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Original Research

# Influence of HEPES buffer on the local pH and formation of surface layer during *in vitro* degradation tests of magnesium in DMEM

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**Abstract**

The human body is a buffered environment where pH is effectively maintained. HEPES is a biological buffer often used to mimic the buffering activity of the body in *in vitro* studies on the degradation behavior of magnesium. However, the influence of HEPES on the degradation behavior of magnesium in the DMEM pseudo-physiological solution has not yet been determined. The research aimed at elucidating the degradation mechanisms of magnesium in DMEM with and without HEPES. The morphologies and compositions of surface layers formed during *in vitro* degradation tests for 15–3600 s were characterized. The effect of HEPES on the electrochemical behavior and corrosion tendency was determined by performing electrochemical tests. HEPES indeed retained the local pH, leading to intense intergranular/interparticle corrosion of magnesium made from powder and an increased degradation rate. This was attributed to an interconnected network of cracks formed at the original powder particle boundaries and grain boundaries in the surface layer, which provided pathways for the corrosive medium to interact continuously with the internal surfaces and promoted further dissolution. Surface analysis revealed significantly reduced amounts of precipitated calcium phosphates due to the buffering activity of HEPES so that magnesium became less well protected in the buffered environment.


*Keywords:* Magnesium; Intergranular corrosion; HEPES; DMEM; Local pH

## 1. Introduction

Biodegradable ceramics or polymers are not really suitable implant materials for applications at the load-bearing sites of the human body, because of a lack of sufficient mechanical properties [1,2], and therefore they are often used to fill up the cavities of the damaged bone tissue [3]. More suitable biodegradable materials for the repair of load-bearing defects are the metallic ones that have higher fracture toughness and ductility than bio-ceramics and higher strength and elastic modulus than bio-polymers. Magnesium and its alloys represent the most interesting biodegradable materials. These materials possess densities and elastic moduli closer to those of the human bone than other metallic biomaterials for permanent implants such as stainless steel, cobalt-chromium alloys and titanium alloys [4], which makes them promising candidates for orthopedic applications at the load-bearing sites of the human body [5,6]. However, advances towards the clinical applications of these materials have been seriously hampered by too rapid degradation and premature loss of mechanical integrity in physiological environments. Although a great deal of research has been directed toward understanding their corrosion behavior and seeking measures to slow down degradation, the underlying corrosion mechanisms of magnesium and its alloys in relation to complex physiological media under *in vitro* and *in vivo* test conditions have not been fully understood. Clearly, further efforts are needed to reveal the nature of the interactions between magnesium and physiological media.

In *in vitro* studies on the degradation behavior of magnesium and its alloys, the choice of a suitable test medium is

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of critical importance. Many types of pseudo-physiological solutions that mimic the composition of body fluids, such as 0.9 wt% NaCl solution, conventional simulated body fluid (c-SBF), revised simulated body fluid (r-SBF), Hank's balanced salt solution, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) have been used. Among these, DMEM is one of the cell culture solutions that has been proven to produce an appropriate physiological condition for *in vitro* degradation tests of magnesium [7–9]. Major inorganic salts in DMEM, such as sodium bicarbonate, turn magnesium ions into magnesium carbonates, resulting in surface passivation [10]. Carbon dioxide also triggers the formation of magnesium carbonates. In the presence of carbon dioxide in aqueous solutions, carbonic acid forms, which is the ingredient for $MgCO_3$ formation [10–12]. The formation of a carbonated layer is thought to encourage further precipitation of the most important inorganic constituents of biological hard tissues—calcium phosphate phases [11], which is of biological and medical significance.

As soon as magnesium is in contact with a simulated physiological solution, corrosion takes place, leading to the changes in the chemistry of the magnesium surface and the surrounding solution. It has been observed during *in vitro* immersion tests that corrosion of magnesium leads to the local formation of hydroxyl ions and their leaching into the surrounding solution, which alters the pH of the solution through local alkalization [13]. In the human body, however, pH cannot increase significantly, as it is actively regulated through various biochemical reactions [14]. Therefore, the addition of a buffer to the immersion solution provides magnesium with a realistic degradation environment, as it closely mimics the *in vivo* situation.

In the *in vivo* situation, the pH of body fluids is regulated 39% by the respiratory system, *i.e.*, the $CO_2$/bicarbonate system, and 61% by biochemical buffers, *e.g.*, proteins (excluding the kidneys that have a long-term buffering effect on pH). In other words, the biochemical buffers play a dominant role in regulating the pH of the physiological environments in the body, relative to the bicarbonate buffering system [15]. Thus, to mimic the *in vivo* environments, the buffer chosen for *in vitro* tests should have a greater buffering capacity than the $CO_2$/bicarbonate system.

