In Situ Production of Polymer-Capped Silver Nanoparticles for Optical Biosensing

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Summary: We report on the preparation and spectroscopic characterization of a novel nanocomposite consisting of silver nanoparticles and a fluorescent polymer obtained by *in situ* ps laser ablation of a silver target. While the polymer is able to detect the fungus *Paecilomyces variotii* by fluorescence quenching, the composite with silver nanoparticles permits an interaction with both cell walls of spores and mycelia without any appreciable reduction of the overall fluorescence quantum yield.

Keywords: biosensors; laser ablation; mycelia; nanocomposite; poly(phenylenethynylene); silver nanoparticles

Introduction

The synthesis and study of nanocomposites consisting of conjugated polymers and nanoparticles of noble metals, particularly silver, has claimed attention for different applications going from nonlinear optics to biosensing.[1–2] Indeed, conjugated polymers are optimal candidates as transducer elements for optical biosensors, as they present specific and interesting optical properties due to the high π-electron delocalization along their backbone. Among them, phenylenethynylenes, which are strongly fluorescent compounds, have been previously proposed for the detection of *Escherichia coli*.[3] Such sensing devices, based on the quenching of the polymer fluorescence, are expected to take considerable advantage from the combination of the detection ability of the organic compound with the well-known bactericide properties of silver.[4–7] In this sense, we have recently demonstrated the combined effect of silver nanoparticles produced by chemical reduction and a phenylenethynylene for the detection and attack of fungus *Paecilomyces variotii*.[8]

The chemical synthesis of nanoparticles functionalized with semiconducting polymers usually involves many steps. In some cases, the interaction with the polymer is not direct, but the nanoparticles must be capped with an intermediate thiol-functionalized molecule with subsequent ligand exchange.[8] In other cases, the chemical reduction approach can require preliminary functionalization of the nanoparticles with the monomer, which is polymerized during a second step, for example by UV photoirradiation.[2] In contrast, physical methods, such as laser ablation, which represents a fast and easy way for the preparation of metal nanoparticles,[9] can be a convenient alternative to standard chemical reduction techniques for all those applications which require a high level of purity. In fact, it is completely free from reaction by-products.

In this work we report on a simple *in situ* method to obtain a nanocomposite of silver nanoparticles functionalized with a fluorescent poly(phenylethynylene) bearing tioesther

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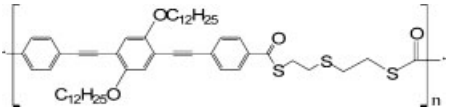

Figure 1.
Chemical structure of pPET3OC12-sqS.

flexible sequences, hereafter named pPET3OC12-sqS (Figure 1). The final goal is the development of a system able both of detecting the fungus *Paecilomyces variotii* by quenching the polymer fluorescence and of attacking the microorganism through the action of silver. The nanocomposites are prepared by direct picosecond laser ablation of a silver target immersed in a chloroform solution of the pure polymer. The ablation wavelength was 1064 nm, in order to avoid possible photodecomposition of the polymer itself.

This paper presents a spectroscopic characterization of the nanocomposite and preliminary results concerning its use for detection of the *Paecilomyces variotii*.

Experimental Part

The synthesis and physicochemical characterization of pPET3OC12-sqS, whose structure is shown in Figure 1, has been reported elsewhere.[10] The polymer was dissolved in spectroscopic grade chloroform with concentration ranging from 0.5 to 1 g/L.

The silver-polymer nanocomposites were obtained by focussing the fundamental (1064 nm) wavelength of a mode-locked Nd-YAG laser (EKSPLA PL2143A: rep. rate 10 Hz, pulse width 25 ps) on a silver target. The pulse energy was 15 mJ and the ablation time ranged from few minutes to one hour. The focussing conditions of the laser beam were maintained constant in all the experiments and the diameter of the laser spot on the silver target was fixed at 1.4 mm. The silver target (99.99%, purchased from Goodfellow) was placed in a 1 cm × 1 cm quartz cuvette and was kept 2 cm in front of the focal plane of the laser beam. We used 2 mL of polymer solution and the liquid column above the target was 2 cm. More details of the experimental set up are described in ref. [11].

We recorded UV-Vis spectra with a double beam spectrophotometer (Lambda19 Perkin Elmer) one day after the preparation of the suspensions. Fluorescence spectra were obtained with a Perkin Elmer LS 50B spectrofluorimeter, by exciting the polymer and the nanocomposite suspensions at 395 nm. Quantum yields (QY) were obtained according to the procedure reported in ref. [12]. Quinine sulfate (QY at 310 nm = 0.54) was used as standard and the fluorescence was excited with 385 nm.

