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Determination of human growth hormone in human serum samples by surface plasmon resonance immunoassay

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ABSTRACT

A surface plasmon resonance immunoassay has been developed to determine human growth hormone (hGH) directly and without pre-treatment in human serum samples. A binding inhibition immunoassay was employed. Antibody concentration, assay buffer and regeneration solution have been optimized in order to reach the best performance and the lower non-specific binding of the matrix components to the sensor surface. The lowest detection limit was 6 ng/mL, with a working range covering the physiological range. Reproducibility of the assay was excellent with both intra-assay and inter-assay relative standard deviations <5%, while a variation of 2.19% was obtained employing different sensor chips. Reutilization of the sensor surface allows its continuous use over 50 measurements with a signal drop <20%. The SPR immunoassay results were validated using enzyme-linked immunosorbent assay (ELISA) showing an excellent correlation ($R^2 = 0.985$). A portable and fully automated system (Sensia SL) was employed in this work. This is the first SPR biosensor assay capable of detecting relevant concentrations of a clinical analyte in serum. This study shows the potentials of this device as a diagnostic tool for the detection of multiple clinical analytes.

1. Introduction

In the last years diagnostics is starting to leave clinical laboratories to come closer to the patient in point-of-care settings as primary care centres, hospital units and homes [1]. Immunoassay techniques have provided a useful tool for rapid diagnostic and monitoring directly at these locations [2]. Biosensors are ideal for these applications as they can reach similar analytical quality as laboratory methods adding some extra features as a short turnaround time, portability and simple use compared to other analytical techniques [1–3]. Surface plasmon resonance (SPR) biosensors [4] have proven the necessary analytical characteristics as specificity, sensitivity, accuracy and precision. They also provide real-time data avoiding labelling steps, thus fulfilling most of the desirable features for a point-of-care analyzer. However, some disadvantages obstruct the development of all types of optical biosensors for clinical use due to matrix effects produced by non-specific binding of the components of such complex samples.

Blood serum is one of the main sources of clinical analytes and it contains thousands of proteins that provide potential information for disease biomarker detection. But it has the drawback of its complexity as analytical matrix. In fact, 90% of its protein content (60–80 mg/mL) consists in a few high abundant proteins that together with its high lipid content hinder the rest less abundant proteins. The dynamic range of serum proteins is 10 orders of magnitude wide, from sub-pg/mL to above mg/mL. This is a serious obstacle for the development of clinical biosensors handling human serum samples, mainly due to non-specific binding to the sensor surface, which would hinder the specific biological interaction.

Human growth hormone (hGH) is a polypeptide hormone, essential for normal growth and development, secreted by the anterior pituitary gland. Circulating hGH consists of a heterogeneous mixture of proteins including a predominant 22 kDa hGH isoform, and other less abundant variants such as the 20 kDa hGH [5]. Selective assays to define the contribution of the different isoforms could be valuable tools for both clinical diagnostics and basic research. Determination of hGH in serum is essential for the diagnosis of disorders in hGH secretion. hGH excess is commonly caused by pituitary tumours which result in disorders as acromegaly or pituitary gigantism when it occurs in childhood. The main causes of hGH deficiency are pituitary malformations or damages. Detection of hGH is also used by sports authorities in doping detection, as hGH is one

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of the most habitual substances used to increase performance in some disciplines [6].

Secretion of hGH is pulsatile, resulting in widely fluctuating levels in blood with peaks of 50–100 ng/mL and minimum levels of 0.03 ng/mL [5]. It is widely accepted, for the diagnosis of hGH deficiency, a cut-off value of 10 ng/mL in response to an appropriate provocative test [7]. Clinical assays for the determination of human growth hormone include bioassays, radioreceptor assays and immunoassays. Radioimmunoassays (RIA), immunoradiometric assays (IRMA), enzyme-linked immunosorbent assays (ELISA) and immunofunctional assays (IFA) have been used for hGH determination due to their sensitivity and high sample throughput. Conventional selective assays are time consuming, require florescent labels or radioactive probes. In addition, most of these assays require facilities, which obstruct their application in settings out of the lab.

