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Polyamine-based spin probes for the study of siliceous structures

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ABSTRACT

Three new spin probes containing polyamine chains (2 or 3 nitrogen atoms) and nitroxide were synthesized. These compounds are stable in aqueous media at pH 5–10, and shape and intensity of their ESR spectra do not depend on pH in this range. The involvement of the spin probes in association in solution results in decrease of the spin mobility which appears as spectral anisotropy. The polyamine spin probes bind to siliceous nanoparticles in solution resulting in a reversible decrease in spectral intensity. These observations open a new way for monitoring silicic acid condensation. Sorption of the spin probes on solid silica gives rise to anisotropic spectra. The polyamine spin probes penetrate into growing cells of diatom algae in a similar manner to polyamine-containing fluorescent dyes.

1. Introduction

Condensation of silicic acid in aqueous medium is actively studied nowadays. Biosilicifying organisms such as diatoms can store a lot of silicon as these single-cell algae build their siliceous exoskeleton (Fig. 1). The structure and properties of biogenic silica are close to those of amorphous quartz glass [1] in spite of formation at ambient temperatures. Study of the biosilicification mechanism is aimed at invention of ecology-friendly approaches to new siliceous and composite materials. Condensation of silicic acid in solution results in sols, gels and solid products [2,3]. Several methods are used to study this process: colorimetric determination of monomeric $Si(OH)_4$ with molybdenum blue assay [2,4], ^{29}Si NMR to measure siliceous structures of various degrees of condensation [5] and IR spectroscopy for studying silanol groups in solid materials [5]. Formation of the primary poly(silicic acid) particles is a very important step in the pathway from silicic acid to siliceous gels and solid materials. These primary particles are poorly visible via light scattering due to their small size and a refractive index close to that of water value. ^{29}Si NMR is not an appropriate method for these objects too because of low sensitivity with the low, natural ^{29}Si content.

Spin probes, which contain moieties capable of interacting with various surfaces, nano- and microparticles [6,7], mesoporous materials [8–12], and membranes [13], allow monitoring of the formation of the corresponding structures and thus permit study of the properties of these objects [6–16]. Electron spin resonance (ESR) gives information about the microenvironment around a spin probe because polarity, viscosity and dynamics of the environment alter ESR spectra [17,18]. The nitroxide in aqueous solution undergoes fast and isotropic spinning. Restrictions to this spinning result in anisotropic broadening of the spectral lines, change of the spectral component amplitudes and shift of the outside components [13,17,18]. Spin probes can be covalently bonded with the materials being studied or they can bear moieties capable of association with active particles by means of ionic, covalent or hydrophobic interactions.

Siliceous materials interact with amines and tertiary amine salts through hydrogen and/or ionic bonds [3] which allows the use of amine containing spin probes in the study of these materials.

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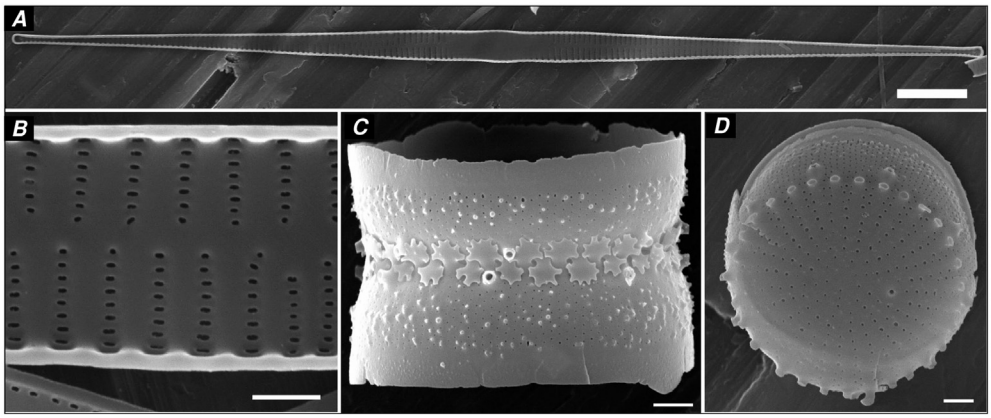

Fig. 1. SEM images of siliceous valves of diatoms *Ulnaria ferefusiformis* Kulikovskiy & Lange–Bertalot (A and B) and *Stephanodiscus meyerii* Genkal & Popovskaya (C and D) from Lake Baikal. Scale bars are 10 μm (A) and 1 μm (B–D).

Investigation of the interaction of these positively charged spin probes with silica gel samples showed that the spin probe is not associated with silica gel surface but placed in surface boundary area [19]. pH-Sensitive spin probes were studied in canals and pores of mesoporous siliceous materials [6]. Nitroxyl spin probes with phenyl and pyridyl moieties were found to be able to interact with hydrophobic and hydrophilic surface domains which allows monitoring of acidity and electric potential in the canals of mesoporous materials. Covalent immobilization of 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-amino-TEMPO) onto siliceous particles was used to study the particles' mobility [20] and to monitor interaction between negatively and positively charged nanoparticles [21].

