

High Precision Measurement of Selenium Isotopic Composition by Hydride Generation Multiple Collector Inductively Coupled Plasma Mass Spectrometry with a ^{74}Se - ^{77}Se Double Spike

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Abstract: A method was developed for high precision measurement of selenium isotopes by hydride generator-multiple collector inductively couple plasma mass spectrometer (HG-MC-ICP-MS) using ^{74}Se - ^{77}Se double spike and modified thiol-cotton purification. Selenium was separated from the sample matrix using thiol-cotton, and $\text{HNO}_3 + \text{H}_2\text{O}_2$ separates Se from organic matter in the thiol-cotton that could interfere with hydride generation. The ^{74}Se - ^{77}Se double spike corrects for isotope fractionation during purification and mass spectrometry analysis. The external reproducibility for standard NIST SRM3149/MH495 and natural samples were 0.1‰ (2σ) and 0.15‰–0.2‰ (2σ) over the course of several months, respectively. Based on the average yield (85%) of Se separation using thiol-cotton, the minimum quantity of Se for isotope analysis was approximately 20 ng. The results of an interlaboratory standard, MH495, $\delta^{82/76}_{\text{SRM3149}} = (-3.44 \pm 0.1)\text{‰}$ (2σ), are in good agreement with previously published data $\delta^{82/76}_{\text{MH495 VS SRM3149}} = (-3.04 \pm 0.5)\text{‰}$ that used a sample-standard bracketing technique. Selenium isotopes were measured in several sedimentary samples with a range from -13.53‰ to 9.03‰ relative to NIST SRM3149. These results suggest a potentially wide application of Se isotopes in environmental, geological, agricultural, and life sciences fields.

Key Words: High precision measurement of Se isotopes; ^{74}Se - ^{77}Se double spike; Thiol-cotton; Hydride generation; Multiple collector inductively coupled plasma-mass spectrometry

1 Introduction

Selenium is an essential trace element for all animals, including humans^[1,2]. In recent years, it has received on-going attention in the fields of geological, environmental, life sciences, agricultural, food, nutrient sciences, and so on^[1–4]. Selenium has six naturally occurring stable isotopes, ^{74}Se , ^{76}Se , ^{77}Se , ^{78}Se , ^{80}Se and ^{82}Se , whose abundance are 0.89%, 9.37%, 7.64%, 23.77%, 49.61% and 8.73%, respectively. Selenium, chemically similar to sulfur, occurs in +6, +4, 0 or

-2 valences in various inorganic compounds and has several organic compounds (e.g. DMS_e)^[5]. The mobility, bioavailability, and toxicity of Se are different with valence state and organic forms^[3–5]. Reactions affecting Se speciation, whether biotic or abiotic, can affect isotope ratios of reactants and products^[6–10]. Selenium isotope systematics has been preliminarily summarized by Dr. Johnson to date^[8]. Selenium isotope ratios can be used as the tracers of pollution source, biogeochemical reactions, and processes. Recent works have confirmed that microorganism and abiotic reduction reactions

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are the main mechanisms of selenium isotope fractionation^[7–10].

The application of Se isotope fraction studies has been inhibited with the difficulties of the precise determination of Se isotope ratio. Krouse *et al.*^[6] first determined ⁸²Se/⁷⁶Se ratio via gas-source mass spectrometry with a precision of approximately ±0.5‰ in the early 1960s. In 1990s, Johnson *et al.* developed a ⁷⁴Se-⁸²Se double-spike technique, and the ^{80/76}Se ratios of ±0.2‰ for N-TIMS were obtained^[7–10]. Subsequently, they produced a preliminary summary of selenium isotope systematics from various geochemical reactions and estimated an 18‰ span of natural ⁸²Se/⁷⁶Se isotope ratios with a precision of ±0.2‰^[7–10]. Recently, Rouxel *et al.* purified Se with a thiol-cotton fiber (TCF) separation technique and measured the isotopes of Se using HG-MC-ICP-MS and standard-sample bracketing (SSB). Subsequently, they reported a precision of ±0.25‰ for ⁸²Se/⁷⁶Se on as little as 10 ng of Se^[11]. Although Rouxel *et al.* used collision and reaction cell technique, the issues of high background and matrix effects such as argides (MAr⁺), dimers (Ar₂⁺), and Se hydride molecular seriously affected the accuracy of Se isotopes. In this study, a method for high precision determination of Se isotope ratios was developed with TCF separation procedure, continuous flow hydride generation system (HG), and a ⁷⁴Se-⁷⁷Se double-spike technique on MC-ICPMS. The external reproducibilities of NIST SRM3149 and MH495 were 0.1‰ (2σ). The reproducibility of natural samples was slightly lower than ±0.2‰ (2σ).

