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Development of standard operation procedures for the manufacture of *n*-octadecyl bonded silicas as packing material in certified reference columns for reversed-phase liquid chromatography

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Abstract

The development of standard operation procedures for the manufacture of a *n*-octadecyl bonded spherical silica packing from partially condensed tetraethoxysilane as silica source is described. The synthesis comprises five intermediate products and six synthesis steps which were examined according to their reproducibility and robustness. The results led to the optimisation of the manufacturing process for a *n*-octadecyl bonded silica. Correlations were drawn between the dynamic viscosity of the poly(ethoxy)siloxane (PES), the synthesis parameters, the resulting pore structural properties and particle size distribution of the silicas. Validated procedures were developed to manufacture spherical porous ultra-pure silicas with a specific surface area of $350\,m^2\,g^{-1} \pm 5\%$ R.S.D., a specific pore volume of $1.0\,ml\,g^{-1} \pm 3.7\%$ R.S.D., an average pore diameter of $12.0\,nm \pm 0.5\%$ R.S.D. and an average particle diameter of 5 μm. Results are presented on trial batches and the final master batch which were both used as packing materials in reversed-phase liquid chromatography (RP-LC) columns. The latter columns were certified and accepted as an HPLC column as reference material (BCR-722) by the European Commission, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium.
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Keywords: Standard operation procedure; Stationary phases, LC; Reference material; Silica, *n*-octadecyl bonded

1. Introduction

Liquid chromatographic methods form the basis of many analytical measurements in industry, including those in the pharmaceutical industry, in food quality, and environmental studies. While automated injection devices, detectors and computing in HPLC have reached a high standard, the HPLC columns still need a substantial improvement with regard to reproducibility and ruggedness. Among all adsorbents employed in chromatography, silica is the dominating and best studied material [1–3]. The major concern of column manufacturers is to achieve a sufficient batch-to-batch reproducibility of the porous microparticulate silica which is employed as column packing. Usually, the micron size spherical porous silica particles are manufactured batch-wise by a multi-step process. During the process beading is achieved, the particle size and particle size distribution are adjusted and the surface and pore structural parameters are controlled to be optimal for the chromatographic application. In order to achieve the desired product quality each step has to be carefully monitored by physico-chemical measurements and finally by chromatographic tests. The variation of the particle size distribution, the pore size distribution, the specific surface area, the surface activity and the porosity of the silica particles have a decisive influence on the chromatographic properties of the HPLC column

Abbreviations: a_s (BET), specific surface are according to Brunnauer, Emmet and Teller; p_d (BJH_{des}), average pore diameter according to Barret, Joyner and Halenda (desorption branch of the nitrogen sorption measurement); v_p (G), specific pore volume according to Gurvitsch; $d_{p(50)}$, particle diameter at 50% of the cumulative particle size distribution; $d_{p(10)}$, particle diameter at 10% of the cumulative particle size distribution; $d_{p(90)}$, particle diameter at 90% of the cumulative particle size distribution; R.S.D., relative standard deviation; dynamic viscosity; α_{exp}, ligand density; α_{OH}, silanol group density; HMDS, hexamethyldisilazane; PES, poly(ethoxy)siloxane; M_n, average molecular weight

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[4]. The aim of this study was to develop a defined and tailored silica packing as a reference material according to a validated procedure by a concerted action of eight partners, of which were four from industry and four from academic institutions. The project was funded by the European Commission, Research Directorate/General (Measurement and Testing, Infrastructure; Contract SMT4 CT95 No. 2026).

Of the commercial HPLC columns *n*-octadecyl (C_{18}) bonded silicas are most frequently applied in HPLC analysis [5]. They are capable to solve the majority of analytical problems. Commercially available stationary phase materials, including Kromasil C_{18} (Eka, Bohus, Sweden) [6], Symmetry C_{18} (Waters, Milford, USA) [7] and Luna C_{18} (Phenomenex, Torrance, USA) [8] were recently examined with respect to the column to column variations in efficiency and retention with high precision but so far no manufacturing process has been published mostly due to proprietary reasons.

The aim of this work was to design and to synthesise 5 μm spherical silica with tailored properties which can be applied as a column packing in reversed-phase HPLC after silanisation. A synthesis procedure was chosen based on Unger et al. [9], and Unger and co-worker [10]. The particular focus of this study was to assess the effect of the synthesis parameters on the physico-chemical properties of the silica and to improve the manufacturing process with respect to reproducibility and ruggedness. As a support a mesoporous silica was targeted with a specific surface area, a_s, of 300–400 $m^2\,g^{-1}$, a specific pore volume, v_p, of 1.0–1.1 $ml\,g^{-1}$, a mean pore diameter, p_d, of 11–12 nm and a mean particle size, $d_{p(50)}$, of approximately 5 μm. These values were chosen because the majority of commercial HPLC packing materials have similar properties.

After manufacture of the native silica, the material should then be subjected to a surface reaction with *n*-octadecyldimethylchlorosilane under defined conditions to achieve a surface concentration, α_{exp}, of approximately 2–3 $\mu mol\,m^{-2}$.

This work forms part of the establishment of a column and test compounds as a reference material for LC, whose chromatographic properties have been defined under specified operating conditions [11]. The principal role of this material will be to provide the ability for a laboratory to check if their operating conditions match those which are specified. The ability to reproduce a standard method is an essential first step in a laboratory's capability to adopt a method developed elsewhere without the need to adjust the conditions and to determine chromatographic values which are directly comparable with results obtained in another laboratory.

The concept of a reference column was heavily debated over the last decade [12,13]. Independent of these arguments of pros and cons, the project was the first time where extensive intra- and inter-laboratory studies were made on the manufacture of a reversed-phase silica as well as on its chromatographic properties in round Robin tests which was never done in such detail before [14].

2. Experimental

The following standard operation procedures (SOPs) were used to prepare the *n*-octadecyl modified silica beads.

