Antibacterial and photocatalytic performance of Silver orthophosphate/Hydroxyapatite composite

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Abstract: The present work reports the synthesis of pure hydroxyapatite and HAp supported Ag3PO4 (Ag3PO4/HAp composites) with varying amounts of silver ions Ag+ with a facile in-situ ion exchange method. XRD results of Ag3PO4/HAp composite reveal the peaks characteristic of pure HAp with additional peaks of silver ions which suggest the deposit of silver particles on the support surface (HAp). The nano-composites Ag3PO4/HAp samples show good adsorption and improved photocatalytic activities in methylene blue MB degradation (more than 70%). Antibacterial study of Ag3PO4/HAp nano-composites showed that the HAp dried at 90°C possesses some antibacterial activity against Escherichia coli and Staphylococcus aureus bacteria. Also, compared to HAp sample, deposition of Ag3PO4 nanoparticles on HAp surface improves the antibacterial performance of the composites.

Keywords: Hydroxyapatite; silver orthophosphate; Gram-positive and Gram-negative bacteria; photodegradation.

Introduction

Nowadays, the textile industries and photographic industries reject vast quantities of polluted wastewater. The purification of water from chemical products and microbial contaminations has become a great environmental challenge. Semiconductor-based photocatalysis seems to be a promising and efficient material to decompose pollutants substances using solar light. Among many photocatalysts, titanium oxide (TiO2) is widely used thanks to its high photocatalytic activity and chemical stability 1. Unfortunately, TiO2 shows good photocatalytic activity only under ultraviolet (UV) (<387nm) 2, which limits its use as a photocatalytic material. Therefore, it is necessary to look for other possible alternative photocatalysts to TiO2. Ag3PO4 was recently found as an alternative photocatalyst material for solar photocatalysis, which can oxidize water and decompose organic dye with excellent photo-oxidation activity 3. The photodegradation performance of Ag3PO4 was found to be higher than that of other classical photocatalysts such as N-TiO2 and BiVO4 4,5. Umezawa and co-workers studied the origin of high photocatalytic performance using the density functional theory based on the first principles calculations indicating that Ag3PO4 had a large dispersion of conduction band facilitating the separation of electron/hole pairs (e-/h+) 6.

However, Silver phosphate can be reduced to metallic silver Ag0 during the photodegradation reaction. Also, the photoinduced electron (e-) cannot react with O2 to form O2- because the conduction band CB position is more positive than that red/ox potential of O2/O2- (E°=−0.33V) 7. Therefore, the photoinduced electrons cannot be combined with O2 to form reduction products, which is necessary for the photodegradation process. To enhance the performance of photoinduced electrons for Ag3PO4 material various methods have been adopted by coupling and constructing a heterogeneous junction between Ag3PO4 and another semiconductor supports such as Ag3PO4/TiO2 8, Ag3PO4/Graphene oxide 9 and Ag3PO4/SnO2 10. Due to its properties such as biocompatibility, bioactivity and biodegradability, Hydroxyapatite HAp, with chemical formula Ca10 (PO4)6(OH)2, is a potential candidate to be used as catalyst support and as a support for inorganic antibacterial agents. HAp supported Ag3PO4 proves to be a promising material for visible light activated photocatalyst.
On the other hand, HAp particles could be contaminated by bacteria during the synthesis or implantation stage. It has been noticed that the HAp does not present any antibacterial activity which limits its use as implant. However, Tin-Oo et al. reported that HAp possesses some antibacterial activity with the broth dilution technique. Hence, the improvement of HAp antibacterial activity has become essential and necessary to ensure the implantation of the HAp biomaterial without any bacterial infections. In this regard, one of the ways to improve the antibacterial activity of HAp is using metal ions to minimize the risk of bacterial contaminations and therefore prevent the growth of pathogens on the implant surface. Various metal ions such as Ag⁺, Zn²⁺ or Cu²⁺ can be used in the coating of the implant to eliminate the bacterial adhesion and growth. One of these ions, which present an excellent antibacterial activity, is Ag⁺ thanks to its substantial toxicity property towards a broad range of pathogens. Ag⁺ ions are also very well known for their antibacterial properties and non-toxic characteristics to human cells at low quantities. The cytotoxicity of Ag⁺ might be due to size, the shape of particles and also to their accumulation inside human cells, leading to their dysfunctions. So, coupling the Ag₃PO₄ and HAp proves to be useful and beneficial to enhance the antibacterial activity of implants if they are used in feeble concentrations.