In preceding *in vitro* degradation tests of magnesium and its alloys, different buffering agents of various concentrations have been added to different pseudo-physiological solutions. As the corrosion behavior of magnesium and its alloys is highly sensitive to the aggressive environment, the type and concentration of buffering agents can dramatically change their degradation behavior. Due to the use of different pseudo-physiological solutions and buffering agents, many inconsistent results have been obtained from *in vitro* and *in vivo* studies on the degradation behavior of magnesium and its alloys, which makes the comparisons between *in vitro* test results and between *in vitro* and *in vivo* test results difficult. HCl-containing buffer systems, for example, have shown their abilities to introduce chloride ions into the solution, which in turn attack the surface layer of magnesium [16]. Phosphate-based buffers alter the chemical properties of the corrosion layer, as they provide phosphate ions in aqueous solutions, thereby producing insoluble salts with magnesium ions and eventually precipitating on the surface [12,17]. In addition, phosphate-based buffers contribute to regulating the pH of the body, although this contribution is often neglected due to their small concentrations in the blood plasma [15]. HEPES (N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic Acid) is one of Good's biological buffers that offers a greater buffering capacity than the bicarbonate buffers [18] and thus could be a suitable candidate to be coupled with the bicarbonate buffers that are present in DMEM. HEPES is water-soluble and atmosphere-independent. It has shown to have negligible affinity to metallic ions found in the blood plasma [19]. Previous studies have provided a basic understanding of the influence of HEPES on the degradation of magnesium in sodium chloride solutions [20,21], but this understanding may not necessarily be applicable to the DMEM solution, because of the differences in the surface layer formed as a result of the interactions between magnesium and the test solution. A better understanding of the influence of HEPES buffering on the degradation behavior of magnesium in the DMEM cell culture medium is of fundamental importance for understanding the correlations between the experimental results obtained from *in vitro* and *in vivo* tests, because body fluids themselves are a buffered environment.

The present research aimed at elucidating the corrosion mechanisms of pure magnesium in the DMEM solutions with and without the HEPES buffer. Degradation tests in a pseudo-physiological condition (Electrolyte=DMEM; T=37 °C; pH=7.45) for 15 s and for up to 3600 s were performed. The morphologies and compositions of surface layers formed were characterized and their correlations with the degradation rate were determined. Potentiodynamic Polarization (PDP) and Open Circuit Potential (OCP) tests were carried out to evaluate the influence of the HEPES buffer in DMEM on the electrochemical behavior and corrosion tendency of magnesium.

## 2. Experimental details

### 2.1. Material

Magnesium powder (of 99.98% purity) with a medium particle size of 90 μm was uni-axially pressed in a cylindrical die at 350 °C and under a pressure of 500 MPa to yield fully consolidated specimens for the research, instead of cast magnesium specimens with inevitable porosity that would affect corrosion behavior. Compacted magnesium pellets with a diameter of 13 mm were cut into slices with a thickness of 8 mm. A copper wire with a waterproof isolation layer was attached to the slices. The conductive specimens were then mounted in an epoxy resin with only the top surface being exposed to the immersion media for degradation tests. The mounted specimens were then ground using SiC grinding paper to 2400 grit and ultrasonically cleaned in acetone for 3 min.

### 2.2. Degradation tests

A corrosion cell operating at 37 °C was used to carry out all the degradation tests. The temperature of the cell was maintained using a thermostatic water bath. DMEM (D1145, Sigma-Aldrich) was used as the base immersion medium. HEPES (391338, Calbiochem) was added to DMEM to reach a concentration of 25 mM (referred to as the HEPES-buffered solution hereafter) to determine the influence of the buffering agent on the degradation behavior of magnesium. The ratio of solution volume to specimen surface area (SV/SA) was 378 ml/cm$^2$, being much larger than the critical value of 67 ml/cm$^2$ [22] in order to prevent ions in the solution from accumulation and the bulk solution from alkalization. Local pH changes during the immersion tests were registered by using a micro pH meter (S220 SevenCompact, Mettler Toledo) placed approximately 1 mm above the specimen surface. Another pH meter was placed over a lateral distance of 90 mm away from the specimen surface to measure the bulk pH, considering the possibility that the bulk pH might not be representative of the pH at the specimen surface and might vary by several pH units [13,23]. Data logging was carried out every 60 s.

### 2.3. Surface analysis

Magnesium specimens were immersed in the DMEM solution and in the HEPES-buffered solution for 15, 300 and 3600 s to determine the effect of the buffer on the morphology and composition of surface layer formed. At these time points, specimens were removed from the solutions, rinsed in ethanol for 30 s and then air dried. The morphologies and chemical compositions of surface layers formed were characterized using a JEOL JSM-6500F Scanning Electron Microscope (SEM) working at an accelerating voltage of 15 kV and equipped with an Energy Dispersive Spectrometer (EDS).

