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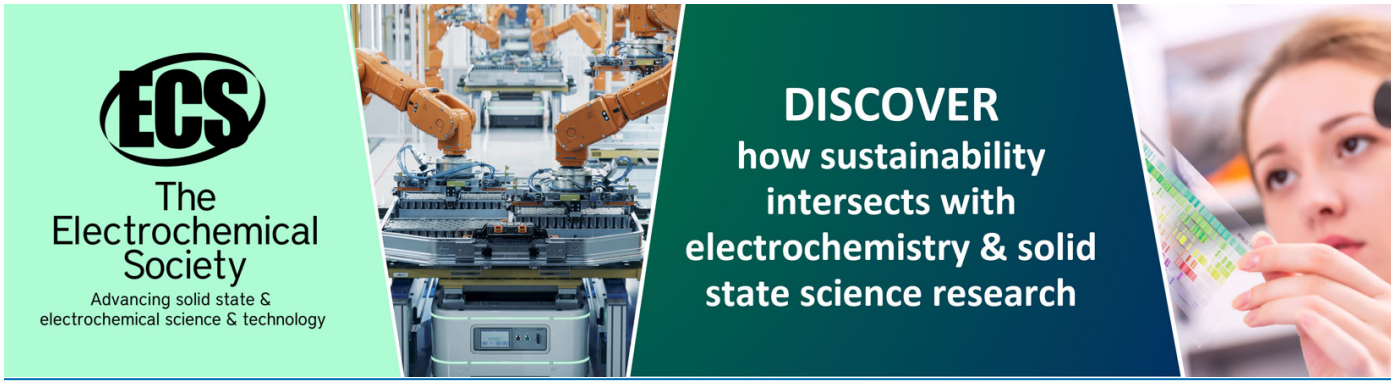

Silver metal nanoparticles study for biomedical and green house applications

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Abstract. Metallic nanoparticles (MNP) with diameters ranging from 2 to 100nm have received extensive attention during the past decades due to their many potential applications. This paper presents a structural and cytotoxicity study of silver metal nanoparticles targeted towards biomedical applications. Spherical Ag MNPs of diameter from 20 to 50 nm have been synthesized. The encapsulation of Ag MNPs inside pH-sensitive polymersomes has been also studied for the development of biomedical applications. A cytotoxicity study of the Ag MNPs against primary prostatic cancer cell line (PPC-1) has demonstrated a high mortality rate for concentrations ranging from 100 to 200mg/L. The paper will discuss the potential for therapeutic treatments of these Ag MNPs.

1. Introduction

Noble metallic nanoparticles (MNPs) with diameter ranging from 2 to 100 nm have received extensive attention during the past decades due to their many potential applications in fields such as catalysis, biomedicine and cancer therapy. The biocidal properties of Ag MNPs have been known for eons, therefore making the study of Ag MNPs even more attractive for biomedical applications[1]. In fact, among noble metals, silver nanoparticles possess the most efficient antibacterial properties with a very broad bactericidal and fungicidal activity spectrum and have been used for the development of new generation of antibacterial burn wound bandage, water filter, antibacterial clothing, etc. In addition, Ag MNPs have a combination of unique physico-chemical properties like chemical stability, catalytic activity, high thermal and electrical conductivity and non-linear optical properties which also make them very appealing for the development of ink and microelectronic applications. The encapsulation of MNPs into biologically friendly water soluble polymer with an extremely low cytotoxicity is a prerequisite for the development of biomedical applications in nanomedicine. In fact, the development of functionalized, nontoxic and biocompatible nanoparticles has focused a lot of interest for applications in cancer diagnostic, drug delivery and anticancer drugs [2] during the last two decades.

The biocidal properties of these Ag NPs have recently been studied against fungi and bacteria that develop on straw in order to protect straw bales that are used in green housing construction [3, 4]. This study has demonstrated the toxicity of these Ag NPs against bacteria, but fungi appear to be a more serious threat, and these Ag NPs need to be combined with another metal or chemical group to enhance their efficiency. In this paper we have studied the toxicity of these Ag MNPs against human cells and cancer cells via MTT assay. The encapsulation of MNPs into polymer with an extremely low cytotoxicity is a prerequisite for the development of biomedical application in nanomedicine. pH-sensitive polymersomes are specially convenient for intracellular delivery, because they are stable vesicles at physiological pH and release the cargo at acidic pH inside the endosomes, releasing the cargo in the cytosol [5]. For these reasons, the encapsulation of these Ag MNPs inside pH-sensitive polymersomes has also been studied for the development of biomedical applications.

