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A Multipurpose Capacitive Biosensor for Assay and Quality Control of Human Immunoglobulin G

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ABSTRACT: We report a flow-injection biosensor system with a capacitive transducer for assay and quality control of human immunoglobulin G (hIgG). The sensing platform is based on self-assembled monolayers (SAMs) of carboxylic acid terminated alkyl-thiols with covalently attached concanavalin A. The electrochemical characteristics of the sensor surface were assessed by cyclic voltammetry using a permeable redox couple (potassium ferricyanide). The developed biosensor proved capable of performing a sensitive label-free assay of hIgG with a detection limit of 1.0 $\mu g\,mL^{-1}$. The capacitance response depended linearly on hIgG concentration over the range from 5.0 to 100 $\mu g\,mL^{-1}$, in a logarithmic plot. Typical measurements were performed in 15 min and up to 18 successive assays were achieved without significant loss of sensitivity using a single electrode. In addition, the biosensor can detect hIgG aggregates with concentrations as low as 0.01% of the total hIgG content (5.0 $\mu g\,mL^{-1}$). Hence, it represents a potential post-size-exclusion chromatography–UV (post-SEC–UV) binding assay for in-process quality control of hIgG, which cannot be detected by SEC–UV singly at concentrations below 0.3% of the total hIgG content.

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KEYWORDS: biosensor; capacitance; concanavalin A; flow injection; human immunoglobulin G; quality control

Introduction

Antibodies (i.e., immunoglobulins) play an important role in biomedical research and development. In parallel, antibody-based therapeutics and in vivo diagnostics are gaining wider approval from many health authorities all over the world (Reichert et al., 2005). Currently, 18 monoclonal antibodies, including 14 unmodified immunoglobulin molecules, have been approved for therapeutic use and over 150 are under clinical development (Carter, 2006). Human immunoglobulin G (hIgG) molecules have been used to treat a variety of disorders such as primary and secondary immunodeficiencies, infections, and inflammatory and autoimmune diseases. Immunoglobulins are glycoproteins composed of two identical heavy- and two identical light chains covalently bonded via interchain disulfide (S–S) linkages to form a Y-shaped molecule. The immunoglobulin molecule has a molecular weight of approximately 150,000 Da and contains three discrete domains: two antigen binding fragments (Fab) at the tips of the Y-shape and one crystallizable fragment (Fc) at its pole (Hamilton and Ambli, 2001). The hIgG molecule contains a total of 2.8% carbohydrate by mass, 2.0% are contained in the Fc section, and whereas the remaining 0.8% is located in the Fab parts (Parkeh et al., 1985).

Classical measurement of hIgG involves techniques such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) (Pålsson et al., 2000). However, they are time-consuming, need labeling, and show a somewhat lower sensitivity (Stacy et al., 1998). The lower sensitivity is not a problem when dealing with process monitoring since then the concentration of the target molecules are often too high for analysis without dilution steps (Mattiasson, 1984). Biosensors have attracted wide-spread attention in recent years for many reasons such as simple instrumentation, fast operation, and high sensitivity and selectivity. In parallel, several biosensors have been developed for measurement of hIgG, including piezoelectric (Chen et al., 2007; Li et al., 2003; Liu et al., 2003; Lu et al., 2000) and piezomagnetic biosensors (Ogi et al., 2006), surface plasmon resonance biosensors (Dong et al., 2008; Suzuki et al., 2002), and electrochemical biosensors such as amperometric biosensors (Bian et al., 2005; Dutra et al., 2000; Messina et al., 2005; Wilson and Nie, 2006), potentiometric biosensors (Li and Gao, 2008), and field-effect transistors (Cid et al., 2008; Kim

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et al., 2008). Furthermore, several online immuno-monitoring systems were successfully developed for the measurement of hIgG based on turbidimetric assays (Fenge et al., 1991; Middendorf et al., 1993), and fluorescence measurements (Reif et al., 1994). In addition, a rapid capillary electrophoresis technique was employed for the determination of the molecular mass of heavy and light chains of hIgG (Lausch et al., 1993).

hIgG preparations for intravenous administration are produced from blood plasma via several steps including cold ethanol precipitation, clear filtration, ultra/diafiltration, several chromatographic steps, and finally virus inactivation/removal steps (Buchacher et al., 1996). Each step can influence the physical and chemical properties of hIgG molecules, which in turn may expose more hydrophobic amino acids at the surface leading to partial aggregation. Additionally, the final hIgG solution will always undergo aggregation with time since the folded state of any protein is not stable in solution (Shortle, 1996). Minimizing the aggregate formation during production is an essential parameter for achieving a long-term stability of the final product. In addition, aggregates can develop undesirable anaphylactoid reactions upon administration, leading to serious side effects (Lebing et al., 2003). Therefore, strict requirements on purity and minimal aggregate content were posed by health authorities.

When dealing with production of monoclonal antibodies (mcAb), a more simple separation sequence is normally applied. Furthermore, mcAbs are today a major fraction of newly developed pharmaceutical drugs, and therefore it becomes increasingly important to have good quality control systems.

The standard method for the determination of aggregate content combines high-performance size-exclusion chromatography (SEC) with UV detection (Buchacher et al., 2001). However, traces of large protein aggregates cannot be detected by UV absorbance due to its limited sensitivity since these aggregates usually exist in low abundance, often smaller than 0.3% of the total hIgG content (Ahrer et al., 2003). Another method combining a light scattering (LS) detector with SEC has successfully detected traces of hIgG aggregates. However, it is susceptible to interference and requires homogeneous distribution of the analyte in the sample. For these reasons, it is not frequently used in pharmaceutical industry (Ahrer et al., 2003). Therefore, advanced bioanalytical methods for quality control of hIgG are required.

