IN VITRO MUTAGENICITY EVALUATION OF IRON OXIDE NANOPARTICLES BY THE BACTERIAL REVERSE MUTATION ASSAY

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ABSTRACT

Iron oxide nanoparticles are becoming highly promising tools for a wide spectrum of biomedical applications. The aim of our work was to determine the potential mutagenic effects of Fe_2O_3 and Fe_3O_4 nanoparticles (NPs), using bacterial reverse mutation assay. This is still the most widely used method for evaluating chemicals and environmental samples for mutagenic activity. In the bacterial reverse mutation assay, two genotypic variants of the *Salmonella typhimurium* strains (TA98 and TA1535) were used. Fe_2O_3 and Fe_3O_4 NPs were incubated with these two strains in four different doses both in presence and in absence of the rat liver metabolic activation system (S9); concurrently, appropriate positive controls were used to validate the test. The assessment of the results was based on the number of reverse mutants. The average number of reverse mutants per plate treated with NPs was less than double compared to negative control. Fe_2O_3 and Fe_3O_4 NPs proved to have no mutagenic effect in the bacterial cellular systems tested, in that they did not significantly increase the number of revertant colonies. Our findings indicate that Fe_2O_3 and Fe_3O_4 NPs are non mutagenic in bacterial reverse mutation assay under the present test conditions. These results are useful to expand our knowledge on the safety of iron oxides NPs.

INTRODUCTION

A variety of metal oxides have been developed as nanoparticles (NPs). Investigation of these NPs is currently a very interesting area of scientific research, due to a wide range of potential applications in cosmetics, electronics and medical diagnostics. A complete list of the potential applications of nanotechnology is too diverse to discuss in detail, but without doubt, one of the greatest values of this technology will be in the development of new and effective medical treatments (Sahoo et al., 2007). Iron oxide NPs have a high potential for use in several biomedical applications – including for example magnetic detection, hyperthermia and magnetic resonance imaging (Sun et al., 2007, Balasubramanyam et al, 2010) – and there have been a few studies related to toxicity examination of nanoparticulate iron oxides.
There are several methods to study the safety of nanomaterials. The bacterial reverse mutation assay is a simple biological test to evaluate the mutagenic potential chemicals and environmental materials (Ames et al., 1975). The aim of this study was to define mutagenic activity of Fe_2O_3 and Fe_3O_4 NPs using *in vitro* bacterial reverse mutation assay.

MATERIALS AND METHODS

Fe_2O_3 and Fe_3O_4 nanoparticles, and dimethylsulphoxide (DMSO) (purity > 99.5%) were obtained from Sigma–Aldrich Co. (St Louis MO, USA). The characteristics of nanoparticles declared by the manufacturer were as follows: Fe_2O_3 nanopowder, 29nm average particle size (TEM), BET surface area > $40m^2/g$; Fe_3O_4 nanopowder, spherical, < 50nm particle size (TEM), BET surface area > $60m^2/g$. The mutagens 2-aminoanthracene (2AA) (purity 96%),

sodium azide (SAZ) (purity > 99.5%), and 4-nitro-1,2-fenilendiamin (NPDA) (purity 98%) were likewise obtained from Sigma–Aldrich Co. *Salmonella typhimurium* tester strains TA98 and TA1535 were supplied by Xenometrix AG (Allschwil, Switzerland).

In this study, different concentrations (16, 63, 500, 1000 μg/plate) of Fe_2O_3 and Fe_3O_4 nanoparticles suspended and ultrasonicated in DMSO (50 μl) were added to an overnight culture (100 μl) and 10/20% of S9 mixture or sodium phosphate buffer (0.1 mM) (500 μl). The mixture was pre-incubated under shaking at 37°C for 30 or 60 minutes, then it was added to top agar (2 ml) containing 10% of histidine/ biotin (0.5 mM) for the tester strains, and was finally poured onto a minimal agar plate. After incubation at 37°C for 48 h, the plates were examined for the number of His+/wild revertant colonies. Viable cells were scored and the bacterial background lawn was observed by light microscopy (Maron and Ames, 1983). In each experiment DMSO was used as a negative control (NC=0) and various diagnostic mutagens: SAZ (1 μg/plate) for TA1535 without S9 mixture, NPDA (4 μg/plate) for TA98 without S9 mixture as well as 2AA (1-2 μg/plate) for both tester strains with S9 mixture were included as positive controls (PC).

