



## NOTE

### Biomaterial Deposition of Magnesium Phosphates: Mixture of $Mg_3(PO_4)_2 \cdot 22H_2O$ and $KMgPO_4 \cdot 6H_2O$

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The magnesium phosphates which are the mixture of  $Mg_3(PO_4)_2 \cdot 22H_2O$  and  $KMgPO_4 \cdot 6H_2O$  was prepared by microbiological method. The main process is substrate hydrolysis by the alkaline phosphatase produced by phosphate-mineralization microbe, in  $Mg^{2+}$  and  $K^+$  environment. The structural characterization by X-ray techniques, energy dispersive X-ray spectroscopy and morphological observations *via* scanning electron microscope showed that the irregular shape of magnesium phosphates have been prepared. And biomineralizing mechanism showed that bacterial body and metabolites play important roles in the process of crystal nucleation, growth and accumulation of magnesium phosphates.

**Keywords:** Magnesium phosphates, Structural, Morphology, X-ray techniques, Scanning electron microscope.

Biomaterialization is the process by which mineral nucleation and growth are regulated *via* the matrix (either cellular or extra cellular) of living organisms<sup>1-3</sup>. The mechanism of controlled crystal nucleation is important for the design of inorganic materials in biological systems<sup>4,5</sup>. In biomineralizing systems, taking the matrix macromolecules as a template, the biogenic minerals finally form the deposition of precise arrays of inorganic crystals involves controlled nucleation at interfaces between the crystals and substrate macromolecules<sup>4,6,7</sup>. Magnesium phosphates are important inorganic materials were used in various domains such as ceramics, cement replacements in various environments, immobilization systems for heavy metal and radioactive ash containment, sewage and wastewater treatment. There stand two synthetic process to prepare magnesium phosphates, including gel method<sup>8</sup> and chemical precipitation<sup>9</sup>. But there are a few papers about the shape and texture of magnesium phosphates by mimicking the biomaterial synthesis. Here, we report the preparation and characterization of magnesium phosphates which were the mixture of  $Mg_3(PO_4)_2 \cdot 22H_2O$  and  $KMgPO_4 \cdot 6H_2O$  by microbiological method. And biomineralizing mechanism of the whole changing process was discussed.

All the reagents and solvents from commercial sources were used without further purification. Double distilled water was prepared in lab. XRD (Bruker, Germany) analysis was carried out on sample at room temperature by a D8-Discover X diffraction meter (40 kv, 40 mA) with Cu ( $\lambda = 1.5406 \text{ \AA}$ )

irradiation at the rate of 0.020s/step in the range of 5-90°. SEM (FEI, Netherlands) with an Genesis 60S energy dispersive X-ray spectroscopy (EDS) spectroscopy system was used to conduct morphological studies and to measure the elemental compositions of the samples. The detector, accelerating voltage and spot size were secondary electron detector, 20 kV and 4, respectively.

**Biomaterial synthesis of magnesium phosphates:** Microbe having OD600 value of 0.8 was used in this study. Cultivation of the organism was conducted in a medium containing 3g/L yeast extract, 5g/L peptone and 1g/L sodium chloride. In general, the harvest microorganism was stored at 4 °C prior to use. After that, 20 mM of substrate was dissolved in a petri dish with 30 mL water and the pH was adjusted to 9 using 18 % hydrogen chloride solution. The solution was added to 200 mL of bacteria solution. The reaction mixture was allowed to stand under static conditions for 10 h at room temperature. The solution pH values were adjusted to 10.5 using 5 % KOH solution. 20 mM of  $MgCl_2 \cdot 6H_2O$  was then added to the reaction mixture. As a result, magnesium phosphates were collected and characterized.

The magnesium phosphates which are the mixture of  $Mg_3(PO_4)_2 \cdot 22H_2O$  and  $KMgPO_4 \cdot 6H_2O$  precipitate induced in bacteria is taken as a more complicated process than the one induced in pure water. In bacteria solution, phosphate-mineralization microbe can produce alkaline phosphatase which constantly decomposed substrate by emzymolysis and obtain  $PO_4^{3-}$

or  $\text{HPO}_4^{2-}$ . In this circular process, the  $\text{PO}_4^{3-}$  or  $\text{HPO}_4^{2-}$  concentration in solution is increased with the continuous depositions of substrate. Phosphate anion ( $\text{PO}_4^{3-}$ ) is obtained when pH is adjusted to 10.5 using 5 % KOH solution. Negatively charged water-soluble organics on the surface of bacterial cells chelate  $\text{Mg}^{2+}$  and  $\text{K}^+$  constantly, inducing higher concentration of  $\text{PO}_4^{3-}$  locally and more  $\text{Mg}^{2+}$  and  $\text{K}^+$  are reacted with  $\text{PO}_4^{3-}$  until crystal precursor concentration increases enough for nucleation and precipitation of magnesium phosphates particles. Small grains are formed in the early precipitation then the small grains pile up together with each other to form larger particles for 24 h due to the microbial cells chelate  $\text{Mg}^{2+}$  and  $\text{K}^+$  until forming different morphology and size of products.

