Preparation, purification and characterization of aminopropyl-functionalized silica sol

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ABSTRACT

A new, simple and "green" method was developed for the surface modification of 20 nm diameter Stöber silica particles with 3-aminopropyl(diethoxy)methylsilane in ethanol. The bulk polycondensation of the reagent was inhibited and the stability of the sol preserved by adding a small amount of glacial acetic acid after appropriate reaction time. Centrifugation, ultrafiltration and dialysis were compared in order to choose a convenient purification technique that allows the separation of unreacted silylating agent from the nanoparticles without destabilizing the sol. The exchange of the solvent to acidic water during the purification yielded a stable colloid, as well. Structural and morphological analysis of the obtained aminopropyl silica was performed using transmission electron microscopy (TEM), dynamic light scattering (DLS) and zeta potential measurements, Fourier-transform infrared (FTIR), ^{13}C and ^{29}Si MAS nuclear magnetic resonance (NMR) spectroscopies, as well as small angle X-ray scattering (SAXS). Our investigations revealed that the silica nanoparticle surfaces were partially covered with aminopropyl groups, and multilayer adsorption followed by polycondensation of the silylating reagent was successfully avoided. The resulting stable aminopropyl silica sol (ethanolic or aqueous) is suitable for biomedical uses due to its purity.

KEYWORDS

aminopropyl silica nanoparticles, surface modification, 3-aminopropyl(diethoxy)methylsilane, silylation in sol

INTRODUCTION

The surface chemistry of silica is a widely investigated subject for silica thin layers and nanoparticles are extensively used in nanotechnology and materials science [1, 2].

2

Nanomedicine is a new field of application for silica nanoparticles (nanocarriers) bringing forth very strict requirements of purity, stability and dispersity. It appears, however, that the surface modification of colloidal silica is still a challenging issue requiring careful consideration of reaction parameters (solvent, reagent, reaction time and temperature).

First, silica nanoparticles with a diameter smaller than 100 nm are more susceptible to form aggregates than larger ones because of the changes in the surface silanol structure. The increasing number of isolated silanol groups results in decreasing amount of hydrogen-bonded molecular water on the silica surface and a lower reactivity towards the silane coupling agents [3].

Another difficulty may arise from the reagent's characteristic. Commonly used silylating agents are (alkoxy)alkylsilanes that bear one to three reactive alkoxy (ethoxy or methoxy) groups and a functional side chain (e.g. aminopropyl-, cyanoethyl, vinyl-). The reactivity of the silane towards the substrate is influenced by the hydrolysis rate of the alkoxy groups [4-5], and the catalytic activity of the functional side chain [6-8]. The presence of other catalysts like water, acid or base usually fastens the rate of the hydrolysis and/or the condensation [9-13]. Depending on the number of reactive alkoxy groups and the presence of catalysts the condensation between silane molecules (bulk phase polycondensation) occurs in parallel with surface modification [14-15]. This polymerization may remain harmless and even undetected in case of the surface modification of a macroscopic substrate, but it may induce irreversible aggregation during the treatment of small nanoparticles.

Thirdly, the purification of surface-modified silica sols may also lead to aggregation. Centrifugation is widespreadly used for the purification of colloids after aminopropyl-functionalization reaction [16-19]. However, the interparticle distance is drastically decreased during centrifugation, which leads to irreversible aggregation if activated molecules – susceptible of polycondensation like alkoxy-silanes – are adsorbed at the surface of the

particles. Ultrafiltration allows the separation of the particles with a moderate increase in concentration. Dialysis, as an apparently ideal separation process, keeps the dilution constant. On the other hand, the time necessary for the separation – also important if reaction may proceed further – is multiple for dialysis in comparison to the two other methods. Therefore the purification process should be optimized together with the reaction parameters if aggregation is to be avoided.

Aggregation, as well as bulk phase polycondensation phenomena are ignored by most authors [14, 20]. Some researchers choose an anhydrous solvent in order to eliminate the effect of polycondensation (DMSO [21], toluene [22], THF [23]). (In this case the hydrolysis of the silylating reagent is hindered.) Therefore the medium of the original sol has to be exchanged. This must be the origin of procedures, where dried silica powders are used, though colloidal silica undergoes irreversible aggregation upon drying [24]. Solvent exchange by continuous distillation seemed to us a suitable alternative, but the stability of the sol could not be preserved during this procedure (in case of toluene).

Our purpose was to elaborate 20 nm diameter aminopropyl-functionalized silica nanoparticles for biomedicinal use. This work is the first step of tailoring new sensor nanoparticles that would be injected into freshly isolated brain slices, where extracellular space is smaller than 64 nm [25]. Hence we undertook the elaboration of an appropriate synthesis procedure, which we could not find in the literature. We used low water content organic solvent (3 V/V%) because water molecules at the surface of the silica nanoparticles play a crucial part in the covalent binding of the silylating agent [3]. Polar organic solvents are, at the same time, more suitable as reaction medium than pure water for keeping bulk phase polycondensation under control [2]. Finally, in view of medicinal application, the use of toxic organic solvents is in preference to be avoided. Ethanol seemed to us an eligible choice for it is the preparation

medium of Stöber silica sols, can be easily exchanged into water after the silylation and before further surface modifications, and is not toxic in low concentration.