A positive response in the test is defined as at least twofold increase in histidine-independent revertant colonies compared to the negative control in both strains (Ames et al., 1975). Each concentration was tested for toxicity and mutagenicity on six plates. The data were represented as mean±standard deviation (SD).

RESULTS

Within the tested concentration range (16, 63, 500 and 1000 μg/plate) neither Fe_2O_3 nor Fe_3O_4 NPs showed to induce any toxic effect, i.e. reduction in the number of revertant colonies and change in the auxotrophic background lawn in tester strains. The positive controls (SAZ, NPDA and 2AA) increased the number of revertant colonies, at least four times vs. vehicle, showing the capability of the system to detect a mutagenic effect.

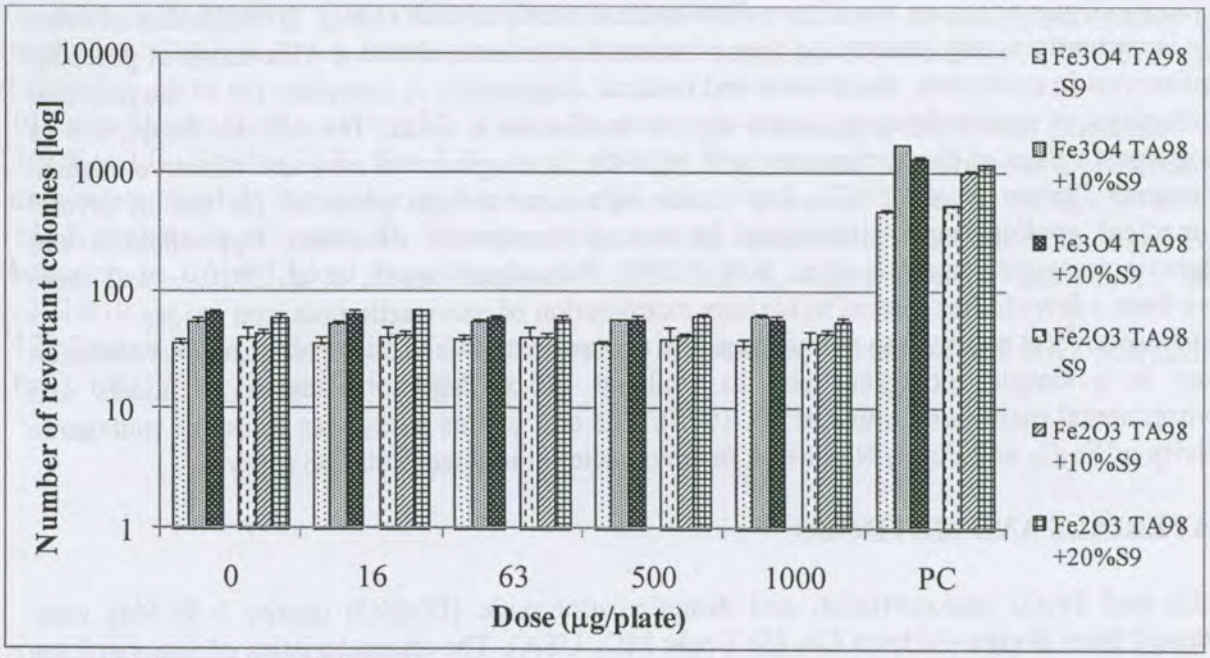

Figure 1 Mutagenic activity of Fe_3O_4 and Fe_2O_3 NPs in *S.Typhimurium* TA98 in presence and in abscence of S9 (Mean+SD). PC: positive controls are follows SAZ (1 μg/plate), NPDA (4 μg/plate) and 2AA (1-2 μg/plate).

The average number of reverse mutants was similar in the groups treated with Fe_2O_3 and Fe_3O_4 NPs and in the negative control. None of the revertant rates was greater than or equal to the twofold of the negative controls, and no concentration-dependent increase was observed (Figure 1 and 2).

