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Zn^{2+} release from zinc and zinc oxide particles in simulated uterine solution

Zhihong Yang [a,b], Changsheng Xie [a,*]

[a] *Department of Material Science and Engineering, Huazhong University of Science and Technology, Hubei, Wuhan 430074, PR China*
[b] *Faculty of Material Science and Chemical Engineering, China University of Geosciences, Wuhan 430074, PR China*

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Abstract

Zn^{2+} release from Zn and ZnO particles with different sizes in simulated uterine solution were investigated by absorbance measurements. The effects of pH and human serum albumin (HSA) on Zn^{2+} release were also studied. The morphology of Zn and ZnO particles was observed by scanning electron microscopy, and the corrosion products of zinc nanoparticles were analyzed by XRD. The results indicate that the maximum release ratios of Zn^{2+} from Zn and ZnO nanoparticles are higher than those from Zn and ZnO microparticles. Zn^{2+} release ratio depends not only on the pH of the simulated uterine solution but also the presence of human serum albumin. It decreases as the pH of the uterine solution increases. The trends of Zn^{2+} release ratios are almost the opposite for solutions with and without HSA. XRD analysis results indicate that zinc oxide is the main corrosion product of zinc particles.

Keywords: Zn^{2+} release ratios; Zn; ZnO; Particles; Intrauterine devices; Human serum albumin; pH; Simulated uterine solution

1. Introduction

Intrauterine devices (IUDs) are effective, long-term reliable and reversible devices for contraceptive use worldwide [1,2]. The antifertility effect is poor for IUDs made of inert materials like steel, plastic and silicone rubber. It can be improved greatly by the addition of copper. The enhanced contraceptive effect is commonly attributed to active copper ions released as a result of copper corrosion in the uterus. Copper-bearing intrauterine devices (Cu-IUDs) as effective contraceptives are being increasingly used worldwide [3]. However, after the insertion of Cu-IUDs, side effects such as pelvic inflammatory disease, pain and bleeding have been observed [4,5]. Concerns about them remain a stubborn obstacle to a wider use of modern IUDs [6].

One possible way to solve the problem of these side effects is the addition of zinc in copper-bearing intrauterine devices. Free zinc (Zn^{2+}) plays an important role in the process of development, cell division and the synthesis of proteins and DNA. It also helps wound healing and tissue repair of the endometrium [7,8]. By preventing uterus injury caused by IUD insertion, free zinc is expected to reduce the incidence of pain and bleeding, and it has been reported that one of the important factors that causes bleeding after Cu-IUDs insertion is the absence of free zinc [9]. Moreover, free zinc can help to prevent the risk of pelvic inflammatory disease since zinc ions have been found to have antibacterial effects [10]. Therefore, when zinc or zinc oxide is introduced into copper-bearing intrauterine devices, side effects like pain, bleeding and pelvic inflammatory disease are expected to be decreased greatly by releasing antimicrobial and restorative Zn^{2+} from zinc or zinc oxide in the uterus.

Compared with zinc and zinc oxide in bulk, zinc and zinc oxide nanoparticles show stronger antibacterial activity [11–13]. It is thus expected that nanoparticles of zinc and zinc oxide will decrease the side effects more effectively than microparticles.

In relation to reducing side effects it is important to demonstrate Zn^{2+} release from Zn or ZnO in human uterine solution. Human uterine solution is a complex mixture and contains a number of biochemical components including serum albumin (HSA). The pH in the uterus varies from one individual to another, with reported values ranging from 6 to 8 [14]. The

* Corresponding author. Tel.: +86 27 8754 3840; fax: +86 27 87543776.
E-mail address: csxie@mail.hust.edu.cn (C. Xie).

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effects of biochemical components, pH, and exposure time on the corrosion of copper in uterine solution have been extensively studied [14,15]. But investigation of Zn^{2+} release from zinc and zinc oxide in simulated uterine solution (SUS) has not been reported. The purpose of the present study was therefore to investigate Zn^{2+} release from Zn and ZnO particles of micro or nano size in simulated uterine solution (SUS). The effects of human serum albumin (HSA) and the pH value of SUS on Zn^{2+} release have also been studied. This work provides a basis for the application of Zn and ZnO nanoparticles in intrauterine contraceptives.