Surface plasmon resonance biosensors allow monitoring of biomolecular interactions as increases in refractive index caused by mass changes at the sensor surface. The coupling of immunoassays with SPR biosensors enables online monitoring of analytes in a fast, simple, direct and reversible way, avoiding labelling and sample preparation steps. In this work, a recently launched commercial sensor, β-SPR (Sensia SL, Spain) based in surface plasmon resonance has been employed to detect hGH in serum samples. This system is portable and incorporates software for data acquisition and instrument control, thus it turns on a useful tool for fast diagnostics in clinical laboratories and point-of-care settings. Recently, this device has proven its usefulness in environmental analysis for the online monitoring of pesticides in natural water samples [8–10].

In this work we report the development and validation of a SPR immunosensor for hGH determination in serum. To our knowledge this is the first biosensor assay for the detection of hGH. Moreover a SPR sensor to detect relevant concentrations of a clinical analyte in serum has not been developed to date [4]. Surface plasmon resonance immunosensor applications that measure clinical samples directly are still unusual, and generally use signal amplification steps, sample pre-treatment or dilution [11–15].

2. Experimental

2.1. Reagents

Purified monoclonal antibody (mAb) hGH-12, that recognizes all hGH isoforms, was obtained and characterized as described before [16]. Recombinant hGH was obtained from Pfizer (Spain). Rabbit and human sera were purchased from Sigma–Aldrich (Steinheim, Germany). Serum samples from patients undergoing stimulation tests for hGH deficiency diagnostic were kindly provided by Dr. M.D. Rodríguez Arnau. Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Mercaptoundecanoic acid, N-hydroxysuccinimide (NHS) and 1-ethyl-3-(-3 dimethyl-amino-propyl)carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (Steinheim, Germany). Organic solvents used in gold chip cleansing process: tricholoroethylene, acetone and ethanol, and piranha solution components H_2SO_4 and H_2O_2 were supplied by Merck (Darmstadt, Germany). Potassium chloride, sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate, used for the preparation of PBST buffer (10 mM phosphate pH 7.4 with 137 mM NaCl, 2.7 mM KCl and 0.05% Tween 20), acetic acid and sodium acetate for the preparation of acetate buffer (10 mM acetate pH 5) and sodium hydroxide and hydrochloric acid, used to adjust the pH of the solutions, were provided by Panreac (Barcelona, Spain). Ethanolamine hydrochloride blocking agent was obtained from Acros Organics (Geel, Belgium). Tween 20 was purchased from Quantum Appligene (Heidelberg, Germany).

2.2. Instrumentation

A commercial surface plasmon resonance biosensor, from Sensia SL (Spain) was used for the SPR measurements. The sensor has two flow cells with a volume of 300 nL each. The device incorporates optics and upgradeable electronic modules as well as computer controlled pumps, valves and injection fluidics. All the measurements were performed by sample injection using the flow delivery system incorporated in the platform that assures the injection for analysis of precise volumes of 220 μL while maintaining a continuous flow of buffer between 10 and 40 μL/min. Further description of this system can be found elsewhere [8].

2.3. Preparation of the sensor surface: immobilization procedure

Specific hGH chip surface was prepared with conventional amino coupling on SPR gold chips. Chips were cleaned with tricholoroethylene, acetone and ethanol, immersed in piranha solution (H_2SO_4/H_2O_2, 3:1) rinsed with water, ultrasonicated for 5 min and dried with N_2. Gold chip was then placed over the flow cells and prism was adhered to the chip using matching refractive index oil. A constant flow speed of 10 μL/min was maintained during the immobilization process. Formation of the carboxyl terminated alkenethiol SAM was carried by adsorption of mercaptoundecanoic acid (0.05 mM solution in ethanol) on the gold sensor surface. After rinse of alkanethiol excess with ethanol, a continuous flow of water was maintained for the next steps of the immobilization process. Then the carboxylic surface was activated with EDC 0.2 M and NHS 0.05 M to form a N-hydroxysuccinimide ester intermediate and immediately after, hGH was covalently coupled via amine groups using two injections with a washing step with HCl 100 mM in between. The unreacted groups remaining on the chip surface were deactivated using ethanolamine 1 M, pH 8.5. Once hGH was immobilized on the sensor surface, a flow of PBST at 20 μL/min was fixed. This procedure ensures that only covalently bound biomolecules remain on the sensor surface. The selectivity of the monolayer was evaluated by studying possible interactions with non-specific antibodies.