### 2.4. Electrochemical tests

The three electrode configuration was adopted to perform the polarization tests. A Saturated Calomel Electrode (SCE) was used as the reference electrode and a platinum mesh as the current electrode. The electrochemical activity of magnesium specimens in the DMEM solutions with and without HEPES at 37 °C were determined by measuring the Open Circuit Potential (OCP) during immersion using a Solartron 1250/1255 potentiostat. Potentiodynamic Polarization (PDP) tests were performed immediately after the OCP tests at an initial potential of −0.2 V *vs* OCP increasing to +0.5 V *vs* OCP at a scan rate of 0.2 mV/s. The pH variation of the solutions with and without HEPES with time was monitored using a micro pH electrode placed approximately 1 mm above the specimen surface.

## 3. Results and discussion

### 3.1. Effect of HEPES on the $Mg(OH)_2$ layer formed

Fig. 1 shows the surface morphology on the cross section of a magnesium specimen. It was observed that initial spherical powder particles were mostly transformed into particles of a hexagonal shape and a fully consolidated microstructure with no structural discontinuities was developed as a result of deformation at 350 °C.

Fig. 2 shows the morphologies of specimen surfaces after immersion in the HEPES-buffered solution for 15, 300 and 3600 s. After immersion for 15 s, the original grinding marks were still visible, indicating that the initial surface layer was yet very thin at this stage (Fig. 2(a)). In the surface layer, the original powder particle boundaries and grain boundaries, indicated by white arrows in Fig. 2(a) (backscattered image), were outlined. Observation of the square box in Fig. 2(a) at a higher magnification revealed that a nanostructured layer with grain sizes between 10–100 nm was formed on the surface and the surface layer contained nano-sized cracks with lengths of less than 100 nm. EDS point scans showed the presence of the elements of magnesium and oxygen on the surface, suggesting that the nano-structured layer was mainly composed of MgO/$Mg(OH)_2$.

After immersion for 300 s, the thickness of the surface layer slightly increased and larger cracks (with a maximum length of 1 μm) appeared mostly at the grain boundaries (Fig. 2(b)). Obviously, such a cracked surface layer could not effectively protect the surface from further corrosion, because in effect it provided pathways for the immersion solution to stay in direct contact with the magnesium surface and thus accelerated the degradation [24]. As a result, the dissolution of magnesium continued, in spite of the formation of the surface layer. During further immersion tests till the final time point (3600 s), these cracks significantly grew almost 500 times (up to a maximum length of 50 μm ) from their initial sizes. These grown surface cracks were mostly developed along the original powder particle boundaries and grain boundaries where an interconnected network of cracks throughout the surface was formed (Fig. 3(a)). Aung and Zhou [25] considered the formation of surface cracks as an indication of a higher dissolution rate in some regions on the surface. A grain boundary can be regarded as an area of defects in the crystal structure with a configuration of dislocations. It is well known that anodic metal dissolution would be accelerated in the vicinity of dislocations [26]. During the immersion tests of magnesium made from

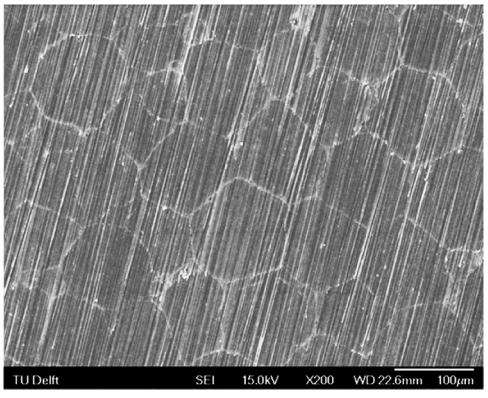


Fig. 1. SEM micrograph showing the surface morphology of a magnesium specimen after compaction.

