# Behavior of copper oxide nanoparticles in gastrointestinal juice

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**Abstract**. In this work, we investigated the behavior of CuO nanoparticles (NPs) in simulated gastrointestinal juices. It was found that CuO NPs agglomerated significantly in the gastric and small intestine phases, with a higher agglomeration in the small intestine than that in the gastric phase, which was consistent with the results on zeta potentials. Dissolution experiment showed that CuO NPs released more $Cu^{2+}$ in the gastric phase (pH = 2.5) than that in the small intestine phase (pH = 7). This was due to much lower pH in gastric juice, leading to higher dissolution of CuO NPs.

## 1. Introduction

With the rapid development of modern science and technology, a new generation of engineering technology represented by nanotechnology was quietly emerging in the early 21st century. Nanomaterial products are everywhere in our daily life. Nanomaterials have special physical and chemical properties, such as small particle size, relatively large specific surface area, excellent optoelectronic and mechanical properties, and thus are widely used in various fields [1]. Among them, metal oxide nanoparticles (NPs) have high application value in agriculture, foodstuffs, chemical materials, and biomedicine due to their unique physicochemical properties [2, 3]. CuO NPs, one of the most extensively applied NPs, were widely used in fields such as agriculture and biomedicine. Meanwhile, CuO NPs are inevitably released into the environment during their production and application [4, 5], and eventually affecting creature survival and human health [6-10]. Therefore, assessing the risks of CuO NPs to human health has become crucial. What is the morphology of CuO NPs, and whether or not the release of CuO NPs is the same in gastrointestinal juices. The dissolution of CuO NPs in gastrointestinal juices is not yet clear. Our scientific hypothesis is that due to the lower pH in gastric juice, more $Cu^{2+}$ will dissolve than in the small intestinal juice. Therefore, this article aims to study potential behaviors of CuO nanoparticles in the simulated gastrointestinal juice.

## 2. Materials and methods

### *2.1 CuO NPs characterization*

1.2 mg of CuO NPs in 40 mL of ethanol was sonicated to prepare a suspension(30 mg/L). The suspension was dropped onto a copper mesh with a 100 microliter pipette and allowed to air dry before being observed by transmission electron microscopy (TEM, H-7650, Hitachi, Japan).

*2.2 Hydrodynamic diameter and zeta potential of CuO NPs.*
100 mg/L of CuO NPs was added in DI water, simulated gastric juice and small intestinal juice, respectively. CuO NPs stock solution was sonicated for 5 min (20 kHz, FB 120, Fisher Scientific, USA). The size and zeta potential were analyzed by Zetasizer (Nano ZS90, Malvern, UK).

*2.3 Dissolution experiment.*
CuO NPs (< 50 nm), bile salts (B8631), pepsin (P7000) and lipase (L3125) were purchased from Sigma-Aldrich (USA).

Stomach stage: 40 ml of 0.1 M PBS buffer solution was added in simulated gastric juice (containing 3.2 g/L pepsin) and the pH was then adjusted to 2.5 (the pH of the suspension was adjusted by adding negligible amounts of NaOH or HCl) to mix with the 0.4 mg of CuO NPs. The mixed solutions were incubated in a shaker for 2 h (120 rpm, at 37 ℃), and the supernatants were obtained by centrifugation (the 40 ml centrifuge tubes were centrifuged at 4000rpm for 10min). The released $Cu^{2+}$ concentration was determined by ICP-MS (NexION 350).

Small intestine stage: Stock solution of small intestinal juice (containing 24 g/L lipase, 53.57 g/L bile salts) was dispersed in 40 ml of 0.1 M PBS buffer solution (pH = 7, adjusted by adding negligible amounts of NaOH or HCl) and mixed with the 0.4mg of CuO NPs. The mixture was incubated in a shaker for 4 h(120 rpm, at 37 ℃), and the supernatants were obtained by centrifugation (the 40 ml centrifuge tubes were centrifuged at 4000 rpm for 10 min). The released $Cu^{2+}$ concentration was determined by ICP-MS.

*2.4 Statistical analysis*
All the data in this study were expressed as mean values. Error bars presented in the results represent the standard deviation. Significant differences among the treatments were analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple range test (P = 0.05) using Statistical Product and Service Solutions Software 20.0 (SPSS 20.0).

## 3. Result and discussion

*3.1 Characterization of CuO NPs*
The morphological characterization of CuO NPs in ethanol was analyzed by TEM. The TEM image showed that the shape of CuO NPs was sphere, the diameter of CuO NPs was 30-50 nm (Fig. 1).

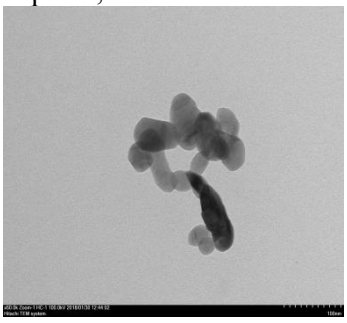

**Fig. 1** The TEM image of CuO NPs. Scale bar is 100 nm.

*3.2 Hydrodynamic diameter and zeta potential of CuO NPs.*
The zeta potentials and size of CuO NPs in DI water, simulated gastric juice and small intestinal juice were shown in Fig.2 A and B. In DI water, the zeta potential of CuO NPs was 14.3 mV. In simulated gastric juice and small intestinal juice, the zeta potential of CuO NPs were -2.47 and -25.26 mV, respectively. In DI water, the size of CuO NPs was 272.5 nm. In simulated gastric juice and small intestinal juice, the size of CuO NPs were 1320.3 and 5371.3 nm, respectively. The addition of bile salts, a strongly hydrophobic substance, to the small intestinal juice made the charge of the solution

highly negative. The results showed that CuO NPs agglomerated significantly in the simulated small intestinal phase compared with that in DI water and simulated gastric juice.

