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RESEARCH ARTICLE

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Selenate—An internal chemical shift standard for aqueous ^{77}Se NMR spectroscopy

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Abstract

The ^{77}Se NMR spectra of selenate were studied under various circumstances, such as concentration, pH, temperature, ionic strength, and D_2O:H_2O ratio, in order to examine its potential as a water-soluble internal chemical shift standard. The performance of selenate as a chemical shift reference and that of other attempted ones from the literature (dimethyl selenide, tetramethylsilane/TMS, and 3-(trimethylsilyl)propane-1-sulfonate/DSS) was also explored. The uncertainty in the resulting chemical shift relative to the effective spectral width is comparable to that of DSS. Compared to the currently prevalent water-soluble external chemical shift reference, selenic acid solution, the properties of internal selenate are much more favorable in terms of ease of use. We have also demonstrated that selenate can be used in reducing media, which is inevitable for the analysis of selenol compounds. Thus, it can be stated that sodium selenate is a robust internal chemical shift reference in aqueous media for ^{77}Se NMR measurements; the chemical shift of this reference in a solution containing 5 *V*/*V*% D_2O at 25°C and 0.15 mol·dm^{-3} ionic strength is 1048.65 ppm relative to 60 *V*/*V*% dimethyl selenide in $CDCl_3$ and 1046.40 ppm relative to the ^{1}H signal of 0.03 *V*/*V*% TMS in $CDCl_3$. In summary, a water-soluble, selenium-containing internal chemical shift reference compound was introduced for ^{77}Se NMR measurements for the first time in the literature, and with the aforementioned results all previous ^{77}Se measurements can be converted to a unified scale defined by the International Union of Pure and Applied Chemistry.

KEYWORDS
chemical shift standard, ppm reference, selenium NMR

1 | INTRODUCTION

The nucleus of ^{77}Se isotope has valuable, yet underutilized properties for nuclear magnetic resonance spectroscopy (spin: ½; natural abundance: 7.63%; relative receptivity: $5.37 \cdot 10^{-4}$ with respect to ^{1}H and 3.15 with respect to ^{13}C; chemical shift range: ~2,800 ppm). The availability of ^{77}Se NMR spectroscopy was demonstrated in the early 1950s,[1,2] then it quickly evolved as a selective method to investigate selenium-containing compounds.[3–6] The biological significance of selenium-containing proteins (selenoproteins) was described early on in the maintenance of physiological redox homeostasis and it has also been proven that the selenocysteine residues are essential for the catalytic activity of such redox enzymes.[7–10] Moreover, our research group has recently found close correlation among the standard redox potential, the acid–base properties and the ^{77}Se chemical shift of selenolate-diselenide redox systems.[11,12] These findings will no doubt lead to a more extensive use of ^{77}Se NMR as a selective analytical tool in the characterization of selenoproteins and organoselenium compounds. Despite the long-standing use of this technique, a reliable and robust chemical shift standard is still an unmet need, as seen in a systematic error between separately reported ^{77}Se chemical shifts in the literature.[12]

In the ^{77}Se NMR literature, chemical shift data are referenced to a relatively large number of compounds(seleninyl chloride,[13] dimethyl selenide,[14,15] selenophene,[16–19] diphenyl diselenide,[20–24] 4,4′-dimethyldiphenyl diselenide,[25] selenium oxide,[26] sodium selenite,[27] selenous acid).[13,28–31] In addition, indication of the measurement parameters (concentration, ionic strength, temperature) is rather the exception than the case. This may cause differences and misunderstanding between various results. It is noteworthy that selenic acid has already been used as a chemical shift reference[13]; however, the measurement conditions were not specified. As we have shown certain measurement parameters can significantly affect the frequency of the selenate signal, the precise knowledge of measurement circumstances can therefore have a crucial effect on the research findings.

There are two compounds that are commonly used as chemical shift reference for ^{77}Se NMR, namely dimethyl selenide[14] and selenous acid.[32] The solvent-, concentration- and temperature-dependence of ^{77}Se chemical shift of dimethyl selenide has been elaborated. Based on literature recommendation, in principle, this reference can be used as an internal standard in nonpolar solvents; however, it is virtually always used as an external reference compound. The disadvantages of this compound are that dimethyl selenide is not water soluble; therefore, the external standard method will cause some inconveniency in aqueous measurements. Furthermore, dimethyl selenide is an extremely volatile and toxic substance.