We used the method of Stöber *et al.* for the preparation of native silica particles [26-27]. After examination of the hydrolysis/condensation properties of several 3-aminopropyl (alkoxy)alkylsilanes (3-aminopropyltriethoxysilane, 3-aminopropyl(diethoxy)methylsilane, 3-aminopropyltrimethoxysilane and 3-aminopropyl(dimethyl)methoxysilane) we chose 3-aminopropyl(diethoxy)methylsilane as the most appropriate for our purpose. An optimized surface modification procedure was established, and the advantages-disadvantages of three purification methods (centrifugation, ultrafiltration and dialysis) examined. Next, thorough analysis of surface modified silica sol was performed using transmission electron microscopy (TEM), small angle X-ray scattering (SAXS), infrared spectroscopy (FTIR), dynamic light scattering (DLS), pH and zeta potential measurements and ^{13}C and ^{29}Si CP-MAS-NMR. The dispersity and stability of the obtained nanoparticles were carefully analyzed during preparation, purification and solvent exchange (to water).

EXPERIMENTAL

Materials

Ethanol (a.r., 99.98%, max. 0.02% water, Reanal), ammonia solution (25%, a.r., Reanal), tetraethyl-ortho-silicate (TEOS, puriss. GC, Sigma-Aldrich), 3-aminopropyl (diethoxy)methylsilane (APDEMS, 97%, Aldrich) and glacial acetic acid (EMSURE, Merck) were used as received. TEOS and APDEMS were kept under argon. Deionized and bidistilled water were used during the experiments.

Preparation of silica sols

Silica sols were prepared according to Stöber et al. [26]. Briefly, 8.2 mL 25% ammonia solution was added to 250 mL ethanol at room temperature under vigorous stirring. After mixing for 5 min 10 mL of TEOS was quickly added and the reaction mixture was allowed to react for 24 hours. Ammonia was then evaporated at 60°C, but the dilution of the sol was carefully maintained. Sol samples were kept at 4°C until use.

Preparation of aminopropyl-functionalized silica sols

In a typical reaction 70 mL of sol (solid content of 14 mg/mL) was diluted with 70 mL of ethanol. 140 µL APDEMS was injected at 60°C and the reaction was stopped 10 minutes later by the addition of 38 µL glacial acetic acid. The as prepared samples were slightly opaque like the native silica sol, with no sign of aggregation or sedimentation for over a month. The separation of unreacted sylilating agent from the functionalized particles was achieved by centrifugation, ultrafiltration or dialysis.

Purification of aminopropyl-functionalized silica sols by centrifugation

20 mL of the aminopropyl-functionalized silica sol was divided into two equal volume fractions in centrifuging tubes. Sedimentation of the particles was achieved at 4500 rpm for 30 minutes. The supernatant still contained silica particles (laser light scattering was observed). The sediment was resuspended in 20 mL fresh ethanol containing 5.5 µl acetic acid and its silica content was measured on 3 times 2 mL samples.

Purification of aminopropyl-functionalized silica sols by ultrafiltration

20 mL of the aminopropyl-functionalized silica sol was poured into a Millipore solvent resistant stirred cell assembled with a polyethersulfone ultrafiltration disc (PBMK Biomax, NMWL of 300 kDa, Ø76 mm). The sol was concentrated to 10 mL by filtration at 2 bar

transmembrane pressure and then washed with 5 times 20 mL of ethanol containing 5.5 μl acetic acid. Finally, the volume of the retentate was adjusted to 20 mL, and its solid content was measured on 3 times 2 mL samples.

Purification of aminopropyl-functionalized silica sols by dialysis

20 mL of the aminopropyl-functionalized silica sol was filled in dialysis membrane tube (Sigma-Aldrich, dialysis tubing cellulose membrane, Ø76 mm, NMWL 12,400). The sample was purified against 3 times 300 mL ethanol containing 82 μl acetic acid (3 days). Thereafter, the solid content of the resulting sol was measured on 3 times 2 mL samples.

Characterization

The solid content of samples was determined on 3×2 mL aliquots of the sol dried at 120°C for 10 hours.

Morphological investigation of the sols was carried out on a MORGAGNI 268(D) (FEI, Eindhoven, Netherlands) transmission electron microscope. Diluted samples were dropped and dried on carbon coated copper grids.

The mean particle diameter and size distribution function of the samples were determined using dynamic light scattering (DLS) at 20 °C with a Malvern Zetasizer Nano ZS (Malvern, Worcs, UK) equipped with a He-Ne laser (λ = 633 nm) and a backscatter detector at fixed angle of 173°. The samples were used as obtained: 14 mg/ml native silica particles in ethanol at neutral pH, and nearly 7 mg/ml aminopropyl silica particles in ethanol containing 0.027 V/V% glacial acetic acid. The presented size distribution functions are intensity-weighted data. Zeta potential was measured also with Zetasizer Nano ZS. For these experiments the samples in ethanol were stabilized using 0.1 M HCl solution instead of glacial acetic acid. The particles were transferred into acidic water by dialysis. The pH of the samples was thereafter

adjusted manually for each measurement by using 0.1 M NaOH solution and a JENWAY 3540 Bench Combined Conductivity/pH Meter.

