Gold nano-particle modified silica monolithic micro-columns for selected chromatographic and biological applications.

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ABSTRACT

Monolithic microcolumns and especially silica monoliths are showing several advantages compared to classical particle packed and organic polymeric monolithic columns: ease of production and functionalisation, excellent mechanical and thermal stability. Morphology of the monolithic columns can easily be tuned by simply changing the compositions of reaction mixtures. High porosity and interconnected flow-through pores ensure low back pressures at higher flow rates so increasing reaction speeds. High salt resistance allows use water based buffer solutions without any swelling of the stationary phase, large biomolecules can be utilised and conditions to prevent denaturation and comformation changes of these biomolecules can be maintained.

Introduction of gold nano-particles on the surfaces of silica monoliths allows increase of the surface areas and alows creation of new, exotic surfaces. Gold shows strong affinity towards thiol groups, which can be found in different biomolecules so utilisation of this phenomena would allow production of micro-reactors and bioreactors in order to mimic biological reactions happening in living organisms and large biological systems. Silica monoliths were synthesised using classical sol-gel process. In order to immobilise gold nano-particles, surfaces of the silica monoliths were amminated using standard silanisation reaction with 3-aminopropyl-methyl-diethoxysilane. 20 nm citrate stabilised gold nano-particles were immobilised on the surfaces afterwards. Depending on the desired application, gold nano-particle modified silica monoliths were functionalised afterwards. Immobilisation of ionic species such as amino acids and small peptides would allow creation of stationary phase for ion chromatography, retention of enzymes and other biologically active molecules would allow to create micro-reactors. Leaving gold nano-particles unmodified would make ideal stationary phase for micro-extraction.

These modified monoliths were characterised using microscopy techniques, such as scanning electron microscopy (SEM) and field emission SEM. They were used to characterise morphology of the monoliths as well as to evaluate the coverage of the surface with gold nano-particles. The fabricated stationary phases were used for selected biological and chromatographic applications (incorporanting classical chromatographic techniques in order to evaluate the performance of these new modified monolithic materials).

1. Background

Glycoproteins – proteins having sugar functionality – influences biological activity and stability and they can serve as bioindicators in biological systems.

Most biopharmaceuticals are based on glycoproteins – increasing need for fast, sensitive and reliable analytical methods:

- to monitor production
- to assure quality control
- to identify and quantify glycoproteins
- to differentiate and separate different glycoforms

✓Lectins have selectivity towards different sugars present in glycoproteins

✓Trap-and-release mechanism – allows glycoprotein separations and preconcentration

✓Lectins can be immobilised on surfaces of different stationary phases – important to maintain their activity

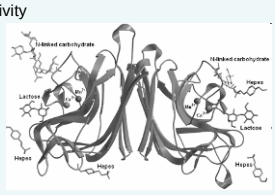


Fig. 1. *Structure of Erythrina cristagalli lectin (ECL) which is selective towards galactose [1]*

Fig. 2. *Flowers of cockspur coral tree - seeds of which are the source of ECL*

2. Experimental approach

Monolith synthesis

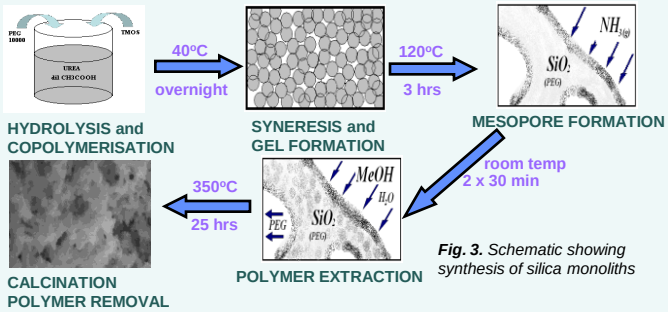


Fig. 3. *Schematic showing synthesis of silica monoliths*

Functionalisation of silica monolits

Conventional silanisation reaction was used to introduce amino groups on silica monolith surfaces.

