Comparative Solubility of Nanoparticles and Bulk Oxides of Magnesium in Water and Lung Simulant Fluids

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EXTENDED ABSTRACT

Introduction:

Nanoparticles, particles with one or more dimension less than or equal to 100 nanometers, can clear airborne smoke particles from the air. Small inhaled particles can deposit in the deep lung (<4 micrometers aerodynamic diameter; AD), evade phagocytosis (< 1 micrometer AD) and enter the interstitial space of the lung. Thus, the solubility of nanoparticles will determine the amount of time particles remain in the interstitial lung space with potential to irritate and injure. Nanoparticles may have smaller size and diffusion layer thickness as well as greater amounts of their mass on the surface and steeper concentration gradients than conventional macrocrystalline (MC) chemicals. We hypothesized that dissolution of particles of nanomaterials would be more rapid than MC particles of the same chemical.

Methods:

Initially, we investigated the solubility and pH and the rate of dissolution of oxides of magnesium (MgO) at room temperature. We compared the relative solubility of magnesium oxide (nanoactive^{TM} MgO (NA^{TM}MgO) and nanoactive^{TM} MgO plus (NA^{TM} MgO plus) (NanoScale Materials Inc, Manhattan, KS) to that of conventional MC MgO (MC MgO). These materials were added to three 500 ml acid washed glass beakers and the contents continuously stirred by magnetic teflon stirring bars. Lung simulant fluids were Hank's Balanced Salt Solution (HBSS; Sigma-Aldrich) and Dulbecco's Modified Eagle's Medium with low glucose (DMEM, Invitrogen). Dissolved magnesium was measured using ICP-AES; Accuris-141, Fisons Instruments, Beverely, MA) and corrected by atomic mass to MgO dissolved.

Results:

Short-term (20 minute) solubility of all 3 oxides of magnesium at room temperature were more rapid with buffers that contained bicarbonate than with distilled, deionized water at room temperature. These data correlated to the bicarbonate concentration of epithelial lining simulant fluids – HBSS and DMEM (ELF) ($p < 0.001$).

Since more MgO dissolved with increasing bicarbonate concentration of simulated ELF, pH of the fluid was measured at the same time as Mg was measured to aid in describing the compound that was formed. The pH of conventional macrocrystalline MgO and both NA MgOs increased from 9.4 to 10.6 as the concentration of MgO to be dissolved in bicarbonate containing solutions increased (Figure 1). The pH increased from 7.4 to 8.4 with DMEM which contained a stronger bicarbonate buffer with the same MgO compounds (data not shown).

