Effect of ZnO nanoparticles addition to PEO coatings on AZ31B Mg alloy: antibacterial effect and corrosion behavior of coatings in Ringer’s physiological solution

Mahya Seyfi*, Arash Fattah-alhosseini†, Mohammadreza Pajohi-Alamotib and Elham Nikoomanzaric

*Department of Materials Engineering, Bu-Ali Sina University, Hamedan, Iran; †Department of Food Hygiene, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran

ABSTRACT
In this paper, the plasma electrolyte oxidation (PEO) technique was applied to modify the AZ31B Mg alloy surface. Effects of various concentrations of ZnO nanoparticles into an electrolyte with nanoparticles of hydroxyapatite (HAp) on the antibacterial and corrosion behavior of coatings were studied. Potentiodynamic polarization tests were done in Ringer’s electrolyte to study the coatings corrosion behavior. Results of XRD indicated that the provided PEO films mostly have phases of HAp, MgO, and Mg3(PO4)2 and ZnO. The results indicated that increasing the concentration of ZnO nanoparticles raised the thickness, roughness, and wetting angle and also enhanced the coatings antibacterial activity. The inhibition percentage of bacterial growth for the specimen with the highest concentration of nanoparticles (4 g/L) after 6 h against E. coli and S. aureus was 23.5% and 45.5%, respectively. The concentration of nanoparticles had no major effect on the porosity size of the ceramic coating. Moreover, adding ZnO nanoparticles declined the corrosion current density and raised the corrosion potential with regard to the metal substrate. The formed coating in the solution containing 1 g/L ZnO nanoparticles had the highest corrosion behavior among all of specimens that led to a 970 multiplication corrosion resistance of AZ31B Mg alloy.

1. Introduction
Mg and its alloys have been widely studied in the late decades because of various properties that make them great choices for biodegradable materials in medical uses [1]. The density of Mg is about 1.74 g/cm³ [2], Young’s modulus of Mg alloys (E = 41–45 GPa) is same as that of bones (E = 3–20 GPa) and can decline the effect of stress shielding in orthopedic uses [3]. In addition, Mg is vital to the metabolism of humans as a cofactor for many enzymes, and ions of Mg (Mg²⁺) are prominent in order to facilitate tissue-healing [4]. The most significant advantage is the Mg alloy is able to be adjusted to resorb after the bone-healing procedure. This could further decline the appeal to carry out a later surgery to delete it [5]. Mg alloys have been widely used in numerous usages like porous scaffolds for bone repair, bone fixation materials, and stents of cardiovascular [6]. In spite of the advantages, the week anti-corrosion properties of Mg and its alloys have restricted its extensive usage, particularly in corrosive media [7]. The corrosion of Mg alloys is too quick and divulges a high attitude for localized mechanisms. Consequently, a quick degeneration of mechanical integrity has been seen in Mg alloys and results in implant failure before tissue has cured. Another matter is the evolution of hydrogen and an alkaline pH shift within corrosion. As the evolution of hydrogen is too quick to absorb tissue, a gas balloon will be observed [8]. There are some solutions to boost the biocompatibility and corrosion behavior [9]:

(i) Optimizing the microstructure and composition like crystalline structure phase, grain size, and the base metal texture through development of manufacturing procedures/approaches.

(ii) In order to enhance the biocompatibility and the corrosion behavior of magnesium-based implants via protective coatings applied on Mg and its alloys.

There are many technologies accessible for coating magnesium and its alloys like PEO [10], anodizing [11], physical vapor deposition [12], chemical vapor deposition [13], laser surface alloying/cladding [14], electroless plating [15]. Among these techniques, PEO is one of the most protective coatings for magnesium alloys. This procedure is an anodic plasma-chemical surface process that are produced via making oxygen plasma in an alkaline electrolyte [16]. By PEO procedure, surfaces of crystalline ceramic can be produced on Mg alloys that show cool hardness and are proper for both: wear and corrosion resistance with no further
process [17]. In the PEO technique, many of the factors such as electrolyte composition [18], additive [19], electrolyte concentration [20], electrical parameters [21–23], temperature of the process [24], and composition of a substrate [25] affect the produced oxide structure. In this field, the electrolytes modification via additive including micro/nanoparticle to produce a micro/nanocomposite coating having specific properties is a good way.

Various particles including Al₂O₃ [26], MoS₂ [27], CeO₂ [28], SiO₂ [29], TiO₂ [30] to improve the corrosion behavior, of SiO₂ [29], Si₃N₄ [31] to improve abrasion behavior and nanoparticles of Ag [32], AgHAp [33], CuO [34,35] to improve the antibacterial performance of PEO coatings produced on Mg and its alloys have been utilized. Among the chemical composition of nanoparticles, HAp is a beneficial and safe option for implants as they are the principal component of human bones [36]. Researchers have shown the existence of HAp nanoparticles not only improves the bioactivity of PEO coatings produced on Mg alloys but also is very effective in boosting its corrosion behavior [36,37].