The particle dimension and shape were evaluated by TEM analysis. TEM samples were obtained by casting few drops of the diluted suspensions on fomvar grids. Due to the fact that chloroform attacks the fomvar film, the suspensions were dried and re-suspended in toluene. The images were recorded with a HRTEM JEOL2010, 200 KV.

For the microbiological tests, cultures on potato dextrose agar of the fungus *Paecilomyces variotii* were incubated for 7 days at 28 ° C. For the fungus detecting assay, 15 μl of a 1 g/L solution in THF of either the polymer or the nanocomposite were added to 1.485 mL of the culture already suspended in water. In this way we obtained a concentration of 10 μg of polymer per mL of fungal suspension. The tests were made in eppendorf vials of 2 mL. Each assay was repeated three times. The suspension was shaked strongly and then incubated statically at 30 °C for two hours. After that, the suspension was centrifugated (5 minutes at 10,000 rpm), it was washed twice with sterile water and then re-suspended in 1.5 mL of sterile water. The interaction with the fungus was observed by Laser Scanning Confocal Microscopy (LSCM, Carl-Zeiss Pascal 5), in dual channel. The obtained micrographs are the sum of the images taken in fluorescence and reflection. The excitation source was the 458 nm line of an Ar laser (200 mW).

Fluorescence lifetime was measured by time-resolving the sample photolumines-

cence with a streak camera (Hamatsu C5680) after excitation with 150-fs long laser pulses, 360 nm in central wavelength, from a kHz-repetition rate parametric amplifier (Lightconversion TOPAS, pumped by Quantronik Integra C femtosecond amplifier). The overall time resolution in the employed configuration is 40 ps. The excitation laser pulses are focused down to a 150 micron spot on the sample. The emission, before being time dispersed in the streak tube, is spectrally dispersed by an Acton 2500i spectrometer, using a 150 grooves/mm grating.

Results and Discussion

Figure 2 shows a TEM image of a nanocomposite obtained by 40 minutes laser ablation with 15 mJ per pulse in a 1 g/L polymer solution. It is a typical example for all the nanocomposites studied in this work. We did not observe any precipitate. This demonstrates that the polymer can stabilize the Ag nanoparticles. This stabilization is expected to take place through coordination between the metal and the sulphur atoms of the polymer tioesther sequences. The metal nanoparticles exhibit a strongly irregular shape and the TEM images show several big polymer-metal aggregates. Due to these characteristics, a statistic to obtain particle mean diameter and dispersivity was not performed. However, we could evaluate that particle diameter ranged from 20 to 30 nm, much larger than the value obtained in analogous samples prepared by chemical reduction ($\cong$ 10 nm).[8]

Figure 3 shows the absorption and fluorescence spectra of a polymer solution and of a suspension. The excitation wavelength was 395 nm. The spectra of the Ag-containing suspension (dashed and dot-dashed curves) are compared with those of the pure polymer before the ablation (continuous and dotted curves). A blue shift of the absorption and fluorescence maxima in the presence of Ag is evident. In particular, the absorption peak corresponding to the polymer conjugated backbone passes from 395 nm to 384 nm in the Ag-containing suspension and the fluorescence maximum shifts from 488 nm to 470 nm.

Due to perfect spectral overlap between the Ag plasmon band and polymer absorption, the effect of Ag nanoparticles is not

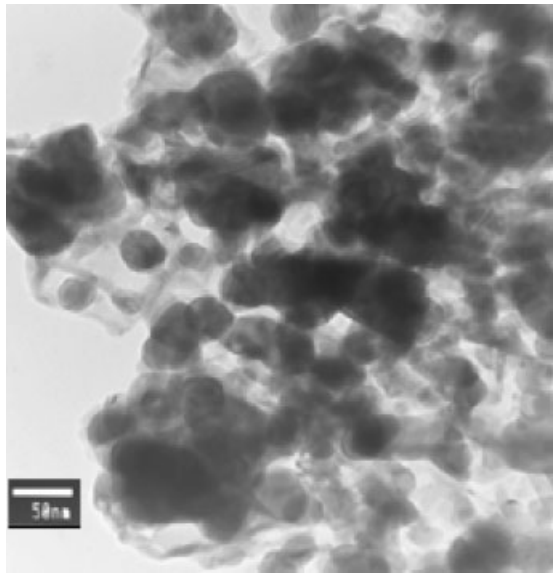

Figure 2.
TEM image of a nanocomposite obtained by 40 minutes laser ablation with 15 mJ per pulse.

