**Development of competitivepseudo*-*ELISA assay based on nanoMIPs for cocaine and its metabolites**

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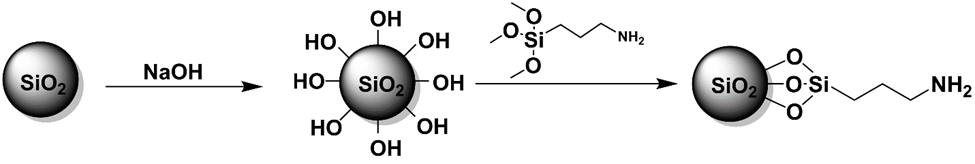
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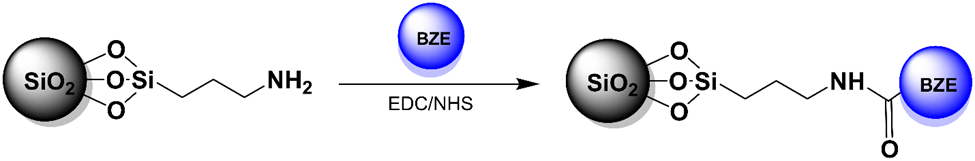
**Supporting Information**

1. **Glass beads modification**

Modification scheme of the surface glass beads with 3-aminopropyltrimethyloxysilane (Figure S1) and immobilisation of the template on the glass beads (Fig. S2) are presented below.



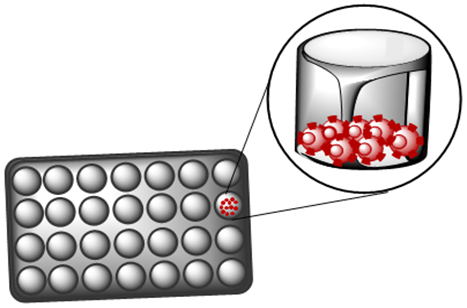
**Figure S1**. Modification of the glass beads with 3-aminopropyltrimethyloxysilane.



**Figure S2**. Immobilization of the template on the modified glass beads.

1. **NanoMIPs immobilisation in the microplates wells**

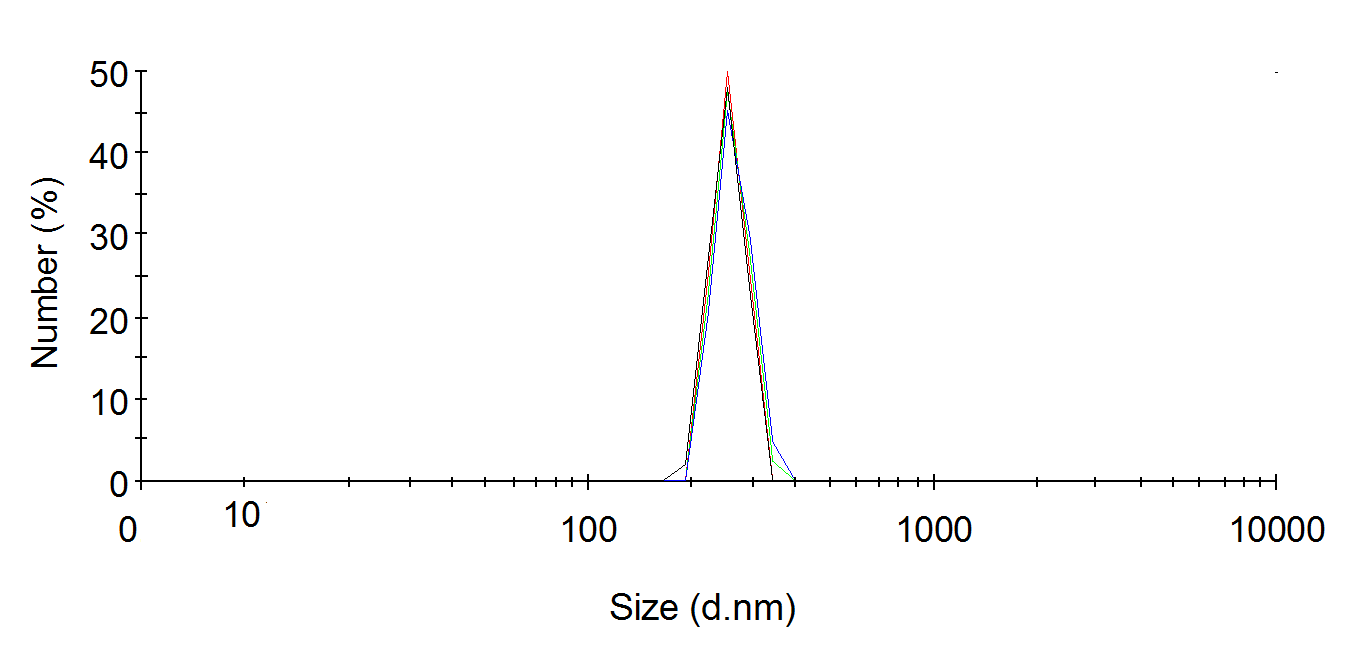
On the Fig. S3 we show a schematic arrangement of nanoparticles in microplate. Imprinted polymer nanoparticles (40 µL, 0.06 mg mL–1) were dispensed into the wells of a 96-well polystyrene microplate, and left to dry overnight at ambient temperature.



