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AMECRYS - *Revolutionising Downstream Processing of Monoclonal Antibodies by Continuous Template-Assisted Membrane Crystallization*

Deliverables D2.1

Report on Preparation of Nanotemplates for mAb Crystallization

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1.0	Initial document creation	08/09/2017	J. Heng, W. Chen, H. Yang
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Summary

Introduction

This report presents the fabrication and the characterisation of nanotemplates for use in mAb crystallisation studies. The objectives of this part of D2.1 are:

1. the preparation of nanotemplates of pore dimension between 3 nm and 20 nm
2. the characterisation of pore dimension and surface area; and
3. to derivitise and modify surface chemistry and characterise for the surface coverage of functional groups.

Two different approaches to synthesis the nanotemplates were undertaken: (i) a top-down approach based on a thermal aging route, and (ii) a bottoms-up approach based on the Stober synthesis route.

The synthesized nanotemplates are characterised to determine physicochemical properties using a range of experimental techniques, such as; N_2 sorption for surface area (BET analysis) and porosity (BJH analysis), dynamic light scattering for particle size, SEM for morphology, zeta potential and FTIR for surface charge and chemical groups.

The design of these nanotemplates receive input from modeling work of ULB (WP5) and also feed into the development of models in that WP from the use of these nanoparticles in crystallisation screening of mAb. The nanotempaltes have also been used for preparation of membranes (WP3).

Preparation and Characterisation of Nanotemplates

Route A: Thermal Aging Route

Background Information

Sol-gel chemistry is often used to synthesise silica nanoparticles and, in particular, tetraethoxyorthosilicate (TEOS) ($Si(OC_2H_5)_4$) is a widely used and studied silica precursor. The silica precursor first undergoes hydrolysis (Scheme 1), where the alkoxy group (-OR) is converted to the hydroxyl group (-OH). The hydrolysed silica compound then undergoes condensation with another silica compound (Scheme 2 & 3) to form siloxane bonds (Si-O-Si).

$$Si(OR)_4 + H_2O \rightarrow HO\text{-}Si(OR)_3 + R\text{-}OH \quad \text{Scheme 1}$$

$$(OR)_3\text{–}Si\text{-}OH + HO\text{–}Si\text{-}(OR)_3 \rightarrow [(OR)_3Si\text{–}O\text{–}Si(OR)_3] + H\text{-}O\text{-}H \quad \text{Scheme 2}$$

$$(OR)_3\text{–}Si\text{-}OR + HO\text{–}Si\text{-}(OR)_3 \rightarrow [(OR)_3Si\text{–}O\text{–}Si(OR)_3] + R\text{-}OH \quad \text{Scheme 3}$$

Gelation is the formation of three-dimensional network of colloidal particle and condensed silica species. The physical characteristics of a gel depends on the size of colloidal particle and extent of cross-linking before gelation. The structure of a gel is determined by the relative rate of hydrolysis and condensation reaction. It has been reported previously that faster hydrolysis and slow condensation reactions results in linear polymeric gels, whereas a slow hydrolysis and fast condensation results in larger, bulkier and more nonuniform polymeric gels.

The synthesis of mesoporous silica nanoparticles was developed during the early 1980s. Pillared clays were the first reported mesoporous materials having pore diameters in the range of 10 – 20 nm. Scientists from Mobil Corporation and Waseda University, Japan later independently reported the preparation of mesoporous surfaces with pore diameter of approximately 4 nm and the structuring the pores with the soft templating approach, where surfactant served as the soft template.

The cooperative self-assembly of the silica and surfactant interaction across the hydrophobic–hydrophilic interface results in mesoscopic order. The resulting mesoporous materials have been produced in different forms (i.e. powders, thin-films and monoliths). The pore size can be controlled by tuning the micellar diameter with process parameters such as reaction temperature, sol aging and sintering methods, type of additives and swelling agents.

