Parallel analysis of glycoproteins using lectin-functionalised monoPLOT columns integrated in micro-fluidic chips

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Glycoprotein isolation, purification and analysis is critical in the production of biologics. Therefore, there is a growing interest in new methods and techniques that allow ultra fast analytical characterisation and detection of glycoproteins and their glycoforms in the biological samples employed within the biopharmaceutical industry. In that context, micro-fluidic devices offer a great potential owing to the very fast analysis they allow, the low sample/reagent consumption, the disposability, and the ease of integration with other analytical systems, among others.

In this work, a micro-fluidic device integrating porous layer open tubular columns based on dimethacrylate monoliths (monoPLOT) and functionalised with *Erythrina cristagalli lectin* (ECL) were used for extraction of selected glycoproteins. Polymer monoliths are very versatile materials as they can be prepared with different porosities, pore sizes, and a wide variety of functionalities using many different precursors and chemistries. The monoPLOT columns were fabricated and functionalised off-chip, and then bonded to the channel walls before final channel lamination. In order to increase the monolith surface area, immobilisation of gold nano-particles (AuNPs) was carried out prior to covalent attachment of the ECL to the AuNPs. Detection was carried out with capacitively coupled contactless conductivity detection (C^4D).

1. Fabrication and characterisation of ECL-modified monoPLOT columns

MonoPLOT columns were prepared by UV initiated polymerisation at 365 nm using an automated "scanning" technique, resulting in a polymer monolith with a thickness of 5 μm.

Composition of polymerisation solution:

Ethylene glycol dimethacrylate (EDMA), 40 % w/w;
1-decanol, 60 % w/w;
2,2-dimethoxy-2-phenylacetophenone (DPA), 1 % w/w (with respect to monomer).

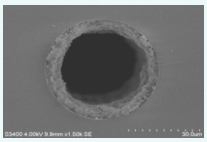


Figure 1. *Scanning electron microscopy (SEM) image of the monoPLOT column cross-section (capillary ID, 50 μm).*

Immobilisation of ECL in monoPLOT columns modified with gold nano-particles (AuNP): monoPLOT A

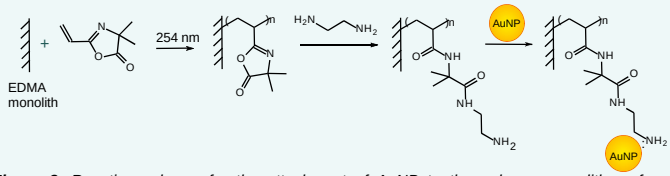
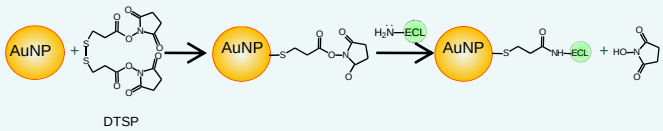


Figure 2. *Reaction scheme for the attachment of AuNP to the polymer monolith surface following photo-grafting with vinyl azlactone. .*



DTSP

Figure 3. *Reaction scheme for immobilisation of ECL via 3,3'-dithiodipropionic acid di(N-hydroxysuccinimide ester) (DTSP) coupling reagent.*

Immobilisation of ECL in "bare" monoPLOT columns: monoPLOT B

EDMA-based monoPLOT column was grafted with vinyl azlactone, and then ECL was immobilised onto the resulting pendant azlactone moieties, followed by blocking with 1 *M* Tris buffer.

