Colloids and Surfaces B: Biointerfaces 66 (2008) 96–102

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Influence of dipalmitoylphosphatidylcholine on the dissolution of Brazilian chrysotile

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ARTICLE INFO

Article history:
Received 24 October 2007
Received in revised form 21 May 2008
Accepted 22 May 2008
Available online 29 May 2008

Keywords:
Dissolution
Brazilian chrysotile
Dipalmitoylphosphatidylcholine
Lung surfactant

ABSTRACT

It is known that Brazilian chrysotile is rapidly removed from the lungs, but quantitative studies about the influence of lung surfactants on chrysotile dissolution have not been investigated. In this work, the chemical behavior of chrysotile and its dissolution in the presence of dipalmitoylphosphatidylcholine (DPPC) were investigated in physiological conditions. The dissolution was investigated through quantification of magnesium and silicon released by chrysotile. At 37 °C, the magnesium concentration is similar to control (without DPPC), which is about 2.0×10^{-4} mol L^{-1}, meaning that the dissolution process is not affected by the presence of this surfactant. The same was observed for silicon. The silicon concentration released by chrysotile is similar in all media tested. It is known that the dissolution mechanisms of brucite and tridymite layers are different. From our results, we propose that under physiological conditions, the mechanism of brucite dissolution is based on its interaction with hydrogen ions and that the mechanism of tridymite dissolution is based on a hydrolysis process.

1. Introduction

Chrysotile is a fibrous hydrated magnesium silicate, $Mg_3Si_2O_5(OH)_4$, consisting of octahedral sheets of brucite, $Mg(OH)_2$, covalently bonded to tetrahedral sheets tridymite, SiO_4 [1]. The crystalline structure is characterized by spiraling of both the tridymite as an inner layer and brucite as an outer layer. Chrysotile is incombustible, chemically stable, thermally insulating; also, the fibrils are strong and very flexible [2,3]. The zeta potential of the fibers is positive in the range of pH 2–11 and the isoelectric point is at pH 11.8 [4]. Due to these properties, it was widely used in numerous industrial applications as, for example, thermal insulation, filtering devices and friction material for brakes. However, the use of chrysotile and other asbestiform minerals has decreased markedly because of health risks associated with inhalation of these materials [1,2,5].

Inhaled particles reach the bronchoalveolar region of the lung that becomes coated by an extracellular fluid which is a complex mixture of lipids and proteins [6], comprising about 90% lipids and 10% proteins by weight. The lipid composition is dominated by phosphatidylcholine-derived molecules (PC, 70–80%) [6], with dipalmitoylphosphatidylcholine (DPPC) being the major PC present (41–70%) [7]. Lung surfactants play a crucial role in respiration. Their primary function is to reduce surface tension at the air–liquid interface in alveoli during respiration, decreasing the energy required to inflate the lungs. DPPC is a zwitterionic surfactant and is the main contributor to reach near zero surface tension at the air–liquid interface in alveoli [6].

In response to the exposure to dust such as silica or chrysotile, there is a progressive increase in the amount of pulmonary surfactant in order to minimize cellular damage [8]. It was reported that the hemolytic activity of asbestos is determined by the surface charge of the fibers in solution. Light and Wei [9] investigated the interaction of DPPC on chrysotile and concluded that the zeta potential was reduced by adding DPPC to fiber suspensions. Jaurand et al. [10] demonstrated that a pre-treatment of chrysotile fibers with DPPC prevents hemolysis of cell membranes. Additionally, Jaurand et al. [11] established by photoelectron spectrometry that DPPC liposomes are adsorbed on chysotile as a bilayer by photoelectron spectrometry. From these results it was concluded that biological molecules present in the lung alveolar lining can be adsorbed on chrysotile fibers. However, quantitative studies about the influence of DPPC on the chrysotile dissolution processes at physiological conditions were not investigated.

The chemical instability of chrysotile in different media has also been studied. Pundsack [12] showed that in aqueous media chrysotile releases Mg^{2+} and OH^- ions to the solution reaching pH 10.33, which is quite similar to the pH 10.37 determined for a suspension of magnesium hydroxide. Choi and Smith [13] investigated the dissolution kinetics of chrysotile in water and observed that

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doi:10.1016/j.colsurfb.2008.05.014

Mg^{2+} and OH^- ion concentrations were temperature-sensitive only at the initial stages of the contact between chrysotile and water. Bales and Morgan [14] showed that the lower the pH, the higher the rate of magnesium release from chrysotile. On the other hand, silica showed no specific pH dependence. Additionally, the authors observed that the anions NO_3^-, Cl^-, HCO_3^- and SO_4^{2-} affected the rate of magnesium release only during the initial 12–24 h of each experiment.

Hume and Rimstidt [15] investigated the dissolution kinetics of chrysotile in dilute HCl solutions for 3 h and observed that the rate of silica release is slower than that of magnesium. The authors estimated that a fiber with 1 μm diameter would dissolve in 9 ± 4.5 months considering the dissolution rate of the silica layer. However, the authors did not discuss the dissolution mechanism of chrysotile. Bernstein et al. [16–18] studied the biopersistence of asbestos in the lungs of rats. The clearance kinetics of chrysotile was shown to be different from those of amphibole asbestos, with chrysotile clearing rapidly from the lung. The authors also reported that chrysotile fibers from Canada, Brazil and California, with fiber lengths longer than 20 μm were removed with a half-life of 16, 1.3 and 0.3 days, respectively, most likely by dissolution and degradation into shorter fibers; however, no dissolution mechanism was described.