Selenous acid is only recommended as an external reference compound and the temperature-, concentration-, solvent- and pH-dependence of the ^{77}Se chemical shift has been elaborated. However, in every work using selenous acid as chemical shift standard the final chemical shift data are referred to 60 *V*/*V*% dimethyl selenide in $CDCl_3$. Since selenite undergoes two protonation processes ($\log K_1 = 8.20 \pm 0.01$ and $\log K_2 = 2.46 \pm 0.06$, these values are in good agreement of the previously described parameters[33]), it is certainly unfit to be used as a reliable internal chemical shift reference in aqueous media where pH changes usually take place. Given the obvious requirements of an internal standard, a reliable and robust ^{77}Se NMR chemical shift reference compound which can be used in aqueous media is a definite precondition for future selenoprotein analysis. Other potential reference compounds from the available organoselenium compounds were thoroughly investigated and excluded due to poor water solubility, high toxicity or having a protonating group in the pH range of interest. Notably, N,N-dimethylselenourea was excluded because of having an acid–base function ($\log K = 4.98 \pm 0.01$), just like dimethyl selenoxide ($\log K = 2.04 \pm 0.03$, in agreement with previous findings[34]) which is also unstable and hygroscopic in pure form.

In this work we introduce selenate and demonstrate its properties as an internal ^{77}Se chemical shift reference for aqueous media. The pH-, temperature-, concentration-, ionic strength-, and solvent-dependence of the ^{77}Se frequency are analyzed in terms of a multilinear regression model. Its chemical stability and reactivity in various media were also determined.

2 | MATERIALS AND METHODS

2.1 | Materials

Selenic acid (40 *m*/*m*%), anhydrous sodium selenate, selenous acid, N,N-dimethylselenourea, dimethyl selenoxide, sodium chloride and seleno-L-cystine were purchased from Sigma (Merck), sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) and dimethyl selenide were obtained from Tokyo Chemical Industry, deuterium oxide (D_2O) and chloroform-d containing 0.03 *V*/*V*% tetramethylsilane (TMS) were purchased from Merck. All the above-mentioned substances were used

without further purification. Deionized water was prepared with a Milli-Q Direct 8 Millipore system.

2.2 | NMR spectroscopy measurements

NMR spectra were recorded on a Varian Unity Inova DDR spectrometer (599.9 MHz for ^{1}H) with a 5 mm $^{1}H\{^{13}C/^{31}P\text{-}^{15}N\}$ pulse field gradient triple resonance probehead at 298.15 ± 0.1 K. The solvent was H_2O: D_2O 95:5 (V/V) unless otherwise stated, ionic strength was adjusted to either 0.15 or 1.0 mol·dm^{-3} unless otherwise stated. The concentration of sodium selenate varied between 0.0025 and 0.5 mol·dm^{-3}. For the stability tests of selenate two samples with different ionic strengths and pH were prepared and stored at room temperature. The ^{77}Se NMR spectra of these samples were recorded at different time points between 1 minute and 130 days. The experimental design to study the above mentioned parameters is presented in Table 1.

The pH values were determined in situ by internal indicator molecules (at ca. 1 mmol·dm^{-3}) optimized for ^{1}H NMR.[35,36] The sample volume was 600 µl and every sample contained ca. 1 mmol·dm^{-3} DSS. Where necessary, the H_2O ^{1}H signal was suppressed with a presaturation sequence; the average acquisition parameters for ^{1}H measurements are: number of transients = 16, number of points = 65,536, acquisition time = 3.33 s, relaxation delay = 1.5 s. The ^{77}Se NMR parameters were as follows: pulse and acquire sequence using 7.25 µs observe pulse, at most the number of transients was between 128 and 65,536 based on the concentration of the selenium-containing compound, number of points = 262,144, maximal spectral width applied = 131578.9 Hz, relaxation delay = 1 s. The ^{77}Se measurements were acquired with a standard s2pul pulse sequence optimized for the ^{77}Se nucleus at pulse width = 14.50 µs (at 60 dB) with ^{1}H decoupling during the pulse and FID acquisition. The presence or absence of the ^{1}H decoupling had no effect on the signal frequency or width.

Based on IUPAC recommendations[37,38] the resulting ^{77}Se NMR spectra were referenced to the ^{1}H NMR signal of TMS (measured in a separate sample at 0.03 V/V% in $CDCl_3$) using the *substitution method*. However, the chemical shift referencing was also carried out relative to the ^{1}H NMR signal of DSS methyl groups present in the same sample using the *substitution method*, in order to determine whether this widely accepted ^{1}H reference can be used instead of TMS.