2. Experimental

Simulated uterine fluid was prepared by mixing the following analytical grade chemicals: NaCl (4.97 g/l), KCl (0.224 g/l), $CaCl_2$ (0.167 g/l), $NaHCO_3$ (0.25 g/l), glucose (0.50 g/l), $NaH_2PO_4{\cdot}2H_2O$ (0.072 g/l) and HSA (0.5 g/l). The pH value was kept at 6.5 by adding dilute hydrochloric acid (analytical grade) or sodium hydroxide solution (analytical grade). The concentration of human serum albumin and the experimental protocol were the same as described previously [16]. About 2 mg of samples (zinc and zinc oxide particles) were incubated in 250 ml of simulated uterine solution, and were dispersed at the start of the incubation using an ultrasonic vibrator for 2 min. Incubations were performed in a rocking bed at $37.0 \pm 0.1\,^{\circ}C$.

Absorbance measurements were employed to measure the concentration of Zn^{2+} released into the SUS. Zn^{2+} release was evaluated by the release ratio of Zn^{2+} from samples at different times. The release ratio is the amount of zinc ion in the SUS to the total amount of zinc contained in the sample.

To investigate the processes involved in corrosion of zinc particles in SUS, the corrosion products of zinc nanoparticles were identified by a RIGAKU D/Max-3B X-ray powder diffractometer with Cu Ka1 incident radiation. The morphology and particle size of Zn and ZnO particles were observed with a SIRION 200 scanning electron microscope.

3. Results and discussion

3.1. Shape and size distributions of the Zn and ZnO powders

Zinc oxide nanoparticles were column shaped, about 50 nm in diameter and 200 nm in length. Zinc nanoparticles, about 30 nm across, agglomerated into larger particles of around 500 nm diameter. For comparison to the nanoparticles of Zn and zinc oxide, zinc oxide microparticles of 1 μm and zinc microparticles of about 40 μm were also investigated.

3.2. Zn^{2+} release from Zn and ZnO particles

3.2.1. The effect of particle size on Zn^{2+} release

3.2.1.1. Zn^{2+} release from Zn particles. Fig. 1 shows Zn^{2+} release from Zn particles in the uterine solution in the presence of HSA. Ultrasonic dispersal of the Zn nanoparticles at the beginning of the experiment leads to initially high Zn^{2+} release ratios.

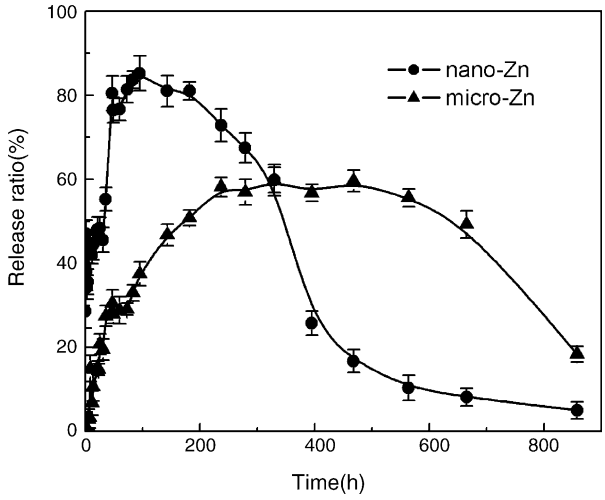

Fig. 1. The effect of particle size on Zn^{2+} release ratios from zinc particles incubated in simulated uterine solution with human serum albumin at 37 °C. Each point represents the mean of three experiments ± S.D.

Fig. 1 indicates that Zn^{2+} release ratios from Zn nanoparticles are higher than those from Zn microparticles in the initial stages; the maximum ratios are 85 and 60%, respectively. The increased corrosion of zinc nanoparticles in comparison with relatively coarse-grained samples is possibly due to higher surface area to volume ratio of the nanoparticles providing an abundance of active zinc atoms on the surface. This is consistent with findings reported in the literature [17].

It is well known that the pH value of SUS has been recognized as an important factor for corrosion processes. In order to examine the relationship between pH and Zn^{2+} release, the pH value of SUS was measured along with release. The results are illustrated in Fig. 2.

