ELECTRONIC SUPPLEMENTARY MATERIAL

Iridium Nanoclusters as High Sensitive-Tunable Elemental Labels for Immunoassays: Determination of IgE and APOE in Aqueous Humor by Inductively Coupled Plasma-Mass Spectrometry

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DESCRIPTION OF THE SUPPLEMENTARY MATERIAL

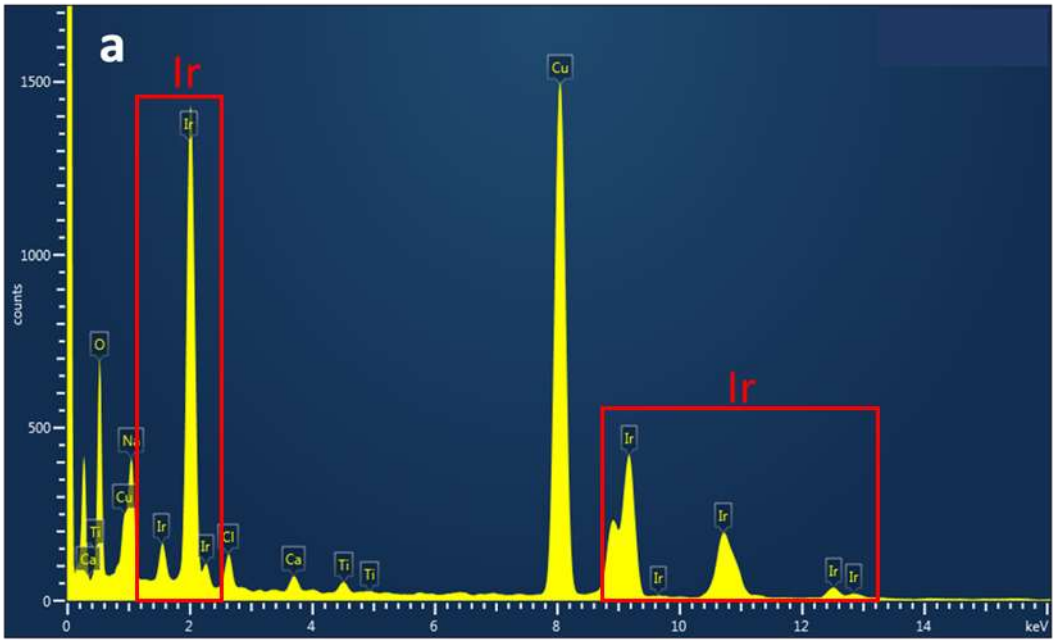
This Electronic Supplementary Material contains details related to the IrNCs characterization as well as one Figure and one Table collecting experimental results discussed at the Results and Discussion section of the article.

First, the elemental characterization of IrNCs by EDX (Figure S1) as well as the calculations followed up to obtain the number of Ir atoms per NC are indicated. Also, a Table with the IgE concentrations determined in human serum samples using both, the proposed methodology and a commercial ELISA kit, was included for method validation purposes.

RESULTS AND DISCUSSIONS

Characterization of IrNCs

IrNCs@citrate and IrNCs@LA were analyzed by EDX in order to confirm the ligand exchange procedure carried out. Figure S1 collects the elemental composition (at selected areas) for the two types of IrNCs. It can be observed that IrNCs@LA contain sulfur, whereas no sulfur was detected in the IrNCs@citrate.



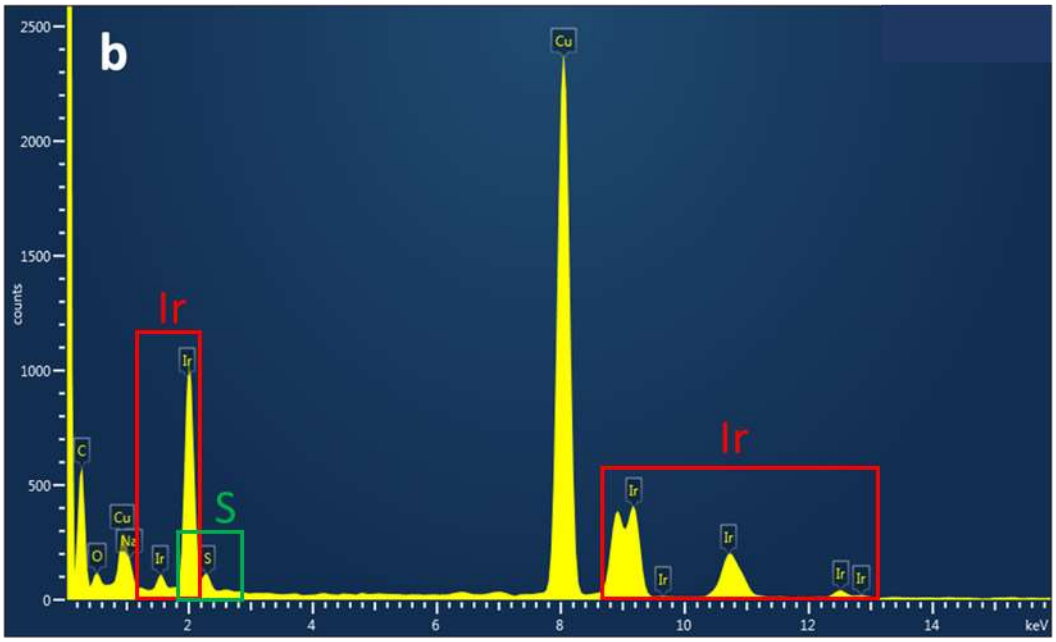


Figure S1. Elemental characterization of IrNCs by EDX. The spectra collect the elemental composition at selected areas for the two types of IrNCs. **(a)** IrNCs@citrate; **(b)** IrNCs@LA.

In order to characterize the solution containing the synthesized IrNCs, it is necessary to determine the Ir concentration as well as to calculate the number of Ir atoms per NC. The iridium concentration was measured by ICP-MS after an acid digestion of the samples, obtaining 0.47 ± 0.02 mM and 0.38 ± 0.04 mM for IrNCs@citrate and IrNCs@LA, respectively. In a face centered cubic cell there are 4 atoms, therefore the mass of a unit cell would be $1.28\ 10^{-21}$ g·cell^{-1}. Thus, the volume of a unit cell is equal to $5.67 \cdot 10^{-23}$ cm^3·cell^{-1} considering the iridium density is 22.5 g·cm^{-3}. The volume of an IrNC can be calculated from its experimental diameter assuming it has spherical shape. Next, dividing the volume of a NC by the volume of a unit cell, the number cells per NC is known. Multiplying the number of cells by the number of Ir atoms in a cell, the number of Ir atoms per NC is calculated, being obtained an average of 274 for IrNCs@citrate and 250 for IrNCs@LA.

 # IgE Determination in serum samples

In order to validate the proposed methodology using IrNCs labels and ICP-MS detection, Table S1 collects the IgE concentration determined in human serum samples using both the IrNCs@LA-based immunoassay and the commercial ELISA kit.

Table S1. IgE concentration determined in human serum samples employing both, the IrNCs@LA immunoprobe by ICP-MS and an ELISA kit. Uncertainties represent the standard deviations of the mean of four independent measurements.

[IgE] in Serum Samples (ng mL^{-1})		
Serum sample	**ELISA kit**	**IrNCs@LA-based immunoassay**
S1	66 ± 4	71 ± 2
S2	204 ± 13	197 ± 11
S3	296 ± 11	297 ± 2