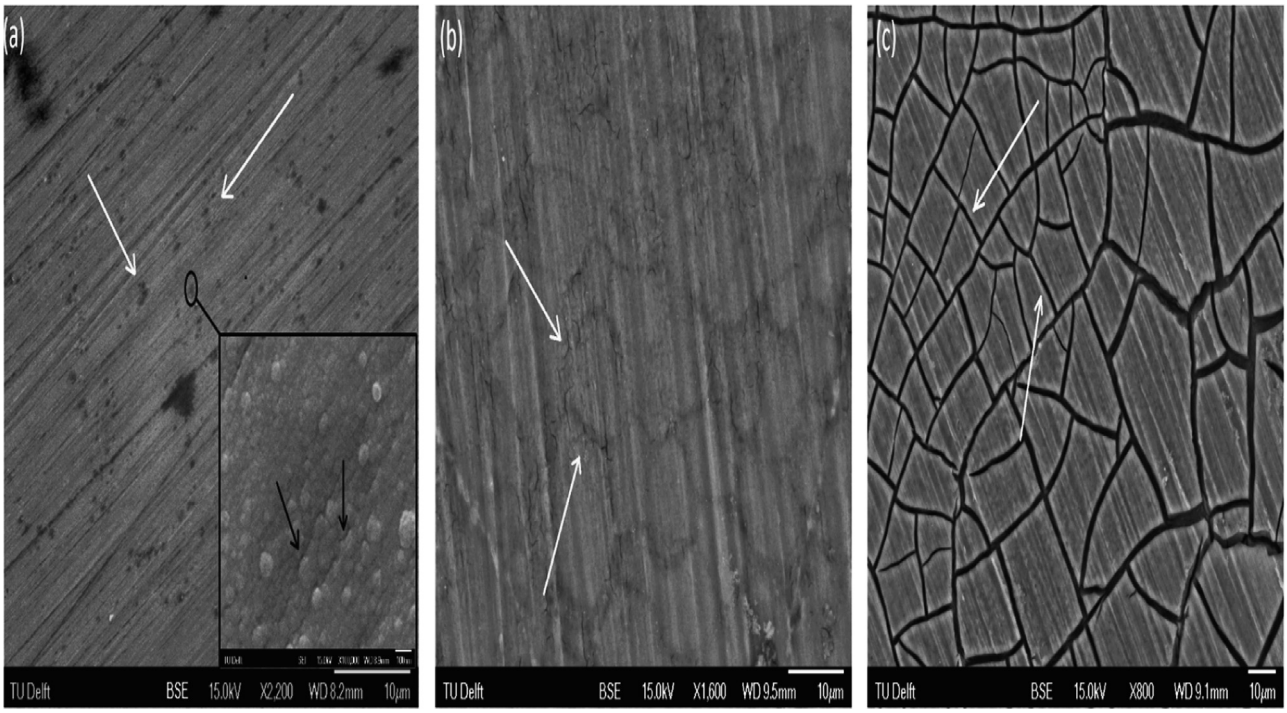


Fig. 2. Surface morphologies of magnesium specimens after immersion in the HEPES-buffered solution for (a) 15, (b) 300 and (c) 3600 s. Black arrows in Fig. 2(a) show the presence of nano-sized cracks in the initial surface layer, while white arrows indicate the formation (Fig. 2(a)) and growth of cracks (Fig. 2(b) and (c)) in the surface layer along the original powder particle boundaries and grain boundaries.

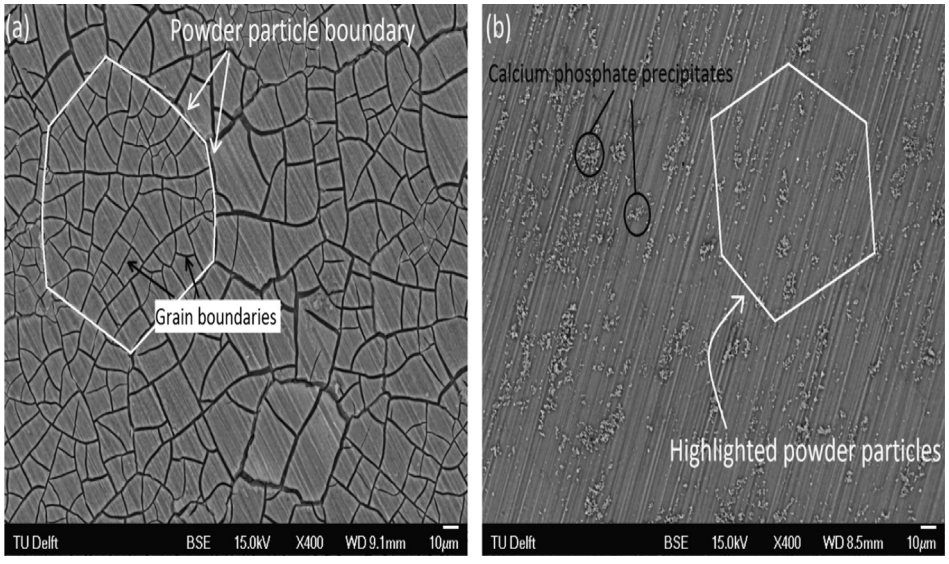


Fig. 3. Surface morphologies of specimens after immersion in (a) the HEPES-buffered solution and (b) the DMEM solution for 3600 s. Black and white arrows in Fig. 3(a) show an interconnected network of cracks along the grain boundaries and original powder particle boundaries, respectively.

powder, the original powder particle boundaries and grain boundaries became anode and preferentially corroded, building up local galvanic coupling with the surrounding grains. Subsequently, magnesium grains became cathode relative to the original powder particle boundaries and grain boundaries and galvanically protected (Fig. 3(a)). This explanation is yet to be confirmed by using the electrochemical microcell technique and scanning vibrating electrode technique to reveal local galvanic coupling between the internal structural boundaries and neighboring grain interior on a micro scale.