SAXS measurements were performed on beamline B1 [28] at HASYLAB at DESY (Hamburg, Germany). The samples (for details see above) were filled into quartz capillaries with 2 mm diameter (Hilgenberg Ltd., Germany). The energy of the incoming X-ray beam was set to 12 keV, and the 2D scattering patterns were collected with a Pilatus 1M hybride-pixel detector (Dectris Ltd., Switzerland) with an exposure time of 10 min. All the measurements were carried out at room temperature. The scattering patterns were corrected for background scattering, and the geometry of the experimental arrangement. The scattering curves were obtained by radial averaging of the patterns and were normalized to the primary beam intensity and corrected for transmission. Finally, the calibration of the curves to absolute units of macroscopic cross section (cm^{-1}) was performed by the use of a glassy carbon standard.

For data evaluation, the solid sphere model with log-normal size distribution was used [29]. The fitting procedures were performed using the SASfit program [30].

The used model function was the following:

$$I(q) = const \int_0^\infty P(R) \times \left(\frac{4R^3\pi}{3} \Delta\rho \frac{sin(qR) - qRcos(qR)}{(qR)^3} \right)^2 dR$$

where $\Delta\rho$ is the electron density contrast of the particles, and P(R) is the size distribution defined as

$$P(R) = \frac{1}{\sqrt{2\pi}} \frac{1}{\sigma R} \exp\left(-\frac{\ln^2 \frac{R}{R_0}}{2\sigma^2} \right)$$

where R_0 and σ is the mean and the width of the size distribution, respectively.

Surface modification of silica nanoparticles was demonstrated by attenuated total reflection infrared (ATR-FTIR) spectroscopy using a Varian Scimitar 2000 FTIR spectrometer (Varian Inc.) equipped with an MCT (mercury-cadmium-telluride) detector and a single reflection ATR unit (SPECAC "Golden Gate") with diamond ATR element. In general, 4 cm^{-1} resolution and records of 128 scans were applied.

The solid-state NMR measurements have been performed on dried silica samples after purification of the reaction mixture with dialysis. Solid-state ^{13}C, ^{29}Si NMR spectra were recorded on a 400 MHz Varian NMR SYSTEMTM using zirconia rotors in Varian/Chemagnetics narrow bore 4.0 mm HX double resonance MAS probe. Cross-polarization at the magic angle spinning (CP/MAS) was applied to enhance 13C and 29Si sensitivity. MAS rates of 7 kHz were chosen and proton high power decoupling (SPINAL scheme) was applied during 20 ms acquisition time. CP contact times of 0.4, 3 ms were applied for ^{13}C and ^{29}Si experiments, respectively. Repetition delays were 60 s in direct polarization (DP) and 5 s in cross-polarization (CP) experiments. Spectral windows of 78kHz were used in ^{13}C- and ^{29}Si-NMR. Carbon chemical shifts are referenced to adamantine (δ = 38.6, 29.5 ppm). ^{29}Si shifts are referred to tetramethylsilane (δ = 0 ppm).
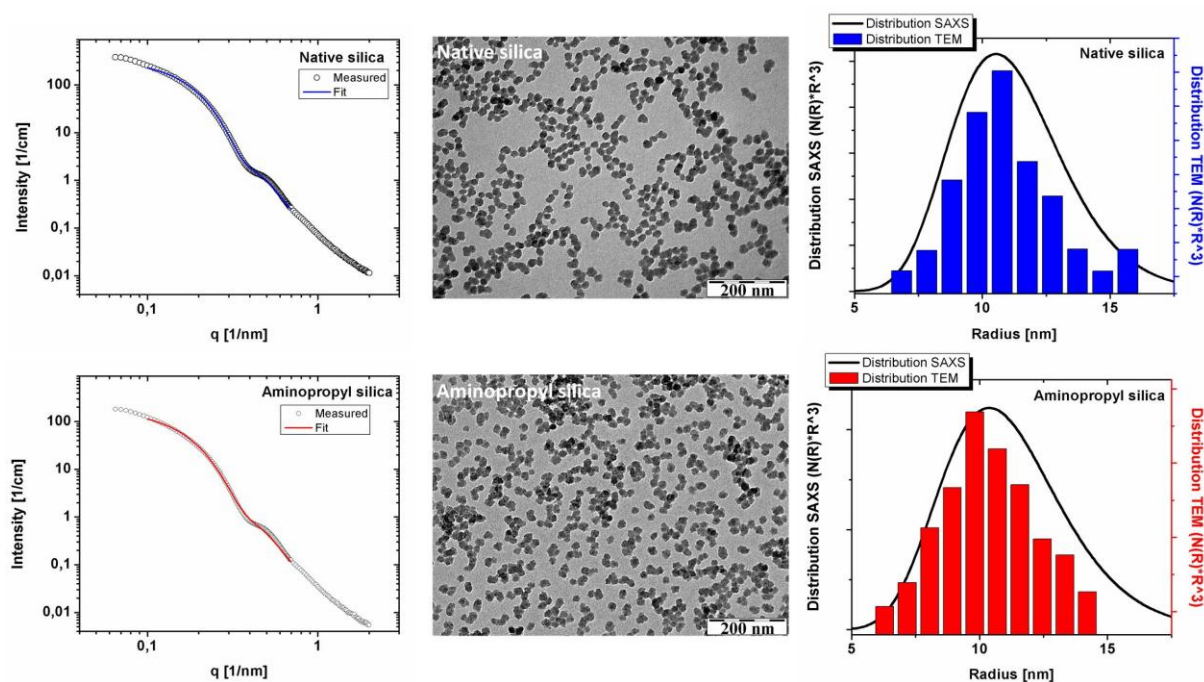
RESULTS AND DISCUSSION