The majority of hIgG detection methodologies are based on the protein A sensing platform, a *Staphylococcus aureus* protein which binds specifically to the Fc part of the IgG molecule (Chen and Lee, 2002). However, in spite of its high selectivity, protein A has some drawbacks such as its high cost and the difficulty to immobilize it in the proper orientation (Denizli and Arica, 2000). To overcome such drawbacks, affinity-binding techniques that utilize concanavalin A (Con A), instead of protein A, have become promising candidates due to the ability of Con A to recognize the carbohydrate moiety of the IgG molecule (Babac et al., 2006). Con A is a tetrameric metalloprotein found in the jack bean (*Canavalia ensiformis*) and can bind to α-glucose and α-mannose with high molecular specificity (Cairo et al., 2002; Gestwicki et al., 2002). Capacitive biosensors have been employed extensively for various applications, including antibody-based detection of proteins and polysaccharides (Jiang et al., 2003a,b; Labib et al., 2009a,b). In the present study, the development of a reusable multipurpose flow-injection biosensor with a capacitive transducer for measurement and quality control of hIgG is reported. The biosensor interface is based on self-assembled monolayers (SAMs) of carboxylic acid terminated alkylthiols with covalently attached Con A. The electrochemical characteristics of the sensor surface were assessed by cyclic voltammetry. Finally, the analytical characteristics of the prepared biosensor were discussed, including the working range, limit of detection, reproducibility, selectivity, and stability.

Materials and Methods

Chemicals

Concanavalin A from jack bean in lyophilized form, thioctic acid, acetonitrile 99.8%, D-fructose, D-maltose, D-mannose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, dextran (average molecular weight: 10, 39, 71.4, 298, 500 kDa), β-cyclodextrin, β-cyclodextrin polymer, glycogen type II from oyster, potassium ferricyanide, and manganese chloride were all from Sigma–Aldrich Chemie (Steinheim, Germany). D-glucose, *N*-(3-dimethylaminopropyl)-*N*'-ethyl-carbodiimide hydrochloride (EDC), and glutaraldehyde solution (50%, w/v) were purchased from Fluka, Sigma–Aldrich GmBH (Steinheim, Germany). hIgG was the product of Octapharma (Stockholm, Sweden). 1-Dodecanethiol was obtained from Aldrich (Deisenhofen, Germany). Alumina slurries with particle diameter of 0.1 μm was purchased from Struers (Ballerup, Denmark). Chromatographic columns were obtained from Pharmacia (Uppsala, Sweden). Sephadex G-25 and G-75 superfine were purchased from Amersham Biosciences AB (Uppsala, Sweden). All other chemicals were of analytical grade. All buffers were prepared with water treated with a Milli-Q system (Bedford, MA). Before use, the buffers were filtered through a Millipore filter with pore size of 0.22 μm and subsequently degassed.

Fabrication of the Biosensing Platform

SAMs via spontaneous adsorption of sulfur-containing compounds on gold electrodes have been widely used to immobilize biorecognition molecules in capacitive biosensors due to its simplicity, versatility, and well insulation and orderliness (Li et al., 2005). Briefly, a gold rod electrode (Ø3 mm, 99.99% purity) was cleaned with piranha solution

(H_2O_2:H_2SO_4, 1:3), then washed thoroughly with distilled water. Next, the electrode was carefully polished with alumina slurries (0.1 and 0.05 μm) on microcloth pads and then rinsed with water and placed in a Teflon holder. Afterwards, the electrode was dried with high-purity nitrogen and plasma cleaned for 15 min (Model PDC-3XG, Harrick Scientific, Pleasantville, NY). The cleaned electrode was immediately immersed in an ethanolic solution of thioctic acid (1%, w/v) and stored overnight at room temperature. Subsequently, it was rinsed with dry acetonitrile and activated with EDC/acetonitrile (1%, w/v) for 5 h. Finally, the electrode was washed with water and incubated with 40 μL of 0.75 mg mL^{-1} Con A in 5.0 mM Tris–HCl buffer (pH 7.4), containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$, overnight at 4°C. The same buffer was further employed as a mobile phase for capacitance measurements. Prior to use, the electrode surface was blocked with 10 mM of 1-dodecanethiol in ethanol and placed in the flow cell.

Cyclic Voltammetry Studies

Cyclic voltammetry experiments were carried out using a potentiostat/galvanostat (Autolab PGSTAT 12, EcoChemie, Utrecht, The Netherlands). Instrument operation and data acquisition were controlled using GPES 4.8 software. All electrochemical measurements were conducted in a standard three-electrode flow cell fitted with a platinum wire and a commercial Ag/AgCl (saturated KCl) electrode as the counter- and the reference electrode, respectively. The gold electrode, either unmodified or covered by different layers, served as the working electrode. The electrolyte solution, 0.1 M KCl containing 0.1 M potassium ferricyanide, was de-aerated with nitrogen (99.999%) before use. Cyclic voltammetry was performed by sweeping the potential between −0.3 and 0.8 V at a sweep rate of 0.1 V s^{-1}. All electrochemical experiments were carried out at room temperature.