2 Experimental

2.1 Instruments and reagents

Chemical reagents, including HNO₃, HCl, HF, H₂O₂ (30%), NaOH, and NaBH₄ (98%, pellets), were of Fisher analytical grade. Milli-Q water and high purity nitrogen were used. The ⁷⁴Se- and ⁷⁷Se-enriched spikes were purchased from ISOFLEX, USA. Selenium standard solutions of NIST SRM3149 and MH495 that was an interlaboratory standard were used as the reference standard. The vessels mainly included 15 ml PFA beaker with covers and Parr Bomb PTFE. All Teflon materials and glassware were cleaned using approximately 8 M subboiling HNO₃ for 30 min and triple rinsed with Milli-Q water.

2.2 Selenium double-isotope spike

The double-spike technique corrects for mass-dependent fractionation during sample preparation and determination. The technique has been widely used in isotope measurement of metallic elements, such as Fe, Cr, Mo, Pb, and so on^[12–15]. The principle of the isotope double spike is described in

detail^[7,14]. In brief, a double-isotope spike with a known isotope ratio is introduced into samples as early as possible in the procedure of sample preparation. An assumed mass fractionation coefficient (β) is calculated according to the known and measured ratio of the double-isotope spike. The true mass fractionation coefficient (β_0) and the isotope ratio of the sample are obtained via an iterative data reduction method that accounts for the added double-isotope spike^[7]. An exponential fractionation law was assumed for the mass fractionation during the sample preparations and measurements. The double spike consists of a mixture of two high purity spikes enriched in ⁷⁴Se (99.7%) and ⁷⁷Se (99.5%). The double-spike mixture has an approximate 1:1 of ⁷⁴Se/⁷⁷Se ratio (⁷⁴Se/⁷⁷Se = 1.05413).

2.3 Sample preparation and chemical separation

2.3.1 Sample digestion

The geological samples of 25–100 mg were digested using HNO₃ + HF + H₂O₂ in Parr bomb PTFE vessels, whereas sulfide was digested in HNO₃. Parr bombs were heated at (150 ± 5) °C for 16–18 h. After cooling, the solutions were transferred to PFA beakers of 15 ml and evaporated to incipient dryness on a hot plate at 70 °C. The evaporated solutions were diluted to 5 ml using 10% HNO₃ and were diluted to 10–20 ml and then were centrifuged.

2.3.2 Measurement of selenium concentration

The sample aliquots of 0.2–1.0 ml were mixed with 0.5 ml HNO₃ and evaporated to incipient dryness at 70 °C. Next, the samples were dissolved with 5 ml of 5 M HCl and then were transferred to 25 ml borosilicate glass tubes with Teflon-lined caps and heated at 95–100 °C for 1 h in an Al block. After cooled, the solutions were diluted to 20 ml with 2 M HCl. The selenium concentrations were determined using HG-MC-ICP-MS.

2.3.3 Selenium isotope double spike

The Se isotope double spike (⁷⁴Se-⁷⁷Se) was added to each sample prior to any separation procedures that could fractionate isotopes. A spike of sample ratio of ⁷⁷Se_{spike}/⁷⁸Se_{sample} was designed approximately two; however, it was observed that a ratio in the range of 1.5–3.5 did not affect the precision. All separation and analysis procedures described above were performed after the spike, and the samples were homogeneously mixed.