**Figure S3**. Immobilisation of nanoMIPs on the microplate wells.

1. **NanoMIPs size**

The determination of size distribution of the nanoMIPs was made using the Dynamic Light Scattering (DLS) technique. The typical size distribution of nanoparticles in aqueous format is shown in Fig. S4.



**Figure S4**. Particles size distribution of nanoMIPs measured by DLS.

1. **Optimisation of the blocking conditions**

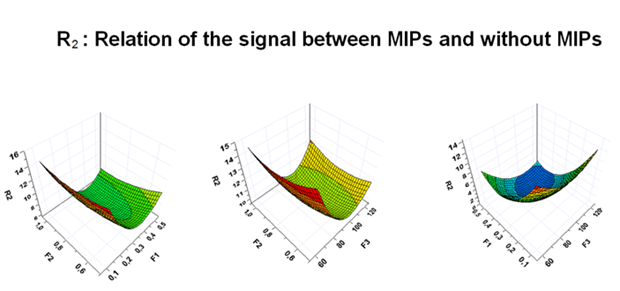
Optimisation of the blocking conditions was performed using response surface methodology for studying the correlation between response and factors. Box-Behnken design (BBD) was employed to evaluate the main interaction and quadratic effects of the protein, the surfactant, and time of incubation of the blocking solution in the ELISA assay. The parameters, specifically, the ranges and constrains for each factor , responses values, design comprising 15 runs and analysis of variance for the response surface quadratic model for optimisation of the blocking solution are presented in Table S1 and Table S2, respectively. Three-dimensional response surface contour plots of the signal between MIPs and wells without MIPs is shown on Figure S6.

