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Solubility of Nickel Oxide Particles in Various Solutions and Rat Alveolar Macrophages

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ABSTRACT

The solubility of five types of commercial nickel oxide particles was determined in different types of solutions, including distilled water, physiological saline, buffered saline, and tissue-culture medium, in order to estimate their solubility in the human respiratory tract. In addition, we examined the solubility of the two types of particles that were the most and least soluble particles of the above five types of nickel oxide, in rat alveolar macrophages cultured in vitro. The solubility of the nickel oxide particles in these solutions varied remarkably with their types, suggesting that, even though they are called as "nickel oxide," their solubilities are different among the manufacturer and the product lot. Their solubilities were also influenced by the types of solution and the existence of carbon dioxide in the atmosphere. The solubility of nickel oxide particles in the alveolar macrophages was significantly larger than that observed in the culture medium without macrophages, but smaller than that observed in the distilled water. These results suggest that the actual solubility of nickel oxide particles in the respiratory tract may be difficult to estimate by the conventional solubility analysis method using distilled water, and that the enhancement of particle dissolu-

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tion by the alveolar macrophages and the depression of particle solubility by the coexisting salt and carbon dioxide should be taken into consideration for the accurate estimation.

Index Entries: Nickel oxide; alveolar macrophages; nickel release; solubility; carbon dioxide.

INTRODUCTION

Major nickel-related human hazards are attributed to inhalation exposure. The increased incidence of cancer in the respiratory tract has been reported among certain groups of nickel refinery workers (*1,2*). Occupational exposure to various nickel aerosols also induces the harmful health effects, including asthma, acute pneumonities, and contact hypersensitivity (*3*). Airborne nickel compounds were detected up to a concentration of 1.5 mg Ni/m^3 in occupational atmosphere (*4*), and nickel metal, soluble compounds, nickel sulfide, and nickel carbonyl are regulated by the American Conference of Governmental Industrial Hygienists to 1, 0.1, 1, and 0.35 mg/m^3, respectively (*5*).

Both in vitro and in vivo experiments have been carried out in relation to the metabolism of nickel compound in the respiratory tract and other organs. One of the authors has reported the difference of nickel concentrations in internal organs, the degree of inflammation, and the clearance rate from the respiratory tract in rats exposed to various kinds of nickel compound particles (*6–10*). Acute and chronic inhalation exposures of nickel compounds caused a decrease in phagocytic and bacteriocidal activities of macrophages without concomitant loss in cell viability in mice and rabbits (*11–13*). In vitro exposure of rabbit alveolar macrophages to nickel chloride at millimolar concentrations induced severe functional deficits—inability to phagocytize latex beads and diminished release of lysozyme (*14,15*).

The toxicity of inhaled particles is related to the dissolution in the respiratory tract. Alveolar macrophages may have an important role in the solubilization of particles in the respiratory tract, since it is well known that a large fraction of inhaled particles is phagocytosed and solubilized by alveolar macrophages (*16*). Up to the present, however, little has been known of the dissolution of nickel compound particles in the airways, especially the solubilization by alveolar macrophages. In the present article, we describe the dissolution of some types of nonstoichiometric nickel oxide (NiO) particles in the different types of model body fluid and by rat alveolar macrophages.

MATERIALS

Five kinds of commercial NiO powder were used in the present study. Their visual colors, chemical compositions, and morphologies are shown in Table 1 and Photo 1. All these samples are called "nickel

Table 1
Nickel Oxide Samples

No.	1	2	3	4	5
Name	Wako	Super fine	Soekawa	INCO	INCO
Color	Black	Grayish blue	Gray	Green	Black
Chemical formula	$NiO_{1.43}$	$NiO_{1.04}$	$NiO_{0.98}$	$NiO_{0.92}$	$NiO_{1.13}$