The known amine containing spin probes have one amino group only which does not allow strong interactions with siliceous materials. We have found [22] that oligomeric propylamines containing 2–3 nitrogen atoms can catalyze condensation of silicic acid and associate with siliceous particles. These propylamines are synthetic analogs of biogenic amines from siliceous frustules of diatom algae [23] and of spermine. Spermine plays an important role in cell division [24]. This work is aimed at synthesis of TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl) [25] derivatives containing two or three amino groups (Fig. 2). We have studied paramagnetic properties of the new probes and composite systems including the probes and solid silica, soluble and gel-like poly(silicic acid) and frustules of the living diatoms after culturing them in the presence of the spin probe.

2. Experimental section

2.1. Reagents and materials

Toluene, acetic and formic acids, aqueous ammonia (25%), 1-methylimidazole, NaOH, $Na_2SiO_3 \cdot 5H_2O$, 1 M HCl, reagents for the molybdenum blue assay (ammonium molybdate, oxalic acid, 4-methylaminophenol sulfate, sodium sulphite, standard silicate solution, hydrochloric acid (35%), and sulphuric acid (98%)) were purchased from Aldrich, Fisher, Panreac, or Acros chemicals and used without further treatment. Polyamines N,N-Bis[3-(methylamino)propyl]methylamine (N3), N^1-[3-(Dimethylamino) propyl]-N^1,N^3-dimethyl-1,3-propanediamine (N3–H) and N,N-Bis [3-((3-methylaminopropyl)methylamino)propyl]methylamine (N5) were synthesized according to the methods in our previous publications [22,26]. Nitroxides TEMPONE [27], and 4-Iodoacetamido-TEMPO [28] were prepared according to the protocols given in those papers. 20 mM buffer solutions were prepared in deionized water by adjusting pH with 0.1, 1 M NaOH and HCl (sodium formate – pH 3, sodium acetate – pH 5, 1-methylimidazole - pH 7, aqueous ammonium solution – pH 10). The following solid siliceous materials were used for

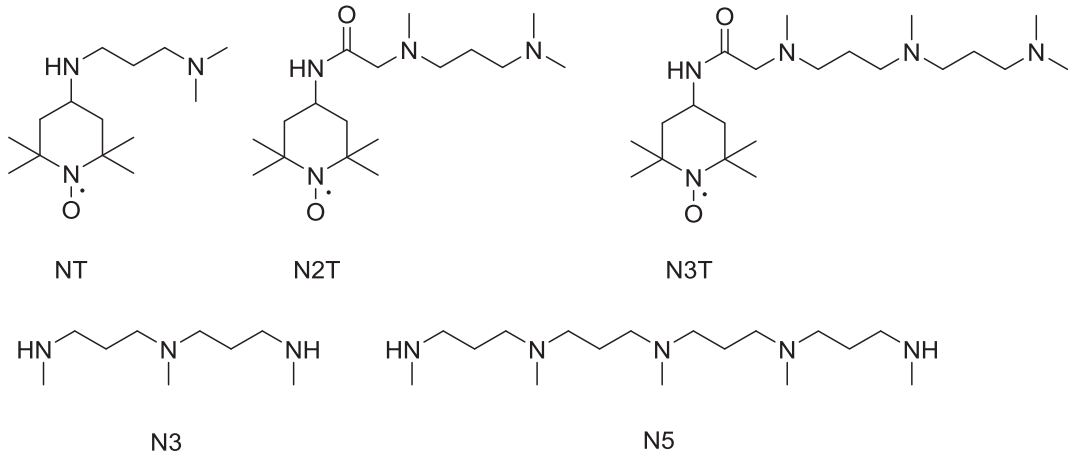

Fig. 2. New spin probes and polyamines.

immobilization of spin probes: chromatography sorbent Panreac (silica gel 60, 63–200 microns) and diatom frustules after alkali etching [29].

2.2. Synthesis of the polyamine spin probes

4-((3-(Dimethylamino)propyl)amino)-2,2,6,6-tetramethylpiperidin-1-oxyl (NT): $NaBH_3CN$ (0.5 g, 7.5 mmol) was added to a stirred solution of TEMPONE (0.85 g, 5.0 mmol) and *N′*,*N′*-dimethylpropane-1,3-diamine (616 μL, 5.0 mmol) in anhydrous methanol (15 mL), and the pH was adjusted with CH_3COOH to 5. After stirring for 27 h at ambient temperature, the solvent was removed under reduced pressure and then H_2O (20 mL) was added, the pH was adjusted with 3% HCl to 3, the mixture was then extracted with $CHCl_3$, and the organic layer was discarded. The pH of the aqueous layer was adjusted with NaOH to 9–10 and extracted with $CHCl_3$ (10 mL × 5). The combined extracts were dried over $MgSO_4$, the solvent was removed under reduced pressure, the residue was purified by column chromatography on Al_2O_3 ($CHCl_3$). The product NT (4.8 mmol, 1.23 g) was obtained as a red oil with a yield of 93%.

