Unveiling the role of β-Ag$_2$MoO$_4$ microcrystals to the improvement of antibacterial activity

Camila Cristina De Foggi$^a$, Regiane Cristina De Oliveira$^b$, Marcelo Assis$^b$, Maria Tereza Fabbro$^b$, Valmor Roberto Mastelaro$^c$, Carlos Eduardo Vergani$^a$, Lourdes Gracia$^d$, Juan Andrés$^d$, Elson Longo$^b$ and Ana Lucia Machado$^a$.

$^a$São Paulo State University (UNESP). Department of Dental Materials and Prosthodontics, 14801-907, Brazil
$^b$Interdisciplinary Laboratory of Electrochemistry and Ceramics, University Federal of São Carlos, P.O. Box 676, 13565-905 São Carlos, SP, Brazil.
$^c$São Carlos Institute of Physics, University of São Paulo, CP 369, São Carlos 13560-970, São Paulo, Brazil
$^d$Department of Physic and Analytical Chemistry, Universitat Jaume I, Campus Riu Sec, E-12071 Castellón, Spain

**Abstract**

Crystal morphology with different surfaces is important for improving the antibacterial activity of materials. In this experimental and theoretical study, the antibacterial activity of β-Ag$_2$MoO$_4$ microcrystals against the Gram-positive bacteria, namely, methicillin-resistant *Staphylococcus aureus* (MRSA), and the Gram-negative bacteria, namely, *Escherichia coli* (*E.coli*), was investigated. In this study, β-Ag$_2$MoO$_4$ crystals with different morphologies were synthetized by a simple co-precipitation method using three different solvents. The antimicrobial efficacy of the obtained
microcrystals against both bacteria increased according to the solvent used in the following order: water < ammonia < ethanol.

Supported by experimental evidence, a correlation between morphology, surface energy, and antibacterial performance was established. By using the theoretical Wulff construction, which was obtained by means of density functional calculations, the morphologies with large exposition of the (001) surface exhibited superior antibacterial activity. This study provides a low cost route for synthesizing β-Ag$_2$MoO$_4$ crystals and a guideline for enhancing the biological effect of biocides on pathogenic bacteria by the morphological modulation.

**Keywords:** β-Ag$_2$MoO$_4$, antibacterial activity, morphology evolution

**Introduction**

Metal molybdates such as A$_x$MoO$_z$ (where A is a monovalent, divalent, or trivalent metal ion) have recently been investigated intensively [1,2,11,12,3–10] owing to their chemical stability and unique crystal structure (layers of molybdenum oxide octahedra separated by the metal ions) [13], which make them suitable for a wide range of applications. Particularly, silver molybdates have been investigated in the fields of lubrication [14,15], humidity and gas sensors [16,17], surface enhanced Raman scattering techniques [18,19], photocatalysis [11–13,20,21], and photoluminescence [20,22,23]. A plethora of synthetic methods have been used to harness these applications, such as hydrothermal [19,24–27], solution-based chemical reaction [18], co-precipitation [23,28], dynamic template route [21], laser annealing [29], and microwave-assisted hydrothermal methods [20,30]. In this context, Ng and Fan [31] have recently reported the preparation of β-Ag$_2$MoO$_4$ crystals with high-index facets via
the delicate tuning of the supersaturation conditions during crystal growth. Additionally, they were able to find a relationship between morphology and photocatalytic activity.

The morphological modulations can be achieved by the different solvents (water, ammonia, and ethanol) used in the co-precipitation synthesis method, as we described previously [28].

As a continuation of our work in this field of research, in this paper, we report the antibacterial activity of $\beta$-Ag$_2$MoO$_4$ microcrystals against gram-positive bacteria, namely, methicillin-resistant *Staphylococcus aureus* (MRSA), and gram-negative bacteria, namely, *Escherichia coli* (*E. coli*). These bacteria are important because they are opportunistic pathogens that are often inherently resistant to antibiotics or capable of rapidly building resistance to many common antimicrobial agents [32]. More significantly, the biological effects of $\beta$-Ag$_2$MoO$_4$ with different morphologies on the bacteria are systematically discussed. Additionally, to gain a deeper understanding of the atomic and electronic structure, and to establish a correlation among the morphology, surface energy, optical properties, and antibacterial activities, we conducted first principle calculations on the basis of density functional theory (DFT) to complement our experimental findings. This study intends to provide a more comprehensive insight into the development of novel biocide with a unique morphology and future potential for use in biological applications.