2.4. SPR immunoassay format

For calibration curves, a set of triplicate hGH standard concentrations in the 10^{-4} to 100 μg/mL range in PBST and blank controls were mixed (1:1) with mAb hGH-12 in PBST. Then, solutions were injected sequentially over the hGH sensor surface at 20 μL/min and SPR signal was monitored in real-time. Calibration curves with mAb hGH-12 concentrations of 0.25, 0.5, 1 and 2 μg/mL in PBST were evaluated in order to find the one that provides the optimal assay sensitivity. Reutilization of the sensor surface was accomplished by an injection of HCl 5 mM regeneration solution at a flow speed of 30 μL/min. SPR signal of each standard was expressed as the percentage of the maximum response [100 × (SPR signal/SPR signal, max)]. The averaged responses of the three standards measured for each concentration were plotted versus the logarithm of hGH concentration and fitted to a four-parameter logistic equation:

$$y = \frac{D + (A - D)}{1 + (x/C)^B}$$

where x is the concentration, y is the response, A is the asymptotic maximum, corresponding to the signal in absence of analyte, B is the slope at the inflection point, C is the inflection point, equivalent to the half inhibitory concentration I_{50} and D is the asymptotic minimum, corresponding to the background signal.

2.5. Sample matrix effects

Experiments to test matrix effects and the conditions to reduce them for improving the regeneration step were carried with rabbit serum, which has similar behaviour in the SPR immunosensor to human serum. Aliquots of rabbit serum were mixed (1:1) with PBST buffer to simulate assay conditions, injected on the sensor without any treatment and recorded the SPR signal.

Twelve PBST buffers were prepared varying simultaneously NaCl concentration (137 and 500 mM), pH (6.5–7.4–8) and Tween 20 concentration (0.05–0.1%). Rabbit serum aliquots were mixed (1:1) with these twelve different assay buffers and were injected on the sensor at 20 μL/min using the same assay buffer to obtain information of non-specific binding of serum matrix components to the sensor surface. Rabbit serum aliquots were mixed (1:1) with mAb hGH-12 to a concentration in the mixture of 0.25, 0.5, 1 and 2 μg/mL using those different assay buffers, injected on the sensor at 20 μL/min and the SPR response was tested to check mAb hGH-12 interaction with the sensor surface in the serum sample matrix. PBST buffer containing 500 mM NaCl, 0.1% Tween 20 and pH 8, hereafter called PBST-S, was maintained as assay buffer for the rest of the measures of serum samples.

Regeneration solutions combining acid or basic pH with a high ionic strength and surfactant Tween 20 were prepared to assess the regeneration of mAb interaction in serum samples. These factors were varied simultaneously, pH (2, 2.3, 3, 11, 11.7, 12), ionic strength (0, 0.5, 1, 2 M NaCl) and Tween 20 (0, 0.05, 0.1%) and were tested to break the interaction after an injection of a (1:1) mixture of rabbit serum with mAb hGH-12 to a concentration of 2 μg/mL in PBST-S.

2.6. Serum sample analysis

To obtain serum calibration curves, triplicate standards of hGH in the 4×10^{-3} to 40 μg/mL range were prepared in human serum by serial dilution of a stock solution of 1 mg/mL of hGH in PBST-S, mixed (1:1) with mAb hGH-12 to a concentration of 2 μg/mL in PBST-S and injected on the sensor at 20 μL/min. Blank controls were prepared equal. Regeneration after the measurement of each sample was accomplished using regeneration solution with 2 M NaCl, 0.1% Tween 20 and pH 11 at 30 μL/min. Standards were analysed with the SPR immunosensor and calibration curves were calculated as described above.

Serum sample volumes of 110 μL were mixed (1:1) with mAb hGH-12 to a concentration of 2 μg/mL in PBST-S and injected in the sensor without any further treatment. All samples were analysed with both the immunosensor and ELISA method to validate the immunosensor results. Sandwich ELISA for hGH determination was developed using mAb hGH-27 as a capture antibody and biotinylated mAb hGH-12 as second antibody as described before [16].

3. Results and discussion

3.1. Characterization of the hGH SPR immunoassay

An inhibition immunoassay format with the antigen immobilized on the biosensor surface was chosen to extend the immunosensor lifespan. Antibody coated surfaces show low performance as antibody affinity could be lost upon immobilization, because they are sensitive to regeneration conditions and due to the random orientation of immobilized antibodies. Inhibition immunoassay allows a direct measure without need of secondary species or fluorescent labels. This format assures immunosensor reusability and stability maintaining its activity intact throughout a long number of measure and regeneration cycles.

