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Bioactivity of Rekagraf Calcium Phosphate

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A synthetic calcium phosphate, labelled as Rekagraf, was synthesized in our laboratory at Universiti Sains Malaysia. The product is part of an up-scaled production of synthetic calcium phosphate for medical and dental uses. This is based on a precipitation process using a chemical reactor. The powder produced fits the ICDD: 09-0432 pattern upon analysis by x-ray diffraction (XRD). The powder was compacted into a pellet at 20 bar and tested for bioactivity and mechanical integrity. Bioactivity test was conducted using a simulated body fluid (SBF) solution and the results were examined by scanning electron microscopy (SEM). SBF test confirmed the growth of apatite layer after 7 days. The hardness test showed a value of 200 Mpa.

Keywords : Hydroxyapatite, Precipitation, Apatite layer, Hardness, Chemical reactor

INTRODUCTION

The name calcium phosphate refers to minerals containing calcium ions (Ca^{2+}) together with orthophosphates (PO_4^{3-}), metaphosphates or pyrophosphates ($P_2O_7^{4-}$), and occasionally hydrogen, or hydroxide ions. Calcium phosphates (CaPs) bioceramics constitute a major family of inorganic materials currently in use for dental and orthopaedic reconstructive surgeries [1].

At the Rekagraf Laboratory, Universiti Sains Malaysia the work is focused to produce this material at a pilot plant scale. Apatites, as well as other CaPs, can also be obtained as the result of the transformation of more soluble, metastable phosphates in a wet environment [2].

As with other precipitation methods, the process follow the sequence of synthesis, aging, centrifuging, drying, grinding and sintering process [3-6].The objective of this method is to produce calcium phosphate materials in large quantities ready for commercialisation to support and supply calcium phosphate for other laboratory research work, as well as for direct application for dental and medical uses.

MATERIALS AND METHODS

Synthesis of Hydroxyapatite

Hydroxyapatite was synthesized using a Syriss Chemical Reactor by adding phosphoric acid, $H_3(PO)_4$ (Merck, 85%) at a pre-determined rate into calcium hydroxide solution, $Ca(OH)_2$ (Fluka, 96%) at 30-80 °C. The pH in the calcium solution was adjusted to be slightly alkaline. Wet-chemical methods use solutions as starting sources.

The advantage that arises from the use of a liquid state precursor, is the source elements are thoroughly mixed at the atomic level [7].

The method used in the Rekagraf Laboratory is an up-scaled method from a laboratory scale method which has been proven to be technologically feasible. The machine used for the synthesis process is a Syriss Reactor. It has a maximum capacity of about 5 to 10 litres.

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This is compared to a production of calcium phosphate at less than 1 litre which is the maximum limit of a laboratory scale production.

After the reaction was completed, aging was carried out for 1-3 days and the process was continued by filtering and drying. It took a minimum of 3 days to obtain the samples in powder form.

Characterization

Compositional characterization

Compositional characterizations of the samples were carried out using x-ray diffraction and x-ray fluorescence. An x-ray diffractometer (D8 Advance, Bruker AXS) was used as the main analytical tool to detect that HA was produced in all the reactions and to detect whether other phases might be present from the reaction. X-ray fluorescence (Rigaku, Rix 3000) was used to obtain chemical analysis of samples and with this value, the Ca/P ratio of samples was calculated.

Mechanical characterization

For this characterization, the value of hardness was determined by using a microhardness machine (Leco, LM 247 AT). The samples were compacted into pellets with a dimension of 13 mm diameter and using 20 bar of pressure.

Biocompatibility in vitro test

The bioactivity of the materials was evaluated based on the ability to induce a bond-like apatite layer on the surface in a simulated body fluid (SBF). The ion concentrations of SBF are shown in Table 1 [8]. The samples were soaked in SBF solution for 1 day, 3 days, and 7 days. Scanning electron microscopy (Supra, Zeiss 35VP) was used to observe the apatite layer on the samples.

TABLE 1
Ion concentrations of SBF

Ion	Ion concentrations (mW)	
	Blood plasma	SBF
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	147.8
HCO_3^-	27.0	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.2-7.4	7.4

RESULTS AND DISCUSSION

Structural Characterization

X-ray diffraction

X-ray diffraction pattern of the Rekagraf calcium phosphate is presented in *Fig. 1.* This