Zinc oxide (ZnO) has an antibacterial behavior which is made by rising the volume fraction of surface owing to its nanometric size [38]. Zn embedded into biomaterials must be able to inhibit bacteria but also show osteogenic activity [39]. Roknian et al. [40] found that the antibacterial properties of PEO coatings produced on pure titanium were highly raised. However, little effort has been made by researchers to adsorb ZnO nanoparticles into the oxide film produced by the PEO procedure on Mg and its alloys. Stojadinović and coauthors [41] indicated that by rising the concentration of ZnO nanoparticles (0–8 g/L), ceramic coating photoactivity increased remarkably. In other investigation, the influence of different concentration in the ZnO nanoparticles on the corrosion and bioactivity behaviors of MgO/ZnO nanocomposite coatings applied on AZ91 was studied by Bordbar-Khiabani et al. [42] The results indicated that by making an increase in the concentration of ZnO nanoparticles from 0 to 4.5 g/L, the corrosion resistance and bioactivity were augmented. Keyhani et al. [43] evaluated the influence of ZnO nanoparticle concentration (0 to 8 g/L) on the corrosion behavior of PEO coatings that were formed on AZ31B alloy in an alkaline solution. They observed that by increasing the concentration of ZnO nanoparticles from 0 to 6 g/L, the percentage of coating porosity was diminished and as a result, the corrosion resistance of the coating increased. On the other hand, by a further increase in the nanoparticles up to 8 g/L, the porosity of the coating increased and the corrosion resistance was decreased. In this study, a comprehensive investigation was carried out to evaluate the effect of various concentrations of ZnO nanoparticles on the corrosion behavior and also the microstructure of the coatings that were produced using the PEO procedure in the solution with nanoparticles of HAp and Na₃PO₄.12H₂O on AZ31B Mg alloy. In addition, the antibacterial effect and also wettability of the surfaces of coated specimens containing different concentrations of ZnO nanoparticles against S. aureus and E. coli were studied.

2. Experimental process

2.1. PEO process

A detailed description of the chemical composition of AZ31B Mg alloy, substrate preparation, and PEO process equipment can be found in previous publication [37]. In order to lead the coating procedure as the anode, specimens of Mg were immersed in the solution having distilled water, Na₃PO₄.12H₂O (provided by Merk co.), KOH (provided by Merk co.), and nanoparticles of HAp that were produced in our last research [37] and various concentration of ZnO nanoparticles (with the mean size of 25 nm, made by US research co.). The related data about the utilized solutions along with the specimens’ code are listed in Table 1. The cooling system was utilized to control the temperature during the coating procedure. For the PEO process, frequency, duty cycle, and current density were 1000 Hz, 50%, and 10 A/dm², respectively.

To measure the surface of HAp and ZnO nanoparticles charge for different pH values (7, 9, and 11), a Zetasizer nano model as a Zeta potential (ζ) device was utilized before the PEO procedure.

2.2. Surface characterization and antibacterial measurement

The composition and surface morphology of PEO coatings were studied using energy dispersive X-ray spectrometer (EDS), X-ray diffraction (XRD), and field emission scanning electron microscope (FESEM). Also, profilometry and contact angle instruments were respectively utilized to carry out surface roughness and wettability of the coatings. A detailed description of the EDS, XRD, FESEM, contact angle, and profilometry instruments can be found in previous publication [16]. It should be noted that the calculation of porosity

| Sample | Na₃PO₄.12H₂O (g/l) | KOH (g/l) | HA (g/l) | ZnO (g/l) | Conductivity (mS/cm²) |
|--------|-------------------|-----------|----------|-----------|----------------------|
| Z1     | 5                 | 3         | 5        | 1         | 11.49                |
| Z2     | 5                 | 3         | 5        | 2         | 11.53                |
| Z3     | 5                 | 3         | 5        | 3         | 11.56                |
| Z4     | 5                 | 3         | 5        | 4         | 11.59                |
size and thickness of coatings was carried out using MIP software.

The measurements of antibacterial were carried out against E. coli (ATCC 25,922) as pathogenic gram-negative bacteria and S. aureus (ATCC25923) as pathogenic gram-positive bacteria in order to evaluate the antibacterial activity of the prepared coatings in various concentrations of ZnO nanoparticles. A detailed description of the antibacterial measurement can be found in previous publication [16]. The inhibition of growth for each bacteria was calculated using Equation (1):

\[
\text{Growth inhibition} \% = \left[ \frac{N_0 - N_R}{N_0} \right] \times 100
\]

Where \( N_0 \) stands for the initial count (CFU/ml) of the inoculated bacteria and \( N_R \) stands for the count of the survived bacteria on the surface of specimen after various contact times [43].

### 2.3. Zn ion release of the coatings

A coupled plasma-mass spectrometer (ICP-MS, ELAN 6100 DRC-e) was utilized to test the Zn\(^{2+}\) release characteristics of the coatings. Before the test, the Zn-incorporated PEO coatings were incubated into 20 mL of Ringer’s electrolyte for various moments (2, 4, and 6 h). After these moments, the released Zn\(^{2+}\) in the distilled water was analyzed by ICP-MS.

### 2.4. Potentiodynamic polarization (PDP) measurements

In order to compare the corrosion resistance in the coated specimens with substrates, the tests of PDP in Ringer’s electrolyte having a composition of 0.86 g/L NaCl, 0.03 g/L KCl and 0.033 g/L CaCl\(_2\).2H\(_2\)O were utilized. The corrosion measurements were evaluated using a µAutolab Type III/FRA2 instrument using a three-electrode compartment, which is a saturated Ag/AgCl as a reference electrode, a Pt rod as a supporting electrode, and a specimen as a working electrode. Prior to carrying out the measurement, the specimens were being immersed in the Ringer’s electrolyte for 1 week under the condition of the open-circuit potential (OCP) in order to reach the condition of steady-state. Thus, after 1 week, the PDP measurements were conducted from −250 mV compared to the OCP with a scan rate of 1 mV/s.

