Genotoxicity analysis of different magnetite-based nanoparticles applied in chemical catalysis processes

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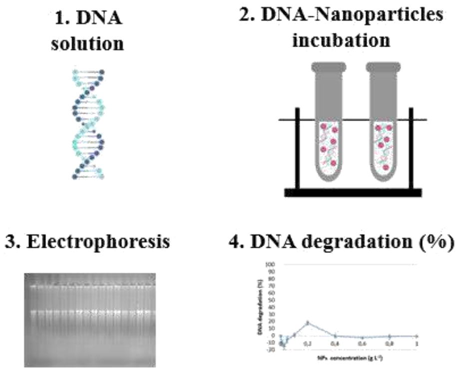

Eco-toxicity is an important consideration in understanding the potential environmental impacts of the release of nanomaterials into the environment. In this study, the potential genotoxicity of nanosized magnetic iron-based oxide particles used in the treatment of contaminated effluents by chemical catalysis processes such as Fenton reaction or photocatalysis was investigated using a simple method for the quantitative evaluation of DNA degradation. The selected nanoparticles were magnetite (Fe_3O_4), silica, citrate, poly (acrylic acid) and polyethylenimine coated magnetite ($Fe_3O_4@SiO_2$, Fe_3O_4@Citratete, Fe_3O_4@PAA, Fe_3O_4@PEI) and two different magnetite nanocomposites with TiO_2 and ZnO. The obtained results highlight the need for caution during the use and disposal of these manufactured nanomaterials, as DNA oxidation effects may be observed at very low concentrations.

Introduction

In the last decade, research has increased with the application of nanomaterials to the treatment of industrial wastewater using different advanced oxidation processes (AOPs) such as heterogeneous oxidation of Fenton or heterogeneous photocatalysis [1,2]. In addition, the use of iron oxide-based magnetic nanoparticles as solid catalysts presents great advantages as unique magnetic properties that allow easy recovery and reuse of the nanocatalyst when applying a magnetic field. However, these heterogeneous processes must ensure the complete retention of nanoparticles to ensure their use in long-term processes and also to avoid possible adverse effects on the environment.

Numerous studies have investigated the potential human health toxicity associated with nanomaterials [3,4]. Nevertheless, the study of the potential ecological risks of the release of nanomaterials into the environment is a growing area of research. In this sense, one of the most noteworthy reported method for the estimation of the nanoparticles potential effects is an *in vitro* assay capable of measuring DNA degradation by electrophoresis [5].

Objectives

This study compares the different magnetite-based nanoparticles genotoxicity results after the direct exposure of the selected nanomaterials with a specific DNA solution to estimate the pro-oxidant effects of these nanoparticles.

Methods

Deoxyribonucleic acid (DNA) from calf thymus (Sigma, Germany) at 0.025 mg mL^{-1} sterile water solution was prepared and stored at 4 °C up to seven days. This DNA solution was incubated in the presence of different concentrations of the selected magnetic nanoparticles (1-0.0125 g L^{-1}). After 1 h at 37°C, an electrophoresis assay was run. A positive control (without nanoparticles) was carried out for each case.

Samples of DNA-nanoparticles solution (10 µL) were mixed with loading buffer (1:4; 25 mg bromophenol blue, 10 mL Tris EDTA 1x buffer and 20 mL of glycerol; pH 8) and the electrophoresis test was performed in a 0.75% (w v^{-1}) agarose gel (Nztech, Portugal) prepared with Tris-Acetate EDTA buffer (TAE; Grisp, Portugal) and 0.03 µL mL^{-1} of GreenSafe Premium (Nztech, Portugal). The test was run for 1.25 h at 150 mV in PowerPac Universal (BioRad, USA). The resulting DNA bands were viewed using a molecular imager GelDOC XR+ (BioRad, USA) and analysed with Image Lab™ Software v5.1.

The results were expressed as the percentage of inhibition of DNA, calculated as follows,

$$\text{DNA degradation } (\%) = 100\text{-}\ \frac{\text{Intensity}_{\text{sample}}}{\text{Intensity}_{\text{DNA control}}} \text{x } 100$$

Where $\text{Intensity}_{\text{sample}}$ is the intensity of the band area of each sample and $\text{Intensity}_{\text{DNA-solution}}$ is the manually measured intensity of DNA solution control band. To ensure the reproducibility of the assay, duplicate of sample-DNA solution was performed for each nanoparticle sample. Also, duplicate of electrophoresis band sample was carried out, obtaining four replicates for each evaluated sample.

Results

The main characteristics of the selected nanoparticles: magnetite (Fe_3O_4), silica, citrate, poly (acrylic acid) and polyethylenimine coated magnetite ($Fe_3O_4@SiO_2$, Fe_3O_4@Citratete, Fe_3O_4@PAA, Fe_3O_4@PEI) and two magnetite nanocomposites with TiO_2 and ZnO are shown in Table 1.

The results of the DNA degradation after the incubation in presence of Fe_3O_4 nanoparticles (1-0.0125 g L^{-1}) are displayed in Figure 1. The obtained results indicated that low concentrations (0.05-0.0125 g L^{-1}) of magnetite nanoparticles present a negative effect on DNA stability.

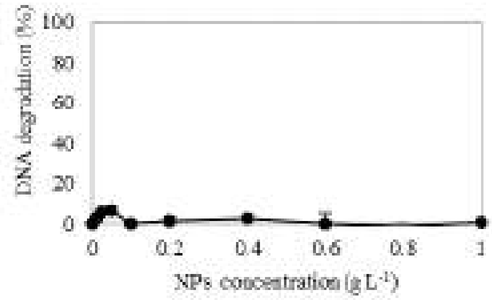

Figure 1. DNA degradation effect of Fe_3O_4 nanoparticles with concentration between 1 and 0.0125 g L^{-1}.

In the case of magnetite coated with different stabilizers, a similar effect was observed. The higher degradation percentages were obtained in the case of citrate coating with 7.2% of DNA degradation for a nanoparticle concentration of 0.0125 g L^{-1}.

In regard to the magnetic nanophotocatalysts (Fe_3O_4/TiO_2 and Fe_3O_4/ZnO), DNA damage alterations were observed not only at low concentrations (0.05-0.0125 g L^{-1}), but also at intermediate concentrations (0.6-0.1 g L^{-1}), reaching DNA degradation values of 17.8% for 0.2 g L^{-1} of Fe_3O_4/TiO_2. Therefore, a nanoparticle concentration effect on the increase of the DNA degradation was detected.

Conclusion

This work contributes to the understanding of the potential effects of the iron-based nanoparticles disposal may have on the environment, through the genotoxicity study of different nanoparticles in a wide range of concentrations. Therefore, this study underscores the importance of further research on the mechanisms and factors that increase toxicity to enhance nanomaterials use and disposal awareness.

Table 1. Characteristics of nanoparticles under study.

Nanoparticles	Characteristics	Size (nm)
Fe_3O_4	Magnetite magnetic nanoparticles	9.4 ± 1.4
Fe_3O_4@SiO_2	Silica coated magnetite nanoparticles	21.5 ± 1.3
Fe_3O_4@PAA	Magnetite stabilized with Poly (acrylic acid)	10.1 ± 2.4
Fe_3O_4@Citrate	Citrate coated magnetite nanoparticles	10.9 ± 3.9
Fe_3O_4@PEI	Polyethylenimine (25 kDa) coated magnetite nanoparticles	8.5 ± 2.3
Fe_3O_4@TiO_2	Titanium dioxide magnetic nanocomposite	9-10
Fe_3O_4@ZnO	Zinc oxide magnetic nanocomposite	15-30

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