This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-96SR18500 with the U. S. Department of Energy.

DISCLAIMER

Adsorption of biometals to monosodium titanate in biological environments

Hobbs DT, Messer RLW*, Lewis JB*, Click DR, Lockwood PE*, Wataha JC*

Savannah River National Laboratory, Westinghouse Savannah River Company, Aiken, SC 29808

*Medical College of Georgia
Augusta, GA 30912-1100

Running Title: Adsorption of monosodium titanate to biometals

Corresponding author:

John C. Wataha DMD, PhD
Medical College of Georgia
Augusta, GA 30912-1100

Phone: (706) 721-2991
Fax: (706) 721-3992
E-mail: watahaj@mail.mcg.edu

Acknowledgement of Financial Assistance/Relationships
No outside financial arrangements or interests exist that would constitute a conflict of interest.

ABSTRACT

Monosodium titanate (MST) is an inorganic sorbent/ion exchanger developed for the removal of radionuclides from nuclear wastes. We investigated the ability of MST to bind Cd(II), Hg(II), or Au(III) to establish the utility of MST for applications in environmental decontamination or medical therapy (drug delivery). **Methods:** Adsoprtion isotherms for MST were determined at pH 7-7.5 in water or phosphate-buffered saline. The extent of metal binding was determined spectroscopically by measuring the concentrations of the metals in solution before and after contact with the MST. Cytotoxic responses to MST were assessed using THP1 monocytes and succinate dehydrogenase activity. Monocytic activation by MST was assessed by TNFα secretion (ELISA) with or without lipopolysaccharide (LPS) activation. **Results:** MST sorbed Cd(II), Hg(II), and Au(III) under conditions similar to that in physiological systems. MST exhibited the highest affinity for Cd(II) followed by Hg(II) and Au (III). MST (up to 100 mg/L) exhibited only minor (< 25% suppression of succinate dehydrogenase) cytotoxicity and did not trigger TNFα secretion nor modulate LPS-induced TNFα secretion from monocytes. **Conclusions:** MST exhibits high affinity for biometals with no significant biological liabilities in these introductory studies. MST deserves further scrutiny as a substance with the capacity to decontaminate biological environments or deliver metals in a controlled fashion.

Key words: Spectroscopy, auranofin, cytokines, monocytes, succinate dehydrogenase

INTRODUCTION

Monosodium titanate (MST) is an inorganic substance first prepared by Lynch, ***et al*** by a sol-gel process.[1] Modification of the original synthetic conditions produces particles that are roughly spherical with diameters ranging from 1-10 μm (Fig. 1). The particles have a 'fuzzy' appearance, which is a manifestation of a highly porous surface. The porosity of the surface extends about 500 nm into the particles, but the cores of the particles are discontinuous from the surface.

MST strongly adsorbs or ion exchanges with a number of metallic species in a variety of aqueous matrices.[2] For example, MST has high affinity for strontium and actinide ions in the presence of a high sodium salt matrix with a free hydroxide concentration in excess of one molar.[3] Testing indicates that the metal ions form strong inner sphere complexes through the oxygen atoms of the titania.[4] Thus, this material is an excellent sorbent/ion exchanger for the separation of the radionuclides (e.g., ^{90}Sr, ^{239}Pu, ^{237}Np and ^{238}U) from waste solutions from spent nuclear fuel reprocessing operations.[5]

The affinity of MST for biometals is largely unstudied, but MST may have utility for metal detoxification in biological or wastewater environments. To investigate metal sorption under conditions relevant to biological systems, we measured adsorption isotherms of MST for three biometal ions: Au(III), Hg(II) and Cd(II). Mercury and cadmium ions are toxic contaminants of both organisms and environmental systems.[6-8] A number of gold compounds have been used as therapeutic agents, particularly for the treatment of rheumatoid arthritis.[9] In the current study, we used loading isotherms to assess the affinity of MST to adsorb these three metal ions at pH 7 in both water (relevant to environmental applications) and a phosphate-buffered saline (relevant to living organisms). For gold, we assessed the reversibility of binding to determine if MST may be a plausible drug delivery device. Finally, we assessed the cytotoxicity and phagocytic activation potential of MST which has not been determined previously, but would be

critical to any biological or environmental application. Our results indicate that MST has binding and toxicological properties that are favorable for biological applications and further study.