2.1. SOP for the production of poly(ethoxy)siloxane (PES)

Poly(ethoxy)siloxane (PES) was prepared by hydrolytic polycondensation of prepolymerised tetraethoxysilane, labelled as TES 40 (Wacker Chemie, Burghausen, Germany). TES 40 is a commercial material with defined properties. The batch-to-batch variability was overcome by the reservation of a 500 l master batch of TES 40. The material was characterised by elemental analysis (silica content of 38.4 wt.%, composition $Si(OC_2H_5)_{2.33}O_{0.835}$) and its average molecular weight ($M_n = 621 \pm 12\,g\,mol^{-1}$; assessed by vapour pressure osmometry, Knauer GmBH, Berlin, Germany). 1300 g TES 40 are poured into a clean and dry 2 l round-bottom flask and then 280 g ethanol (99.7–100%, Promochem, Wesel, Germany) are added while stirring at position 8 (IKA RCT basic, 750–850 rpm) with a Teflon covered magnetic stir bar. When the mixture of TES 40 and ethanol was mixed homogeneously 60.0 g of 0.10 $mol\,kg^{-1}$ hydrochloric acid (Titrisol, Merck KGaA, Darmstadt, Germany) are added over a period of 10–12 min using a dropping-funnel in order to initiate the condensation. After stirring for another 30 min, the 2 l round-bottom flask is connected to the rotary evaporator and a temperature of 140 °C (oil-bath, measured by an external thermometer) is applied in order to remove the ethanol. Then the vacuum is reduced to 30 mbar ($\Delta p = 1$ mbar). The system is kept at 30 mbar and 140 °C for 5 h in order to completely remove the ethanol.

2.2. SOP for the production of silica hydrogel beads

There was a need of a specific instrumentation to carry out the conversion of PES into silica hydrogel beads. This implied the type of flask, the type of stirring plate and the type of stirring device. The optimum device was assessed by an extensive study before the project was started. One thousand grams of PES and 60 g dimethylformamide (DMF, 99%, Merck, Darmstadt, Germany) are mixed in a 1 l beaker. At the same time 2.700 l of deionised water and 1.500 l *iso*-propanol (p.a., Merck, Darmstadt, Germany) are mixed in a 5 l flask. Then the PES/DMF mixture is added to the water/*iso*-propanol mixture. The stainless steel stirrer is adjusted to the flask, the drilled stopper including the funnel is connected and finally the stirrer is attached to the drilling jig. The stirrer engine (FL-RD 20 V1, Fluid, Lörrach, Germany) is switched on and the revolution rate is increased from 330 rpm up to 1800 ± 5 rpm within 1 min. Then the mixture is emulsified for 5 min. After 5 min, 100 ml of 14.7 $mol\,kg^{-1}$ ammonium hydroxide (ammonia, 25%, Merck, Darmstadt, Germany) are added at once and then the mixture is stirred for another 5 min at the same speed. Then the speed is

reduced down to 1300 rpm and stirring is continued for another 25 min. After 25 min the suspension is poured into a 10 l glass-beaker and allowed to sediment for 24 h. After 24 h the supernatant dispersion is decanted and the residue suspended in 8 l of deionised water. Again, the particles are allowed to sediment for 24 h. This last step is repeated once.

After the third sedimentation the supernatant dispersion is decanted and the residue suspended in 2 l of deionised water. Afterwards the suspension is filtered using a porcelain Buechner-funnel (diameter: 270 mm) with a round filter paper (No. 589/3, 250 mm, Schleicher-Schuell, Dassel, Germany) and washed with 3 l of deionised water at room temperature. The deionised water had an electrical resistivity of 15 MΩ cm.

2.3. SOP for the production of silica xerogel beads

A heatable magnetic stirrer (IKA RCT basic), an electronic contact thermometer (IKA ETS 300) and a Teflon covered stirring shaft are used to age the native silica. The silica hydrogel particles (amount approximately 300 g) are suspended in 3 l of deionised water by adding the particles to the water under stirring using the electronic stirrer (IKA RW 20, position I/5, 250 rpm) at room temperature. Then 35 ml of 14.7 mol kg^{-1} ammonium hydroxide (ammonia, 25%, Merck, Darmstadt, Germany) is added using a graduated cylinder (100 ml). After that the suspension is heated to 70 °C (contact thermometer: 70 °C; heatable magnetic stirrer: 250 °C). In order to reach a pH of 7.0–7.5 (which was measured by a pH-meter, type Handylab 1, Schott, Germany) it was necessary to heat the suspension for 8 h at 70 °C. If the pH is outside of the specified range, particularly to the alkaline pH notable changes in the pore structural properties of the final product will occur. Finally the suspension is filtered once more using a porcelain 270 mm Buechner-funnel with a filter paper (No. 589/3, Schleicher-Schuell, Dassel, Germany) of 250 mm outer diameter, washed with 5 l of deionised water, then with 2 l of *iso*-propanol (p.a., Merck, Darmstadt, Germany) and with 1 l of methanol (p.a., Merck, Darmstadt, Germany). Finally the silica hydrogel is dried for at least 4 h at 150 °C in a vacuum oven (<20 mbar) in order to convert it into a xerogel. Exceeding the drying time is not critical with regard to the properties of the material.

2.4. SOP for the calcination of silica xerogel beads

The porcelain-dish (o.d. = 20 cm, depth of the bed was 3 cm) with the dry, evenly distributed silica is placed in a furnace (N 100, Nabertherm, Bremen, Germany) and calcined according to the following temperature program:

- heat-up time: 1 °C min^{-1} to 650 °C;
- calcination time: 300 min at 650 °C;
- cooling down time: to room temperature, approximately 20 h.

The amount of silica subjected to calcination was between 180 and 250 g.

2.5. SOP for the rehydroxylation of silica xerogel beads

Six hundred milliliters of 5 mol kg^{-1} hydrochloric acid (Merck, Darmstadt, Germany) is poured into in a 1000 ml three-neck round-bottom flask using a funnel. The round-bottom flask is equipped with a Dimroth-condenser, a stirrer-bearing, a stirring-shaft and a stirring motor (IKA RW 20). The calcined silica is added in small portions while stirring (position I/3.5, 175 rpm). After adding the silica, the stirrer-speed is raised for a few minutes to I/6 (300 rpm) to suspend the whole silica. The total amount of silica applied was 150–200 g and depended on the yield of the previous synthesis steps. Due to the large excess of hydrochloric acid, the amount of silica was not crucial. Afterwards the speed is lowered back to position I/3.5 (175 rpm). The flask is placed into a oil-bath (electronic-thermometer: 150 °C; heater: 200 °C). The mixture is then refluxed for 12 h. After 12 h the oil-bath is removed and the suspension is allowed to cool down for 30 min. The silica is filtered off onto a glass-filter funnel (G4, Schott, Mainz, Germany) and washed with about 60 l of deionised water. After washing with water, the silica is washed twice with 300 ml of methanol to avoid clotting during drying. Finally the silica is dried in a vacuum-oven (Vacutherm, Heraeus, Hanau, Germany) at 80 °C and 12 hPa for 4 h and at 150 °C and 12 hPa for 15 h, using a porcelain-dish (o.d. = 20 cm) and stored in 500 ml bottles or directly silanised. Storage time should not exceed 1 month.

