Long-wavelength homogeneous fluoroimmunoassay for the veterinary antibiotic monensin using nile-blue doped silica nanoparticles

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The present method describes for the first time the use of nile blue-doped silica nanoparticles (NPs) as labels to develop a long-wavelength homogeneous fluoroimmunoassay for a hapten, more specifically the veterinary antibiotic monensin. The use of silica NPs as labels has been previously described for the determination of macromolecules [1], but their application to the determination of low molecular weight antigens has been scarce up to date.
Dye-doped silica NPs have several advantages when used as labels compared to organic fluorophores, such as: 1) an enhanced sensitivity since a single NP label contains a high number of fluorophore molecules and 2) the photostability of luminescent organic fluorophores can be improved owing to the protection that the silica matrix confers to the fluorophore molecules. In addition, the use of long-wavelength fluorophores to obtain dye-doped silica NPs provides to these labels with a suitable spectral selectivity since the presence of background signals from the sample matrix can be avoided.

The NPs used in the method reported here have been synthesized according to a reverse-micelle microemulsion sol-gel method previously described [2]. These NPs do not have any moiety suitable for its coupling to biomolecules being necessary the use of a functionalization reaction using 3-aminopropyltriethoxysilane (APS) as reagent for the introduction of amino groups. The use of APS alone gives rise to aggregated NPs but this effect can be overcome using a silane reagent containing phosphonate groups, which reduces the aggregation of NPs. The functionalized NPs are bound to the carboxylic acid group of monensin via a carbodiimide reaction in the presence of N-sulfohydroxysuccinimide and a water soluble carbodiimide reagent. The obtained tracer was purified by washing and centrifuging the NP suspension. The reaction of the synthesized tracer with anti-monensin antibodies originated a fluorescence quenching, which is hindered in the presence of monensin. This can be explained owing to competition phenomena between monensin and the tracer and the fluorescence is recovered when the tracer is free in solution. The method has a dynamic range of 0.1-10 ng mL^{-1}.

REFERENCES

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