Fig.1. SAXS and TEM analysis of native and aminopropyl silica nanoparticles

The aminopropyl-functionalized silica sol prepared by our new method and stabilized with acetic acid has a slightly opalescent aspect similar to that of the original silica sol. Its solid content is 7 mg/ mL. TEM images of the native and surface-modified sols (Fig.1) prove that polycondensation was successfully avoided at the chosen reaction parameters. However, larger amount of sylilating agent or longer reaction time leads to the appearance of polycondensed silica bridges between the nanoparticles and the sol becomes visibly more opaque.

SAXS is capable to characterize the size distribution and aggregation state of nanoparticles, hence it is widely applied to describe silica nanoparticles [31]. The SAXS curves of native and aminopropyl modified silica sols (purified by dialysis) reveal that both systems are monodisperse with average diameter around 20 nm based on the fits applying the solid sphere model with log-normal size distribution (Table 1). The latter is in good agreement with the results from TEM investigations (Fig.1). At high q-values ($q=(4\pi/\lambda)\sin\theta$, where λ is the wavelength of the used X-ray radiation and θ is the half of the scattering angle), the curves

deviate from that of the solid particles due to the porous structure of Stöber silica sols, while the low-q parts confirm that aggregates are not formed in the investigated systems.

On the other hand, the mean hydrodynamic diameter of the original sample increased by cca. 10 nm according to DLS measurement in consequence of the surface modification. (Fig.2). The increase of the hydrodynamic diameter indicates an enlarged Stern layer moving together with the nanoparticle (since the size of the „solid" part is unchanged according to SAXS investigation). This is concordant with the fact of surface modification.
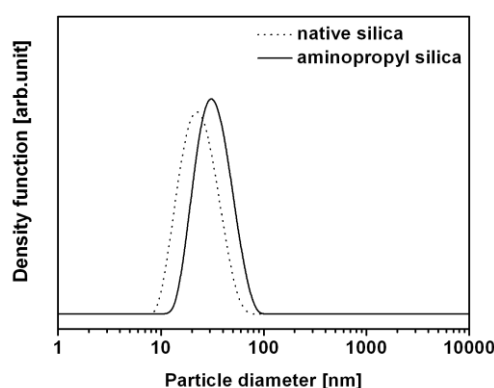
Fig.2. DLS analysis of native (Z-average: 21.6 nm, PdI: 0.122) and aminopropyl silica sols in ethanol (Z-average: 30.3 nm, PdI: 0.125, purified by dialysis)

According to the ATR-FTIR spectrum (Fig.3), the native silica obtained by the Stöber method contains some ethoxy groups on the surface proved by C-H stretching and bending vibrational bands around 3000-2800 and 1450-1350 cm^{-1}, respectively. After silylation, some minor changes can be witnessed in the spectrum of aminopropyl silica sol due to partial coverage of the silica nanoparticle surface with aminopropyl groups. The two broad bands at 3386 and 3260 cm^{-1}, and the band at 1583 cm^{-1} can be assigned to the N-H stretching vibration and to the NH_2 deformation mode of free amino groups, accordingly [32-33]. The covalent binding of the silylating agent onto the silica surface was confirmed by the changes in the spectral

