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Sensitive targeted multiple protein quantification based on elemental detection of Quantum Dots

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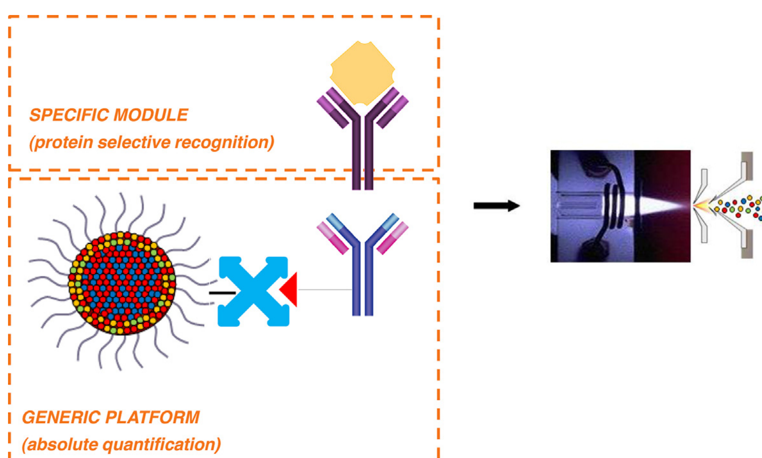
HIGHLIGHTS

- Novel generic platform for multi-parametric quantification of proteins.
- QDs labeling and ICP-MS detection allow significant analytical signal amplification.
- ICP-MS mass balances information provided an internal validation of the immunoassay.
- Multiparametric determination of 5 proteins in human serum samples.
- ICP-MS reduced matrix effects as compared to other conventional detection techniques.

GRAPHICAL ABSTRACT



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ABSTRACT

A generic strategy based on the use of CdSe/ZnS Quantum Dots (QDs) as elemental labels for protein quantification, using immunoassays with elemental mass spectrometry (ICP-MS), detection is presented. In this strategy, streptavidin modified QDs (QDs-SA) are bioconjugated to a biotinylated secondary antibody (b-Ab_2). After a multi-technique characterization of the synthesized generic platform (QDs-SA-b-Ab_2) it was applied to the sequential quantification of five proteins (transferrin, complement C3, apolipoprotein A1, transthyretin and apolipoprotein A4) at different concentration levels in human serum samples. It is shown how this generic strategy does only require the appropriate unlabeled primary antibody for each protein to be detected. Therefore, it introduces a way out to the need for the cumbersome and specific bioconjugation of the QDs to the corresponding specific recognition antibody for every target analyte (protein). Results obtained were validated with those obtained using UV–vis spectrophotometry and commercial ELISA Kits.

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As expected, ICP-MS offered one order of magnitude lower DL (0.23 fmol absolute for transferrin) than the classical spectrophotometric detection (3.2 fmol absolute). ICP-MS precision and detection limits, however turned out to be compromised by procedural blanks. The full analytical performance of the ICP-MS-based immunoassay proposed was assessed for detection of transferrin (Tf), present at the low ng mL^{-1} range in a complex "model" synthetic matrix, where the total protein concentration was 100 μg mL^{-1}. Finally, ICP-MS detection allowed the quantitative control of all the steps of the proposed immunoassay, by computing mass balances obtained, and the development of a faster indirect immunoassay format where the plate wells were directly coated with the whole protein mixture sample.
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1. Introduction

Quantum Dots (QDs) are inorganic nanoparticles with outstanding photoluminescent properties [1]. They are very attractive as highly valuable novel fluorophores to improve bioanalytical applications based on biolabeling and bioimaging methodologies [2,3]. So far, however, such methods are still far from being well established for quantitative bioanalytical measurements. In fact, the use of QDs as labels for reliable biomolecule quantification requires a strict control of the synthesis and the surface-modification processes [4]. In this context, previous works demonstrated that inductively coupled plasma mass spectrometry (ICP-MS) can play a pivotal role in the assessment of CdSe/ZnS QDs synthesis [5], solubilization and bioconjugation for their eventual use in reliable quantitative bioassays [6–9].

