most impact on the final hydroxyapatite particle size.

Material and methods

To conduct this test, 0.67 M ammonium hydrogen phosphate solution was added dropwise to ***1 M calcium nitrate tetrahydrate solution for 1 hour while properly stirring. The pH was*** adjusted in the range of 1-13 using ammonia solution and nitric acid. The hydroxyapatite suspension was then transferred to a Teflon container and treated to hydrothermal treatment at various temperatures and periods as shown in Table 1. Finally, the solution was filtered, and the hydroxyapatite powder was washed three times with deionized water and ethanol (volume ratio 1:1), followed by a 10-hour oven drying at 60°C. A mortar was used to grind the obtained powder into fine powder.

- **Experimental design**

Response surface methodology (RSM) with a central composite design (CCD) was used to examine the effects of variables like pH, hydrothermal temperature, and time while assessing the particle size of produced hydroxyapatite as the response. The independent variables' upper and lower bounds are displayed in Table 1 below. The number of experiments in an experiment designed using CCD is determined by the equation (1):

$$Y=2x+2x+C\ x \qquad (1)$$

Where Cx is the number of times the central point's (Mehta, Mondal et al. 2017) is repeated, x is the number of variables, and Y is the number of experiments. In this study, there are three

variables, three canter points, one repeat, and three variables overall. A total of 17 experiment were produced by the central composite design according to Table 2.

Table 1. Experimental parameters and their levels

Sample	parameter	(+1)	(0)	(-1)	-alpha*	+alpha
A	Temperature	100	130	160	70	190
B	Time	4	7	10	1	13
C	pH	4	7	10	1	13

Table 2. Table 2. Design of CCD and its actual values

Run	A	B	C	Crystal size (nm)
	Temp (°C)	Time (h)	pH	Actual
1	100	4	4	60.23
2	160	10	10	45.48
3	70	7	7	28.76
4	130	7	13	46.81
5	160	4	4	84.10
6	130	7	1	-
7	100	10	4	65.15
8	160	4	10	38.98
9	130	7	7	45.82
10	130	7	7	46.88
11	130	1	7	44.10
12	160	10	4	89.00
13	100	10	10	31.59
14	130	13	7	56.18
15	100	4	10	29.18
16	190	7	7	71.15
17	130	7	7	44.77

The chemical structure of hydroxyapatite produced under various conditions was investigated using Fourier infrared spectroscopy (FTIR, Perkin Elmer Spectrum 100). The spectra were taken in the 400-4000 cm-1 region. The shape and size of nano hydroxyapatite particles were determined using a Transmission electron microscope (TEM, FEI Tecnai Spirit BioTWIN).

Result and discussion

Figures 1a and b show the FTIR results. Peaks of hydroxyl and phosphate groups in hydroxyapatite were found in this assay. The bending vibration of phosphate (PO_4^{-2}) is relevant for the bands at 465, 559.51, and 598.92 cm^{-1}, whereas the stretching vibration of phosphate is related for the bands at 962.18, 1022.58, and 1088.45 cm^{-1}. The broad peaks seen in the bands 3300-3450 and 11367 cm^{-1} are caused by the bending vibration of water's hydroxyl group (OH^{-1}). Water may be included in the hydroxyapatite structure due to its

synthesis in an aqueous environment. The stretching vibration of OH^- ions in the hydroxyapatite lattice is due to two weak peaks at 3570 and 631.70 cm^{-1} (Wang, Zhang et al. ***2006). Furthermore, FTIR results revealed that all phosphate and hydroxyl functional*** groups are in an alkaline environment. The presence of hydroxyl groups in the hydroxyapatite network reduces at low pH, and the presence of calcium carbonates is seen in the bands 1420-1450 and 1864 cm^{-1}. Furthermore, the strength of hydroxyapatite-associated peaks rises with increasing temperature and reaction time. The FTIR bands produced from hydroxyapatite nanoparticles are fully compatible with previous studies (Manoj, Subbiah et al. 2015).

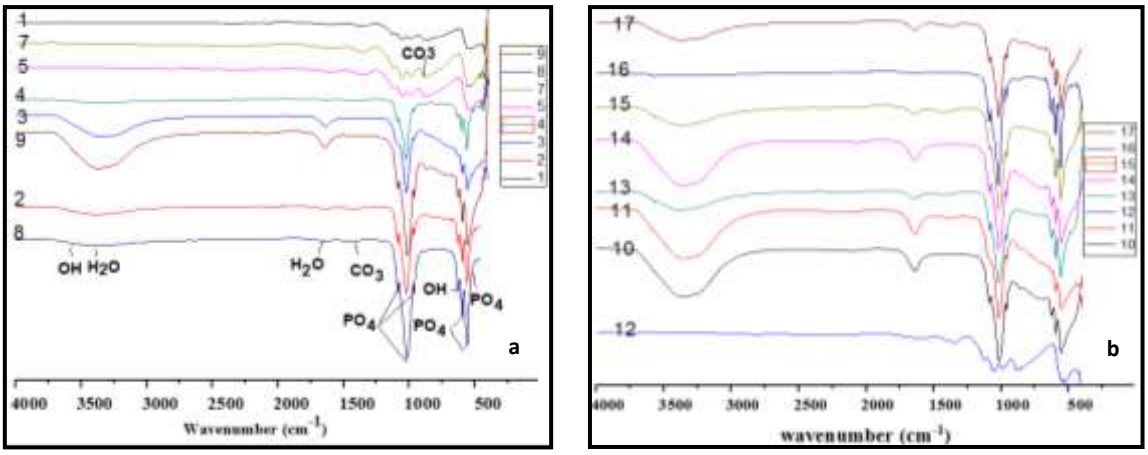

Figure 1. FTIR spectra of HAp powders of different conditions: (a) 1-9 and (b) 10-17