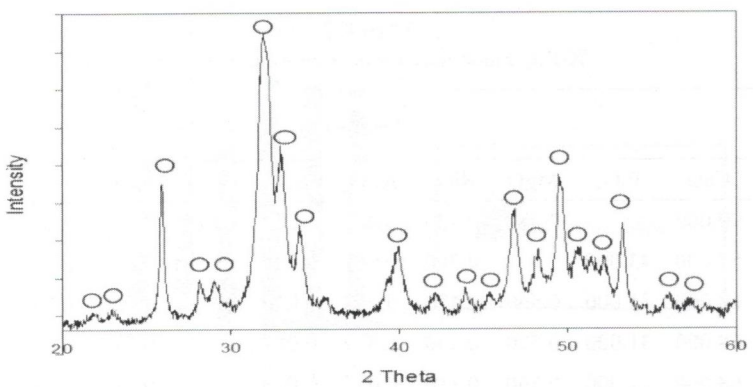

Fig. 1: XRD Pattern of as-synthesized Rekagraf Hydroxyapatite. The symbols (○) indicate ICDD 09-0432, $Ca_{10}(PO_4)_6(OH)$

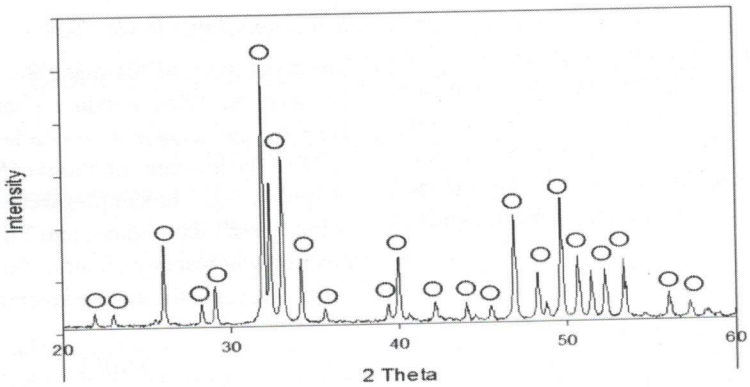

Fig. 2: XRD pattern of Rekagraf hydroxyapatite sintered at 1000 °C. The symbols (○) indicate ICDD 09-0432, $Ca_{10}(PO_4)_6(OH)_2$

result illustrates the typical XRD pattern of hydroxyapatite powder.

Fig. 2 shows the sintered Rekagraf hydroxyapatite, which was sintered at 1000 °C. It is noted that the sintered bodies have relatively sharp XRD reflections than the as-synthesized samples. Sharp and narrow peaks refer to a good crystallinity and can be indexed as the hexagonal hydroxyapatite lattice with the lattice parameters a=b=9.418 Å, c=6.884 Å, α=β=90.0, γ=120.0 and space group P63/m which is in close agreement with the standard values for HA (ICDD, 09-0432). These results confirm that the precipitation method adopted allows one to obtain pure hydroxyapatite of high crystallinity [6].

X-Ray fluorescence

In order to complement the XRD result, tests were carried out using x-ray fluorescence (Table 2). The samples tested were calcium hydroxide, $Ca(OH)_2$ the raw material, commercial hydroxyapatite (Fluka, 90%) and 3 samples of Rekagraf hydroxyapatite which were synthesized with the same quantity of the raw materials and method but on a different day.

The results show that the calcium phosphate ratio for the three Rekagraf samples are nearly equal to the standard calcium phosphate ratio for hydroxyapatite which is 1.67. It is slightly different depending on the adjustable dropping rate of the chemical solutions. Surprisingly, the commercial hydroxyapatite shows a Ca/P value far from the standard value.

TABLE 2
X-Ray Fluorenscence data by weight percent

Sample	Results (wt%)										Ca/P ratio
	CaO	P_2O_5	MgO	SiO_2	Al_2O_3	SO_3	K_2O	MnO	Fe_2O_3	SrO	
$Ca(OH)_2$	99.000		0.580	0.120	0.089	0.018	0.015	0.016	0.057	0.039	
HA COM	57.000	43.000		0.300	0.091	0.021		0.013	0.040	0.038	1.02
Rekagraf 1	68.000	31.000	0.560	0.120	0.058	0.020		0.020	0.048	0.032	1.69
Rekagraf 2	68.000	31.000	0.520	0.140	0.079	0.017		0.012	0.048	0.032	1.69
Rekagraf 3	68.000	32.000	0.560	0.110	0.077	0.018		0.014	0.047	0.032	1.64

Mechanical Characterization

The values of hardness for Rekagraf samples are shown in Table 3.

Based on the diameter data before and after sintering it is shown that the sample shrank after the sintering process. The thickness and weight of the samples also become reduced after the sintering process. The sintered samples tested for microhardness give values of hardness above 200 Mpa. The average result of hardness for the samples can be illustrated by the graph in *Fig. 3*.

In Vitro Biocompatibility Test

The objective of the in vitro biocompatibility test is to determine whether the sample can form an apatite layer on the surface. It is because most bioactive materials will develop an apatite layer on their surfaces in the living body and then bond to the bone through this apatite layer [9]. Micrographs in *Fig. 4* show Rekagraf samples after 1 day and 7 days of soaking in SBF solution. The sample was also soaked in SBF solution for 3 days but the micrograph is similar to the sample 1 day after soaking in SBF solution (*Fig. 4(a)*) where no apatite layer was formed. After 7 days of soaking in SBF solution, an apatite layer was formed on the Rekagraf samples. Previous studies showed that for apatite-wollastonite (A-W) glass ceramics soaked in SBF, the surface was covered with a layer of the apatite phase after 7 days of immersion [10-11]. Based on this, Rekagraf samples can be classified as bioactive.