MATERIAL AND METHODS

Titanate-MST Loading Isotherms

Monosodium titanate (MST, $NaTi_2O_5H$) was supplied as an aqueous suspension containing approximately 15 wt.% solids (Optima Chemical Group, Douglas GA). The pH of the aqueous suspension measured 11.7. Organic content of the suspension was less than 500 ppm. For all experiments, we adjusted the pH of the MST suspension to 7.0-7.5 using reagent grade nitric acid (Fisher).

Experiments to measure MST-metal ion adsorption were performed using deionized, distilled water (Milli-Q Element water purification system). Stock metal ions solutions were prepared in water or phosphate buffered saline (PBS) from $HAuCl_4.3H_2O$ (Sigma-Aldrich), $Cd(NO_3).4H_2O$ (Fisher), or $Hg(NO_3)_2.H_2O$ (Fisher). Loading isotherms were determined by mixing the pH-adjusted MST slurry and the metal stock solutions in clean plastic bottles. Control bottles containing no MST confirmed no sorption of the metals by the bottles. We varied the phase ratio of MST solids to metal salt solution from 15 to 3300. After combination, the mixtures of MST and metal salt solutions were sealed and incubated with shaking for 48 h at 20 ± 2°C (LabQuake Model #4002110 shaker, Barnstead /Thermolyne). After 48 h, the suspension was filtered through a syringe filter with a 0.45 µm polyethersulfone filter membrane. Filtrates were placed in clean plastic sample bottles and analyzed for gold or cadmium content using inductively-coupled plasma-emission spectroscopy (ICP-ES) or mercury by means of cold vapor atomic absorption spectroscopy (CVAA).

For gold, additional adsorption and desorption experiments were done to assess the potential of MST as a drug delivery device. First, we determined loading of a second gold compound used in the treatment of arthritis, auranofin (AF, triethylphosphinegold(I) tetraacetatothioglucose, Alexis Biochemicals) in PBS at phase ratios of 100 and 18.6 mL/g of MST. Second, we assessed the ability of Au(III) to desorb from the MST as follows. We

adjusted the pH of the MST slurry to a pH of 4.24 to promote initial adsorption of Au(III). MST (0.10 g) was combined with 10 mL of a 3.0 mM solution of $HAuCl_4$ in water, with the pH of the mixture adjusted to 4.35 with 0.1 N NaOH. The mixtures were sealed and incubated for 48 h as described above. The suspensions were centrifuged to remove the MST-Au product, then suspended this product in 10 mL of PBS and adjusted the pH to 7.0. These mixtures were sealed and incubated with shaking for an additional 48 h, after which the MST solids were removed from the PBS and the PBS and assayed for gold as described above.

Loading isotherms were constructed by plotting the amount of metal bound to the MST (mmol metal ion/mmol MST) versus the final concentration (mM) of metal in the MST-metal salt mixture. Approximate loading capacities were determined graphically by noting the concentration at which increases in metal loading did not result in increased MST loading and noting the amount of metal ion loaded onto the MST at that concentration. Errors in the points defining the loading isotherms were determined using standard methods of error propagation from the uncertainly in metal ion measurements. In some cases, saturation was not observed.

Cell-culture/Cellular Activity

Cytotoxic responses to the MSTs were assessed by exposing human monocytic cells (THP1, ATCC TIB 202) to suspensions of MST in cell-culture medium ranging from 0.1 to 100 mg/L for 24-72 h, after which cellular mitochondrial activity was estimated via succinate dehydrogenase (SDH) activity. Monocytic cells were chosen as prime targets of MST in any therapeutic application (e.g., blood decontamination) or because they would be exposed in tissues loaded with MST used as drug delivery devices. Furthermore, monocytes were used to assess the ability of the MST particulate to cause phagocytic activation (see next paragraphs).

THP1 monocytes were cultured in RMPI 1640 with 10% fetal bovine serum, with 2 mmol/L glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin, all at pH 7.4 (Gibco BRL). Cells (250,000 cells/mL in 0.2 mL) were plated in 96-well format (n = 8), adding MST from concentrated stocks and incubating for 24-72 h at 37° C, 5% CO_2, and 100% relative humidity.

After incubation, SDH activity was assessed via the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, Sigma, 1252) method as previously published. Controls received no MST, and SDH activity of controls were used as a basis for normalizing SDH activity of the MST-exposed cultures. MST concentrations of interest were compared with the controls using 2-sided t-tests ($\alpha = 0.05$).