Immobilization of the biological receptor involves the formation of a monomolecular film of the biological recognition element in a controlled and stable manner. Self-assembled monolayers (SAMs) provide an excellent method for this purpose, due to the simplicity and reliability of the process, the reproducibility of the surface upon regeneration and to the flexibility for the incorporation of different biomolecules [17]. Covalent attachment of the recognition element to the sensor surface was achieved via carbodiimide coupling between free amino groups in the protein and carboxyl end of the SAM [18].

hGH concentration and immobilization buffer pH were adjusted to obtain the optimal immobilization conditions that produces the best sensor performance. hGH concentrations of 10, 25, 50 and 100 μg/mL were employed and 10 mM acetate buffer pH was changed from 4, 4.5, 5, 5.5 and 6. Optimal immobilization conditions were found to be hGH 50 μg/mL in 10 mM acetate buffer pH 5. The response of the immunosensor was assessed using different mAb hGH-12 concentrations: 0.1, 0.5, 1, 2.5 and 5 μg/mL. Concentrations lower than 0.25 μg/mL produce signals too low to maintain the desired precision of the assay. The negligible response of non-specific antibodies confirms the selectivity of the monolayer.

The experiments were carried in an inhibitive immunoassay format. This format consists in the addition of a constant amount of antibody to all the samples. Further antibody binding to the immobilized antigen is inhibited by the presence of the analyte, thus producing decreasing SPR signals as the analyte concentration increases. Immunoassay analytical characteristics as detection limit and working range are influenced by antibody concentration, since lower antibody concentrations are saturated by little amounts of the analyte. Therefore, optimization of the immunoassay requires finding a compromise between a low antibody concentration, which provides a better assay sensitivity, and a concentration high enough to generate a quantifiable signal in the SPR immunosensor. A set of calibration curves were obtained using concentrations of 0.25, 0.5, 1 and 2 μg/mL of mAb hGH-12. Calibration standards including concentrations ranging from 10^{-4} to 100 μg/mL hGH and blank samples were mixed with the mAb and injected in the sensor. Fig. 1a shows the SPR signal decreases in response to increasing hGH concentrations. Injection of HCl 5 mM solution disrupts completely the interaction, which allows the sensor reutilization as the SPR signal returns to the initial value after each sample measurement. Analysis cycle, including regeneration, takes 20 min. Calibration curves were calculated using mean triplicate measurements of each hGH concentration. The limit of detection was 4 ng/mL, determined as the analyte concentration inhibiting 3 times the standard deviation of blank samples. The linear working range (18–542 ng/mL) comprises the concentrations producing an inhibition from 20 to 80% of the maximum SPR signal and the I_{50}, 91 ng/mL, is the concentration producing 50% of the maximum SPR signal.

3.2. Determination of hGH in human serum samples

The development of optical biosensors for clinical applications has been hindered by matrix effects caused by high molecular weight species present in any complex biological samples. The applications of SPR biosensors using serum are unusual or generally require pre-treatment or dilution of samples. The aim of this work was to achieve hGH determination in serum directly without dilution or any pre-treatment.

Sample matrix components can affect the immunoassay by binding to the sensor surface, producing a non-specific response that hides the real interaction and prevents adequate sensor regeneration, reducing the device shelf-life. These species can additionally interfere the binding of the antibody to the immobilized antigen in such a way that the specific signal is reduced. In inhibition immunoassays, matrix components can affect the interaction

