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Nine surface plasmon resonance assays for specific protein quantitation during cell culture and process development

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ABSTRACT

Quantitation of protein is essential during pharmaceutical development, and a variety of methods and technologies for determination of total and specific protein concentration are available. Here we describe the development of a streamlined assay platform for specific quantitation assays using surface plasmon resonance (SPR) technology. A total of nine different assays were developed using similar conditions, of which eight assays were for quantitation of different human blood plasma proteins (IgG, IgG1–4 subclasses, IgA, transferrin, and albumin) from a chromatography-based IgG plasma process. Lastly, an assay for monitoring the concentration of a recombinant monoclonal antibody during 13 days of CHO cell culturing was developed. Assay performances were compared with enzyme-linked immunosorbent assay (ELISA), nephelometry, ARCHITECT, and Cobas c501. SPR assays were shown to have higher sensitivity than analysis using nephelometry, ARCHITECT, and Cobas and to have significantly lower analysis and hands-on time compared with ELISA. Furthermore, the SPR assays were robust enough to be used for up to 12 days, allowing specific protein concentration measurement of a sample to be completed at line within 10 min. Using the same platform with only few varied parameters between different assays has saved time in the lab as well as for evaluation and presentation of results.

The detection and quantitation of protein is essential for pharmaceutical preparations, enzyme kinetics, and the monitoring of protein yield and purity at each step of a protein purification procedure. There are two main approaches for protein concentration determination available today: (i) total (unspecific) protein measurements as listed in European Pharmacopoeia [1] (Bradford, bicinchoninic acid, biuret, Kjeldahl, and high-performance liquid chromatography) and (ii) specific immuno-based assays such as enzyme-linked immunosorbent assay (ELISA)[1], nephelometry, immunoturbidimetric assay, radial immunodiffusion (RID), and surface plasmon resonance (SPR). Methods to determine total protein concentration detect particular amino acids by either absorbance or interactions of dyes or peptide bonds. Specific quantitation of proteins often gives more reliable information compared with total assays; however, because antibody-based quantitation tends to imply more hands-on work and longer analysis time than total assays, the specific assays are sometimes seen as complementary analyses. ELISA is widely used but laborious if not set up with pipetting robots [2]. The traditionally performed RID technique is time-consuming and often not sensitive enough [3]. Using nephelometry for the specific protein quantitation is automated, saving hands-on time. Nephelometric assays allow for rapid determination of concentrations in a large number of samples with an intra-assay variation of approximately 2.5% [3,4]. The choice of method will be influenced by the amount of protein available, the detection limit of the method, the ease of use, and the time required to complete the method.

The increasing demands to characterize low levels of impurities that remain in pharmaceutical preparations have placed a focus on development of high-sensitivity analytical techniques that are relatively fast, convenient to use, and possible to integrate in the production process for at-line measurements (reviewed in Ref. [5]). Conventional immunochemical binding assays often operate in the concentration range of 10^{-9} to 10^{-12} mol/L. A drawback with highly sensitive analytical procedures is that any nonspecific responses dramatically change the analytical outcome. One way to compensate for this is to introduce a secondary antibody to increase the specificity. Another approach is to take advantage of

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[1] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; RID, radial immunodiffusion; SPR, surface plasmon resonance; UV, ultraviolet; IgGsc, IgG subclasses; LOQ, limit of quantitation; RU, response units; mAb, monoclonal antibody; CV, coefficient of variation; HSA, human serum albumin; BSA, bovine serum albumin; IVIG, intravenous administration; TBS, The Binding Site.

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SPR sensor surfaces that have been designed specifically for the purpose of reducing any nonspecific binding to an absolute minimum. Yet another approach is to develop assays that are sensitive enough to dilute the sample by several orders of magnitude, thereby significantly reducing the influence of nonspecific binding. The presence of impurities in a biotechnical production process might vary considerably over time. Therefore, it is often important to analyze samples quickly and at line to allow for adjustment of conditions in the ongoing production protocol. Likewise, it would be desirable to monitor protein concentration in the downstream processes, not only broadly via ultraviolet (UV) absorbance at 280 nm or by conductivity of the eluting buffer but also by more specific means. The process analytical technology (PAT) initiative by the Food and Drug Administration has increased the development toward on-line immunoassays for process control [5,6].

In the current study, we have focused on SPR analysis and, more specifically, on the use of Biacore systems. In these systems, binding events are monitored in real time using label-free assay formats. One of the interacting molecules, here an antibody, is immobilized to a semi-fluidic dextran matrix on a sensor surface. The other molecule is then injected in solution over the surface (see Fig. 1A). As molecules in the injected sample bind to the antibody immobilized on the surface, a change in SPR response is detected (Fig. 1B). The change in response level is proportional to the change in mass at the surface.

SPR assays are extensively used for characterization of binding events (e.g., binding specificity and kinetic studies of protein interactions) and also for screening and characterization of small molecules. SPR quantitation assays were first described by Fagerstam and coworkers in 1992 [7], and although a variety of individual quantitation assays have been published since that time, this type of assay has not gained as much attention as the SPR characterization assays. Nevertheless, published examples include quantitation of basic fibroblast growth factor [8], CTLA-4 fusion protein [9], and serum antibodies against darbepoetin alfa (Aranesp) and epoetin alfa (Epogen) [10]. Helmerhorst and coworkers reviewed SPR quantitation assays in a more diagnostic context for detection of numerous biomarkers [11]. A relatively recent SPR assay to quantitate transferrin in patient sera showed a limit of detection of 20 ng/ml and correlation with immunoturbidimetric assay [12].

