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Rapid C-reactive protein determination in whole blood with a White Light Reflectance Spectroscopy label-free immunosensor for Point-of-Care applications

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ABSTRACT

The fast and accurate determination of C-reactive protein (CRP) in human whole blood samples employing a white light reflectance spectroscopy sensing platform is demonstrated. Each assay cycle was completed in 12 min, including 5-min reaction with the diluted whole blood samples, 3-min with the biotinylated anti-CRP antibody, and 4-min with streptavidin solution. The effect of whole blood on the assay performance was evaluated, and it was found that dilutions as low as 50-times could be employed. Thus, taking into account that the assay had a detection limit of 2.2 μg/L CRP in 50-times diluted sample, whole blood concentrations as low as 110 μg/L could be determined. In addition, due to the implementation of a two-step sandwich immunoassay, high-dose hook-effect was avoided. The accuracy of the assay was determined through recovery experiments by spiking whole blood samples with exogenous CRP. Mean recovery values ranging from 92 to 108% were obtained, indicating the accuracy of measurements performed with the developed immunosensor. The excellent analytical characteristics, the short analysis time and the compact instrument size support the applicability of the immunosensor developed for point-of-care CRP determinations.

1. Introduction

CRP is a biomarker widely used in clinical practice to detect infections and inflammatory conditions ranging from injury to autoimmune diseases [1]. CRP is an acute phase protein whose concentration in human serum could increase from normal values (<5 mg/L) to concentrations up to 10 mg/L, within 6–12 h after the inflammation onset, and reach values higher than 100 mg/L after 48–60 h [2]. Persistently high CRP blood concentrations are indicative of non-reversible conditions and they have been correlated to high mortality rates amongst hospitalized patients [2]. CRP serum levels are also used to distinguish between bacterial and viral infections, since its concentration increase abruptly in bacterial but not in viral infections. Thus, its determination could help to reduce the unnecessary administration of antibiotics, which are usually administered when CRP serum levels exceed 20 mg/L [2–4]. In these cases, the monitoring of CRP levels is also implemented to evaluate the efficacy of the antibiotic treatment [2–5]. CRP is also the best marker (as compared to other inflammation markers such as interleukin-6 and soluble tumor necrosis receptors) for the diagnosis of neonatal sepsis (with a cut-off value of 5 mg/L), which is one of the major causes of neonatal morbidity and mortality caused from exposure to bacteria either prior or after their delivery [2]. Moreover, mildly elevated CRP serum levels (1–3 mg/L) have been correlated with high risk for cardiovascular events [2,6,7], and therefore CRP determination has been used in prevention programs to identify patients that run such a risk [8–10].

The clinical significance of CPR has boosted the development of numerous methods for its quantitative determination in human biological fluids, mainly blood serum or plasma. The majority of these methods are immunochemical [10,11], with a wide range of formats such as, standard 96-well plate ELISA assays [12],

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ultra-high sensitivity sensors [13], automated large-volume clinical analyzers [14], and Point-of-Care (PoC) portable instruments [15]. The latter could be employed for on-site determination of CRP levels in decentralized first-aid units, during the transfer or right after the admission of patients to hospitals, as well as to monitor response to therapy with antibiotics. The employment of label-free sensing approaches is beneficial for point-of-care determinations since they allow for direct and therefore faster analysis as compared to labelled ones. Thus, label-free CPR immunosensors based on different transduction principles either optical [13,16–18] or electrochemical [19] have been developed.

In a previous report [20], we demonstrated the simultaneous determination of two biomarkers related with the prognosis of a cardiovascular event, CRP and D-dimer, in human blood plasma samples. This was achieved by combining a White Light Reflectance Spectroscopy (WLRS) sensing platform with a biochip spotted on spatially discrete areas with the respective analyte-specific antibodies. Measurements were performed by computer-controlled mechanical movement of the biochip with respect to the reflection probe used for the white light illumination of the biochip surface and collection of the reflected light. Due to different concentration ranges of the two analytes in the human blood plasma and the binding characteristics of the respective antibodies, CRP was directly detected through a 20-min reaction with the calibrators or the diluted human plasma samples, whereas a two-step immunoassay was employed for D-dimer.