- **Experiment analysis using the surface response methodology (RSM)**

According to Table 2, a total of 17 experiments were designed using Design Expert software (version 11.0.0). Analysis of variance (ANOVA) was performed to determine the model's significance and the influence of each parameter. Parameters with a significance value of less than 0.05 (p-value0.05) were chosen (Kehoe 2008). The quadratic model was selected as the best model by the ANOVA test, with a p-value < ***0.0001, R^2=0.972, and AdjR^2=0.95. The pH factor has the major impact on the*** size of hydroxyapatite nanoparticles, according to p-value < 0.0001. Whenever the result, as the pH increases, the size of the particle decreases**.**

Equation 2 shows the second-order polynomial equation for hydroxyapatite particle size obtained from a quadratic regression model on the tested variables.

$$\text{Crystal size} = ٧٠.٢٦٠٧ + ٠.٥٣٣٦٧ * \text{Temp} - ٠.٥٦٦١٤ * \text{Time} - ١٦.٥٢٧٠٥ * \text{pH} - ٠.٠٣٣٣٧١ * \text{Temp} * \text{pH} + ٠.١٠٤٢٥ * Time^{٢} + ١.٠٤١٦٤ * \text{pH}^{٢} \quad (2)$$

The response surface graph for the combined variables is shown in Figure 2.

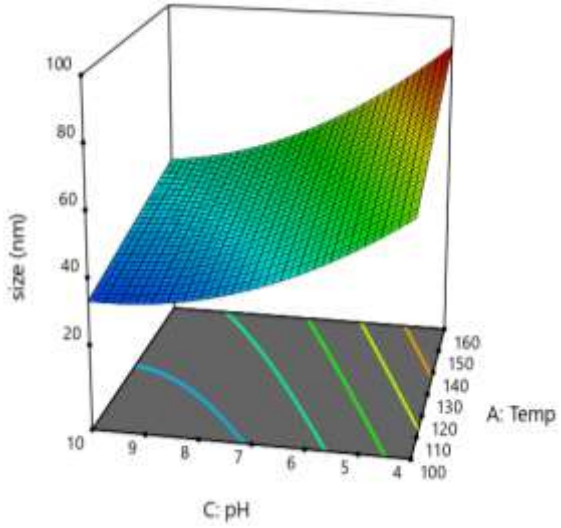

Figure 2. 3D surface response plot for the effect of pH and temperature on hydroxyapatite particle size at 10 h

Figure 3 illustrates the TEM results as well as the shape and size of hydroxyapatite nanoparticles at optimal conditions of 130 °C, 10 h, and pH=10. The TEM images demonstrated that the particles' shape is spherical and their size is around 38 nm.

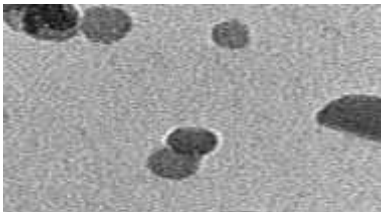

Figure 3. TEM images of hydroxyapatite nanoparticles at optimum conditions, 10 hours, 130 °C, pH=10.

Conclusion

The hydrothermal approach was used to synthesize hydroxyapatite nanoparticles powder from calcium and phosphate sources ($Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$). The quadratic model was chosen as a significant model using the RSM method and the CCD design, with a correlation coefficient (R^2=0.972) and an adjusted coefficient ($AdjR^2$=0.95). The FTIR analysis and experimental design outcomes were evaluated. The findings revealed that pH is a more critical variable in hydroxyapatite powder nanoparticle size. The smallest hydroxyapatite particles were achieved at pH=10, temperature 130 °C, and time 10 hours.

ACKNOWLEDGMENT

The authors wish to thank Universiti Malaysia Sabah for the postgraduate student fee scheme support and to the Ministry of Higher Education Malaysia for providing funding on Transdisciplinary Research Grant Scheme, TRGS0001-SG-2/2014.

References

Cai, Y., et al. (2007). "Role of hydroxyapatite nanoparticle size in bone cell proliferation." Journal of Materials Chemistry **17**(36): 3780-3787.

Kehoe, S. (2008). Optimisation of hydroxyapatite (HAp) for orthopaedic application via the chemical precipitation technique, Dublin City University.

Manoj, M., et al. (2015). "Influence of growth parameters on the formation of hydroxyapatite (HAp) nanostructures and their cell viability studies." Nanobiomedicine 2: 2.

Mehta, D. and S. George (2013). Hydroxyapatite: Synthesis and Characterization, ICMF.

Mehta, D., et al. (2017). "Synthesis of hydroxyapatite nanorods for application in water defluoridation and optimization of process variables: advantage of ultrasonication with precipitation method over conventional method." Ultrasonics sonochemistry 37: 56-70.

Sandukas, S., et al. (2011). "Osteoblast adhesion to functionally graded hydroxyapatite coatings doped with silver." Journal of Biomedical Materials Research Part A 97(4): 490-497.

Singh, R. K., et al. (2014). "Novel hybrid nanorod carriers of fluorescent hydroxyapatite shelled with mesoporous silica effective for drug delivery and cell imaging." Journal of the American Ceramic Society 97(10): 3071-3076.

Wang, Y., et al. (2006). "Hydrothermal synthesis of hydroxyapatite nanopowders using cationic surfactant as a template." Materials Letters 60(12): 1484-1487.