2.6. SOP for the silanisation of silica xerogel beads

One hundred grams of rehydroxylated, dry silica (150 °C and 12 hPa, 15 h) is poured into a previously evacuated and nitrogen-flushed 1000 ml three-neck round-bottom flask (Schott, Mainz, Germany), equipped with a Dimroth-condenser with a drying tube, a 500 ml dropping-funnel, a stirrer-bearing, a stirring-shaft and a stirring motor. Four hundred milliliters of dry toluene is added to the silica under stirring. The flask is placed into a preheated oil-bath (electronic-thermometer: 130 °C; heater: 200 °C). The flask is heated until the toluene refluxes and 16.4 g (0.24 mol) of imidazole (Sigma–Aldrich, Steinheim, Germany) is added while still stirring. After 10 min of stirring, a solution of 86.4 g (0.24 mol) melted *n*-octadecyldimethylchlorosilane (Wacker Chemie, Burghausen, Germany) in 200 ml toluene is added to the stirred silica over approximately 15 min using the dropping-funnel. The suspension is then stirred under reflux for 1 h. After 1 h the oil-bath is lowered and the suspension allowed to cool down for 30 min while stirring. In most cases, a cluster of crystals of imidazole hydrochloride is formed. The suspended silica is decanted from the crystals and the solution is filtered through a 1000 ml glass-filter funnel. The silanised, filtered silica is sequentially washed with 200 ml of toluene (Merck, Darmstadt, Germany), 300 ml

of methanol (Merck, Darmstadt, Germany), 300 ml of methanol/water (50/50, v/v) and finally washed with 250 ml of methanol. The silica beads are subjected to THF-treatment by pouring it into a 1000 ml, three-neck round-bottom flask, equipped with a Dimroth-condenser, a stirrer bearing, a stirring shaft and a stirring motor. Five hundred milliliters of tetrahydrofurane (SeccoSolv, Merck, Darmstadt, Germany) is added and the suspension is stirred (speed: II/3, 500 rpm), under reflux, for 20 min (electronic-thermometer: 80 °C; heater: 150 °C). After removal of the oil-bath, the hot suspension is filtered through a glass-filter funnel (G4, Schott, Mainz, Germany) and washed with 300 ml tetrahydrofurane and twice with 300 ml methanol. Finally, the product is dried in a vacuum-oven (40 °C, 12 hPa, 12 h).

2.7. Characterisation methods

2.7.1. Viscosity measurements

The measurements were carried out using a Carri-Med CSL 500 Rheometer, Leatherhead, UK. A cone/plate geometry was chosen (4 cm cone diameter at an angle of 1°). Three hundred seventy microliters of the sample were introduced on the plate, and a 10 min measurement of shear stress from 0 to 20 N m^{-2} was performed at 25 °C.

2.7.2. Nitrogen sorption

Adsorption and desorption isotherms were measured at 77 K on a Quantachrome Autosorb 6B (Quantachrome Corporation, Boynton Beach, FL, USA) using nitrogen. The samples were out-gassed at 423 K and 1 mPa for 14 h before adsorption measurements.

2.7.3. Determination of surface hydroxyl group concentration

The silanol group concentration, α_{OH}, was calculated from the mass loss between 200 and 1000 °C measured by thermal gravimetry [15]. Twenty to 50 mg of the silica samples were heated up from room temperature to 1000 °C in a thermo balance (L81, Linseis, Selb, Germany) with a heating rate of 10 K min^{-1}.

2.7.4. Determination of metal impurities

The metal impurities (Al, Zn, Mg, Ti, K, Na, Li, Pb, Cu, Ni and Ca) were determined by atomic absorption spectroscopy (AAS type 5100 ZL, Perkin-Elmer, Wellesley, USA), optical emission spectroscopy (ICP-OES type Liberty Series II, Varian, Darmstadt, Germany) and neutron activation analysis (reactor TRIGA MARK II, Department of Nuclear Chemistry, Mainz, Germany).

3. Results and discussion

The synthesis of the packing material comprised nine discrete steps which are listed in Table 1. The scheme is given in Fig. 1. Generally, 29 PES batches were synthesised by four different partners and were individually converted into hydrogel and xerogel beads, respectively. Twenty-five hydrogel batches as well as xerogel batches were produced. The selection of the individual batches for blending to a master batch prior to HCl treatment and silanisation took place according to the following criteria:

- specific surface area of 350 m^2 g^{-1} ± 5% R.S.D.;
- specific pore volume of 1.0 ml g^{-1} ± 3.7% R.S.D.;
- average pore diameter of 12.0 nm ± 0.5% R.S.D.

Due to the low yield of the size classification step, 10 batches were rehydroxylated and silanised individually and blended again prior to packing of the columns.

Table 1
Synthesis and post-synthetic steps of a 5 μm spherical particulate silica material

No.	Step	Comment
1	Synthesis of poly(ethoxy)siloxane (PES); acceptance criterion: the dynamic viscosity η = 40–50 mPa s	Acid catalysed condensation and hydrolysis of monomers, control of viscosity
2	Converting PES into silica hydrogel beads	Stirring process, base catalysed
3	Washing, aging, drying and blending of the individual batches	Removal of impurities, conversion of hydrogel into xerogel
4	Calcination; acceptance criteria of xerogel batches: v_p = 1.0 ml g^{-1} ± 3.7% R.S.D. a_s (BET) = 350 m^2 g^{-1} ± 5% R.S.D.	Densification of material, removal of remaining functional groups from monomer
5	Blending	Production of a homogeneous master batch out of accepted individual batches
6	Size classification	Narrowing the particle size distribution
7	HCl treatment	Removal of inorganic impurities, increase of surface silanol group concentration
8	Silanisation	Surface modification
9	Packing and testing of columns	Application of material in HPLC

The acceptance criteria of the xerogel beads were set after the individual batches were characterised by nitrogen sorption.