2. Experimental

2.1. Synthesis

The synthesis of Ag NPs was carried out under air and is based on non-hydrolytic sol-gel method developed elsewhere [6, 7] and has been reported elsewhere [3]. The Ag NPs were encapsulated inside pH-sensitive polymersomes composed by the co-polymer $POEGMA_{20}$-$PDPA_{90}$ (poly oligo(ethylene glycol) methacrylate co-poly(2-(diisopropylamino)ethyl methacrylate) [8] following the protocol described by Chen et al. [9]. Briefly, the co-polymer dissolved in DMF (dimethylformamide) was mixed with the Ag NPs dissolved in methanol and dodecanethiol. Then water was added slowly to form the polymeric vesicles. The organic solvent was removed by dialysis and the sample was purified by centrifugation.

2.2. Characterization

XRD data were collected using a Bruker D8 Discover instrument equipped with a LynxEye detector. Cu (ka1=1.54056) radiation selected by a Ge (1 1 1) monochromator was used. Crystallite size analysis from the XRD data was carried out using full profile Scherrer methods in TOPAS, with a fundamental parameters peak shape. Transmission electron microscopy (TEM) studies were carried out on a probe corrected Titan G2 80-200 kV operating at 200 kV in STEM mode and a Tecnai 10, Philips (Netherlands) to assess the size, surface topology and morphology of Ag NPs and assembled Ag-polymeric nanoparticles, respectively. X-ray photoelectron spectroscopy (XPS) analysis was carried out on a Kratos Analytical Axis UltraDLD photoelectron spectrometer equipped with Al Ka X-ray source.

2.3. Cytotoxicity assays

Different concentrations of nanoparticles were studied via MTT assay (M). Primary prostatic cancer cell line (PPC-1) were seeded on day 0 at a density of 1000 per well in 96-well microtiter plates. On day 1, silver nanoparticles at different concentrations were added and incubation was continued for 48 hours. After 48 hours, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well (0.5 mg/ml; Sigma Aldrich), and plates were maintained at 37°C for 2 h. The medium was then discarded, and DMSO was added to each well to lyse the cells. Absorbance was measured using a multiwall spectrophotometer (Tecan, microplate reader). All MTT assay were repeated twice.

3. Results and Discussion

3.1. Structural characterizations

The XRD pattern of the prepared Ag MNPs (figure 1a) shows that silver nanoparticles are highly crystalline with the face-centred cubic structure of silver metal (JCPDS File No 87-0720) and no secondary phase of silver oxide structure is visible. From the XRD pattern an average crystallite size of 58 nm was calculated using the Scherrer method.

The morphology and size of Ag MNPs were also studied by (S)TEM. The HAADF-STEM electron micrograph of spherical silver nanoparticles is shown in figure 1b and gives an overview of the nanoparticles. Before dispersing it on a carbon coated copper grid for TEM observations, the solution had to be sonicated at room temperature for 2 min. STEM images revealed that particles are easily dispersed in ethanol and surfactant-free. The silver nanoparticle diameter ranges from 20 to 50 nm with a few bigger particles of around 100nm in size suggesting that the average crystallite size suggested by XRD is not misleading.