### 3. Results and discussion

#### 3.1. Voltage-time plots

Figure 1 shows the experimentally PEO voltage-time response plots obtained for electrolytes having distinct concentrations of ZnO nanoparticles. Based on the time of the process, the PEO voltage-time response plots consist of three different zones of anodic oxidation, micro-discharge oxidation, and micro-arc oxidation [44]. As can be observed in Figure 1, the slope of the voltage-time diagram in the first step of the coating procedure is almost the same, and the breakdown voltage of specimen Z1, Z2, Z3, and Z4 is almost equal to 299, 306, 308, and 312 V. Based on the model of Ikonopisov, the relationship between the electrical conductivity of an electrolyte (k) and voltage and can be proposed as Equation (2) [45]:

\[
V_b = a_b + b_\log(1/k)
\]

Where \( V_b \) is the voltage of breakdown, \( a_b \) and \( b_\) are, respectively, the constant values for substrate and composition of electrolyte [45]. As can be observed in Table 1, the electrical conductivity increases via a very small amount by increasing the concentration of ZnO nanoparticles, so a very small decrease in the breakdown voltage due to increasing the concentration of ZnO nanoparticles can be attributed to increased

![Figure 1](image-url)
3.2. Characterization of the coatings surfaces

Figure 2 indicates the morphology of ceramic coatings produced by means of the PEO procedure at distinct concentrations of ZnO nanoparticles at two magnifications of 100X and 1000X. The porosity was observed on the surface of all oxide coatings. By producing a dielectric breakdown phenomenon, in the existence of an electric field between the cathode and the anode, the anionic components within the solution move toward the anode and come into the discharge channels. Simultaneously, the substrate metal oxidizes under existing status (local pressure and high temperature) after melting and entering the channel of discharge. The oxidized material then exits the channels of discharge and settles on the surface. As a result of the release of gases produced through the discharge channels, porosities are produced [48]. As can be seen in Figure 2, no remarkable changes in the microstructure of the coatings are seen at high magnification (1000X) owing to the rise in the concentration of ZnO nanoparticles. The size of the pores in coatings produced at concentrations of 1, 2, 3, and 4 g/L ZnO nanoparticles are 7, 6.6, 6.7, and 6.6 μm, respectively. Thus, adding distinct concentrations of ZnO nanoparticles did not make a major change in the size of surface porosity. The reason for this might be because of the very small change in voltage of breakdown and the final voltage because of adding nanoparticles by distinct concentrations. Nevertheless, the microstructure of the coatings gradually varies at 100X magnification. In addition to crater-like porosity, the nodular structure has been seen in the surface morphology of specimens with nanoparticles of 3 and 4 g/L ZnO.

Figure 3 shows the cross-sectional images of formed coatings at concentrations of 1, 2, 3, and 4 g/L ZnO nanoparticles. There is a good relation between all the coatings and the substrate. The average final thickness for Z1 and Z2 specimens was 18.04 and 18.22 μm, respectively. This shows that the addition of 1 g/L ZnO nanoparticles does not have a major effect on rising the thickness of the coating. While by making an increase the concentration of nanoparticles, the thickness of the coating will increase. The mean final thickness for the samples of Z3 and Z4 was 26.31 and 26.73 μm, respectively. The reason for this increase in thickness in the samples of Z3 and Z4 can also be ascribed to the behavior related to the diagram of voltage-time. As can be observed in the diagram of voltage-time, the curve of voltage-time for the samples of Z3 and Z4 comes into the third step of micro-arc oxidation much faster than the diagrams for the Z1 and Z2 samples. Thus, the longer the third coating step for specimens with 3 and 4 g/L ZnO, in which high-intensity microorganisms are produced, might be effective in rising the thickness of the coatings. In addition, in the last part, it was seen that the diagram of voltage-time for the specimen containing 3 and 4 g shifted upward in comparison to the diagram of the specimen containing 2 g/L nanoparticles of ZnO. Making an increase in the voltage of the coating refers to creating more heat and energy in the discharge channels. More proper conditions are produced for reactions of chemical and electrochemical and more products are deposited on the surface owing to the more energy and heat input and this can leads to an increase in the growth rate of the coating.

3.3. Phase and elemental analyses of PEO coatings

Figure 4 indicates the XRD pattern for produced coatings at various concentrations of ZnO nanoparticles. In the diffraction pattern of all specimens, peaks related to the Mg phase are seen, the reason for which can be ascribed to the porous nature of the coatings. In addition, phases of MgO and Mg₃(PO₄)₂ were also seen in the XRD pattern of all specimens. These two phases were produced within the PEO procedure using the following reactions on the substrate of AZ31B [49,50].

\[ \text{Mg} \rightarrow \text{Mg}^{2+} + 2e^- \]  
\[ \text{Mg}^{2+} + 2\text{OH}^- \rightarrow \text{Mg(OH)}_2 \]  
\[ \text{Mg(OH)}_2 \rightarrow \text{MgO} + \text{H}_2\text{O} \]  
\[ 3\text{Mg}^{2+} + 2\text{PO}_4^{3-} \rightarrow \text{Mg}_3\text{(PO}_4)_2 \]
Peaks of ZnO were not seen in the XRD pattern of specimens Z1, Z2, and Z3. This might be owing to the low concentration of nanoparticles within these specimens. However, in the Z4 specimen, the peak related to the phase of ZnO was recognized in the XRD pattern. In addition, the HAp phase was present in the diffraction pattern of all specimens that was owing to the existence of HAp nanoparticles in the solution. The existence of HAp and ZnO phases shows the neutral entry of nanoparticles into the coating. This means that participated nanoparticles of HAp and ZnO in the production of the coating with no production of a new phase and could not melt under the effect of high discharge energy and then reacted with molten MgO in order to produce a new phase. Stojadinović [41], Bordbar-Khiabani [42], and Keyvani [43,51] reported the inert entry of ZnO particles into the PEO oxide coating.

Table 2 provides the results of EDS analysis for coatings that have various concentrations of ZnO nanoparticles, indicating that the coatings are mostly consisted of magnesium, phosphorus, calcium, oxygen, and zinc.
Magnesium is the major element in the composition of the coatings derived from the substrate, whereas the existence of P and Ca shows the major role of HAp nanoparticles in the formation of the coating. The zinc fraction in the specimens increases relatively with the concentration ZnO nanoparticles in the solution. By rising the concentration in nanoparticles of ZnO from 1 to 4 g/L, the amount of Zn in the oxide coating increased from 0.3% wt. to 1.13% wt.