**Results and Discussion**

The $\beta$-Ag$_2$MoO$_4$ microcrystals were synthesized by using different solvents (water, ammonia, and ethanol) and the co-precipitation method. The experimental method is described in the Supplementary Material (SM) section. The samples were structurally characterized by x-ray (XRD) diffraction to evaluate the order/disorder at
long range. Figure 1 shows the XRD for the samples of silver molybdate. It was observed that all compounds presented a structure type assigned to a cubic spinel with the Fd3m space group, which is in agreement with the *Inorganic Crystal Structure Database* (ICSD) card 238013 [20]. The lattice parameters for the \( \beta \)-Ag\(_2\)MoO\(_4\) phase are \( a = b = c = 9.3170 \) Å and \( a = b = c = 90^\circ \). Moreover, Figure 1 shows clearly that there was no other additional peak; that is, there was no undesirable secondary phase. Figure 1 shows the cubic spinel structure type that was composed by distorted octahedral clusters \([\text{AgO}_6]\) with Oh symmetry for the Ag sites, and distorted tetrahedral clusters \([\text{MoO}_4]\) with Td symmetry for the Mo sites. The crystallinity degree of a structure (order/disorder), that is, the organization at long range is directly dependent of the manner of the ions organize themselves into the structure, being that it is totally dependent on the chemical environment in which the material is formed. Therefore, the change of solvent, or the addition of any species in the synthesis of the material may corroborate a change in the order/disorder. A factor related to structural order/disorder effects at long-range can be found by the analysis of the full width at half maximum (FWHM) of the most intense peak of the XRD patterns, related to the plane (311) of the \( \beta \)-Ag\(_2\)MoO\(_4\). The samples obtained in water, alcohol and ammonia, have FWHM values of 0.18, 0.21 and 0.23° respectively, showing that the samples synthesized with alcohol and ammonia have a lower degree of order than the samples obtained in water.

*Figure 1*:

**Figure 1:** XRD patterns of the \( \beta \)-Ag\(_2\)MoO\(_4\) microcrystals synthesized in water, alcohol and ammonia.
Figure 2: Raman (2a) and FT-IR (2b) spectra of the $\beta$-Ag$_2$MoO$_4$ microcrystals synthesized in water, alcohol and ammonia.

Raman spectroscopy is a technique complementary to XRD for estimating structural order/disorder at short-range. Figure 2 shows the experimental spectra obtained for all samples. The $\beta$-Ag$_2$MoO$_4$ belongs to the point-group symmetry $O_h^7$ with the centrosymmetric inversion, which indicates five active Raman modes ($A_{1g}$, $E_g$, and $T_{2g}$) obtained from the decomposition of point $\Gamma$ ($\Gamma = A_{1g} + E_g + 3T_{2g} + T_{1g}$) [20]. Figure 2a shows the spectra for the samples obtained in different solvents. The spectra of all samples show four characteristic peaks centered at 276, 355, 761, and 873 cm$^{-1}$. The first Raman mode at 276 cm$^{-1}$ is associated with a transition $E_g$, which refers to external structure vibrations on the [AgO$_6$] clusters [20]. The Raman mode at 355 cm$^{-1}$ was associated with the transition $T_{2g}$ caused by the O-Mo-O asymmetric bending vibrations [20,33]. Both the 761 and 873 cm$^{-1}$ modes are associated with the $T_{2g}$ and $A_{1g}$ transitions, respectively, and were caused by the asymmetric and symmetric O-Mo-O vibrations [20,33]. Figure 2b shows shows FT-IR for the samples of $\beta$-Ag$_2$MoO$_4$. In the FT-IR spectra of the samples the band at 827 cm$^{-1}$ is observed related to a asymmetrical stretching of the O-Mo-O bonds of the [MoO$_4$] tetrahedral clusters [11,34]. Other vibrations are not observed because they appear below 400 cm$^{-1}$, outside the limit of the equipment used.