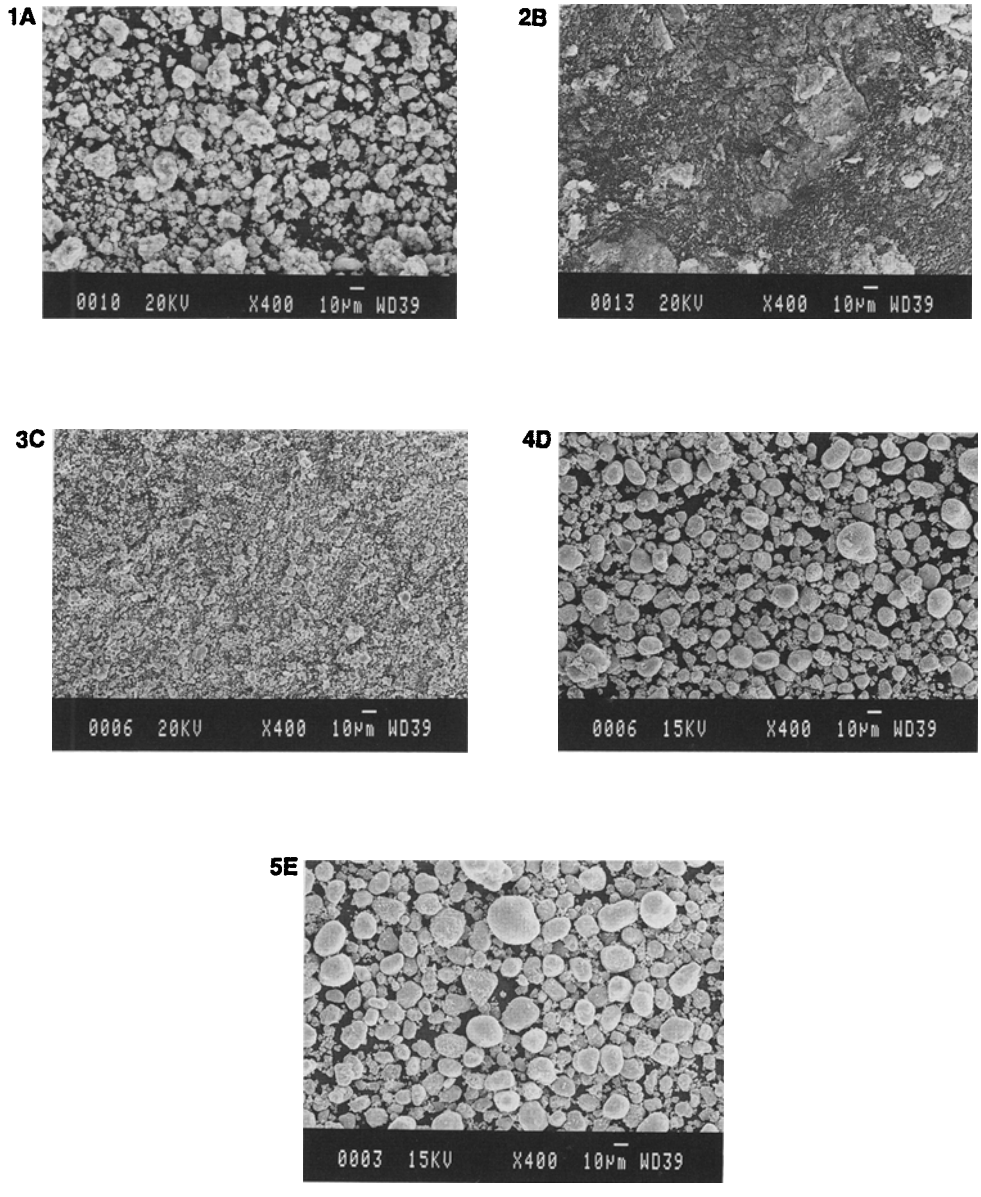

Photo 1. Scanning electron micrographs of nickel oxide particles.

oxide," but their colors, chemical compositions, and forms are different from each other. Nickel and oxygen contents in the samples were determined by atomic absorption spectrometry for nickel and by inert gas fusion method for oxygen, independently. Most of nickel in the black-colored samples was determined as Ni(III) and that in the other color samples was Ni(II). Among them, No. 2 NiO are composed of very fine particles, whose size is almost in the submicron order.

EXPERIMENTAL

The solubility of NiO particles was evaluated in the following four experimental systems.

Solubility in a Closed System

In a 150-mL container, 500 mg of NiO particles (Nos. 1–5) were mixed with 100 mL of distilled water or saline. The particle suspension solution was then stirred up to 316 h at 30–32°C, under the closed condition without free contact with carbon dioxide in the air. After stirring, sample solutions were filtrated with a 0.22-μm membrane filter (Millipore Ltd.) to separate the dissolved nickel from the NiO particles. The nickel concentration in the filtrates was determined by atomic absorption spectrometry directly for the distilled water samples and, after removing other salts by an ion-exchange method, using a chelating form of resin for saline samples (*17*).