Results and Discussion

In the thermal aging route, organosilane is subject to heating at 120 ^oC under acidic environment to form closely packed nanoparticles with individual diameter of approximately 20 nm (Figure 1). Grinding and sieving are performed to produce and fractionate nanotemplates to diameter less than 32 µm (for membrane synthesis in WP3). The pore size of nanotemplate can be tuned between 4 nm and 17 nm by varying the thermal aging condition (Figure 2). Specifically, increasing the quantity of hydrochloric acid in the thermal aging mixture can increase the pore size of the final nanotemplate (Figure 3).

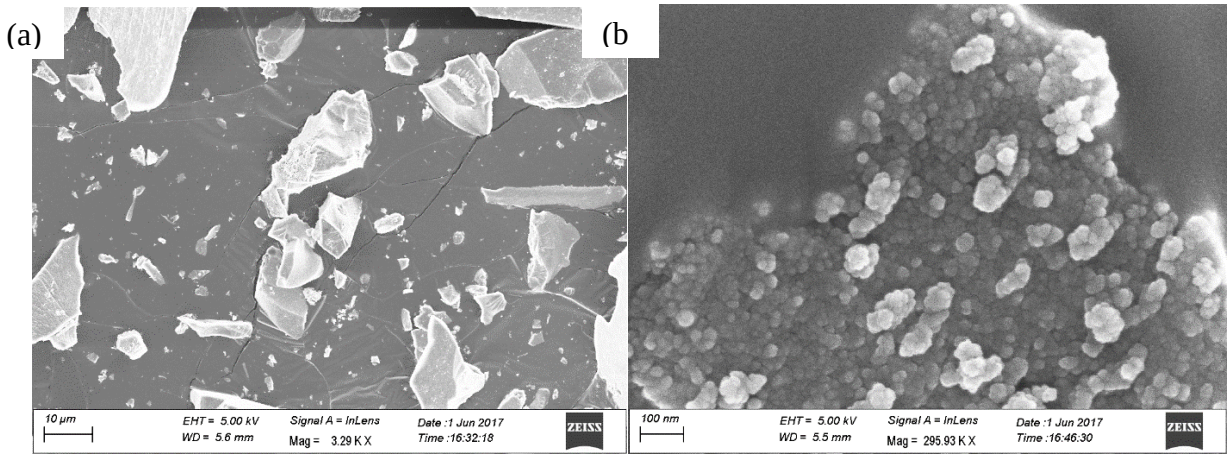

Figure 1. (a) SEM image of 3D nanotemplate obtained with Method A and, (b) higher resolution image of the nanotemplates synthesized.