4-(2-((3-(Dimethylamino)propyl)(methyl)amino)acetamido)-2,2,6,6-tetramethylpiperidin-1-oxyl (N2T) and 4-(2-((3-((3-(dimethylamino)propyl)(methyl)amino)propyl)(methyl)amino)acetamido)-2,2,6,6-tetramethylpiperidin-1-oxyl (N3T): The mixture of anhydrous K_2CO_3 (1.4 g, 10 mmol), 4-Iodoacetamido-TEMPO (0.68 g, 2.0 mmol) and corresponding propylamine (2.0 mmol) in acetone (20 mL) was stirred for 48 h. The inorganic precipitate was filtered off, the solvent was removed under reduced pressure and the residue was purified by column chromatography on Al_2O_3 ($CHCl_3$). The product N2T (1.3 mmol, 0.43 g) was obtained as red oil with a yield of 66%. The product N3T (1.0 mmol, 0.40 g) was obtained as a red oil with a yield of 51%.

Characterizations of NT, N2T and N3T are described in detail in the Supporting Information (SI) (Figs. 1–12 in SI).

2.3. Association of propylamines in the presence of spin probe

To study the association of oligopropylamine (N5) in the micelles the following procedure was performed: 30 mM N5 solution was prepared at different pH values using 1 M HCl and an aliquot of the spin probe (final concentration of 0.1 mM) was added. Measurements were performed within a few hours and also a day after spin probe addition.

2.4. Condensation of $Si(OH)_4$ with a spin probe

Sodium silicate solution (20 mM) was used as a precursor of $Si(OH)_4$ in this study. The desired pH (5.5, 7 and 10) was adjusted with 1 M HCl over 30–60 s using predetermined volumes of the acid. The spin probe (0.1 mM) was added to the $Si(OH)_4$ solutions just after the pH was adjusted. Concentration of orthosilicic acid (in the form of monomer and dimer) was measured by the molybdenum blue colorimetric method [2,4].

OSCM-1 and OSCM-2 precipitates were synthesized as follows: solution of $Si(OH)_4$ (100 mM), N3–H solution (100 mM), solution of the spin probe N3T (5 mM) and water were mixed to a final concentrations 10 mM, 10 mM, 0.02 (OSCM-1) or 0.1 mM (OSCM-2), respectively. The pH was adjusted to the desired pH 7 with 1 M HCl over 30–60 s using predetermined volumes of the acid. The turbid solution was left overnight, then centrifuged, washed with cold water and lyophilized.

The gel of polysilicic acid (100 mM) was prepared in the presence of the spin probe (0.1 mM) at pH 7, the gel was allowed to stand for three days, washed with water three times and freeze-dried giving sample abbreviated as SSG.

2.5. Sorption on silica and diatom valves

Sorption of the spin probe N3T to the surface of silica and diatom valves was performed from an aqueous solution at different concentrations of N3T (50, 5 and 0.5 mM) using 125 μL of a solution of the spin probe to 10 mg of material left to stand for a day. Then the samples were centrifuged, washed with water three times while being sonicated, and dried under vacuum.

2.6. Diatom cultivation

A clonal culture of the diatom *U. ferefusiformis* was isolated from phytoplankton of Listvennichny Bay, Lake Baikal, Russia. The diatoms were cultivated in DM medium [30] with silicon content of 0.11 mM (in the form of sodium silicate). The cultivation details are described in Ref. [31]. The spin probe NT was added into cultural medium bringing it to 0.003 mM concentration. The diatom growth was continued for 30 days. New portions of the spin probe and sodium silicate solution (half of the initial concentration) were added to the medium on the 7-th and 14-th days of the experiment. The cells obtained were collected by centrifugation (3000 g), washed with water three times and dried under vacuum.

2.7. Methods

^1H NMR spectra were recorded at 300 or 500 MHz, and ^{13}C NMR spectra were recorded at 75 or 125 MHz, as indicated next to each NMR analysis. To confirm structure of nitroxides, N_2D_2 (5–10 mg) was added into NMR tube with nitroxide solution in CD_3OD, and the sample was left for 12 h before recording the spectra. ^1H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak. IR spectra were acquired on an FT-IR spectrometer and are reported in wave numbers (cm^{-1}). Reactions were monitored by TLC on Al_2O_3 carried out using UV light or iodine as the visualizing agent. Column chromatography was performed on Al_2O_3 with eluent $CHCl_3$. HRMS were recorded on a double-focusing, high resolution mass spectrometer equipped with high performance toroidal ESA.

The surface area and porosity of the samples were measured by sorption of nitrogen using the instrument “Sorbtometr M” (Katakon, Novosibirsk). ESR spectra were recorded with an FT X-band Bruker ELEXSYS E580 spectrometer (X-wave range 9.4 GHz). The precision of the measurement of the g-factor was ±0.0002. CW ESR spectra were recorded at the following conditions: amplitude modulation 0.3 G, modulation frequency 100 kHz, averaged scans 5 (solid samples), receiver gain 30 dB (solutions) and 60 dB (solid samples), time constant 0.02 s, conversion time 0.06 s, field range 100 G/centre field 3348 G (solutions) and field range 300 G/center field 3410 G (solid samples), microwave power 0.6325 mW at room temperature. The concentrations of paramagnetic centers were calculated by the established method [32] with the use of diphenylpicrylhydrazyl as a standard.