To assess the ability to MSTs to activate or modulate secretory responses of monocytes, MSTs (1-30 mg/L) were exposed to THP1 monocytes (250,000 cells/mL) for 72 h (n = 3), after which medium was collected, centrifuged (1000 x ***g***) to remove cells and titanates, and tested for TNFα content (an inflammatory cytokine secreted from activated monocytes) using enzyme-linked immunosorbent assays (ELISA, R&D Systems, detection limit 4 pg/mL). Negative controls received no MST, and positive controls received 1 µg/mL of lipoloysaccharide (LPS, ***E. coli***, serotype 0111:B4, Sigma) for the last 6 h of titanate exposure. To assess the ability of the MST to modulate activated monocytic secretion, MSTs were added to cultures as above (n = 3), with LPS added for the last 6 h. In all conditions, the amount of secreted TNFα was expressed as a percentage of the +LPS controls, and conditions were compared using ANOVA and Tukey post-hoc analysis ($\alpha = 0.05$).

RESULTS

Adsorption

Results indicated that MST adsorbs Au(III), Hg(II) and Cd(II) from aqueous and PBS solutions and pH conditions between 7.0 and 7.5 . The capacity of the MST for Cd(II), Hg(II) and Au(III) varied considerably (Figs. 2-4). The highest capacity (0.35 mmol/mmol or 1.8 mmol/g) was that determined for Cd(II) dissolved in water (Table 1). In PBS the loading capacity (0.15 mmol/mol or 0.75 mmol/g) was much lower than that measured in water. The lower capacity reflects the much lower concentration of Cd(II) in PBS (0.18 mM) compared to that in water (2.0 mM).

Loading capacities for Hg(II) were $\geq$0.018 and 0.052 mmol/mmol MST in water and PBS, respectively. Loading capacities for Au(III) were $\geq$0.09 and 0.039 mmol/mmol MST in water and PBS, respectively. Unlike Cd, the concentrations of Hg(II) and Au(III) in PBS proved higher than those in water . We also measured the sorption of Au(I) upon contact of a PBS solution containing auranofin with MST at two different phase ratios. Gold sorption increased from 10% to 36% upon an increase in the quantity of MST contacted with the auranofin solution (Table 2).

Desorption

Because gold compounds have been used medicinally, we studied the desorption of Au(III) from MST to assess the potential of using MST as a drug delivery agent. Au(III) showed little tendency to desorb (1.2 - 2.3%) in an aqueous environment; however, 21% desorption occurred after 48 h in PBS.

Biological response

The MSTs themselves caused low cytotoxicity in THP1 monocytes (Fig. 5). Succinate dehydrogenase activity remained about 70% regardless of time of exposure (up to 72 h) or concentration of the MSTs (up to 100 mg/L). However, the SDH activity dropped by 20-30% at MST exposure concentrations of 1-10 mg/L, regardless of exposure time. These drops were reversed at concentrations above 50 mg/L, particularly at 48 and 72 h. The MSTs did not

activate TNFα secretion from the THP1 monocytes at any concentration tested (Fig. 6). Furthermore, MSTs at concentrations up to 30 mg/L did not alter LPS-induced TNFα secretion.

DISCUSSION

Adsorption isotherms for Cd(II), Hg(II) and Au(III) confirmed that each of these metal ions are sorbed by MST under conditions similar to those present in physiological environments (Figs. 2-4). The shapes of the adsorption isotherms were typical for those reported for the sorption of metal ions on metal oxides.[11] At low sorbate (metal ion) concentrations, the concentration of the metal ion loaded onto the MST increased linearly with the equilibrium concentration of metal ion in solution. With increasing solution concentration, sorbate loading increased rapidly. As the equilibrium sorbate concentration approach the initial sorbate concentration, the sorbate loadings approached a saturation value or loading capacity for the particular ion with MST at the the experimental conditions.

Based on the measured loading capacities, the relative affinity of MST for the three metal ions were; Cd(II) > Hg(II) > Au(III) (Figs. 2-4, Table 1). We observed this order in both water and PBS. For Hg(II) and Au(III) in water, we could not determine exact values for loading capacities as the loading values continued to rise at the highest phase ratios tested. Thus, the loading capacities are equal to or greater than the values reported in Table 1.