features in the Si-O-Si stretching region from 1200 cm^{-1} to 900 cm^{-1}. The changes in intensity of the shoulder band around 1220 cm^{-1} (demonstrated by fitted Gaussian-Lorentz bands in Fig.3) can be related to changes in Si-O-Si bonding angle and implicit to the chemical bonding structure of the silica surface [34].
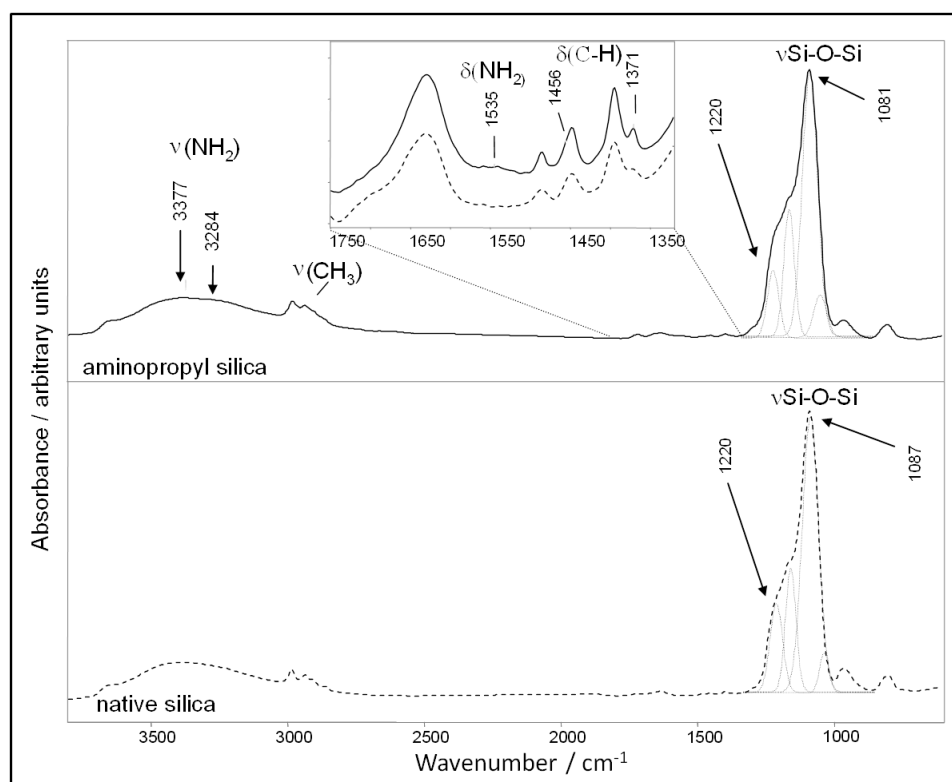

Fig.3. IR analysis of native and aminopropyl silica. In the offset spectra bands belonging to deformation modes of aminopropyl moieties are depicted. Dotted lines represent the fitted bands of Si-O-Si stretching in the region of 1250-1000 cm^{-1}.

We used the combination of solid-state ^{13}C- and ^{29}Si-MAS NMR spectroscopy to verify the covalent surface modification of the bulk dried aminopropyl silica samples. Figures 4-5 show the spectra acquired in these investigations. In ^{13}C-CP/MAS (Fig.4) the signals of the surface modifier are visible only. The resonances of the C–H groups in the aliphatic chemical shift region (δ 10–70 ppm) appear well resolved. They were readily identified according to their

characteristic chemical shifts. Direct polarization (DP) proton decoupled [29]Si-MAS and ^{1}H–^{29}Si-CP/MAS NMR provides information about the polysiloxane network of the dried silica (Fig.5). The DP experiment verifies the [29]Si resonance of the bulk silica [Q4] Si(IV)-O(4) at δ −110 ppm. Enchanced by the CP experiment peaks centered at (δ −18, −91, −100 ppm) are close to hydrogens and are assigned to the [T2] $H_{2-3}C(2)$–Si(IV)–O(2) and [Q2], [Q3] HO–Si(IV)–O(2–3) silicon sites [35]. According to the DP experiment the extent of surface modification is less than 1mol%.
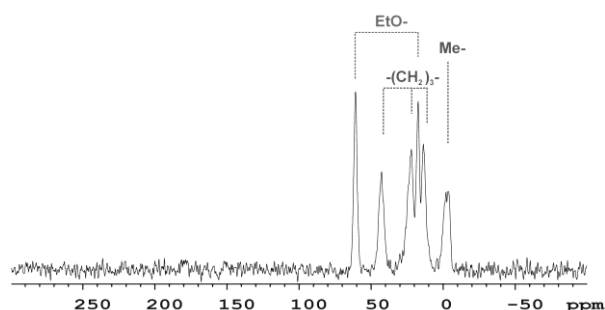

Fig.4. ^{13}C-CP/MAS NMR (100.56 MHz) experiment of dried aminopropyl silica nanoparticles
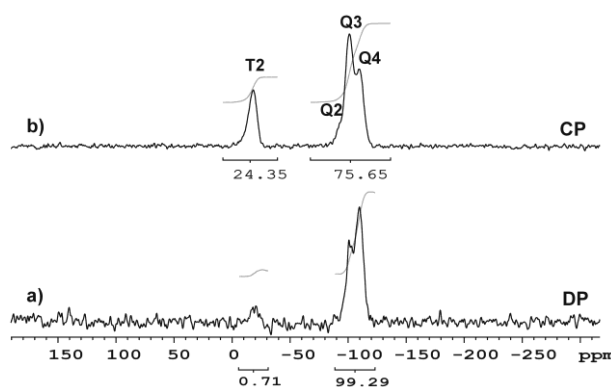

Fig.5. a) direct polarization and b) cross-polarization ^{29}Si-NMR (79.45 MHz) experiments of dried aminopropyl silica nanoparticles

Stabilization of the reaction mixture

13

Our preliminary experiments pointed out that the silylation reaction proceeding further at room temperature – as well as at 4°C – leads inevitably to the aggregation of silica nanoparticles during the purification step (either centrifugation, ultrafiltration or dialysis).