TABLE 3
Dimension and hardness result for Rekagraf samples

No	Code sample	Before sintering			After sintering			Hardness, Hv	Hardness, MPa
		Dimension, mm		Weight, g	Dimension, mm		Weight, g		
		Diameter	Thickness		Diameter	Thickness			
1	B	12.100	4.800	0.672	8.810	3.570	0.622	255.22	250.3
2	F	12.100	5.200	0.691	9.020	3.850	0.563	239.00	234.4
3	A	12.100	4.800	0.682	9.380	3.820	0.627	211.66	207.6
4	C	12.150	4.900	0.673	8.740	3.570	0.612	263.860	258.8
5	D	12.100	4.900	0.672	8.840	3.640	0.619	250.300	245.5
6	E	12.100	4.900	0.684	9.120	3.690	0.627	246.000	241.3

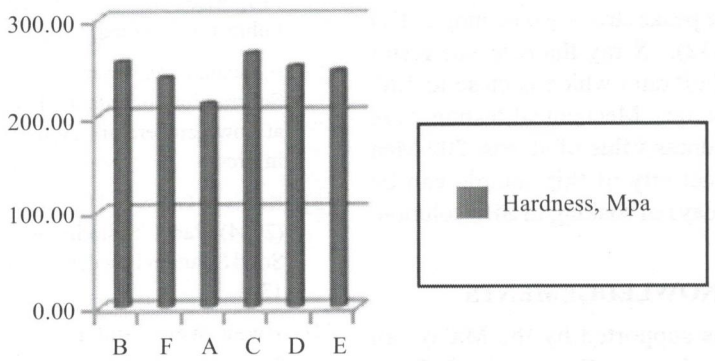

Fig. 3: The hardness (Mpa) of Rekagraf samples

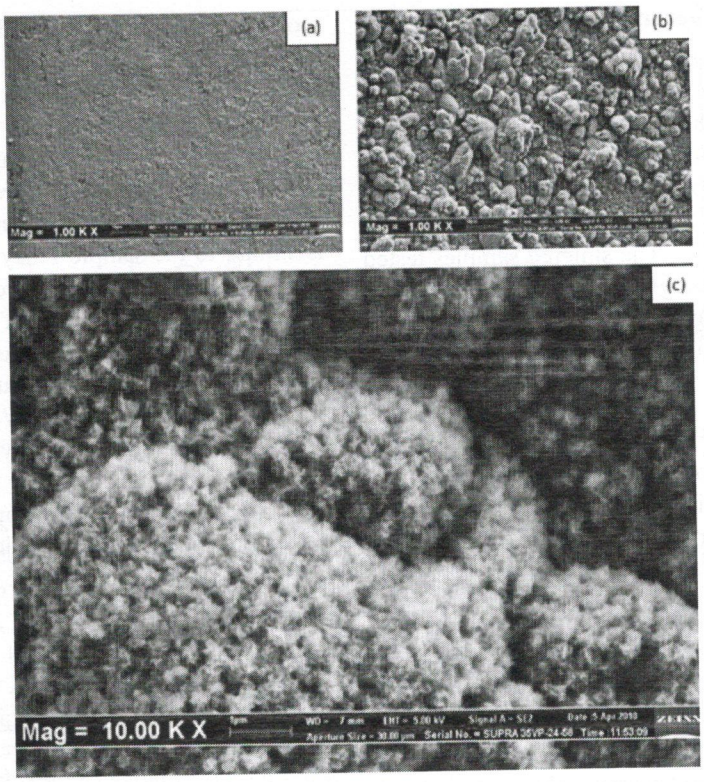

Fig. 4: Scanning electron microscopy (SEM) (a) soaking in SBF solution for 1 day with magnification 1.0 K X. (b) soaking in SBF solution for 7 days with magnification 1.0 K X (c) soaking in SBF solution for 7 days with magnification 10.0 K X.

CONCLUSIONS

It can be shown that the high quality of the Rekagraf samples can be validated by the compositional characterization, mechanical characterization and in-vitro testing. The XRD pattern showed no contamination in the Rekagraf sample with the peaks strongly matching ICDD pattern (09-0432). X-ray fluorescence result provides the Ca/P ratio which is close to 1.67 for hydroxyapatite. Mechanical testing gave an average hardness value of at least 200 Mpa (*Fig. 3*). Bioactivity of this sample can be proven after 7 days of soaking in SBF solution.

ACKNOWLEDGEMENTS

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