Capacitance Detection System

A schematic drawing of the system setup is provided in Figure 1. Briefly, the modified electrode was inserted as the working electrode in a specially constructed four-electrode flow cell with a dead volume of 10 μL. A platinum foil and a platinum wire served as the counter and the reference electrode, respectively. An external reference (Ag/AgCl) electrode was placed in the outlet stream to correct for the potential drift of the platinum wire. The electrodes were connected to a potentiostat. A fast data acquisition unit (575 measurement and control system, Keithley Instruments, Cleveland, OH) was connected between the potentiostat and a personal computer. A sampling frequency of 50 kHz was determined by an internal clock in the data acquisition unit. The rest potential was 0 mV versus the reference Ag/AgCl electrode throughout the experiment. A potential pulse of 50 mV was applied and the current transient evoked by the potentiostatic step was sampled. The potentials of the platinum and the Ag/AgCl reference electrodes were compared before the potentiostatic step and the computer adjusted the potential so that the potentiostat behaved as if the Ag/AgCl reference had controlled the working electrode. The mobile phase was pumped with a peristaltic pump (Model M-312 Gilson, Villiers-le-bel, France) at a flow rate of 50 μL min^{-1}. All injections were performed via a 250-μL loop.

Capacitive Monitoring of hIgG

A highly purified hIgG fraction was obtained from a commercial preparation by desalting on Sephadex G-25

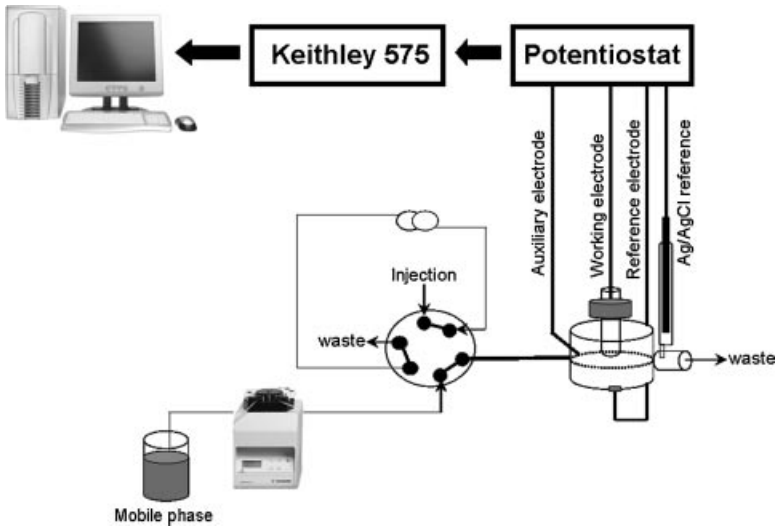

Figure 1. Schematic drawing of the experimental setup.

followed by lyophilization. A series of hIgG concentrations ranging from 1.0 ng mL^{-1} to 1.0 mg mL^{-1} in 5.0 mM Tris–HCl buffer (pH 7.4), containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$ was prepared to be subsequently measured by the capacitive biosensor. Before loading the aliquot to be tested to the flow cell, the sugar binding sites of the immobilized Con A were blocked with an injection of 0.1 M glucose (18 mg mL^{-1}) to eliminate the effect of nonspecific binding. Subsequently, the sample aliquot was injected and the corresponding ΔC_{hIgG} was calculated, where ΔC_{hIgG} is the capacitance change due to the binding between hIgG and Con A. The system was regenerated with 0.1 M glucose after each sample injection. Finally, a calibration curve was plotted and the concentrations showing a linear agreement with the corresponding ΔC_{hIgG} were determined.

Preparation of hIgG Aggregates

To test the feasibility of the proposed biosensor for the detection of hIgG aggregation, hIgG aggregates were prepared via cross-linking with glutaraldehyde according to the method described by Wisdom (2002), with slight modifications. Briefly, 10 mg of the lyophilized hIgG was reconstituted in 1.0 mL of 10 mM sodium phosphate buffer (PB), pH 7.4, then 0.1 mL of 1% (w/v) glutaraldehyde solution was added and the solution was shaken gently for 2 h at room temperature. Thereafter, the solution was extensively dialyzed against four changes of 1 L distilled water over 24 h. The dialyzed solution was subjected to SEC, using an XK-10 column (10 mm × 810 mm) packed with 70 mL of Sephadex G-75 superfine. The same buffer was used as an eluent at a flow rate of 0.5 mL min^{-1}. The extent of polymerization was monitored using a fast protein liquid chromatography (FPLC) system (Bio-Rad, Munich, Germany). A high molecular weight fraction was collected and lyophilized. A parallel control experiment was performed to determine the retention time of the monomeric hIgG. Thus, the hIgG aggregate was easily detected by its relatively short retention time.

Capacitive Detection of hIgG Polymerization

The purpose of this work was to develop a sensitive method for monitoring of process-induced aggregation of hIgG. The lyophilized hIgG aggregate was reconstituted in 5.0 mM Tris–HCl buffer (pH 7.4), containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$, yielding a final concentration of 1.0 mg mL^{-1}. A series of hIgG aggregate concentrations ranging from 1.0 ng mL^{-1} to 1.0 mg mL^{-1} was measured by the capacitive biosensor, following the same previously mentioned steps for monitoring of monomeric hIgG. Moreover, the system was subsequently regenerated with 0.1 M glucose after each sample injection.