Theoretical investigations based on first principle calculations by ab-initio and quantum-chemical simulations have been increasingly used to complement the experimental findings, provide valuable information of the electronic, structural, and energetic properties, and simulate and predict the morphology of the materials [20,33,35]. Thus, by using this combined experimental and theoretical approach,
previous studies determined that the most stable morphology for the $\beta$-Ag$_2$MoO$_4$ can occur when the surface energies are 1.90, 1.28, and 3.46 J/m$^2$ for the (001), (011), and (111) faces, respectively [28,33].

Considering that Ag$_2$MoO$_4$ acquires different morphologies according to the synthetic method employed [28,30], in this study, the morphologies of the $\beta$-Ag$_2$MoO$_4$ samples were investigated by field emission scanning electron microscopy (FE-SEM), and are of fundamental importance to understanding the morphological evolution process and changes in the surfaces of the crystals with the variations of the used solvents. The $\beta$-Ag$_2$MoO$_4$ samples that were obtained by using different solvents are shown in Figure 3. The predominant morphologies obtained experimentally display the exposed (001), (011), and (111) surfaces, which support the hypothesis that these surfaces are stabilized by interacting with the solvent molecules. This observation suggests that the Wulff shape of $\beta$-Ag$_2$MoO$_4$ is closely related to the chemical environment.

<Figure 3>

Figure 3: FE-SEM images, morphologies, and facets of the $\beta$-Ag$_2$MoO$_4$ samples obtained by using different solvents: (a) water, (b) alcohol, and (c) ammonia.

For the following discussion on the relationship between the morphology-antibacterial activity of the $\beta$-Ag$_2$MoO$_4$ samples to be valid, it is assumed that the structure and facet composition of the crystals are preserved in the time scale of the experiments. The Wulff crystal representation of the optimized $\beta$-Ag$_2$MoO$_4$, and the available morphologies that would be obtained by assuming different values for the surface energies of the three facets are displayed in Figure 4. By analyzing Figure 4, it can be seen that the experimental and theoretical morphologies are in good agreement:
(a) when water was used as the solvent, the surface energy of the (111) surface decreased from 3.46 to 1.28 J/m²; (b) when ammonia was used as the solvent; the surface energy of the (111) surface decreased from 3.46 to 1.28 J/m², and the surface energy of the (001) surface decreased from 1.90 to 1.5 J/m²; (c) when ethanol was used as the solvent, the surface energy values of both the (111) and (001) surfaces decreased to 1.28 J/m².

*Figure 4*

**Figure 4:** Map of available β-Ag$_2$MoO$_4$ morphologies based on Wulff crystal construction as function of surface energy values.

The minimum inhibitory concentrations (MICs), which are defined as the lowest concentrations required for complete growth inhibition (no visible growth by visual inspection), and the minimum bacterial concentrations (MBCs), which are defined as the lowest concentrations that do not result in bacterial growth on plates, were determined for the β-Ag$_2$MoO$_4$ microcrystals that were synthesized in different solvents (water, ammonia, and ethanol). The tests were performed according to the standard methods described in Clinical Laboratory Standards Institute (CLSI) [36]. The microorganisms evaluated in this study were MRSA (ATCC 33591) and *E. coli* (ATCC 8739). In the microbiological test results shown in Figure 5, the MIC value was the same as the MBC value for MRSA for the three solvents used in the synthesis of the β-Ag$_2$MoO$_4$. Similar findings were observed for *E. coli*.

