# Total synthesis of isotopically enriched Si-29 silica NPs as potential spikes for isotope dilution quantification of natural silica NPs

Marcell Pálmai [a,b], Roland Szalay [c], Dorota Bartczak [b], Zoltán Varga [a,*], Lívia Naszályi Nagy [a], Christian Gollwitzer [d], Michael Krumrey [d], Heidi Goenaga-Infante [b]

[a] Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences (IMEC RCNS HAS), H-1117 Budapest, Magyar Tudósok körútja 2., Hungary

[b] LGC Limited, Queens Road, Teddington, Middlesex, TW11 0LY, UK

[c] Institute of Chemistry, Eötvös Loránd University, H-1117 Budapest, Pázmány Péter sétány 1/A., Hungary

[d] Physikalisch-Technische Bundesanstalt (PTB), Abbestr. 2-12, 10587 Berlin, Germany

[*] Corresponding author: Tel.: +36-1-382-6833, Fax: +36-1-382-6427,

varga.zoltan@ttk.mta.hu

**Abstract**

A new method was developed for the preparation of highly monodisperse isotopically enriched Si-29 silica nanoparticles ($^{29}$Si-silica NPs) with the purpose of using them as spikes for isotope dilution mass spectrometry (IDMS) quantification of silica NPs with natural isotopic distribution. Si-29 tetraethyl orthosilicate ($^{29}$Si-TEOS), the silica precursor was prepared in two steps starting from elementary silicon-29 pellets. In the first step Si-29 silicon tetrachloride ($^{29}$SiCl$_4$) was prepared by heating elementary silicon-29 in chlorine gas stream. By using a multistep cooling system and the dilution of the volatile and moisture-sensitive $^{29}$SiCl$_4$ in carbon tetrachloride as inert medium we managed to reduce product loss caused by evaporation. $^{29}$Si-TEOS was obtained by treating $^{29}$SiCl$_4$ with absolute ethanol. Structural characterisation of $^{29}$Si-TEOS was performed by using $^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopy and Fourier-transform infrared (FTIR) spectroscopy. For the NP preparation, a basic amino acid catalysis route was used and the resulting NPs were analysed using transmission electron microscopy (TEM), small angle X-ray scattering (SAXS), dynamic light scattering (DLS) and zeta potential measurements. Finally, the feasibility of using enriched NPs for on-line field-flow fractionation coupled with multi-angle light scattering and inductively coupled plasma mass spectrometry (FFF/MALS/ICP-MS) has been demonstrated.

# 1. Introduction

Following changes in regulation and standardisation related to the use and commercialisation of nanomaterials, mostly in cosmetic and food industries (e.g. Regulations (EC) No 1223/2009 and No 178/2002 of the European Parliament), the demand for robust approaches to NPs characterisation in a complex matrix is growing rapidly. Colloidal silica (E551) is widely used as surfactant in different consumer products including wines, juices, toothpastes etc. The negatively charged silica particles electrostatically bind to positively charged proteins and facilitate the formation of flocs which float to the top (creaming) or settle to the bottom (sedimentation).

The large-scale usage of colloidal silica creates distinct sources for potential human exposure for decades. Therefore, investigations focusing on the characterisation of silica NPs in different complex matrices (e.g. biological systems) are urgently needed. In the last few years the popularity of on-line field-flow fractionation (FFF) coupled with inductively coupled plasma mass spectrometry (ICP-MS) in the characterisation of various nanomaterials has increased significantly [1-8]. FFF-ICP-MS allows NP sizing, size-based elemental speciation and quantification of the nano-sized fraction in the presence of complex matrix components. Recently, Menendez-Miranda and co-workers used asymmetric flow FFF (AF4) coupled on-line to fluorescence and ICP-MS detectors for the separation and identification of quantum-dot populations (polymer-coated CdSe/ZnS) of different composition [1]. They managed to provide a time-resolved nanosize separation of the eventual species present in the heterogeneous nanoparticle suspensions. Hoque et al. developed and applied an analytical method to determine the size and concentration of 1 to 80 nm sized Ag NPs in wastewater samples [5].