According to the literature, the addition of an acid decelerates polymerization because the protonated form of the silylating molecule (quaterner ammonium ion) is inactive towards the surface silanol groups – its catalytic effect is hindered [2]. It has been proven by Naviroj et al. that only a moderate surface modification of silica could be achieved in the presence of hydrochloric acid [36].

We observed, however, that the addition of acetic acid maintains the *status quo*: the surface modification reaction (as well as bulk phase polycondensation) is stopped and the dispersity of the sol preserved (through the protonation of surface aminopropyl groups). This fact has not been described in the literature before.

Size distribution functions of DLS measurements indicate that the stability of the aminopropyl-functionalized silica sol is affected by the quantity of glacial acetic acid added to it (Fig.6). Beyond 1 molar equivalent of the silylating agent no aggregation occurs within 30 minutes after the reaction has been stopped. The addition of 1 equivalent acetic acid stabilizes the reaction mixture as long as several weeks hence separation of unreacted silylating molecules can be realized under quasi-stationary conditions. The chosen concentration of acetic acid is maintained during all types of purification processes: 0.027 V/V% glacial acetic acid is added to the fresh solvent (ethanol or water) used. Repeated DLS analysis does not detect any change in the particle size and size distribution function during the 3 months period.
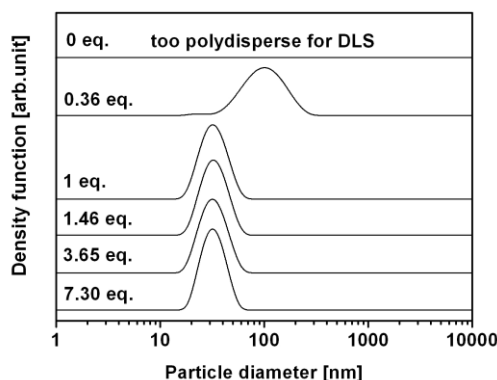
Fig.6. DLS size distribution function of the reaction mixture after addition of 0-7.3 molar equivalent of acetic acid (related to APDEMS). Measurements were carried out 15 minutes after the addition of acetic acid. (0.36 eq.: Z-average: 88.61 nm, PdI: 0.161, 1 eq.: Z-average: 31.28 nm, PdI: 0.051, 1.46 eq.: Z-average: 31.76 nm, PdI: 0.049, 3.65 eq.: Z-average: 31.05 nm, PdI: 0.055, 7.30 eq.: Z-average: 31.25 nm, PdI: 0.049)

Comparison of purification techniques

The dispersity of the final sol depends strongly on the purification technique applied (Fig.7). Centrifugation and redispersion leads to the appearance of large aggregates (see curve 'centrifuged II' of the sediment after redispersion in Fig.7). In effect sedimentation of 20 nm diameter aminopropyl silica particles is still incomplete after 30 minutes of centrifugation because of small particles' slow sedimentation rate (see curve 'centrifuged I', the size distribution function of the supernatant in Fig.7). During this time condensation occurs between the remaining silanol groups at the surface of the particles causing an irreversible aggregation. The solid content of the sol sample purified by centrifugation and diluted to its original volume is 1.8 mg/mL. This indicates a net recovery of 26% (recovery values are evaluated on 20 mL of samples).

Ultrafiltration is found to be an effective method of purification for our reaction mixture. It is of moderate time and solvent consumption and only small quantity of aggregates is formed

according to DLS study. The net recovery of this purification method is 54% (solid content of 3.75 mg/mL). The membrane fouling, however, becomes significant at larger amount of sample (50 mL for our experimental arrangement) bringing forth a markedly increased separation time. For larger samples, therefore, another separation method is required.

Dialysis, though time and solvent consuming, is an optimal separation method for this silylation reaction mixture, when its volume exceeds 50 mL. A net recovery of nearly 100% is obtained (solid content of 7.0 mg/mL) and no sign of aggregation is detected.
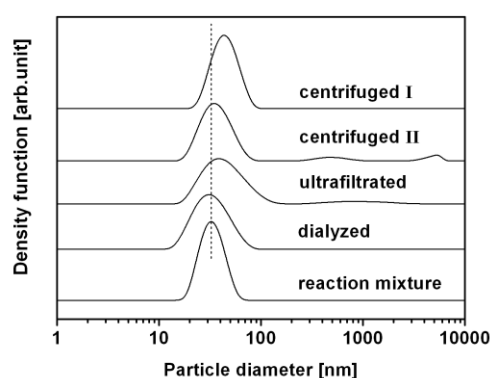

Fig.7. Comparison of size distribution functions after various purification processes. Reaction mixture (Z-average: 32.01 nm, PdI: 0.044) was divided into three portions and centrifugation (I – supernatant (not ultrasonicated, Z-average: 42.35 nm, PdI: 0.062) II – sediment (ultrasonicated, Z-average: 39.75 nm, PdI: 0.290) redispersed in original volume of ethanol), ultrafiltration (Z-average: 41.80 nm, PdI: 0.216) and dialysis (Z-average: 30.32 nm, PdI: 0.125) were carried out.