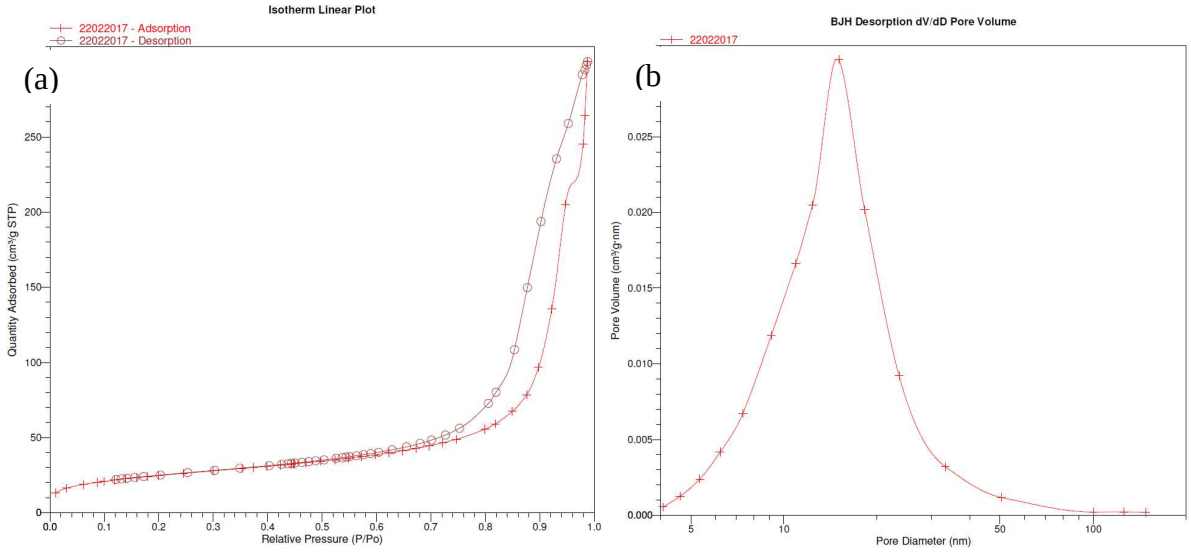

Figure 2. (a) Nitrogen adsorption and desorption isotherm for 3D nanotemplate obtained with Method A and (b) Pore size distribution of the same sample.

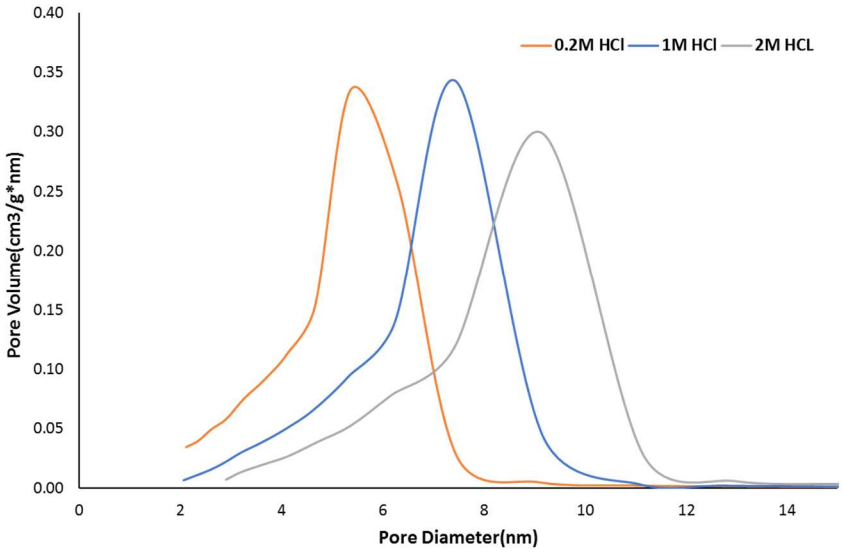

Figure 3. The effect of hydrochloric acid concentration on the pore size of the 3D Nanotemplates, obtaining pores of 5.5 nm, 7.3 nm and 9.4 nm (peak value).

Route B: Stober Route

Background Information

In 1968, Stöber et al. reported the synthesis of monodisperse spherical silica nanoparticles with diameter between 50 and 2000 nm by reacting tetraesters of silicic acid in a saturated alcoholic solutions of ammonia. Since then, the Stöber method has been one of the most cited methods for synthesising silica nanoparticles. Various studies have found that the diameter of Stöber silica nanoparticle can be tuned with the concentrations of ammonia and water, reaction temperature, addition rate of silica precursor.

Results and Discussion

Spherical silica nanotemplates have been synthesized with the Stöber process, in which the organosilane molecules react under a basic condition at room temperature. Particle size measurements by SEM and DLS indicate monodisperse nanoparticles with diameter between ~100 nm and 600 nm have been obtained (Figure 4: example of nanotemplate with diameter ~ 500 nm). SEM and DLS measurements also confirm the monodispersity of the particle size (Figures 4 and 5). Pores can be created with surfactant-assisted coating of another layer of organosilane on the nanotemplate. BJH measurements show that pores with diameter of 4 nm has been achieved (Figure 6).