In contrast, after immersion tests in DMEM without the buffer for 3600 s, the original powder particles could still be identified using SEM backscattered imaging. These are highlighted in Fig. 3(b) for comparison with the surface morphology after immersion in the HEPES-buffered solution also for 3600 s (Fig. 3(a)). From the comparison, it is clear that in

DMEM without HEPES, interparticle and intergranular corrosion did not take place and the surface was less electrochemically active. It also suggests that the degradation of magnesium in the HEPES-buffered solution would proceed considerably faster than that in DMEM.

It is well known that the pH of a solution strongly influences the nature and stability of the protective hydroxide layer [11,16]. The hydroxide layer is more stable in an alkaline solution [16]. The higher degradation rate of magnesium in the HEPES-buffered solution could be associated with the limited alkalization of the solution on account of the buffering activity of HEPES. During the immersion tests, the bulk pH of both of the electrolytes (DMEM and HEPES-buffered DMEM) remained constant at 7.45, confirming that the very large SV/SA ratio indeed prevented the solutions from bulk alkalization, as observed by Yang and Zhang [22]. Of more interest were the changes of the local pH. It was found that while the bulk pH remained stable during the immersion tests, the local pH changed to alkaline values, as shown in Fig. 4. The local pH of DMEM continuously increased from 7.45 to 7.65 in 3600 s, exceeding the pH range of 7.4–7.6 in the normal physiological environments, while only a short positive shift with a maximum pH value of 7.55 in the local pH of the HEPES-buffered solution occurred and quickly the local pH returned to the initial value of 7.45. It demonstrated that the local alkalization indeed took place despite the large SV/SA ratio and the alkalization remained local throughout the tests without affecting the pH of the whole solution. It is likely that the local pH measurements are affected by the distance between the pH electrode placement and the corroding surface. In other words, the local pH measurements of the solution at closer spots, *e.g.*, a few micrometers away from the surface, might have shown even higher values [13]. The exact correlation of the local pH with the distance from the magnesium surface, yet to be established by means of pH micro-sensors, is of major significance, because it is the microenvironment at the solid–liquid interface that mainly determines the corrosion behavior of magnesium.

From Fig. 4, it is clear that the local alkalization was less severe for magnesium in the HEPES-buffered solution than in DMEM. This suggests that HEPES neutralized an excess

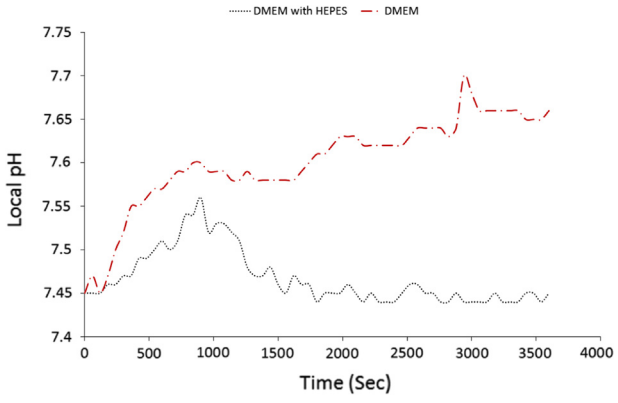


Fig. 4. Local pH variations with time during immersion tests in the DMEM solution and in the HEPES-buffered solution.

number of local $OH^-$ ions in the solution and provided a neutral environment, which would enhance the degradation rate. Other researchers proposed that a complex might have formed between $Mg^{2+}$ and HEPES, which effectively removed Mg cations from the solution, thereby disturbing the equilibrium and leading to further dissolution [11], although such a complex probably would not form at the physiological concentration of $Mg^{2+}$ ions [11]. Therefore, the lower local pH in the HEPES-buffered solution might have had a dominant effect on the degradation of magnesium. In the present immersion experiments, adding HEPES to DMEM increased the degradation rate of magnesium through intense dissolution at the original power particle boundaries and grain boundaries, causing the formation of initially nano-sized and then micro-sized cracks in the surface layer, as the degradation proceeded further. The hierarchy of the cracks built up an open structure of the surface layer, which allowed the immersion solution to stay in direct contact with the magnesium surface, thereby promoting further degradation. As a result, the magnesium surface became less protected and exhibited a stronger tendency of corrosion in the HEPES-buffered solution.