Results and Discussion

Electrochemical Characterization of the Biosensing Interface

Proper insulation of the electrode surface is crucial for the design of capacitive biosensors (Berggren et al., 1999). The presence of defects in the layer covering the electrode surface can allow the contacting solution to reach the electrode surface and consequently, increases its capacitance due to the high polarity of water. Cyclic voltammetry, in the presence of a permeable redox couple (potassium ferricyanide), is a valuable method for probing the degree of insulation of the electrode surface. The reason is that the electron transfer between the redox couple and the gold electrode can occur either by tunneling across the layer covering the electrode surface or through defects (holes) in this layer (Bharathi et al., 2001). As shown in Figure 2, the degree of insulation (blocking) of the electrode surface increased for each additional layer immobilized on the electrode surface. The clean gold electrode exhibited reversible peaks for the oxidation and reduction of the redox couple during cycling of potential between −0.3 and 0.8 V at a scan rate of 0.1 V s^{-1} (Fig. 2, curve a). Self-assembly of thioctic acid caused a significant reduction of the redox currents, indicating a considerably reduced penetration of the conducting ions (curve b). As can be seen in curve c, the immobilization of Con A did not cause a significant change in the redox currents. Finally, treatment with 1-dodecanethiol reduced the redox currents substantially, probably because they can penetrate down to the electrode surface, thereby blocking the direct access of

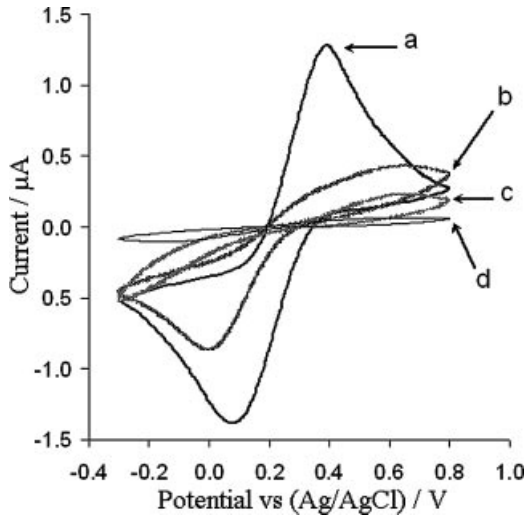

Figure 2. Cyclic voltammograms recorded in a solution of 100 mM KCl containing 100 mM $K_3[Fe(CN)_6]$ using: (a) bare gold electrode; (b) gold electrode coated with thioctic acid; (c) the same as (b) with subsequent immobilization of Con A; (d) as in (c) after treatment with 1-dodecanethiol. The scan range was from −0.3 to 0.8 V (vs. Ag/AgCl), at a scan rate of 0.1 V s^{-1}.

the conducting ions. The electrochemical results demonstrate the good insulation of the electrode surface and hence, it can be further utilized for capacitive measurement of hIgG.

Capacitive Detection of hIgG

The principle of capacitive biosensors is based on the theory of the electrical double layer. A metal electrode immersed in an electrolyte solution resembles a capacitor in its ability to store charges. The capacitance at the electrode/solution interface can be described to be built up of a series of several capacitances according to Figure 3. The first capacitance involves the SAMs of thiol (C_{SAM}). The second capacitance comprises the protein layer (C_p), whereas the third capacitance represents the double layer (C_{DL}) caused by the solvated ions of the liquid forming a space charge according to the following equation:

$$\frac{1}{C} = \frac{1}{C_{SAM}} + \frac{1}{C_p} + \frac{1}{C_{DL}} \quad (1)$$

Measurements were made by applying a potential pulse of 50 mV every minute and recording the current transients evoked by the potential step according to the following equation:

$$i(t) = \frac{u}{\mathrm{Rs}\exp(-t/\mathrm{Rs}\,C)} \quad (2)$$

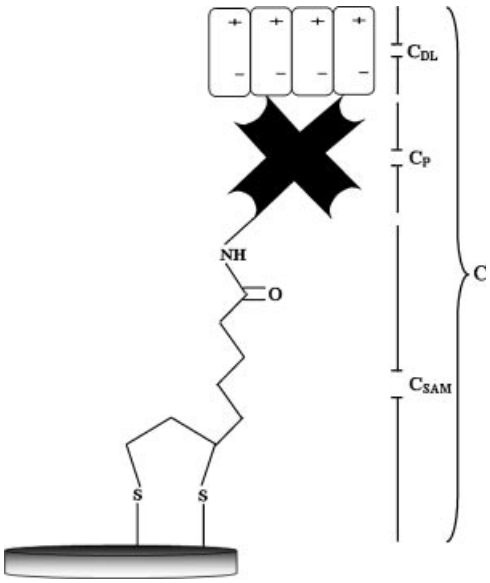

Figure 3. Schematic representation of the contribution of the layers that cover the electrode surface toward the total capacitance.

where $i(t)$ is the current at time t, u is the applied pulse potential, Rs is the resistance of recognition layer, t is the time elapsed after the potential pulse was applied, and C is the total capacitance measured at the working electrode/solution interface. Taking the logarithm of Equation (2), the values of Rs and C can be calculated by the computer software, PotStat, Pascal 7.0, employing the linear relationship between $\ln(i)$ and t (Berggren et al., 1999). Capacitance measurements were performed by inserting the protein-modified electrode as the working electrode in a four-electrode flow-injection manifold cell. As shown in Figure 4, the binding between hIgG and the Con A immobilized on the electrode surface brought about a decrease in capacitance. The average value of the capacitance change (ΔC_{hIgG}) was calculated according to the following formula:

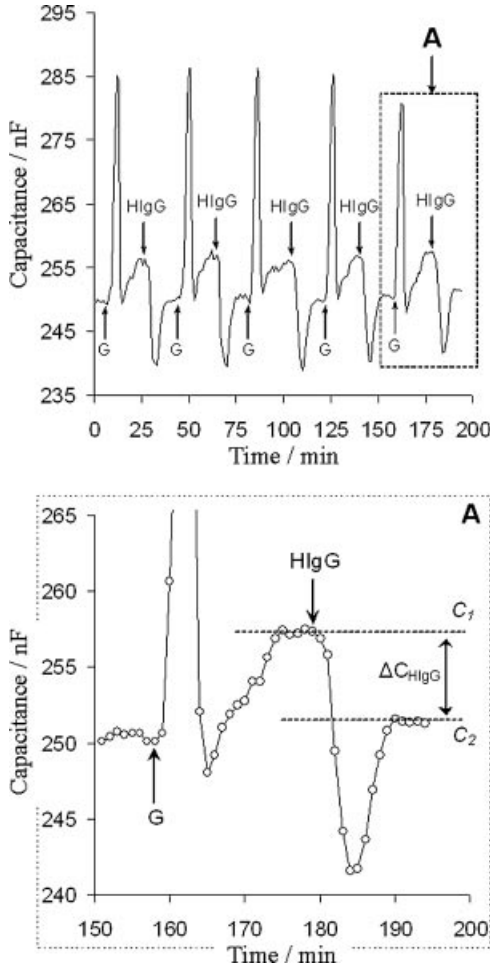

Figure 4. Capacitive response of Con A-coated biosensor in continuous repetitive measurements of 100 μg mL^{-1} hIgG in 5.0 mM Tris–HCl buffer (pH 7.4), containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$, at a flow rate of 50 μL min^{-1}. The system was repeatedly regenerated with 1.0×10^{-1} M glucose (G). Only five repeated assay cycles are shown here. In section A: typical capacitive response obtained upon loading glucose, followed by an injection of the tested hIgG; where C_1 and C_2 represent the baselines after an injection of glucose and hIgG, respectively. ΔC_{hIgG} represents the change in capacitance due to the binding between hIgG and the immobilized Con A, and equals $C_1 - C_2$.

$\Delta C_{hIgG} = C_1 - C_2$, where C_1 and C_2 represent the average of five consecutive baseline values before and after the injection of hIgG, respectively. The system was subsequently regenerated with 0.1 M glucose after each sample injection.

Analytical Performance

Examination of Figure 5 reveals that the detection of hIgG is linear from 5 to 100 $\mu g\,mL^{-1}$, with the regression equation of $y = 4.2291x + 10.591$ ($R^2 = 0.9986$), where y is the change in capacitance in nanofarad and x is the logarithm of hIgG concentration in $mg\,mL^{-1}$. The detection limit was 1.0 $\mu g\,mL^{-1}$ at a signal-to-noise ratio of 3.0. The relative standard deviation (RSD) values were between 5.1% and 6.9% at the tested hIgG concentrations. The device showed a sensitivity of 4.2 nF $(mg\,mL^{-1})^{-1}$, corresponding to the slope of the straight line fitted for the linear range of the inset in Figure 5. The response time was about 15 min. Regeneration ability of the biosensor was confirmed by prompt return to baseline after introduction of 0.1 M glucose subsequent to challenge with 5.0–100 $\mu g\,mL^{-1}$ of hIgG. Furthermore, up to 18 successive assays were achieved without significant loss of sensitivity using a single electrode, suggesting that the developed biosensor is highly reusable and stable in continuous-flow assays. A major advantage of the developed platform is that it can be repeatedly regenerated with a carbohydrate solution via competitive displacement. Such kind of regeneration does not alter the stability and/or activity of the immobilized biorecognition element as compared to other systems that depend on low pH or concentrated salt solutions for regeneration. Consequently, the system can be repeatedly regenerated without any noticeable effect on its sensitivity.

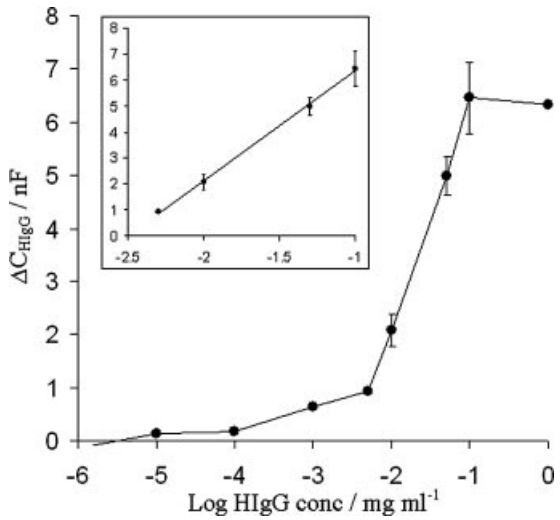

Figure 5. Calibration curve showing the relationship between the capacitance changes and varying concentrations of hIgG obtained with the Con A-modified biosensor. The inset shows the linear range of capacitance changes versus the logarithm of hIgG concentration from 5.0 to 100 $\mu g\,mL^{-1}$. Symbols denote mean values of triplicate measurements of capacitance, and the error bars indicate the standard deviations. Capacitance values are represented by mean values and standard deviations of all measurements.