Aminopropyl silica sol in water

The solvent exchange from ethanol to water is performed during the purification of the reaction mixture by either ultrafiltration or dialysis. Distilled water containing acetic acid is used to replace the alcoholic medium. The obtained sol is transparent; TEM and DLS analysis

do not show significant aggregation (Fig.8). DLS study gives a mean particle diameter of 37.4 nm. The aqueous aminopropyl silica sol is stable at 4ºC for at least three months.
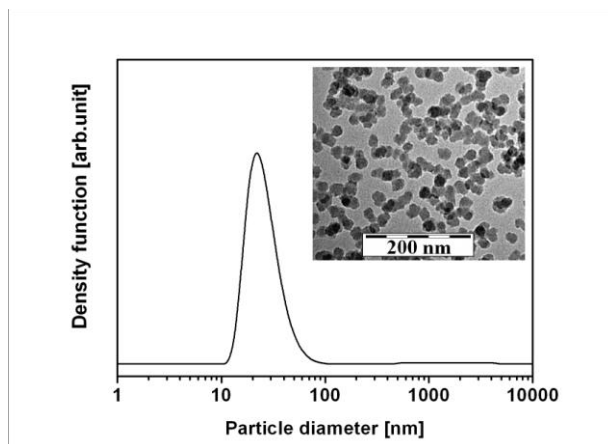

Fig.8. DLS and TEM analysis of aminopropyl silica in water (Z-average: 37.40 nm, PdI: 0.239)
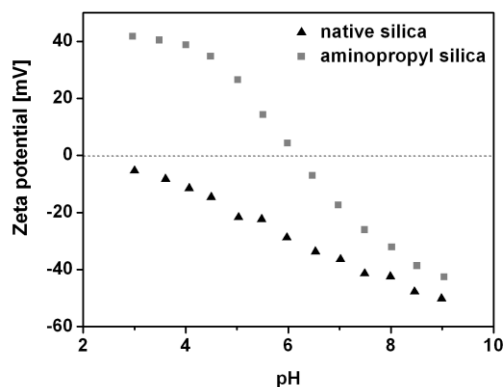

Fig.9. Zeta potential of native and aminopropyl silica as a function of pH. The pH of native silica and aminopropyl silica was set to 3 with 0.1 M HCl during solvent exchange into water. 0.1 M NaOH solution was used for titration.

Fig.9. shows the zeta potential (surface charge) of the native and the aminopropyl modified silica nanoparticles as a function of pH. The surface charge of native silica nanoparticles

becomes more and more negative by increasing the pH due to the dissociation of surface silanol groups in agreement with previous measurements [37-39]. The surface charge of the aminopropyl-modified silica nanoparticles becomes positive below pH 6.2 (isoelectric point) due to the protonation of surface amino groups. This value is, however, lower than was found previously in the literature for 150 nm diameter silica nanoparticles prepared from 3-aminopropyl-triethoxysilane [40]. We assume that this difference is mainly due to the incomplete surface modification of our silica nanoparticles. The inversion of the surface charge indicates, on the other hand, that the major part of the original silanol groups has been converted into aminopropyl groups.

4. CONCLUSIONS

A new synthesis method has been developed for the preparation of very small (20 nm diameter) aminopropyl silica nanoparticles destined for biomedicinal use. Original silanol groups on the native Stöber silica surfaces were partially converted into aminopropyl groups, and the covalent binding was proven by FTIR and NMR investigations. The dispersity of the original sol was maintained during the preparation and purification (ultrafiltration or dialysis) process according to combined TEM-SAXS and DLS study.

It is an important fact that the silylation reaction making use of a divalent silylating agent has become controllable by the addition of acetic acid after appropriate reaction time [2, 14, 15]. The elaborated process is furthermore suitable for scaling up (potential use in biomedicine): it is a one-bottle synthesis method, where no solvent exchange is needed between the preparation of the native sol and the surface modification reaction (avoidance of spontaneous aggregation of silica nanoparticles [20]). It has been proven that centrifugation is not a convenient separation technique for such small nanoparticles [16-19], while either

ultrafiltration or dialysis preserve the dispersity of the sol sample even during solvent exchange to water.

Nanoparticles prepared by our simple, cheap and "green" (environmentally friendly) method meet the requirements of most delicate applications in a biological medium. Our future purpose will be to continue the surface modification of the presented aminopropyl silica to obtain bifunctional silica nanoparticles and, in a further step, elaborate sensor nanoparticles [41].

REFERENCES

[1] R.K. Iler, The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry; John Wiley and Sons, Inc., New York, 1979.

[2] E.F. Vansant, P. Van Der Voort, K.C. Vrancken, Characterization and Chemical Modification of the Silica Surface, Elsevier, Amsterdam, 1995.

[3] M. Iijima, M. Tsukada, H. Kamiya, J. Colloid Interface Sci. 307 (2007) 418.

[4] N. K. Pham, D. Fullston, K. Sagoe-Crentsil, Aust. J. Chem. 60 (2007) 662.

[5] M-C.B. Salon, M. Bardet, M.N. Belgacem, Silicon Chem. 3 (2008) 335.

[6] K.C. Vrancken, K. Possemiers, P. Van Der Voort, E. F. Vansant, Colloids Surf. A 98 (1995) 235.

[7] H. Salmio, D. Brühwiler, J. Phys. Chem. C 111 (2007) 923.