Multiple linear regression was carried out by MedCalc 12.5.00 software (MedCalc Software Ltd., Ostend, Belgium).

2.3 | pH-potentiometric titrations

A 716 DMS Titrino automatic titrator (Metrohm) with a Metrohm 6.0234.110 combined pH glass electrode was used for the potentiometric titrations, under automatic PC (personal computer) control. The pH-potentiometric

TABLE 1 The investigated parameters and the exact measurement parameters (each line corresponding to an investigated parameter) applied to characterize selenic acid ^{77}Se chemical shifts

		Other measurement parameters				
Investigated parameter	**Investigated range**	**pH**	***T* (K)**	**Selenate *c* (mol·dm^{-3})**	***I* (mol·dm^{-3})**	***V/V*% D_2O**
pH	1.36–11.68	NA	298	0.05	0.15	5
	0.66–13.09	NA	298	0.333	1.00	5
T (K)	293–328 (6 measurement points)	8.78	NA	0.333	1.00	5
selenate *c* (mol·dm^{-3})	0.0025–0.5 (9 measurement points)	~7	298	NA	1.5	5
I (mol·dm^{-3})	0.15–1 (7 measurement points)	~7	298	0.05	NA	5
V/V% D_2O	5–100 (11 measurement points)	~7	298	0.333	1.00	NA
stability	stability observed throughout 130 days	11.50	298	0.05	0.15	5
		8.78	298	0.333	1.00	5

Note. pH ~ 7 refers to the native pH of aqueous solutions of sodium selenate and sodium chloride.

system was calibrated using pH 1.68, 4.01, 6.87, 9.18 aqueous National Bureau of Standards (NBS) buffer solutions. Constant temperature ($25 \pm 0.1°C$) was provided by a thermostated double-walled glass cell. Difference titrations were carried out in the absence (blank) and presence of ligands. First 2 ml of 0.1 mol·dm^{-3} HCl was titrated with 0.1 mol·dm^{-3} KOH. Constant ionic strength of 0.15 mol·dm^{-3} was provided by the presence of KCl. Then, a ligand was added to the same volume of HCl solution and was subsequently titrated with KOH. The initial concentration of the ligands varied between 10 and 20 mmol·dm^{-3} in the titrations. Nonlinear parameter fitting with Origin 8 provided the protonation constants from the volume differences.

3 | RESULTS

3.1 | pH dependence

Protonation of selenate significantly changes the electron structure of the species and therefore has a high perturbing effect on the chemical shift modelled described by the following equation:

$$\delta_{obs}(pH) = \frac{\delta_L + \sum_{i=1}^{2} \delta_{H_iL} \times 10^{\log\beta_i - i\cdot pH}}{1 + \sum_{i=1}^{2} 10^{\log\beta_i - i\cdot pH}}, \qquad (1)$$

where δ_L is the chemical shift of an unprotonated ligand (L), δ_{H_iL} values stand for the chemical shifts of successively protonated ligands, and β_i is the cumulative protonation macroconstant; log henceforth refers to the base 10 logarithm. Note that under normal aqueous conditions ($0 < pH < 14$) only one protonation step of selenate takes place, thus in such a monoprotic case, in Equation 1 i = {1}.

However, as selenic acid is a strong diprotic acid (even stronger than sulfuric acid), even the first protonation step of selenate ions only takes part below pH = 3.5. To obtain the sensitivity of ^{77}Se nuclei in selenic acid to acid–base processes, the complete ^{77}Se-NMR pH titration was carried out at two different ionic strengths, namely, 0.15 mol·dm^{-3} and 1 mol·dm^{-3}; the concentration of selenate ions was 0.05 mol·dm^{-3} and 0.33 mol·dm^{-3}, respectively. The measured samples all contained DSS, therefore the ^{1}H methyl DSS chemical shifts were also observed relative to TMS; the resulting titration curves are shown in Figure 1. We did not fit a titration curve onto the obtained data points in order to determine the protonation constants, since the low pH plateaus of the first protonation steps are not visible under the measurement conditions. Note that the ^{1}H methyl DSS signal chemical shift varies below pH 3.5 only because of the changing water resonance frequency (from previous results not shown: the H_2O ^{1}H chemical shift changes considerably below pH 2 and slightly above pH 13). Since the *substitution method* calculation makes use of the solvent deuterium frequency, it is expected that the otherwise pH-insensitive DSS signal will show a change in chemical shift when referred to TMS. In order to demonstrate the pH-insensitivity of DSS, an experiment was carried out where acetone and DSS were titrated together and the stable chemical shift of acetone can be seen when referred to internal DSS (Figure 1).