### 3.2. *Effect of HEPES on the precipitation of calcium phosphates*

After immersion in DMEM for 3600 s, colonies of agglomerated spherical precipitates were observed on specimen surfaces (Fig. 3(b)). EDS line scan analysis revealed that the spherical precipitates contained the elements of calcium and phosphorus (Fig. 5). Physiological solutions are known for inducing the formation of calcium phosphate precipitates on the magnesium surface owing to super-saturation [27]. These precipitates are thought to be mainly amorphous calcium phosphate phases agglomerated to form spherical particles [28]. The calculated *Ca*/P ratio of spherical precipitates was around one, meaning that these precipitates were deficient in calcium. The contribution of magnesium cations in the DMEM solution to spherical agglomerates might be the cause for the calcium deficiency, as magnesium cations could react with calcium and phosphate ions in the solution to form insoluble precipitates [22]. These precipitates have shown to improve the biocompatibility and osteoconductivity of magnesium-based implants, in comparison with hydroxyapatite [29].

By contrast, the surfaces of specimens after immersion in the HEPES-buffered solution for 3600 s showed no visible calcium phosphate precipitates (Fig. 3(a)). It is generally acknowledged that the alkalization of the solution as a result of an increase in $OH^-$ concentration through reduction reactions at the specimen surface encourages the precipitation of calcium phosphate phases [22,30]. Therefore, the lower local pH almost without solution alkalization, caused by the HEPES buffer, must have prevented the calcium phosphate precipitation from taking place. The Tris buffer, often used to regulate the pH of the simulated body fluid (SBF), is notorious for limiting the precipitation of calcium phosphates [31]. It is believed that the Tris buffer lowers the local pH and also forms

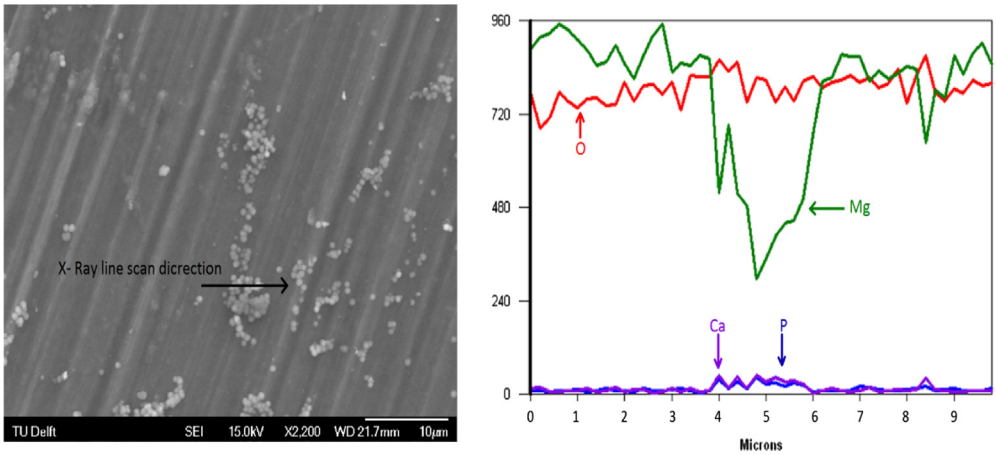


Fig. 5. EDS line scan on magnesium specimen surface after immersion in DMEM for 3600 s. White spherical particles (left image) were calcium phosphate agglomerates precipitated directly from the solution.

soluble complexes with calcium ions, which further reduces the concentration of free $Ca^{2+}$ required to form calcium phosphate precipitates [31]. In the HEPES-buffered solution used in the present study, however, such complexes with calcium ions would unlikely form [32]. Thus, the tendency of the HEPES-buffered solution to precipitate calcium phosphates was reduced, as the local pH of the solution was maintained at a lower level by the buffer [33].

### 3.3. Corrosion behavior of magnesium during PDP and OCP tests

The corrosion tendency of magnesium in the DMEM with and without the HEPES buffer was determined by performing OCP tests for 3600 s (Fig. 6(a)). The OCP value of magnesium in DMEM was considerably elevated to more positive values, shortly after immersion (from −1.76 V to −1.62 V after 500 s) and increased with time (−1.54 V after 3600 s), indicating the immediate formation and maturation of the protective layer. Such a strong shift to positive values was however not observed for magnesium in the HEPES-buffered solution; OCP increased smoothly and gradually from −1.86 V to −1.83 V after 3600 s. After 500 s, the OCP value of magnesium in the HEPES-buffered solution was 200 mV smaller than that in the DMEM solution and after 3600 s the difference became even larger (300 mV). It suggested that magnesium surface was better protected by a hydroxide layer in DMEM at the early stage of immersion and the initially formed layer was more rapidly matured, as compared to that in the HEPES-buffered solution [20]. This is in line with the observations of the surface layer of magnesium in the presence of HEPES (Figs. 2 and 3), where cracks appeared such that the surface was continuously exposed to the immersion solution. The multiple fluctuations of the OCP curve of magnesium in DMEM (Fig. 6(a)) could be an indication of metastable surface breakdown. A previous study conducted by Xin and Chu [34] on the effect of the TRIS buffer on magnesium corrosion showed a similar indication for pitting at the early stages of immersion. However, such an indication was absent in the OCP curve of magnesium in the HEPES-buffered solution, as shown in Fig. 6(a).