Non-isotopic standard immunoassays are widely considered as one of the methods of choice for the quantification of target proteins in complex mixtures as they offer a combination of sensitivity, specificity and cost-effectiveness. Nowadays, UV–vis spectrophotometry is the reference detection technique in ELISA immunoassays. In this context, recent developments in biofunctionalized nanoparticles (NPs) have allowed significant improvements, including better sensitivity, specificity, multiplexing ability and response time [10,11]. In addition, the use of NPs as labels allows easy coupling to optical, electrochemical, piezoelectric, electrical, and mechanical devices as transduction means [12,13]. In most of those applications, the well-established specificity and high affinity of biotin (b) noncovalent interaction with streptavidin (SA) provide the basis for developing selective assay systems, particularly useful for antibodies [14]. However, covalent modification of either carboxyl or amine groups in the constant fraction of the primary antibodies can adversely affect their binding properties. In these cases, a general strategy consists of the use of a SA-coated NP labeled with biotinylated secondary antibodies (b-Ab_2) able to recognize the primary one (Ab_1), which in turn targets specifically the protein under study. Such functionalization has been already reported for QDs in some bioanalytical applications [15–17]. A rough estimation of the CdSe/ZnS QDs-SA stoichiometry using gel electrophoresis has been reported [18]. Interestingly, the b–SA system could be established in this way as a linker system for detecting different targets (e.g., proteins), providing a significant signal amplification along with an additional degree of flexibility [19,20].

On the other hand, ICP-MS has been successfully introduced in the latest years as a complementary tool for sensitive protein detection [21] and quantification at the trace and ultratrace levels [22]. One of the most promising applications in this sense relies on the use of element-labeled immunoassay strategies [23]. Several elemental labeling strategies have been applied for that purpose [24]. Among them, the use of NPs as elemental labels for antigens or antibodies stands out as a most promising strategy since that allows the introduction of hundreds of atoms per protein molecule. Of course, such "element amplification" can significantly lower the protein detection limit by ICP-MS. Several metal NPs, including AuNPs [25,26], AgNPs [27], Ag deposited on AuNPs [28], PbS-NPs [29] or even metal oxides, have been tested for this purpose so far. However, QDs appear to exhibit superior properties as elemental labels due to the possibility to provide complementary fluorescence, a feature which can be critical to control possible NP degradations [8] and to open the door to imaging applications. In addition, CdSe QDs allow multiplexing analysis, labeling each Ab with one of the numerous isotopes available (i.e., 8 from Cd and 5 from Se). Surprisingly, the number of reported immunoassays based on QDs as elemental labels for protein quantification is very limited so far [30–32]. A common disadvantage of all such reported strategies is the need of individual and tedious optimization of the QDs bioconjugation to every specific target (antigen/protein) or primary antibody. On the other hand, a remarkable advantage of ICP-MS over conventional spectrophotometric detection in quantitative immunoassays is ICP high tolerance to sample matrix (matrix effects can produce a severe influence on the luminescent emission of bioconjugated QDs as demonstrated by Trapiella-Alfonso et al. [33] and reported by Giesen et al. [23].

We describe here the development and in-depth characterization of a generic immunoassay platform, based on QDs and ICP-MS detection, for "amplified" protein quantifications. As compared to previous work using QDs as labels for ICP-MS immunosassays [30], the here proposed approach rules out the need for specific bioconjugation procedures for each target protein. Moreover, the sandwich-type immunoassay format selected in this new work allows us to further improve the sensitivity and selectivity of the determination. The flexibility and the robustness of the proposed approach have been tested for the multiparametric determination of serum proteins [34]. Results were validated using the mass balances attained by ICP-MS and after their critical comparison to those obtained by commercial ELISA kits. Finally, the analytical performance characteristics have been assessed for transferrin using both sandwich and indirect immunoassay formats.

2. Material and methods

2.1. Reagents, solutions and materials

All experiments were carried out with analytical grade chemical reagents used as received without further purification. Deionized ultrapure water (18.2 MΩ cm^{-1}) was obtained with a Milli-Q system (Millipore, Bedford, MA).

CdSe/ZnS Qdot® 565 streptavidin conjugate (QDs-SA) was acquired from Invitrogen (Karlsruhe, Germany). Agarose D1 Medium EEO from Pronadisa (Madrid, Spain), Tris-Borate EDTA Buffer (TBE) from Sigma–Aldrich (St. Gallen, Switzerland), glycerol 85% from Merck (Darmstadt, Germany) and bromophenol blue from Sigma–Aldrich were used in gel electrophoresis (GE). Iminobiotin agarose and spin columns screw caps were purchased from Thermo Fisher Scientific (Bremen, Germany). Sodium chloride from Merck, ammonium acetate from Panreac (Barcelona, Spain) and acetic acid (≥99.5%) from Sigma–Aldrich. Plastic syringes (1 mL) from BD (NJ, USA). Ammonium bicarbonate (grade

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