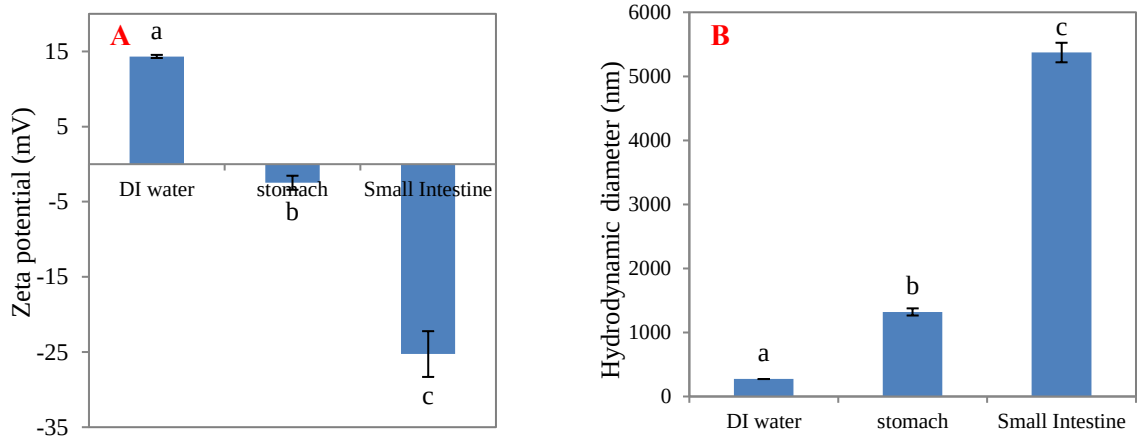


**Fig. 2** The zeta-potential (A) and size (B) of CuO NPs in DI water, simulated gastric juice and small intestinal juice, respectively. The different letters among different treatments indicate the significant differences, which were analysed by Duncan's test ($P$ = 0.05) using SPSS 20.0. Error bars represent standard errors of the mean ($n$ = 4).

*3.3 CuO NPs in Simulated Gastrointestinal Juice*

As shown in Figure 3, the dissolution of CuO NPs in simulated gastrointestinal juices. CuO NPs in gastric juice dissolved $Cu^{2+}$ with time, and dissolved about 30 mg/L $Cu^{2+}$ at 2 h. The dissolution reached equilibrium and 75 mg/L $Cu^{2+}$ was dissolved at 16 h (Fig. 3A). As shown in Fig. 3B, about 6.5 mg/L of $Cu^{2+}$ was released at 4 h, and the dissolved $Cu^{2+}$ increased continuously and reached the maximum (7.7 mg/L) at 48 h. In summary, the $Cu^{2+}$ dissolved in the simulated gastric juice was much higher than that in the small intestinal juice. This was due to the lower pH in the simulated gastric juice (pH = 2.5) than that in the simulated small intestinal juice (pH = 7), more $Cu^{2+}$ was released.

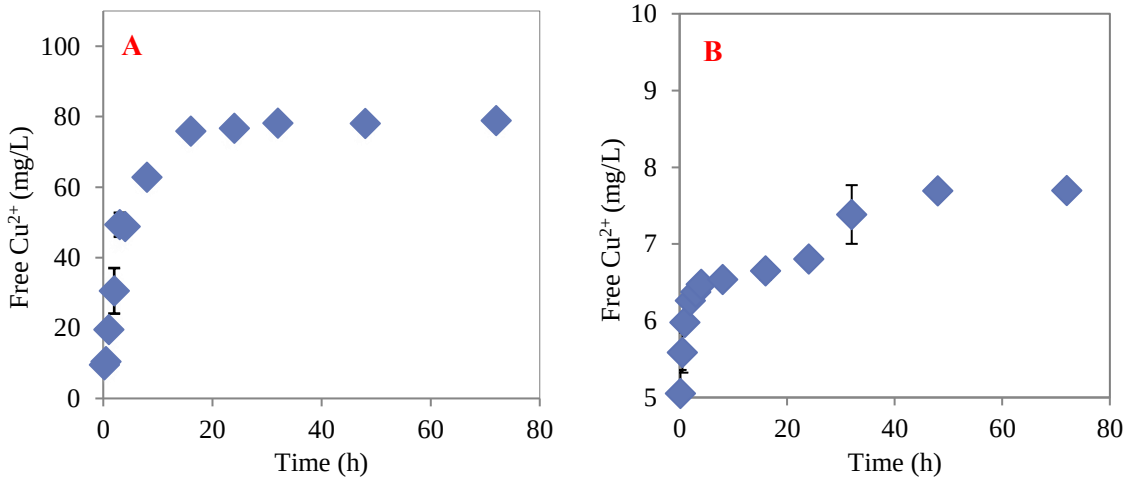


**Fig. 3** Dissolution of CuO NPs (100 mg/L) in simulated gastric juice (A) and small intestinal juice (B).

## 4. Conclusions

CuO NPs aggregated significantly during the stomach and small intestine phase and even more strongly in the small intestine, which was consistent with zeta potentials. Dissolution experiment showed that CuO NPs released more $Cu^{2+}$ in the gastric juice than that in the small intestinal juice and

reached dissolution equilibrium (75 mg/L) at 16 h. In small intestinal juice, CuO NPs reached dissolution equilibrium (7.7 mg/L) at 48 h.

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