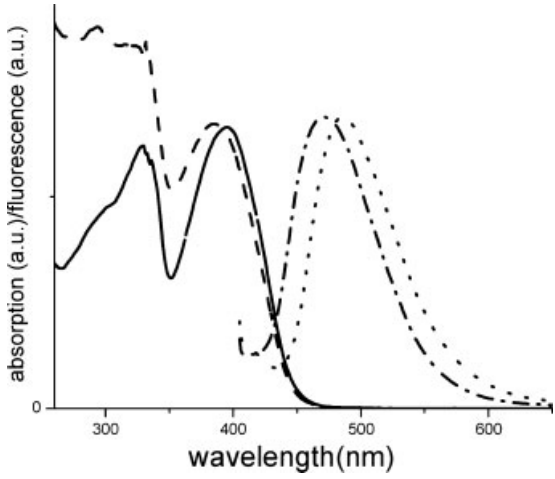

Figure 3.
Fluorescence and absorption spectra of a solution of the pure polymer in chloroform (dotted and continuous curves, respectively) and of an Ag-containing suspension (dot-dashed and dashed curves, respectively).

directly observable in the 350–450 nm region of the spectrum. However, the increase of the absorption below 350 nm in the case of the Ag containing sample, although not thoroughly clarified, is to be related to the presence of silver in the suspension, being observed also in the spectra of Ag nanoparticles in pure chloroform.

The measurement of QY did not give any evidence of reduction, due to the presence of the metal, at least within the experimental error, which is around 20%.

Further studies on the fluorescence properties of the nanocomposite were performed by fluorescence lifetime spectroscopy. Figure 4a,b shows the fluorescence spectra obtained by excitation of the polymer solution (Figure 4a) and of the nanocomposite suspension (Figure 4b) with femtosecond pulses at 360 nm. The blue shift of the fluorescent emission is confirmed. Also in this case the fluorescence maximum is at 488 nm in the polymer solution and shifts down to 470 nm in the Ag-containing suspension. Figure 4a,b also shows the behaviour of both samples with different incident fluencies. No fluency dependence of the spectra was detected in the whole accessible range, from as low as a few $\mu J/cm^2$/pulse, up to tens of mJ/cm^2/pulse, when coherent nonlinear effects can start to appear. Figure 5 shows the low-

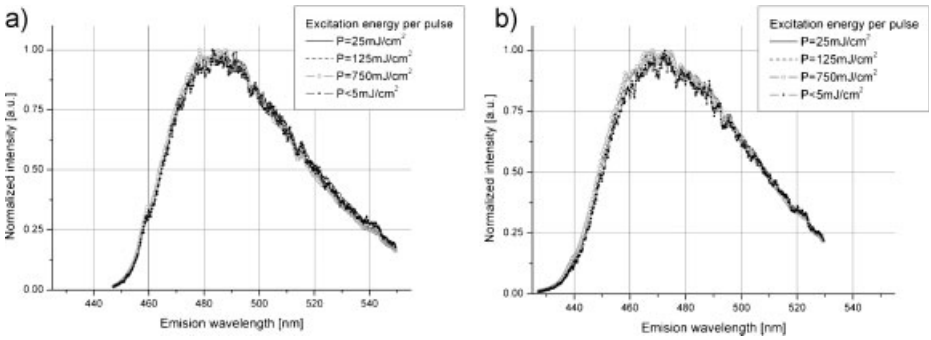

Figure 4.
Time integrated emission spectra obtained by 360 nm femtosecond excitation and different fluencies with: a) a solution of the pure polymer in chloroform; b) the suspension of Figure 2.

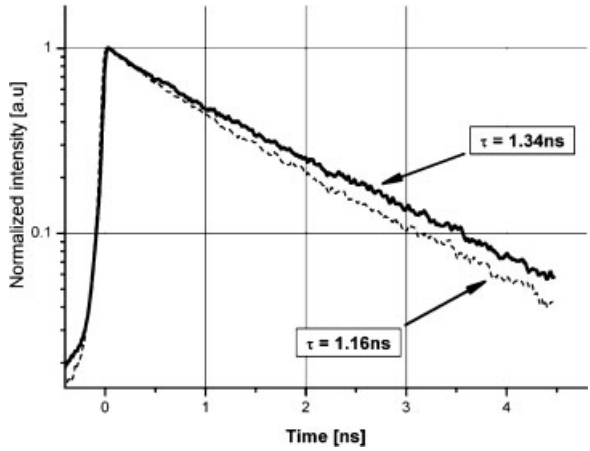

Figure 5.
Low fluence (<5 mJ/cm^2) fluorescence lifetime spectroscopy under femtosecond excitation at 360 nm of: a) a solution of the pure polymer in chloroform (dashed grey curve); b) the suspension of Figure 2 (continuous black curve).

fluency fluorescence lifetime results corresponding to the samples of Figure 4. The difference in the decay time and QY in absence (grey curve) and in presence (black curve) of silver is of the order of 15%, a difference large enough to be detected, but low enough to confirm the negligible effect of silver addition to the fluorescence properties of the polymer.