*Figure 5*

**Figure 5:** Summary of log$_{10}$ CFU/mL MRSA values (a) and *E. coli* (b) obtained for sub-inhibitory concentrations of the β-Ag$_2$MoO$_4$ synthesized in water, ammonia, and ethanol. Control MRSA = 8.8 (±0.71) log$_{10}$ CFU/mL; control *E. coli* = 7.3 (±0.20) log$_{10}$ CFU/mL.
When the microcrystals were synthesized with ethanol, the MIC/MBC value (31.25 μg/mL) for MRSA was lower than that observed for the microcrystals synthesized with ammonia (62.50 μg/mL). Additionally, the bactericidal activity of both β-Ag₂MoO₄ microcrystals was higher than that exhibited by the samples synthesized in water, which required higher concentration (250 μg/mL) to inhibit the MRSA growth. Figure 5a shows that, in comparison with the control (8.8 ± 0.71 log₁₀ CFU/mL), at half of the MICs/MBCs values, the MRSA growth was reduced by approximately 4 logs for the β-Ag₂MoO₄ microcrystals synthesized by using ethanol (to 4.3 ± 0.07 log₁₀ CFU/mL), 5 logs for the microcrystals synthesized in ammonia (to 3.9 ± 0.06 log₁₀ CFU/mL), and 4 logs when water was used as the solvent (to 4.3 ± 0.07 log₁₀ CFU/mL).

For *E. coli*, the microcrystals synthesized using ethanol exhibited a lower MIC/MBC value (0.49 μg/mL) than that observed for the microcrystals synthesized in ammonia (1.95 μg/mL) and water (3.91 μg/mL). The incubation of the bacteria in the presence of half of the microcrystal MICs produced a reduction of growth of approximately 5 logs for the β-Ag₂MoO₄ microcrystals, in comparison with the control (7.3 ± 0.20 log₁₀ CFU/mL) and regardless of the solvent used in the synthesis (Figure 5b).

Oliveira et al. found different morphologies of Ag₂MoO₄ according to the synthesis used. When ethanol was employed, there was improvement in its antibacterial activity against the same *E. coli* strain [30]. In this study, in addition to observing better anti-*E. coli* activity for the sample synthesized in ethanol, we also tested, for the first time, the efficiency against MRSA, which is a highly pathogenic bacterium.

It is worth noting that, for the three β-Ag₂MoO₄ microcrystals obtained in water, ammonia, and ethanol, the concentrations required to kill *E. coli* were always significantly lower than those required to kill MRSA. These findings can be attributed,
at least in part, to the different cell wall structures. The Gram positive bacteria, namely, MRSA are composed of a cytoplasmic membrane and a thick, overlying peptidoglycan network (10-40 nm) composed of repeating units of a disaccharide-multipeptide building block that are polymerized and cross-linked to create a continuous network that envelops the cell [37]. This peptidoglycan network has several layers and contains mainly carboxyl, amide and hydroxyl functional groups [38], and teichoic acids (TAs). Two distinct types of TAs have been identified: wall teichoic acids (WTAs) that are linked to and embedded into the peptidoglycan, and lipoteichoic acids (LTAs) extending into and anchored to the cell membrane (cytoplasmatic membrane) [39]. On the contrary, TAs were not found in gram-negative bacterial cells, such as E. coli [38,39]. Additionally, although gram-negative bacteria contain an outer membrane, they only have a single peptidoglycan layer that is thin (3-6 nm) and located in the periplasmatic space between the outer membrane and the inner (cytoplasmatic) membrane [37]. Given that the cell wall is crucial to the mechanical and chemical integrity of the cells, because it protects them from the external environment and stress, all the mentioned differences between the gram-positive and gram-negative bacteria may have contributed to the higher activity of the synthesized \( \beta-\text{Ag}_2\text{MoO}_4 \) microcrystals against E. coli, in comparison to MRSA.

From Figures 6a, 6b, and 6c, we can see that the (111) surfaces of \( \beta-\text{Ag}_2\text{MoO}_4 \) were Mo, O, and Ag ion-terminated, while the (001) and (011) surfaces were O- and Ag ion-terminated. In the (111) surface, the Ag cations were coordinated to three oxygen anions to form \([\text{AgO}_3.3V_0^2]\) complex clusters, and the Mo cations were coordinated to only one oxygen anion (disfavoring its stability). The (011) surfaces exhibited the Ag cations surrounded by four oxygen anions, which resulted in the \([\text{AgO}_4.2V_0^2]\) complex clusters, while in the (001) surfaces, the silver cations were coordinated to five oxygen...
anions and formed $[\text{AgO}_5.V_0^x]$ complex clusters (more stable for the electron/hole recombination processes). In addition to the atomic configuration of the exposed facets, the solvent used in the synthesis had a great effect on the reactivity and stabilization of the surfaces.