Quality Control of hIgG

Aggregation of hIgG is a major problem in pharmaceutical industry because of the possible loss of potency and development of serious side effects upon administration (Lebing et al., 2003). Although the aggregates usually exist at extremely low concentrations, often smaller than 0.3% of the total hIgG content, they may have an immense impact on the quality of the product (Ahrer et al., 2003). Furthermore, traces of large hIgG aggregates cannot be determined by SEC combined with UV detection (SEC–UV) alone. As can be seen in Figure 6, the sensor response toward the hIgG aggregate was greater than that toward the monomeric counterpart, over the whole detection range. This result agrees with previous studies, which stated that the interaction between Con A and glycol conjugates occurs by means of biospecific molecular recognition and depends on the molecular mass of the glycol conjugate and its binding affinity to Con A (Aslan et al., 2004; Lee and Pérez-Luna, 2005). The developed biosensor was able to detect very low concentrations of the hIgG aggregate, down to 5.0 $\mu g\,mL^{-1}$, with relatively high sensitivity, taking into consideration that immunoglobulin preparations normally have concentrations ranging between 50 and 165 $mg\,mL^{-1}$ of hIgG. Hence, the system can detect hIgG aggregates with concentrations down to 0.01% of the least total hIgG content. Nevertheless, the system cannot detect the aggregates in the hIgG preparation directly. It can only detect aggregation after removal of the monomeric hIgG via SEC–UV. Therefore, the authors believe that the developed capacitive biosensor can be employed as a post-SEC–UV

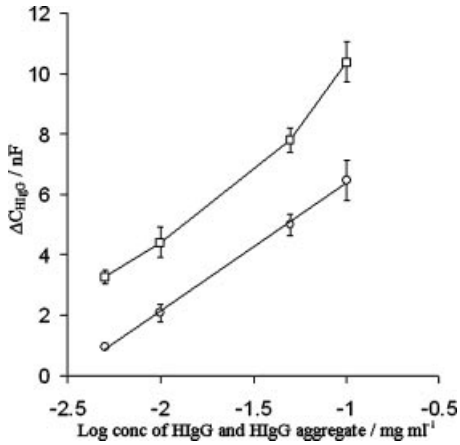

Figure 6. Calibration curves of hIgG (○) and hIgG aggregate (□) by the capacitive biosensor. Symbols denote mean values of triplicate measurements of capacitance. Capacitance values are represented by mean values and standard deviations of all measurements.

affinity assay for the detection of hIgG aggregation, combining high sensitivity with excellent reusability.

Assay Precision and Fabrication Reproducibility

A series of 10 repetitive measurements of 100 $\mu g\,mL^{-1}$ hIgG in 5.0 mM Tris–HCl buffer (pH 7.4), containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$, was used to estimate the assay precision. The series yielded a mean integration of the capacitance response of 6.5 nF, corresponding to a relative activity of 98%, with an RSD of 6.6%, as shown in Figure 7. The fabrication reproducibility was estimated from the response of five different electrodes, prepared under the same conditions, to 100 $\mu g\,mL^{-1}$ hIgG in the same buffer. The RSD of the capacitance response was 9.2%. Such small signal variation reflects the good precision of the developed immunoassay as well as a reasonable electrode-to-electrode reproducibility of the fabrication protocol.

Selectivity of the Developed Biosensor

Con A is a protein that has different binding affinities for various sugar molecules (Ibey et al., 2005). To investigate the selectivity of the developed biosensor, 12 interfering substances with known binding affinities to Con A were tested and triplicate measurements of the capacitive response to 100 $\mu g\,mL^{-1}$ of each substance were performed. Small molecular mass sugars (D-fructose, D-mannose, D-maltose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, β-cyclodextrin) did not cause any significant change in capacitance due to their small sizes. It is worth noting that the capacitive detection is restricted to either analytes capable of directly increasing the thickness of the dielectric layer above the electrode surface by virtue of their large size, or those which can change the conformation of the immobilized biorecognition element (Bontidean et al., 2003, 2004). Even if small carbohydrates will not give rise to measurable disturbances by creating capacitive signals, presence of such low molecular weight compounds might disturb the assays since they might displace the glycoprotein bound by Con A. On the other hand, large molecular mass sugars (dextran 10, 71.4, 298, 500 kDa, β-cyclodextrin polymer, glycogen) brought about significant changes in capacitance. However, these changes are still smaller than those produced by the monomeric or polymeric hIgG. This is due to the higher hydrophobicity of immunoglobulins compared to the tested carbohydrates, which in turn caused larger decrease in capacitance upon replacing the more conducting flow buffer. Furthermore, such carbohydrates do not normally contaminate the immunoglobulin solution.

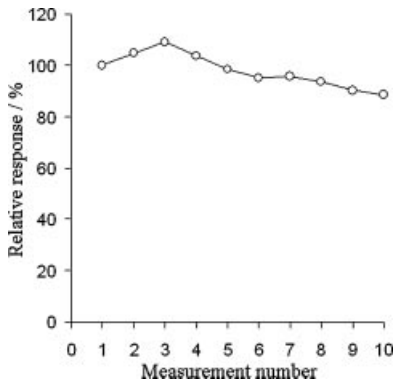

Figure 7. Stability of the prepared competitive glucose biosensor after repeated measurements of 100 $\mu g\,mL^{-1}$ of hIgG in 5.0 mM Tris–HCl buffer (pH 7.4), containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$.

If low molecular weight carbohydrates are present in a cultivation broth to be analyzed, one can relatively simply remove the low molecular weight compounds by integrating an on-line dialysis step in the flow injection assay (Håkanson et al., 1993). With these precautions taken, one can compensate for a low selectivity of the Con A and still get a good sensitivity and selectivity under the conditions the assays are carried out.

Other merits of the developed Con A-based biosensor include its ability to quantify aggregates of hIgG which is less feasible by using anti-hIgG-based assays since the aggregation usually occurs through the Fab part, thus leaving only the Fc part available for detection. The quantification of IgG aggregates needs to be done after separation of monomeric and multimeric IgG using, for example, a size-exclusion chromatographic step (a post-column detection).