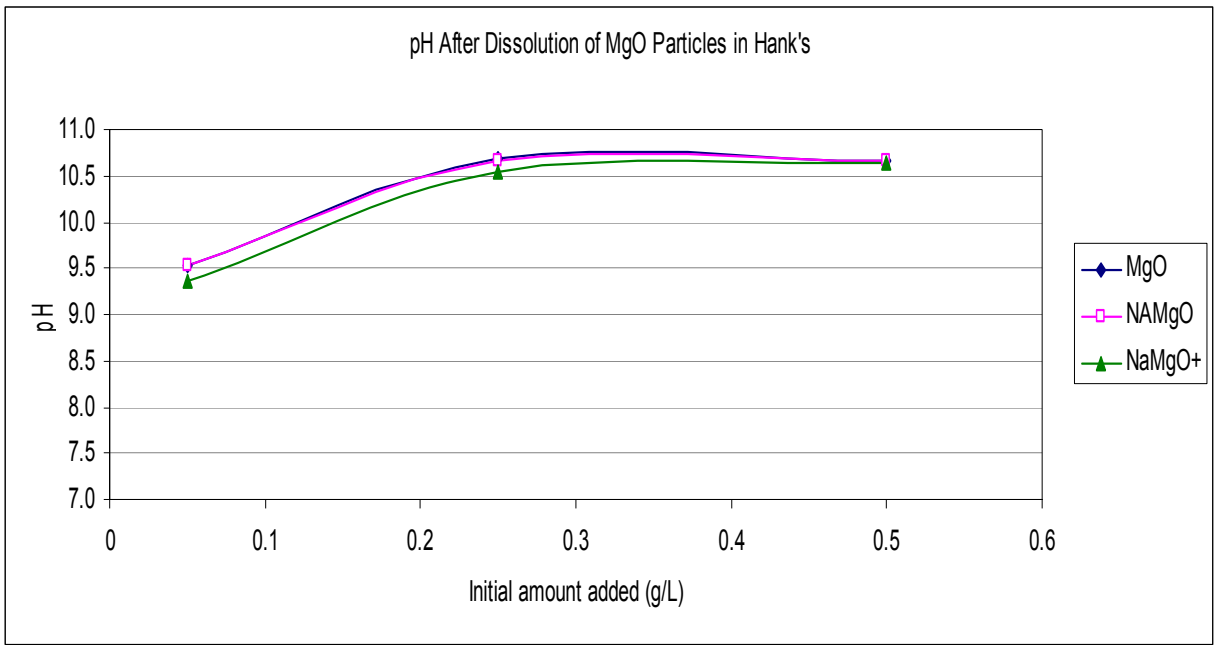

Figure 1.

The pH is shown as a function of MgO (g/L; 1000 mg/L) added to Hanks Balanced Salt solution (HBSS) used as a simulant epithelial lining fluid (ELF). Materials examined were conventional macrocrystalline magnesium oxide (MgO), Nanoactive ™ MgO (NA ™ MgO) and NA ™ MgO

Plus (NA ™ MgO+). Samples were taken at the time of peak solubility during the 14 day dissolution study. Each point represents a mean of 2 replicates. The pH increased from 9.4 at 50 mg MgO/L to 10.6 at 250 mg/L MgO between 50 and 250 mg MgO/L. No change was noted by going to 500 mg/L. The increase in pH suggests the formation of a compound with a higher pH than the lung stimulant fluid whose buffer was bicarbonate. This compound was likely a carbonate. Magnesium carbonate trihydrate, nesquehonite fulfills the requirements for stability at room temperature 22-25°C or human body temperature (37°C) and human body P_{CO2} (near 40 torr).

Solubility of MgO in DMEM was greater than that of HBSS at 37°C, although this difference is less after 96 hours of dissolution (Figure 2).