2.3.4 Chemical separation of Se with TCF

Selenium is separated by one of two chemical methods: hydride separation^[7,9,10] or TCF separation^[11]. Because the

former is a relatively more expensive^[10] and more complicated process than the later, requiring a higher degree of experimental skills, TCF separation was used in this study. Detailed separation procedures are as follows: TCF of (0.145 ± 0.005) g was weighed and loaded into polypropylene tubes or glass columns. The TCF was washed with 3 ml of water and conditioned with 1 ml of 6 M HCl followed with 1.0 ml 0.8 M HCl. And then samples were loaded onto TCF in 0.83 M HCl at a flow rate of approximately 1.0–1.5 ml min⁻¹. The TCF was washed using 2.0 ml of 6 M HCl, followed by 3.0 ml H₂O. After pure H₂O was passed through, the TCF was squeezed to remove most of the remaining water. The TCF was moved to 15 ml polypropylene centrifuge tubes. A total of 1 ml of a well-mixed solution of HNO₃ (0.5 ml) + 30% H₂O₂ (0.2 ml) + H₂O (0.3 ml) was added to the TCF, and the tubes were placed in a boiling-water bath for 20 min to completely desorb Se. And then, 3.5 ml of H₂O was added and the tubes were centrifuged for 20 min at 8000 rpm, then the supernatant was carefully decanted into a 15 ml PFA beaker. A total of 1.0 ml HNO₃ was added and then the samples were evaporated to 80 μl at 75 °C. A mixed solution of HNO₃ and H₂O₂ was repeatedly added and then evaporated to approximately 80 μl to remove the unidentified organic matter. When the sample residue consisted of a colorless tiny spot of less than 5 μl, the process was stopped. The samples were dissolved in 5 ml 5 M HCl, transferred into 25 ml borosilicate glass tubes with Teflon caps, and heated at 95–100 °C for 1 h in an Al block on a hot plate. After it was cooled, a high purity N₂ flow was bubbled through the sample solutions for 15 min to remove volatile Br species. The sample solutions were diluted exactly with (2.0 ± 0.1) M HCl for Se isotopes measurement.

The average recovery of Se for the TCF procedure is higher than 85%, depending on the quality of TCF batch. A sample introduction volume of 5 ml and 4 μg l⁻¹ of Se had a response of 1.2–1.5 V with a high precision with as little as 20 ng of Se.

2.4 Hydride generation system and mass spectrometer

The samples were introduced into MC-ICP-MS as SeH₂ via an on-line continuous-flow hydride generator (HG system). A detailed description is given in literature^[10,11]. In this study, the sample, with a flow rate of 0.25 ml min⁻¹, reacted with sodium borohydride (0.3% NaBH₄) in 0.3% NaOH to form the volatile of SeH₂. After the SeH₂ was stripped from solution, a carrier gas of He carried the SeH₂ from a gas-liquid separator and through PTFE filter and Teflon tubing into the ICP torch.

The measurements of selenium isotopes were performed using two double focusing Nu instruments HR MC-ICPMS (Wrexham, North Wales, UK); one located in the Department of Geology at the University of Illinois at Urbana-Champaign, and the other at Key Lab of Isotopic Geology, the Ministry of

Land and Resources, China. These instruments have a double focusing system, 12 Faraday cups, and 3 ion counters. Nine Faraday cups were used simultaneously during determining selenium isotopes: H6, H4, Ax, L2, L3, L5 correspond to ⁸²Se, ⁸⁰Se, ⁷⁸Se, ⁷⁷Se, ⁷⁶Se and ⁷⁴Se, respectively. The Faraday cups of L4, H2, H5 correspond to ⁷⁵As, ⁷⁹Br, and ⁸¹Br, respectively. The Ge signal was detected by an ion counter (IC0), and the intensity potential was acquired according to a measured conversion coefficient between the ion counter and the Faraday cup. The Se concentration in samples was determined in the range of 4–6 μg l⁻¹ with a ⁷⁸Se signal intensity of 1.2–2.0 V. The background signal was measured for 2 min by on-peak zero (OPZ), followed immediately by the measurements of Se and Ge. To avoid memory effects from the double spike, the system was rinsed between two measurements with a 2 M HCl blank solution until the normal background was reached.