Solubility in an Open System

The solubility of Nos. 4 and 5 NiO particles was determined in five types of solutions at a concentration of 200 or 800 μg/mL. The solutions used were distilled water, saline, Hank's balanced salt solution (HBSS), Eagle's minimum essential medium (EMEM), and EMEM containing 10% fetal calf serum (EMEM-FCS). The particles' suspension solutions were kept at 37°C in air containing 5% carbon dioxide for 24 h and then filtrated with a 0.22-μm membrane filter. The concentration of nickel in the filtrates was measured by atomic absorption spectrometry after removing other salts in the solutions by the ion-exchange method using a chelating form of resin.

Dissolution of Nickel Oxide Particles by Alveolar Macrophages

In Vitro Loading and In Vitro Culture System

Female wistar rats, weighing 200–250 g and aged 10–15 wk, were used as donors of the alveolar macrophages. The animals were killed by exsanguination under anesthesia. The lungs were lavaged three times

with phosphate-buffered saline (PBS), and the lavage fluid was centrifuged to separate the cell fraction from the supernatant. After washing three times with EMEM, the recovered cells were resuspended in EMEM-FCS to a concentration of 5×10^5 cells mL^{-1}, and 2 mL of the cell suspension were cultured at 37°C in the atmosphere of 95% air and 5% carbon dioxide. Two hours after the incubation, the culture supernatant containing nonadherent cells was discarded and replaced by fresh medium. Then, NiO particles (Nos. 4 and 5) were introduced into the medium to concentrations of 200 or 800 μg/mL, and the cells were incubated for 24 h. In a preliminary experiment, we confirmed by the trypan-blue exclusion method that the viability of the macrophages was not influenced in the range of particle concentration and culture time used here. At the end of incubation, the culture supernatant was collected, and the dish was washed two times with PBS. The supernatant and washing solution were filtrated with a 0.22-μm membrane filter. Then the adherent macrophages were lysed with 1 mL of 10% sodium dodecylsulfate. The amount of nickel in the lysed cell fraction and the filtrate were determined by atomic absorption spectrometry. The experiment described above was also carried out without alveolar macrophages as a blank test. The ratios of the nickel amount dissolved in macrophages and released extracellularly to that taken up by macrophages were calculated by the following equation:

$$R = (F - FB) / (F - FB + AM/N) \quad (1)$$

in which R is the ratio of the nickel amount released extracellulary to that taken up my macrophages, F, FB, and AM are the nickel amount in the filtrate, in the filtrate in the blank experiment, and in the adherent macrophages, respectively, and N is the ratio of the number of adherent macrophages to the total number of cultured macrophages.

Dissolution of Particles by Alveolar Macrophages

In Vivo Loading and In Vitro Culture

In this experiment, the administration of NiO particles to the macrophages was carried out in vivo by intratracheal instillment to the lung as reported previously (*18*). In brief, 0.5 mL of the saline containing 6–8 mg NiO particles (Nos. 4 and 5) as administered to the rat lung under halothane anesthesia. After 24 h, the alveolar macrophages were collected by the same procedure used in the above in vitro loading and in vitro culture experiment. After the preincubation of 2 h, the culture supernatant containing nonadherent cells and free particles was discarded and replaced by fresh medium. The adherent cells continued to be cultured at 37°C for 24 h. The amount of nickel uptake by macrophages and the release from macrophages were determined by the same procedure as the in vitro loading experiment. The ratio of the nickel

amount dissolved in macrophages and released extracellularly to that taken up by macrophages was calculated by the following equation:

$$R = F / (F + AM/N) \quad (2)$$

in which R is the ratio of the nickel amount released extracellularly to that taken up by macrophages, F and AM are the nickel amount in the filtrate and in the adherent macrophages, and N is the ratio of the number of adherent macrophages to the total number of cultured macrophages.

RESULTS

Figures 1 and 2 show the solubility of the five kinds of NiO particles in distilled water and saline under the closed system. The solubility of NiO particles Nos. 1 and 5, whose color was black, were larger than those of other samples. The amount of dissolved nickel was larger in the distilled water than in the saline.

Figure 3 shows the solubility of NiO particles of Nos. 4 and 5 in the five different solutions under the open system. The order of the solubility of No. 5 NiO particles was: distilled water > Saline > HBSS > EMEM > EMEM-FCS. The solubilities of No. 4 NiO were remarkably smaller than those of No. 5. They were 0.1 μg/mL in the distilled water and saline, and at an undetectable level in the other solutions.

