Molecular characterization of drug nanocarriers based on Plasmon Enhanced Spectroscopies: Fluorescence (SEF) and Raman (SERS)

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Introduction

Metallic nanoparticles are ideal materials for developing novel analytical methods, since they have shown excellent optical properties due to excitation of Localized Surface Plasmon (LSP), which result in strong absorption bands and an enhancement of local electromagnetic fields [1]. Such near-field enhancement is profited by molecular spectroscopic techniques such as Raman and fluorescence, consequently improving their corresponding detection sensitivities. Thus, Surface-enhanced Raman Scattering, SERS [2], and Surface-enhanced Fluorescence, SEF [3], have become appreciated high-sensitivity label-free detection methods, with imaging capabilities [4]. In fact, interaction of the antitumoral drug emodin with silver nanoparticles has been previously studied in our group [5]. On the other side, metallic nanoparticles are also useful as drug nanocarriers [6]. Here we present our recent developments in applying SEF (and SERS), using silver colloids as plasmonic substrates, to the detection of different drugs: a) the emodin (EM) embedded in porous silicon (PSi), a biodegradable material of interest in drug delivery [7], and b) the NSAIDs indomethacin (IM) and ketorolac (KT) in solution.

Experimental methods

Silver colloids (50 nm mean diameter silver nanoparticles) were prepared using hydroxylamine hydrochloride as reduction agent [8]. PSi matrices, with mean pore size of 60 nm, were obtained by electrochemical attack of silicon in a solution of HF in ethanol [9]. All fluorescence and Raman experiments were recorded on a Renishaw Raman *in Via* Microscope system, with laser excitations at 325, 442, 532 y 785 nm. In the case of emodin experiments, atmospheric and vacuum conditions at room temperature were used to infiltrate emodin-silver nanoparticles complexes into PSi matrices. The drug was loaded after adsorption on metal surface, alone, and bound to bovine serum albumin (BSA). Methanol and water were used as solvents.

Results

While we verified that pristine emodin didn´t penetrate the PSi channels (usually PSi requires a previous functionalization to be able to load drugs), the system emodin-Ag nanoparticles did, as we confirmed through SEF spectra of cross-section of porous silicon layers taken with 1 μm spatial resolution [9]. A maximum fluorescence enhancement factor of 24 was obtained when protein was loaded bound to albumin, and atmospheric conditions of inclusion were used. A better penetration was obtained using methanol as solvent when comparing with water. Complexes of emodin remain loaded for 30 days after preparation without an apparent degradation of the drug, although a decrease in the enhancement factor was observed.

Before loading PSi with indomethacin or ketorolac it is necessary to have a deep knowledge of physicochemical and spectroscopic properties of the drugs in solution. As there is a lack of spectroscopic data about IM and KT, we have carried out a deepest investigation of the absorption and fluorescence properties (steady state and time resolved) of IM and KT in different solvents as well as a correlation of them with the corresponding Raman spectra obtained in solution. Additionally, as the drugs delivered orally have to survive encounters with various pHs (2 in stomach and above 8 in duodenum), we have also studied the spectra in the pH range of 1–9, in order to get data about their stability in these conditions because it is essential for a better understanding of the physiological processes involved. Moreover, we have also characterized the corresponding spectra after addition of a silver colloid to the solutions, aiming to find the optimal experimental conditions for getting SERS and SEF spectra of both drugs in solution.

Conclusions

SEF (and SERS) could be employed as label-free high sensitivity detection techniques to *probe* drug delivery and drug release. The joint use of both techniques provides information about the molecular species adsorbed on silver surface at different pHs values. Emodin adsorbed on a silver colloid has been loaded in a PSi silicon matrix without previous surface functionalization.

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