Characterization of X-ray irradiated cell culture media by means of Surface-Enhanced Raman Spectroscopy

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Cell culture media (CCM) are an essential part of the cell culture processes guaranteeing optimal cell growth [1]. It is also known that CCM are not stable and can modify their composition due to chemical reactions. Monitoring CCM is important for avoiding degradation that can negatively influence cellular processes [2] and also because it can give useful information during the interaction of cells with external agents. In this context, to examine cell media during cell exposure to ionizing radiation is particularly interesting since it would enable to obtain useful information on the complex processes occurring during the interaction between cells, cell culture media and radiation. Nowadays a wide spectrum of analytical techniques is available for monitoring variations in CCM composition [2], including optical spectroscopy and nuclear magnetic resonance (NMR) spectroscopy [3]. In particular, Surface enhanced Raman spectroscopy (SERS) has been already adopted for the detection of trace melamine in CCM for protein pharmaceutical manufacturing [4], for monitoring CCM degradation [5, 6] and pH changes [7].
The proposed contribute is about the study by SERS of the effects of X-ray radiation on two different kinds of Dulbecco's modified eagle media, used as representative CCM, namely DMEM-A and DMEM-B. DMEM samples were exposed to different X-Ray doses (0, 2, 4, 6, 8, 10 Gy). DMEM-B samples in contact with cells were also investigated. The SERS measurements were performed by using a Jobin-Yvon system from Horiba Scientific ISA (Edison, NJ, USA) described in details elsewhere [8] and commercial SERS substrates. SERS measurements were performed by placing a volume of 1 µl for each DMEM samples on the substrate. SERS spectra collected from complex media typically show a smeared background signal. In order to enhance the signal readability and attenuate background and noise components, an automatic numerical treatment based on the wavelet algorithm was used [2]. In order to determine the basic vibrational modes that contribute to the Raman signal, the spectra were analyzed in terms of convoluted Lorentzian functions by using a best-fit peak-fitting routine of GRAMS/AI program based on the Levenberg-Marquardt nonlinear least-square method. The raw spectra detected for all the investigated samples are quite noisy and show a large unfeatured background, as expected (data not shown), efficiently removed by the data analysis procedure. In Figure 1a a typical denoised spectrum obtained for unexposed DMEM-B samples not in contact with cells are shown. The large number of modes observed and labeled in the figure is related to the complex content of DMEM samples and can be assigned to different vibrational modes of proteins, lipids and other CCM components. Details about the assignments of the main peaks can be found in Refs 5 and 6. The positions and relative intensities of the observed modes are in agreement with what expected for CCM and can be used to analyze medium components. In Figure 1(b) a representative spectrum detected for unexposed (0 Gy) DMEM-B samples in contact with cells is shown and the main peaks are labelled. Specific peaks due to cells are observed (red Lorentzian peaks) and labelled in addition to CCM-related features. SERS spectra detected from DMEM samples irradiated with X-rays show significant differences for increasing X-ray doses. Also changes related to contact with cells can be observed, in agreement with the results reported in Ref. 7. The obtained results confirm the potentials of SERS as effective tools for cell culture media monitoring.

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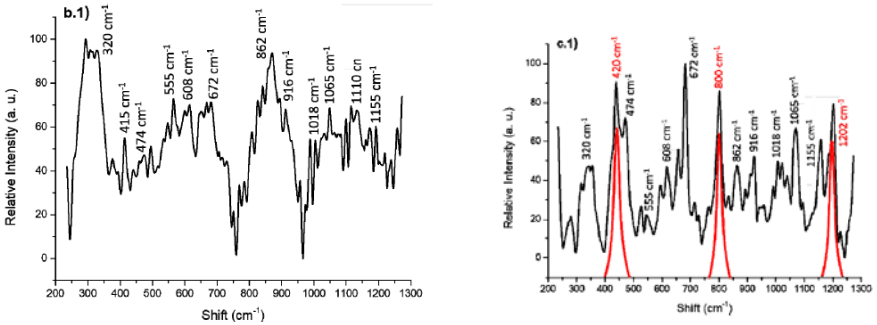

Figure 1(a). SERS spectrum for unexposed DMEM-B; (b) SERS spectrum for a DMEM-B sample after contact with cells (200-1300 cm-1 region). Red curves and labels indicate cell-related features.