The theoretical capacity for MST based on the complete exchange of all sodium ions is 5.0 milliequivalents per gram of MST. For a divalent cation such as Cd(II) or Hg(II) and a trivalent cation such as Au(III), the theoretical capacity of the MST is 2.5 and 1.8 mmol/g I respectively, Thus, under the conditions tested in the current study, Cd(II) loaded to about 70% of its theoretical capacity in water and 45% in PBS. Similarly Hg(II) loaded to ca. 36% of its theoretical capacity in water and 16% in PBS and Au(III) loaded to ca. 27% and 12% of its theoretical capacity in water and PBS, respectively. Given these relative loading values, we conclude that MST exhibited a higher affinity for Cd(II) and similar affinities for Hg(II) and Au(III). This trend is consistent with the relative strength of metal-oxygen bonds. In general, Cd(II) forms stronger bonds with oxygen than either Hg(II) and Au(III).[12]

We attributed the trend of reduced loading capacity of the metals in PBS compared to water

to the formation of complex ions in the PBS solution. In water, the predominant metal ion species is the aquo ion, $M(H_2O)_6^{n+}$. In the PBS solution, the presence of chloride and phosphate will produce complex ions, such as the tetrachlorodiaquo ion, $MCl_4(H_2O)_2^{n-}$. For example, Cd(II), Hg(II) and Au(III) are known to form stable chloride complexes in aqueous solutions.[12] Since we observed lower loading of the metals in PBS compared to water (Table 1), we suggest that the complexed form of the metals exhibited lower affinity for adsorption or ion exchange with the MST compared to the aquo form of the metal ion.

Metal loadings typically measure between 1% and 10% of the theoretical capacity upon contact of MST with high level nuclear waste solutions.[13] The measured metal loading values in the current study were 25 – 70% and 12 – 45% of the theoretical capacity in water and PBS solution, respectively. The relatively high metal loadings indicate that MST exhibits high affinity for Cd(II), Hg(II) and Au(III) under near neutral pH and the presence of a buffered saline solution. Thus, we believe that MST may be ideally suited to remove metals from solution in physiological systems.

Testing also showed that the MST exhibits an affinity for auranofin, which is one of several gold compounds that are used therapeutically. This compound contains gold in the monovalent oxidation state, Au(I). Au(I) is bound to a phosphorus of the triethylphosphine ligand and a sulfur of the tetraacetatothioglucose ligand. Unlike tests with Au(III), higher uptake of gold was observed in the PBS (23%) solution than that measured in water (11%). This result suggests that the auranofin may interact with the PBS solution to produce a species that exhibits higher affinity for sorption or ion exchange with the MST.

In addition to metal removal, MST may also serve as a means to deliver therapeutic metals. To test this possible application, we sorbed Au(III) onto MST at a pH of 4, isolated the solids and then contacted the Au-loaded MST solids in separate tests with water adjusted to a pH of 7.3 and a PBS solution (pH = 7.3). In both tests, a portion of the gold desorbed from the MST and dissolved into the test media. Desorption of the gold proved higher in PBS (21%)

compared to water (1.2 - 2.3%). Since the test featured only a single contact time for desorption, we do not know if desorption would increase with longer exposure time. However, based on these results we conclude that release of metal ions can be accomplished with a metal-loaded MST. Our results are promising, given that Au(III) is not used therapeutically because its systemic toxicity is too high.[14]

MST showed mild cytotoxicity at concentrations between 0.1 and 50 mg/L (Fig. 5), but the recovery of SDH suppression for all three exposure times suggested some artifact. SDH suppression was slightly more pronounced with time, as might be expected. The apparent reversal of SDH suppression at 50 and 100 mg/L could have been from clumping of the particles, thereby reducing surface area and effective concentration. In any case, the SDH suppression was not severe by most standards used in these types of assays,[15] and was not sufficient to trigger or suppress LPS-triggered TNFα secretion (Fig. 6). Thus, preliminary results were positive overall for further investigation into possible use of MST in biological applications.

In summary, the current study demonstrated a potential application of monosodium titanate (MST) as a scavenger of biometals such as Cd(II) or Hg(II), and as a drug delivery source for Au(III). Although preliminary, tests for biological toxicity were favorable for further development of these unique materials.

ACKNOWLEDGEMENTS

The authors thank H. L. Thacker and the staff of the Analytical Development Section of the Savannah River National Laboratory for performing many of the metal adsorption experiments and the determination of metal concentrations, respectively. We also thank the Medical College of Georgia for their financial support of our SRNL-MCG collaboration.