The previous experimental results (i.e. spectral blue shift and lack of quantum yield reduction in the presence of Ag) are consistent with a decrease of the polymer conjugation as a consequence of the coordination with the metal. Indeed, such coordination with large and strongly irregular metal nanoparticles can cause defects in the polymer chain, which in turn can interrupt the electronic free path. The reduced polymer conjugation also corresponds to a narrower localization of the exciton and a consequent reduction of the

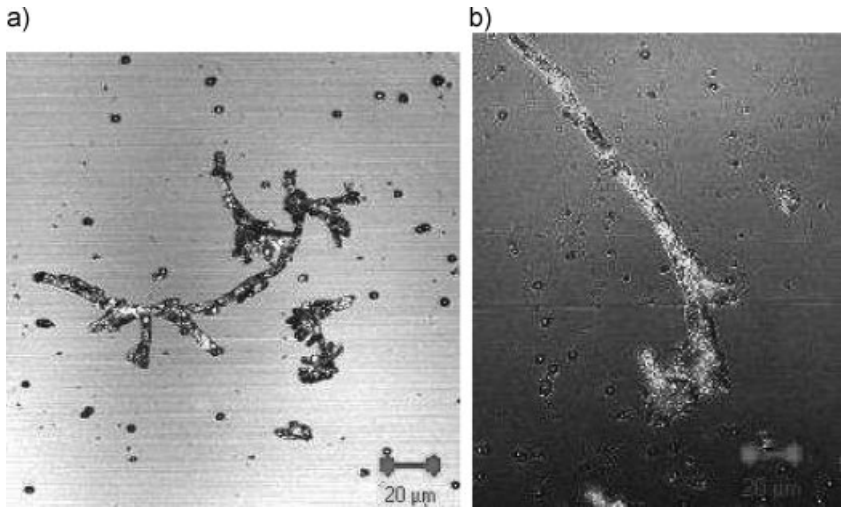

Figure 6.
Laser Scanning Confocal Microscopy image of: a) a solution of the pure polymer in chloroform; b) the suspension of Figure 2 after contact with P. Variotii. Green color: emitting part of the samples.

probability of non radiative de-excitation of the molecule through the metal, with no appreciable change of the QY with respect to the case of pure polymer samples.

The spectral blue shift had not been observed with nanocomposites prepared by following a fully chemical route.[8] However, in that case, the presence of dodecanthiol as the capping agent for the Ag nanoparticles with no direct coordination of the polymer with the metal, and the reduced particle dimensions impaired significant effect of the metal on the polymer conjugation.

The interaction of our samples with fungus *Paecilomyces variotii* is illustrated in Figure 6a,b, which shows the fluorescence images obtained by LSCM of a solution containing the pure polymer and the fungus (Figure 6a) or the nanocomposite and the fungus (Figure 6b). The green colour of the images indicates the fluorescent species and highlights the interaction with the fungus (which itself does not emit). The pure polymer, apparently, interacts only with the cell wall of the mycelium of *Paecilomyces variotii*, while the Ag-containing nanocomposite interacts strongly with both spores (small globular structures in the picture, which represent the reproductive part of the fungus) and cell walls of mycelium, thus increasing its potential application as a biosensors.

Conclusions

The paper presents a novel metal-polymer nanocomposite obtained by ps laser ablation of a silver target in a chloroform solution of the organic compound. The polymer, which is a fluorescent poly(phenylene ethynylene) bearing tioesther flexible sequences, could stabilize the silver nanoparticles most likely through coordination of sulfur atoms with the metal. This method is faster and easier than analogous fully chemical preparation routes and guarantees a higher level of purity of the final composite, being free from reaction by-products.

The direct interaction of the polymer chain with the metal and the large dimensions and irregular shape of the nanoparticles causes a blue shift of the absorption and fluorescence spectra of the nanocomposite with respect to pure polymer solutions. This blue shift was explained in terms of defect formation and consequent reduction of the conjugation length. This effect can also explain the negligible influence of the metal on the polymer QY, which can be related to a narrower localization of the exciton.

Preliminary tests of the nanocomposite for the detection of the fungus *Paecilomyces variotii* showed that this novel material is able to detect the fungus efficiently, by quenching of the polymer fluorescence.[8] Moreover, while tests performed with the pure polymer showed that the organic compound interacts only with the cell wall of mycelium, we found that the Ag-containing nanocomposite exhibits a strong affinity to both spores and mycelium of the fungus. In this sense, the use of silver, which preserves the fluorescent properties of the poly(phenylene ethynylene), seems to be promising for the development of a biosensors. Further experimental tests are in progress to investigate the expected antimicrobial properties.

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