USE OF NILE BLUE-DOPED SILICA NANOPARTICLES AS LABELS IN HETEROGENEOUS IMMUNOASSAYS FOR ANTIBIOTIC DETERMINATION

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The use of silica nanoparticles (NPs) as labels in immunoassays with optical detection has very interesting features: 1) transparency of silica to visible light, 2) almost negligible photo-bleaching phenomena of encapsulated organic dyes, 3) stability of silica matrix at medium-term, since it is not susceptible to microbiological degradation and, 4) porosity or swelling changes do not happen at moderate pH variations. In addition, the spectral selectivity of conventional fluorophores can be enhanced by using long-wavelength emitting organic compounds, such as cyanines, thiazines and oxazines, since their emission is not overlapped by static background signals from sample matrix, which usually happen at shorter wavelengths. Nile blue-doped silica NPs, synthesized using a simple reverse-micelle method[1], have been assayed for analytical purposes.

The use of these NPs is described for the first time to develop heterogeneous immunoassays for the determination of the veterinary antibiotic monensin. Two different formats, with antigen and antibody capture, have been assayed using black and shallow Proxy-plate 96-well microplates as solid supports. The first assay relies on the immobilization of anti-sheep IgG previously to the incubation of sheep anti-monensin antibodies. Then, a mixture of monensin and tracer (monensin bound to nile blue-doped silica NPs) is added and, after subsequent incubation and washing steps, the fluorescence of the bound tracer fraction is measured onto the dry surface of the well. The second assay format, relies on the competition of the monensin present in the samples with a monensin-BSA conjugate, which has been previously immobilised onto the well surface, for the active sites of anti-monensin antibodies. In both instances, the fluorescence signal obtained can be correlated to the analyte concentration. Preliminary studies have shown that the best results are achieved with the antibody capture heterogeneous immunoassay. The use of NPs as labels allows a decreased number of steps to perform this assay compared to those required for the development of ELISA assays, since the use of enzyme conjugate and substrate solutions is avoided.

[1] Godoy-Navajas, J., Aguilar Caballos, M.P., Gomez-Hens, A. *J. Fluoresc.*, **2010,** 20, 171.