The following values were obtained from the ESR spectra:

- the main values of the tensor of hyperfine constants ($A_{||}$ and $A_{\perp}$) were calculated by simulation of ESR spectra with a program published in Ref. [33], the hyperfine interaction (HFI) was limited to the second-order term and the main axes of the g-tensor and the HFI tensors coincide (Table 2 and SI, Fig. 20);
- rotational correlation time τ_c (s) was calculated according to [34,35]:

$$\tau_c = 6.5 \cdot 10^{-10} \Delta H_0 \left(\sqrt{\frac{I_0}{I_{-1}}} - 1 \right)$$

Where ΔH_0 (in Gauss) is the line width of the central line and I_0 and I_{-1} are the line heights of central and high field lines, respectively.

3. Results and discussion

The new polyamine spin probes were prepared using two methods (Fig. 3). The nitroxide NT was synthesized from TEMPONE by reductive amination according to a modified procedure developed by Rosen et al. [36]. The mixture of TEMPONE and *N*,*N*-dimethyl-1,3-diaminopropane was treated with $NaBH_3CN$ in anhydrous methanol. The spin probes N2T and N3T were prepared in moderate yields via alkylation of the corresponding polyamines with convenient spin label 4-Iodoacetamido-TEMPO. The reaction proceeds unselectively giving a mixture of products of alkylation onto different nitrogens in polyamine. The nitroxides N2T and N3T were isolated from these mixtures using chromatography on Al_2O_3.

ESR spectra of the spin probes in aqueous medium in 0.01–1 mM concentration look like isotrope triplets (Fig. 13 in SI). The shape and intensity of the spectra were independent of pH over the range pH 3–10 for over 2 days (Fig. 4, Figs. 14 and 15 in SI). The signal did not change visibly after 82 days (Fig. 15A–C in SI) and its intensity was still 95% of its initial value. The isotropic parameters for synthesized spin probes are presented in Table 1. Rotational correlation time τ_c is $(1.0 \pm 0.5) \cdot 10^{-10}$ s for all spin probes in the aqueous solutions.

The condensing of spin probes into aggregates which is accompanied with increase in microviscosity can result in limitation of the probe mobility at room temperature. This becomes apparent in anisotropy of the ESR spectrum. Polyamines containing five and more nitrogen atoms in the chain are capable of aggregating in aqueous medium giving submicrometer drops which are stable in alkali and neutral area but dissociate at low pH under amine protonation [37]. We expected that the spin probes with polyamine tails could be involved in these aggregates resulting in spectral changes. The results obtained (SI, Fig. 16) show anisotropy of the ESR spectrum in the presence of N5 polyamine. As expected, decrease of intensity of the third spectral component is more pronounced at high pH values.

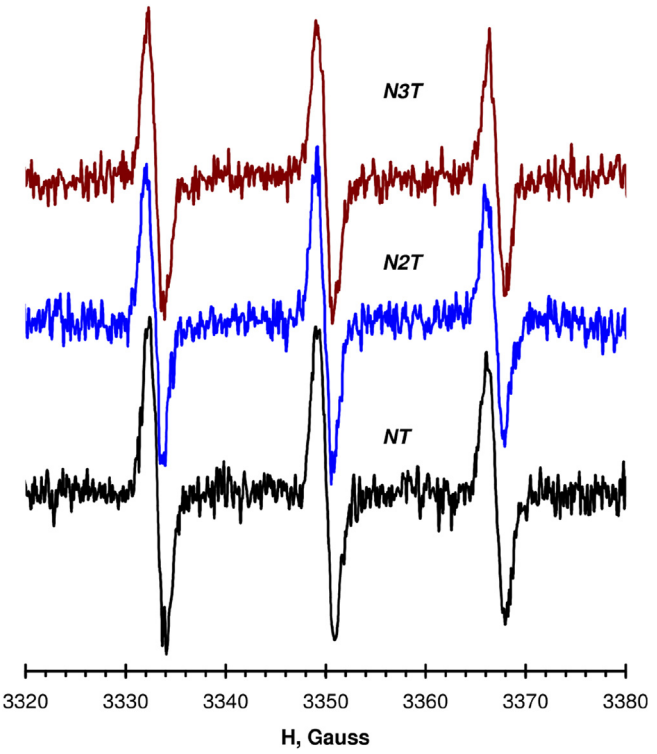

Fig. 4. ESR spectra of the spin probes (0.1 mM solutions) at pH 7 in 1-methylimidazole buffer.