The developed biosensor can be easily regenerated by administering a pulse of glucose solution without affecting the sensor sensitivity. When protein A and anti-hIgG-based biosensors are used, regeneration requires harsh conditions due to the strong binding between such bioelements and hIgG molecules, thus compromising the regeneration ability of the biosensor.

Conclusions

Quality and quantity of proteins during bioreactor operations have arrived at center stage with respect to manufacturing and regulatory compliance in the biotechnology industry. The data collected in this study demonstrate the suitability of the developed biosensor for the analysis of hIgG, combining high sensitivity with excellent reproducibility and regeneration ability. Furthermore, it can be employed as a post-SEC–UV-binding assay, providing a promising tool for in-process evaluation of the antibody purity and lot-to-lot consistency. In comparison with the SEC–UV alone or the SEC-LS, the proposed biosensor shows a better sensitivity for the detection of antibody aggregation with minimal interference.

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References

Ahrer K, Buchacher A, Iberer G, Josic D, Jungbauer A. 2003. Analysis of aggregates of human immunoglobulin G using size-exclusion chromatography, static and dynamic light scattering. J Chromatogr A 1009:89–96.

Aslan K, Lakowicz JR, Geddes CD. 2004. Nanogold-plasmon resonance-based glucose sensing. Anal Biochem 330:145–155.

Babac C, Yavuz H, Galaev IY, Pişkin E, Denizli A. 2006. Binding of antibodies to concanavalin A-modified monolithic cryogel. React Funct Polym 66:1263–1271.

Berggren C, Bjarnason B, Johansson G. 1999. Instrumentation for direct capacitive biosensors. Instrum Sci Technol 27:131–139.

Bharathi S, Nogami M, Ikeda S. 2001. Novel electrochemical interfaces with a tunable kinetic barrier by self-assembling organically modified silica gel and gold nanoparticles. Langmuir 17:1–4.

Bian C, Xia S, Xu Y, Chen S, Cui Z. 2005. A micro amperometric immunosensor based on two electrochemical layers for immobilizing antibody, Sens. 2005 IEEE, p 416–419.

Bontidean I, Ahlqvist J, Mulchandani A, Chen W, Bae W, Mehra RK, Mortari A, Csoregi E. 2003. Novel synthetic phytochelatin-based capacitive biosensor for heavy metal ion detection. Biosens Bioelec 18:547–553.

Bontidean I, Mortari A, Leth S, Brown NL, Karlson U, Larsen MM, Vangronsveld J, Corbisier P, Csoregi E. 2004. Biosensors for detection of mercury in contaminated soils. Environ Pollut 131:255–262.

Buchacher A, Biesert L, Helmich B. 1996. Requirements to a 4th generation IGIV characteristics of octagam; product overview. Hämostaseologie 16:74–77.

Buchacher A, Iberer G, Jungbauer A, Schwinn H, Josic D. 2001. Continuous removal of protein aggregates by annular chromatography. Biotechnol Prog 17:140–149.

Cairo CW, Gestwicki JE, Kanai M, Kiessling LL. 2002. Control of multivalent interactions by binding epitope density. J Am Chem Soc 124:1615–1619.

Carter PJ. 2006. Potent antibody therapeutics by design. Nat Rev Immunol 6:343–357.

Chen J, Lee CS. 2002. On-line post-capillary affinity detection of immunoglobulin G for capillary zone electrophoresis. J Chromatogr B 768:105–111.

Chen H, Jiang J-H, Li Y-F, Deng T, Shen G-L, Yu R-Q. 2007. A novel piezoelectric immunoagglutination assay technique with antibody-modified liposome. Biosens Bioelectron 22:993–999.

Cid CC, Riu J, Maroto A, Rius FX. 2008. Carbon nanotube field effect transistors for the fast and selective detection of human immunoglobulin G. Analyst 133:1005–1008.

Denizli A, Arica Y. 2000. Protein A immobilized microporous polyhydroxyethyl methacrylate affinity membranes for selective sorption of human-immunoglobulin G from human plasma. J Biomater Sci Polym Ed 11:367–382.

Dong Y, Wilkop T, Xu D, Wang Z, Cheng Q. 2008. Microchannel chips for the multiplexed analysis of human immunoglobulin G-antibody interactions by surface plasmon resonance imaging. Anal Bioanal Chem 390:1575–1583.

Dutra RF, Coelho GD, Silva VL, Ledingham WM, Filho JLL. 2000. A reusable amperometric biosensor based on a novel silver-epoxy electrode for immunoglobulin detection. Biotechnol Lett 22:579–583.

Fenge C, Fraune E, Freitag R, Scheper T, Schügerl K. 1991. On-line monitoring of monoclonal antibody formation in high density perfusion culture using FIA. Cytotechnology 6:55–63.

Gestwicki JE, Cairo CW, Strong LE, Oetjen KA, Kiessling LL. 2002. Influencing receptor-ligand binding mechanisms with multivalent ligand architecture. J Am Chem Soc 124:14922–14933.

Hamilton RG, Ambli D. 2001. The human IgG subclasses. Darmstadt, Germany: CalBioChem-NovaBioChem.

Håkanson H, Kyroelaeinen M, Mattiasson B. 1993. Portable system for continuous ex vivo measurements of lactate. Biosens Bioelec 8:213–217.

Ibey BL, Beier HT, Rounds RM, Coté GL. 2005. Competitive binding assay for glucose based on glycodendrimer-fluorophore conjugates. Anal Chem 77:7039–7046.