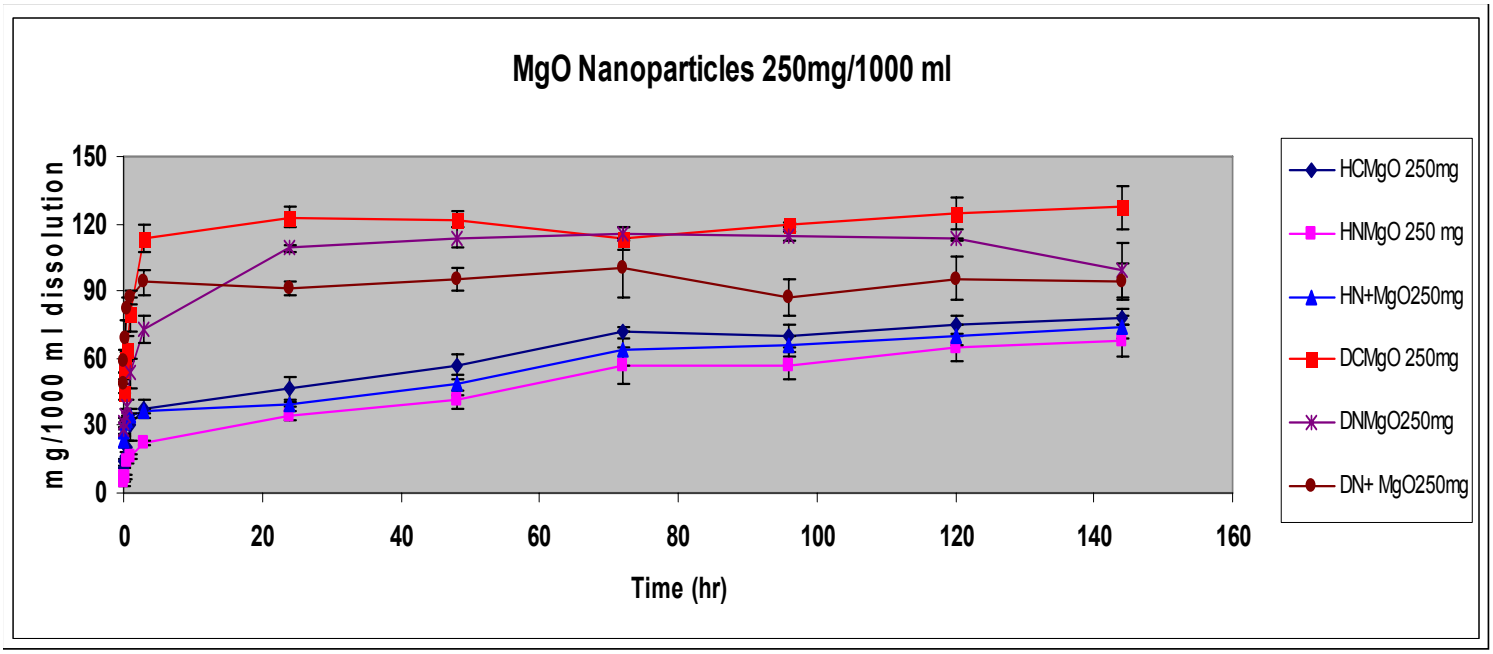

Figure 2.

Solubility of MgO in mg/L is shown as a function of time of dissolution (hr) for macrocrystalline (MC), nanoactive (NA) ™ MgO and NA ™ MgO Plus. Samples were taken from 3 minutes to 144 hours. Comparison was made between Hanks Balanced Salt Solution (HBSS; HN) and Dulbecco's Modified Eagle's Medium (DMEM; D), simulant fluids for lung epithelial lining

fluid (ELF). Data are shown as means of 3 results ± standard errors of means. Solubility for DMEM was greater than that of HBSS alone at 37°C, although this difference is less after 96 hours of dissolution.

There were only modest differences (1.5- to 2-fold) between solubilities of MgO in HBSS or DMEM at room temperature (22 to 25°C) and their respective solubilities at 37°C (Figure 3). At 250 mg/L MgO, solubility of all particles in HBSS + 5% CO_2 exceeded solubility in HBSS alone after 3 hours (Figure 3). The MgO particles incubated with DMEM alone or incubated with 5% CO_2 were similar to HBSS and 5% CO_2, implying an upper limit of solubility from bicarbonate (Data not shown).

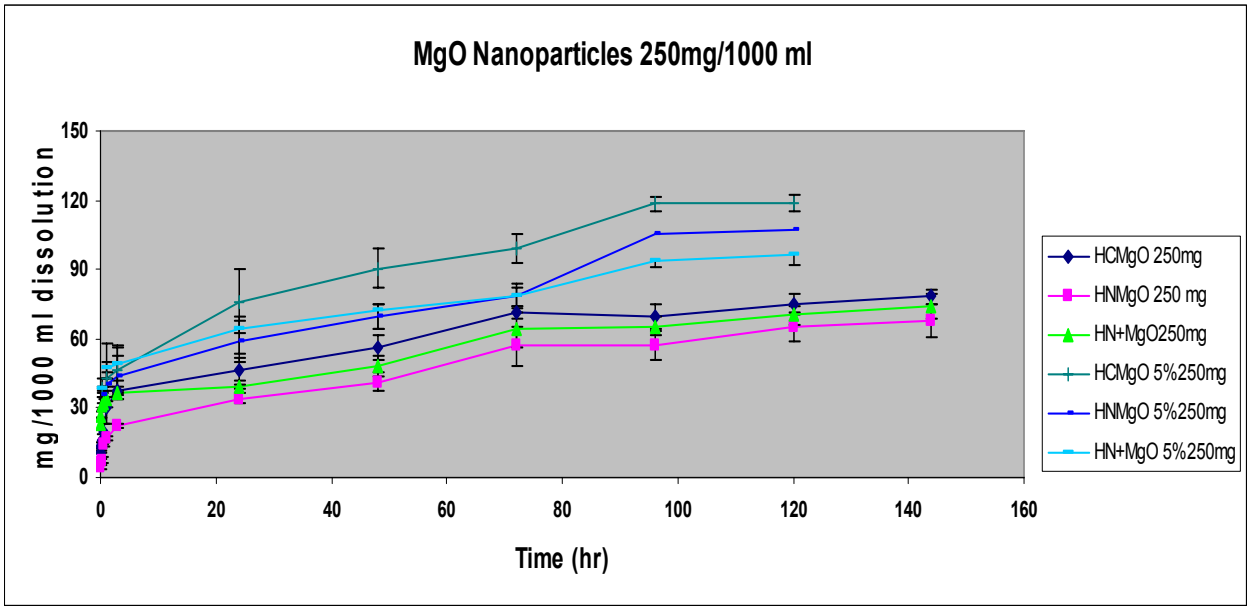

Figure 3.