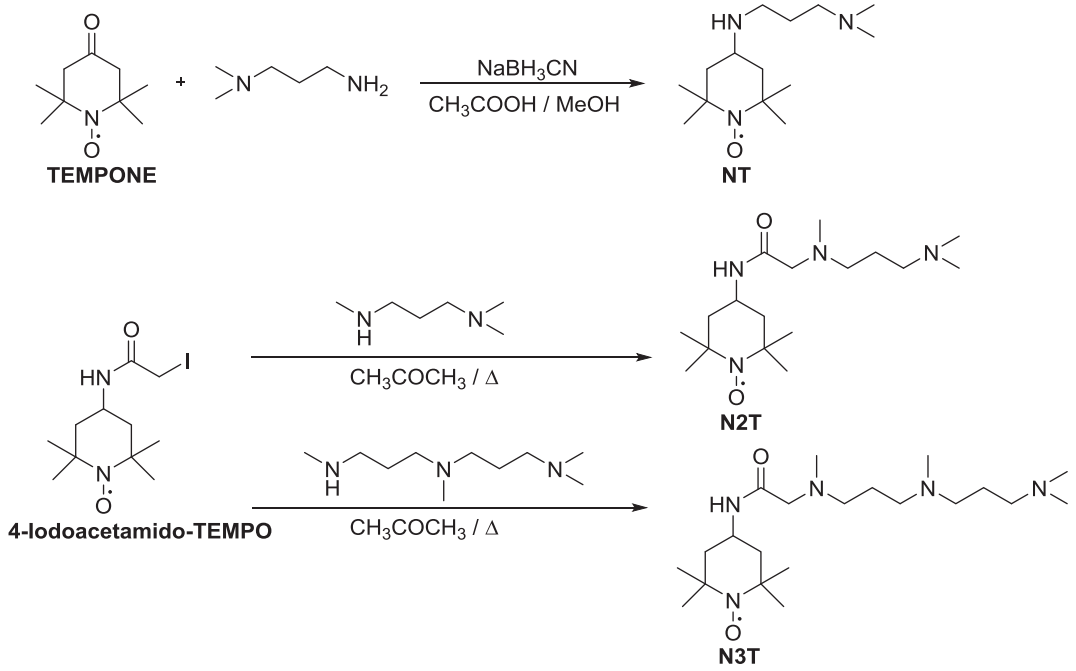

Fig. 3. Synthesis of the polyamine spin probes.

Table 1
Parameters of isotropic spectra for synthesized spin probes at pH 5.

Parameters	N3T	N2T	NT
g	2.0057	2.0055	2.0058
ΔH_0, G	1.83	1.77	1.47
I_{+1}	0.198	0.195	0.690
I_0	0.182	0.202	0.695
I_{-1}	0.152	0.157	0.587
a_N, G	17.07	17.04	16.84

The next objects studied with new spin probes were siliceous nanoparticles which are obtained in aqueous solutions during condensation of silicic acid. These particles are formed when sodium silicate in neutralized with acid at silicon concentrations of more than 3 mM. $Si(OH)_4$ condensation proceeds till equilibrium concentration 2–3 mM giving rise to soluble submicrometer particles, gels or precipitates depending on pH and starting concentration.[2,3] We used 20 mM solutions of silicic acid at pH 5.5–10. These conditions allow us to obtain stable solutions of siliceous nanoparticles after several hours of the reaction. pH 5.5 corresponds to the pH in silica deposition vesicles of the diatoms - intracellular vesicles in which parts of siliceous frustules are synthesized [38].

The introduction of the N3T probe into a solution of silicic acid at pH 7 results (Fig. 5) in a gradual decrease of the signal intensity without change of its shape. The intensity decrease was also observed at pH 10 for NT and N2T probes (Fig. 6 and SI, Fig. 17). The signal intensity was not changed at pH 5.5 (Fig. 6). The concentration of unreacted silicic acid (in monomeric and dimeric forms) can be measured with the use of the molybdate method [2,4]. The

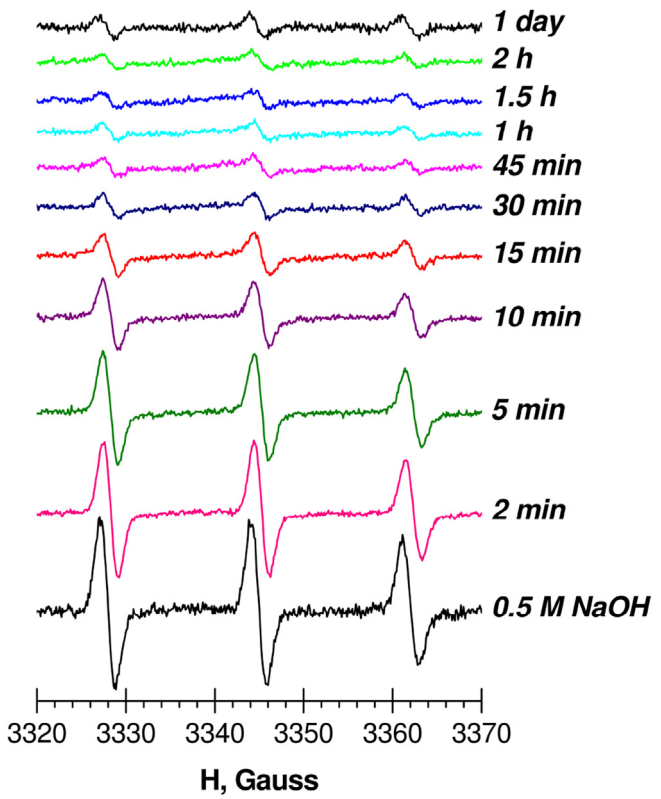

Fig. 5. ESR spectra obtained during condensation of silicic acid (20 mM) in the presence of N3T probe (0.1 mM) at pH 7. 0.5 M NaOH curve corresponds to 1:1 addition of 1 M NaOH after a day of the reaction. Intensity of the ESR signal was twice increased for 0.5 M NaOH curve.