**Figure 6**

**Figure 6**: Mechanism of free radical formation, associated with the predominant face of the $\beta$-$\text{Ag}_2\text{MoO}_4$ crystal obtained in (a) water, (b) ammonia, and (c) ethanol.

A semiconductor’s antibacterial activity mechanism is mainly attributed to the oxidative stress caused by the $\text{OH}^*$, $\text{O}_2^-$, and $\text{O}_2\text{H}^*$ (reactive species) in contact with the bacterial cell wall [40,41], when $[\text{AgO}_3.3V_0^x]$, $[\text{AgO}_4.2V_0^x]$, $[\text{AgO}_5.V_0^x]$, $[\text{AgO}_6]^x$, and $[\text{MoO}_4]_d^x$ complex clusters, from which the $\beta$-$\text{Ag}_2\text{MoO}_4$ semiconductor is formed, are excited (Equations 1-7).

$$[\text{MoO}_4]_o^x + [\text{MoO}_4]_d^x \rightarrow [\text{MoO}_4]_o^\prime + [\text{MoO}_4]_d^\prime$$ ..........................................................(1)

$$[\text{AgO}_6]^x + [\text{AgO}_5.V_0^x] \rightarrow [\text{AgO}_6]^\prime + [\text{AgO}_5.V_0^\prime]$$ ..........................................................(2)

$$[\text{AgO}_6]^x + [\text{AgO}_3.V_0^x] \rightarrow [\text{AgO}_6]^\prime + [\text{AgO}_3.V_0^{\prime\prime}]$$ ..........................................................(3)

$$[\text{AgO}_6]^x + [\text{AgO}_4.V_0^x.V_0^x] \rightarrow [\text{AgO}_6]^\prime + [\text{AgO}_4.V_0^x.V_0^\prime]$$ ..........................................................(4)

$$[\text{AgO}_6]^x + [\text{AgO}_4.V_0^x.V_0^x.V_0^x] \rightarrow [\text{AgO}_6]^\prime + [\text{AgO}_4.V_0^x.V_0^{\prime\prime}]$$ ..........................................................(5)

$$2[\text{AgO}_6]^x + [\text{AgO}_3.V_0^x.V_0^x.V_0^x] \rightarrow 2[\text{AgO}_6]^\prime + [\text{AgO}_3.V_0^x.V_0^x.V_0^\prime]$$ ..........................................................(6)

$$2[\text{AgO}_6]^x + [\text{AgO}_3.V_0^x.V_0^x.V_0^x.V_0^x] \rightarrow 2[\text{AgO}_6]^\prime + [\text{AgO}_3.V_0^x.V_0^x.V_0^{\prime\prime}]$$ ..........................................................(7)

The $[\text{AgO}_3.V_0^x]$, $[\text{AgO}_5.V_0^{\prime\prime}]$, $[\text{AgO}_4.V_0^x.V_0^\prime]$, $[\text{AgO}_4.V_0^x.V_0^{\prime\prime}]$, $[\text{AgO}_3.V_0^x.V_0^x.V_0^\prime]$, and $[\text{AgO}_3.V_0^x.V_0^x.V_0^{\prime\prime}]$ complex clusters transfer a hole for water, which decomposes into a hydroxyl radical and a proton ($\text{OH}^*$ and $\text{H}^\prime$).
Simultaneously, \([\text{AgO}_4\text{V}_0^x\text{V}_0^*]\), \([\text{AgO}_3\text{V}_0^x\text{V}_0^*\text{V}_0^*]\), \([\text{AgO}_3\text{V}_0^x\text{V}_0^*\text{V}_0^*\text{V}_0^*]\), \([\text{MoO}_4]\)^{′}\), and \([\text{AgO}_6]\)^{′} transfer an electron to the oxygen molecule \((\text{O}_2)\), which produces \(\text{O}_2^\prime\) that interacts with the proton and forms the \(\text{O}_2\text{H}^\ast\) radical. Figures 6a, 6b, and 6c illustrate the formation of these radicals.

