Determination of the stoichiometry CdSe/ZnS quantum dot to antibody in bioconjugates by asymmetric flow field-flow fractionation coupled on-line to elemental mass spectrometry (ICP-QQQ)

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Introduction and Purpose

The development of sensitive bioanalytical applications of engineered metal nanoparticles demands for analytical tools able to guide the functionalization of their inorganic-core with biomolecule recognition units (such as antibodies, proteins and/or aptamers) as well as characterize and control this bioconjugation reaction of the nanoparticles to such recognition units, which are specific for the target biomolecules of interest.[1] Among the different metal nanoparticles popular today, photoluminescent quantum dots (QDs) stand out due to their exceptional and tunable fluorescent properties which enable their use as labels for biomedical applications, such as cell biology and immunoassay. However, such methods based on QDs are still not very reliable for quantitative measurements.

The analytical potential of QDs to be used as labels for the quantitative analysis of biomolecules will require for the control of the bioconjugation reaction and the determination of the number of antibodies or recognition units attached per nanoparticle.[2] In this context, the hybridation of the asymmetric flow field-flow fractionation (AF4), which is one of the most promising separation techniques able to achieve size-dependent separation of nanoparticles and biomolecules, with an inductively coupled plasma-mass spectrometry (ICP-MS) could provide invaluable capabilities and information to achieve the intended purpose.

Experimental Methods

In this work, the AF4 coupled on-line to ICP-MS is proposed as a powerful diagnostic tool for QDs bioconjugation studies. In particular, the determination of QDs stoichiometry in bioconjugates (between a monoclonal IgG antibody (Ab) and CdSe/ZnS core-shell QDs, which are surface-coated with an amphiphilic polymer) has been monitored by such hyphenated technique. Experimental conditions have been optimized searching for an appropriate separation between the sought bioconjugates from the eventual free QDs and antibodies excesses employed during the bioconjugation reaction. Moreover, an ICP-MS equipped with a Triple Quadrupole was selected as elemental detector to enable sensitive and reliable simultaneous quantification of the elemental constituents, including elements difficult to be quantified by ICP-MS such as sulfur, of the QD-Ab bioconjugates, and the free QDs and Ab.

Results and Conclusion

The hybrid AF4-ICP-QQQ technique used provided nanoparticle size-based separation and elemental detection analysis that turned out not only to investigate in depth the bioconjugation process but also to determine and quantify the stoichiometry QD:Ab in different bioconjugates assessed (including a commercially available sample of QD conjugates). That is a key challenge in future development of bionanoanalytical applications of such fluorescent QDs.

References

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