Small-angle neutron scattering studies of alginate as biomineralizing agent and scale initiator

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ABSTRACT

Alginate is an important part of the extracellular polymeric substances excreted by microorganisms giving rise to biofouling on reverse osmosis membranes in desalination plants for water treatment. We present a small-angle neutron scattering study on aggregation of alginate when exposed to an aqueous salt solution simulating the reverse osmosis concentrate of a wastewater reclamation plant. We explored the behavior of alginate as dissolved molecule and as grafted to the surface of gold nanoparticles thereby simulating the condition of alginate attached at the surface of a membrane. The behavior of dissolved and grafted alginate was different when exposed to the simulation solution. The dissolved alginate aggregates to a gel like structure whereas the grafted alginate induces formation of 0.2 μm large particles. These particles form stable composites within few seconds with volume fraction of about half mineral and half gold nano-particles.

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1. Introduction

Reverse osmosis (RO) technology of producing potable water from pretreated wastewater and seawater has to overcome the severe problem of membrane biofouling and scaling. In the course of operation the permeability of membranes declines thereby limiting the economic feasibility of the process. Unlike seawater, scaling of calcium phosphate during wastewater effluent desalination is a particular problem as no effective antiscalant is presently available. Quite generally RO desalination permits gaining potable water with yields of about 80% and 50% from wastewater and seawater, respectively [1,2]. The feed for RO treatment contains salt molecules as well as biomolecules excreted from microorganisms as extracellular polymeric substances (EPS). These extracellular polymeric substances are proteins and polysaccharides forming a biofilm at the surface of the membrane, which induces the inorganic salts to formation of minerals such as calcium phosphate and calcium carbonate. This type of fouling is considered the "Achilles heel" of the membrane process because microorganisms multiply and produce the EPS over time [3].

We systematically studied the interaction of EPS organic and salt molecules in a simulated secondary effluent (SSE) solution using the technique of small-angle neutron scattering (SANS). The SSE solution is modelling the concentrate of secondary-treated domestic wastewater effluents at 80% stage of recovery; the ionic profile of the SSE solution was designed based on the chemical composition of secondary treated effluents of the Shafdan wastewater plant in Israel and was described previously [4]. The concentrations and activities of the ions in SSE solution are presented in the Appendix A2 (Table A2), and preparation of SSE solution was described previously [4]. SANS studies on two prominent members of EPS, namely the proteins BSA and lysozyme in SSE solution were already published [5,6]. The main result of these studies is that both proteins induce instantaneously a mineral formation of μm large composite colloids of mineral and protein. The mineral part consisted of calcium phosphate as well as of calcium carbonate. According to [6] the surface of a 30 Å large gold nanoparticle (GNP) was coated by BSA in order to better mimic the biofouling at the surface of a membrane. We found an immediate (within a few seconds) mineralization of 0.2 μm large composite colloids when mixing the coated GNPs with the SSE solution. The BSA coated onto GNPs induces a smaller amount of precipitated mineral compared

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to that of dissolved BSA, probably because the protein surface was partially exposed to the mineral solution.

The use of SANS is novel in the field of biofouling. It is a non-destructive method, which can follow the process of nucleation and growth in steps of several seconds and characterize particles of size at the range of 10 Å to several μm. An important property of neutrons is the easy change of scattering contrast when performing the experiments in H_2O and D_2O. The exchange between hydrogen and deuterium in water leads to large differences in scattering contrast and allows the identification of organic and inorganic components by contrast matching. This method of contrast variation is continuously applied in our present research.

In this paper a SANS study on the influence of alginate, an EPS component, on mineralization is presented. Two configurations of alginate will be studied, namely dissolved and bound to gold nanoparticles (AA-GNP), thereby simulating alginate bound at the membrane. In a first step alginate molecules and AA-GNP were dissolved in DI water in order to determine the radius of gyration and molar mass, whereas in a second step mixing of alginate with SSE was performed using the stopped-flow technique implemented at the sample position of the SANS instrument and connected with their control routines. Under such conditions we could measure the interaction of alginate and the salts in a controlled manner a few seconds after mixing.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate (99.99%) and alginate from brown algae were purchased from Sigma Aldrich. Sodium citrate was purchased from MERC, sodium carbonate from Frutarom and tannic acid from Riedel-de Haen. Ultra-pure water with specific resistance of 18.2 MΩ × cm was used in all experiments. Heavy water was purchased from ARMAR (Europa) GmbH with 99.8% as D_2O.