**Table S1** Factors and responses in Box-Behnken experimental design

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Level** |  |
| **Factor** | **Low (-1)** | **Medium (0)** | **High (+1)** |
| F1: BSA concentration (%) | 0.1 | 0.3 | 0.5 |
| F2: Tween 20 (%) | 0.5 | 0.75 | 1 |
| F3: Incubation time (min) | 60 | 90 | 120 |
| **Responses** | **Constraints** |  |  |
| R1: Relation of the signal between MIPs and NIPs | Maximize |  |  |
| R2: Relation of the signal between MIPs and without MIPs | Maximize |  |  |
| |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | |  | **Blocking solution** | | | | | |  | **Factors** | | **Responses** | | | | **Exp No** | **F1 (%)** | **F2 (%)** | **F3 (min)** | **R1** | **R2** | | 1 | 0.10 | 0.50 | 90.00 | 2.55 | 9.57 | | 2 | 0.50 | 0.50 | 90.00 | 2.31 | 3.99 | | 3 | 0.10 | 1.00 | 90.00 | 2.45 | 10.50 | | 4 | 0.50 | 1.00 | 90.00 | 1.64 | 3.39 | | 5 | 0.10 | 0.75 | 60.00 | 2.65 | 14.76 | | 6 | 0.50 | 0.75 | 60.00 | 2.40 | 8.03 | | 7 | 0.10 | 0.75 | 120.00 | 3.39 | 12.24 | | 8 | 0.50 | 0.75 | 120.00 | 1.94 | 2.04 | | 9 | 0.30 | 0.50 | 60.00 | 3.13 | 9.36 | | 10 | 0.30 | 1.00 | 60.00 | 2.63 | 6.48 | | 11 | 0.30 | 0.50 | 120.00 | 2.10 | 3.76 | | 12 | 0.30 | 1.00 | 120.00 | 3.13 | 2.17 | | 13\* | 0.30 | 0.75 | 90.00 | 1.50 | 1.64 | | 14\* | 0.30 | 0.75 | 90.00 | 1.47 | 1.40 | | 15\* | 0.30 | 0.75 | 90.00 | 1.26 | 1.65 | | \*Center points in the design | | |  |  |  | | | | |

**Table S2** Analysis of variance for the response surface quadratic model for optimizationof the blocking solution

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Sum of squares** | **Df** | **Mean square** | ***F*-values** | **P-values** |
| Model | 260.14 | 6 | 43.36 | 36.81 | 0.000 |
| F1: BSA concentration | 109.67 | 1 | 109.67 | 101.31 | 0.0002 |
| F2: Tween 20 | 2.14 | 1 | 2.14 | 1.98 | 0.2185 |
| F3: Incubation time | 42.41 | 1 | 42.41 | 39.18 | 0.0015 |
| F1F1 | 76.84 | 1 | 76.84 | 70.99 | 0.0004 |
| F1F2 | 0.58 | 1 | 0.58 | 0.54 | 0.4952 |
| F1F3 | 3.01 | 1 | 3.01 | 2.78 | 0.1563 |
| F2F2 | 2.01 |  | 2.01 | 1.85 | 0.2315 |
| F2F3 | 0.42 | 1 | 0.42 | 0.38 | 0.5624 |
| F3F3 | 36.45 | 1 | 36.45 | 33.68 | 0.0021 |



**Figure S5**. Three-dimensional response surface contour plots of the signal between MIPs and wells without MIPs.

1. **Optimisation of the conjugate concentration**

To choose the best concentration of the blocking solution blank assay was made. The difference in absorbance between signal from the well with and without nanoMIPs together with absorbance value were analysed (Table S3). The selection of the HRP conjugate dilution 1:100 was based on the higher absorption values for the wells containing MIPs (1.728 ± 0.016 a.u.) that allowed to achieve higher sensitivity of the assay.

**Table S3.** Absorbance at 450 nm of the dilutions of the HRP-BZE

|  |  |  |  |
| --- | --- | --- | --- |
| **HRP-BZE** | | | |
| **Dilutions** | **MIPs** | **Without MIPs** | **ABS MIPs/ABS without MIPs** |
| **1:100** | 1.728 ± 0.016 | 0.880 ± 0.014 | 1.964 |
| **1:200** | 0.964 ± 0.039 | 0.475 ± 0.015 | 2.029 |
| **1:400** | 0.561 ± 0.007 | 0.246 ± 0.002 | 2.280 |
| **1:800** | 0.201 ± 0.001 | 0.116 ± 0.001 | 1.733 |

1. **Pseudo-ELISA for measurement the cocaine in blood serum using nanoMIPs prepared in water**

A sample of human serum was spiked with several concentrations of cocaine and tested using the ELISA. The results are shown in Fig. S7. It shows only a weak response for cocaine in the concentration range between 10-12 and 10-9 M, which can be explained by high interference from the blood serum matrix. It was decided that nanoMIPs for the measurement of such small molecules as cocaine in the complex biological samples should be made in organic solvent in order to ensure higher affinity of the interactions.

**Figure S6**. Competitive pseudo-ELISA assay for COC in human serum based on nanoMIPs made in water polymerisation.