In the present report, CRP is determined in whole blood samples without any pre-treatment apart from dilution with assay buffer aiming at PoC applications, e.g., emergency departments. The direct determination of CRP in whole blood combined with a short assay duration are key factors in the pace to reduce the time between sampling and bioanalytical result, and thus surpass the need to wait for the central laboratory to perform the CRP determination in blood serum or plasma, in order, for example, to administrate antibiotics [21]. The shortened sampling to result time will thus lead to swift medical decisions when treating patients with acute infection symptoms with pronounced positive effect on their life. To reduce the assay duration while preserving the assay analytical sensitivity in the current study, a two-site sandwich immunoassay format employing a biotinylated reporter antibody in combination with streptavidin was selected, as it is schematically depicted in Fig. 1a. In addition, the CRP determinations were executed using a miniaturized USB-powered and controlled reader accompanied by a small size peristaltic pump, thus addressing the PoC needs.

2. Materials and methods

2.1. Reagents

C-Reactive Protein (CRP) from human fluids and affinity purified polyclonal goat antibody against human CRP (code GC019) were from Scripps Laboratories (San Diego, CA). (3-Aminopropyl)triethoxysilane (APTES), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Succinimidyl-6-(biotinamido)hexanoate (EZ-Link NHS-LC-Biotin) was from Pierce (Rockford, IL). Biotinylation of the goat anti-CRP antibody was performed following a previously published procedure [22]. The calibration of CRP standard solutions was performed using a commercially available ELISA kit (hs-CRP; DIAsource Immunoassays S.A., Louvain-la-Neuve, Belgium). Peripheral blood samples from anonymous donors were collected by the NCSR "Demokritos" infirmary after approval from the Centre's Ethics Committee and informed consent of the donors. The blood was collected by finger-pricking in heparinized glass capillary micro hematocrit tubes (internal diameter 1.15 mm, length 75 mm) purchased from Vitrex Medical A/S (Herlev, Denmark). After transferring the blood to Eppendorf tubes, 10 μL were obtained and diluted with assay buffer prior to the assay. The fluidic compartments and the docking station were designed and manufactured by Jobst Technologies GmbH (Freiburg, Germany).

2.2. Instrumentation

The measurement system consisted of: a) FR-pOrtable system (ThetaMetrisis, Athens, Greece) accommodating the light source, the reflection probe, and the spectrometer, in a device with dimensions of $30 \times 11 \times 4.4\,cm^3$ (Fig. 1b & c), and b) a custom-made computer controlled peristaltic micropump based on the CPP1-150 unit of Jobst Technologies GmbH, for continuous delivery of reagents at a constant rate of 20 μL/min. A custom designed docking station that provides for: a) correct alignment with the microfluidic module, b) accurate positioning of the antibody zone with respect to the reflection probe, and c) complete protection from external light fluctuations was employed in all measurements (Fig. 1c).

2.3. Biochip preparation and assay performance

A 1000-nm thick silicon dioxide layer was grown by wet oxidation at 1100 °C over standard Si wafers (⟨100⟩) which were then diced to obtain 5 mm x 15 mm chips. The SiO_2/Si chips were hydrophilized by oxygen plasma treatment in a Reactive Ion Etcher for 30 s and then immersed in a 2% (v/v) aqueous APTES solution. After incubation for 20 min, they were washed with distilled water, dried under a N_2 stream and cured for 20 min at 120 °C. The APTES-modified chips were then functionalized with the anti-CRP antibodies by spotting a solution with concentration 200 mg/L prepared in carbonate buffer, pH 9.2, using the BioOdyssey Calligrapher™ microarray spotter (Bio-Rad Laboratories, Hercules, CA). The chips were incubated overnight in a controlled humidity chamber (average humidity 75%) and then they were washed with Tris-HCl 10 mM, pH 8.25, 9 g/L NaCl (washing solution) and immersed in blocking solution (10 g/mL BSA in 0.1 M $NaHCO_3$, pH 8.5) for 2 h at room temperature. Finally, the biofunctionalized chips (biochips) were washed with washing solution and distilled water, and dried under nitrogen flow. The biochips were placed onto the docking station and the fluidic module was applied. Once all the fluidic connections were secured, assay buffer (Tris-HCl 50 mM, pH 7.8, 5 g/L BSA, 0.5 g/L bovine IgG, 9 g/L NaCl) was run over the biochip. Then, the CRP calibrators in assay buffer or 50-times diluted with assay buffer whole blood, as well as the diluted whole blood samples were introduced and run for 5 min. After that, a solution containing 5 mg/L of biotinylated anti-CRP antibody in assay buffer was run for 3 min, followed by running for 4 min a 5 mg/L streptavidin solution in the same buffer. The biochip surface was regenerated by running 0.1 M glycine/HCl buffer, pH 2.5, for 3 min, followed by washing and equilibration with assay buffer prior to the next run.

