

Appendix.

CONTENTS: Narrative on “Materials and Methods” and “Result”, 3 Figures, and 2 Data Tables.

1. Preparation of NiO suspension

After NiO particles sterilizing in the autoclave (Panasonic, MLS-3751L, Japan) at 120°C for 30 min, nano and micro NiO particles were suspended in 9% normal saline at concentrations of 0.24 mg/ml respectively. Then, the suspension of nano NiO was diluted to appropriate concentrations (0.015 and 0.06 mg/ml). Suspensions were sonicated with an ultrasonic homogenizer in ice-bath for 30 min at 750 W (Cole-Parmer, CP750, USA) and oscillated for 5 min to avoid NiO particles agglomeration before intratracheal instillation.

2. Endotoxin assay of NiO samples

To verify whether suspensions of NiO particles were contaminated by endotoxin, the Limulus Amebocyte Lysate (LAL) assay was performed with ToxinSensor Gel Clot Endotoxin Assay Kit (GenScript, USA). Twenty endotoxin free tubes were added 0.1 ml Limulus Amebocyte Lysate (LAL) solution, then 0.1 ml endotoxin free water, E. coli endotoxin standard, physiological saline, 0.24 mg/ml nano and micro NiO suspensions were transferred into tubes, dividing into negative, positive, physiological saline, nano and micro NiO groups respectively. Tubes were incubated at 37°C for 1 h, and completely reversed. The increased viscosity or turbidity (negative result) indicated that the endotoxin levels were lower than 0.25 EU/ml, while the gel kept intact as a positive reaction.

Our results showed that the endotoxin levels were lower than 0.25 EU/ml in micro and nano NiO suspensions, namely negative results.

3. NiO particles characterization

The particle sizes and distribution of NiO were measured by scanning electron microscope (SEM) (Jeol, Jsm-7610 F, Japan). The results indicated

that most particles had a uniformly scattered situation. Average sizes of nano NiO was 20 nm and micro NiO was 1 μm on the powder (Fig. 1A and B).

The crystal structure of NiO particles were characterized by X-ray diffractometer (Shimadzu, XRF-1800, Japan). The results showed that structure of NiO particles phase identification of the NiO particles was sphere (Fig. 2).

We examined hydrodynamic sizes of nano NiO particles in exposure medium by Malvern Instruments Zetasizer Nano ZS90 after sonicate 30 min (Worcestershire WR, UK). As shown in Fig. 3, hydrodynamic sizes of nano NiO was 244.5 nm in exposure medium, which indicated that nano NiO agglomerated slightly due to their high surface activity.

Chemical composition of NiO particles were detected by inductively coupled plasma mass spectrometry (Agilent, 7500i, USA). The results indicated that the purity of nano and micro NiO particles was better than 99.90% (Table 1).

Specific Surface area of NiO particles were detected by brunauer emmett teller method (Beishide, 3H-2000PS2, China). The specific surface area of nano NiO was 60 m^2/g and micro NiO was 20 m^2/g . The other physicochemical characteristics were showed in Table 2.

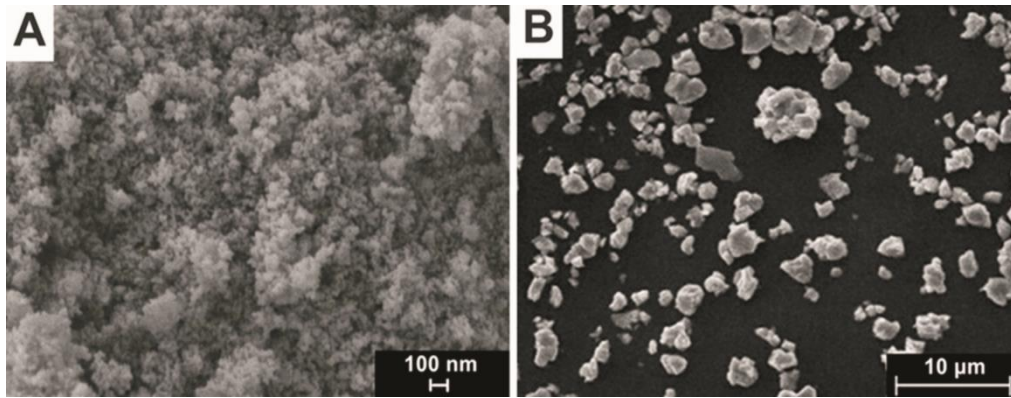


Fig. 1. Scanning electron microscope photograph. (A) Nano NiO and (B) micro NiO.