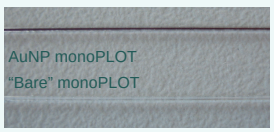


Figure 4. *Photograph of gold nano-particle modified and "bare" monoPLOT columns.*

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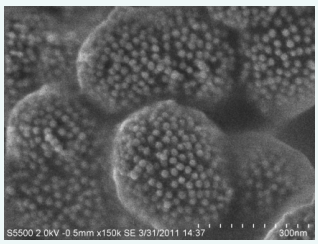

Figure 5. *Field emission (FE)-SEM image of 20 nm AuNPs attached to monoPLOT column surface.*

2. Integration of ECL-modified monoPLOT columns into micro-fluidic devices

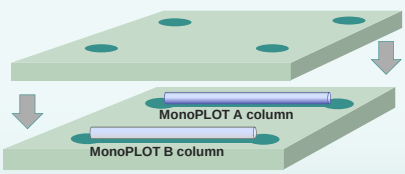

Figure 6. *Scheme showing the integration of monoPLOT columns fabricated off-chip into micro-fluidic channels created in poly(methyl methacrylate) (PMMA) films by CO_2 laser ablation.*

Pressure sensitive adhesive (PSA) films were used to bond the PMMA layers containing the inlet/outlets (top) and the micro-fluidic channels (bottom) once the monoPLOT columns were bonded to the channels.

Channel dimensions:

50 mm x 400 μm x 500 μm

MonoPLOT column dimensions:

51 mm length, OD 365 μm, ID 100 μm

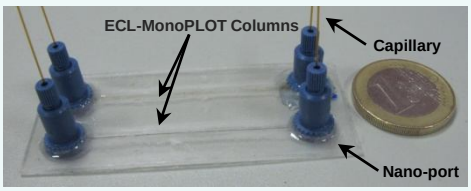

Figure 7. *Resulting micro-fluidic chip with commercial nano-ports for connection of chip to external pumps via fused silica capillaries.*

3. Trap and release studies on ECL affinity monoPLOT columns

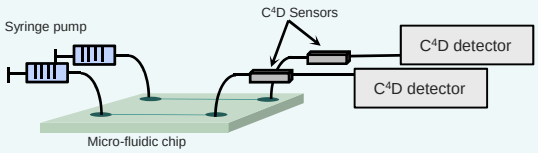

Figure 8. *Scheme depicting the analytical system employed.*

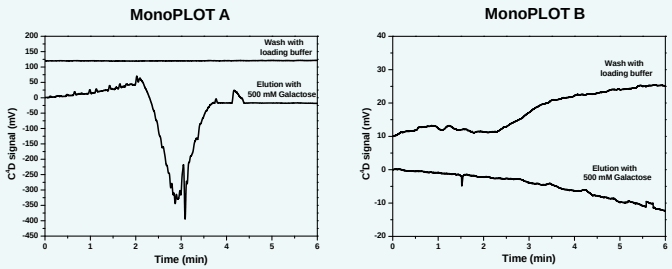

Figure 9. *Parallel flow C^4D measurements of washing and elution steps following injection of 20 μg/mL desialated transferrin (10 μL injection) in monoPLOT A and B columns. Conditions: flow rate: 1 μL/min; loading buffer: 10 mM Tris , pH 7.4, containing 150 mM NaCl, 1 mM Ca^{2+} and 1 mM Mn^{2+}; eluting buffer: 500 mM galactose prepared in loading buffer. C^4D parameters: frequency, 75kHz; excitation voltage, 10 $V_{peak\text{-}to\text{-}peak}$.*

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4. Conclusions

- The integration of monoPLOT columns into micro-fluidic devices for on-chip parallel extraction of a selected glycoprotein was demonstrated in standards.
- The monoPLOT column functionalised with AuNPs showed very promising results in terms of columns capacity for application in on-chip extraction of glycoproteins. However, further optimisation of the sample injection conditions is still needed.
- In principle, the fabrication of monoPLOT columns off-chip allows higher column-to-column (or channel-to-channel) reproducibility as columns can be prepared in the same batch and then chopped to the desired length.
- The generation of close-to-zero backpressures in monoPLOT columns allowed their integration in polymeric micro-fluidic chips generally operating at low backpressures, in contrast to higher-cost micro-fluidic platforms fabricated in silicon or glass. Moreover, that eased the connection of the micro-fluidic device to the macro-world as it allowed the use of standard nano-ports commercially available.
- Future work will involve the fabrication of micro-fluidic chips with extra number of channels allowing a higher number of binding affinity and selectivity studies to be performed in parallel.

6. Acknowledgements

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