Analysis of element distribution maps was used to investigate the distribution of elements at the cross-sectional area of the coating. Figure 5 reveals the distribution of the main constituent elements in the cross-sectional area of the coating that contains 4 g/L nanoparticles of ZnO (specimen Z4). The main elements of ceramic coatings are O, Zn, P, Ca as well as Mg. The sources of Ca, P, and Zn are the solutions that contain nanoparticles of HAp, sodium phosphate salt, and nanoparticles of ZnO, respectively. Of course, it should

| Sample | Zn (at. %) | Ca (at. %) | P (at. %) | O (at. %) | Mg (at. %) |
|--------|-----------|------------|-----------|-----------|------------|
| Z1     | 0.3       | 2.47       | 4.82      | 59.77     | 32.64      |
| Z2     | 0.4       | 2.43       | 4.82      | 59.76     | 32.59      |
| Z3     | 0.7       | 1.59       | 3.57      | 61.13     | 33.05      |
| Z4     | 1.13      | 3.54       | 5.96      | 56.96     | 32.41      |
be noticed that in the chemical composition of the substrate 0.96 wt. % the element of Zn can be seen. For nanoparticles to be entered into the coating, they need to move to the interface of the coating/electrolyte. Electrophoretic forces and mechanical mixing are able to help distribute the particles in the interface of the coating/substrate. Figure 6 indicates the zeta potential of HAp and ZnO nanoparticles at various values of pHs. The pH range of the solution is between 11 and 12, so the zeta potential of both HAp and ZnO nanoparticles in the alkaline electrolyte (pH> 7) is negative, so the surface of both nanoparticles is negatively charged as soon as it comes into the electrolyte and then there is a strong electric field between the anode and the cathode of the procedure, it causes the nanoparticles to be driven to the positive pole (substrate). Mechanical mixing speed is also one of the effective factors in the distribution of particles in the joint of the substrate/coating. Asgari et al. [52] concluded that at low rates of mechanical mixing, the electrolyte stirring power is not sufficient for particles to come into the coating, while the high rate of electrolyte stirring causes the particles to move away from the surface of the substrate due to high turbulence. It should be noticed that the electrolyte stirring rate was the same for all coatings and it was 100 rpm. Figure 5(a) shows that the distribution of oxygen in the coating is increased in comparison to the substrate. The reason for this can be ascribed to the production of ceramic coatings through the oxidation procedure. The results of element distribution for Zn also show that more nanoparticles are distributed in the middle part of the coating. The EDS line scan analysis (Figure 5(b)) was used in order to have a more accurate investigation of the distribution of elements.

Based on the linear analysis of the cross-section, the content of Mg declined by moving from the substrate to the surface of a coating. The existence of magnesium and oxygen in the vicinity of the surface of the substrate gave an increase to the phase of MgO. The content of Zn was initially increased and then decreased. This is completely consistent with the observed results in the element distribution map. Necula et al. [53] reported that particles can be handed over the sites of coating growth via distinct pathways of transport (e.g., cracks, short-circuit channels, and open pores), while other particles might be deflected back by some heat waves, shock waves, and/or gas bubbles produced within spark discharge. At the locations of coating growth, with the local heating produced by sparking events, the newly produced coating can be quite soft and particles might get entrapped and be molten into it as the spark extinguishes and the site is cooled by the solution. Thus, particles are embedded in the oxide film as it is growing.

A few researchers have seen a high fraction of nanoparticles just on the outer part of the porous film, noting that at the last times the size of the porosity increases that raises the entry of nanoparticles on the outer part of the porous film [54,55]. However, in
3.4. Wettability

The most important feature of an implant that affects the biological system is the wetting of its surface. An implant having a hydrophilic surface (low contact angle) in contact with blood and biological electrolyte can raise uptake of protein and cellular response and better integrate with hard and soft tissue in addition to accelerating the heal surrounding wounds and osseointegration.

Figure 7(a) shows taken images of water droplets sprayed on the substrate and formed coatings at various concentrations of ZnO nanoparticles. The contact angle of distilled water with the substrate is about 64 degrees. Z1 specimen shows a contact angle of 56 degrees that shows the hydrophilicity of the coating. In addition, the contact angle increased and the wetting of the coating decreased by rising concentration of nanoparticles. The Z4 sample has the highest contact angle of about 90 degrees in comparison to all other samples. Parameters affecting the wettability of surfaces include roughness of the surface and chemical composition. Figure 7(b) shows the relation between the roughness and the wettability angle of the samples. As can be seen, the amount of roughness and wetting angle of both ones augmented with rising the concentration of nanoparticles from 1 to 4 g/L. The reason for this might be ascribed to the entrapment of air in the porosity of rougher surfaces that inhibits water from producing a hydroxyl bond with the surface [57–59].

3.5. Antibacterial performance

Figure 8 indicates the comparing of the capability of the specimens to control the bacteria after 6 h. As can be seen in Figure 8, the number of colonies declined by creating a ceramic coating with nanoparticles of ZnO in comparison to the control sample showing that the nanocomposite coating containing nanoparticles of ZnO reduces the antibacterial effect of the ceramic coating. For
Figure 7. (b) Pictures of wettability measurement for substrates and oxide coatings, (b) Correlation between wettability angle and roughness.

Figure 8. Pictures from antibacterial testing for coated substrates.
In a quantitative study, the percentage of inhibition in the bacterial growth for each specimen at intervals 2, 4, and 6 h was calculated and reported in the bar chart in Figure 9. According to the results, increasing the concentration of ZnO from 1 to 4 g/L during 2 h exposing the specimens with the bacteria had no effect on the percentage of growth inhibition of E. coli, as for S. aureus the percentage of growth inhibition was 7.9%, reached 16.5% (Figure 9).