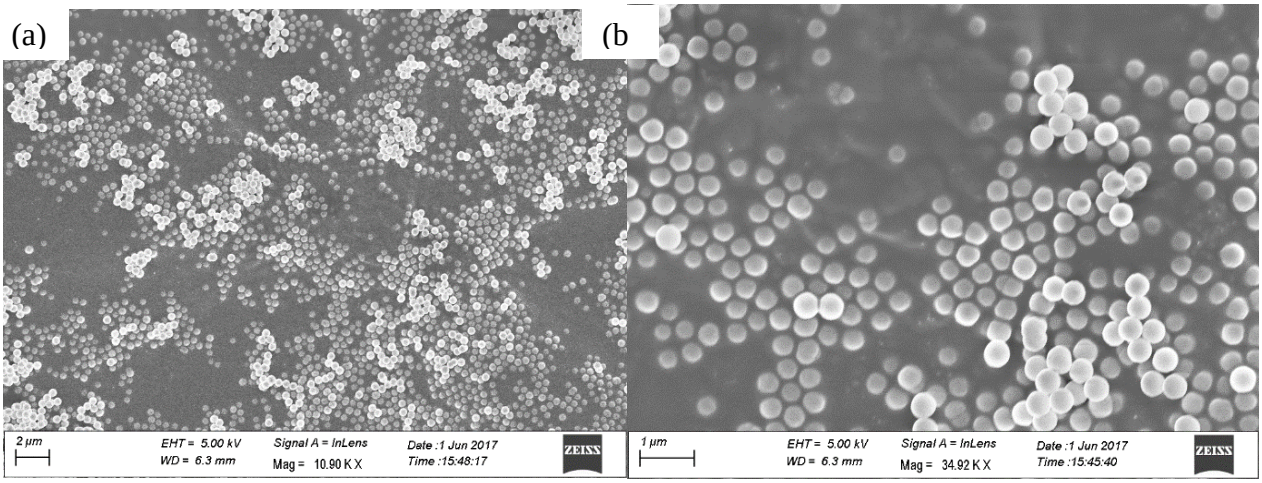

Figure 4 (a) SEM image of nanoparticles obtained with the Stöber route and (b) high resolution SEM image of the same nanoparticles.

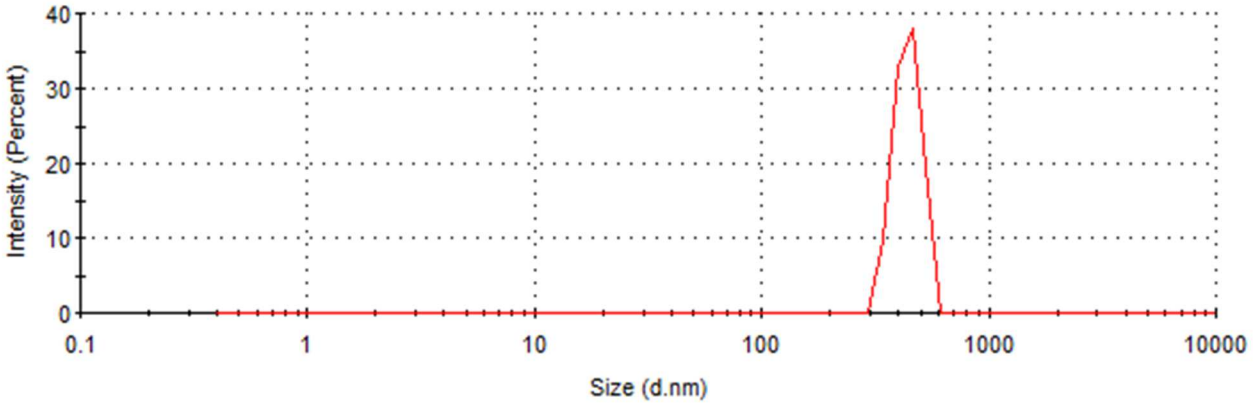

Figure 5. DLS data showing the hydrodynamic diameter of nanoparticles obtained with the Stöber route.