The present work aims to synthesize the nanocomposites of HAp supported Ag₃PO₄ with varying amounts through a facile in-situ ion exchange method as well as silicon-substituted HAp (SixHAp) by the wet chemical precipitation method. The synthesized nano-composites were analysed by XRD technique. In order to understand the effect of the support on the Ag₃PO₄ photocatalysis, the photodegradation of MB under visible light irradiation was investigated. The effect of Ag₃PO₄ deposited on HAp surface on the antibacterial activity of HAp was studied through disk diffusion method by measuring the zone inhibition for each sample against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus).

**Materials and methods**

**Synthesis of samples**

Pure Hydroxyapatite (HAp), as a support, was synthesized via conventional aqueous precipitation method as it was briefly dealt in our previous paper. Briefly, 0.925g of calcium hydroxide Ca(OH)₂ was dissolved in 25ml of deionized water DI and stirring for 15min at room temperature. Concurrently, 0.720g of phosphoric acid (H₃PO₄) was dissolved in 25ml of DI water and stirring for 15min and added drop by drop, under stirring, to the already prepared solution of Ca(OH)₂. The pH of the slurry remained higher than 10 (no ammonia was added). The resultant mixture was stirred for 2h and then matured over 48h at ambient temperature. The as-prepared precipitate was filtered and dried at 90°C for 24h.

The Ag₃PO₄ nanoparticles were prepared by the liquid-solid method, and 0.408g of silver nitrate (Riedel-de Haën, 98.5-100%) directly added under stirring to 20ml of an already prepared (NH₄)₂HPO₄ (LOBA CHEMIE) solution (5.4g/L). After 2h of agitation, the resulting paste was filtered and dried at 80°C for 12h. On the other hand, to synthesize Ag₃PO₄/HAp nano-composites, 0.5g of the biomaterial supports (HAp) was added to 20ml of DI water and aged for 10min. Then, 15ml of nitrate silver precursor, containing the desired quantity of AgNO₃ (0.05, 0.1 and 0.15g), was mixed with HAp precursor and kept under stirring for 4h at room temperature and matured over 12h. The yellow product suspensions were removed by filtration, and the remaining particles were dried at 80°C for 12h to remove the residual water. For reference, the samples were labelled as Ag₃PO₄/HAp-1 with 5.8% (weight percentage) of Ag, Ag₃PO₄/HAp-2 (10.6 wt. % of Ag) and Ag₃PO₄/HAp-3 (14.6 wt. % of Ag) and the amount of AgNO₃ added is 0.05g, 0.1g and 0.15g respectively.

**Powders characterization**

The identification of crystallites phases of all samples was investigated by Philips X’Pert PRO X-ray diffractometer (XRD) using CuKα radiation (λ = 1.5406 Å) at 40 kV and 30 mA. The XRD patterns were collected in the 20 range of 10–60° with a step of 0.02°. The morphological characteristics of as-synthesized nanoparticles were carried out by scanning electron microscopy (SEM, Hitachi, S-3400N).

**Photocatalytic measurements**

The as-prepared photocatalysts were evaluated through the photodegradation of the MB organic pollutant in aqueous solution under visible light irradiation (36W LED lamp). 50 mg of each photocatalyst was dispersed into 50ml MB solution dye (20mg/l), and then the result suspensions were stirred in the dark for 30min to establish adsorption-desorption equilibrium between MB and photocatalysts. After irradiation time, the MB aqueous solution was sampled and centrifuged to eliminate the photocatalysts. The remaining concentration of MB was measured using a UV–vis spectrophotometer (Jenway, Serial: 67XX) and the decolorization efficiency (%) was calculated by the following equation (1):

\[
\text{Decolorization} \% = \left( \frac{C_0 - C}{C_0} \right) \times 100\% = \left( \frac{A_0 - A}{A_0} \right) \times 100\%
\]