Table 2 shows the nickel amount taken up by macrophages, and the amount of nickel that was dissolved in alveolar macrophages and released to the culture medium, in the in vitro loading and in vitro culture system. The uptakes of nickel by alveolar macrophagess were dependent on the concentrations of particles administered and independent of the kind of NiO particles. The percentages of the nickel amount released extracellularly to that taken up by macrophages was approx 7% for the No. 5 NiO particles, whereas < 0.1% for the No. 4 NiO particles.

Table 3 shows the nickel release from alveolar macrophages in the in vivo loading and in vitro culture system. The amounts of nickel taken up by the alveolar macrophages were less than those in the in vitro loading system. There was no difference in the nickel uptakes by the alveolar macrophages between NiO particles Nos. 4 and 5. The rate of nickel release from alveolar macrophages was approx 4% of the amount of nickel in the alveolar macrophages for the No. 5 NiO particles and 0.1% for the No. 4 NiO particles.

DISCUSSION

The possible factors affecting the solubility of the particulate matters may be their size and chemical state. Regarding the solubility of the NiO particles used here, their chemical state may be more important than their size, because the solubility of the No. 2 NiO particles, which are

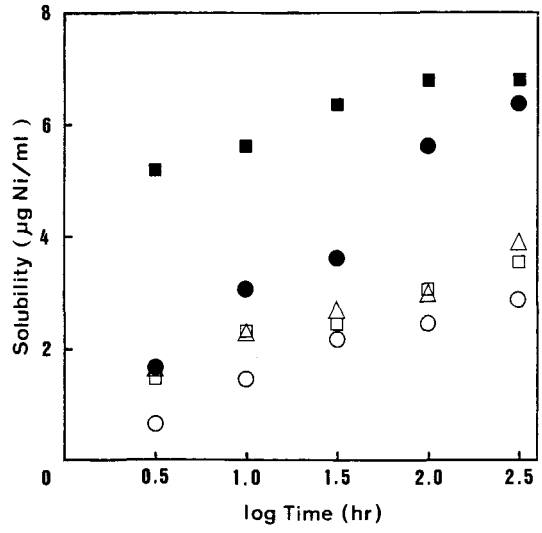

Fig. 1. Solubility of nickel oxide in distilled water in closed system.

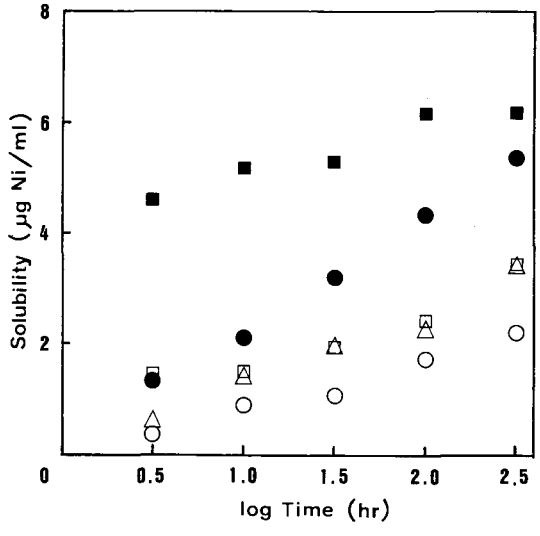

Fig. 2. Solubility of nickel oxide particles in saline in closed system.

very much finer than other samples, was nearly equal to or even less than that of other samples. The solubilities of the NiO particles of Nos. 1 and 5, whose color was black, were larger than those of the other samples. Most of nickel in the Nos. 1 and 5 of NiO particles was determined as Ni(III), and that in the other NiO particles was determined as Ni(II). Therefore, these results may indicate that the solubility of Ni(III) is larger than that of Ni(II).