2.4. Data processing and assay evaluation

For the monitoring of the biomolecular adlayer thickness and the calculation of the CRP concentration in the sample, a dedicated software was implemented (Fig. 1d). The software acquires the reflectance signal from the embedded spectrometer in real-time (integration time 15 ms; 1 spectrum per second). From the collected reflectance spectra, after their normalization with respect to the dark and reference spectra (spectrum from a plain Si wafer substrate of known reflectivity), the effective biomolecular layer thickness was determined implementing the Levenberg-Marquart algorithm [22]. As this conversion is done by the software, the evolution of the effective biomolecular layer thickness during the

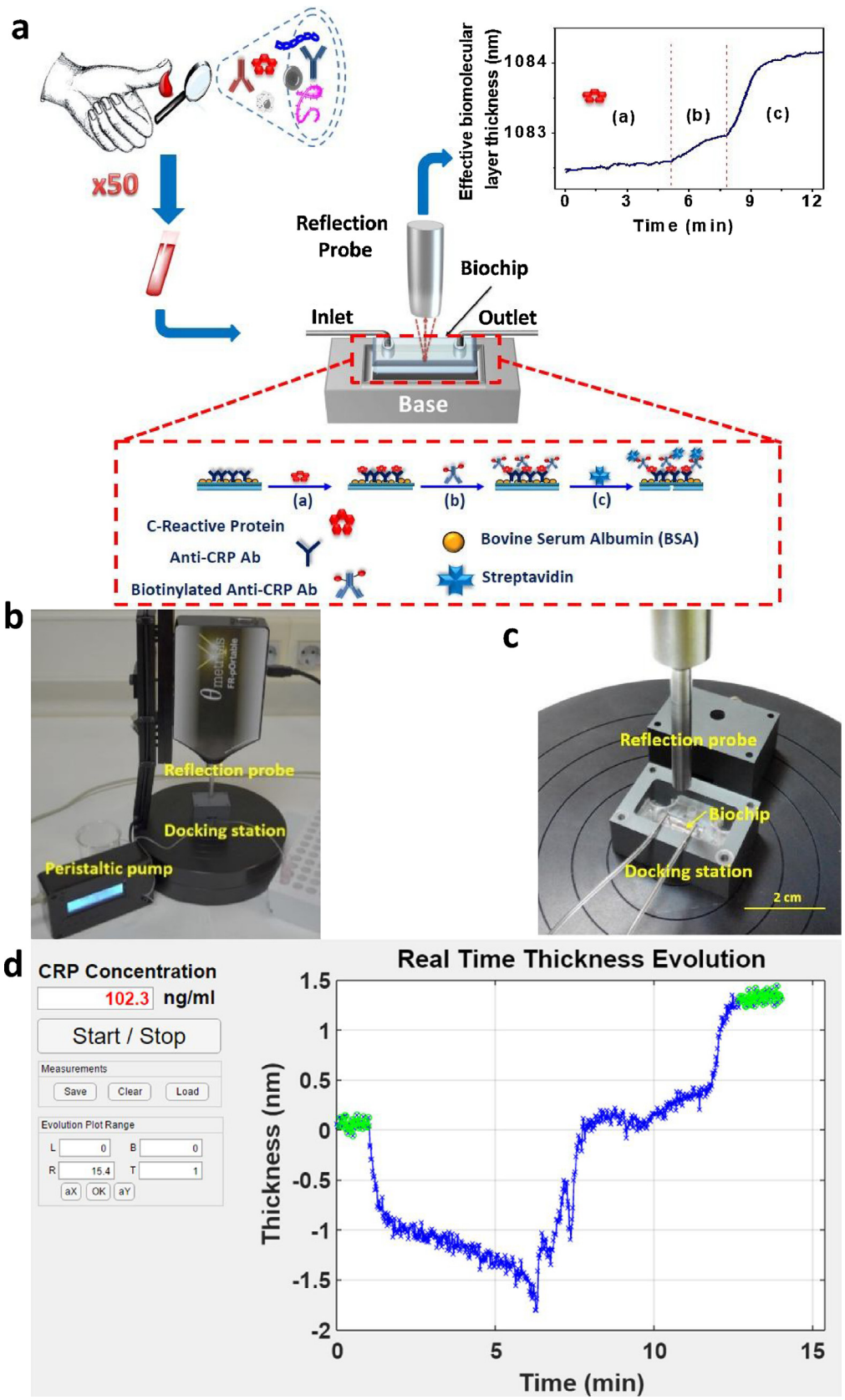

Fig. 1. (a) Schematic depicting sample collection (upper left side), optical set-up (middle), assay procedure (bottom) and real-time signal acquisition (upper right side). (b) Photograph of the biochip assembled with the docking station and the jacket. (c) Photograph of the whole system. (d) Screenshot of the software controlling the measuring apparatus. The user has just to press the Start/Stop button to perform the assay and the measurement. The software automatically calculates the concentration of the CRP in the sample according to an already loaded calibration curve taking into account the signal difference between the two green points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

course of the binding reactions taking place onto biochip surface could be monitored in real time (Fig. 1d). Taking into account the noise signal is 0.02 nm, the lowest value of effective biomolecular layer thickness that can be distinguished from the noise is 0.06 nm. The CRP concentration in the samples was determined using the linear regression equation originating from the linearization of the calibration curve obtained using the responses of calibrators prepared in a human whole blood sample with CRP concentration of approximately 75 μg/L (as determined in the corresponding blood serum by a commercially available CRP ELISA kit). The response of the human whole blood sample used for the preparation of calibrators was subtracted from those received from the rest of the calibrators. Assay