In addition, Figure 9 indicates that the percentage of inhibition of bacterial growth increased by increasing the concentration of nanoparticles from 1 to 4 g/L and increasing the exposing time from 2 to 6 h so that the Z4 specimen had the highest growth inhibition percentage at 6 h for S. aureus (45%) and E. coli (23.5%). As can be seen in Figure 9, in comparison to E. coli (gram-negative bacteria), S. aureus (gram-positive bacteria) seem more susceptible. Higher susceptibility of gram-positive bacteria can be highly related to the differences in the structure of metabolism, degree of contact, cell physiology, or cell wall [60]. Chemical composition is widely accepted that to play a pivotal role in the antibacterial activity of coatings [61]. It is crystal clear that MgO and ZnO can play a major part in bacterial eradication [62, 63]. It has been concluded that Zn-embedded coatings can decline bacterial adhesion and prevent bacterial growth by producing reactive oxygen species (ROS) in light or even dark condition and releasing a high concentration of zinc ions [64]. It should be noticed that the content of Zn2+ ion release was tested for 2, 4, and 6 h and no release of Zn2+ ion was seen for all specimens, so the antibacterial mechanism of the coatings does not consider as release-killing.

Thus, one of the most probable antibacterial mechanisms of ZnO embedded coatings is the production of ROS (such as .OH, H2O2 and O2−) that is harmful to bacteria. ROS attacks the cell membrane, and the coenzyme A on the cell membrane is hurt, resulting in inhibition of respiration dependent on the intact cell membrane, reduction or loss of cellular respiration activity, and eventually cause the control of the bacterial growth [65]. It was observed that the content of Zn in the ceramic coating increased by raising the concentration of ZnO nanoparticles within the electrolyte in Section 3.3. This can also raise the possibility of ROS formation on the coating. Thus, the antibacterial property of the coatings also increased by raising the concentration of nanoparticles. In addition to ROS, the mechanism of

![Figure 9](image-url)
contact bacterial properties of coatings.

3.6. Assessing corrosion performance

PDP measurement was used in order to find out the effect arising from the concentration of ZnO nanoparticles on the corrosion behavior of ceramic coatings produced by the PEO procedure. The graph of the PDP measurement is indicated in Figure 10. The same electrochemical behavior is also seen for all of the four coatings. As can be seen in Figure 10, the PDP curve of all coatings shifts to a more noble potential as well as less corrosion current density, showing an enhancement in the corrosion behavior of the coatings in comparison to AZ31B Mg alloy. Some electrochemical data such as corrosion potential and corrosion current density are obtained from the PDP curves and are listed in Table 3. The Stern-Geary equation can be used in order to calculate the content of polarization resistance [66]:

\[ R_p = \frac{\beta_a \beta_c}{2.3(\beta_a + \beta_c)I_{corr}} \]  

(7)

In Equation (7), \( \beta_a \) and \( \beta_c \) are called the slope of the anodic and cathodic branches and \( R_p \) polarization resistance, respectively. The percentage of porosity in the coating \( (P) \) can be obtained via substituting the data that was obtained from the PDP plot in Equation (8) [67]:

\[ P = \left( \frac{R_{ps}}{R_p} \right) \times 10^{-\frac{\Delta E_{corr}}{0.057}} \times 100 \]  

(8)

In Equation (8), \( R_{ps} \) and \( R_p \) show the corrosion resistance of the substrate and the coating, \( \beta_a \) is the slope of the anodic branch of the substrate and \( \Delta E_{corr} \) shows the corrosion potential difference between the substrate and the coating, respectively. According to the obtained results, it was found out that sample of Z1 has the least corrosion current density (8.2 nA/cm²) and thus has the highest corrosion resistance (3.59 MΩ.cm²) and sample of Z2 has the highest corrosion current density (25.49 nA/cm²) and as a result has the least corrosion resistance (0.67 MΩ.cm²). Porosity is one of the most important defects in coatings that plays a crucial role in the corrosion resistance of coatings, as the corrosive electrolyte can penetrate into the coating through these porosities and eventually reach the substrate by destroying the coating [68]. As can be seen in Table 3, the calculated porosity percentage is shown using Equation (8). As can be seen, the sample of Z1 has the lowest percentage of porosity and so the best corrosion behavior. The addition of 2 g/L nanoparticles of ZnO resulted in a coating with the highest percentage of porosity. In addition, it can be observed that by increasing the concentration of nanoparticles from 2 to 4 g/L, the percentage of coating porosity declined and as a result, polarization resistance increased. This reveals that the addition of nanoparticles using concentrations above 2 g/L results in a denser coating.

One of the most effective parameters in the corrosion resistance of coatings is the wetting angle. Researchers have proved that these two parameters are highly related to each other and raise the corrosion resistance of the coating by rising wetting angle [69]. However, such a trend was not seen in this study. Although the sample of Z1 had the least wetting

| Table 3. Extracted electrochemical data from the potentiodynamic polarization curve of substrates and the produced coatings at different concentrations of ZnO nanoparticles. |
|---|---|---|---|---|---|---|
| Sample | \( E_{corr} \) \( (\text{V}_{Ag/AgCl}) \) | \( i_{corr} \) \( (\text{nA/cm}^2) \) | \( \beta_a \) \( (\text{mV/dec}) \) | \( \beta_c \) \( (\text{mV/dec}) \) | \( R_p \) \( (\text{MΩ.cm}^2) \) | \( P \) \( (\%) \) |
| Substrate | -1.215 | 26,500 | 98.37 | 79.30 | 0.0037 | - |
| Z1 | -0.331 | 8.20 | 136.6 | 237.98 | 3.59 | 0.10 |
| Z2 | -0.327 | 25.42 | 84.77 | 74.78 | 0.67 | 0.59 |
| Z3 | -0.378 | 19.71 | 103.00 | 155.66 | 1.26 | 0.29 |
| Z4 | -0.303 | 10.35 | 118.07 | 108.77 | 2.97 | 0.12 |

Figure 10. PDP curves for substrates and coated specimens at different concentrations of ZnO.
angle, it indicated the highest corrosion resistance to the corrosive solution of Ringer. The reason for the better corrosion behavior in the sample of Z1 can be ascribed to the least percentage of porosity and surface roughness among the specimens.