Where C₀ and A₀ were the initial concentration and initial absorbance of MB respectively, while C and A were the concentration and absorbance after the irradiation of visible light respectively.
In an attempt to understand the effect of the silver percentage on the antibacterial activity compared to HAp as a control, the antibacterial properties were carried out against two kinds of bacteria strain: Staphylococcus sharp and intense peaks indicate the high purity (S. aureus) and Escherichia coli (E. coli) as Gram-positive and Gram-negative strains, respectively. The used pathogens were isolated from the nutrition, health and environment laboratory; by the microbiology team, at Ibn Tofail University in Morocco. The test evaluation is performed by the disk diffusion method. The disks of filter paper (Woltman n°1); with a diameter of 5mm; emerged for 24h in the solutions containing the samples already sterilized by tyndallization (at 60 °C over 30 min then 5-10 °C over 30 min). The diffusion technique was performed using the Müller-Hinton Agar (MHA). The zone plates were carried out by filling the used agar into Petri dishes, then 1cm³ of the 10⁸ (cfu cm⁻³) suspension of each tested species was poured and spread over the MHA medium previously prepared simple (the bacterial culture was 24 hours old). The as-prepared disks (already emerged) were deposited on the medium culture and incubated for 24h at 37°C. The culture of the blank sample investigated in the same conditions, and the same nutrient broth medium is used for all tests. All of the tests were repeated ten times, and their average represented the results. The obtained results were performed by measuring the width of the inhibition zone (cm).

Figure 1. XRD diffraction data of (a) HAp support, pure Ag₃PO₄, Ag₃PO₄/HAp-1 and (b) Ag₃PO₄/HAp-1, Ag₃PO₄/HAp-2 and Ag₃PO₄/HAp-3 composites
Results and discussion

Characterization of products

The phase and the crystallinity of resultant products were investigated by XRD diffraction analysis. Figure 1 shows the XRD patterns of samples. Fig 1-a represents the XRD patterns of pure hydroxyapatite support (HAp) dried at 90°C, pure Ag₃PO₄ and Ag₃PO₄/HAp-1 composite. Fig 1-b shows just the XRD patterns of three composites samples.

All XRD patterns were indexed based on JCPDS card n° 06-505 for Ag₃PO₄ and JCPDS Card n° 9-432 for hydroxyapatite support Ca₁₀(PO₄)₆(OH)₂. The important diffraction peaks characteristics of HA apatite were observed located at 25.89° - 32.13° - 33.00 and 34.15° corresponding to (002), (211), (112) and (300) planes, respectively. XRD pattern of pure HAp did not show the presence of any other materials as a secondary phase. The broad peaks of pure HAp reveal the presence of a significant amount of amorphous phase, which is in good accordance with the literature. On the other side, the eight peaks located at 29.77°, 33.39°, 36.65°, 42.50°, 47.82°, 52.71°, 55.06° and 57.30° correspond to the (200), (210), (211), (220), (310), (222), (320) and (321) planes of pure Ag₃PO₄ nanoparticles, respectively. Therefore, the sharp and intense peaks indicate the high crystallinity and crystallinity of Ag₃PO₄ particles. Lattice parameters and crystallite size of the synthesized Ag₃PO₄ particles were calculated using the Full Prof program and Scherrer equation, respectively. The mean estimated crystallite size and lattice parameters were 0.56 Å, and a=6.0139 Å, respectively and the structure of Ag₃PO₄ microcrystal was determined as a body-centred cubic structure with P-43n space group (JCPDS no 06-0505). Thus the XRD analysis reveals also that the as-prepared sample of silver nitrate is pure Ag₃PO₄ with no detected impurities phases. After impregnation of metallic silver, all composites samples show both the peaks characteristic of HAp and Ag₃PO₄, suggesting the successful deposit of Ag₃PO₄ microcrystals on the HAp support surface and confirming their coexistence. As the amount of AgNO₃ increase, the characteristic peaks of Ag₃PO₄ nano-crystals become increasingly intense.

The mean crystallite size (L) of Ag₃PO₄ particles was calculated from the XRD data using the Scherrer equation as follow (2):

\[ L = \frac{k\lambda}{\beta\cos \theta} \]

Where L = crystallite size, K = the shape factor equal to 0.9, \( \lambda = \) wavelength of Cu Kα radiation equal to 1.5406 Å, θ = half of the diffraction angle and \( \beta = \) full width at half maximum (FWHM), the Scherrer equation is relative to a single peak (210).