Jiang D, Tang J, Liu B, Yang P, Shen X, Kong J. 2003a. Covalently coupling the antibody on an amine-self-assembled gold surface to probe hyaluronan-binding protein with capacitance measurement. Biosens Bioelectron 18:1183–1191.

Jiang D, Tang J, Liu B, Yang P, Kong J. 2003b. Ultrathin alumina sol-gel-derived films: Allowing direct detection of the liver fibrosis markers by capacitance measurement. Anal Chem 75:4578–4584.

Kim JP, Lee BY, Hong S, Sim SJ. 2008. Ultrasensitive carbon nanotube-based biosensors using antibody-binding fragments. Anal Biochem 381:193–198.

Labib M, Hedström M, Amin M, Mattiasson B. 2009a. A capacitive immunosensor for detection of cholera toxin. Anal Chim Acta 634:255–261.

Labib M, Hedström M, Amin M, Mattiasson B. 2009b. A capacitive biosensor for detection of staphylococcus enterotoxin B. Anal Bioanal Chem 393:1539–1544.

Lausch R, Scheper T, Reif OW, Schlösser J, Fleischer J, Freitag R. 1993. Rapid capillary gel electrophoresis of proteins. J Chromatogr A 654:190–195.

Lebing W, Remington KM, Schreiner C, Paul HI. 2003. Properties of intravenous immunoglobulin (IGIV-C, 10%) produced by virus inactivation with caprylate and column chromatography. Vox Sang 84:193–201.

Lee S, Pérez-Luna VH. 2005. Dextran-gold nanoparticle hybrid material for biomolecule immobilization and detection. Anal Chem 77:7204–7211.

Li J, Gao H. 2008. A renewable potentiometric immunosensor based on Fe_3O_4 nanoparticles immobilized anti-IgG. Electroanalysis 8:881–887.

Li J, He X, Wu Z, Wang K, Shen G, Yu R. 2003. Piezoelectric immunosensor based on magnetic nanoparticles with simple immobilization procedures. Anal Chim Acta 481:191–198.

Li JS, Wu ZS, Wang H, Shen GL, Yu RQ. 2005. A reusable capacitive immunosensor with a novel immobilization procedure based on 1,6-hexanedithiol and nano-Au self-assembled layers. Sens Actuators B 110:327–334.

Liu Y, Yin F, Long Y, Zhang Z, Yao S. 2003. Study of the immobilization of alcohol dehydrogenase on Au-colloid modified gold electrode by piezoelectric quartz crystal sensor, cyclic voltammetry, and electrochemical impedance techniques. J Colloid Interface Sci 258: 75–81.

Lu H-C, Chen H-M, Lin Y-S, Lin J-W. 2000. A reusable and specific protein A-coated piezoelectric biosensor for flow injection immunoassay. Biotechnol Prog 16:116–124.

Mattiasson B. 1984. Immunochemical assays for process control: Potentials and limitations. Trends Anal Chem 3:245–250.

Messina GA, Torriero AAJ, De Vito IE, Olsina RA, Raba J. 2005. Continuous-flow/stopped-flow system using an immunobiosensor for quantification of human serum IgG antibodies to *Helicobacter pylori*. Anal Biochem 337:195–202.

Middendorf C, Schulze B, Freitag R, Scheper T, Howaldt M, Hoffmann H. 1993. On-line immunoanalysis for bioprocess control. J Biotechnol 31:395–403.

Ogi H, Motohisa K, Matsumoto T, Mizugaki T, Hirao M. 2006. Wireless electrodeless piezomagnetic biosensor with an isolated nickel oscillator. Biosens Bioelectron 21:2001–2005.

Pålsson E, Nandakumar MP, Mattiasson B, Larsson P-O. 2000. Miniaturised expanded-bed column with low dispersion suitable for fast flow-ELISA analyses. Biotechnol Lett 22:245–250.

Parkeh RB, Dwek RA, Sutton BJ, Fernandes DL, Leung A, Stanworth D, Rademacher TW, Mizuochi T, Taniguchi T, Matsuta K, Takeuchi F, Nagano Y, Miyamoto T, Kobata A. 1985. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 316:452–457.

Reichert JM, Rosensweig CJ, Faden LB, Dewitz MC. 2005. Monoclonal antibody successes in the clinic. Nat Biotechnol 23:1073–1078.

Reif OW, Lausch R, Scheper T, Freitag R. 1994. Fluorescein isothiocyanate-labeled protein G as an affinity ligand in affinity/immunocapillary electrophoresis with fluorescence detection. Anal Chem 66:4027–4033.

Shortle D. 1996. The denatured state (the other half of the folding equation) and its role in protein stability. FASEB J 10:27–34.

Stacy AR, Bell GD, Newell DG. 1998. The value of class and subclass ELISAs and antibody specificity in monitoring treatment of *Helicobacter pylori*. In: Gasbarrini G, Petrolani S, editors. Basic and clinical aspects of H. pylori infection. Berlin: Springer, p 159.

Suzuki M, Ozawa F, Sugimoto W, Aso S. 2002. Miniature surface-plasmon resonance immunosensors—Rapid and repetitive procedure. Anal Bioanal Chem 372:301–304.

Wilson MS, Nie W. 2006. Electrochemical multianalyte immunoassays using an array-based sensor. Anal Chem 78:2507–2513.

Wisdom GB. 2002. Alkaline phosphatase labeling of IgG antibody. In: Walker JM, ed. The protein protocols handbook. 2nd ed. Totowa, NJ: Humana Press, p 343–344.