To simplify practical aspects of analysis, we wanted to use the same platform for quantitation of all different proteins of interest during plasma process development as well as cell culturing. We used SPR to develop new concentration assays for eight different human blood plasma-derived proteins: IgG, IgG subclasses (IgGsc, 1–4), IgA, transferrin, and albumin. Samples from a chromatography-based IgG plasma process were analyzed. In another context, the IgG assay was also set up for quantitation of a recombinant CHO cell-derived monoclonal IgG1 antibody analyzing crude samples from 13 days of cell culturing. In addition, the SPR assays were compared with ELISA, nephelometry, and immunoassays using Cobas and ARCHITECT, techniques that also measure specific protein concentration.

Materials and methods

Antibodies

Antibodies used were anti-human albumin, Ab399 (Abcam), anti-human IgG (Fc) (Human Antibody Capture Kit, GE Healthcare), anti-human IgG1 (Fc), clone HP 6070 (Invitrogen, Life Technologies), anti-human IgG2 (Fc) clone 52G1, Ab1933 (Abcam), anti-human IgG3 (Fab2), clone HP 6050 (The Binding Site), anti-human IgG4 (pFc), clone HP 6025 (The Binding Site), rabbit anti-transferrin polyclonal antibody (in-house GE Healthcare),

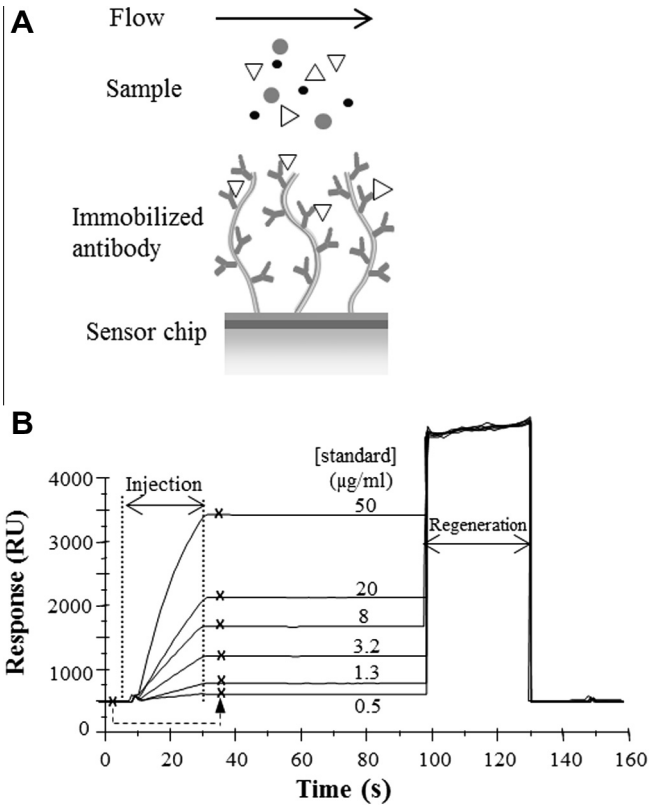

Fig.1. Schematics of an SPR quantitation analysis. (A) Antibody is immobilized on a sensor chip. Sample is injected and specific protein binds to the antibody surface, resulting in a sensorgram and a response. (B) Sensorgram showing responses (marked X) from standard curve points; the higher the response, the higher the concentration. Each sample injection is followed by regenerating the surface, removing samples bound, and restoring the baseline.

and anti-IgA, I167 (Cygnus Technologies). Antibodies also tested were IgG1 Ag 502, IgG2 Ag 504, IgG3 Ag 506, IgG4 Ag 508, anti-human IgG2, and MAB1308 (all from Millipore), anti-human IgG1 and MH1015 (Invitrogen, Life Technologies), and IgA and ab91025 (Abcam).

Reference materials

Reference materials used were International Reference Material ERM-DA470k/IFCC (EDQM, France), Human Serum Protein Calibrator (Dako, Agilent Technologies), and WHO International Standard Immunoglobulins G, A and M, 67/086 (NIBSC, UK).

SPR reagents

SPR reagents used were Series S Sensor Chip CM5, HBS-EP+ buffer (10 mM Hepes, 150 mM NaCl, 3 mM ethylenediaminetetraacetic acid, and 0.05% Surfactant P20, pH 7.4), amine coupling kit, 10-mM acetate buffers pH 4.5 and 5.0, and 10 mM glycine solutions pH 1.5 and 2.0 (all from GE Healthcare, Sweden).

SPR analysis

All SPR quantitation assays were developed as direct binding assays (Fig. 1) using a Biacore T200 system (GE Healthcare, Sweden). HBS-EP+ buffer was used as sample and analysis buffer. Analysis temperature was 25 °C, and sample compartment temperature was set to 15 °C. Immobilizations of antibodies were made using the amine coupling kit according to the manufacturer's instructions, including test of suitable immobilization pH. One flow