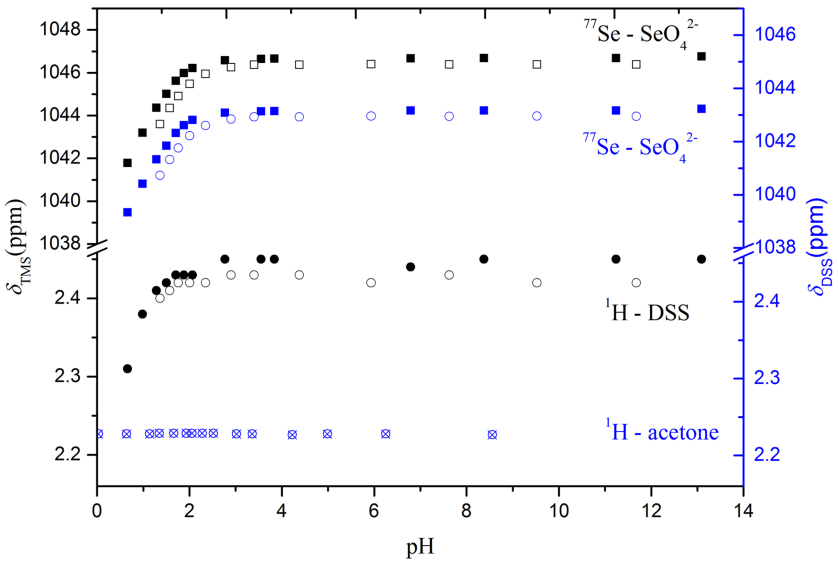

FIGURE 1 Visible in black, the pH-dependence of ^{77}Se and ^{1}H (methyl) TMS-referred chemical shifts of selenate and DSS, respectively. Solid plot points were recorded at 1 mol·dm^{-3}, while empty plot points were recorded at 0.15 mol·dm^{-3} ionic strength. Visible in blue, the pH-dependence of ^{77}Se DSS-referred chemical shifts of selenate, using the DSS within each sample. Solid plot points were recorded at 1 mol·dm^{-3}, while empty plot points were recorded at 0.15 mol·dm^{-3} ionic strength. Crossed empty points show the DSS-referred ^{1}H chemical shifts of acetone in a pH titration experiment under the same conditions as the above

3.2 | Temperature

The temperature dependence of the ^{77}Se chemical shift of selenate^{-} was studied from 293 to 328 K (Figure 2). In the knowledge of pH dependence, a slightly basic medium was chosen to avoid the perturbing effect of pH. The concentration of the selenate ions was 0.33 mol·dm^{-3} at 1.0 mol·dm^{-3} ionic strength.

3.3 | Ionic strength

The ^{77}Se and ^{1}H chemical shift values were recorded for solutions with different ionic strength at 0.15 and 1.0 mol·dm^{-3} and constant selenate concentration (0.05 mol·dm^{-3}). The results are shown in Figure 3.

3.4 | Concentration

The variation of analyte concentration has a relatively small effect on the ^{77}Se chemical shift of selenate and likewise on the ^{1}H (methyl) chemical shift of DSS; the result of the measurements is plotted in Figure 4. Two stock solutions, one with 0.5 mol·dm^{-3} sodium selenate and 1 mmol·dm^{-3} DSS, the latter with 1.5 mol·dm^{-3} sodium chloride, were used to obtain samples with different selenate and DSS