Fig. 6(b) shows the potentiodynamic polarization curves of magnesium in the DMEM solution and in the HEPES-buffered solution. The corrosion potential of magnesium in DMEM (−1.53 V) showed a more positive value than in the HEPES-buffered solution (−1.77 V), meaning that the surface was more effectively protected by the initially formed surface layer. As a consequence, the corrosion current densities in both cathodic and anodic regions (before surface breakdown) were higher in the HEPES-buffered solution (Fig. 6(d)). In DMEM, however, the corrosion current density suddenly increased drastically at relatively low anodic over-potentials (+50 mV *vs* corrosion potential), as a result of the surface breakdown. On the contrary, no such surface breakdown occurred for magnesium when HEPES was added as a buffer to DMEM and with increasing over-potential the corrosion current remained at a higher but constant level. Fig. 6(c) depicts the measured values of the local pH as a function time in the PDP tests. With increasing anodic potential, a local acidic environment developed over the surface of magnesium and its magnitude was significantly higher in DMEM. Increasing anodic potential caused the rapid dissolution of magnesium, leading to a large amount of $Mg^{2+}$ migrating to the solution. Baes and Masmer [35] showed that the high concentration of $Mg^{2+}$ cations would eventually react with water and produce protons that would acidify the local environment (Eq. (1)).

$$4Mg^{2+}\ (aq)+4H_2O\ (l)\rightarrow Mg_4(OH)_4^{4+}\ (aq)+4H^+\ (aq) \quad (1)$$

Subsequently, the surface layer would become more unstable in the presence of a higher concentration of protons and eventually breakdown would take place according to the following reaction

$$Mg(OH)_2\ (S)+2H^+\ (aq)\rightarrow 2H_2O\ (l)+Mg^{2+}\ (aq) \quad (2)$$

The local acidification as a result of the hydrolysis of magnesium cations was first mentioned by Robinson and George [36] and then adopted to explain the degradation mechanisms of magnesium [37]. However, other studies suggested that the possible anodic acidification of magnesium surface would not be significant enough to influence the pH of