The morphologies of as-prepared photocatalysts were characterized by scanning electron microscopy (SEM), and the representative images are shown in Figure 2. It can be seen that the Ag₃PO₄ nanoparticles exhibit both a nanorod and irregular polyhedra morphologies with a smooth surface (Fig 2.a).
The particles size varies than from 1.5 to 9 microns (Fig. 2.a). In Fig. 2.b-d, we can see the numerous bright particles with a mean particle size of 1–2.5 microns are deposited onto the surface of the support (dark surface) suggesting the deposition of Ag$_3$PO$_4$ nano-particles on HAp surface. Based on the above SEM images, we can infer that the use of hydroxyapatite support promotes better dispersion and enhances stronger contact with Ag$_3$PO$_4$. Hence, the anti-agglomeration of Ag$_3$PO$_4$ nanoparticles can be realized in the samples, which is favourable to the reduction of the particle size of Ag$_3$PO$_4$ and therefore beneficial to the photocatalytic process.

Figure 3 shows the photocatalytic performance of as-prepared samples to decompose MB dye after exposing to visible light irradiation. The decolorization of MB dye was measured by absorbance at 664 nm which is largely taken as a reference to evaluate the decolorization rate of MB organic dye. Furthermore, the absorbance of MB at 291 nm could be used to extract information about the degradation stage of the entire dye. For the HAp sample, and according to Figure 3, it can be seen that the MB concentration remains practically unchanged under visible light irradiation in 120 min. In addition, the pure Ag$_3$PO$_4$ photocatalyst exhibits very good photocatalytic activity under visible irradiation, and 98% of MB is decomposed after 60 min reaction time. The photocatalytic activity of Ag$_3$PO$_4$ particles depends on the balance between the size and morphology. Xiang et al reported that the Ag$_3$PO$_4$ nanorods exhibit good photocatalytic activity for dye degradation. Obviously, the good photocatalytic activity can be explained by the nanorod and irregular polyhedra morphology of pure Ag$_3$PO$_4$ nanoparticles.

![Figure 3](image-url) 
**Figure 3.** degradation curves for MB using HAp, Pure Ag$_3$PO$_4$, Ag$_3$PO$_4$/HAp-1, Ag$_3$PO$_4$/HAp-2 and Ag$_3$PO$_4$/HAp-3

Compared with pure HAp, the Ag$_3$PO$_4$/HAp nano-composites show a superior photodegradation rate of MB, which was due to the deposited Ag$_3$PO$_4$. It should also be noted that the photocatalytic activity of Ag$_3$PO$_4$/HAp composites increase when the concentration of silver ions increase. Under visible irradiation, Ag$_3$PO$_4$/HAp-3 show higher photocatalytic performance compared to other silver hydroxyapatite composites with a decolorization rate of MB of 70.30% in 120 min. However, the decolorization rate of Ag$_3$PO$_4$/HAp-1 and Ag$_3$PO$_4$/HAp-2 are only 50% and 55% in 100 min respectively. We can observe that the decolorization rate of Ag$_3$PO$_4$/HAp-1 and Ag$_3$PO$_4$/HAp-2 is higher than that of Ag$_3$PO$_4$/HAp-3 until 100 min and vice versa at 120 min. Moreover, a small amount of silver ion could improve the photocatalytic activity due to the slow release of deposited silver ions on the HAp support by photogenerated electrons which leads to the rapid carrier (electrons and holes) separation under visible light irradiation.

**Antibacterial assessment**

The antibacterial activities of HAp, pure Ag$_3$PO$_4$ and Ag$_3$PO$_4$/HAp samples were investigated by measuring the zone inhibition (ZoI) against E. coli and S. aureus strains (Table 1). The antibacterial efficiency of the test is evaluated by measuring the diameter of ZoI around the disk. When the ZoI increases the antibacterial efficiency becomes more critical. Results show that the samples have acted differently on the two bacteria. According to the obtained results, it can be seen that the E. coli strain is less responsive than S. aureus ones to all studied
compounds (Figure 4). This difference in response might be due to the difference in the cellular wall structure of Gram-positive and Gram-negative strain.23,24

Table 1. Average diameter (cm) of zone inhibition.