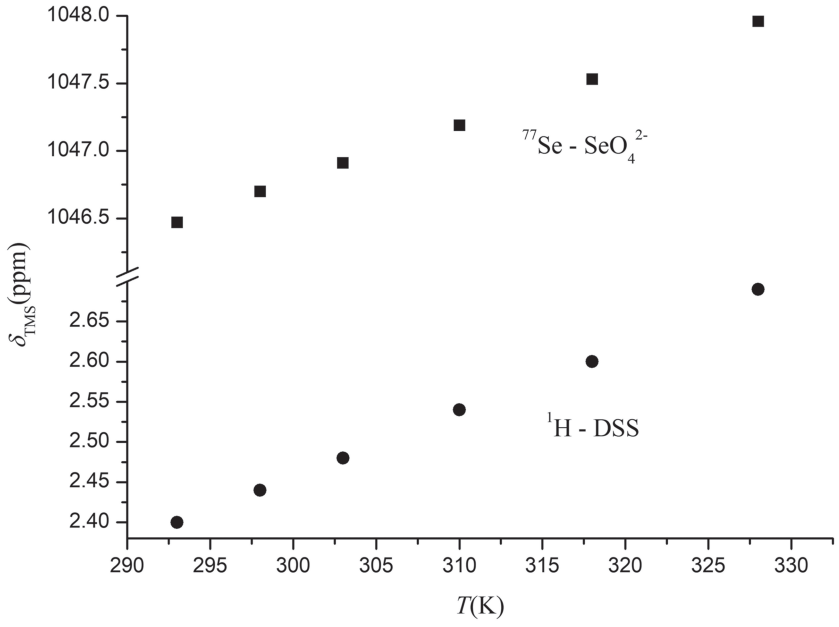

FIGURE 2 The temperature dependence of ^{77}Se and ^{1}H (methyl) chemical shifts of selenic acid and DSS, respectively. The chemical shift values were referred to the ^{1}H signal of 0.03 V/V % TMS in $CDCl_3$

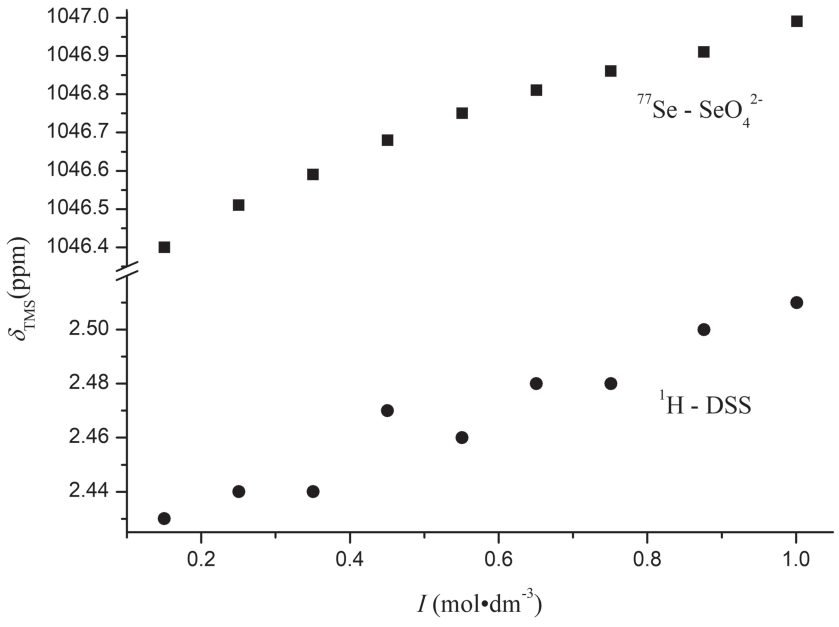

FIGURE 3 The perturbing effect of ionic strength on ^{77}Se and ^{1}H (methyl) chemical shifts of selenic acid and DSS, respectively. The chemical shift values were referred to the ^{1}H signal of 0.03 V/V% TMS in $CDCl_3$

concentration at a constant ionic strength of 1.5 mol·dm^{-3}.

3.5 | D_2O saturation

Water and deuterium oxide are similar in their main physico-chemical properties; however, due to their different relative permittivity values, they can exert a perturbing effect on the chemical shifts. Thus, H_2O/D_2O mixtures with different component ratios may have a significant effect on the chemical shift. D_2O content of the solvent mixture was changed from 5 to 100 *V*/*V*%; the results are plotted in Figure 5.

3.6 | Stability

Selenate-containing solutions at two different ionic strengths in slightly basic media were stored in NMR tubes at room temperature for more than 4 months. The effect of the time elapsed after preparing the solutions on