REFERENCES

1. Lynch R, Dosch R, Kenna B, Johnstone J, Nowak E. The Sandia Solidification Process- a Broad Range Aqueous Solidification Method. IAEA Symposium on the Management of Radioactive Waste. Vienna, Austria. 1976;306-72.

2. Lynch RW (ed.). Sandia Solidifcation Process Cumulative Report. Technical Report SAND-76-0105, Sandia Laboratories, Albuquerque, NM, January 1976.

3. "Strontium and actinide removal from alkaline high-level waste solutions", Hobbs, D. T.; Pulmano, R. L.; Bronikowski, M. G.; Edwards, T. B.; Thacker, H. L.; Blume, M. S., Savannah River Technology Center, Westinghouse Savannah River Company, Aiken, SC, USA. Proceedings of the International High-Level Radioactive Waste Management Conference, 9th, Las Vegas, NV, United States, Apr. 29-May 3, 2001 (2001), 304-308.

4. "Mechanisms of Strontium and Uranium Removal from High-Level Radioactive Waste Simulant Solutions by the Sorbent Monosodium Titanate," Duff, M. C.; Hunter, D. B.; Hobbs, D. T.; Fink, S. D.; Dai, Z.; Bradley, J. P.; Environ. Sci. Technol. 2004; 38(1), 5201 – 7.

5. "Radiochemical Separations for the Pretreatment of High-Level Nuclear Waste Solutions at the Savannah River Site," Hobbs, D. T.; Peters, T. B.; Duff, M. C.; Barnes, M. J.; Fink, S. D.; Walker, D. D.; Proceedings of ICEM03; 9th International Conference on Environmental Remediation and Radioactive Waste Management; Oxford, England; ICEM03-4536, 1 – 6, ASME; 2003.

6. Goyer RA. Toxic effects of metals. In: Klaassen CD, Amdur MO, Doull J (eds.). Casarett and Doull's Toxicology. New York: Macmillan; 1986:582-635.

7. USHDSS Public Health Service. Toxicological Profile for Mercury, March 1999, Appendix A.

8. Drummond JL, Liu Y, Wu T-Y, Callas MD. Particle versus mercury removal efficiency of amalgam separators. J Dentistry 2003;31:51-8.

9. Simon LS. CMARDS in the treatment of rheumatoid arthritis: current agents and developments. Int J Clin Pract 2000;54:243-9.

10. Wataha JC, Hanks CT, Sun ZL. In vitro reaction of macrophages to metal ions from dentla

biomaterials. Dent Mater 1995;11:239-45.

11. Kinniburgh, D. G.; Jackson, M. L., "Cation Adsorption by Hydrous Metal Oxides and Clays" in ***Adsorption of Inorganics at Solid-Liquid Interfaces***, Anderson, M. A.; Rubin, A. J., Eds., Chapter 3, Ann Arbor Science Publishers, Ann Arbor, Michigan, 1981

12. Cotton, F.A and Wilkinson, G., 3rd Ed, ***Advanced Inorganic Chemistry***, Interscience, New York, 1972.

13. Hobbs, D. T.; Bronikowski, M. G.; Edwards, T. B.; Pulmano, R. L. Final Report on Phase III Testing of Monsodium Titanate Adsorption Kinetics. Technical Report WSRC-TR-99-00134, Westinghouse Savannah River Company, Aiken, SC, May 28, 1999.

14. Gromer S, Arscott LD, Williams Jr. CH, Schirmer RH, Becker K. Human placenta thioredoxin reductase. J Biol Chem 1998;273(32):20096-101.

15. Hanks CT, Wataha JC, Sun ZL. In vitro models of biocompatibility: a review. Dent Mater 1996;12:186-93.

Table 1. Adsorption Capacities of MST for Cd(II), Hg(II) and Au(III)

	H20 (mmol/mmol)	**H20 (mmol/g)**	**PBS (mmol/mmol)**	**PBS (mmol/g)**
Cd(II)	0.35	1.8	0.15	0.75
Hg(II)	>0.18	>0.90	0.052	0.26
Au(III)	>0.090	>0.45	0.039	0.20

Table 2. Sorption of Gold onto MST from a PBS Solution of Auranofin

Phase ratio[a] (mL/g)	% Removed	Loading (mmol/mmol)	Loading (mmol/g)
100	10	5.6E-05	2.8E-04
20	36	1.9E-04	9.4E-04

