(BIO)ANALYTICAL STRATEGIES FOR ASSESING NANOPARTICLES TOXICITY

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In the last years, nanoparticles (NPs) have received a great attention for their use and applicability in many new consumer products. A recent estimate suggests that more than 1000 NP-containing consumer products are currently on the market. In addition, their ability to advance science with novel analytical and medical tools also make them relevant to both physical and life sciences.

NPs are generally defined as spheres with a diameter between 1 and 100 nm. This small size gives them unique properties, especially because their specific surface area is larger and their reactivity is increased or different compared to bulk materials. These specific features are often linked with a potential toxicity. The products and applications of NPs include electronics, optics, textiles, medical applications, cosmetics, food packaging, water treatment technology, fuel cells, catalysts, biosensors and agents for environmental remediation. As a result of these applications, exposure of NPs to the environment and humans are becoming increasingly widespread. Consequently, different metals in the form of NPs have gained an increasing access to tissues, cells and biological molecules within the human body.

To date, the impact of NPs exposure to human health and the environment is not fully assessed. Research efforts to evaluate the toxic potential of NPs have presented some serious and far-reaching challenges and there remains an urgent need for well-designed studies that will generate data so that risk assessments for NPs can be conducted.

In this communication, we present different analytical and bioanalytical strategies to study the interaction of different NPs, including AgNPs, TiO_2NPs, SeNPs and quantum dots (Cd/Se), with cells and living organisms in order to assess their potential toxicity. To correlate any toxic reaction with a NP type, it is indispensable to investigate if NPs are attached to the cell surface or if they enter cells. If NPs are found in cells, their localization in different compartments such as endosomes, lysosomes, mitochondria, the nucleus or the cytosol, may also provide some answers regarding their potential toxicity. For these purposes, we have used ICP-MS and microscopic techniques. Our results show that the degree of internalization as well as the cellular localization is highly dependent on the type and size of the NP studied, rather than the type of cell.

We have also carried out bioanalytical assays to evaluate specific cellular pathways or cellular responses to NPs exposure including cell viability, cell proliferation and cell migration assays, and flow cytometry-based methods to determine the degree of apoptosis after exposure or changes in the cell cycle pattern. Additionally, we have used mass spectrometry-based high throughput discovery platforms for the identification of key proteins involved in the molecular mechanisms related to cell-NPs interaction.

Our results have shown that while some of the NPs induce a general stress response and result highly toxic at relatively low concentrations, other NPs exert their toxicity through specific pathways. In this case, such NPs could represent promising chemotheraputic agents when used under controlled conditions and modified with targeting ligands.

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SISTEMAS ANALÍTICOS TOTALES EN SUPERFICIES TERMOCRÓMICAS

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El desarrollo de soluciones integradas sensibles, versátiles y baratas es uno de nuestros retos. En particular, esta comunicación presenta el desarrollo de sistemas analíticos totales basados en la tecnología de etiquetaje de discos compactos, empleando plataformas y lectores de disco convencionales como única instrumentación. El recubrimiento termocrómico de estos discos les confiere propiedades muy atractivas para el desarrollo de nuevos sistemas de análisis. Esta tecnología presenta además un conjunto de características superiores a las mostradas por

los sistemas sensores basados en la tecnología CD, DVD o Blu-ray, admitiendo la funcionalización química y derivatización física de la superficie del disco. Además, permite irradiar selectivamente el área de la superficie de sensado que contiene el ensayo, acelerando así el proceso de lectura y reduciendo el volumen de los registros. Por otro lado, dada la elevada potencia del láser (40 mW, 780 nm), alrededor de 40 veces mayor que la de los lectores/grabadores de discos convencionales, permite incrementar la magnitud de las señales registradas. Otras de las prestaciones que hacen interesante a la tecnología desarrollada es la ausencia de limitaciones ópticas, superando ampliamente las prestaciones de los discos compactos estándar. De esta forma, las propiedades ópticas del resto de la plataforma no interfieren en el proceso de irradiación y lectura del disco. Este hecho amplía las posibilidades de efectuar nuevos diseños y aplicaciones analíticas que incluyen diversas etapas. Asimismo, a partir de discos de etiquetaje se obtienen plataformas de ensayo con un elevado grado de sofisticación mediante procedimientos muy sencillos. El uso de esta tecnología permite realizar ensayos en materiales con propiedades mejoradas para el sensado (silicio, PMMA, PDMS, poliestireno, nitrocelulosa, metales, geles), permitiendo desarrollar sistemas totales de análisis aplicados en el ámbito de las ciencias de la vida.

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KINETIC-SPECTROSCOPY THREE-DIMENSIONAL CHEMILUMINISCENCE: A NEW APPROACH IN LUMINISCENCE.

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The time-resolved chemiluminescence elucidates the kinetic characteristics of the chemiluminescence emission. This technique is based on conventional flow-injection analysis and provides the analyst with the whole time profile for the chemiluminescence signal, thereby facilitating the resolution of complex mixtures of analytes.

Using a back-thinned CCD spectrometer to obtain chemiluminescence spectra in this context has the advantage that it affords the acquisition of whole spectral signals in a few milliseconds. This solves the traditional limitation imposed by the fact that chemiluminescence emission usually lasts only a few seconds and is thus nearly impossible to measure with conventional means. However, modern spectrometers allow several spectra to be recorded while the chemiluminescence signal develops. The kinetic–spectroscopic 3D chemiluminescence technique acquires a certain number of 2D spectra during signal development that are subsequently assembled into a 3D spectrum with the software CLTotal[1].

We used two different types of software to obtain 3D kinetic–spectrometric profiles. BWSpec 3.26_41, which was run on the CCD spectrometer via a USB 2.0 interface, was used to acquire and store 2D spectra, as well as to export them as ASCII files. The software affords spectral acquisition at variable times and its timeline acquisition mode allows users to record and save (as ASCII files) a preset number of 2D spectra one by one.

Once 2D spectra were obtained, we used the custom-made software CLTotal, developed by our research group, to produce and process 3D spectra. CLTotal is based on software developed in the 1990s for a similar purpose using 3D fluorescence spectra. CLTotal allows the user to import previously acquired 2D chemiluminescence spectra and assemble them into a single 3D spectrum. The 3D spectrum can then be subjected to various graphical and mathematical treatments. However, the most interesting use of this new software is to obtain 2D spectra by following different trajectories in the 3D spectrum. For example, a horizontal cut allows an emission spectrum at a specific time to be obtained; similarly, a vertical cut provides a kinetic measurement at a specific wavelength. The user can also perform cuts along straight lines of variable slope or non-linear cuts according to a mathematical equation. The 2D spectra thus obtained are kinetic–spectroscopic spectra.

To demonstrate the potential of kinetic–spectroscopic measurements for the simultaneous determination of analytes with overlapped spectra, this methodology was applied to the simultaneous determination of benzo(a)pyrene and benzo(k)fluoroanthene.

Polycyclic aromatic hydrocarbons (PAHs) are well-known pollutants resulting from incomplete combustion of hydrocarbons. These substances have aroused much interest on account of their potential deleterious effects on human health. PAHs usually reach water through