In analytical chemistry and biochemistry, where high accuracy is required, isotope dilution mass spectrometry (IDMS) is employed [9, 10]. Elements with two or more stable isotopes

may be quantified by IDMS, which uses an artificially enriched isotope (called spike) as an internal standard. So far its application in the field of nanotechnology has been very limited due to the lack of isotopically enriched nanomaterials. Isotope dilution based methodology is needed for the certification of materials and standards, as well as for the provision of reference values to intercomparison studies.

In this paper we describe the synthesis and characterisation of isotopically enriched like-for-like [29]Si-silica NPs starting from elementary silicon-29 pellets. The total synthesis of the precursor was necessary since [29]Si-TEOS is not commercially available. For the NP preparation, a basic amino acid catalysis route was used to get particles with narrow size distribution [11]. Finally, the morphology, size distribution and surface charge of the synthesized NPs were characterised for using transmission electron microscopy (TEM), small angle X-ray scattering (SAXS), dynamic light scattering (DLS), zeta potential measurements and on-line field-flow fractionation coupled with multi-angle light scattering and inductively coupled plasma mass spectrometry (FFF/MALS/ICP-MS).

## 2. Experimental

### 2.1. Materials

Silicon-29 (99.73 % isotopic enrichment, STB Isotope), chlorine gas (in cylinder, 99.8 %, Linde), ethanol (a.r., 99.98 %, max. 0.02 % water, Reanal), pentane (reagent grade, 98 %, Sigma-Aldrich), L-arginine (reagent grade, ≥98 %, TLC, Sigma-Aldrich) and cyclohexane (G.R., 99.99 %, Lach-Ner) were used as received. For the preparation of [29]Si-TEOS carbon tetrachloride (a.r., Reanal) and triethylamine (puriss., 99 %, Reanal) were refluxed on calcium hydride (95+ %, Aldrich), and then distilled prior to use. High purity deionized water (18.2 MΩ·cm) was used during the particle preparation.

### 2.2. Preparation of [29]Si-TEOS

$^{29}$SiCl$_4$ was prepared in a quartz reactor tube (0.8 cm i.d.), equipped with a gas inlet, and attached to the top joint of a water-cooled condenser via a bent glass tube. The above water-cooled condenser and a second coolant-jacketed (working at -18 °C) condenser were fitted to each neck of a two-neck flask. 15 ml of carbon tetrachloride and a magnetic bar were placed into the flask. All subsequent operations were carried out under moisture-free conditions. Then Si-29 pellets (1.0 g) were heated by a Bunsen burner under continuous stream of chlorine gas (dried with cc. sulfuric acid) so as to maintain a red-yellow glowing. The $^{29}$SiCl$_4$ formed was condensed and collected into the ice-cooled flask containing CCl$_4$. After all Si-29 pellets have been burnt up (ca. ½ h), the excess of chlorine was purged out of the solution by heating at 55 °C under a slow inert gas stream. After the disappearance of the yellowish colour of dissolved chlorine, the $^{29}$SiCl$_4$/CCl$_4$ solution was transferred to a dropping funel, and added dropwise (ca. 1 h) into a two-neck flask containing a mixture of 10 mL of ethanol and 10 ml of CCl$_4$. The reaction mixture was stirred for another 1 h and HCl purged out by argon was trapped in two successive gas-washing bottles filled with known volumes of 2 M NaOH. At the end of the reaction, the NaOH solutions were unified and back-titrated in order to estimate the residual acidity. Hydrogen chloride dissolved in the reaction mixture was scavenged by slow addition of a 1:1 (*V/V*) mixture of triethylamine and CCl$_4$ (ca. 5 mL) until the medium turned into basic. The voluminous precipitation of triethylammonium chloride formed upon neutralization was filtered off, the volatiles were removed *in vacuo*, and then the residue was taken up in 10 mL of *n*-pentane. After filtration and evaporation, the purity of the product allowed the preparation of NPs without further purification step. Yield: 5.0 g (69%). $^1$H NMR ($\delta$/ppm in TMS/CDCl$_3$ solution): 1.24 (t, 12 C$\underline{H}_3$), 3.86 (d-q, 8 C$\underline{H}_2$); $^{13}$C NMR: 18.1 (d, $\underline{C}$H$_3$), 59.2 (s, $\underline{C}$H$_2$). NMR heteronuclear coupling constants: $^3J(^{29}$Si$^1$H$) = 3.0$ Hz, $^3J(^{29}$Si$^{13}$C$) = 2.4$ Hz. IR: 3000-2800 $\upsilon$(C-H), 1500-1250 $\delta$(C-H), 1071 $\upsilon_a$(SiO-C), 780 $\upsilon$(Si-O) cm$^{-1}$.