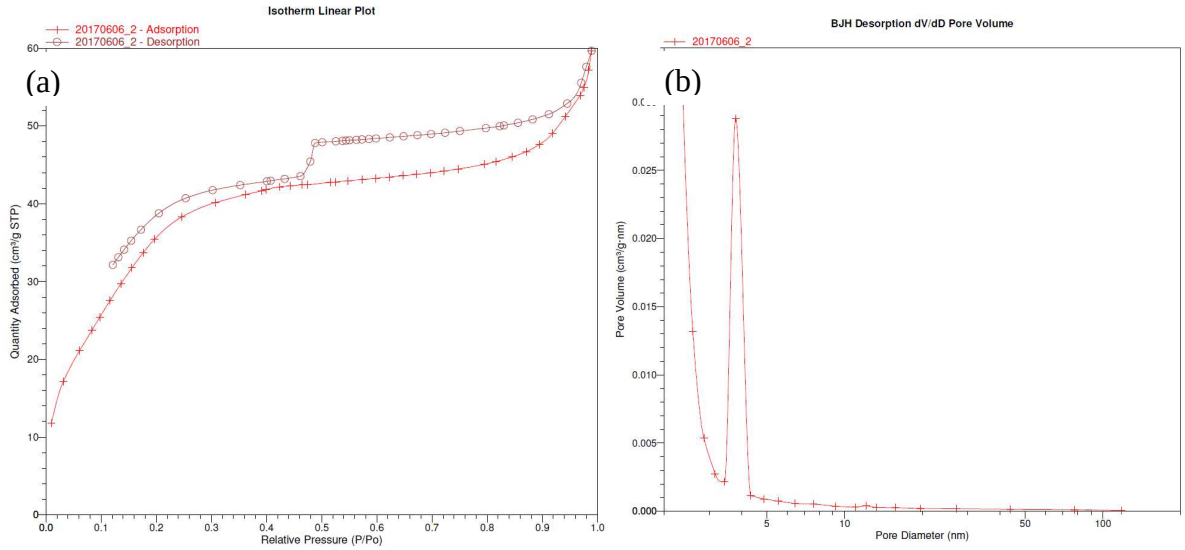

Figure 6. (a) Nitrogen adsorption and desorption isotherm for nanotemplate obtained with the Stöber route and (b) Pore size distribution of the same sample determined by BJH.

Surface Chemistry Modification and Characterisation

Route A: Thermal Aging Route

Background Information

Grafting is the post-synthesis functionalisation of silica nanotemplate, which is achieved by reacting organo-silanes or chloro-silanes with the free silanol groups on the surface of silica nanotemplate in an inert organic solvent at elevated temperature. Grafting allows the original structure of ordered mesoporous silica phase to be retained, but the pore diameter can be reduced depending on the size of attached organic moiety and the extent of functionalisation.

Results and Discussion

The surface property of nanotemplates from the thermal aging route has been modified with silanisation under reflux condition. A typical reaction involves the nanotemplate suspended in an inert organic solvent (e.g. toluene) and the organosilane that bears the target functional group (e.g. chloro, amine, phenyl and methyl). Under reflux, the organosilane reacts with the hydroxyl group on the surface of the original nanotemplate to form covalent bond (i.e. –Si-O-Si-). The silanisation with phenyl- and chloro-bearing organosilanes can increase the zeta potential of nanotemplate from -28 mV to -19 mV (Figure 7), changing the surface property of the nanotemplate.

The percentage coverage of functional groups on the silica nanoparticles will be determined by comparing the zeta potential of standard silica nanoparticles made from tetraethyl orthosilicate (TEOS) with those made from the organosilanes of the specific functional groups. The synthesis of the nanotemplates prepared with organosilanes of the specific functional groups is still in progress. To give an estimate of the coverage, phenyl and chloro groups are assumed to have zeta potential of

0 mV. The coverage of phenyl and chloro groups was hence estimated as 18% and 32% respectively. Figure 8 shows the FTIR result for the functioned nanotemplate, confirming the presence of functional groups of chloro and phenyl. The silanisation of nanotemplate with methyl and amine groups is still in progress.