2.2. Alginate

Alginate is a naturally occurring hydrophilic polysaccharide obtained from the various species of brown seaweed (*Phaeophyceae*) [7]. The structural unit formula of alginate is depicted in Fig. 1. It is a linear copolymer formed from β-1,4-linked ᴅ-mannuronic acid (~61%) and α-1,4-linked ʟ-guluronic acid (~39%). These monomers are often arranged in homopolymeric blocks separated by regions approximating an alternating sequence of the two acid monomers. Characteristic parameters of alginate are compiled in Table 1. Alginate is soluble in water only in basic conditions [8,9]. At pH 3 it is completely protonated and therefore not charged. It becomes deprotonated above pH 5 along with taking a stretched
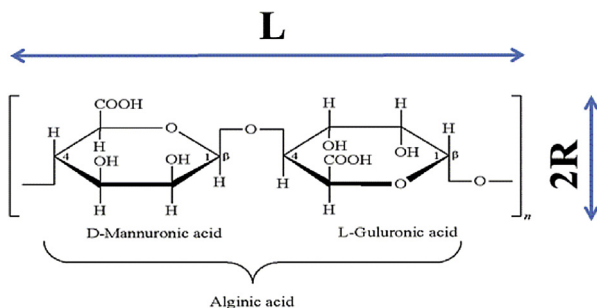
conformation due to electrostatic repulsions of the dissociated carboxyl groups [10,11]. The coherent scattering length density ρ and the incoherent scattering cross-section $d\Sigma/d\Omega_{inc}$ were determined from parameters listed in Ref. [12]. We also give some relevant parameters of calcium phosphate and carbonate [5].

2.3. Alginate coated gold nanoparticles (AA-GNPs)

Gold nanoparticles of 30 Å diameter were synthesized following the Turkevich method [13] in the same manner as reported in our earlier publication [6]. The citrate capped gold nanoparticles which had relative volume fractions of 1.4% and 98.6% of gold and citrate, respectively (Table 1 in Ref. [6]) were modified by adsorption of alginate [14]. The same procedure was used for the synthesis of gold nanoparticles in deuterated water.

2.4. Small-angle neutron scattering (SANS)

The small-angle neutron experiments (SANS) were performed at the classical pin-whole instruments KWS 1 and 2 operated at the FRM II in Garching [15,16]. The measurements were performed at the full available range of momentum transfer $Q = (4\pi/\lambda)\sin(\delta/2)$ (δ scattering angle) between $10^{-3} \leq Q[Å^{-1}] \leq 0.2$ with neutrons of λ = 7 and 9.73 Å wavelength and half width $\Delta\lambda/\lambda$ of 10% and 20% in order to allow the characterization of scattering centers such as colloids, particles or precipitates of size in the range of $10^3 \geq \Lambda[Å] \geq 10$. The experiments were usually performed with a neutron beam area of about 1 cm² at the sample position. One series of experiments was performed using refractive lenses of non-spherical shape in order to increase the sample area to 20 cm² and thereby the neutron intensity at the sample by the order of one magnitude [17].

The scattering law is expressing the scattered intensity as macroscopic cross-section $d\Sigma/d\Omega(\underline{Q})$ in absolute units of cm^{-1}, i.e. scattering per unit volume. For dilute solutions such as in the present study $d\Sigma/d\Omega(\underline{Q})$ is written as [18].

$$\frac{d\Sigma}{d\Omega}(\underline{Q}) = \left[\Phi V \Delta\rho^2\right] F(\underline{Q}) \tag{1}$$

$d\Sigma/d\Omega(\underline{Q})$ is a function of the scattering vector (or momentum transfer) $\underline{Q}$ which becomes a scalar for particles of isotropic shape or if randomly oriented. The scattering originates from diffraction at heterogeneities in the sample such as chemical composition and/or mass density and describes the scattering as product of form factor F(Q), particle volume fraction Φ, particle volume V, and scattering contrast $\Delta\rho^2$. The parameter $\Delta\rho = [\rho_P - \rho_S]$ represents the difference of the coherent scattering length density of particle (ρ_P) and solvent (ρ_S), in our case water. The shape of scattering intensity is determined by the form factor F(Q) which we formulate as approximate expressions valid in limited Q regimes according to

$$F(Q) = \begin{cases} 1 & \rightarrow Q = 0 \\ \exp\left[-(R_g Q)^2/3\right] & \rightarrow Q < 1/R_g \\ Q^{-\alpha} & \rightarrow Q > 1/R_g \end{cases} \tag{2}$$

The size of the particles is expressed by the radius of gyration defined as $R_g^2 = \int r^2 d_3\underline{r}/V$ (volume of the particle $V = \int d_3\underline{r}$) and which determines the valid Q range of the various approximations. The relationship between R_g and size of the particles depends on their shape. For spherical shape particles one has $R = \sqrt{5/3}R_g$ [18]. The extrapolated scattering at Q = 0 in Eq. (1) is determined according to

$$\frac{d\Sigma}{d\Omega}(0) = \Phi V[\rho_{Water} - \rho_P]^2 \tag{3}$$

Fig. 1. Structural formula of alginate. The dimensions L and R are discussed below (Eq. (6)).