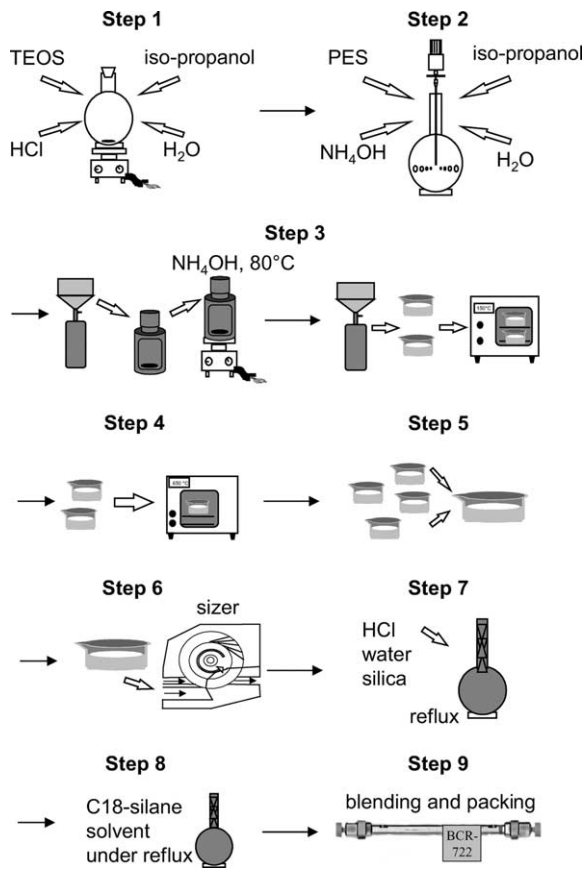

Fig. 1. Scheme of the manufacturing process for a C_{18} bonded packing material for liquid chromatography. The synthesis comprise: PES synthesis (step 1); conversion of PES into hydrogel beads (step 2); washing, aging and drying (step 3); calcination (step 4); blending of individual batches into a master batch (step 5); size classification (step 6); rehydroxylation (step 7); silanisation (step 8); blending and packing of material into HPLC columns (step 9).

3.1. Synthesis of poly(ethoxy)siloxane (PES)

The manufacturing process of the silica microparticulate packing material started with the acid catalysed hydrolysis and condensation (see step 1 in Table 1) of the silica precursor TES 40 (Wacker Chemie, Burghausen, Germany). TES 40 is a commercially available prepolymerised tetraethoxysilane with a silicondioxide content of approximately 40% (tetraethylorthosilicate, TEOS, possesses about 28%, also termed TES 28). The obtained product, poly(ethoxy)siloxane, was characterised by its dynamic viscosity. The dynamic viscosity of the PES increased with increasing amounts of the catalyst hydrochloric acid according to Fig. 2.

The critical step in the processing of the PES is the conditions at the removal of ethanol which are given in the respective SOP. Minor changes in the temperature, the duration of treatment and the pressure lead to significant changes in the dynamic viscosity and thus impair the reproducibility of η. As there was a significant influence of the dynamic viscosity of the PES on the average particle size and particle size distribution (see Section 3.2) after conversion into beads, the target value for the viscosity was set to 40–50 mPa s to obtain beads of approximately 5 μm size. Four different laboratories took part in the production of PES batches. The results presented in Table 2 show the large variations in dynamic viscosities of the PES batches. Although laboratory 1 obtained PES batches with good reproducibility (4.6% relative standard deviation), the mean value was too high. The other laboratories synthesised PES batches with acceptable mean dynamic viscosity but with very high R.S.D.-values (up to 30%).

The SOP for making the PES was clearly defined. The results, however, showed that the participating laboratories did not follow carefully the instructions. In order to avoid variations in the properties for the conversion of the PES into silica hydrogel beads the identical equipment was applied by all partners. Despite the large variations, it was decided to further process all PES charges without blending of batches.

3.2. Conversion of PES into silica hydrogel beads

In the second step of the silica production, the poly(ethoxy)siloxane was converted into silica hydrogel beads in a stirring process under basic conditions (see step 2 in

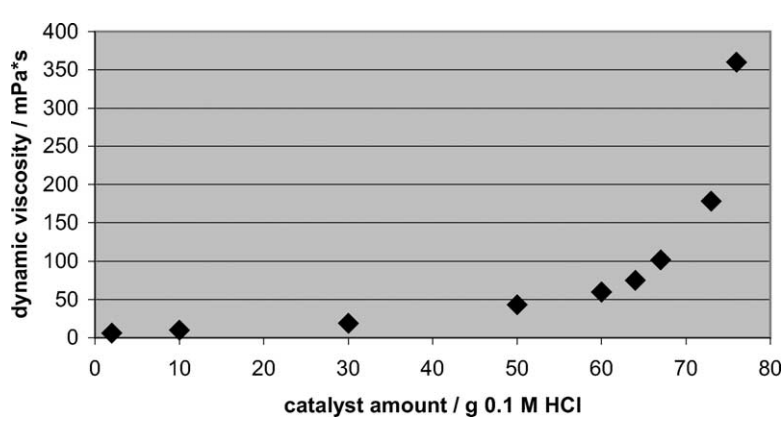

Fig. 2. Dependence of the dynamic viscosity of PES batches on the catalyst amount in the reaction solution (trial batches).

Table 2
Dynamic viscosities and mean values of individual PES batches produced by four different laboratories

Laboratory	Batch	Dynamic viscosity (mPa s)
1	PES-1	61.05 ± 1.21
	PES-2	60.38 ± 1.27
	PES-3	32.97 ± 0.55
	PES-4	36.07 ± 0.59
	PES-5	39.53 ± 0.72
	Mean (R.S.D.)	46.0 ± 13.6 (29.6%)
2	PES-6	64.94 ± 2.11
	PES-7	71.04 ± 3.17
	PES-8	72.77 ± 2.04
	PES-9	68.36 ± 1.95
	PES-10	67.10 ± 2.39
	Mean (R.S.D.)	68.84 ± 3.11 (4.5%)
3	PES-11	46.72 ± 1.25
	PES-12	38.00 ± 0.64
	PES-13	62.15 ± 1.83
	PES-14	55.47 ± 1.76
	PES-15	45.62 ± 1.16
	PES-16	48.90 ± 1.05
	PES-17	49.08 ± 1.45
	PES-18	42.71 ± 0.98
	PES-19	40.22 ± 0.78
	PES-20	48.23 ± 1.08
	PES-21	55.73 ± 1.46
	PES-22	44.43 ± 0.78
	PES-23	52.50 ± 1.21
	PES-24	46.73 ± 0.94
	PES-25	50.77 ± 1.21
	Mean (R.S.D.)	48.48 ± 6.28 (12.9%)
4	PES-26	41.64 ± 0.77
	PES-27	36.81 ± 0.70
	PES-28	59.14 ± 1.46
	PES-29	48.72 ± 1.08
	Mean (R.S.D.)	46.58 ± 9.69 (20.8%)
1–4	Mean (R.S.D.)	47.65 ± 8.36 (17.5%)