From Fig. 2, it can be observed that the pH value decreases initially and then increases until a steady state is reached. The mechanism of this effect is not well understood. It could be

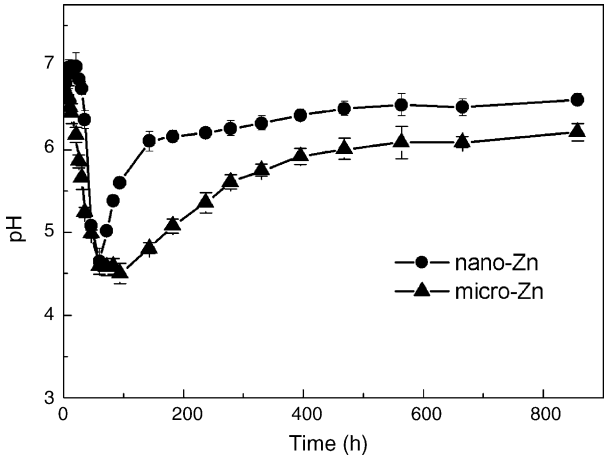

Fig. 2. The variation of pH of simulated uterine solution containing human serum albumin in which Zn particles of nano and micro sizes were incubated at 37 °C. Each point represents the mean of three experiments ± S.D.

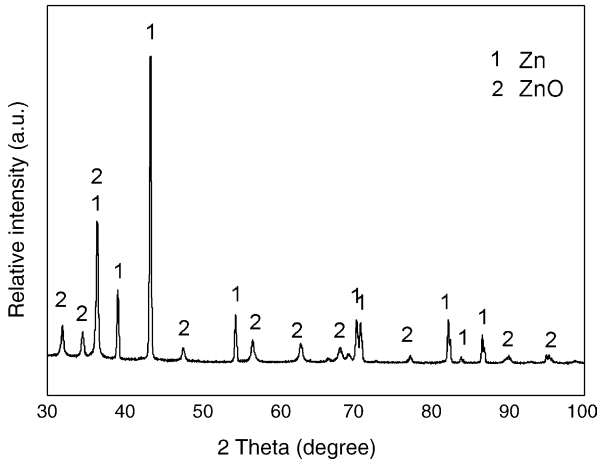

Fig. 3. XRD analysis of products of Zn nanoparticles after incubation in simulated uterine solution for 30 days at 37 °C.

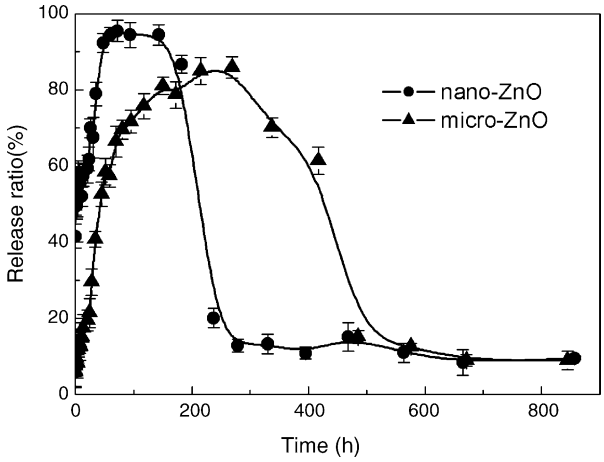

Fig. 4. The effect of particle size on Zn^{2+} release ratios from zinc oxide particles incubated in simulated uterine solution containing human serum albumin at 37 °C. Each point represents the mean of three experiments ± S.D.

interpreted that slow reactions occur among the complicated negative and positive ions in the uterine solution, and are responsible for variation of the pH value.

Figs. 1 and 2 indicate that the trends of Zn release ratio and pH of the SUS with time are almost opposite. For the nanoparticles, the Zn^{2+} release ratio increases initially to a maximum value at about 95 h, while the pH value decreases to a minimum at the same time. Later the Zn^{2+} release ratio declines to less than 20% while the pH rises to more than 6.0 until a steady state is obtained. An explanation of the relationship between the pH values and Zn release ratios may be found in the reactions occurring in the solution.

In order to investigate the course of these reactions, about 200 mg of Zn nanoparticles were incubated in SUS for 30 days under the same conditions as above. The corrosion products were then removed and analyzed by X ray diffractometry. Fig. 3 shows the XRD data for the product of Zn nanoparticles after incubation in SUS. It indicates that some Zn phases have been transformed into ZnO phases in the course of the reactions. The main reaction equations are as follows [18–20]:

$$Zn^{2+} + 3H_2O \rightarrow ZnO + 2H_3O^+$$

$$ZnO + 2H^+ \rightarrow Zn^{2+} + H_2O$$

It is often suggested that the formation of ZnO in solution occurs through a dissolution–precipitation mechanism. The active dissolution of zinc involves two consecutive one-electron charge transfer reactions with Zn^+ as intermediate, followed by the formation of Zn^{2+} ions. The Zn^{2+} produced can undergo a complexation reaction with water to yield ZnO_2^{2-} ions until a critical concentration is reached at which ZnO precipitates [20].