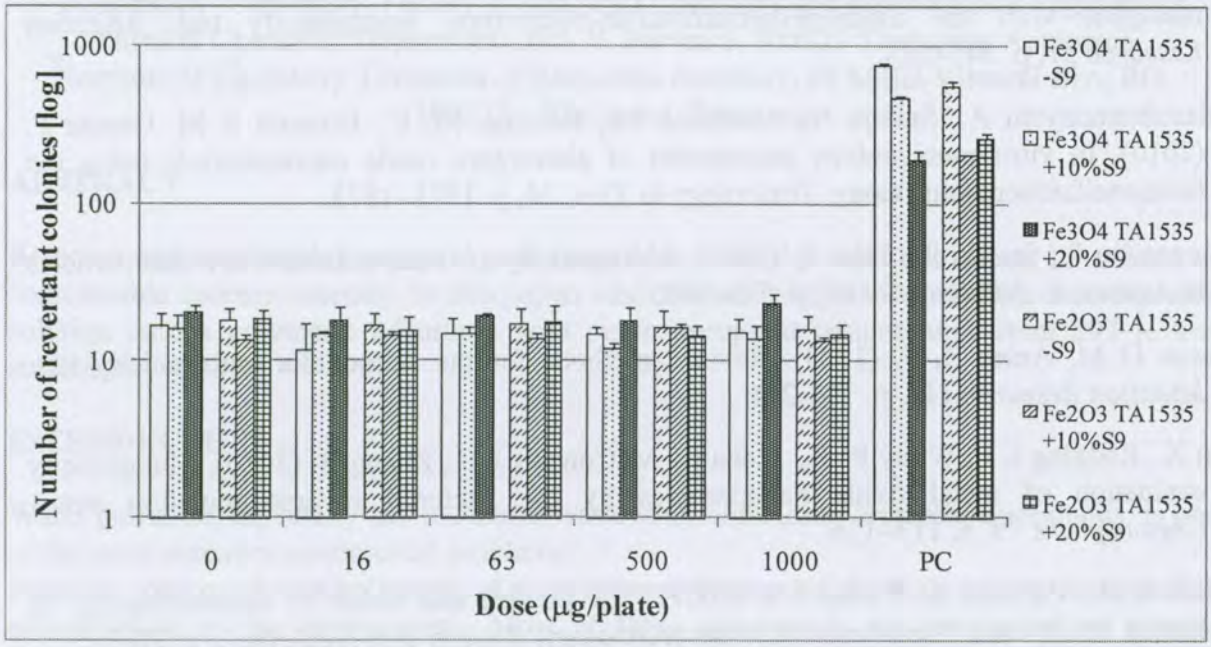

Figure 2 Mutagenic activity of Fe_3O_4 and Fe_2O_3 NPs in *S. typhimurium* TA1535 in presence and in abscence of S9 (Mean+SD). PC: positive controls or diagnostic mutagens are follows SAZ (1 µg/plate), NPDA (4 µg/plate) and 2AA (1-2 µg/plate).

The results were identical in absence and in presence of the rat liver metabolic activation system (S9). In the negative control group of each tester strain, the average number of reverse mutants was within the range of the historical control data of our laboratory, and the positive controls showed significant mutagenicity. These results demonstrated that Fe_2O_3 and Fe_3O_4 NPs are not mutagenic to the bacterial strains TA98 and TA1535.

CONCLUSION

The bacterial reverse mutation assay was conducted with Fe_2O_3 and Fe_3O_4 nanoparticles. Our results indicated that iron oxide nanoparticles were not toxic and not mutagenic on bacterial cells of the TA98 and TA1535 *Salmonella typhimurium* strains. Moreover, mutagenic activity of Fe_2O_3 and Fe_3O_4 NPs did not appear even in the presence of a cytochrome P450-based metabolic activation system (S9 mixture).

This implication is in accord with literature data on the mutagenic potential of metal oxide NPs. The mutagenic activity of Al_2O_3, Co_3O_4, TiO_2, and ZnO NPs to *S. typhimurium* TA97a and TA100 was found to be negative in the absence and presence of S9 mixture (Pan et al., 2010). On the contrary, iron-platinum (FePt) NMs tested in the Ames test (using Salmonella TA98, TA100, TA1535, TA1537 and E. coli WPA2uvrA strains) with and without S9 mixture were mildly positive in the TA100 strain without S9 mixture (Maenosono et al., 2007).

Iron oxide nanoparticles are an important group of nanomaterials with extensive potential of biomedical applications, e. g. as contrast agents in magnetic resonance imaging. Because of

such biomedical applications of iron oxide NPs, more detailed toxicological investigations are necessary to determine their potential toxic and genotoxic effects.

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