Table 1). After washing and sedimentation (step 3), small amounts of fines (particles with $d_p < 1\,\mu m$) were found. The aging process was performed at elevated temperature (70 °C) and resulted in the densification and stiffening of the silica framework. However, alkoxy groups from the starting silane still remained even after the conversion of the hydrogel into the xerogel by drying. This is evidenced in the comparison of the differential thermo analysis (DTA) signals measured during a thermogravimetric (TG) experiment of a native silica gel and the same sample after calcination and rehydroxylation (see Fig. 3). The sharp exothermic peak at 370 °C which only appeared for the as-synthesised material was assigned to the combustion of organic groups, i.e. the uncondensed alkoxy groups of the precursor. Therefore, calcination had to be applied.

For the development of the appropriate procedure, it was necessary to investigate various dependencies of synthetic parameters and resulting material properties. Both, the dynamic viscosity of PES and the stirring speed during polycondensation directly influenced the particle size, particle size distribution and the pore structural parameters of the porous silica beads, i.e. trial batches. This is shown for the parameters like the specific surface area, a_s (BET), the specific pore volume, v_p (G), and the mean pore diameter, p_d (BJH_{des}) in Figs. 4 and 5 for a wider range of viscosities. At constant stirring speed, the mean particle diameter, $d_{p(50)}$, increased with increasing dynamic viscosity of the PES (samples A–C in Table 3). It is clearly seen that the pore diameter is very much affected by the dynamic viscosity. The higher the viscosity the tighter the gel network of the prepolymerised PES. This led to smaller mesopores in the fully condensed hydrogel beads. When the viscosity of the PES was kept constant, the pore structural parameters remained nearly unchanged (samples D–G). By varying the stirring speed it was possible to fine-tune to a desired average

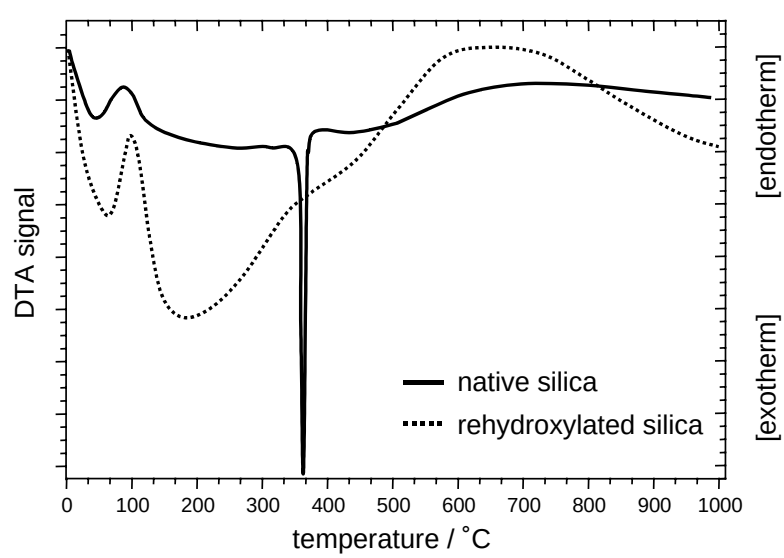

Fig. 3. Differential thermal analysis (DTA) signal of native and rehydroxylated silica.

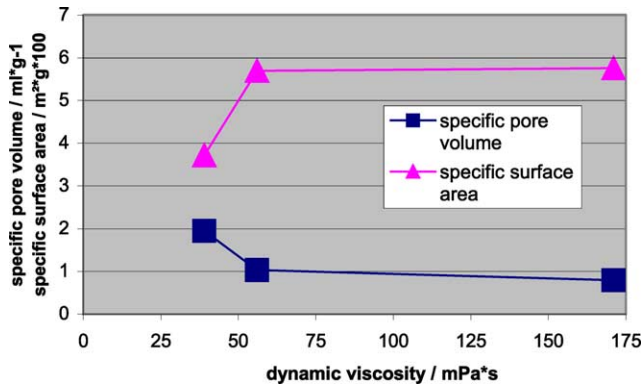

Fig. 4. Dependence of the specific surface area and specific pore volume of silica beads on the dynamic viscosity of the starting poly(ethoxy)siloxane.

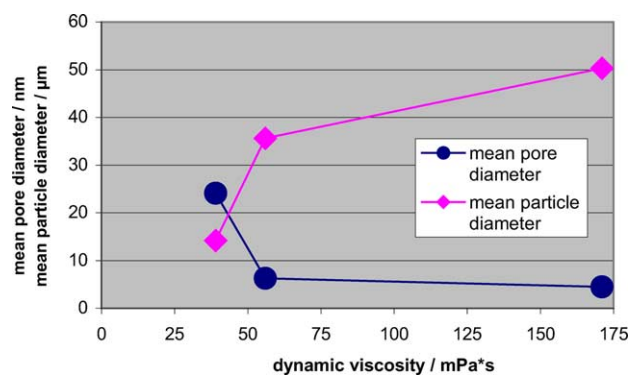

Fig. 5. Dependence of the mean pore and mean particle diameter of silica beads on the dynamic viscosity of the starting poly(ethoxy)siloxane (trial batches).

particle size. After these preliminary results, the procedure was adapted to give approximately 5 μm sized beads.

The synthesis procedures were up-scaled to a 5 l batch and the influence of the synthesis parameters on the materials properties were assessed by factorial design experiments as well as the ruggedness of the procedure by choosing two operators (data not shown) [16] which led to the proposed standard operation procedures (see Section 2). The individual PES batches in Table 2 were converted into hydrogel beads, washed and aged according to the SOPs. The synthesis procedure as described in Section 2.2 is limited by the size of the stirring device to a volume of 5 l. Each synthesis performed at this scale yields approximately 200 g of dry silica beads (individual batches).