2.5 Data procession

The first step of data procession was to correct the interferences such as Ar₂⁺, SeH⁺, GeH⁺, AsH⁺ and so on. And then the corrected ratios of ⁷⁴Se/⁷⁸Se, ⁷⁶Se/⁷⁸Se, ⁷⁷Se/⁷⁸Se, ⁸⁰Se/⁷⁸Se and ⁸²Se/⁷⁸Se were iteratively reduced to separate the ⁸²Se/⁷⁶Se and ⁸²Se/⁷⁸Se ratios in the sample ratio from the added double spike. All Se isotope ratios of NIST SRM3149 were reported in the per mil notation (‰):

$$\delta^{82/76}\text{Se} (\text{‰}) = [({}^{82}\text{Se}/{}^{76}\text{Se})_{\text{sample}} / ({}^{82}\text{Se}/{}^{76}\text{Se})_{\text{std}} - 1] \times 1000$$

3 Results and discussion

3.1 Analysis of selenium standard solution

Although there are no formal consensus isotope references of Se, the samples of NIST SRM3149 tends to be the ‘zero’ reference for Se isotope analysis^[16]. The external precision of 2-standard deviation of the ⁸²Se/⁷⁶Se ratio for unprocessed NIST SRM3149 was 0.08‰ (*n* = 42) during 6-month period. The external precision of unprocessed interlaboratory standard of MH495 was 0.04‰ (*n* = 9) and the $\delta^{82/76}\text{Se}_{\text{SRM3149}}$ was (−3.44 ± 0.1)‰. The external precision on repeated preparations of TCF processed standards over a 6-month period was better than 0.1‰ (2σ) (*n* = 10). The isotope ratios of the processed standards were in agreement with the unprocessed standards: $\delta^{82/76}\text{Se}_{\text{SRM3149}} = (-0.02 \pm 0.1)\text{‰}$, $\delta^{82/76}\text{Se}_{\text{SRM3149}} = (-3.44 \pm 0.1)\text{‰}$ for SRM3149 and MH495, respectively. The accuracy of $\delta^{82/76}\text{Se}_{\text{SRM3149}}$ MH495 was better than that of $\delta^{82/76}\text{Se}_{\text{SRM3149}} = (-3.04 \pm 0.5)\text{‰}$ reported by Carignan and Wen^[16]. These results show that the precision obtained via a double-spike technique is higher than that of the SSB method in determination of Se isotopic compositions.

3.2 Interferences of isotope and matrix effects

The interferences of Se isotopes are mainly from two sources^[11,17]: (1) the instrumental background, resulting from the Ar carrier gas and Ni cone; (2) matrix effects, resulting from an unidentified dissolved organic matter and incompletely removed hydride-forming elements such as As, Ge, Sb. Nickel oxide ($^{58}\text{Ni}^{16}\text{O}^+$) is formed due to ablation of the Ni cone, but the Ni oxide signal was generally less than 0.005 mV in the Nu plasma MC-ICP-MS. A large $^{40}\text{Ar}^{40}\text{Ar}^+$ dimer signal is generally above 1 mV (Table 1). Argon and Cl form a polyatomic ion that can interfere with ^{77}Se . To ensure a constant $^{40}\text{Ar}^{37}\text{Cl}^+$ background signal, the HCl concentration was always titrated to (2.0 ± 0.05) M HCl. Although Kr, which is impurity in the Ar gas, can affect the signal of ^{82}Se , it was negligible in this study. Stable Se isotopes signals were obtained by OPZ.

Thiol-cotton fibers completely adsorb Se(IV) under acidic condition, and then release the adsorbed Se by destruction of the TCF with HNO_3 ^[11]. However, the two inevitable issues occur during the procedures of Se separation and purification with TCF: (1) unidentified dissolved organic matter that produced in the process of decaying TCF can interfere with the formation of Se hydride^[7,10,11]; (2) TCF does not effectively remove Ge and As^[11], which interfere the determination of Se isotopes (Table 2). The interferences from Ge and As were evaluated by doping double-spiked standard solutions (NIST SRM3149) with As and Ge. Figure 1 shows that the $\delta^{82/76}\text{Se}$ shifts with changes in concentration ratios of As/Se and Ge/Se. To achieve the precision of $\pm 0.1\%$ (2σ), the ratios of As/Se and Ge/Se should be controlled to be

less than 1.5 and 0.05, respectively. However, because As can cause relatively positive contribution to the ratios of $^{82}\text{Se}/^{76}\text{Se}$ and $^{78}\text{Se}/^{76}\text{Se}$ while Ge was negative in an iterative data reduction program, the actual ratio range of As/Se and Ge/Se can reach 4 and 0.1 for Se isotopes measurement, which was found to be negligible for the accurate measurement of Se isotopes.