repeatability was assessed by analyzing three control samples prepared in a whole blood sample spiked with CRP concentrations so as their final concentration to be 2.5, 7.5 and 12.5 μg/L. These samples were diluted 50-times with assay buffer and run 3 times within the same day to determine the intra-assay repeatability, and for at least 20 runs in 5 different days to determine the inter-assay repeatability. To determine the assay accuracy, recovery tests were run. For this purpose, three blood samples from anonymous donors were spiked with known concentrations of CRP and the analyte concentration was determined both prior to and after the addition. All samples were diluted 50-times prior to analysis. To calculate the percent recovery values, the initial concentration of each sample was subtracted from the concentrations determined in the spiked samples; this difference which corresponds to added CRP amount determined was divided by the actual CRP amount added to get the percent recovery values according to the formula:

$$\%\text{Recovery} = (\text{Added CRP amount determined}/\text{Added CRP amount}) \times 100.$$

3. Results and discussion

3.1. Sensor biofunctionalization

The immobilization of anti-CRP antibodies onto the chips surface was based on their physical adsorption after chemical modification of the chips with APTES. APTES, as all silanes, reacts with surfaces that contain hydroxyl groups to form siloxane bonds with simultaneous loss of ethanol molecules [21]. To create the hydroxyl groups onto the SiO_2 surface of the chips used in the present study, a short treatment in oxygen plasma is employed. Then, the chips are immersed in an APTES solution, where silanization is initiated by the hydrolysis of its ethoxy terminal groups. This is the ideal situation where an APTES monolayer is formed with all of its amine-groups directed away from the surface. Usually the APTES layer formed includes also multilayers due to polymerization between APTES molecules, as well as, APTES molecules that have been attached with their amine group to the hydroxyl groups of the surface [23]. The APTES layer could be used to covalently attach the antibodies through its amine groups or, as in our case, physical adsorption of antibodies could be performed.