As was expected by the viscosity variation of the individual PES batches, the pore diameters of the silica gel batches scattered widely. Mainly three groups of pore diameters were found at around 11.4, 13.2 and 15.5 nm and one outlier having a pore size of approximately 9 nm. Due to the targeted mean pore diameter of 12.0 ± 0.5 nm, the batches being out of the range were not further used after the calcinations step.

3.3. Calcination of the native silica beads

Calcination (step 4 in Table 1) was a necessary procedure to achieve silica materials with homogeneous surface properties. In addition, ethoxy groups which remained from the poly(ethoxy)siloxane even after the conversion of the hydrogel into the xerogel were removed by applying temperatures between 370 and 400 °C (see Fig. 3).

Depending on the calcination temperature, a significant change in the pore structure took place. Treatments up to 500 °C led to an activation of the surface, i.e. the specific surface area increased. At higher temperatures, the specific pore volume and the pore size diminished. Thus, calcination was used to fine-tune pore structural parameters of the products. The calcination process was also accompanied with a severe reduction of the surface hydroxyl (silanol) groups which condensed upon heating to form water molecules leaving surface siloxane bonds. A rehydroxylation post-treatment was applied to reverse the latter reaction. However, exceeding a certain calcination temperature the dehydroxylation of the silica surface became slow or even irreversible by hydrochloric acid treatment due to the sintering of the material. It was found out, that the maximal calcination temperature after which the silica could still be rehydroxylated lied approximately at 750 °C for this silica type [17] (Table 4).

Fig. 6 shows the dependence of the silanol group concentration of calcined silica batches on the calcination temperature and the followed rehydroxylation and silanisation step of the calcined silicas. The silanol group concentration of calcined and rehydroxylated silicas was calculated from TG experiments whereas ^{1}H NMR was employed to assess the silanols of silanised silicas after exchanging the surface silanol protons by deuterated trifluoro acetic acid according to a procedure developed by Holik and Matêjková [18].

Table 3
Influence of the stirring speed and the dynamic viscosity on the mean particle diameter and pore structural parameters of silica xerogel beads (trial batches)

Sample	η (mPa s)	Stirring speed (rpm)	$d_{p(50)}$ (μm)	a_s (BET) ($m^2\,g^{-1}$)	v_p (G) ($ml\,g^{-1}$)	p_d (BJH_{des}) (nm)
A	39	1250	14.2 ± 0.7	372 ± 17	1.95 ± 0.06	24.1 ± 0.7
B	56	1250	35.6 ± 1.8	570 ± 17	1.03 ± 0.03	6.3 ± 0.2
C	171	1250	50.3 ± 2.5	576 ± 17	0.79 ± 0.02	4.5 ± 0.2
D	72	1000	43.4 ± 2.2	557 ± 17	1.20 ± 0.04	7.4 ± 0.2
E	72	1100	40.0 ± 2.0	554 ± 17	1.25 ± 0.04	6.8 ± 0.2
F	72	1200	38.9 ± 1.9	576 ± 17	1.20 ± 0.04	6.8 ± 0.2
G	72	1300	35.6 ± 1.8	581 ± 17	1.25 ± 0.04	7.4 ± 0.2

Table 4
Pore structural parameters of individual native silica xerogels prepared by three laboratories

Batch	a_s (BET) ($m^2 g^{-1}$)	v_p (G) ($ml g^{-1}$)	p_d (BJH) (nm)	Yield (g)
Xerogel-1	383.0	1.132	11.53	201.0
Xerogel-2	354.1	1.168	11.44	207.0
Xerogel-3	267.9	1.134	15.35	192.0
Xerogel-4	265.7	1.136	15.69	202.0
Xerogel-5	286.6	1.147	15.46	211.0
Mean 1–5	311.5 ± 53.7	1.143 ± 0.015	13.89 ± 2.20	202.6 ± 7.2
R.S.D.	17.2	1.305	15.85	3.5
Xerogel-6	329.0	1.260	13.32	330.4
Xerogel-7	318.4	1.126	13.22	332.9
Xerogel-8	334.1	1.241	13.20	339.1
Xerogel-9	337.4	1.231	13.11	325.6
Xerogel-10	317.6	0.846	9.01	335.0
Mean 6–10	327.3 ± 9.0	1.141 ± 0.173	12.37 ± 1.88	332.6 ± 5.0
R.S.D.	2.8	15.152	15.21	1.5
Xerogel-11	328.4	1.076	13.18	99.2
Xerogel-12	330.5	1.089	13.27	122.5
Xerogel-13	310.8	1.101	13.17	238.4
Xerogel-14	332.7	1.046	11.56	227.9
Xerogel-15	326.0	1.070	13.10	239.4
Xerogel-16	361.5	1.137	11.52	205.5
Xerogel-17	352.3	1.105	11.40	205.6
Xerogel-18	351.2	1.103	11.45	218.3
Xerogel-19	347.4	1.105	11.60	207.0
Xerogel-20	335.7	1.055	11.47	234.9
Xerogel-21	342.2	1.093	11.44	237.1
Xerogel-22	414.9	1.323	11.43	220.2
Xerogel-23	345.3	1.096	11.41	243.9
Xerogel-24	336.9	1.061	11.45	266.0
Xerogel-25	338.7	1.072	11.38	240.5
Mean 11–25	343.6 ± 23.3	1.102 ± 0.065	11.92 ± 0.79	213.8 ± 45.1
R.S.D.	6.8	5.940	6.61	21.1
Mean 1–25	333.9 ± 32.0	1.118 ± 0.090	12.41 ± 1.56	235.3 ± 62.0
R.S.D.	9.6	8.081	12.55	26.4

The native silicas have the batch number corresponding to their parent PES batches of Table 2.