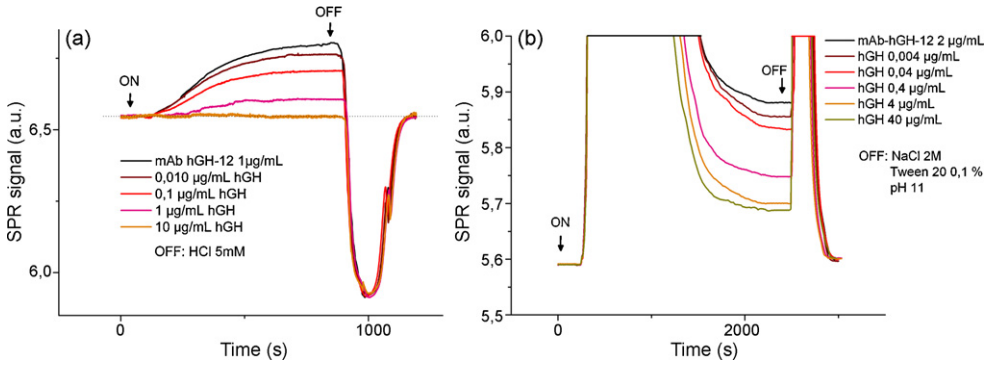

Fig. 1. SPR response to the binding of different hGH concentrations and subsequent regeneration cycles during the performance of binding inhibition tests in (a) PBST and (b) serum.

between the antigen and the antibody in the pre-incubated mixture causing a decrease in assay sensitivity. These matrix effects are a challenge not only for SPR biosensors but also for almost any type of biosensor device, so different strategies have been proposed to reduce their impact, among which stand out the development of surface coatings exhibiting high protein resistance [19]. The influence of assay buffer conditions as pH and ionic strength have been studied, and they have proved that an adequate assay buffer can reduce dramatically the non-specific binding from matrix components [20].

Preliminary tests carried to assess non-specific binding of matrix components present in serum show a large non-specific signal which is far exceeding the specific signal and therefore it will mask any result. The effect of assay buffer on non-specific binding of serum proteins to the sensor surface was studied varying three factors: ionic strength, pH and surfactant Tween 20. Non-specific binding was markedly reduced at high pH in all cases, with an additional decrease at high ionic strength. A high concentration of Tween 20 caused a moderated reduction in non-specific binding as well. An elevated ionic strength contributes to minimization of electrostatic interactions and surfactants as Tween 20 prevent protein aggregation and adsorption. PBST buffer containing 500 mM NaCl, 0.1% Tween 20 and pH 8 (PBST-S) produces a reduction in non-specific binding of 88% compared with previous PBST buffer, showing the best behaviour regarding to non-specific binding.

The response in the SPR immunosensor of mAb hGH-12 at concentrations of 0.25, 0.5, 1 and 2 μg/mL was tested to assess the antigen–antibody interaction in those different buffers. PBST-S has demonstrated the best performance in non-specific binding while maintaining 90% of the antibody signal obtained in PBST. Inhibition immunoassay was neither influenced in these buffer conditions.

Regeneration of the sensor surface after each measurement in serum samples was not achieved in the same conditions used in buffer samples. In this case, regeneration comprises disruption of antigen–antibody binding and desorption of non-specific bound serum proteins. Several regeneration solutions were prepared in order to attain the complete regeneration of the surface using mild conditions that make possible its prolonged use over multiple measurement cycles. Complete regeneration was accomplished using a regeneration solution with 2 M NaCl, 0.1% Tween 20 and pH 11 that was maintained for the rest of the measurements carried out in human serum.

Serum samples were measured using the optimal PBST-S buffer. The fixed amount of antibody in the inhibition assay was raised to 2 μg/mL to compensate the signal reduction due to matrix interference and to enhance the response against the matrix residual background. This increase in antibody concentration leads to a slight decrease in the sensitivity of the assay but it still remains within the physiological concentration range. The measures in serum samples were carried out with the same flow parameters, but after each interaction, the running buffer needs some additional time to wash the surface and to reach a stable signal, enlarging the measure and regeneration cycle to 45 min for the simultaneous determination of two samples. Calibration standards in the 4×10^{-3} to 40 μg/mL range and blank samples were analyzed. Calibration curves were calculated similar to previous experiments. Fig. 1b shows SPR response to different samples containing increasing amounts of hGH and the complete regeneration of the sensor surface. Calibration curve and analytical characteristics of the assay are shown in Fig. 2.

This SPR immunoassay is useful for the determination of a clinical analyte as hGH in serum samples at concentration levels from 6 ng/mL to 1.3 μg/mL that cover the lower part of the physiological range of 30–100 ng/mL, in a direct and fast way without sample pre-treatment or dilution using a small sample volume of 110 μL.

