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Chemical properties of polyamines with relevance to the biomineralization of silica†

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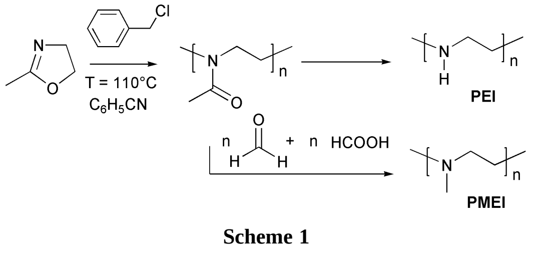
Polyamines mimicking substances which occur naturally in biosilicas have been synthesized and show an accelerating effect on silica condensation, which depends on the chemical nature, the architecture (linear or branched), and the degree of polymerization.

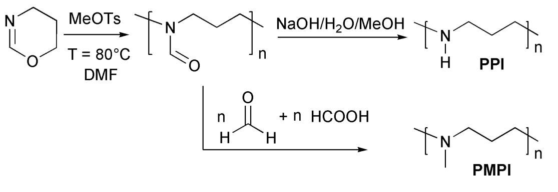
Important bioorganic components taking part in the biomineralization process of silica have been identified recently.[1] For example, polysaccharides[1] and proteins (silicatein from sponges,[2] silaffin from diatoms[3,4]) have been isolated from biosilica minerals. For the biomineralization of silica in diatoms, also simple polyamines as polypropylene imine derivatives play an important role.[5] Sumper has developed a model to explain the pattern formation in diatom shells by phase separations occurring in silica solutions of these polyamines.[6] We describe the synthesis of polyamines modelled on the naturally occurring substances as well as their acid-base behaviour and their influence on the kinetics of silica condensation.

In order to model the polyamines found in diatoms, which are all linear and have a relatively low degree of polymerization P_n of 10 to 20 monomer units, we have synthesized polyamines by ring-opening polymerization of oxazolines or 1,3-oxazines,[7] respectively, and subsequent hydrolysis.

Linear polyethylene imine (PEI) was prepared by polymerisation of methyloxazoline (Scheme 1). The prepolymer polyacetylethylene imine has a molecular weight of approximately 830 g mol^{-1} corresponding to a degree of polymerisation P_n = 8–9. Linear polypropylene imine (PPI) can be prepared with essentially the same chemistry, with 1,3-oxazine[8] used as monomer (Scheme 2). The corresponding prepolymer has a P_n of 12–13 (determined by NMR spectroscopy). The prepolymers are subsequently either hydrolysed by aqueous sodium hydroxide, yielding linear PEI or PPI, respectively, or are subjected to a combined hydrolysis/Leukart–Wallach reaction, yielding the methylated variants, PMEI or PMPI, respectively. Both polymer-analogous reactions do not change P_n. The hydrolysis is almost complete as no residual acetyl groups can be detected by ^{1}H NMR. The degree of methylation was verified by means of NMR spectroscopy to be larger than 99% for PMEI as well as for PMPI. 1,5,9,14,18,22-hexaazadocosane is prepared by Michael addition of acrylonitrile to spermine.[9]



Scheme 1



Scheme 2

It is expected that the pH of a polyamine-silica solution strongly influences the condensation behaviour. We therefore characterized the acid-base behaviour of the polyamines used in this study by collecting titration curves.[10,11]

Furthermore, we found that the solutions of polyamines used in the condensation experiments (see below) always show an initial pH of 4.3 to 4.8. The system polyamine-silica thus is strongly buffering the solution. It is noteworthy that this buffering pH is close to the pH found in silica deposition vesicles.[12]

There are significant differences in the titration curves for the various polyamines (Fig. 1). Linear PEI shows two distinct steps in the titration curve. At high pH, the majority of the amino groups is neutral; protons start to bind to the chain independently up to a situation where approximately one half of the amino groups are protonated. At this point, a polyamine chain tends to minimize its free energy by protonation of only

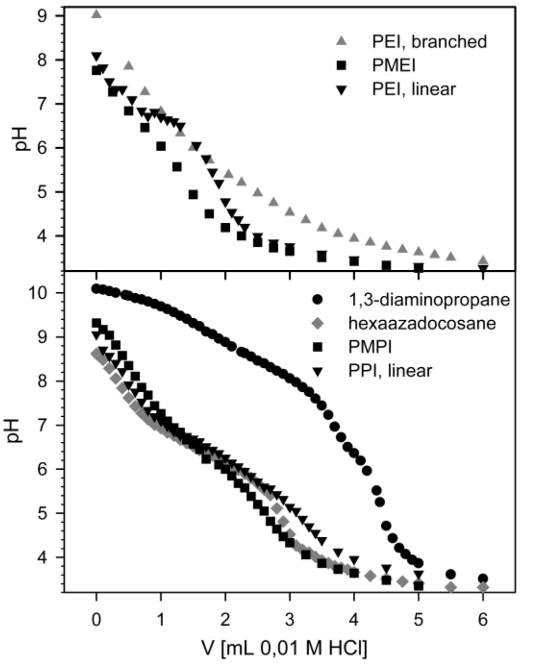


Fig. 1 Titration curves for the polyamines (c = 8.6 · 10^{-4} mol L^{-1} amino groups); top: ethylene imines, bottom: propylene imines.