3.2. CRP assay development

The detection of CRP using the WLRS method was previously demonstrated in the context of a dual-analyte assay aiming to the simultaneous determination of CRP and D-dimer in human blood plasma samples [20]. A direct assay was selected for CRP based on 20-min reaction of calibrators or samples with the immobilized onto the biochip antibody. Nevertheless, it was found that the assay detection limit could be improved up to 5000-times by employing a biotinylated-reporter antibody and streptavidin for signal enhancement. Thus, in the present study we first determined the maximum signal that could achieved following this assay format by prolonging each one of the assay steps so as to achieve plateau values. As shown in Fig. 2a for a 100 μg/L CRP calibrator, regarding the first assay step, i.e., the reaction of analyte molecules in the sample with the immobilized onto the biochip antibody, maximum plateau values were achieved after about 45–50 min of reaction, while the second step, i.e., the binding of biotinylated reporter anti-CRP antibody onto immunoadsorbed CRP molecules, is completed in about 30 min, and the last one, i.e., the binding of streptavidin to biotinylated reporter antibody, needs also approximately 30 min. To shorten the assay time, several combinations of duration for each step were tested. For the first step, duration times of 5, 10, 20 and 50 min were tested, keeping the duration of both the second and third step at 30 min. Then, for a set duration of 5 min for the first step, duration times of 3, 6, 15 and 30 min were tested, for a set duration of 30 min for the third step. Finally, by employing reaction duration of 5 and 3 min for the first and the second step, respectively, a reaction duration of 4, 10, 20 and 30 min, was tested for the third step. The results are presented in Fig. S1 of Supplementary content. As shown, when the duration of the first reaction step was reduced from 50 to 5 min, the signal decreased by approximately 25%. By keeping, the first reaction step duration at 5 min and reducing the second step duration from 30 to 3 min, an additional 35% signal reduction was obtained. Finally by reducing, the third step duration from 30 to 4 min, a 40% signal drop was observed. Taking it overall, the reduction of the assay duration from 110 to only 12 min, resulted in a signal decrease of approximately four times. The real-time sensor responses obtained following the selected assay protocol with CRP calibrators prepared in assay buffer with concentrations ranging from 2 to 1000 μg/L are depicted in Fig. 2b. As shown, concentrations as low as 2 μg/mL could be distinguished from the zero calibrator which is well below the cut-off values for healthy individuals (<5 mg/L).

3.3. Effect of whole blood on CRP assay

Due to fact that in the sensing system used, both the incident and the reflected light has to pass through the medium layer in order to reach the surface or the spectrometer, respectively, the real-time signal is expected to be affected by the presence of coloured, particulate or other components of the medium that could adsorb and/or scatter either the incident or the reflected light. To access the effect of whole blood on the CRP measurements, a whole human blood sample with CRP concentration of approximately 75 μg/L (as determined in the corresponding serum by a commercially available CRP ELISA kit) was spiked with different amounts of CRP so as after 50, 100, 200 and 500-times dilution with assay buffer the final concentration to be in all cases 100 μg/L. Taking into account that a CRP concentration of 75 μg/L in serum corresponds to a whole blood concentration of approximately 40 μg/L [24], the contribution of the endogenous CRP to the final concentration of spiked samples is not significant (0.8, 0.4, 0.2 and 0.08 μg/L for the 50, 100, 200 and 500-times diluted sample, respectively). The responses obtained from these samples are depicted in Fig. 2c. As shown, even at 500-times dilution the introduction of the diluted blood led to a decrease in the signal as compared to assay buffer (area from arrow 1–2), that was much more prominent for samples that have been diluted 50 or 100-times. To distinguish the response due to the binding reaction from the that of the blood matrix, the biochip surface was washed out (for approximately 3 min) with assay buffer after running of the diluted blood and prior to the introduction of the biotinylated anti-CRP antibody solution. This enable us to determine the signal increase due to the two last immunoassay steps that are identical for all blood dilutions tested and also equal to the respective sig-

