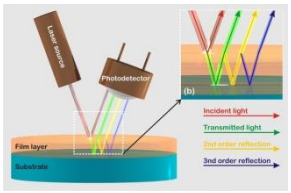
ThetaMetrisis APPLICATION NOTE #025

Real-time monitoring of bioreactions in whole blood



Introduction:

Biomolecular interactions play a key role in many biological and biochemical processes and are widely explored as methods for biodiagnostic applications. WLRS is introduced for the real-time and label-free monitoring of biomolecular interactions on biofunctionalized chip surfaces providing certain advantages[1] against other label-free methods in terms of ease of use, reproducibility, Limit of Detection, specificity and cost. In this application note, the fast and accurate immunochemical determination of C-reactive protein (CRP) in human whole blood samples[2] employing the WLRS sensing platform is demonstrated. CRP is a biomarker widely used in clinical practice to detect infections and inflammatory conditions ranging from injury to autoimmune diseases.

Means & Methods:

For the CRP assay, a goat polyclonal anti-CRP antibody was immobilized on aminosilanized chips with dimensions of 5X15 mm. The measurement system used is an FR-pOrtable system combined with a microfluidic module and a docking station for accommodation of the biochips and a miniaturized peristaltic pump for the reagents supply. The assay consists of three steps: a) running the samples for 5 min, b) supplying a biotinylated goat anti-CRP antibody for 3 min, and c) supplying a streptavidin solution for 4 min. All steps are monitored in real-time and the CRP concentration in the sample is calculated based on pre-determined calibration curve through appropriate application software.

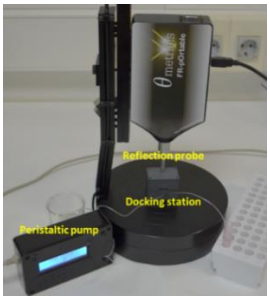


Figure 1. Measurement system

Results:

A typical CRP calibration curve is shown in fig. 2a. 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, fig. 2b. Thus, taking into account that the assay had a LOD of 2 μg/L in assay buffer; whole blood concentrations as low as 100 μg/L could be determined. On the other hand, CRP concentrations as high as 500 mg/L could be determined.

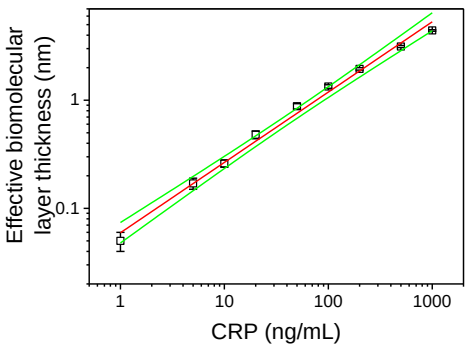


Figure 2a): Typical CRP calibration curve obtained with the 10-min assay. Each point is the mean value of 4 replicate measurements ± SD.

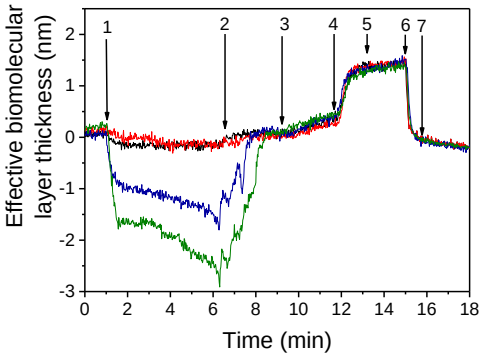


Figure 2b): Real-time responses from a blood sample spiked with CPR at different concentrations: 50 (green line), 100 (blue line), 200 (red line) and 500-times (black line) with assay buffer.

Conclusions:

The developed sensing system provides sensitive quantitative determinations of CRP in whole serum samples and can be performed by non-experts.

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[1] G. Koukouvinos, P. Petrou, D. Goustouridis, K. Misiakos, S. Kakabakos, I. Raptis "Development and bioanalytical applications of a White Light Reflectance Spectroscopy label-free sensing platform" Biosensors 7 (2017) 46

[2] G. Koukouvinos, D. Goustouridis, K. Misiakos, S. Kakabakos, I. Raptis, P. Petrou "Rapid C-reactive protein determination in whole blood with a White Light Reflectance Spectroscopy label-free immunosensor for Point-of-Care applications" Sens. Act. B 260 (2018) 282