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Surface-enhanced Raman and fluorescence joint analysis of soil humic acids

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ABSTRACT

Surface-enhanced Raman scattering (SERS) and surface-enhanced fluorescence (SEF) combined emissions were used in this work to the analysis of humic acids (HA). This study examined HA structure at different pH and HA concentrations and assessed the structural differences taking place in HA as a result of various amendment trials. Raman and fluorescence emissions behave in opposite ways due to the effect of the metal surface on the aromatic groups responsible for these emissions. The information afforded by these techniques can be successfully employed in the structural and dynamic analysis of these important macromolecules. The surface-enhanced emission (SEE) spectra, that is the sum of the Raman and the fluorescence emissions, were acquired by using both *macro-* and *micro-*experimental configurations in order to apply imaging and confocal Raman and fluorescence spectroscopy techniques on the analysis of HA.

1. Introduction

Humic substances (HS) are the most important organic components of soil and the most widely distributed organic products of biosynthesis on the face of the earth [1]. In addition to the quantitative importance of HS, these substances act as a sink and source of C, they influence the soil fertility through the release of nutrients and affect the fate of contaminants leading to soil remediation [2,3]. Although HS are widespread in soils and water, during a long time their structure remained unknown, but in the last years the knowledge on these compounds has notably increased [2,4–6]. HS are divided into two main groups: humic acids (HA) and fulvic acids (FA) each displaying a different solubility in acidic media [7].

The inherent complexity of HS has seriously limited the application of more traditional optical spectroscopies, such as Raman and fluorescence spectroscopy, in structural and dynamic studies of HS. Fluorescence spectroscopy has been used so far in the characterisation of HA by several authors [8–11]. However, the high complexity of HS limits the application of this technique to these compounds. Raman spectroscopy is a technique, which has been widely applied, in structural studies of organic molecules and biological compounds [12]. However, normal Raman spectroscopy cannot be employed in the structural characterisation of HS due to the intense fluorescence emission, which overlaps the Raman bands.

Over recent years, several optical spectroscopy techniques (Raman, IR and fluorescence) have undergone a renaissance due to the notable characteristics of metal nanostructures (MNs) where localised surface plasmon (LSP) can occur. LSP leads to a remarkable local electromagnetic field enhancement owing to the high absorption of light in the vicinity of metal nanoparticles and thus induces a huge enhancement of

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spectroscopic signals, mainly in the case of emission signals of molecules placed in the proximities of the surface [13–15].

Raman scattering is greatly enhanced as a consequence of LSP. For this reason, the surface-enhanced Raman scattering (SERS) technique can be successfully applied to the study of highly fluorescent molecules in water, due to the fluorescence quenching occurring on the metal surface via a charge-transfer mechanism [16]. This effect enables Raman to be applied to the characterisation of HS [17–20].

SERS spectra can provide important information on the HS structure in function of factors such as the humification degree [21], extraction and purification processes [22], the soil amendment trials [23], HS extraction fractions [17,24] and pH [25]. The growing importance of SERS in the analysis of HS has recently been highlighted in several reviews dealing with techniques applied to the analysis of HS [26–28]. SERS has also been used to examine pesticide–HA interaction [29]. However, SERS is not a quantitative technique because of effects such as the resonance Raman and the adsorption on surfaces [30], although these characteristics can also serve to study structural and dynamic processes undertaken by macromolecules in the presence of surfaces [31].