3.3 Selenium isotopic composition of several natural samples

Selenium isotope ratios were measured on geological samples that had Se concentrations ranging from several mg kg^{-1} to 3%. The most samples were carbonaceous shale with high organic matter content (up to 47%) and a comparatively complex matrix. Replicate measurements were nearly consistent and yielded a precision of $\pm 0.20\%$ for the $^{82}\text{Se}/^{76}\text{Se}$ ratio. Figure 2 shows that the $\delta^{82/76}\text{Se}$ and $\delta^{82/78}\text{Se}$ are within the error range along the theoretical mass fractionation line, which indicates that the Se isotopic composition can be determined without interference and the results are reliable. However, considerable variations of the $\delta^{82/76}\text{Se}$ ratios were observed in a few samples, such as ytbcoal and ytbfault (Table 2); this was attributed to the nonhomogeneity of the sample powder. In these samples, $\delta^{82/76}\text{Se}$ ratios ranged from -14.20% to 11.37% . This represents the largest Se isotopic variations reported to date and suggests a potentially wide application of Se isotopes in the geological, environmental, agricultural, and life field.

Table 1 Faraday collectors array for measurement of Se isotopes and the blank signals of interfering species

Collectors	L5	L4	L3	L2	Ax	H2	H4	H5	H6
Mass	74	75	76	77	78	79	80	81	82
Se isotopes	^{74}Se		^{76}Se	^{77}Se	^{78}Se		^{80}Se		^{82}Se
Isobaric interferences	$^{38}\text{Ar}^{36}\text{Ar}^+$ $^{74}\text{Ge}^+$ $^{58}\text{Ni}^{16}\text{O}^+$	$^{40}\text{Ar}^{35}\text{Cl}^+$ $^{38}\text{Ar}^{37}\text{Cl}^+$ $^{38}\text{Ar}^{36}\text{ArH}^+$ $^{75}\text{As}^+$ $^{74}\text{GeH}^+$	$^{40}\text{Ar}^{36}\text{Ar}^+$ $^{38}\text{Ar}^{38}\text{Ar}^+$ $^{76}\text{Ge}^+$ $^{75}\text{AsH}^+$ $^{60}\text{Ni}^{16}\text{O}^+$	$^{40}\text{Ar}^{37}\text{Cl}^+$ $^{40}\text{Ar}^{36}\text{ArH}^+$ $^{76}\text{SeH}^+$ $^{76}\text{GeH}^+$ $^{61}\text{Ni}^{16}\text{O}^+$	$^{40}\text{Ar}^{38}\text{Ar}^+$ $^{77}\text{SeH}^+$ $^{62}\text{Ni}^{16}\text{O}^+$	$^{79}\text{Br}^+$ $^{78}\text{SeH}^+$	$^{40}\text{Ar}^{40}\text{Ar}^+$ $^{80}\text{Kr}^+$ $^{79}\text{BrH}^+$	$^{40}\text{Ar}^{40}\text{ArH}^+$ ^{81}Br	$^{82}\text{Kr}^+$ $^{81}\text{BrH}^+$ $^{12}\text{C}^{35}\text{Cl}_2^+$ (?)
Blank signal (mV)	4	2	32	5	11	5	5500	140	2

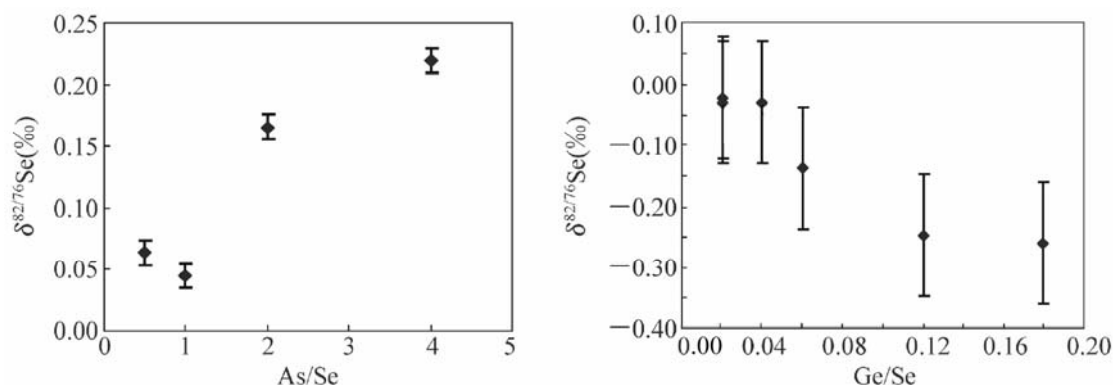


Fig.1 Se isotope ratios using ^{74}Se - ^{77}Se double spike for NIST SRM3149 standard doped with As and Ge