3.3. Reproducibility

The immunoassay reproducibility was studied by the assessment of the intra- and inter-day variability. Triplicate samples with hGH concentrations of 4×10^{-3}, 4×10^{-2}, 4×10^{-1} and 4 μg/mL were prepared in serum and measured on 3 different days to assess within-day and day-to-day variation. Table 1 shows the intra- and inter-assay coefficients of variation of different samples. Mean intra-assay values were below 3% and inter-assay mean value was

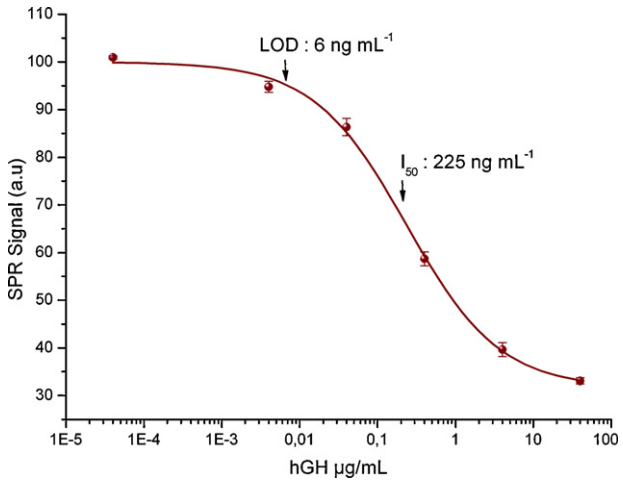

Fig. 2. Standard calibration curve of hGH in serum. Each point shows the mean value of three replicate measurements.

Table 1
Performance of intra- and inter-assay variations for the SPR determination of triplicate measurements of hGH over a 3-day period.

	hGH concentration (μg/mL)				Mean
	0.004	0.04	0.4	4	
Intra-assay variation (R.S.D.%)					
Day 1	1.22	2.10	2.52	3.71	2.39
Day 2	0.57	1.22	0.81	3.16	1.44
Day 3	0.37	1.83	3.24	4.92	2.59
Inter-assay variation (R.S.D.%)					
3-Day period	0.24	1.71	2.48	3.06	1.87

1.87%. Furthermore, all the individual coefficients of variations were below 5%. All these values were by far within the accepted variability values for analytical methods.

The variability of the assay using different sensor chips was also tested. Triplicate standard curves were measured under identical conditions using three different sensor surfaces prepared at different times. Fig. 3 shows the three calibration curves for the three sensor chips tested. The chip-to-chip reproducibility is excellent, showing a mean coefficient of variation of 2.19% with I_{50} values varying from 226 to 235 ng/mL.

3.4. Reusability

Together with reproducibility, biosensor reusability is a key point of the immunoassay robustness. The reusability is related to the stability of the sensor surface throughout a number of measurements. Reutilization of an immunosensor is accomplished regenerating the sensing surface by the complete dissociation of the antigen–antibody complex without affecting the affinity of the immobilized molecule. Antigen–antibody interactions are caused by electrostatic, Lewis acid–base and van der Waals forces, which can be disrupted by different strategies. Essentially, regeneration conditions for immunosensors can be guided by experience in affinity chromatography elution conditions. In most cases, regeneration is carried by extreme pH solutions as HCl or glycine pH 1–3 or NaOH pH 10–11, but high ionic strength, detergents and non-polar water-diluted solvents have been used as well. Solutions combining several of these additives can be used to regenerate resistant interactions or to minimize the risk of bioreceptor damage [21].

The stability of the immunosensor upon regeneration was tested by measuring the variation of the maximum SPR signal produced by blank samples introduced in each calibration or sample series.

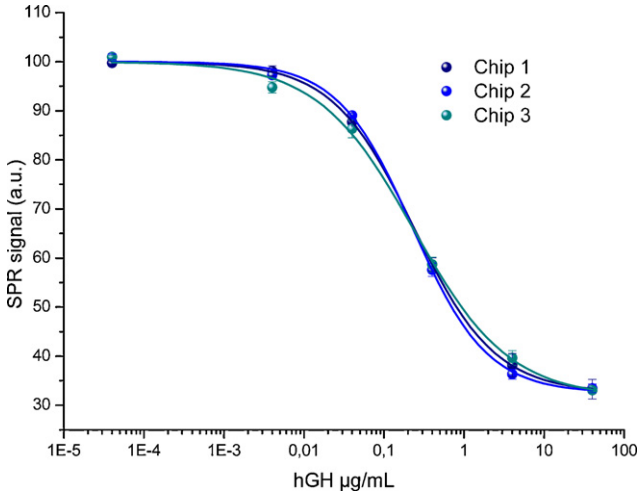

Fig. 3. Standard calibration curves of hGH for three different batches of sensor chips. Each point shows the mean value of three replicate measurements.