In this paper, Brazilian chrysotile dissolution was studied in physiological conditions using Tris-buffer at 37 °C (310 K), which provides a system with pH 7.4, comparable to the buffer capacity and ionic strength found in extracellular lung fluid. Also, the chemical behavior of chrysotile in this medium was discussed. Dipalmitoylphosphatidylcholine was added during the dissolution experiments in order to establish the influence of this surfactant on the chrysotile dissolution process after short times of contact.

2. Experimental

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC (Sigma–Aldrich, St. Louis, MO, USA, 99% purity) was used as received. Highly purified 18.2 MΩ cm water was used in the preparations of all solutions. Brazilian chrysotile type 5RL from Cana Brava mine was supplied by SAMA Mineração de Amianto Ltda, Minaçu, GO. All other reagents used were analytical grade.

2.1. Chrysotile surface treatment

Prior to use, chrysotile fibers were washed on a 250-mesh sieve under tap water for 5 min, suspended in distilled water, submitted to ultrasound (25 kHz) for 15 min, filtered and dried at approximately 100 °C for 24 h. This treated chrysotile was named "sonicated chrysotile". The surface area of this sonicated chrysotile was measured by BET (N_2 adsorption) as 18 $m^2\,g^{-1}$.

2.2. Preparation of unilamellar vesicles

To prepare unilamellar vesicles, 0.191 g of DPPC were dissolved in a chloroform/methanol mixture (2:1 v/v, total volume 7.5 mL) [19]. The solvent was removed by rotary evaporation at a temperature of 53 ± 1 °C, depositing a thin lipid film on the wall of a 250-mL round bottom flask. Traces of residual solvent were removed by using vacuum for 1 h. 40 mL of prewarmed water or Tris–HCl buffer (50 mmol L^{-1}, pH 7.4, 0.11 mol L^{-1} of KCl) were added to dissolve the lipid film and vigorously vortexed at 53 ± 1 °C for 1 h. After this time, the multilamellar dispersion obtained was left standing at room temperature for 1 h. In order to produce unilamellar vesicles, multilamellar dispersions were transferred to a membrane extruder system at 53 ± 1 °C. These dispersions were forced under argon flow to pass (10 times) through a 100-nm diameter porous membrane. This procedure resulted in unilamellar vesicles with diameter of approximately 150 ± 1 nm for vesicles prepared with water and 165 ± 1 nm for those prepared with Tris–HCl buffer and KCl, as determined by dynamic light scattering measurements. The final phospholipid concentration [20] in this preparation was 4×10^{-3} mol L^{-1}, and subsequent dilutions were prepared from this initial preparation. Each preparation was used within 1 week.

2.3. Adsorption experiments

Adsorption experiments were carried out using the batch method. 0.010 g of sonicated chrysotile were weighed and suspended in sealed Erlenmeyer flasks with 5 mL of buffer solution containing unilamellar vesicles of DPPC, at various concentrations ranging from 3.5×10^{-5} to 4.1×10^{-4} mol L^{-1}. Also, isotherms were obtained without pH and ionic strength control. Suspensions were maintained in a thermostated incubator, under shaking, for 10 min and left standing at constant temperature (298, 310 or 328 K) for 2 h. After this time, chrysotile was removed from the suspensions by filtration. The residual concentration of surfactant in the filtrate was measured by the modified ammonium ferrothiocyanate method [20]. This method is based on a complex formation between ammonium ferrothiocyanate and phospholipids that allows measurements of phospholipids in the range of 15–150 nmol. The complex formed was extracted with chloroform. After the extraction, absorbance measurements were made at 480 nm using an HP 8453 UV–vis spectrophotometer (Palo Alto, CA, USA). The amount of surfactant adsorbed on the fibers was calculated from the difference between initial and final surfactant concentrations.

2.4. Dissolution kinetics

The silicon and magnesium concentrations of the samples were measured by inductively coupled plasma optical emission spectroscopy using a PerkinElmer Optima 3000DV ICP OES (Norwalk, CT, USA). Samples for ICP OES were prepared as follows: 0.010 g of sonicated chrysotile was suspended in sealed Erlenmeyer flasks with 5 mL of buffer solution containing 4.1×10^{-4} mol L^{-1} of unilamellar vesicles of DPPC. The DPPC-free samples were named control. To prevent diffusion effects, the samples were kept at 85 rpm in an incubator at 37 ± 1 °C (310 K), under shaking. The silicon and magnesium concentrations were measured as a function of contact time (from 0.5 to 24 h). After each time, the chrysotile was removed from the suspensions using a 250-mesh sieve and the suspensions were filtered to avoid the presence of residual chrysotile fibrils. Drops of HCl were added to the samples before analysis. Triplicate experiments were performed.

3. Results

Fig. 1 shows the adsorption isotherms for DPPC vesicles on sonicated chrysotile at different temperatures, with and without pH and ionic strength control. The adsorption is related to an electrostatic interaction of the electronegative charge of DDPC with the electropositive surface charge of chrysotile. Fig. 1a shows that the higher the temperature the higher the adsorbed amount plateau, reaching 10 mg g^{-1} (298 K), 13 mg g^{-1} (310 K) and 18 mg g^{-1} (328 K). The presence of different phases of the lipid at different temperatures explains the temperature dependence of DPPC adsorption on chrysotile. According to Jackson et al. [21], the gel–liquid crystalline phase transition temperature for DPPC bilayer is 315 K (42 °C). At 328 K, with DPPC bilayers in the liquid crystalline state, DPPC adsorption on chrysotile is higher than that obtained at