4. Conclusions

In this study, the influence of concentration of ZnO nanoparticles as an additive on properties such as microstructure, antibacterial activity, and corrosion behavior of coatings that were formed on AZ31B Mg alloy was studied. Although rising the concentration of ZnO nanoparticles resulted in a very small rise in the electrical conductivity of the solution and a very small decrease in the breakdown voltage of the specimens, but it influenced the behavior of the voltage-time diagram in the second and third stages of coatings. When the concentration of oxide nanoparticles increased, the thickness of the specimens also increased, but no significant difference was seen in the surface microstructure of the samples. XRD results also indicated that the coating consisted of MgO, Mg3(PO4)2, HA, and ZnO phases. As the concentration of nanoparticles increased, the amount of wetting angle, roughness, and also weight percentage of Zn increased. Studies on the antibacterial properties of coatings revealed that increasing the concentration of ZnO nanoparticles increased the antibacterial activity of the samples. The sample containing 4 g/L ZnO nanoparticles had the best antibacterial activity in 6 h and caused a 23.5% decrease in the growth of E. coli and a 45% decrease in the growth of S. aureus. Studying the effect of ZnO nanoparticle concentration on the corrosion behavior of coatings indicated that the sample containing 1 g/L nanoparticle of ZnO had the least porosity and so the least corrosion current density and the best corrosion resistance that can raise the corrosion resistance of the substrate to 970 times more than before.

Disclosure of potential conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID

Arash Fattah-alhosseini http://orcid.org/0000-0001-9560-0049

References

[1] Sanchez AHM, Luthringer BJC, Feyerabend F, et al. Mg and Mg alloys: how comparable are in vitro and in vivo corrosion rates? A review. Acta Biomater. 2015;13:16–31.

[2] Staiger MP, Pietak AM, Huadmaj J, et al. Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials. 2006;27(9):1728–1734.

[3] Wu G, Ibrahim JM, Chu PK. Surface design of biodegradable magnesium alloys — a review, Surf. Coatings Technol. 2013;233:2–12.

[4] Chakraborty Banerjee P, Al-Saadi S, Choudhary L, et al. Magnesium Implants: prospects and Challenges. Materials (Basel). 2019;12(1):136.

[5] Xi Z, Wu Y, Xiang S, et al. Corrosion resistance and biocompatibility assessment of a biodegradable hydrothermal-Coated Mg–Zn–Ca alloy: an in vitro and in vivo study. ACS Omega. 2020;5(9):4548–4557.

[6] Zhang L, Zhang J, Chen C, et al. Advances in microarc oxidation coated AZ31 Mg alloys for biomedical applications. Corros Sci. 2015;91:7–28.

[7] Fattah-alhosseini A, Sabaghi Joni M. Effect of KOH concentration on the microstructure and electrochemical properties of MAO coated Mg alloy AZ31B. J. Materi. Eng. Perform. 2015;24(9):3444–3452.

[8] Gu X-N, Li -S-S, Li X-M, et al. Magnesium based degradable biomaterials: a review. Front. Mater. Sci. 2014;8(3):200–218.

[9] Agarwal S, Curtin D, Duffy B, et al. Biodegradable magnesium alloys for orthopaedic applications: a review on corrosion, biocompatibility and surface modifications. Mater Sci Eng C. 2016;68:948–963.

[10] Fattah-alhosseini A, Chaharmahali R, Babaei K. Effect of particles addition to solution of plasma electrolytic oxidation (PEO) on the properties of PEO coatings formed on magnesium and its alloys: a review. J. Magnes. Alloy. 2020;8(3):799–818.

[11] Guo X, An M, Yang P, et al. Effects of benzoatriazole on anodized film formed on AZ31B magnesium alloy in environmental-friendly electrolyte. J Alloys Compd. 2009;482(1–2):487–497.

[12] Durdu S. Characterization, bioactivity and antibacterial properties of copper-Based TiO2 bioceramic coatings fabricated on titanium. Coatings. 2018;9(1):1.

[13] Ishizaki T, Hieda J, Saito N, et al. Corrosion resistance and chemical stability of super-hydrophobic film deposited on magnesium alloy AZ31 by microwave plasma-enhanced chemical vapor deposition. Electrochim Acta. 2010;55(23):7094–7101.

[14] Liu F, Ji Y, Sun Z, et al. Enhancing corrosion resistance of Al-Cu/AZ31 composites synthesized by a laser cladding and FSP hybrid method. Mater Manuf Process. 2019;34(13):1458–1466.

[15] Zhao H, Huang Z, Cui J. A new method for electroless Ni–P plating on AZ31 magnesium alloy. Surf Coat Technol. 2007;202(1):133–139.

[16] Toorani M, Aliofkhazraei M, Roughdasm A. Microstructural, protective, inhibitory and semiconducting properties of PEO coatings containing CeO2 nanoparticles formed on AZ31 Mg alloy. Surf Coat Technol. 2018;352:561–580.

[17] Molaee M, Fattah-alhosseini A, Babaei K. Improving the wear resistance of plasma electrolytic oxidation (PEO) coatings applied on Mg and its alloys under the addition of nano- and micro-sized additives into the electrolytes: a review. J. Magnes. Alloy. 2021. DOI:10.1016/j.jma.2020.11.016.

[18] Ghasemi A, Raja VS, Blawert C, et al. The role of anions in the formation and corrosion resistance of the plasma electrolytic oxidation coatings, Surf. Coatings Technol. 2010;204(9–10):1469–1478.
[19] Fattah-alhosseini A, Chaharmahali R. Enhancing corrosion and wear performance of PEO coatings on Mg alloys using graphene and graphene oxide additions: a review. Flat Chem. 2021;27:100241.