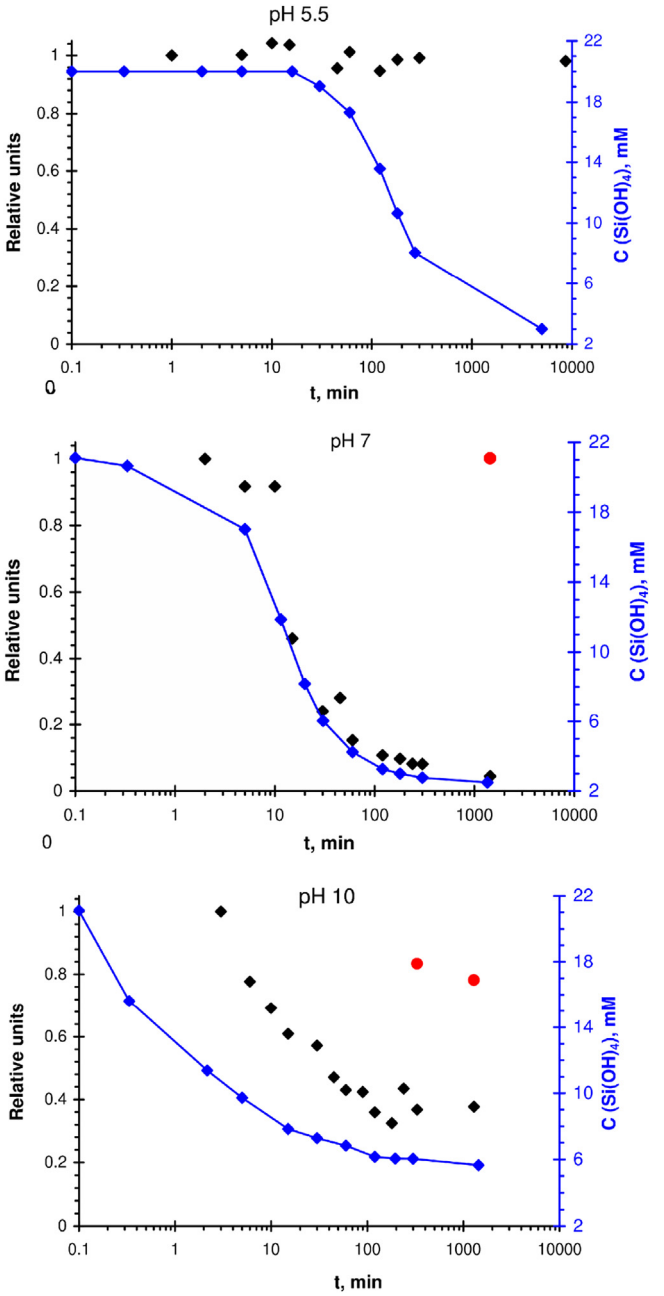

Fig. 6. Dependence of relative values of double integrals from ESR spectra intensity (black points) and free silicic acid concentration (blue lines) on time. Starting $Si(OH)_4$ concentration was 20 mM, N3T - 0.1 mM. Red points correspond to the double integrals after addition of NaOH in 0.5 M concentration to the condensation products. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

data presented in Fig. 6 and SI, Fig. 17 show correlated change of $Si(OH)_4$ concentration and intensity of ESR signal at pH 7 and 10 but condensation at pH 5.5 does not affect the ESR spectra. The most plausible reason for the intensity decrease is the interaction between the spin probe and siliceous particles by means of hydrogen and/or ionic bonds between amine and silanol groups. This interaction considerably retards the probe motion which results in the intensity decrease [18]. The amino groups at pH 5.5 are mostly protonated which prevents interaction with Si–OH groups and the probe remains in a free state. An alternative explanation is that some kind of the probe destruction under some reaction occurs on

the surface of siliceous particles. The latter hypothesis was verified by the addition of 1 M NaOH to the solutions after a day of the reaction up to 0.5 M concentration. The alkali destroys siliceous particles and prevents any interactions with the spin probe because silanol groups are ionized at these conditions and amino groups are deprotonated. The alkali action resulted (Figs. 5 and 6 and SI, Fig. 17) in near full recovery of the probe signal which is the evidence of the probe stability in the interaction with siliceous particles.

Interaction of the new spin probes with solid silica was studied using four systems: silica precipitated from a solution in the presence of probe, siliceous chromatography sorbent, siliceous frustules of diatoms and living diatom *U. ferefusiformis* after culture in the presence of spin probe (Table 2). Anisotropy spectra typical for free radicals immobilized on a solid phase were observed in all these cases (Figs. 7–9 and SI, Figs. 18 and 19). The presence of ESR signal from the diatom biomass shows that the polyamine spin probes penetrate into growing cells of diatoms in a similar manner to polyamine-containing fluorescent dyes [31]. TEMPONE containing compounds show triaxial anisotropy of g-tensor ($g_{xx} \neq g_{yy} \neq g_{zz}$) and tensor of hyperfine interactions ($A_{xx} \neq A_{yy} \neq A_{zz}$) [17,18]. In our cases the poor resolution of the lines in the "perpendicular" area of the ESR spectra of the spin probes immobilized on surface and inside siliceous materials can be caused by various static and dynamic reasons of anisotropic origin. The observed averaging and broadening of the lines is probably resulted from slow rotation of spin probe. In this case we can estimate correlation time τ_c from the following consideration: the rotation rate (τ^{-1}) is high enough for averaging lines in the "perpendicular" area of the ESR spectra but is not enough for the averaging in the "parallel" area. Comparing our results with the TEMPONE data [18] we estimated τ_c for silica immobilized spin probes as $5 \cdot 10^{-9}$–$7 \cdot 10^{-8}$ s.