## 2.3. Preparation of [29]Si-silica NPs

Silica NPs with nearly 20 nm average particle diameters were synthesized according to Hartlen et al. in a 20 ml scintillation vial (VWR Scientific) [11]. Briefly, 9.1 mg of L-arginine was added to 6.9 mL of water at room temperature under vigorous stirring (300 rpm). Then 0.45 mL of cyclohexane was overlayered on the arginine solution and the reaction mixture was heated to 60 °C. 0.55 mL of [29]Si-TEOS was injected into the cyclohexane phase and it was allowed to react for 20 h. Finally cyclohexane was removed under reduced pressure.

## 2.4. Characterisation

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 250 MHz (for protons) NMR spectrometer.

IR absorption spectrum was recorded using a Varian Scimitar 2000 FTIR spectrometer (Varian Inc.) equipped with an MCT (mercury-cadmium-telluride) detector and a single reflection attenuated total reflection (ATR) unit (SPECAC "Golden Gate") with diamond ATR element. In general, 4 cm$^{-1}$ resolution and records of 128 scans were applied.

Morphological investigations of the NPs were carried out on a MORGAGNI 268(D) (FEI, Eindhoven, Netherlands) transmission electron microscope. Diluted sample was dropped and dried on a carbon coated copper grid.

SAXS measurements were performed at the four-crystal monochromator beamline of Physikalisch-Technische Bundesanstalt supplemented by the SAXS setup of Helmholtz-Zentrum Berlin at the synchrotron radiation facility BESSY II (Helmholtz-Zentrum Berlin, Germany) [12, 13]. The sample-to-detector distance was 2.2 m, the X-ray energy was set to 8 keV, and the sample was filled into borosilicate glass capillary with 1.0 mm nominal diameter (Müller & Müller OHG, Berlin, Germany). 2D scattering patterns were collected

with a vacuum-compatible large-area pixel detector Pilatus 1M (Dectris Ltd., Baden, Switzerland) [14]. The scattering curves were obtained by radial averaging of the 2D patterns, and the size distribution was calculated by non-linear least squares fitting of the form factor of a homogeneous sphere and taking into account the polydispersity by a Gaussian size distribution [15].

DLS measurements were performed using a W130i Dynamic Light Scattering System (High Wycombe, UK). Low volume disposable plastic cuvette was used for the DLS measurements (UVette, Eppendorf Austria GmbH, Austria), and data evaluation was performed using the iSize 2.0 software. Volume weighted distributions were used to characterize the NP sample.

Zeta potential measurements were performed on a Malvern Zetasizer Nano ZS (Malvern, Worcs, UK) equipped with He-Ne laser ($\lambda$=633 nm) and backscatter detector at fixed angle of 173°. The pH of the sample was measured using a JENWAY 3540 Bench Combined Conductivity/pH Meter.

Asymmetric flow field flow fractionation was applied using a metal-free AF2000 MT system (Postnova, Landsberg, Germany). A regenerated cellulose (RC) membrane was used with 10 kDa molecular weight cut off and a spacer of 350 mm. Milli-Q water (filtered through 0.1 mm membrane) was used as the FFF carrier. Manual injection was performed using a 21.8 µL loop and 4 minutes focusing time (tip flow 0.2 mL min$^{-1}$, focus flow 1.5 mL min$^{-1}$). The samples were vortexed for 30 s prior to injection to ensure homogeneous distribution of suspended particles. A constant cross-flow of 2 mL min$^{-1}$ was applied and the flow rate to the detector was kept at 0.5 mL min$^{-1}$. The fractograms are shown including the focus step to prove that there is no silica eluting during this step. The eluate from the channel was mixed via a t-piece with an acidic solution containing Ge as internal standard prior to it entering the ICP-MS equipped with a Micromist nebuliser and Scott spray chamber. The internal standard was used to correct instrumental drift. Analysis of Si-29 silica NPs was performed with post-