According to the Wulff crystal representation of the optimized \(\beta\)-\text{Ag}_2\text{MoO}_4\) (Figure 4), and the FE-SEM images presented in Figure 3, when \(\beta\)-\text{Ag}_2\text{MoO}_4\) is synthetized using water, ammonia, and ethanol as the solvents, the morphologies 1, 2, and 3, respectively, are preferably obtained. This change of morphology is accompanied by the appearance of the \((001)\) surface. When water is used as the solvent, this face is not present. However, when ammonia is used as a solvent, the \((001)\) surface appears in the morphology. Finally, for the microcrystals synthesized in ethanol, this surface becomes more apparent. The results of the microbiological tests (MIC/MBC values and Figure 5) revealed that the antibacterial efficiency of the \(\beta\)-\text{Ag}_2\text{MoO}_4\) microcrystals varied with the solvent used in the synthesis, and increased in the following order: water < ammonia < ethanol. Because the appearance of the \((001)\) surface increased the antibacterial efficiency, we propose that the observed mechanism of \(\beta\)-\text{Ag}_2\text{MoO}_4\) for the inactivation of the MRSA and \(E.\) coli was mainly caused by the presence of the \([\text{AgO}_5\text{V}_0^x]\) complex clusters.

In the \([\text{AgO}_4\text{V}_0^x\text{V}_0^*]\) and \([\text{AgO}_3\text{V}_0^x\text{V}_0^x\text{V}_0^*]/[\text{AgO}_3\text{V}_0^x\text{V}_0^*\text{V}_0^*]\) clusters, internal electron-hole recombination can occur, while in the \([\text{AgO}_5\text{V}_0^x]\) complex clusters, the vacancy is separated from the electron because the electron is located in an \([\text{AgO}_6]\)^{′}\)organized complex cluster. This justifies the fact that the \([\text{AgO}_5\text{V}_0^x]\) complex clusters are more reactive and thus more effective for antibacterial applications.

Moreover, according to the Wulff crystal representation of optimized \(\beta\)-\text{Ag}_2\text{MoO}_4\), which is shown in Figure 4, these faces have low surface energy \((E_{\text{suf}} = 1.28\)
J/m²), as determined by the *ab initio* calculations. Therefore, they are more easily polarized, and able to generate OH∗, O₂’, and O₂H*, which are responsible for cell death.

**Conclusion**

We established a facile approach for the synthesis of β-Ag₂MoO₄ microcrystals with different morphology by means of the co-precipitation method and by using three different solvents (water, ammonia, and ethanol). All products exhibited powerful bactericidal capability against gram-positive bacteria, namely, MRSA, and gram-negative bacteria, namely, *E. coli*. The biocidal power increased in the following order: water < ammonia < ethanol. The relationship between the morphology of the different β-Ag₂MoO₄ microcrystals synthesized with different solvents, and their biological effect, was constructed systematically by combining experimental techniques and first-principle calculations. The morphologies were calculated by the Wulff crystal constructions based on first principle calculations using three surfaces: (001), (111), and (011). It was concluded that, morphologically, the larger presence of the (001) surface enhanced the antibacterial activity against the MRSA and *E. coli* bacteria. We are firmly convinced that the present study opens up the possibility for extensive investigation, not only for antimicrobial activity, but also for other applications such as the photocatalytic activity of β-Ag₂MoO₄, which can be controlled by tuning their morphology. Additionally, we expect that the results and concepts presented in this work can be extrapolated to the morphology-controlled synthesis of other materials.

ACKNOWLEDGMENT
The authors are grateful to FAPESP (FAPESP-CDMF: 2013/07296-2 and FAPESP 2015/03654-7), CNPq (304190/2013-6) and CAPES (for financially supporting this research). J.A. acknowledges the following Spanish research funding institutions: PrometeoII/2014/022 and ACOMP/2015/1202 projects (Generalitat-Valenciana), Ministerio de Economía y Competitividad (CTQ2015-65207-P), Programa de Cooperación Científica con Iberoamérica (Brasil) of Ministerio de Educación (PHBP14-00020), and Ministerio de Economía y Competitividad, “Salvador Madariaga” program, PRX15/00261.