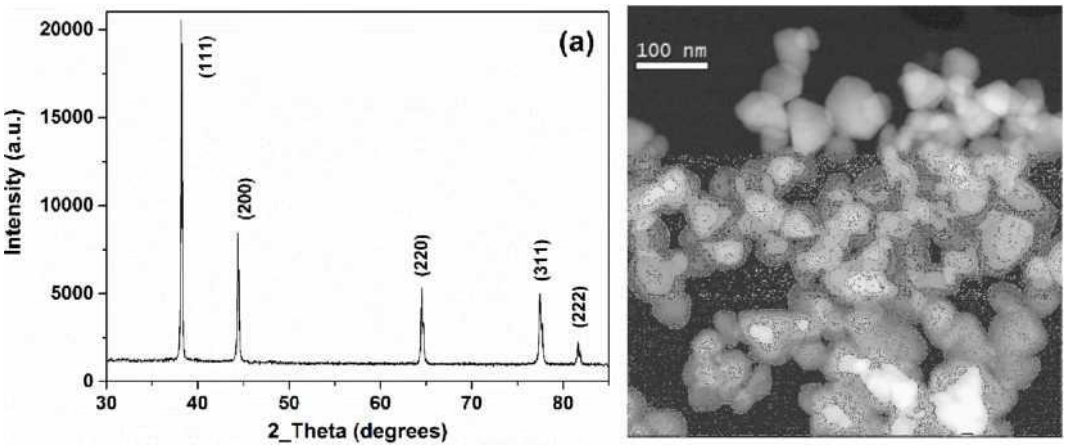

Figure 1. (a) XRD pattern of Ag metal nanoparticles, (b) STEM image overview of Ag MNP

The biocidal properties of these Ag MNPs have recently been reported [10], however, it has also been observed that the culture medium consisting of many amino acids that contain Sulphur groups shielded these biocidal properties due to the attachment of these molecules on the surface of the metal Ag NPs [4]. The metallic character of the surface promotes the bonding of Sulphur groups on the surface of Ag MNPs. To prevent such an attachment and shielding, the possibility of encapsulating these Ag MNPs with diameters around 10-50 nm inside pH-sensitive polymersomes composed by the co-polymer POEGMA-PDPA (poly oligo (ethyleneglycol) methacrylate co-poly(2-(diisopropylamino)ethyl methacrylate) [8] was studied. The formation of the hybrid metallic/polymeric nanoparticles was then investigated using TEM.

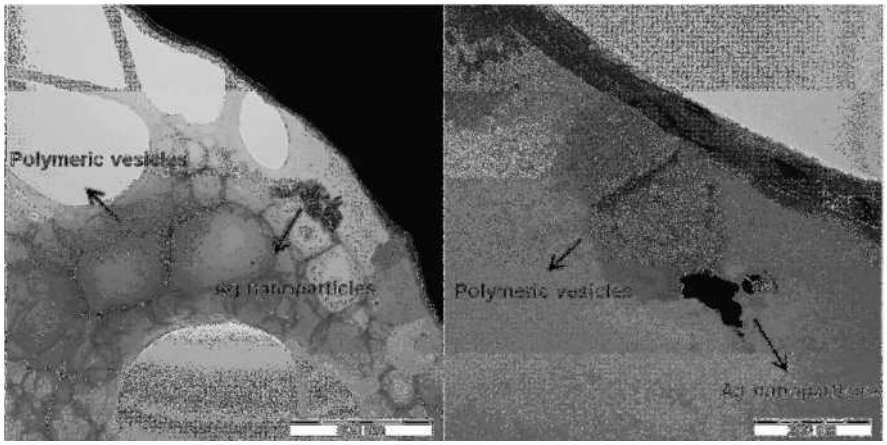

Figure 2. TEM micrographs of Ag NPs embedded into co-polymer POEGMA-PDPA vesicles. The Ag NPs are attached on the surface of the polymeric vesicles

TEM study shows that Ag MNPs are mainly localized outside of the polymer shell (Fig 9) and the Ag MNPs agglomerate on the surface of the vesicle. This study shows that the Ag MNPs need either to be functionalized to promote their encapsulation inside the polymer shell or a specific polymer containing sulfur or thiol groups should be used. We plan to test a surfactant that contains thiol groups that can attach on the metallic surface of the Ag MNPs. The encapsulation of the metal nanoparticles appears to be very important to enhance the efficiency of the metal nanoparticles for cancer therapy. The encapsulation inside other types of polymersome or inside gelatin shell will also be tested.