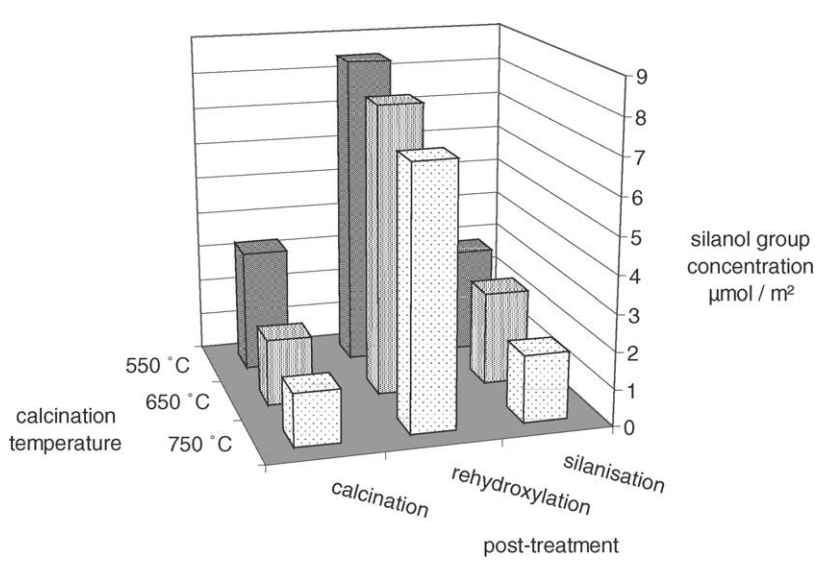

Fig. 6. Silanol group densities of parent silica samples calcined at different temperatures and subjected to rehydroxylation and silanisation (trial batches).

As can be seen from Fig. 6, the silanol group concentration decreased with increasing calcination temperature. After rehydroxylation, the samples formerly treated at higher temperature could not reach their fully hydroxylated state ($\alpha_{OH} \sim 8\,\mu mol\,m^{-2}$ [19]). A calcination temperature of 650 °C was chosen for the standard operation procedure, because the resulting materials properties were a compromise between favourable surface chemistry and desired pore structure. This means that the pore structural properties of the silica were fine-tuned by calcination at 650 °C while retaining a fairly high surface hydroxyl concentration for further surface modification steps.

The pore structural parameters of the calcined individual batches are shown in Table 5. The acceptance criteria were set after the individual batches were characterised by nitrogen sorption. The mean specific surface area of the batches had a relative standard deviation of approximately ±5% and the specific pore volume had a similar R.S.D.-value of ±3.7%, whereas the mean pore diameter showed a very small relative standard deviation of ±0.4%. An intercomparison test on the determination of the specific pore volume v_p and the specific surface area a_s (BET) using nitrogen sorption experiments at 77 K was performed with one selected batch of silica xerogel between six partners. The results were: specific pore volume $v_p = 1.00\,ml\,g^{-1} \pm 2\%$ R.S.D. and specific surface area a_s (BET) $= 300\,m^2\,g^{-1} \pm 3.0\%$ R.S.D.

Table 5
Pore structural parameters of individual calcined silica xerogels prepared by three laboratories

Batch	a_s (BET) ($m^2\,g^{-1}$)	v_p (G) ($ml\,g^{-1}$)	p_d (BJH) (nm)
Xerogel-1*	372.9	1.084	11.44
Xerogel-2*	384.5	1.107	11.40
Xerogel-3	290.1	1.135	15.64
Xerogel-4	286.0	1.134	15.58
Xerogel-5	309.8	1.142	15.59
Xerogel-6	358.5	1.267	13.10
Xerogel-7	368.3	1.220	13.12
Xerogel-8	353.7	1.221	13.29
Xerogel-9	356.5	1.215	13.15
Xerogel-10	337.3	0.836	9.00
Xerogel-11*	337.2	1.051	11.40
Xerogel-12*	331.5	1.043	11.56
Xerogel-13	314.6	1.062	13.15
Xerogel-14*	343.7	1.029	11.44
Xerogel-15	329.6	1.037	13.01
Xerogel-16*	349.9	1.026	11.38
Xerogel-17*	349.0	1.048	11.40
Xerogel-18*	393.5	1.181	11.49
Xerogel-19*	354.8	1.074	11.50
Xerogel-20*	343.9	1.028	11.48
Xerogel-21*	344.3	1.047	11.42
Xerogel-22*	353.8	1.074	11.47
Xerogel-23*	354.6	1.069	11.41
Xerogel-24*	362.0	1.079	11.48
Xerogel-25	355.0	1.058	11.39
Mean values of accepted batches (denoted with '*')	355.3 ± 16.9	1.066 ± 0.039	11.44 ± 0.05
R.S.D.	4.8	3.670	0.45
Mean values of all batches	345.4 ± 25.9	1.091 ± 0.090	12.25 ± 1.59
R.S.D.	7.5	8.208	12.97

The xerogels have the batch number corresponding to their parent native silica batches (Table 4).

3.4. Blending of the individual batches to a master batch

The blending (step 5 in Table 1) of the individual batches of Table 5 that met the criteria (specific surface area of $350\,m^2\,g^{-1} \pm 5\%$ R.S.D., specific pore volume of $1.0\,ml\,g^{-1} \pm 3.7\%$ R.S.D., average pore diameter of 12.0 nm ± 0.5% R.S.D.) was performed using a EIRICH-Intensivmischer (Typ R02, Maschinenfabrik Eirich, Hardheim, Germany). 3.0 kg of blended silica was obtained.

3.5. Size classification

Due to the beading process by stirring, the particle size distribution was relatively broad. The span of the distribution was characterised by the particle size at 10 and 90% of the cumulative distribution curve ($d_{p(10)}$, $d_{p(90)}$), respectively. The ratio $d_{p(90)}/d_{p(10)}$ holds for the broadness of the distribution and should be smaller than 2 in order to achieve good packing properties. Batches produced according to the proposed SOP possessed $d_{p(90)}/d_{p(10)}$-values of 2.5–4.0 and therefore had to be subjected to sizing.

The size classification of the master batch (calcined and blended) yielded a material with a narrow size distribution (step 6 in Table 1). The broadness and mean value of the latter was adjusted by varying the parameters of the air elutriation equipment (Zig-Zag siever, type 100 MZR, Alpine GmbH, Augsburg, Germany). The particle size distribution was assessed by Coulter Counter (Coulter Multisizer, AccuComp 1.19 software). The particle size distribution (Fig. 7) showed a gaussian-like distribution with a peak maximum at 6 μm. The particle specifications of the calcined and blended master batch are summarised in Table 6.