The elemental analysis of samples composition was performed using EDS (Fig. 1) to confirm the presence of elemental O, P, K<sup>+</sup> and  $\text{Mg}^{2+}$  in the sampled magnesium phosphates. The XRD of materials analysis further confirms that the diffraction diagram indicated that the crystal formed was the mixture of  $\text{Mg}_3(\text{PO}_4)_2 \cdot 22\text{H}_2\text{O}$  and  $\text{KMgPO}_4 \cdot 6\text{H}_2\text{O}$  (Fig. 2). The standard XRD patterns of the reported structures of  $\text{Mg}_3(\text{PO}_4)_2 \cdot 22\text{H}_2\text{O}$  and  $\text{KMgPO}_4 \cdot 6\text{H}_2\text{O}$  used are JCPDS No. 44-0775 and JCPDS No. 35-0812, respectively. The characteristic peaks of these two compounds appeared in the X-ray diffraction pattern of samples.

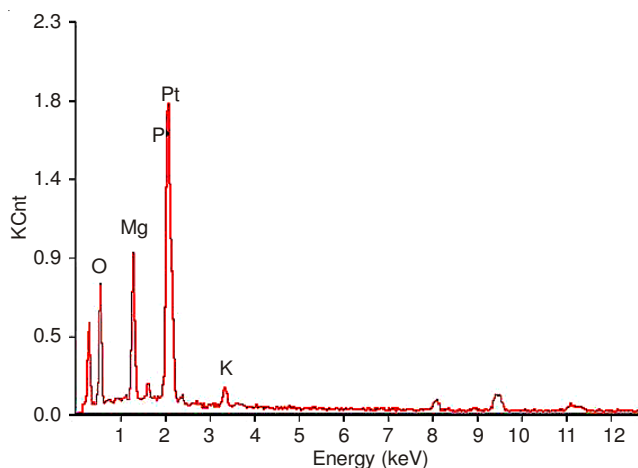


Fig. 1. EDS spectrum of magnesium phosphates

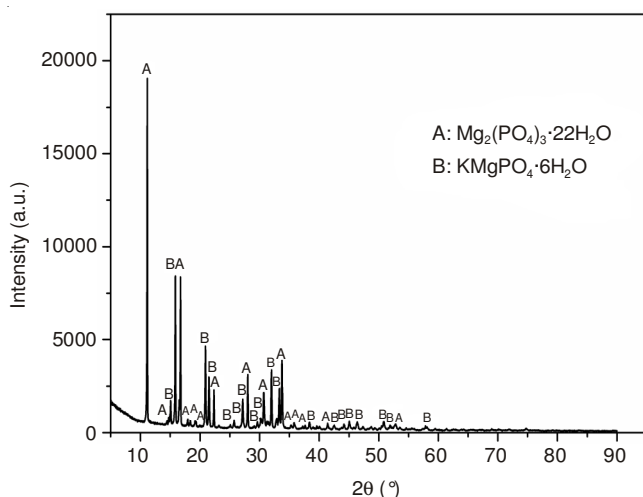


Fig. 2. XRD patterns of magnesium phosphates

The SEM images show that the cells adhered to these dimentation and formed a mineralization system (Fig. 3). It is clearly observed that the image of magnesium phosphates particles, their irregular shapes are found in the samples. The size of the irregular shapes are not uniform, the length ranges from 2 to 3  $\mu\text{m}$ .

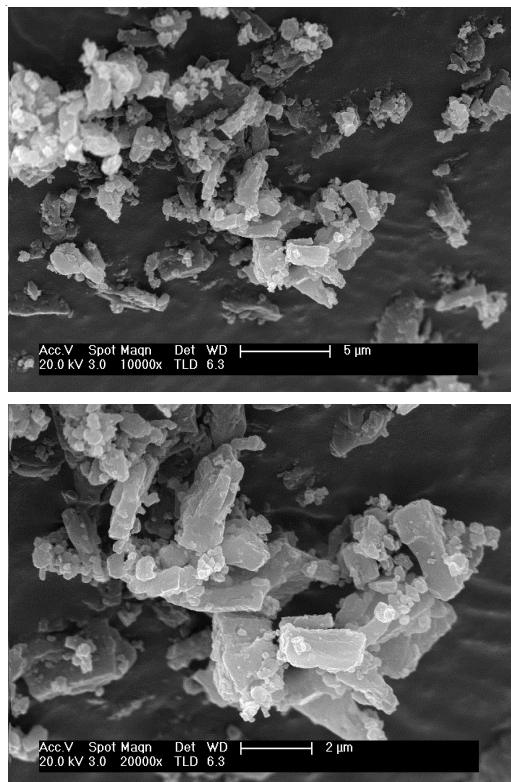


Fig. 3. Scanning electron micrograph of magnesium phosphates

## Conclusion

In this work we have shown that magnesium phosphates which are the mixture of  $\text{Mg}_3(\text{PO}_4)_2 \cdot 22\text{H}_2\text{O}$  and  $\text{KMgPO}_4 \cdot 6\text{H}_2\text{O}$  were successfully synthesized *via* microbiological technique. The chemical compositions and structures of samples were characterized with XRD and SEM, revealing crystal morphologies of magnesium phosphates were irregular shapes with not uniform size.

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