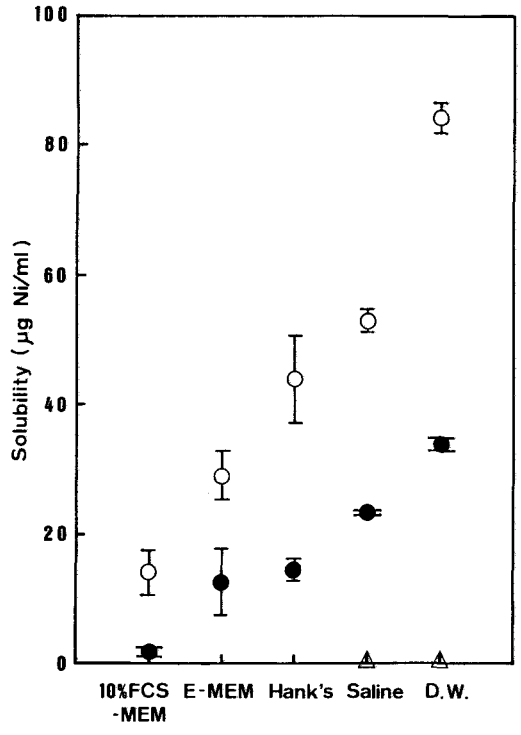

Fig. 3. Solubility of nickel oxide particles in different types of solutions in open system.

Table 2
The Amount of Nickel Taken Up by and Released from the Alveolar Macrophages in the In Vitro Loading and in In Vitro Culture Systems

	Uptake by Mϕ μgNi/10^6 cell	Release from Mϕ μgNi/10^6 cell	Ratio of R to U, %
No. 4 800 μg/mL	127.7 ± 26.8	0.1 ± 0.1	0.1 ± 0.1
No. 4 200 μg/mL	15.6 ± 1.2	ND[a]	ND[a]
No. 5 800 μg/mL	106.7 ± 14.0	7.4 ± 4.4	6.9 ± 4.3
No. 5 200 μg/mL	16.1 ± 8.9	1.1 ± 1.1	6.8 ± 2.8

[a]ND = Not detected.

The solubility of NiO particles in the distilled water and saline was larger in the open system than in the closed system. The distilled water and saline do not have buffered action. The pH of the solution decreased with time, which may be attributable to the dissolution of carbon dioxide in the atmosphere into the solution. This may be the reason for the enhanced solubility of NiO particles in the open system. As shown in

Table 3
The Amount of Nickel Taken Up by and Released from the Alveolar Macrophages in the In Vivo Loading and in In Vitro Culture Systems

	Uptake by Mϕ μgNi/10^6 cell	Release from Mϕ μgNi/10^6 cell	Ratio of R to U, %
No. 4	21.5 ± 19.2	0.1 ± 0.1	0.1 ± 0.1
No. 5	19.2 ± 13.2	0.6 ± 0.3	3.7 ± 1.2

Figs. 1–3, the solubilities of NiO particles were larger in distilled water than those in the other solutions regardless of the kinds of particles. In general, the solubility is suppressed by the coexisting salts, amino acids, and proteins in the solution. This is why the solubility of NiO particles is significantly larger in the distilled water. These results clearly indicate that, in order to evaluate the solubility of NiO in a living body by the in vitro method as in the present study, it is essential to consider the chemical state of particles, the chemical property of the model body fluid, and the existence of carbon dioxide in the atmosphere.

The ratios of the nickel amount released from the alveolar macrophages was approx 4–7% of the total amount of nickel taken up by macrophages for the No. 5 NiO particles, and 0–0.1% for the No. 4 NiO particles, depending on their experimental systems used. On the other hand, the amount of nickel taken up by the alveolar macrophages was almost similar between the Nos. 4 and 5 NiO. This suggests that the difference in the chemical composition between NiO particles Nos. 4 and 5 affects the dissolution of particles in the alveolar macrophages and subsequent extracellular release of nickel, but does not change the phagocytosis of particles by alveolar macrophages.

One of the authors has reported (*10*) that redistribution of nickel and the effect on the respiratory tract following the inhalation of the various types of NiO in rats. In this study,the black NiO (the same as the particle No. 5 in the present study) was more rapidly redistributed to the other organs, such as liver and kidney, than the green NiO (the same as the particle No. 4 in the present study), suggesting the higher solubility of the black NiO in the respiratory tract. These results are very consistent with our present findings. The solubility of NiO particles in the rat alveolar macrophages, or more exactly, the dissolution of the particles in the macrophages and the subsequent release to the medium were significantly less than the solubility estimated in the distilled water and larger than that observed in EMEM with FCS. Since a large fraction of inhaled particles was phagocytosed and solubilized by broncho-alveolar macrophages, the information given by the present experimental systems using the cultured macrophages is more realistic than the conventional chemical method with the distilled water, in order to predict the solubility of particles in the respiratory tract.

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