[a] Volume of PBS solution (mL) divided by weight of MST (g)

Figures/Figure Legends

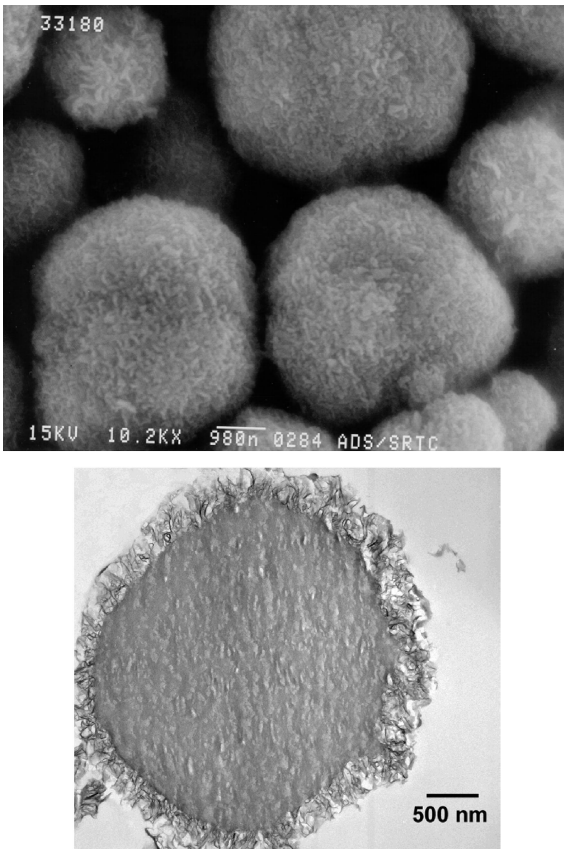

Fig. 1. Scanning electron microscope (***SEM, top***) and transmission electron microscopic (***TEM, bottom***) images of monosodium titanate (MST) particles. Bars on each micrograph indicate scale in nanometers (nm). The MST particles are roughly spherical with diameters ranging from 1-10 μm (SEM image). The particles exhibit a homogenous and amorphous inner core surrounded by an outer fringe region with a fibrous or appearance (TEM). See Reference 3 for details concerning microscopic analyses of the MST particles).

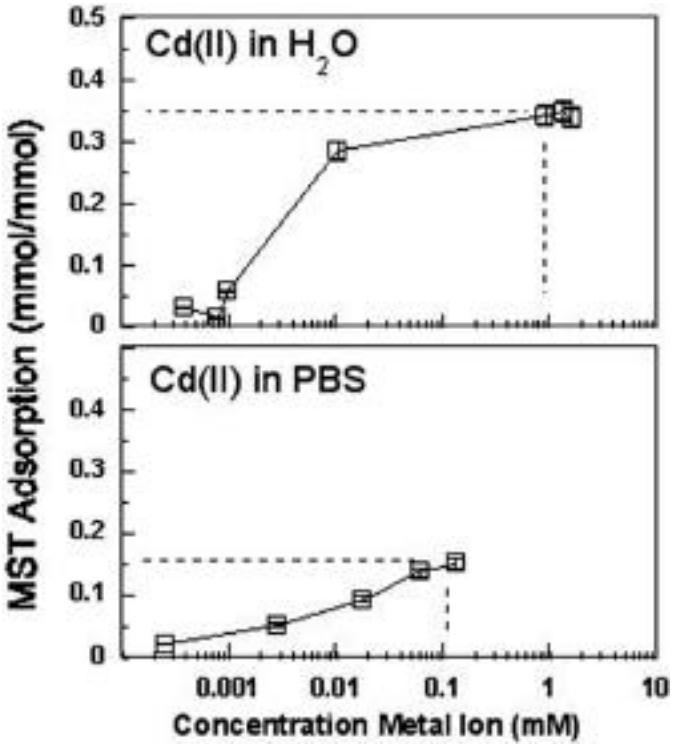

Fig. 2. Loading isotherms for Cd(II) onto monosodium titanate (MST) particles in water (top) or phosphate buffered saline (PBS, bottom) as a function of Cd(II) concentrations (mM). Adsorption was expressed as mmol of adsorbed Cd(II) per mmol of MST. Errors bars indicate errors in measurements calculated from standard error propagation formulae based on known uncertainties in the measurement of Cd(II). Dashed lines estimate MST saturation (horizontal line) and saturating concentration (vertical line).