Table 2 Determination results of Se isotope ratios in several nature samples using a ^{74}Se - ^{77}Se double spike

Sample	Sample type	Se concentration (mg kg^{-1})	$\delta^{82/76}\text{Se}$ (‰ Duplicate)			Mean $\pm 2\sigma$
Cp0A	Carbonaceous shale	2588	9.01	9.13	8.95	9.03 \pm 0.18
CP0D	Carbonaceous mudstone	26054	7.88	7.73	7.72	7.78 \pm 0.17
Gysum1	Carbon-bearing gysum	16.4	3.23	3.28		
ytfault	Carbonaceous shale	5859	11.37	10.39		
ytfcoal	Carbonaceous shale	2622	-13.19	-13.19	-14.20	-13.53 \pm 1.16

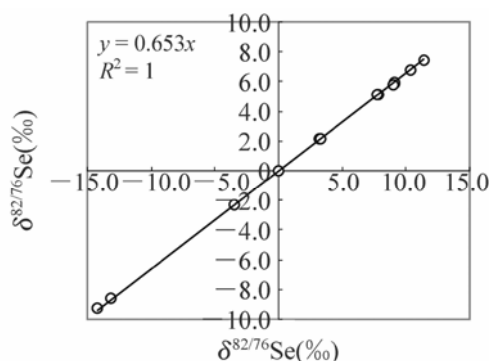


Fig.2 Comparison between theoretical relationship of $\delta^{82/76}\text{Se}$ and $\delta^{82/78}\text{Se}$ (theoretical line: $y = 0.661x$) and their correlation for standard solution (NIST SRM3149 and MH495) and a suite of samples with TCF separation

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References

- [1] Rayman M P. *The Lancet*, **2000**, 356: 233–241
- [2] Wang J, Liu C-Q, Lu H, Wei C, Zhou T. *Chinese J. Anal. Chem.*, **2007**, 35(6): 814–818
- [3] Zhu J M, Wang N, Li S H, Li L, Su H C, Liu C X. *Science of Total Environment*, **2008**, 392: 252–261
- [4] Zhu J M, Zuo W, Liang X B, Li S H, Zheng B S. *Applied Geochemistry*, **2004**, 19: 461–467
- [5] Frankanberger W T, Benson S. *Selenium in the Environment*. Marcel Dekker Inc, New York, 1994: 1–416
- [6] Krouse H R, Thode H G. *Can. J. Chem.*, **1962**, 40: 367–375
- [7] Johnson T M, Herbel M J, Bullen T D, Zawislanski P T. *Geochim. Cosmochim. Acta*, **1999**, 63: 2775–2783
- [8] Johnson T M. *Chemical Geology*, **2004**, 204: 201–214
- [9] Johnson T M, Bullen T D. *Geochim. Cosmochim. Acta*, **2003**, 67: 413–419
- [10] Ellis A S, Johnson T M, Herbel M J, Bullen T D. *Chem. Geol.*, **2003**, 195: 119–129
- [11] Rouxel O, Ludden J, Carignan J, Marin L, Fouquet Y. *Geochim. Cosmochim. Acta*, **2002**, 66: 3191–3199
- [12] Dideriksen K, Baker J A, Stipp S L S. *Geochim. Cosmochim. Acta*, **2006**, 70: 118–132
- [13] Ellis A S, Johnson T M, Bullen T D. *Science*, **2002**, 295: 2060–2062
- [14] Christopher S, Nagler T F, Kramers J D. *Geochem. Geophys. Geosyst.*, **2001**, 2: paper No. 2000GC000124
- [15] Thirlwall M F. *Chemical Geology*, **2002**, 184: 255–279
- [16] Carignan J, Wen H J. *Chemical Geology*, **2007**, 242: 347–350
- [17] Inagaki K, Takatsu A, Nakama A, Eyama S, Yarita T, Okamoto K, Chiba K. *Anal. Bioanal. Chem.*, **2006**, 385: 67–75