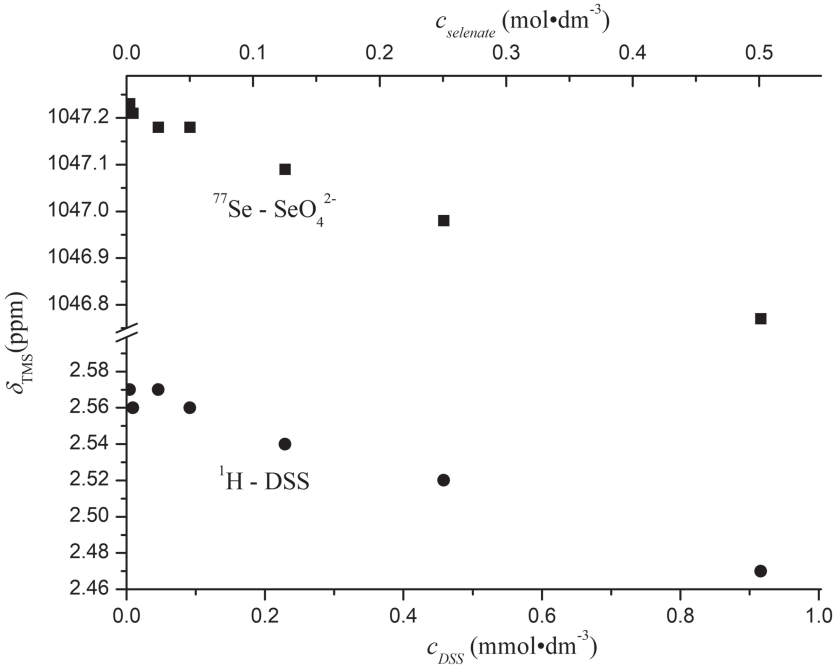

FIGURE 4 The concentration dependence of ^{77}Se and ^{1}H (methyl) chemical shifts of selenate and DSS, respectively. The chemical shift values were referred to the ^{1}H signal of 0.03 V/V% TMS in $CDCl_3$

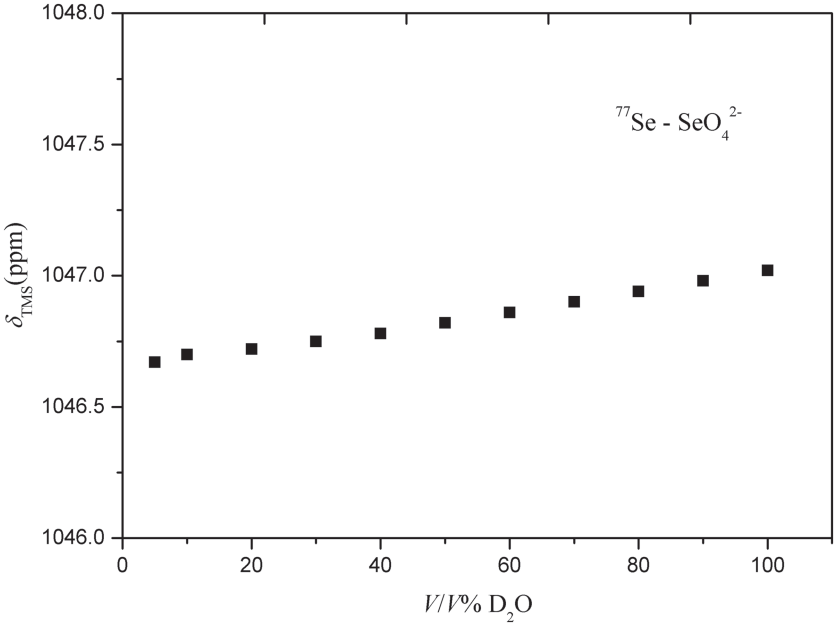

FIGURE 5 The effect of the D_2O content of the solvent on the ^{77}Se chemical shifts of selenate. The chemical shift values were referred to the ^{1}H signal of 0.03 V/V% TMS in $CDCl_3$

the ^{77}Se chemical shift was one order of magnitude lower than that of any other measurement parameter.

3.7 | Redox reactivity

Since selenium-containing proteins and amino acids are sensitive to oxidation by air, it is necessary to ensure a reductive medium during their analysis. This is usually achieved by adding a reducing agent like dithiothreitol (DTT) to the mixture. Selenium is at the most oxidized (+6) state in selenate ion. In a reductive medium with DTT an undesirable side-reaction may occur, disrupting NMR measurements; namely, selenate may turn into a reduced state, for example, selenite. To demonstrate the resistance of selenate to reduction by DTT, a solution containing a diselenide compound (selenocystine), DTT as reducing agent, and selenate were investigated (Figure 6). Based on the spectra no selenite was detectable, while DTT reduced selenocystine to its reduced form, selenocysteine. Thus selenate is a stable, suitable chemical shift reference for selenopeptide measurements, even in the presence of DTT.