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† Electronic Supplementary Information (ESI) available: detailed information about the synthesis and the kinetic investigations. See http://www.rsc.org/suppdata/cc/b3/b310201g/

every second amino group, to avoid nearest neighbor interactions of ionic sites. When then an additional amine site is to be protonated, it has two nearest neighbors which are already protonated and two direct pair interactions have to be overcome. The pK_b for the remaining amino groups therefore is significantly reduced.[11] The branched PEI (purchased from Aldrich) shows a smooth titration curve without a plateau, because there is a large number of different amino groups, which all have different connections to their next neighbors due to the irregular branching scheme. Therefore, for branched PEI no distinct pK_b but an almost continuous distribution of pK_b values for all the different amino groups is expected.[10,11] PMEI shows a titration curve similar to that of linear PEI. There is only a small shift, indicating a higher basicity of the PMEI.

For the polypropylene derivatives, *i.e.* PPI, PMPI and hexaazadocosane, the respective titration curves are very similar. All show two distinct steps as expected for linear polyamines.[11] The shift of the titration curves reflects the differences in the basicity between PPI and PMPI. 1,3-diaminopropane shows a significantly different titration curve, because the amino groups in this compound are primary amino groups, while all other compounds have mostly secondary amino groups. Furthermore, in 1,3-diaminopropane there is only one neighbouring amine group present, while in the polymeric and oligomeric amines, most amino groups have two neighbours.

Polyamines have been shown to influence the condensation of silicic acid[13] and the flocculation of the primary particles to form larger aggregates.[10] We investigated the kinetics of the silicic acid condensation reaction in the presence of different polyamines via the molybdate method, which detects monomeric and dimeric silicic acid.[1,14] Polyamine solutions were prepared by dissolving the amine in 10^{-5} M hydrochloric acid so that the amine concentration was 10 mg L^{-1}. A fresh solution of silicic acid was then mixed with the solution containing the amine. The final pH of the reaction mixture was between 4.3 and 4.8 for the polyamines and 6 for diaminoethane and 1,3-diaminopropane.† The results for the polyamines with ethyleneimine-building blocks are shown in Fig. 2.

A strong accelerating effect is found for the 1,2-diaminopropane and linear PEI having a molecular weight comparable to that of the polyamines isolated from diatoms.[5] The effect is less pronounced for the high molecular mass branched PEI and methylated PMEI.

The results for the polyamines with propyleneimine building blocks show qualitatively similar results (Fig. 3). The strongest acceleration is found for the 1,3-diaminopropane, followed by hexaazodocosane and the linear PPI. The acceleration effect again is much smaller for the methylated variant (PMPI). The difference between hexaazodocosane, which can be regarded as a PPI with $P_n = 6$ and the linear PPI ($P_n = 12$–13) indicates that the chain length influences the accelerating effect.

Linear PPI (linear PMPI) appears to be less effective in accelerating the silicic acid condensation than linear PEI (linear PMEI, respectively). However, the chain lengths of the polypropylene amines are larger than those of the polyethylene derivatives, and in view of the results described above, this could well be the reason for the observed differences.

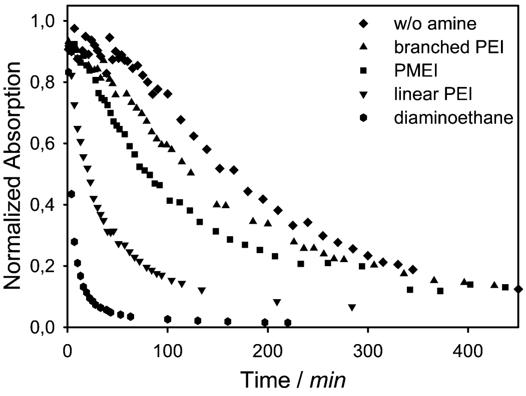

Fig. 2 Condensation kinetics of silicic acid in solutions containing 10 mg L^{-1} polyamine: Amines with ethylene imine building blocks. Plot of the normalized absorption of the molybdatosilicate as a function of time.

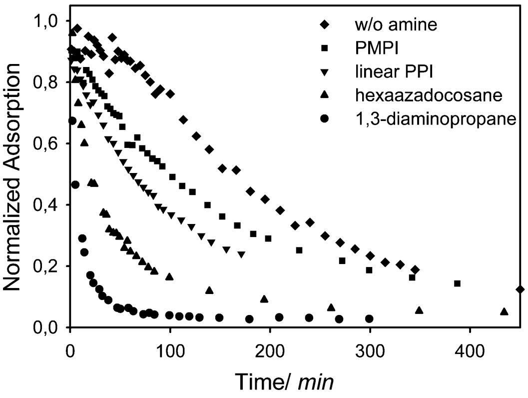

Fig. 3 Condensation kinetics of silicic acid in solutions containing 10 mg L^{-1} polyamine: Amines with propylene imine building blocks. Plot of the normalized absorption of the molybdatosilicate as a function of time.

In summary, polyamines modelled on naturally occurring substances have been synthesised and investigated with respect to their effect on silicic acid condensation. The accelerating effect depends on the chemical nature (polyethylene or polypropylene imine, degree of methylation), the architecture (linear or branched) and the degree of polymerization of the polyamine. Commercially available PEI with high molecular mass and a branched architecture, which was used in other studies,[12] is not a good model for the biological polyamines, as its chemical behaviour is significantly different from that of linear PEI and PPI. Among the polyamines investigated, PMPI is closest to the naturally occurring polyamines. Its acceleration effect is smaller than that of non-methylated PPI or PEI. Obviously, nature does not use the system accelerating the silica condensation most efficiently, but rather uses a slower system, probably to provide sufficient time for the formation of the intricate macrostructures.

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