The specific sorption of polyamine chains on the silica materials proceeds by the interaction of donor nitrogen atoms with silanol groups. Surface density of the silanol groups on silica does not exceed 5 nm^{-2} and depending on conditions of synthesis, drying and storage this value can decrease due to condensation of the Si–OH groups [39,40]. Taking into account that three silanol groups are necessary for full interaction of N3T with the siliceous surface, we can expect that the unrestricted disposition of the probe requires that it be within 1 nm^2 of the surface. The increase of the probe content results in incomplete binding with the surface and more unrestricted rotation of the spin. Multilayer sorption is also possible in this case which would increase the mobility of spin probes too. This results in the absence of pronounced hyperfine structure in the case of concentration of the immobilized probe more than 1 spin/nm^2 (Table 2, samples Dia1-1, Dia1-2, Dia2-1, Dia2-2, Dia3-1 and Dia3-2). Chromatography sorbent (Table 2, SG-Y samples) is an exception: the clear hyperfine structure is not visible at 0.02 spin/nm^2. Possibly, the high surface area in this sample is obtained with narrow pores and spin probes in these

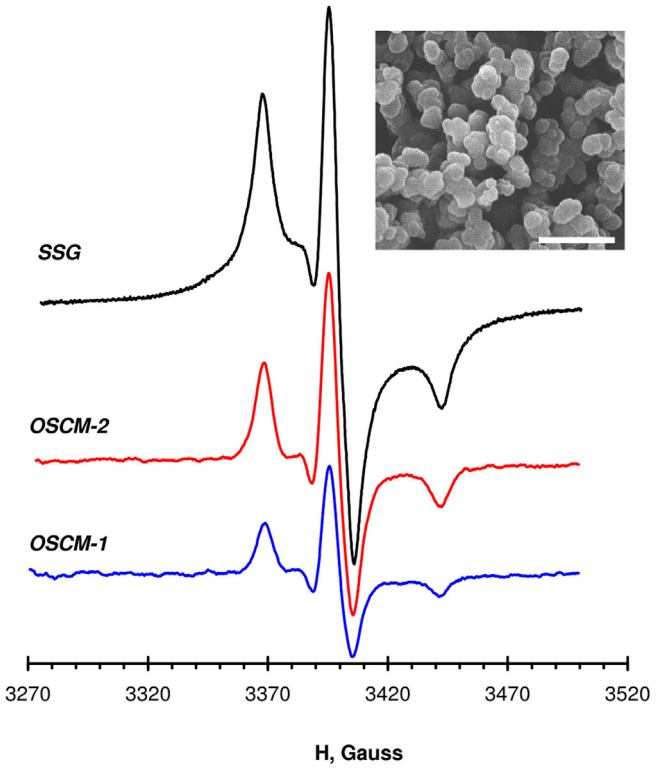

Fig. 7. ESR spectra of the samples obtained by silicic acid condensation in the presence of N3T probe. Intensity of OSCM-1 and OSCM-2 spectra was amplified by the factor of 5. Insertion – SEM image of OSCM-1 precipitate, scale bar represents 2 μm.

Table 2
Parameters of ESR spectra of N3T probe immobilized on solid siliceous materials.

Sample[a]	Surface area, m^2/g	Concentration of the immobilized probe			$g_{\perp}$[b]	$g_{\parallel}$[b]	$A_{\perp}$[b]	$A_{\parallel}$[b]
		$N \cdot 10^{-19}$, spin/g	mmol/g	spin/nm^2				
Dia1-1	60	21.00	0.3414	3.43	–	–	–	–
Dia1-2	60	13.00	0.2117	2.12	–	–	–	–
Dia1-3	60	1.70	0.0282	0.28	2.0074	2.0025	7.6	34.9
Dia2-1	305	54.00	0.9011	1.78	–	–	–	–
Dia2-2	305	29.00	0.4812	0.95	–	–	–	–
Dia2-3	305	1.90	0.0310	0.06	2.0073	2.0030	7.8	37.2
Dia3-1	19	3.90	0.0644	2.04	–	–	–	–
Dia3-2	19	3.90	0.0655	2.07	–	–	–	–
Dia3-3	19	1.30	0.0223	0.71	2.0073	2.0028	7.4	37.0
OSCM-1	–	2.20	0.0361	–	2.0075	2.0026	7.3	36.8
OSCM-2	–	7.80	0.1289	–	2.0075	2.0023	7.2	37.1
SSG	–	3.20	0.0531	–	2.0075	2.0022	7.0	37.8
SG-1	530	220.00	3.6882	4.19	–	–	–	–
SG-2	530	13.00	0.2202	0.25	–	–	–	–
SG-3	530	1.30	0.0210	0.02	–	–	–	–
U. ferefusiformis (with NT probe)	–	0.37	0.0062	–	2.0077	2.0024	7.0	36.5