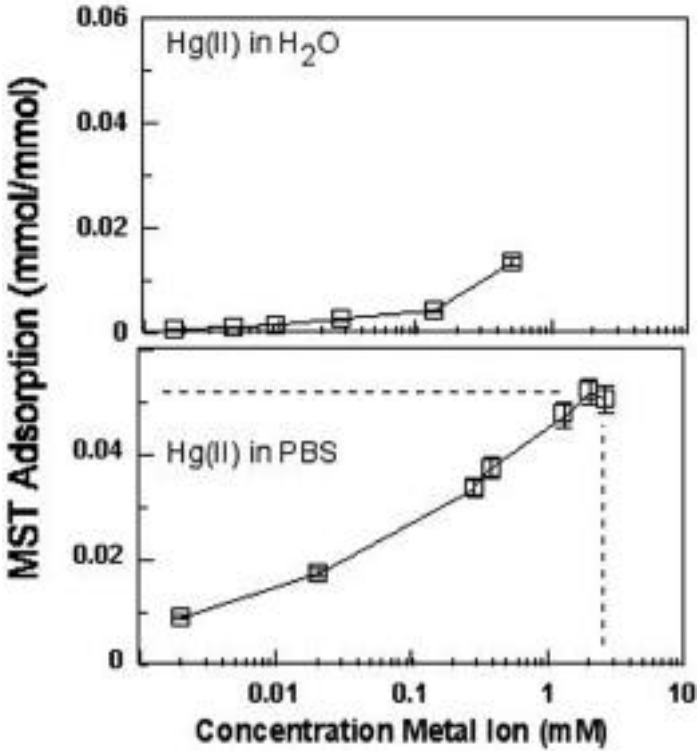

Fig. 3. Loading isotherms for Hg(II) onto monosodium titanate (MST) particles in water (top) or phosphate buffered saline (PBS, bottom) as a function of Hg(II) concentrations (mM). Adsorption was expressed as mmol of adsorbed Hg(II) per mmol of MST. Errors bars indicate errors in measurements calculated from standard error propagation formulae based on known uncertainties in the measurement of Hg(II). Dashed lines estimate MST saturation (horizontal line) and saturating concentration (vertical line). No adsorption saturation was observed in the water condition.

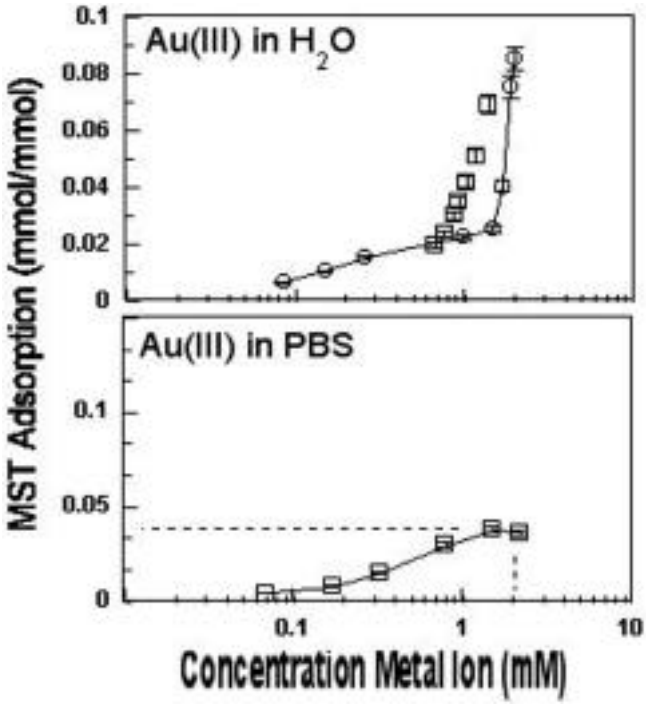

Fig. 4. Loading isotherms for Au(III) onto monosodium titanate (MST) particles in water (top, two separate experiments shown) or phosphate buffered saline (PBS, bottom) as a function of Au(III) concentrations (mM). Adsorption was expressed as mmol of adsorbed Au(III) per mmol of MST. Errors bars indicate errors in measurements calculated from standard error propagation formulae based on known uncertainties in the measurement of Au(III). Dashed lines estimate MST saturation (horizontal line) and saturating concentration (vertical line). No adsoprtion saturation was observed in the concentrations tested for the water condition.