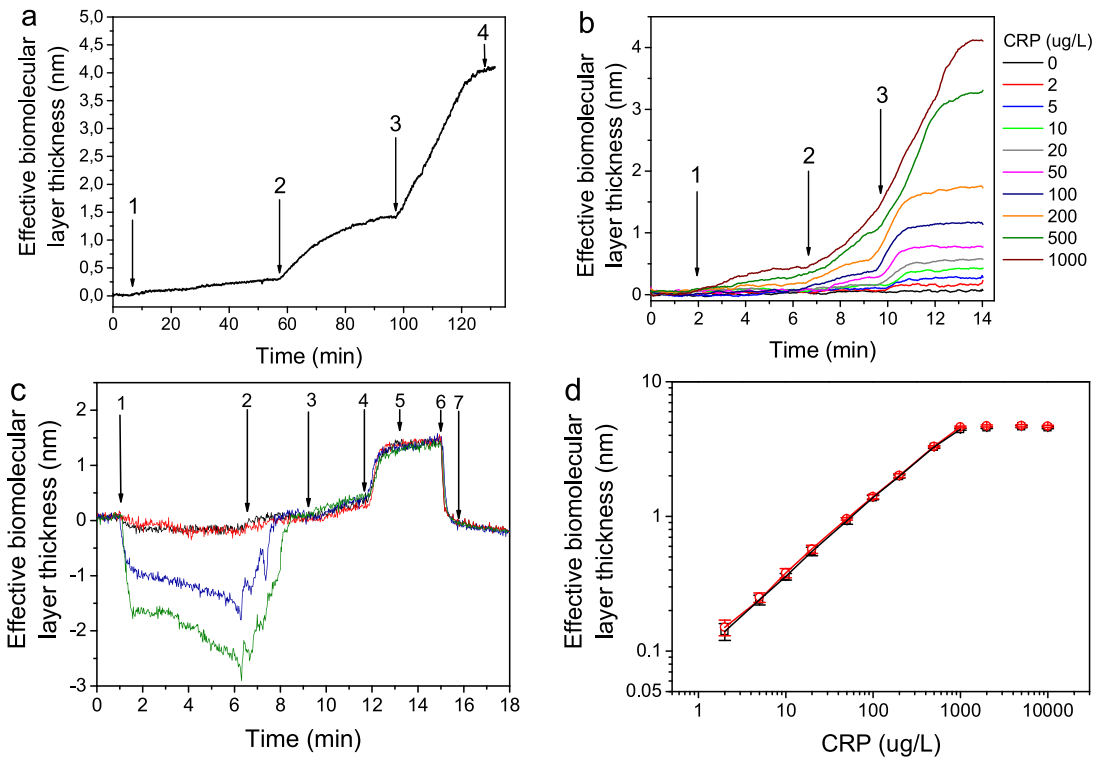

Fig. 2. (a) Real-time response obtained upon running over a biochip functionalized with an anti-CRP antibody a 100 μg/L CRP calibrator (arrow 1–2), a 5 mg/L biotinylated anti-CRP antibody solution (arrow 2–3), and a 5 mg/L streptavidin solution (arrow 3–4). (b) Real-time responses obtained for CRP calibrators with concentrations ranging from 1.0 to 1000 μg/L when the calibrators prepared in assay buffer were run over the biochip for 5 min (arrow 1–2), followed by 3-min reaction with the biotinylated anti-CRP antibody (arrow 2–3) and 4-min reaction with streptavidin (arrow 3 to end). (c) Real-time responses obtained from a blood sample spiked with CPR at different concentrations so as the final concentration to be 100 μg/L after blood dilution 50 (green line), 100 (blue line), 200 (red line) and 500-times (black line) with assay buffer. The sequence of solutions run over the biochip are as follows: assay buffer: start to arrow 1; diluted human blood: arrow 1–2; assay buffer: arrow 2–3; biotinylated anti-CRP antibody solution: arrow 3–4; streptavidin solution: arrow 4–5; assay buffer: arrow 5–6; regeneration: arrow 6–7; washing buffer: arrow 7 to end of run. (d) Typical CRP calibration curve obtained with the 12-min assay using calibrators prepared in assay buffer (black squares) or in whole blood (red circles). In the latter case, all calibrators were diluted 50-times with assay buffer. Each point is the mean value of 4 replicate measurements ± SD. Solid lines correspond to linear regression curves. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nal changes observed during analysis of a 100 μg/L CRP calibrator prepared in buffer. Thus, whole blood dilutions as low as 50-times could be used. It should be noted that this washing step is not necessary, since the overall signal change determined taking into account the difference in the effective biomolecular layer thickness after the completion of the assay to that prior to the introduction of the sample is the same for all the blood dilutions tested and with that determined for the calibrators prepared in buffer. Regarding the implementation of less diluted whole blood samples, it was found that for dilutions ranging from 2 to 20, the net signal corresponding to different CRP calibrators was gradually decreased as the dilution decreased from 20 to 2-times, signifying an negative effect of the whole blood on the immobilized antibody activity.