4 | DISCUSSION

All of the recorded ^{77}Se and ^{1}H (methyl) chemical shift values of selenate and DSS are collated in Table 2. In order to compare the utility of selenate as an NMR chemical shift reference its relative uncertainty in chemical shift given as a percentage of the ^{77}Se chemical shift range (2,800 ppm) was compared to that of DSS and the ^{1}H chemical shift range (46 ppm).

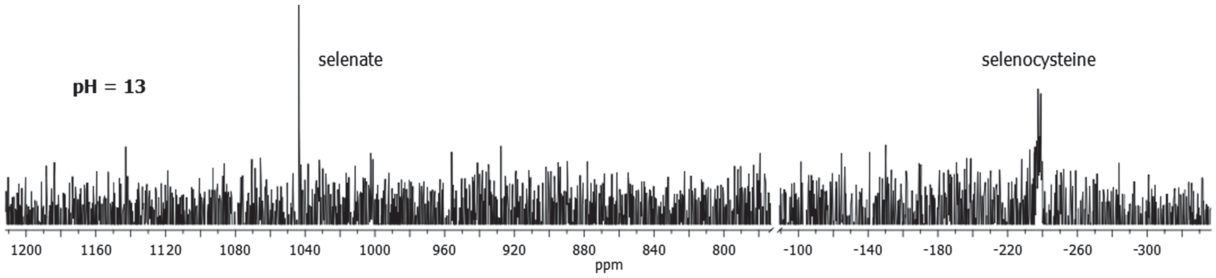

FIGURE 6 ^{77}Se NMR spectrum of a solution containing selenocystine, dithiothreitol and sodium selenate. The reduction of the diselenide bridge was successful as the NMR signal of the diselenide is missing at ca. 250 ppm.[12] The lack of a selenite ^{77}Se NMR signal at 1200 ppm. Milne[32] indicates the redox stability of selenate under such media

TABLE 2 The chemical shift change of ^{77}Se and ^{1}H (methyl) chemical shift of selenate and DSS, respectively, upon varying certain measurement parameters in the given ranges

Parameter	Range investigated	$\Delta\delta^{^{77}Se}_{SeO_4^{2-}}$	Relative error	$\Delta\delta^{^{1}H}_{DSS}$	Relative error
T (K)	293–328	1.49	0.053%	0.29	0.63%
c (mol·dm^{-3})	0.0025–0.5	0.46	0.016%	0.10	0.22%
I (mol·dm^{-3})	0.15–1	0.59	0.021%	0.08	0.17%
pH	1.36–11.68	2.78	0.099%	0.03	0.07%
	0.66–13.09	4.97	0.178%	0.14	0.30%
V/V% D_2O	5–100	0.35	0.013%	0.02	0.04%
t (days)	127	0.05	0.002%	0.02	0.04%
	133	0.08	0.003%	0.01	0.02%
Reliable pH intervals					
pH range	**Ionic strength**	$\Delta\delta^{^{77}Se}_{SeO_4^{2-}}$	**Relative error**	$\Delta\delta^{^{1}H}_{DSS}$	**Relative error**
3.40–11.68	$I = 0.15$ **mol·dm^{-3}**	**0.03**	**0.001%**	**0.04**	**0.09%**
3.55–13.09	$I = 1.0$ **mol·dm^{-3}**	**0.11**	**0.004%**	**0.11**	**0.24%**

Note. The relative error is calculated by expressing the chemical shift change as a percentage relative to the typical NMR chemical shift range of the nucleus in question. The data above pH 3.5 are highlighted as reliable pH intervals, as this is the interval where pH has no significant effect on the chemical shift of the reference.

A multiple linear regression model was fitted to the data above pH 3.5 as an acceptable approximation for a deterministic model to investigate the significant measurement parameters (Table 3). Using a significance level of 0.05 it was revealed that the following parameters had no significant contribution to the linear model above pH 3.5: pH, selenate concentration and time elapsed after sample preparation.

Neglecting the parameters with insignificant perturbing effects, the following equation arises to determine selenate ^{77}Se chemical shifts in any custom measuring environment falling into our range of investigation:

$$\delta_{\mathrm{TMS}} = 1034.8 + 0.0390 \cdot T(\mathrm{K}) + 0.434 \cdot I\left(\mathrm{mol \cdot dm^{-3}}\right) + 0.0019 \cdot V/V\%\mathrm{D_2O} \quad (2)$$

The value of the adjusted coefficient of determination ($R^2 = 0.8935$) and the fact that every measured ^{77}Se chemical shift value was reproducible with a maximum of 0.22 ppm error also verify the results. The random errors of determination showed a normal distribution.