Supplementary Material

Experimental Methods

Synthesis and Characterizations

The samples were synthesized as described by Fabbro et al [28]. The samples were characterized using x-ray diffraction (XRD) with a D/Max-2500PC diffractometer (Rigaku, Japan) involving CuKα radiation (λ =1.54056 Å) in the 10-70° 20 range at a scan rate of 0.01° min⁻¹. X-ray photoelectron spectroscopy (XPS) analyses were obtained by Omicron-Scienta X-ray photoelectron spectrometer with Al Kα radiation. Micro-Raman spectroscopy was carried out by using an iHR550 spectrometer (Horiba Jobin-Yvon, Japan) with a charge-coupled device (CCD) detector and an argon-ion laser (MellesGriot, USA) operating at 633 nm with 200 mW power. The spectra were measured in the 50-1,500 cm⁻¹ range. The microscopy images were analyzed using field emission gun scanning electron microscopy (FEG-SEM) on an FEI instrument (Model Inspect F50) operating at 5 kV.
Computational Methods

The Vienna ab initio Simulation Package (VASP) [42–44] was used to perform the first-principle calculations. The Kohn-Sham equations were solved by using the generalized gradient approximation (GGA) in the Perdew-Burke-Ernzerhof (PBE) functional to determine the electron exchange and correlation contributions to the total energy [45,46]. The electron-ion interaction was described within a plane wave basis set by the projector augmented wave (PAW) method [47]. The (001), (011), and (111) surfaces of β-Ag₂MoO₄ were characterized by an unreconstructed slab model using a calculated equilibrium geometry (a = 9.454 Å, and u(O) = 0.2351) and a (3×3×1) Monkhorst-Pack special k-point grid. A vacuum spacer of 15 Å was introduced in the z-direction such that the surfaces would not interact with each other [33].

\[ E_{surf} = \frac{E_{slab} - nE_{bulk}}{A_{slab}} \]

\( E_{surf} \) is defined as the total energy per repeating cell of the slab (\( E_{slab} \)) minus the total energy of the perfect crystal per molecular unit (\( E_{bulk} \)) multiplied by the number of the molecular units of the surface (\( n \)), and divided by the surface area per repeating cell of the two slab sides. To confirm the convergence of the total energy with respect to the slab thickness of different surface models, \( E_{surf} \) was calculated for several low-index planes. The equilibrium shape of a crystal can be calculated by the classic Wulff construction [48], which minimizes the total surface free energy at a fixed volume, and provides a simple correlation between the surface energy (\( E_{surf} \)) of the (hkl) plane and its distance (\( rhkl \)) in the normal direction from the crystallite center.

Microbiological Tests
Microorganisms and culture conditions: Two reference strains from the American Type Culture Collection (ATCC) were used in this study to evaluate the antibacterial activity of the samples. Methicillin-resistant *Staphylococcus aureus* (MRSA – ATCC 33591) and *Escherichia coli* (*E. coli* – ATCC 8739) were maintained in a TSB medium (Acumedia Manufacturers, Inc. Baltimore, MD) and frozen at -80 °C until use. The strains were thawed and planted on Mueller Hinton Agar (MHA) plates (Acumedia Manufacturers, Inc. Baltimore, MD), which were kept in an oven at 37 °C for 24 h-48 h until isolated colonies were formed. The bacterial inoculum was prepared as described previously [49].

Antibacterial effects of β-Ag₂MoO₄ samples obtained in different solvents: Susceptibility tests were performed according to the Clinical and Laboratory Standards Institute broth microdilution method (document M7-A7-2006) for bacteria, with modifications. The bacteria were incubated to two-fold serial dilutions of each of the β-Ag₂MoO₄ solutions in 96-well microtiter plates for 48 h at 37 °C. The material concentrations ranged from 2.000 to 0.061 μg/mL. As a positive control, the microorganisms were incubated with TSB without the addition of microcrystals, while the negative control consisted of an uninoculated culture medium. After incubation, the MIC was determined by visual inspection, and the MBC was determined by inoculating 25 μL aliquots, taken from each 96-well serial dilution and inoculated onto the MHA plates. A CFU/mL count was performed after the plates incubated at 37º C for 24h-48h. Microbiological experiments were performed on three different occasions, in triplicate.