According to the literature [21–24], in addition to ZnO, the products may include $Zn(OH)_2$ and $Zn_5(OH)_8Cl_2$ due to the Cl^- contained in SUS. However, the quantities of other products were too low to be examined by XRD. The possible reaction equations are as follows:

$$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2$$

$$Zn(OH)_2 \rightarrow ZnO + H_2O$$

$$Zn(OH)_2 + 4Zn^{2+} + 6OH^- + 2Cl^- \rightarrow Zn_5(OH)_8Cl_2$$

Therefore, with increasing pH, ZnO and other possible corrosion products like $Zn(OH)_2$ and $Zn_5(OH)_8Cl_2$ are expected to increase and be deposited on the surface of zinc particles according to the reactions listed above. The protective effects of ZnO and other corrosion products on the particle surface prevent the further corrosion of the Zn. Thus, Zn^{2+} release rates decrease as the pH of the uterine solution increases. Moreover, HSA that is combined with Zn^{2+} denatures and precipitates with time, leading to the decrease of Zn^{2+} concentration as well. Therefore, when the pH rises to a certain value, the Zn^{2+} release ratio begins to decline, and this may account for the maxima of the curves in Fig. 1.

3.2.1.2. Zn^{2+} release from ZnO particles. The curves of solubilization of ZnO particles are similar to those of Zn particles discussed above, and are shown in Figs. 4 and 5. The release ratios increase initially as the pH decreases, but decrease as the pH rises above 6. The highest release ratios of Zn^{2+} are 95% for ZnO nanoparticles and 86% for microparticles in the test solution containing HSA. The main reaction equation in SUS is as follows:

$$ZnO + 2H^+ \rightarrow Zn^{2+} + H_2O$$

The high specific surface area of a nanoparticle causes most of the ZnO molecules to be distributed on the surface of the particle and in an active state, making it easier for the oxygen atoms to combine with hydrogen. Subsequently, Zn^{2+} in a ZnO particle is readily released into solution via the above mechanism. So the Zn^{2+} release rate from zinc oxide nanoparticles would be expected to be faster than that from microparticles in the initial stages. According to the reaction equation, the Zn^{2+} release rate will also decrease as the pH of the solution increases. This would account for the observation that Zn^{2+} release ratios decline with increasing pH at longer time.

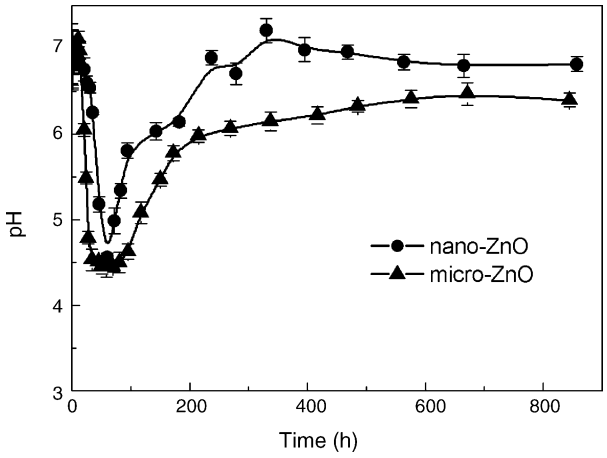

Fig. 5. The variation of pH of simulated uterine solution containing human serum albumin in which ZnO particles of nano and micro sizes were incubated at 37 °C. Each point represents the mean of three experiments ± S.D.

Zinc oxide or zinc would be useful in reducing the side effects of the insertion of Cu-IUDs (injury to the uterus, bleeding and pelvic inflammation) by releasing antimicrobial and restorative Zn^{2+}. Nanoparticles of zinc or zinc oxide would be expected to be most effective.

3.2.2. *The effect of human serum albumin on Zn^{2+} release and pH*

In order to investigate further the effect of HSA on Zn^{2+} release, simulated uterine solution containing zinc sulfate was prepared. Zn^{2+} concentration and pH were measured in the $ZnSO_4$ SUS with or without HSA under the same conditions as mentioned above. The initial Zn^{2+}concentration in the $ZnSO_4$ SUS was 0.65 μg/ml. Zn^{2+} release ratios from zinc and zinc oxide nanoparticles and Zn^{2+} concentrations in the $ZnSO_4$ SUS solution are shown in Figs. 6 and 7.