[8] S. Dire, V. Tagliazucca, E. Callone, A. Quaranta, Mater. Chem. Phys. 126 (2011) 909.

[9] W. Yoshida, R.P. Castro, J-D. Jou, Y. Cohen, Langmuir 17 (2001) 5882.

[10] I.A. Rahman, M. Jafarzadeh C.S. Sipaut, Ceram. Int. 35 (2009) 1883.

[11] M. Marini, B. Pourabbas, F. Pilati, P. Fabbri, Colloids Surf. A 317 (2008) 473.

[12] H. Du, P.D. Hamilton, M.A. Reilly, A. d'Avignon, P. Biswas, N. Ravi, J. Colloid Interface Sci. 340 (2009) 202.

[13] I.J. Bruce, T. Sen, Langmuir 21 (2005) 7029.

[14] A. Campo, T. Sen, J-P. Lellouche, I.J. Bruce, J. Magn. Magn. Mater. 293 (2005) 33.

[15] W. Yoshida, R.P. Castro, J-D. Jou, Y. Cohen, Langmuir 17 (2001) 5882.

[16] P. Vejayakumaran, I.A. Rahman, C.S. Sipaut, J. Ismail, C.K. Chee, J. Colloid Interface Sci. 328 (2008) 81.

[17] F. Branda, B. Silvestri, G. Luciani, A. Costantini, F. Tescione, Colloids Surf. A 367 (2010) 12.

[18] M-L. Ho, J-M. Hsieh, C-W. Lai, H-C. Peng, C-C. Kang, I-C. Wu, C-H. Lai, Y-C. Chen, P-T. Chou, J. Phys. Chem. C 113 (2009) 1686.

[19] G. Shen, A. Horgan, R. Levicky, Colloids Surf. B 35 (2004) 59.

[20] L. Yuan, Q. Tang, D. Yang, J.Z. Zhang, F. Zhang, J. Hu, J. Phys. Chem. C 115 (2011) 9926.

[21] L. Ye, R. Pelton, M.A. Brook, Langmuir 23 (2007) 5630.

[22] A. Penkova, M.J.M. Blanes, S.A. Cruz, M.A. Centeno, K. Hadjiivanov, J.A. Odriozola, Microporous Mesoporous Mater. 117 (2009) 530.

[23] J-F. Lin, W-B. Wang, C-C. Ho, J-H. Jou, Y-F. Chen, W-F. Su, J. Phys. Chem. C 116 (2012) 1955.

[24] J. Depasse, A. Watillon, J. Colloid Interface Sci. 33 (1970) 430.

[25] R.G. Thorne, C. Nicholson, Proc. Natl. Acad. Sci. USA 103 (2006) 5567.

[26] W. Stöber, A. Fink, E. Bohn, J. Colloid Interface Sci. 26 (1968) 62.

[27] A. Deák, B. Bancsi, A.L. Tóth, A.L. Kovács, Z. Hórvölgyi, Colloids Surf. A 278 (2006) 10.

[28] H.G. Haubold, K. Gruenhagen, M. Wagener, H. Jungbluth, H. Heer, A. Pfeil, H. Rongen, G. Brandenberg, R. Moeller, J. Matzerath, P. Hiller, H. Halling, Rev. Sci. Instrum. 60 (1989) 1943.

[29] A. Guinier, G. Fournet, Small.Angle Scattering of X-Rays, John Wiley & Sons, Inc., New York, 1955.

[30] J. Kohlbrecher, User guide for the SASfit software package, http://kur.web.psi.ch/sans1/SANSSoft/sasfit.html

[31] D.L. Greena, J.S. Linb, Y-F. Lamc, M.Z-C. Hud, D.W. Schaefere, M.T. Harris, J. Colloid Interface Sci. 266 (2003) 346.

[32] G. Socrates, Infrared and Raman Characteristic Group Frequencies, John Wiley & Sons, Inc., New York, 2004.

[33] Ch. Weigel, R. Kellner, FreseniusZ. Anal. Chem. 335 (1989) 663.

[34] N. Nagai, H. Hashimoto, Appl. Surf. Sci. 172 (2001) 307.

[35] I-S. Chuang, G.E. Maciel, J. Phys. Chem. B 101 (1997) 3052.

[36] S. Naviroj, S.R. Culler, J.L. Koenig, H.Ishida, J. Colloid Interface Sci. 97 (1984) 308.

[37] C.H. Lee, S.H. Park, W. Chung, J.Y. Kim, S.H. Kim, Colloids Surf. A 384 (2011) 318.

[38] S-C. Shen, W.K. Ng, L. Chia, Y-C. Dong, B.H.R. Tan, Mater. Res. Bull. 46 (2011) 1665.

[39] C. Kneuer, M. Sameti, E.G. Haltner, T. Schiestel, H. Schirra, H. Schmidt, C-M. Lehr, Int. J. Pharm. 196 (2000) 257.

[40] Z. Wu, H. Xiang, T. Kim, M-S. Chun, K. Lee, J. Colloid Interface Sci. 304 (2006) 119.

[41] Patent WO/2010/007451