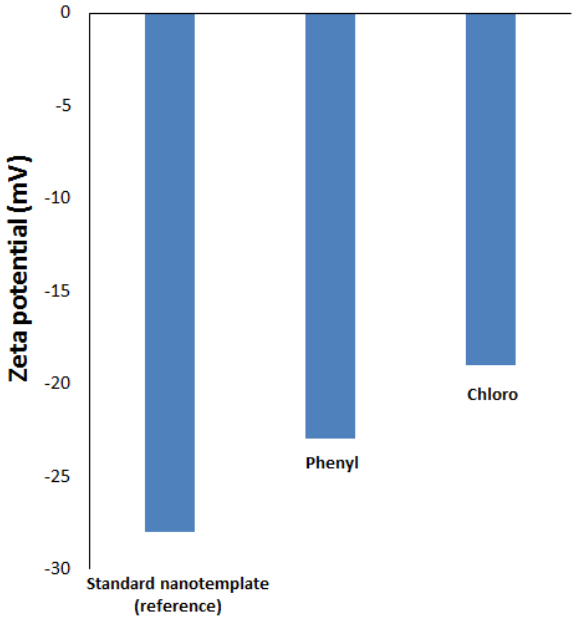

Figure 7. Zeta potential of standard 3D Nanotemplates and silanised nanotemplates with phenyl and chloro functionalities.

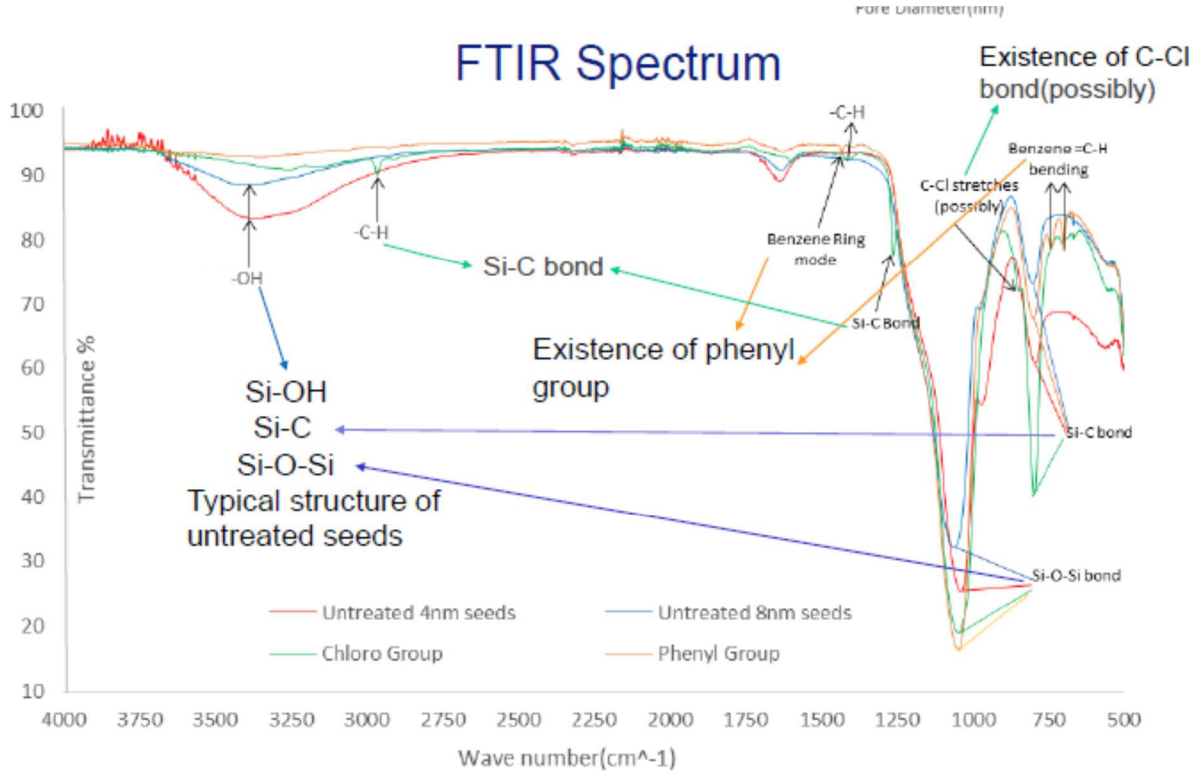

Figure 8. FTIR spectrum for non-functionalised and functionalised 3D Nanotemplates.