[a] DiaX-Y: siliceous frustules of *S. meyerii* (*X* – *1 and 3*) *and U. ferefusiformis* (*X* – *2*), Y – corresponds to the probe amount *per* frustules, 0.625, 0.0625, 0.00625 mmol/g for Y = 1, 2 and 3 respectively. OSCM-1 and OSCM-2 – precipitates obtained by condensation of silicic acid in the presence of polyamine N3. SSG – freeze-dried gel of silicic acid. SG-Y – chromatography sorbent with immobilized probe, Y corresponds to the probe amount *per* sorbent, 0.625, 0.0625, 0.00625 mmol/g for Y = 1, 2 and 3 respectively.
[b] Data were specified with help of the computer modeling.

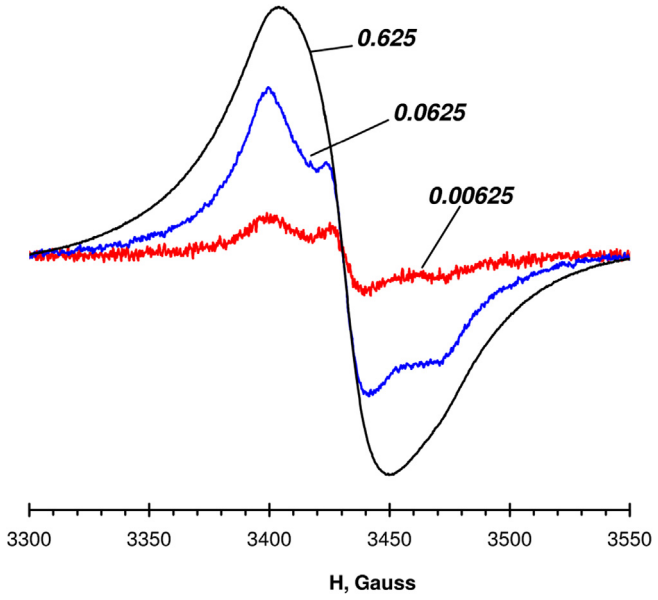

Fig. 8. ESR spectra of N3T probe after sorption on chromatography sorbent. Numbers correspond to the probe amount *per* sorbent, mmol/g. Intensity of 0.625 mmol/g data is decreased tenfold.

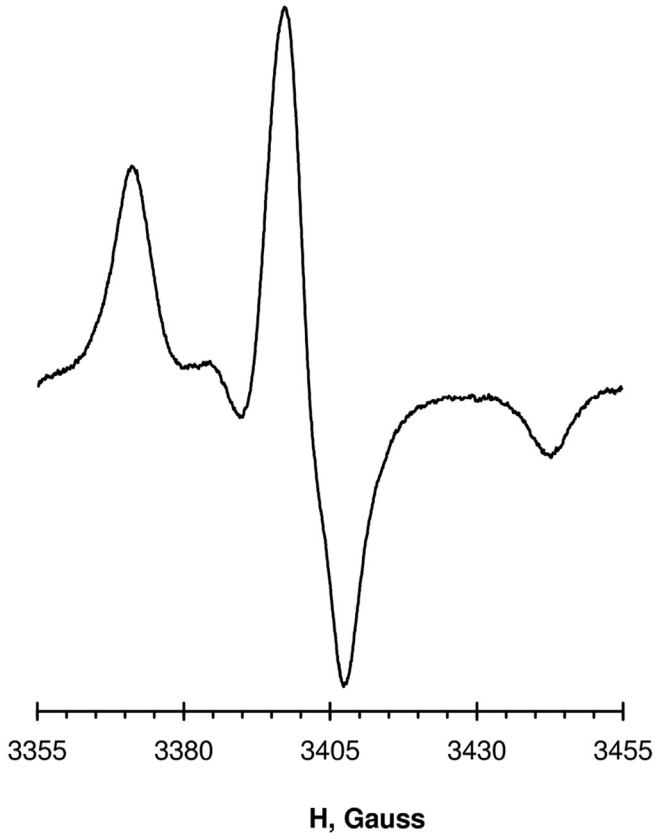

Fig. 9. ESR spectra of diatom algae *U. ferefusiformis* cultivated for one month in the presence of NT probe.

pores are close to each other giving rise to an exchange interaction which suppresses the hyperfine structure of the spectra.

4. Conclusion

We have synthesized three new spin probes which contain polyamine fragments with two or three nitrogen atoms. These compounds are stable in aqueous media at pH 5–10 for a long time, and shape and intensity of their ESR spectra do not depend on pH in this range. The involvement of the spin probes in aggregates in solution results in decrease of the spin mobility which appeared in spectral anisotropy. The polyamine spin probes aggregate with siliceous nanoparticles in solution, which is accompanied by a reversible decrease in spectral intensity. These observations open a new way for monitoring silicic acid condensation. Sorption of the spin probes on solid silica gives rise to anisotropic spectra and parameters of these spectra give information about structure of pores in siliceous materials. The ability of polyamine spin probes to penetrate into growing cells of diatoms allows to use these substances as a new tool for study biosilicification processes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micromeso.2017.01.010.

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