Figs. 6 and 7 show that the $ZnSO_4$ curves are similar to those of zinc and zinc oxide nanoparticles. The changes in Zn^{2+} concentration and pH of the $ZnSO_4$-containing solution are independent of the dissolution process of zinc or zinc oxide particles. Therefore, from the curves for $ZnSO_4$ in Figs. 6 and 7, it can be concluded that the presence of HSA affects the Zn^{2+} concentration and the pH value of SUS.

The first small peaks in pH occur after about 34 h in Fig. 7a and 11 h in Fig. 7b. Corresponding to the first peaks of pH, Fig. 6a shows that the Zn^{2+} release ratio and the Zn^{2+} concentration without HSA appear to reach a minimum. However, Fig. 6b shows that the Zn^{2+} release ratio still goes up and the Zn^{2+} concentration remains constant in the presence of HSA. These results suggest that HSA causes an acceleration of Zn^{2+} release from zinc and zinc oxide, which is consistent with previous reports [25,26].

At longer time, in the absence of HSA, the pH falls to low values (pH 4.6–4.5) until it stabilizes as observed in Fig. 7a, resulting in the Zn^{2+} release ratio and Zn^{2+} concentration rising to high and stable values as seen in Fig. 6a. However, in the presence of HSA, the pH falls to a minimum at about 60 h and rises again until it stabilizes at a value greater than 6.5 (Fig. 7b).

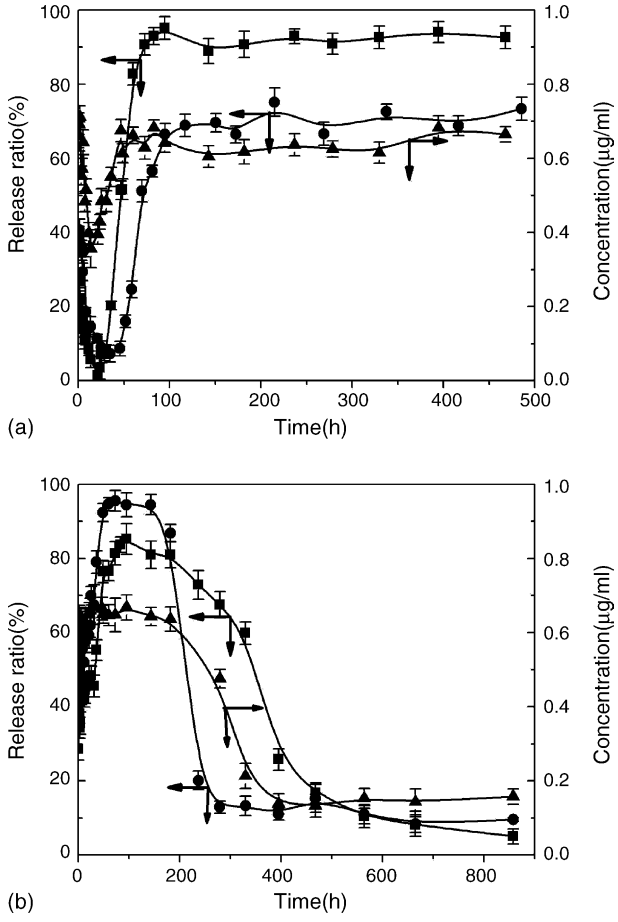

Fig. 6. Effect of human serum albumin on Zn^{2+} release ratio from nanoparticles (zinc and zinc oxide nanoparticles) incubated in simulated uterine solution; and Zn^{2+} concentration in simulated uterine solution containing zinc sulfate at 37 °C. (a) Without HSA; (b) with HSA. (■) Zn^{2+} release ratio from Zn nanoparticles; (●) Zn^{2+} release ratio from ZnO nanoparticles; (▲) Zn^{2+} concentration in uterine solution containing $ZnSO_4$. Each point represents the mean of three experiments ± S.D.

Contrary to the change in pH, the Zn^{2+} release ratio increases, passes through a maximum and decrease to low values as shown in Fig. 6b.

These results reveal that HSA has a strong effect on Zn^{2+} release ratio and pH. The mechanism by which HSA affects pH is not clear. According to a previous report [16], the presence of albumin at the concentration used here (0.5 g/l) is high enough to produce changes in the chemistry of the simulated uterine solution, masking the effect of other compounds and altering the pH in the uterine fluid.