The surface-enhanced fluorescence (SEF) technique has not been employed as much as SERS because of charge-transfer effects, which take place on the surface and which, in turn, lead to an actual quenching of the fluorescence. SEF effect is also induced by LPR near a metal surface [32,33]. However, the net effect seems to vary depending on the distance between the fluorophore and the surface, as well as on the intrinsic quantum yield of the fluorophore [34]. In general, the fluorescence is not quenched if the molecule is relatively far from the surface. The estimated optimal distance for a good SEF enhancement is achieved above 100 Å [35,36]. At distances further than this value, SERS and SEF signals can be simultaneously emitted and registered for molecular species placed in the vicinity of MNs [36–38]. Since the average size of HA is larger than this value [39], the fluorophores included in the HA structure could be good candidates to give rise to intense SEF + SERS joint emission spectra, without the intrinsic quenching observed on a surface. This is possible due to the fact that the HS cross-sections of either Raman or fluorescence are of the same order on MNs, and thus, a surface-enhanced emission (SEE) is seen for HS adsorbed on these substrates (Fig. 1, bottom). Furthermore, the SEF emission affords information on the HA macromolecules adsorbed onto a surface,

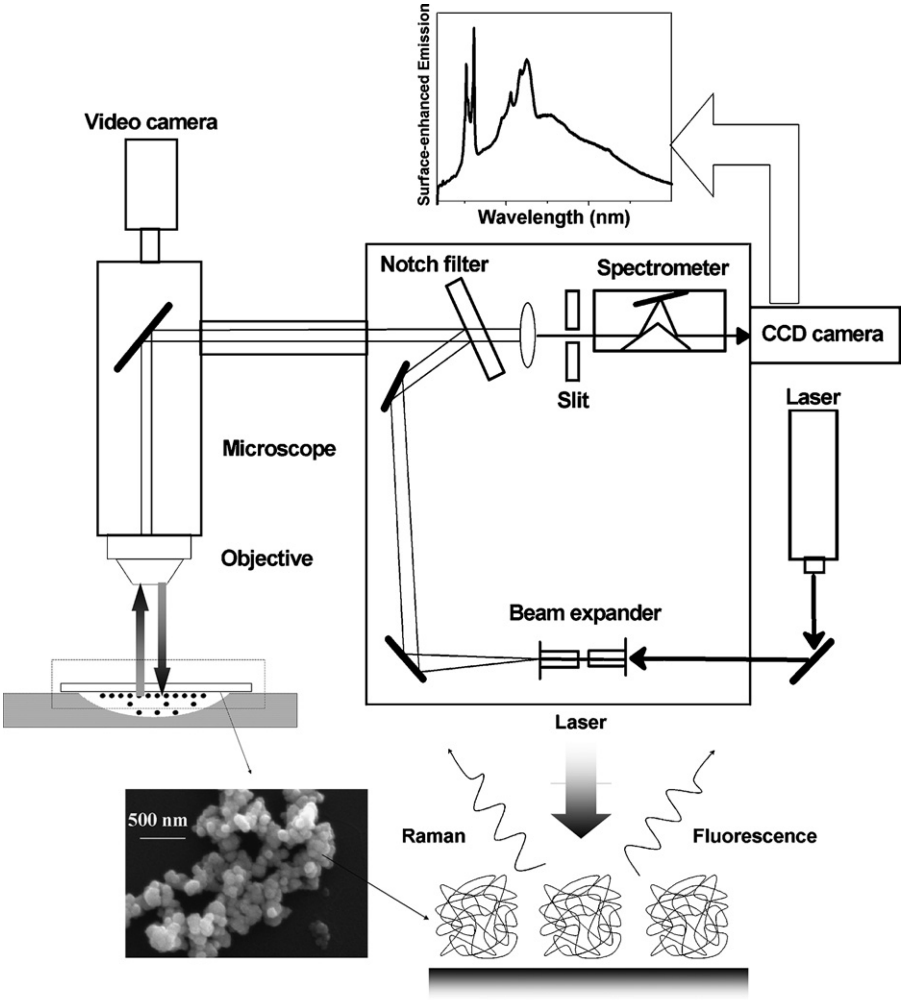

Fig. 1 – Top: experimental set-up for SEE measurements. Bottom left: a SEM image of typical Ag nanoaggregates. Bottom right: scheme displaying the adsorption of HA micelles and the emission of Raman a fluorescence combined process.