[20] Attarzadeh N, Kazemi A, Molaei M, et al. Multipurpose surface modification of PEO coatings using tricalcium phosphate addition to improve the bedding for apatite compounds. J Alloys Compd. 2021;877:160275.

[21] Heydarian A, Atapour M, Hakimizad A, et al. The effects of anodic amplitude and waveform of applied voltage on characterization and corrosion performance of the coatings grown by plasma electrolytic oxidation on AZ91 Mg alloy from an aluminate bath. Surf Coat Technol. 2020;383:125235.

[22] Zhuang JJ, Song RG, Xiang N, et al. Effect of current density on microstructure and properties of PEO ceramic coatings on magnesium alloy. Surf Eng. 2017;33(10):744–752.

[23] Su P, Wu X, Guo Y, et al. Effects of cathode current density on structure and corrosion resistance of plasma electrolytic oxidation coatings formed on ZK60 Mg alloy. J Alloys Compd. 2009;475(1–2):773–777.

[24] Al Bosta MMS, Ma K-J. Influence of electrolyte temperature on properties and infrared emissivity of MAO ceramic coating on 6061 aluminum alloy. Infrared Phys Technol. 2014;67:63–72.

[25] Cakmak E, Tekin KC, Malayoglu U, et al. The effect of substrate composition on the electrochemical and mechanical properties of PEO coatings on Mg alloys. Surf Coat Technol. 2010;204(8):1305–1313.

[26] Wang Y, Wei D, Yu J, et al. Effects of AI2O3 Nano-adhesive on performance of Micro-arc oxidation coatings formed on AZ91D Mg alloy. J Mater Sci Technol. 2014;30(10):984–990.

[27] Lou B-S, Lee J-W, Tseng C-M, et al. Mechanical properties and corrosion resistance evaluation of AZ31 magnesium alloys by plasma electrolytic oxidation treatment: effect of MoS2 particle addition. Surf Coat Technol. 2018;350:813–822.

[28] Ur Rehman Z, Uzair M, Lim HT, et al. Structural and electrochemical properties of the catalytic CeO2 nanoparticles-based PEO ceramic coatings on AZ91 Mg alloy. J Alloys Compd. 2017;726:284–294.

[29] Lu X, Blawert C, Huang Y, et al. Plasma electrolytic oxidation coatings on Mg alloy with addition of SiO2 particles. Electrochim Acta. 2016;187:20–33.

[30] Liang J, Hu L, Hao J. Preparation and characterization of oxide films containing crystalline TiO2 on magnesium alloy by plasma electrolytic oxidation. Electrochim Acta. 2007;52(14):4836–4840.

[31] Lou B-S, Lin -Y-Y, Tseng C-M, et al. Plasma electrolytic oxidation coatings on AZ31 magnesium alloys with Si3N4 nanoparticle additives. Surf Coat Technol. 2017;332:358–367.

[32] Necula BS, Fratila-Apachitei LE, Berkani A, et al. Enrichment of anodic MgO layers with Ag nanoparticles for biomedical applications. J Mater Sci Mater Med. 2009;20(1):339–345.

[33] Yazici M, Gulec AE, Gurbuz M, et al. Biodegradability and antibacterial properties of MAO coatings formed on Mg-Sr-Ca alloys in an electrolyte containing Ag doped hydroyapatite. Thin Solid Films. 2017;644:92–98.

[34] Zhang D, Han Q, Yu K, et al. Antibacterial activities against Porphyromonas gingivalis and biological characteristics of copper-bearing PEO coatings on magnesium. J Mater Sci Technol. 2021;61:33–45.

[35] Chen J, Zhang Y, Ibrahim M, et al. In vitro degradation and antibacterial property of a copper-containing micro-arc oxidation coating on Mg-2Zn-1Gd-0.5Sr alloy. Colloids Surf B Biointerfaces. 2019;179:77–86.

[36] Yang J, Lu X, Blawert C, et al. Microstructure and corrosion behavior of Ca/P coatings prepared on magnesium by plasma electrolytic oxidation. Surf Coat Technol. 2017;319:359–369.

[37] Chaharmahali R, Fattah-alhosseini A, Esfahani H. Increasing the in-vitro corrosion resistance of AZ31B-Mg alloy via coating with hydroxyapatite using plasma electrolytic oxidation. J Asian Ceram Soc. 2020;8(1):39–49.

[38] Xie Y, He Y, Irwin PL, et al. Antibacterial activity and mechanism of action of Zinc oxide nanoparticles against Campylobacter jejuni. Appl Environ Microbiol. 2011;77(7):2325–2331.

[39] Zhang R, Liu X, Xiong Z, et al. Novel micro/nanstructured TiO2/ZnO coating with antibacterial capacity and cytocompatibility. Ceram Int. 2018;44(8):9711–9719.

[40] Rokni M, Fattah-alhosseini A, Gashiti SO, et al. Study of the effect of ZnO nanoparticles addition to PEO coatings on pure titanium substrate: microstructural analysis, antibacterial effect and corrosion behavior of coatings in Ringer’s physiological solution. J. Alloys Compd. 2018;740:330–345.

[41] Stojadinović S, Tadić N, Radić N, et al. MgO/ZnO coatings formed on magnesium alloy AZ31 by plasma electrolytic oxidation: structural, photoluminescence and photocatalytic investigation. Surf Coat Technol. 2017;310:98–105.

[42] Bordbar-Khiabani A, Yarmand B, Mozafari M. Enhanced corrosion resistance and in-vitro biodegradation of plasma electrolytic oxidation coatings prepared on AZ91 Mg alloy using ZnO nanoparticles-incorporated electrolyte. Surf Coat Technol. 2019;360:153–171.