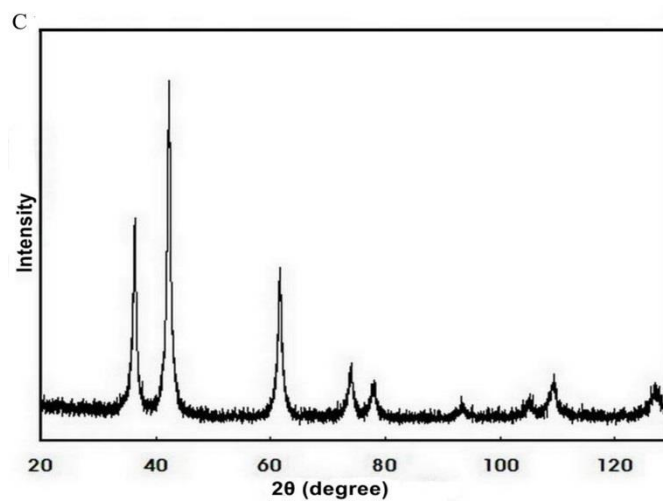


Fig. 2. X-ray diffraction spectra of NiO particles.

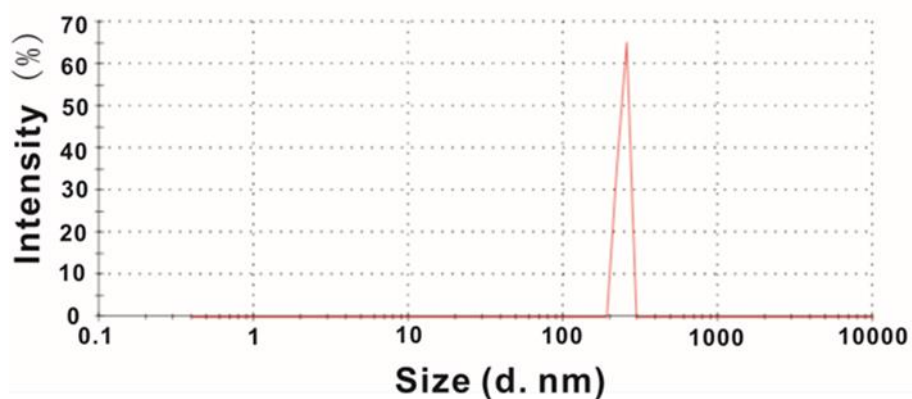


Fig. 3. Size distribution by intensity of nano NiO in exposure medium from dynamic light scattering measurements.

Table 1. Chemical Composition of NiO particles

Chemical Composition	Nano NiO (Wt%)	Micro NiO (Wt%)
NiO	≥ 99.9	≥ 99.9
Al	0.001	0.001
Fe	0.001	0.001
Ca	0.002	0.002
Mg	0.001	0.001
Cu	0.001	0.001
Mn	0.001	0.002
Na	0.001	0.001
Co	0.001	0.001
Si	0.002	0.002
Pb	0.001	0.001
K	0.001	0.001
N	0.001	0.001
C	0.020	0.003
S	0.002	0.002
F. O	0.070	0.050

Table 2. Physicochemical properties of NiO particles

	Particle size (nm)	Purity (%)	Specific surface area (m ² /g)	Volume density (g/cm ³)	Crystallinity	Color
Nano NiO	20	≥99.9	60	0.45	sphere	black
Micro NiO	1000	≥99.9	20	1.75	sphere	black