Based on these results, calibrators with CRP concentration ranging from 100 μg/L to 500 mg/mL were prepared starting from a whole blood sample with CRP concentration of approximately 75 μg/L (as determined in the corresponding blood serum by a commercially available CRP ELISA kit) which was considered as zero calibrator. Taking into account that the concentrations of analytes present mainly in serum or plasma are approximately 1.8-times higher than the respective in whole blood, the endogenous CRP levels in the blood sample used as matrix for the preparation of calibrators is lower than 40 μg/L [24]. The calibration curve obtained using 50-times diluted whole blood calibrators was compared to that obtained with calibrators prepared in buffer. As depicted in Fig. 2d, the two calibration curves where superimposed. The detection limit (LOD) of the assay was calculated as the concentration corresponding to +3SD of 20 replicate measurements of zero calibrator and was 2.1 μg/L in buffer and 110 μg/L in whole blood. Respectively, the limit of quantification (LOQ), defined as the concentration corresponding to +10SD of 20 replicate measurements of zero calibrator, was 8 μg/L in buffer and 400 μg/L in whole blood. The linear response dynamic range extended up to 1000 μg/L in both the buffer and the diluted whole blood sample which corresponded to 50 mg/L in the whole blood sample. The calibration curve was linearized at the log–log scale resulting in a linear regression equation of: $\log Y = -0.56(\pm 0.01)\log X - 0.98(\pm 0.01)$, $r^2 = 0.998$. This equation was used to determine the CRP concentration at the whole blood samples ($X = 10^{(\log Y + 0.98)/-0.56)}$), where X is the CRP concentration (μg/L) and Y the effective biomolecular layer thickness in nm. It should be noted that for CRP concentrations higher than 1000 μg/mL and up to 10000 μg/L in the diluted sample (from 50 to 500 mg/L in whole blood), plateau signal values were reached without any indication of high dose hook effect. This way, the biochip developed could cover the wide range of CRP concentrations in human blood samples.

3.4. Analytical evaluation

The whole blood CRP assay repeatability and accuracy was evaluated. The intra-assay coefficient of variation (CV) values ranged from 3.7 to 7.3%, whereas the inter-assay CV values from 4.8 to 9.1%, proving the good repeatability of the determinations performed with the developed sensor. The accuracy of the assay was evaluated through recovery experiments. The percent recovery values

Table 1
Comparison of the proposed sensor with commercially available POC devices for the determination of CRP in whole blood.

Analyser	Method	Sample volume	Analysis time	Working range	Instrument weight
Smart (Eurolyser)	Immunoturbidimetric assay	5 μL	4 min	2.0–240 mg/L	3.4 kg
CUBE (Eurolyser)					2.4 kg
Afinion™(Allere)	Immunometric membrane flow-through assay	1.5 μL	3–4 min	5–200 mg/L	5 kg
Nycocard® (Allere)		5 μL	3 min	8–200 mg/L	0.54 kg
QuikRead go® (Orion Diagnostica)	Immunoturbidimetric assay	20 μL	2 min	5–200 mg/L	1.3 kg
i-CHROMA™ (SYCOmed)	Fluorescence solid-phase sandwich immunoassay	10 μL	3 min	2.5–300 mg/L	1.2 kg
Microsemi (Horiba)	Immunoturbidimetric assay	10 μL	4 min	2.0–230 mg/L	19 kg
AQT90 Flex (Radiometer)	Solid-phase sandwich immunoassay	2 mL	13 min	5–500 mg/L	35 kg
Innovastar® (Diagnostic Systems GmbH)	Immunoturbidimetric assay	10 μL	7 min	5–400 mg/L	4 kg
Spinit® (biosurfit SA)	Immunoassay with Surface Plasmon Resonance detection	5 μL	4 min	4–200 mg/L	4 kg
Proposed sensor	Solid-phase sandwich immunoassay	2 μL	12 min	0.4–50 mg/L	1.5 kg