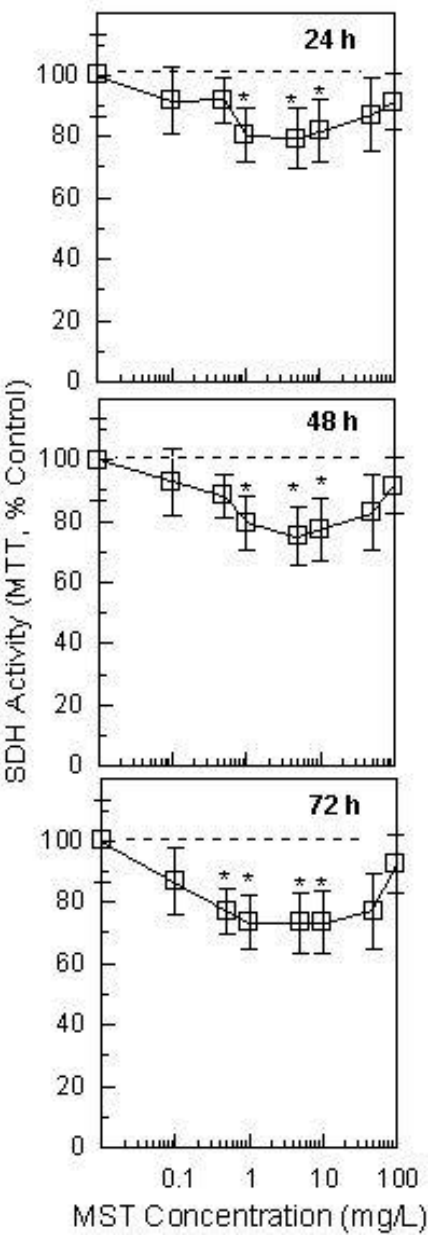

Fig. 5. Response of human monocytic cells (THP1) to monosodium titanate (MST) particle suspensions ranging from 0.01-100 mg/L in cell-culture medium. Cellular mitochondiral response was estimated after 24, 48, or 72 h using succinate dehydrogenase (SDH) activity and the MTT assay. SDH activity was expressed as a percentage of controls without MST (dashed lines, 100%). Error bars represent one standard deviation (n = 8 per condition). Asterisks indicate differences from the controls at each concentration (2-sided t-tests, $\alpha = 0.05$).

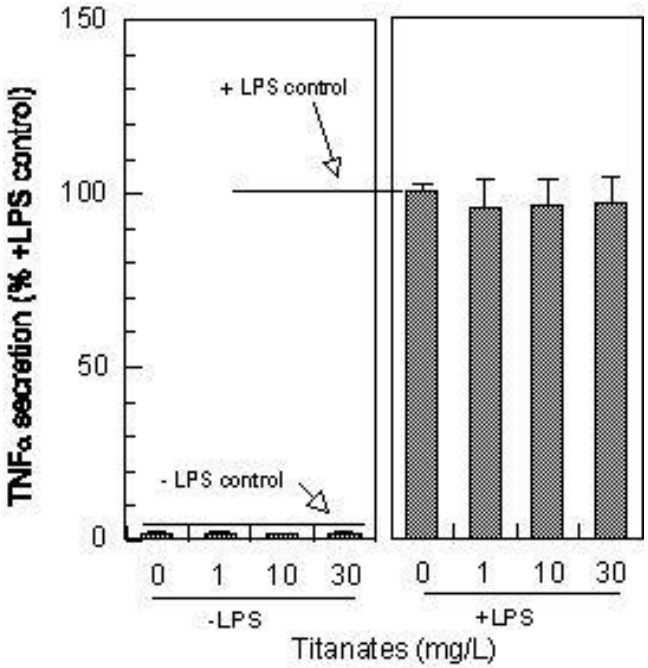

Fig. 6. Secretory response of human monocytic cells (THP1) to monosodium titanate particle suspensions ranging from 1-30 mg/L. THP1 cells were exposed to the titanates for 72 h, with or without lipopolysaccharide (LPS, 1 µg/mL) activation for the last 6 h. TNFα was measured in cell-culture medium supernatants using standard ELISA techniques. TNFα secretion was expressed as a percentage of +LPS controls (100%). Error bars (not always visible) represent standard deviations (n = 3). Within +LPS or –LPS groups, there were no statistical differences between titanate concentrations (ANOVA, Tukey post hoc, $\alpha = 0.05$).