channel addition of 2.5% (v/v) nitric acid in $H_2$-mode monitoring $^{28}Si$, $^{29}Si$, and $^{72}Ge$ using an ICP-MS Agilent 7700 collision cell instrument (Agilent Technologies, Japan).

## 3. Results and discussion

To our best knowledge, the synthesis of pure $^{29}Si$-labelled silicon tetrachloride, tetraalkoxyde and silica NPs has not yet been published. The preparation of $^{29}Si$-TEOS was performed in a two-step synthesis as a modified combination of conventional methods described earlier in literature for isotopically non-enriched TEOS [16, 17]. First, the elementary silicon-29 was converted to its tetrachloride upon heating in chlorine gas, and then treated with abs. ethanol to give the silane ester.

The conventional synthetic methods mentioned in the literature are described for the preparation of much larger quantities (100-500 g), wherein the product loss caused by evaporation is negligible. We aimed to optimize the synthetic method for 1 g of $^{29}Si$ pellets as starting material. Preliminary experiments conducted on native Si powder have shown that only low over-all yields can be obtained (30%) on the small scale. We meant to ameliorate this result before the use of the high-cost isotopically enriched silicon-29. For this purpose we performed three modifications over the traditional synthetic methods of $SiCl_4$ and TEOS in an easy-to-build laboratory equipment (Fig. 1).

One of them included the dilution of the volatile ($SiCl_4$; b.p. 57.65 °C) and moisture-sensitive $^{29}SiCl_4$ in an inert medium thus increasing the sample volume and decreasing the product loss due to evaporation. Carbon tetrachloride (b.p. 76.72 °C) was our choice as solvent exhibiting chemical inertness, the required boiling pont, and the unlimited miscibility with silicon tetrachloride.

The second modification was the use of a multistep (+12 °C, -12 °C, -18 °C) cooling system in order to inhibit the volatilization of $^{29}$SiCl$_4$/CCl$_4$ solution caused by gas stream and, simultaneously, prevent too much chlorine gas to get absorbed in the mixture.

The third change was the use of triethylamine as a proton scavenger to neutralize residual hydrogen chloride dissolved in the mixture after ethanolysis. In the literature the strongly basic sodium ethoxide is used commonly for this purpose. To avoid excessive basification the use of a pH indicator (e.g. congo red) is recommended, however, after the neutralization and filtrating off the sodium chloride as by-product, the removal of the indicator necessitates an extra purification step. In our approach triethylamine was selected as a base which forms insoluble ammonium chloride salt with HCl. In order to avoid the large excess of triethylamine, when all the SiCl$_4$/CCl$_4$ was added to the ethanol/CCl$_4$ mixture, the system was heated up under continued argon stream to purge out the dissolved HCl into a known volume of 2 M NaOH solution. The result of the back-titration of NaOH yielded the amount of HCl actually flushed out, and only the remainder was neutralized in the reaction mixture (with 10% of triethylamine excess). After filtration and evaporation of volatiles (CCl$_4$, EtOH, triethylamine), the precipitation of traces of the ammonium salt could be readily accomplished by taking up the raw product in some n-pentane. With these simple modifications we managed to synthesise $^{29}$Si-TEOS with good over-all yield (69 %). The as-prepared $^{29}$Si-TEOS was used without further purification (i.e. distillation) and we found that it was suitable for the preparation of silica NPs.