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>E. Coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyapatite support</td>
<td>0.85 [a]</td>
<td>1 [ab]</td>
</tr>
<tr>
<td>Ag$_3$PO$_4$ microcrystals</td>
<td>1.1 [c]</td>
<td>1.25 [ab]</td>
</tr>
<tr>
<td>Ag$_3$PO$_4$/HAp-1</td>
<td>1 [c]</td>
<td>1.15 [ab]</td>
</tr>
<tr>
<td>Ag$_3$PO$_4$/HAp-2</td>
<td>1.1 [b]</td>
<td>1.25 [ab]</td>
</tr>
<tr>
<td>Ag$_3$PO$_4$/HAp-3</td>
<td>1.4 [d]</td>
<td>1.5 [ab]</td>
</tr>
</tbody>
</table>

The same letter means that the samples do not differ significantly at the 5% threshold.

![Figure 4](image)

Figure 4. histograms of diameter average of zone inhibition of as-prepared samples.

A. Z. Alshemary et al. and N. Iqbal et al. reported that the HAp has no antibacterial effect against E. coli bacteria strain using the disk diffusion method while Archan et al. found that there is no antibacterial activity against S. aureus using the same method. According to Tin O-o study, the HAp microcrystals possess antibacterial activity using the broth dilution technique. In the present study, it can be seen that the HAp support acted differently and it presents a low antibacterial activity against both bacteria strain. The zones inhibition for E. coli and S. aureus were 0.85 and 1.00 cm respectively. Moreover, the E. coli bacteria are less affected by antimicrobial agent Ag$_3$PO$_4$ than S. aureus. In the literature, Ag$_3$PO$_4$ is well known for its antibacterial property. The silver ions release around the disk kill the pathogens and thus limit the growth of microorganisms.

On the other hand, the ZoI was observed for three HAp supported Ag$_3$PO$_4$ samples against E. coli and S. aureus strains. The average inhibition zones increase when the percentage of Ag ions increases for two studied pathogens. The average ZoI of Ag$_3$PO$_4$/HAp-1, Ag$_3$PO$_4$/HAp-2 and Ag$_3$PO$_4$/HAp-3 for E. coli were 1, 1.1 and 1.4 cm respectively. Ag$_3$PO$_4$/HAp-1, Ag$_3$PO$_4$/HAp-2 and Ag$_3$PO$_4$/HAp-3 showed zone inhibition of 1.15, 1.25 and 1.5 cm toward S. aureus. Our results are in good accordance with the authors who studied the effect of silver ions Ag$^+$ on E.coli and S. aureus.23,27-31 The antibacterial properties of silver ions Ag$^+$ were ascribed to the high surface/volume ratio of silver particles, which gives more efficiency and enhances their antibacterial activity.32 Feng et al. and Song et al. suggested the mechanism explaining the influence of the silver ions on cell bacteria.33,34 The Ag$^+$ ions attack the bacterium proteins at its cell wall causing cellular problems and then separate the cytoplasm from the bacterial cell wall. The silver ions may attach to bacterial DNA and RNA and thus prevents bacterial replication leading finally to the death of the cell. Mocanu et al. report that the silver ions attack preferably the respiratory chain and cell division.
Conclusion

Nano-hydroxyapatite HAp and Ag3PO4 supported hydroxyapatite Ag3PO4/HAp were successfully synthesized. XRD data of HAp reveal the diffraction peaks typical of pure HAp apatite. The diffraction spectra of Ag3PO4/HAp composites show the coexistence of HAp phase and silver ions. Also, coupling Ag3PO4 with hydroxyapatite support reveals a high photocatalytic activity to decompose methylene blue MB dye comparing to pure HAp. Antibacterial tests show that the Ag3PO4/HAp particles have good antibacterial activity against E. coli and S. aureus strains. Consequently, our composites mainly Ag3PO4/HAp, could be used in dental and surgical applications as a bone implant or coating metallic implants.

References

19- Gopal Panthi, Rosa Ranjit, Hak-Yong Kim, Deependra Das Mulmi, Size-dependent optical and antibacterial properties of Ag3PO4 synthesized by facile precipitation and colloidal approach in aqueous solution, Optik 156, 2018, 60-68.
21- Y. Bi, S. Ouyang, N. Umezawa, J. Cao, et J. Ye, Facet Effect of Single-Crystalline Ag 3 PO

22- Xiaoting Hong, Xiaohui Wu, Quiyun Zhang, Mingfeng Xiao, Gelin Yang, Meirong Qiu, Guocheng Han, Hydroxyapatite supported Ag3PO4 nanoparticles with higher visible light photocatalytic activity, Applied Surface Science 258, 2012, 4801-4805.