In order to achieve consistent chemical shift data with previous literature as well, the chemical shift of selenate was determined with respect to the more commonly used ^{77}Se chemical shift standards, such as TMS, DSS and dimethyl selenide. The results are shown in Table 4.

The ^{77}Se chemical shift of selenate ions in aqueous solution was investigated by changing six measurement parameters. Of these six parameters the pH proved to have the largest effect on the selenate signal, however the selenate protonation occurs with a logK < 2. Therefore the effect of pH on the signal is insignificant above pH = 3.5; at lower pH values the expected chemical shift can be predicted based on the titration curves. It is noteworthy that the most commonly used aqueous chemical shift reference, DSS, also undergoes protonation at such pH values. The chemical shift error caused by protonation relative to the spectral width is comparable for the two materials.

TABLE 3 Results of the multiple linear regression applied to data at pH > 3.5

Constant	**1,034,795**		
parameter	coefficient	standard deviation	*p* value
T (K)	0.0390	0.0028	<0.0001
c (mol·dm^{-3})	−0.080	0.088	0.3626
I (mol·dm^{-3})	0.434	0.033	<0.0001
pH	−0.0057	0.0074	0.4493
V/*V*% D_2O	0.00193	0.00063	0.0033
t (days)	−0.00036	0.00049	0.4678

Note. The parameters with a *p* value lower than 0.05 were accepted as statistically significant.

TABLE 4 ^{77}Se NMR chemical shift of selenate ions (5 V/V% D_2O, T = 25°C, I = 0.15 mol·dm^{-3}) with respect to the currently used chemical shift references for ^{1}H and ^{77}Se

^{77}Se NMR chemical shift of selenate with respect to		
^{1}H signal of 0.03 *V*/*V*% TMS in $CDCl_3$	^{1}H methyl signal of 1 mmol·dm^{-3} DSS in H_2O containing 5% D_2O	^{77}Se signal of 60 *V*/*V*% dimethyl selenide in $CDCl_3$
1046.40 ppm	1043.96 ppm	1048.65 ppm

The temperature coefficient in a linear regression model was found to be 0.042 ppm/K while keeping other parameters constant; on the other hand in a multivariate statistical analysis it proved to be 0.039 ppm/K. This value is in agreement with those of the two ^{77}Se chemical shifts introduced so far, i.e. dimethyl selenide (0.025 ppm/K)[14] and selenous acid (0.094 ppm/K).[32] The concentration, ionic strength and D_2O content of the solvent affect the selenate chemical shift by one order of magnitude lower, compared to the two measurement conditions mentioned above. Based on a multiple linear regression analysis, the selenate concentration is not a significant parameter, while ionic strength and solvent composition have a significant effect in the model.

Selenate ion-containing aqueous solutions at various ionic strengths and extreme basic pH values were stored at room temperature for 130 days, under which the ^{77}Se NMR spectrum showed no change. Compared to the currently known water-soluble reference, selenous acid, the properties of selenate are much more favorable. It has been shown experimentally that selenate salts can be used even in reducing media required for the analysis of diselenide/selenol compounds, since selenate reacts neither with thiol-containing reducing agents nor with the selenium-containing substance to be measured. Compared to the uncertainty in DSS methyl chemical shift relative to the common ^{1}H spectral width, the uncertainty of selenate chemical shift relative to the common ^{77}Se spectral width is comparable or lower in case of each six investigated measurement parameter.

5 | CONCLUSION

In this work sodium selenate was introduced as an internal chemical shift standard for ^{77}Se NMR measurement of aqueous solutions. The perturbing effect of pH, temperature, ionic strength, selenate concentration and D_2O content on the chemical shift was investigated. Selenate

proved to be a suitable internal chemical shift reference above pH 3.5, where the following parameters have significant effect on the chemical shift based on a multiple linear regression model: temperature, ionic strength and D_2O content. Moreover, selenate was found to be stable under the reductive conditions of dithiothreitol, the strong reducing agent, therefore selenate is suitable for investigation of diselenide compounds that require reduction prior measurement. Based on our results, sodium selenate is the most suitable internal chemical shift reference for aqueous ^{77}Se NMR in the literature.

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PEER REVIEW

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