Route B: Stober Route

Background Information

An alternative to grafting for functionalising silica nanotemplate is co-condensation, which is achieved by reacting TEOS directly with trialkoxyorganosilanes. This method leads to the organic functional groups attached covalently to the pores on a mesoporous surface, but avoids the issue of reduction in pore diameter as in the case of grafting. In addition, the organic functional groups are more homogeneously distributed on the pore compared to the grafting.

Results and Discussion

The surface property of nanotemplatse can be controlled via the co-condensation method, in which the organosilane with the target functional group (e.g. amine, phenyl and methyl) is added directly into the original organosilane during the formation of nanotemplate. Zeta potential measurements show that the surface charge of nanotemplate vary over a wide range (– 63 mV to 19 mV) indicative of the type and quantity of organosilane introduced to the nanoparticle in the co-condensation process (Figure 9). Assuming nanotemplates fully covered with methyl and phenyl groups have a zeta potential of 0 mV, the coverage of methyl and phenyl groups was estimated as 24% and 35% respectively. The co-condensation with chloro-bearing organosilanes is still in progress.

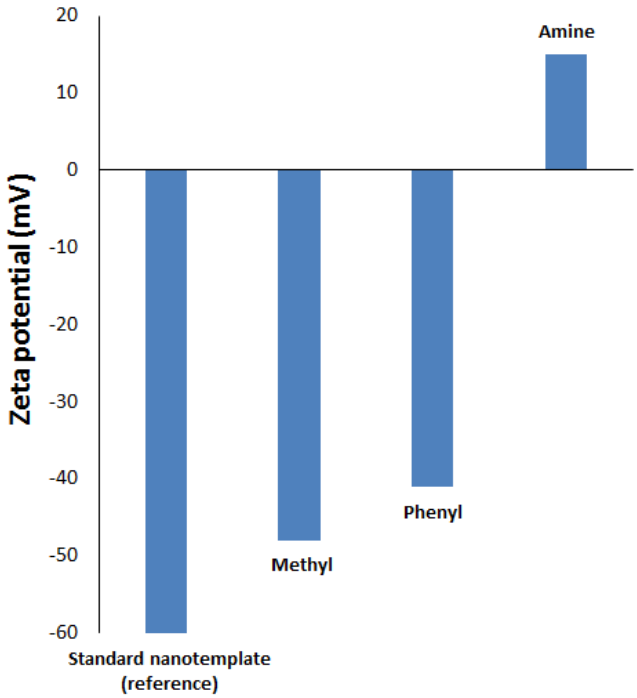

Figure 9. Zeta potential of the nanoparticles obtained from Method B and functionalised nanoparticles with methyl, phenyl and amine functionalities.

Summary

Silica nanotemplates have been synthesised with controllable particle size, pore size and surface property through two separate routes: the thermal aging method and Stöber method (Table 1). Both routes are robust and can be scaled up to produce tens of grams of nanotemplates (<7 days). The characterisation of particle size has been performed with dynamic light scattering (DLS) and scanning electron microscopy (SEM). The surface area and pore size of the synthesized nanotemplates have been determined with nitrogen sorption measurements (Brunauer–Emmett–Teller (BET) & Barrett, Joyner, Halenda (BJH)). The surface chemical change has been confirmed via zeta potential measurement.

Table 1. Summary of nanotemplate properties (achievable range).

	Method A: Thermal Aging route	Method B: Stöber route	Particle Characterisation
Particle size	<32 µm	100 nm to 600 nm	SEM & DLS
Pore size	4 nm to 17 nm	4 nm	BET & BJH
Surface area	108 m^2/g to 812 m^2/g	5 m^2/g to 66 m^2/g	BET & BJH
Surface charge	– 34 mV to – 19 mV	– 63 mV to 19 mV	Zeta potential
Functional group coverage*	Phenyl: ~ 18% Chloro: ~ 32%	Phenyl: ~ 35% Methyl: ~ 24%	

*Estimation only. Accurate characterisation still in progress.