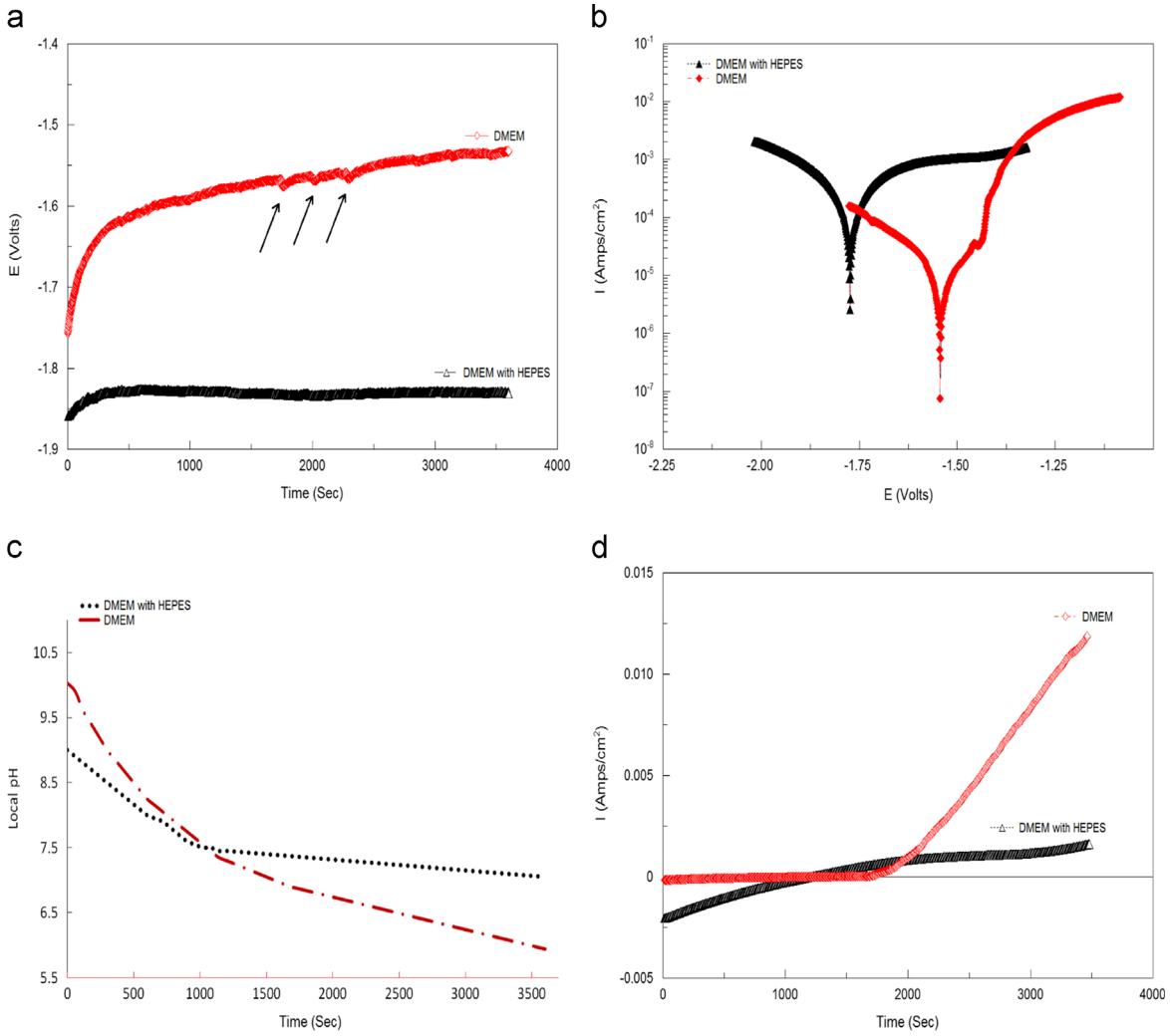


Fig. 6. (a) OCP, (b) potentiodynamic polarization curves of magnesium in the DMEM solution and in the HEPES-buffered solution, (c) changes in local pH and (d) corrosion current density during the PDP tests.

the solution [38]. In the present study, high acidification of the local environment was detected during anodic polarization. The cation hydrolysis could be even more significant inside an isolated pit or a crack where the anodic dissolution was dominant and therefore the concentration of $Mg^{2+}$ might be extremely high. Fig. 6(c) shows that the cation hydrolysis became more significant with increasing anodic current density. This indicates that the magnitude of the local acidification is dependent on the activity of the local anodes, *e.g.*, grain boundaries. In other words, the stronger is the galvanic coupling, the greater the local acidification on the local anodes. HEPES neutralized an excess number of protons and thus limited the magnitude of surface acidification, as shown in Fig. 6(c). The surface layer could be more immune to protons as a result of the buffering activity of HEPES. Highly aggressive anions such as chlorides could also be responsible for the surface breakdown, because they would eventually attack and destroy the protective layer [6]. Since the solutions used in the present study were identical with respect to the base chemical composition (*e.g.*, chloride ions) and the only difference between the two solutions was the presence and absence of the HEPES buffer, it was most likely that the buffering activity of HEPES influenced the anodic dissolution of magnesium.

## 4. Conclusions

In the present research, immersion tests, electrochemical tests and surface characterization were performed to develop an understanding of the effect of the buffering activity of HEPES in DMEM on the degradation behavior of magnesium. This understanding is of fundamental importance, because it will help understand the correlations between experimental results obtained from *in vitro* and *in vivo* tests. It will allow the further research to be focused on other major factors, such as the circulation of pseudo-physiological solutions that continuously carry ions away in a dynamic manner to account for the differences between static *in vitro* test results and *in vitro* test results. The following conclusions have been drawn from the present research.

(1) With the addition of the HEPES buffer to DMEM, the local pH close to the magnesium surface was largely maintained, leading to intense intergranular and interparticle corrosion and thus a higher degradation rate of magnesium.
(2) A higher dissolution rate of magnesium at the original powder particle boundaries and grain boundaries resulted in an interconnected network of cracks in the surface layer, thereby providing pathways for the immersion solution to stay in direct contact with the magnesium surface and promoting further dissolution.
(3) In the HEPES-buffered solution, the cathodic and anodic current densities in the PDP tests were higher and the OCP values were more negative due to the presence of surface cracks, as compared to those in DMEM without the buffer.
(4) The precipitation of calcium phosphates in the HEPES-buffered was limited due to the buffering activity of HEPES.
(5) High acidification of the local environment was detected during anodic polarization. It was derived that HEPES neutralized an excess number of protons and thus limited the magnitude of surface acidification.
(6) The addition of the HEPES buffer to the DMEM solution used in *in vitro* studies on the biodegradation behavior of magnesium provided a harsher environment for magnesium to resist rapid corrosion, but it is necessary, as the body fluids are also a buffered environment.

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