Solubility of MgO in mg/L is shown as a function of time of dissolution (hr) for macrocrystalline (MC), nanoactive (NA) ™ MgO and NA ™ MgO Plus. Samples were taken from 3 minutes to 144 hours. 5% CO_2 samples were analyzed at 15 minutes, 30 minutes and 1 hour and hourly thereafter to preserve CO_2 contact with the media; these samples were terminated at 120 hours because of bacterial contamination. Comparison was made between Hanks Balanced Salt Solution alone (HBSS alone; HN) and HBSS incubated in the presence of 5% CO2 (H 5% CO_2) both conditions simulating epithelial lining fluid (ELF). Data are shown as means of 3 samples ±

standard errors of means. Solubility for HBSS incubated in the presence of 5% CO_2 was greater than that of HBSS alone at 37°C.

This difference was present only at 12-15 minutes for DMEM; at all other times there were no apparent differences. At the 50 mg/L concentration, differences were not present either HBSS or DMEM and the same simulant fluids with 5% CO_2 added.

Dissolution of MgO at 37°C for NA ™ MgO Plus was more rapid than for NA ™ MgO or MC MgO at 125 mg/500 ml (250 mg/L) for both HBSS (Figure 4) and DMEM through 24 minutes.

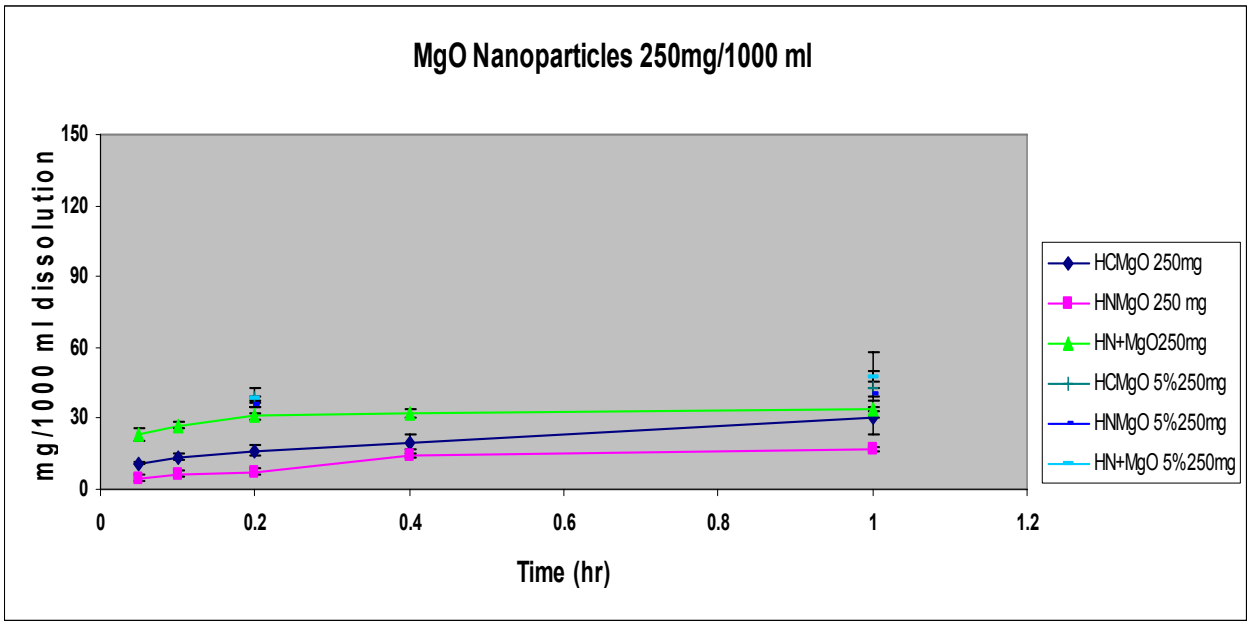

Figure 4.