As one of the transition metals, zinc readily combines with HSA [27,28]. Initially, HSA adsorbs onto the surface of zinc or zinc oxide particles and forms a layer. Then a complex is formed by the binding of zinc and HSA through C–N groups [29]. This complex moves into solution, which leads to the dissolution of zinc or zinc oxide. The sites where the complex moved from become new adsorption sites for HSA. Repetition of the process enhances the dissolution rate of zinc or zinc oxide particles.

At longer time, a red floccular precipitate forms in the SUS, probably consisting of denatured HSA in combination with

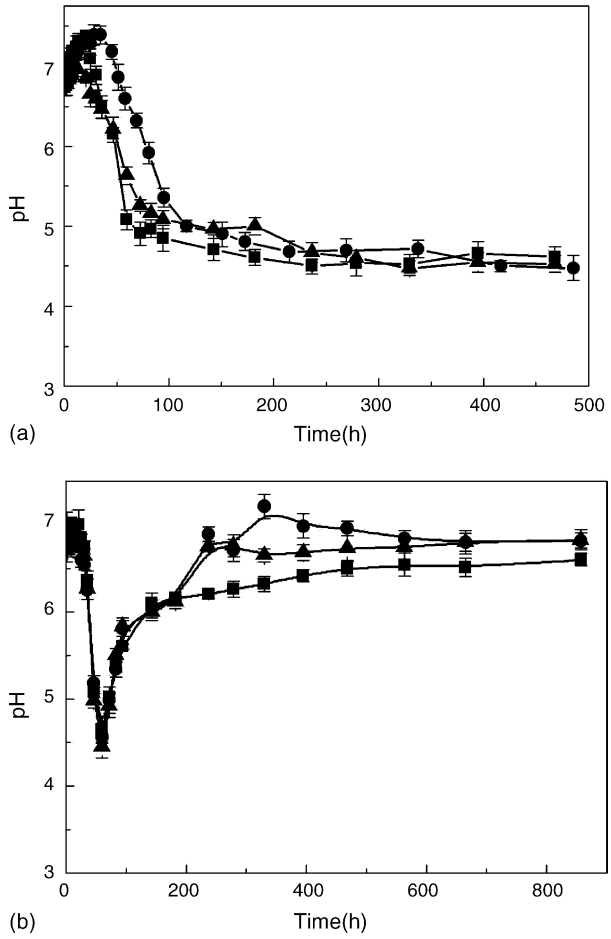

Fig. 7. Effect of human serum albumin on pH of nanoparticle-dispersed simulated uterine solution and simulated uterine solution (SUS) containing zinc sulfate at 37 °C: (a) without HSA; (b) with HSA. (■) Zn nanoparticles-dispersed SUS; (●) ZnO nanoparticles-dispersed SUS; (▲) SUS containing zinc sulfate. Each point represents the mean of three experiments ± S.D.

Zn^{2+}. This precipitation will result in a decrease in Zn^{2+} concentration in SUS. In this way it may be appreciated that HSA accelerates Zn^{2+} release in the initial stages, but retards it later.

4. Conclusions

1. Zn^{2+} release rates from zinc or zinc oxide nanoparticles are faster than those from microparticles in the initial stages. This may be due to the larger surface area of the nanoparticles. The highest Zn^{2+} release ratios are 95% for ZnO nanoparticles and 85% for Zn nanoparticles in simulated uterine fluid containing HSA; these are higher than those of the respective microparticles (86% for ZnO microparticles and 60% for Zn microparticles).
2. The main corrosion product of Zn nanoparticles is ZnO. In addition to ZnO, the products may include $Zn(OH)_2$ and $Zn_5(OH)_8Cl_2$, probably forming a protective film on the surface of Zn particles which prevents Zn^{2+}release from the centre of the zinc particle.
3. The pH affects Zn^{2+} release from zinc and zinc oxide particles. The higher the pH, the lower is the release ratio. Evolution of the pH depends on both incubation time and the presence of HSA, suggesting that changes take place in the chemistry of the simulated uterine solution.
4. HSA has strong effects on Zn^{2+} release ratio. In the absence of HSA in the SUS, Zn^{2+} release ratios from zinc and zinc oxide decrease rapidly to a minimum and then increase until a steady state is reached. In the presence of HSA, Zn^{2+} release ratios increase, pass through a maximum and then decrease to a low value. The results suggest that by binding to Zn^{2+}, HSA is efficient in accelerating Zn^{2+} release in the initial stages, but retards Zn^{2+} release later, probably when it denatures and precipitates.

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