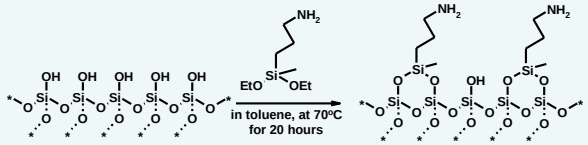
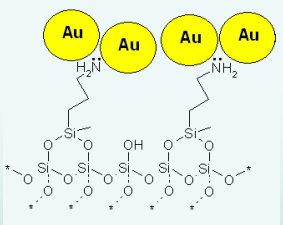


Fig. 4. *Reaction scheme for surface functionalisation*



20nm citrate stabilised gold nano-particles were adsorbed on the aminated surfaces from aqueous solutions

Fig. 5. *Silica surface with immobilised gold nano-particles*

Amino group reactive linker was covalently attached to the surfaces of gold nano-particles

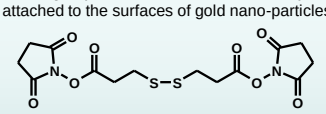


Fig. 6. *Structure of* 3,3'-dithiodipropionic-acid-di-(N-hydroxysuccinimide-ester) – amino group reactive spacer

ECL was immobilised via formation of amide bond

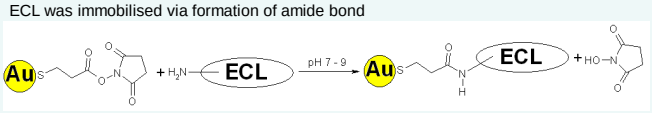


Fig. 7. *Reaction scheme for iimobilisation of ECL onto modified gold nano-particles*

Monolith characterisation

Morphology of synthesised silica monoliths was characterised using Scanning Electron Microscopy (SEM) techniques. Covalent attachment of the monolith to the column wall can be seen. Immobilised gold nano-particles can be seen as white spots. Surface coverage can be evaluated from this.

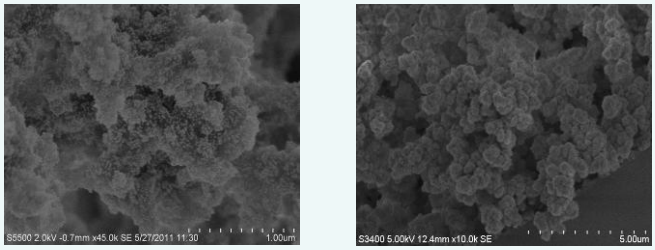


Fig. 8. *SEM images of gold nano-particle modified (on the left) and bare (on the right) silica monoliths*

Glycoprotein analysis

✓Simple mixtures of two glycoproteins were analysed: ribonuclease B **(RNaseB)** having terminal mannose and desyalilated transferrin **(desTF)** having terminal galactose.

✓Trap-and-release mechanism was exploited – competetive elution. Retained glycoprotein was eluted with free sugar.

✓Samples were analysed using RP microHPLC in gradient mode: in-house synthesised laurylmethacrylate-co-ethylene glycol dimethacrylate (LMA-co-EGDMA) monolith, 197x0.1mm; Gradient: A = 5% ACN + 0.1%TFA, B = 90%ACN + 0.1%TFA. In 10 minutes from 0% B to 100%

3. Results

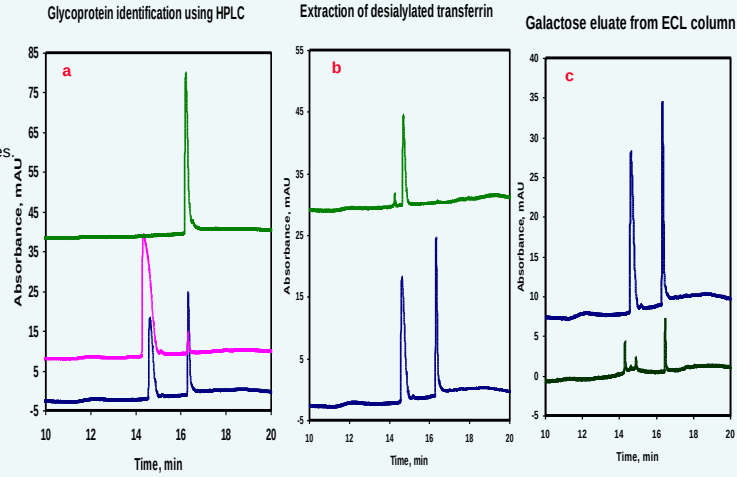


Fig. 9. *Chromatograms of glycoproteins: a – identification (green – desTF, pink – RNaseB, navy – mixture), b - extraction (green – RNaseB, navy – mix), c – elution (navy – mix, green – desTF)*

4. Conclusions

Functionalised silica monoliths provide novel and versatile stationary phases

ECL modified monoliths can successfully be applied in glycoprotein analysis

9. Acknowledgements

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