In order to confirm the nature of the surface of the Ag NPS, XPS measurements have been performed [3]. This study was performed 6 months after the synthesis and only binding energy peak from Ag metal were detected. Figure 3 provides the XPS spectra obtained for Ag 3d5/2, the

photoelectron peak corresponds to metal Ag and confirms the metallic nature of the surface of the Ag NPs. No photoelectron peak corresponding to silver oxide was detected. Due to the high surface to volume ration present in nanoparticles, any oxidation of the Ag NPs surface would have been detected by XPS.

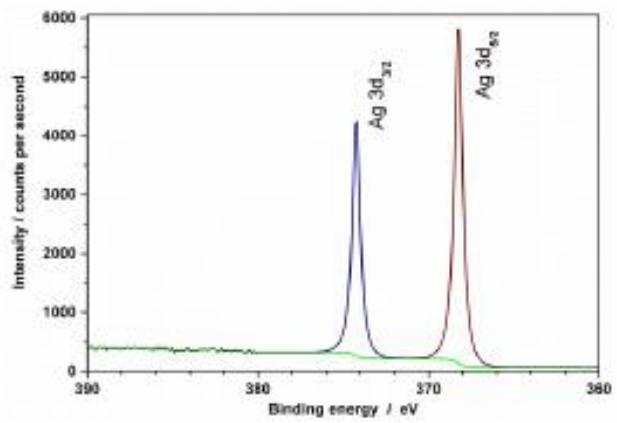

Figure 3. XPS spectra obtain on Ag NPs

3.2. Toxicity study

The toxicity of these Ag MNPs on Human Embryonic kidney (HEK 293) cells[11] as already been tested showing a moderate toxicity [3]. HEK cells are very commonly used for testing the toxicity of metal nanoparticles and the toxicity study has shown that mortality rate is over 50 % only for a concentration of silver nanoparticles of high concentration, which makes them a suitable material for biomedical application. Therefore, a similar cytotoxicity study has been performed on cancer cells and more particularly on the primary prostatic cancer cell line (PPC-1).

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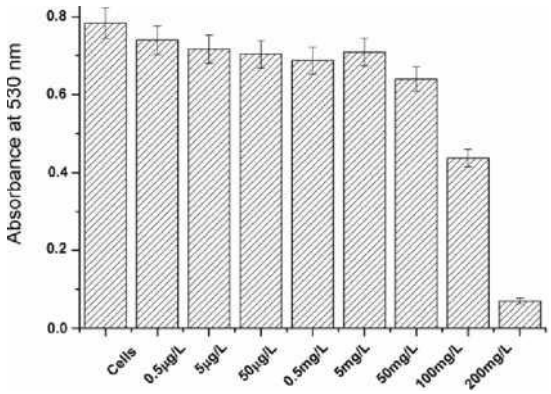

Figure 3. Toxicity test of silver metal nanoparticles on PPC-1 cells

Figure 3 shows MTT assays performed on Ag MNPs solutions of concentration ranging from 0.5 mg/L to 200 mg/L. Figure 3 shows the mean ± SEM of duplicate measurements of a representative sample of three independent experiments. This toxicity study on PPC-1 cells shows that mortality rate is over 44 % for a concentration of silver nanoparticles of 100 mg/L and shows a higher mortality rate (over 91 %) for a concentration of 200 mg/L. These results demonstrate that these Ag MNPs are potential candidates in the treatment of cancer, but their functionalization is needed.

4. Conclusions

In summary, we have reported on the synthesis of spherical silver nanoparticles with average diameters ranging from 20 to 50 nm for biomedical applications. The biocidal properties of these Ag MNPs have recently been demonstrated and the study of their encapsulation into pH-sensitive polymersomes was then performed for their potential use in cancer therapy. It has been shown that stabilisation of the metallic surface of the Ag MNPs requires the use of specific polymersomes that contain Sulphur or thiol groups. The cytotoxicity study shows that at a concentration of 100mg/L the mortality rate is over 44 % and increased to 91 % for a concentration of 200mg/L (200mg/mL). The synthesized Ag MNPs exhibit noteworthy cytotoxicity and show highly effective apoptotic activity against primary prostatic cancer cell line (PPC-1). These Ag MNPs therefore have high potential in therapeutic treatments and may be helpful for the development of anticancer therapeutics via their encapsulation into the appropriate polymersome.

Acknowledgement

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