[43] Keyvani A, Zamani M, Fattah-Alhosseini A, et al. Microstructure and corrosion resistance of MAO coatings on AZ31 magnesium. Mater Res Express. 2018;5(8):86510.

[44] Laleh M, Roughagham AS, Shahrami T, et al. Effect of alumina sol addition to micro-arc oxidation electrolyte on the properties of MAO coatings formed on magnesium alloy AZ91D. J Alloys Compd. 2016;685:376–383.

[45] Fakhri Nabavi H, Aliofkhazraei M, Sabour Roughagham A. Morphology and corrosion resistance of hybrid plasma electrolytic oxidation on titanium. J. Alloys Compd. 2016;685:376–383.

[46] Aliofkhazraei M, Gharabaghi RS, Teimouri M, et al. Ceria embedded nanocomposite coating fabricated by plasma electrolytic oxidation on titanium. J. Alloys Compd. 2016;685:376–383.

[47] Duan H, Du K, Yan C, et al. Electrochemical corrosion behavior of composite coatings of sealed MAO film on magnesium alloy AZ91D. Electrochim Acta. 2006;51(14):2898–2908.

[48] Kaseem M, Fatimah S, Nashrah N, et al. Recent progress in surface modification of metals coated by plasma electrolytic oxidation: principle, structure, and
performance. Prog Mater Sci. 2020;100735. 
[50] Ma X, Blawert C, Höche D, et al. A model describing the growth of a PEO coating on AM50 Mg alloy under constant voltage mode. Electrochim Acta. 2017;251:461–474.
[51] Keyvani A, Zamani M, Bahamirian M, et al. Role of incorporation of ZnO nanoparticles on corrosion behavior of ceramic coatings developed on AZ31 Magnesium alloy by plasma electrolytic oxidation technique. Surf Interfaces. 2021;22:100728.
[52] Asgari M, Allofkhazraei M, Darband GB, et al. Evaluation of alumina nanoparticles concentration and stirring rate on wear and corrosion behavior of nanocomposite PEO coating on AZ31 magnesium alloy. Surf Coat Technol. 2017;309:124–135.
[53] Necula BS, Apachitei I, Tichelaar FD, et al. An electron microscopical study on the growth of TiO2–Ag antibacterial coatings on Ti6Al7Nb biomedical alloy. Acta Biomater. 2011;7(6):2751–2757.
[54] Lu X, Blawert C, Zheludkevich ML, et al. Insights into plasma electrolytic oxidation treatment with particle addition. Corros Sci. 2015;101:201–207.
[55] Bahramian A, Raaessi K, Hakimizad A. An investigation of the characteristics of Al2O3/TiO2 PEO nanocomposite coating. Appl Surf Sci. 2015;351:13–26.
[56] Shokouhfar M, Allahkaram SR. Formation mechanism and surface characterization of ceramic composite coatings on pure titanium prepared by micro-arc oxidation in electrolytes containing nanoparticles. Surf Coat Technol. 2016;291:396–405.
[57] Patel SB, Hamlekhan A, Royhman D, et al. Enhancing surface characteristics of Ti–6Al–4V for bio-implants using integrated anodization and thermal oxidation. J Mater Chem B. 2014;2(23):3597.
[58] Gökhan Demir A, Furlan V, Lecis N, et al. Laser surface structuring of AZ31 Mg alloy for controlled wettability. Biointerphases. 2014;9(2):29009.
[59] Braem A, Van Mellaert L, Mattheys T, et al. Staphylococcal biofilm growth on smooth and porous titanium coatings for biomedical applications. J Biomed Mater Res Part A. 2014;102(1):215–224.
[60] Hu H, Zhang W, Qiao Y, et al. Antibacterial activity and increased bone marrow stem cell functions of Zn-incorporated TiO2 coatings on titanium. Acta Biomater. 2012;8(2):904–915.
[61] Ghilini F, Pissinis DE, Mišnā A, et al. How functionalized surfaces can inhibit bacterial adhesion and viability. ACS Biomater Sci Eng. 2019;5(10):4920–4936.
[62] Leung YH, Ng AMC, Xu X, et al. Mechanisms of antibacterial activity of MgO: non-ROS mediated toxicity of MgO nanoparticles towards Escherichia coli. Small. 2014;10(6):1171–1183.
[63] Qi K, Cheng B, Yu J, et al. Review on the improvement of the photocatalytic and antibacterial activities of ZnO. J Alloys Compd. 2017;727:792–820.
[64] Leśniak-Ziółkowska K, Kazek-Kęsik A, Rokosz K, et al. Electrochemical modification of the Ti–15Mo alloy surface in solutions containing ZnO and Zn3(PO4)2 particles. Mater Sci Eng C. 2020;115:111098.
[65] Deng Y, Li Z, Tang R, et al. What will happen when microorganisms “meet” photocatalysts and photocatalysis? Environ Sci Nano. 2020;7(3):702–723.
[66] Stern M, Geabry AL. Electrochemical Polarization. J Electrochem Soc. 1957;104(1):56.
[67] Gu Y, Bandopadhyay S, Chen C, et al. Effect of oxidation time on the corrosion behavior of micro-arc oxidation produced AZ31 magnesium alloys in simulated body fluid. J Alloys Compd. 2012;543:109–117.
[68] Chaharmahali R, Fattah-alhossein A, Babaei K. Surface characterization and corrosion behavior of calcium phosphate (Ca-P) base composite layer on Mg and its alloys using plasma electrolytic oxidation (PEO): a review. J. Magnes. Alloy. 2021;9(1):21–40.
[69] Gnedenkov SV, Sinebryukhov SL, Egorkin VS, et al. Wetting and electrochemical properties of hydrophobic and superhydrophobic coatings on titanium, colloids surfaces a physicochem. Eng. Asp. 2011;383(1–3):61–66.