3.6. Rehydroxylation

The material synthesised according to the above procedures was after-treated because the loss of silanol groups

Table 6
Particle size classification data of the master batch (blended and calcined individual batches)

Parameter	Value
Mean (μm)	6.028
Median ($d_{p(50)}$) (μm)	5.927
$d_{p(10)}$ (μm)	4.564
$d_{p(90)}$ (μm)	7.462
$d_{p(90)}/d_{p(10)}$	1.635
Yield (g)	970

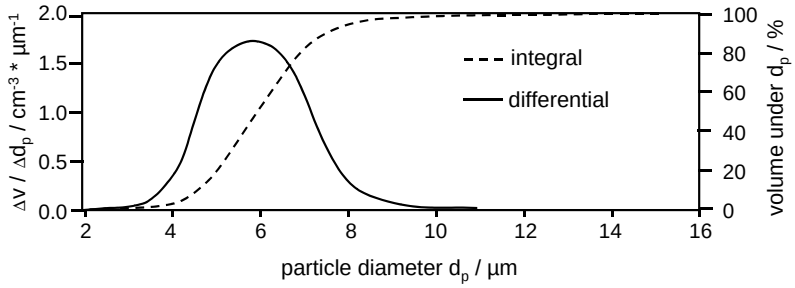

Fig. 7. Particle size distribution curves of the silica master batch.

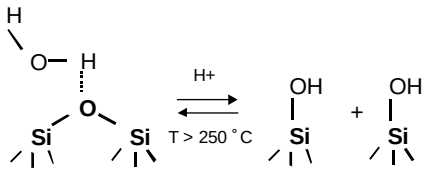

Fig. 8. Rehydroxylation of siloxane groups.

due to calcination was significant and contaminations of the material by metals was observed (abrasion from sizing equipment). The treatment with hydrochloric acid (step 7 in Table 1) tackled both problems, as siloxane bonds were converted to silanol groups according to Fig. 8 and metal impurities were dissolved [20].

In the course of the development of the optimal rehydroxylation procedure the impact of HCl treatment time on the residual metal content was investigated (Table 7). It can be seen from Table 7 that the metal content, mainly of iron and nickel, increased after sizing and was reduced to its original value by treatment with hydrochloric acid treatment for 12 h. Due to the low sizing yield 10 batches were sequentially rehydroxylated and silanised.

3.7. Silanisation

The impact of the post-treatment procedures, as well as their conditions on the chromatographic properties, using standard test mixtures for the characterisation of the prepared RP 18 HPLC columns was already reported [17]. The objective of this study was to subject a high-purity silica to various extraction, dehydroxylation and rehydroxylation processes prior to silanisation with *n*-octadecyldimethylmonochlorosilane and *n*-octadecylmethyldimethoxysilane. A limited number of studies had so far been reported in this field, mainly performed on traditional but not high-purity silicas [21–24].

No dependence between the post-treatment and the ligand density and carbon content, respectively, could be detected in our studies. Moreover, endcapping with hexamethyldisilazane (HMDS) had no effect on the carbon content of the silica silanised with *n*-octadecyldimethylmonochlorosilane and imidazole [25]. Therefore, the endcapping was not established as a standard operation procedure.

The rehydroxylated silica batches were subjected to silanisation (step 8 in Table 1) according to the SOP, i.e. 10 batches were prepared. The average carbon content of the silanised silica batches amounted to (19.0 ± 0.5)% (Table 8). As a result, the C_{18} ligand density amounted to approximately (2.95 ± 0.08) $\mu mol\,m^{-2}$.

3.8. The final master batch

The physico-chemical properties of the final master batch are listed in Table 9. The material was packed into stainless steel columns (150 mm × 4 mm) by the slurry technique and tested under chromatographic conditions using defined testing methods [14].

Table 7
Metal content (measured by ICP-OES) of silica xerogels before and after the treatment with hydrochloric acid at different treatment times

Element	Unsized silica (ppm)	Sized silica (ppm)	After 2 h HCl treatment (ppm)	After 12 h HCl treatment (ppm)
Fe	<1	50	6	<1
Ni	<1	4	<1	<1
Zn	<1	2	<1	<1
Cu	<1	<1	<1	<1
Pb	<1	<1	<1	<1
Co	<1	<1	<1	<1
Al	<1	<1	<1	<1

Table 8
Carbon contents of individually silanised silica batches prior to blending and packing into columns

Batch	Carbon content (%)
C_{18} silica-1	18.7
C_{18} silica-2	19.2
C_{18} silica-3	19.5
C_{18} silica-4	18.3
C_{18} silica-5	18.5
C_{18} silica-6	18.4
C_{18} silica-7	19.5
C_{18} silica-8	19.2
C_{18} silica-9	19.6
C_{18} silica-10	18.9
Mean	19.0 ± 0.5
R.S.D.	2.6

Table 9
Specifications of the C_{18} bonded silica master batch

Parameter	Value	R.S.D.-value (%)	Used technique
Specific surface area ($m^2 g^{-1}$)	355.3 ± 16.9	4.8	N_2 sorption
Specific pore volume ($ml g^{-1}$)	1.066 ± 0.039	3.7	N_2 sorption
Mean pore diameter (nm)	11.44 ± 0.05	0.4	N_2 sorption
Carbon content (%)	19.0 ± 0.5	2.6	Elemental analysis
Ligand density ($\mu mol m^{-2}$)	2.95 ± 0.07	2.6	Elemental analysis
Mean particle size (μm)	6.028 ± 0.31	5.0	Coulter Counter

The aim of this paper was to publish the full details of a manufacturing process for the preparation of a spherical silica packing material. The target specifications of this C_{18} bonded material were outlined but the materials properties were not certified according to the rules of the IRMM as the focus of the overall work was on the certification of the packed column and its test solutions. In the case that a new master batch is needed to produce new CRM columns the established SOPs should be used in order to provide a similar material with the same homogeneous surface chemistry. We suggest this approach for comparability reasons and due to the fact that the certified chromatographic properties were mainly affected by the silica surface chemistry, e.g. type of silica, hydroxyl state and ligand density.

4. Conclusion

The synthesis of poly(ethoxy)siloxane was found to be the crucial step in the manufacturing process. Although high reproducibility within one laboratory could be achieved with trial batches, the materials produced in several laboratories showed properties, e.g. dynamic viscosity, which scattered widely. The following procedures (beading process, aging, calcination and rehydroxylation) led to a homogeneous silica material with closely defined pore structural parameters. The average particle size could be adjusted by sizing technology and the surface chemistry was optimised for the subsequent modification with chlorosilanes. The obtained silica beads were successfully employed as packing material in reversed-phase LC and after the evaluation of the certification campaign accepted as an HPLC column as a certified reference material by the European Commission.

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