ranged from 92 to 108% (Table S1 of Supplementary content), thus verifying the accuracy of the assay.

The developed sensing system was compared to commercially available point-of-care (PoC) systems for qualitative determination of CRP in whole blood based on literature [2,5,21,25,26], as well as data provided from the manufacturers of these systems. In Table 1 are listed the sample volume, analysis time, working range, and the instrument weight, along with the assay operation principle used in each case. The table, contains a CRP blood assay that can be run in two different instruments manufactured by Eurolyser Diagnostica GmbH, as well as two PoC systems commercialized by Allere. In all cases, the sample preparation time is a few minutes, capillary blood is used and the procedure can be performed by non-experts. The analysis time of the developed sensor is a bit longer than most of the systems, but not so long to make the application at the point-of-care impractical, especially if the good assay repeatability and accuracy are taken into account. The whole blood volume required is lower that most of the systems, a feature that is important for the use of such a system in pediatric departments. The LOQ of the proposed method is lower than those of the commercially PoC systems, providing a dynamic range that covers normal values but also patients with cardiovascular or acute inflammation incidents. In addition, the foreseen fully integrated system weight (<1.5 kg, including the pump, the docking station and the optical system) supports its portability.

Although, the developed solution, both the sensor and the measurement set-up, has not yet reach the maturity and automation level of other existing PoC methods, the presented bioanalytical results support its suitability for point-of-care determinations with all the characteristics required by the medical doctors and the health system. Additional developments are needed, prior to its implementation in the real conditions of a hospital. These advancements are mainly the standardization of blood sampling and dilution procedure as well as the delivery of solutions required for the assay, possibly in form of a cartridge that would contain the necessary reagents [25]. In addition, the shelf-life of the biofunctionalized chips as well as of the stored into the cartridge solutions are critical parameters for the logistics of the hospitals and should be defined. Other issues to be considered are the user-device interface and the management of the data with respect to central laboratory data management system. The measurement set-up currently employed is based on the FR-pOrtable platform of ThetaMetrisis that is capable for on-site measurements thanks to its small size and low power requirements (it is powered from the USB cable). Through careful selection of the microfluidic pump, the total power needs could be covered from the USB port of the computer/tablet. In addition, the software exploits the state of the art models and algorithms implemented in the standard software of FR-tools but it has been developed to meet the needs of the particular application such as a) minimum configuration prior to use, b) single button operation, and c) visualization of the results and calculation of the actual CRP concentration based on the stored calibration curve. Thus, the Technology Readiness Level (TRL) of the particular measurement set-up has surpassed 4 (technology validated in lab). On the other hand, the biochip has been further matured and its Technology Readiness Level is closer to TRL5 (technology validated in relevant environment).

4. Conclusions

A fast assay for the determination of CRP in whole human blood based on a white light interference spectroscopy sensor is presented. To reduce the analysis time a three-step assay was employed, including 5-min reaction with the CRP calibrator or sample, 3-min reaction with the biotinylated anti-CRP antibody, and 4-min reaction with streptavidin. Regarding whole blood analysis, it was found that dilutions as low as 50-times with assay buffer could be used without effect on the assay outcome. Thus, taking into account the limit of detection and assay dynamic range determined with calibrators in buffer, concentrations of CRP in whole blood ranging from 400 μg/L to 50 mg/L could be determined. In addition, the assay developed was precise (intra- and inter-assay CVs less than 10%) and accurate (%recovery range 92–108%). Taking into account the small size and low cost of both the biochip and the instrumentation, the presented fast whole blood CRP assay is suitable for on-site CRP determinations at the point-of-care.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.snb.2018.01.008.

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