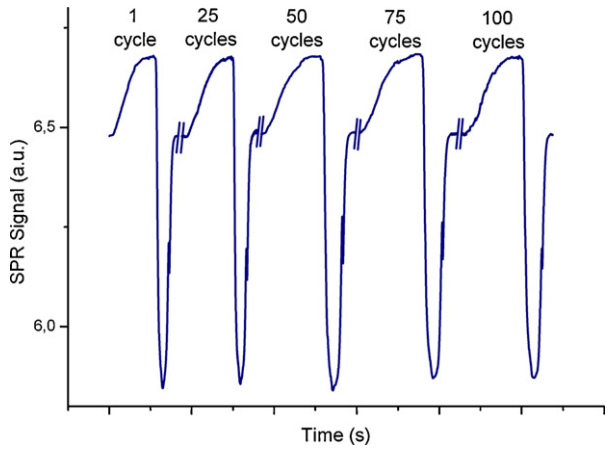

Fig. 4. Sensorgram showing the specific SPR response throughout successive measurement and regeneration cycles.

For hGH immunoassay in PBST, mild acid solution HCl 5 mM provides a complete regeneration of the biosensor surface allowing its stable use throughout above 100 measurement cycles within each flow cell. Stability of the sensor was tested in 10 series of daily measurements, making possible its continuous use with a maximum SPR signal drop of 7% of the initial value (Fig. 4). For the measurements carried out with serum samples an inferior reusability is observed as a predictable consequence of the matrix effects described above. Harsher regeneration conditions were employed to disrupt antigen–antibody binding and to desorb non-specific bound serum proteins. Complete regeneration of the biosensor surface is accomplished by the use of a solution containing NaCl 2 M, Tween 20 0.1% at pH 11. Although harsher regeneration conditions were needed, regeneration performance remained appropriate and sensor stability was maintained throughout more than 50 measurement cycles during 5 consecutive days with a maximum SPR signal drop of 17% of the initial value. These slight variations on the signal do not have effect over assay reproducibility since all the measurements are always normalized to the maximum SPR signal.

3.5. Comparison with ELISA analysis of real samples

The accuracy of hGH determination by SPR immunoassay was evaluated by comparison with ELISA method. Ten real samples from hGH deficiency provocative tests were analysed by both methods, among them five revealed concentrations under the SPR immunoassay detection limit (6 ng/mL). The five remaining samples above this level showed an excellent agreement between both methods ($R^2 = 0.985$). These data reveal that the SPR immunoassay method proposed here is capable to help hGH deficiency diagnosis identifying the samples under the cut-off value (10 ng/mL) of hGH deficient patients and accurately quantifying the samples above the cut-off level of non-deficient patients.

4. Conclusions

The SPR immunoassay method developed in this work demonstrates the usefulness of the β-SPR biosensor for the detection of a relevant clinical analyte as hGH in real serum samples. The method makes possible a fast, label-free and real-time determination using reduced sample volumes. This portable SPR immunosensor is able to determine the analyte directly and without any pre-treatment in serum samples. To our knowledge this is the first biosensor assay for the determination of hGH and the first application of a SPR biosensor for the direct detection of an analyte in serum samples at

relevant concentrations without dilution or sample pre-treatment [4].

The robustness of the method was verified with the excellent reproducibility together with the appropriate regeneration performance. The sensor signal remained stable during more than 50 measurement cycles throughout 5 days of continuous operation. Reproducibility of the method was proven by the low coefficients of variation for intra- and inter-assay precision and low chip-to-chip variation. The validation of hGH determinations in serum samples from stimulation tests for hGH deficiency diagnosis was accomplished by ELISA method. The good correlation of the data demonstrates the application of the method as a valuable tool for fast diagnostics in clinical laboratories and point-of-care settings. This study paves the way for the development of a portable diagnostic tool for the detection of multiple clinical analytes in biological samples and monitoring of protein disease biomarkers.

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