TEM image proves that silica NPs synthesized from $^{29}$Si-TEOS are well-dispersed (Fig. 2). The size distribution evaluated from SAXS measurement has a mean value of 19.44 nm with a standard uncertainty of 0.21 nm (k=1). The full width at half maximum of the distribution is 4.95 nm with a standard uncertainty of 0.52 nm (k=1), which indicates a highly monodisperse nanoparticle system. The latter is in good agreement with the results from TEM and DLS

investigations (Fig. 3). The zeta potential (surface charge) of the obtained particles is -40.9 mV (pH 9.2) which corresponds to good colloidal stability.

Preliminary experiments were performed by FFF/MALS/ICP-MS to investigate the applicability of the $^{29}$Si-silica NPs as spikes for IDMS. Fig. 4 shows the FFF-ICP-MS fractograms of the (A) natural (prepared by same method using isotopically non-enriched TEOS) and (B) $^{29}$Si-silica NPs and demonstrates that in both cases the NPs are eluting in one well resolved peak. We observed a slight difference in the elution times that we attribute to the slight difference in particles size, variations in the membrane condition and channel back pressure. Fig. 4 (A) shows a small intensity $^{29}$Si peak according to natural abundance of silicon isotopes ($^{28}$Si 92.23 %, $^{29}$Si 4.67 %, $^{30}$Si 3.1 %, $^{32}$Si trace). The fractogram of the spike NPs (Fig. 4 (B)) demonstrates the predominance of Si-29 in the spike NPs. The similarity between natural (non-enriched) and spike NPs will be investigated in a further work.


## 4. Conclusions

In summary, a simple method has been developed for the synthesis of $^{29}$SiCl$_4$ and $^{29}$Si-TEOS with good over-all yield (69 %). The synthesized $^{29}$Si-TEOS was suitable for the preparation of silica NPs by a basic amino acid catalysis route [11]. Other synthesis routes (e.g. Stöber) are also presumed to yield silica NPs under identical conditions than for native TEOS [18]. The particles prepared by our method will be invaluable for use as internal standards in applications such as the development of reference methodology (e.g. IDMS) for the accurate quantification of particles with natural isotopic composition. Although the development of reference methodology (e.g. based on IDMS quantitation) is out of the scope of this paper, it can be anticipated that such methodology will be invaluable for the characterisation of reference materials and the development of traceable methods for particle counting (e.g.

based on single particle ICP-MS). Therefore, ongoing studies in our laboratory are fully exploring the potential of the developed spikes for IDMS method development in complex matrices.

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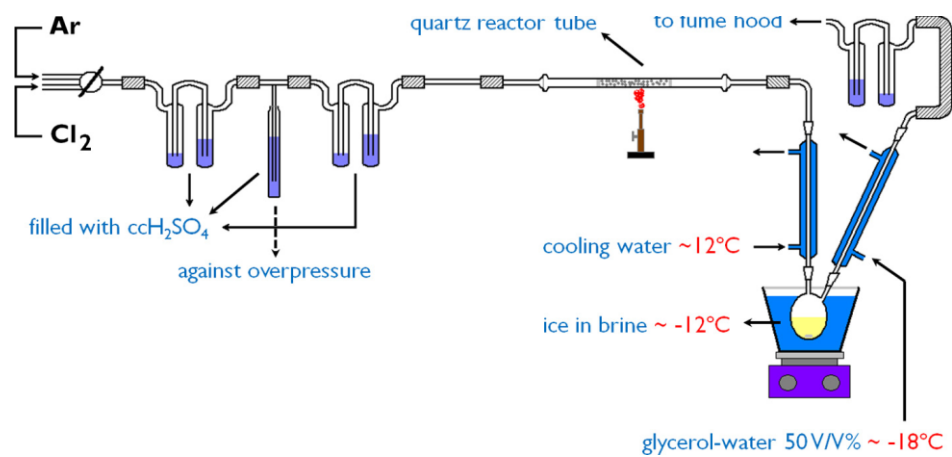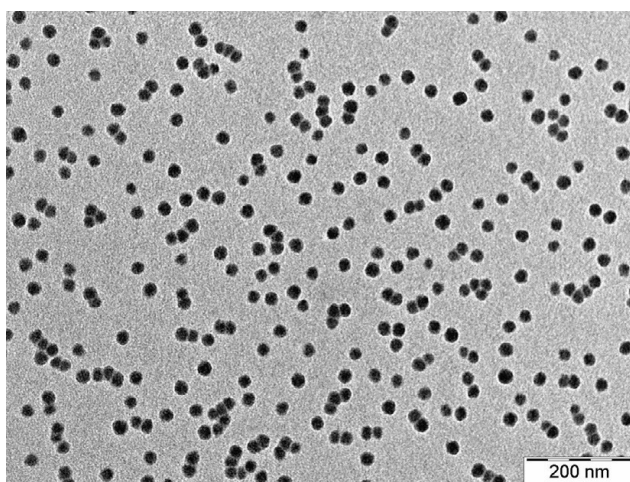Fig. 1. Apparatus scheme for the preparation of $^{29}SiCl_4$