Solubility of MgO in mg/L is shown as a function of time of dissolution for the first hour (hr) of a 120 (HBSS + 5% CO_2 to preserve CO_2 contact with media) to 144 hour study (HBSS alone) for macrocrystalline (MC), nanoactive (NA) ™ MgO and NA ™ MgO Plus. Comparison was made between Hanks Balanced Salt Solution alone (HBSS alone; HN) and HBSS incubated in the presence of 5% CO_2 (H 5% CO_2). Both conditions simulate lung epithelial lining fluid

(ELF). Data are shown as means ± standard errors of means. Solubility for HBSS incubated in the presence of 5% CO_2 was greater than that of HBSS alone at 37°C. Dissolution of MgO at 37°C for NA ™ MgO Plus was more rapid than NA ™ MgO or MC MgO at 250 mg/L for both HBSS and DMEM through 24 minutes of the first hour. Data are shown as means of 3 samples ± standard errors of the means.

Differences between the respective particles of either DMEM or HBSS with 5% CO_2 added were less apparent before 24 hours. After 24 hours MC MgO + 5% CO_2 exceeds both the NA MgOs + 5% CO_2 solubilities for both HBSS and DMEM (Figure 1). At 50 mg/L these differences were not present..

Discussion:

The correlation of short term solubilities of MgO to bicarbonate level in lung simulant fluid predicted the higher solubilities in DMEM than in HBSS at room temperature and 37°C – human body temperature (Figure 2.). The increase in pH while MgO dissolved (Figure 3) suggested the formation of a compound with a higher pH than the lung simulant fluid with bicarbonate causing the dissolution. Increased pH and the relation of bicarbonate to MgO dissolution suggest that the high pH compound might be a carbonate. Magnesium carbonate trihydrate, nesquehonite fulfills the requirements for stability at human body temperature (37°C) and body pCO_2 (~40 torr). Magnesium oxide, magnesium hydroxide, magnesite, Landsfordite, and hydromagnesite all lack sufficient solubility to account for the data, or stability at either human body temperature or P_{CO2}. Interestingly, magnesium hydroxide in water has a pH of ~10.5. Minimal differences were noted between solubilities of MgO at room temperature and 37°C for HBSS or DMEM, indicating that increasing temperature over this limited range had minimal or no effect on solubility in either MC or the NA MgOs.

The lessening of differences in solubility at later times (Figure 2) suggested the possibility of maximum solubility at earlier times in the lung simulant fluid that contained higher bicarbonate concentrations. Increased solubility of MgO at 37°C in DMEM relative to HBSS indicated that bicarbonate determined solubility at human body temperature and at room temperature. At 37°C and 5% CO_2, simulated ELF increased rate of solubility for HBSS (Figure

5), indicating that the higher bicarbonate from the 5% CO_2 at human body temperature increased MgO solubility at a level of bicarbonate below expected for human ELF at the end of expiration. At the higher bicarbonate concentration, this increased solubility was present only to 12-15 minutes in DMEM, indicating that there was sufficient bicarbonate present in DMEM alone to maximize MgO solubility, except at the earliest times.

NA™ MgO Plus dissolves more rapidly at 37°C that did NA™ MgO or MC MgO through 24 minutes of dissolution (Figure 4). This trend reflects NA™ MgO Plus's small nanocrystalline size and high surface activity in both simulant ELFs. These results suggested that the dissolution would be rapid and persistence short for the MgO particles deposited in deep lung that avoided macrophage phagocytosis and entered the interstitial portion of the lung.

MC MgO's increase in solubility for both HBSS and DMEM (Figure 2.**)** at >24 hours would have been expected to have less effect on early clearance, but would supplement overall clearance of MgO. The delay in response may have reflected the time needed for initial fragmentation of the crystalline agglomerates. During the time when MC MgO had higher dissolution, there appeared to be either a smaller diffusion layer reflecting higher crystal convexity, a higher concentration gradient reflecting less intra-crystal stagnation, or both. Alternatively, nanocrystals could have formed necks and re-aggregated, which would have been expected to slow their solubility. These data cannot distinguish among the alternatives that affect later clearance which would be needed at higher level fugitive exposures.

Nanocrystalline structure causes more rapid early dissolution (Figure 4). This would be expected to be most important if exposures were low level and clearance rapid. Alternatively, if exposures were higher and most clearance took longer to accomplish, bulk dissolution driven by bicarbonate would be expected to be more important and the dissolution by all crystalline forms – nanocrystalline and macrocrystalline would provide as rapid clearance as possible. In either case, risks of health effects especially with NA ™ MgO plus in the presence of substantial bicarbonate concentrations in lung simulant fluids would be minimal whether exposures were relatively low or somewhat higher. This research was partially funded through the award of a contract from the Marine Corps Systems Command to M2 Technologies Inc.