Results and discussion

Surface compositions (XPS)
XPS survey spectra (S1) were similar among the three samples tested, and demonstrated the presence of the elements Ag, Mo, O and C. In the three samples, the silver spectrum was similar, with the 3d5/2 peak around 368 eV and the 3d3/2 peak around 374 eV. The energy difference between the peaks is about 6 eV. The data found are in accordance with the literature [50–52].

S1: XPS survey spectra of the β-Ag2MoO4 microcrystals synthesized in water, ethanol and ammonia.

A summary of the data obtained by XPS analysis showed no significant differences between the three synthesized samples. The binding energy of all the samples were similar for the analyzed elements.
<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Ethanol</th>
<th>Ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>284.4 eV (C-C)</td>
<td>284.7 eV (C-C)</td>
<td>284.7 eV (C-C)</td>
</tr>
<tr>
<td></td>
<td>286.0 eV (C-(O,N))</td>
<td>286.1 eV (C-(O,N))</td>
<td>286.2 eV (C-(O,N))</td>
</tr>
<tr>
<td></td>
<td>287.9 eV (C-O-C;C=O)</td>
<td>288.2 eV (C-O-C;C=O)</td>
<td>288.5 eV (C-O-C;C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>530.1 eV (O-(Mo,Ag))</td>
<td>530.1 eV (O-(Mo,Ag))</td>
<td>530.1 eV (O-(Mo,Ag))</td>
</tr>
<tr>
<td></td>
<td>531.4 eV (C=O-OH)</td>
<td>531.4 eV (C=O-OH)</td>
<td>531.6 eV (C=O-OH)</td>
</tr>
<tr>
<td></td>
<td>532.9 eV (C-O)</td>
<td>532.9 eV (C-O)</td>
<td>532.8 eV (C-O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>533.9 eV (C-(OH,H,O))</td>
</tr>
<tr>
<td></td>
<td>367.9 eV (3d_{5/2})</td>
<td>367.8 eV (3d_{5/2})</td>
<td>367.9 eV (3d_{5/2})</td>
</tr>
<tr>
<td></td>
<td>373.8 eV (3d_{3/2})</td>
<td>373.7 eV (3d_{3/2})</td>
<td>373.8 eV (3d_{3/2})</td>
</tr>
<tr>
<td></td>
<td>231.9 eV (3d_{3/2})</td>
<td>231.8 eV (3d_{3/2})</td>
<td>231.8 eV (3d_{3/2})</td>
</tr>
<tr>
<td></td>
<td>234.9 eV (3d_{5/2})</td>
<td>234.8 eV (3d_{5/2})</td>
<td>234.8 eV (3d_{5/2})</td>
</tr>
</tbody>
</table>

**S2**: Summary of the XPS data of the $\beta$-Ag$_2$MoO$_4$ synthesized in water, ethanol and ammonia.

**References**


[10] C.H.B. Ng, W.Y. Fan, Uncovering Metastable $\alpha$-Ag2MoO4 Phase Under Ambient Conditions. Overcoming High Pressures by 2, 3-Bis (2-pyridyl)


https://doi.org/10.1111/febs.13959.


https://doi.org/10.1021/nn300934k.


https://doi.org/10.1103/PhysRevB.54.11169.
[44] G. Kresse, D. Joubert, From ultrasoft pseudopotentials to the projector

https://doi.org/10.1103/PhysRevLett.77.3865.

Singh, C. Fiolhais, Atoms, molecules, solids, and surfaces: Applications of the
generalized gradient approximation for exchange and correlation, Phys. Rev. B.


[48] G. Wulff, Xxv. zur frage der geschwindigkeit des wachstums und der auflösung

A.L. Machado, Tuning the Morphological, Optical, and Antimicrobial Properties
of α-Ag2WO4 Microcrystals Using Different Solvents, Cryst. Growth Des. 17

Oxygen functional groups and electrochemical capacitive behavior of
incompletely reduced graphene oxides as a thin-film electrode of supercapacitor,

(2014) 90.