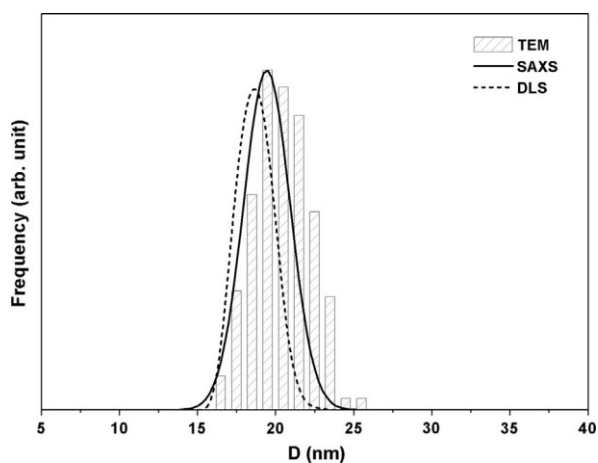Fig. 2. TEM analysis of $^{29}Si$-silica NPs



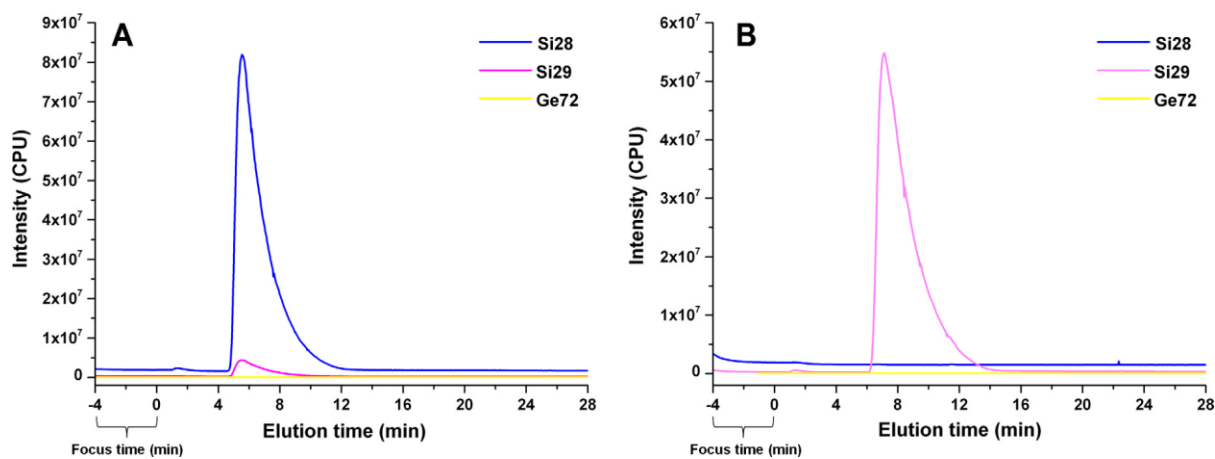Fig. 3. Size distributions of $^{29}Si$-silica NPs calculated from TEM, SAXS and DLS

measurements

Fig. 4. FFF-ICP-MS analysis of (A) natural and (B) [29]Si-silica NPs. Regenerated cellulose with 10 kDa MWCO was used with Milli-Q water as carrier and a detector flow of 0.5 mL min^{-1}. Ge was used as internal standard to correct for drift of the Si signal.