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# Interactions between silica nanoparticles and phospholipid membranes

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ABSTRACT

Silica nanoparticles (SNPs) are widely used for biomedical applications. However, their parenteral administration may induce hemolysis. Molecular mechanisms leading to this effect are still controversially discussed. We therefore used a combination of biophysical techniques to investigate the interaction of hemolytic and non-hemolytic SNPs with model phospholipid membranes.

*Methods:* Interaction of SNPs with membranes was studied using a dye-leakage assay, dynamic light scattering (DLS), isothermal titration calorimetry, and solid state nuclear magnetic resonance.

*Results and discussion:* The dye leakage assay revealed that only hemolytic, negatively charged SNPs, but not non-hemolytic positively charged SNPs, destabilized POPC based phospholipid bilayers. Interaction of SNPs with lipid vesicles leading to particle agglomeration was demonstrated by DLS. Isothermal titration calorimetry confirmed the interaction between negatively charged SNPs and phospholipids, which is characterized by an exothermic reaction enthalpy $\Delta H^0_{SNP}$ of −0.04 cal/g at 25 °C. Calorimetric titrations at different temperatures revealed a molar heat capacity change of zero. This finding excluded a contribution of electrostatic interactions. Mechanistic insight was provided by solid state phosphorus-31 NMR and deuterium NMR measurements.

*Conclusions:* Our results demonstrate that electrostatic interaction between hemolytic SNPs and model phospholipid membranes is negligible. SNPs induce membrane destabilization and adsorptive processes induced by agglomeration of phospholipid vesicles. The interaction is driven by van der Waals forces at the level of the hydration layer on the vesicles surface.



## 1. Introduction

Engineered nanomaterials are increasingly used for biomedical applications [1]. Examples include liposomal carriers [2] or polymer based particles for drug delivery or drug targeting [3]. Different routes of intake are possible for nano-scaled drug delivery systems (DDS) including skin penetration, oral absorption, inhalation, or intravenous (i.v.) injection [4]. Intravenous administration is in most cases the preferred application route for nanoparticulate DDS because no physiological barriers such as the gut wall or the skin need to be crossed. However, upon i.v. injection, nanoparticles have direct contact with blood constituents such as immune cells, erythrocytes and plasma proteins. Depending on their physico-chemical properties, interactions with these blood components will take place within a few seconds [1]. A major safety concern thereby is the interaction with red blood cells. Many types of SNPs are known to induce hemolysis. This adverse interaction has to be avoided, or at least minimized, to allow for safe parenteral drug delivery [5].

SNPs are frequently used in pharmaceutical technology. For example, fumed silica (marketed as Aerosil®) is used as flowing agent in solid dosage forms since almost 50 years. Recently, mesoporous SNPs were proposed as parenteral DDS or imaging agents [6,7]. However, various studies demonstrate hemolytic effects for some types of SNPs [8–11]. For example, Yu et al. have compared different preparations of SNPs in the concentration range of 10 to 500 μg/mL. Depending on the surface charge, geometry and porosity, all particles showed 20% to 100% hemolysis at elevated particle concentration [10]. Hemolysis has also been described for crystalline silica [12–14]. Very high (i.e. non-physiological) particle concentrations may indeed induce forced membrane destabilizations. Alkhammash et al. have investigated dye leakage from 400 nm liposomes composed of DOPC induced by silica nanoparticles and found that 50 nm SNPs caused leakage at a particle:lipid weight ratio of 8.0 [15]. Similar findings were reported for 16 nm SNPs, where the ratio of particle:lipid of 10.0 caused leakage from DOPC vesicles [16]. In contrast to these reports, mesoporous spherical MCM-41 silica particles with particle diameters between 100 and 300 nm showed low hemolytic activity [9]. This was attributed to the low surface density of silanol groups on the surface of these particles. Indeed, anionic amorphous SNPs with high surface silanol content used in the same study were hemolytic. Surface modification of the latter particles by introduction of

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primary amines led to different findings. These positively charged SNPs were shown to be non-hemolytic [9]. However, recent study on membrane interactions of mesoporous SNPs showed that positively charged particles bind to and disrupt negatively charged bilayers composed of DOPE/DOPG [17]. Thomassen at al. have studied the influence of SNPs agglomerates compared to single particles on hemolysis and found that larger, low density agglomerates of SNPs resulted in decreased hemolysis when compared to similar concentrations of individual SNPs of the same size of the aggregates [11]. These studies were confirmed by experiments where amorphous silica nanoparticles with primary diameter of 15 nm agglomerated and caused 20% dye leakage from model membranes [18]. Using a quartz-crystal microbalance, the authors showed tight binding to lipids. However, the particles did not damage the plasma membrane as confirmed by electrochemical impedance spectroscopy. Several mechanisms of hemolysis were proposed and discussed in literature [9]. Silanol groups (Si-OH) are believed to induce hemolysis by electrostatic interaction with membrane proteins or the quaternary ammonium of phosphatidylcholine present in the cell membrane leading to membrane destabilization (Fig. 1). Based on this assumption, Depasse and Warlus have shown that tetramethylammonium ions have a strong affinity for silica nanoparticles. Since tetraalkylammonium groups are present in the outer leaflet of the RBC membrane, they concluded that this interaction is responsible for lysis [19].

Apart from the surface silanol concentration, the relative proportion of strained and unstrained siloxane rings on the surface of silica nanoparticles seems to play an important role with respect to hemolysis [21]. When brought into an aqueous environment, the siloxane bonds of the strained three membered rings are distorted and reactive oxygen species (ROS) are formed, which may result in hemolysis. However, to the best of our knowledge, oxidative stress has not been found in base-catalyzed silica nanoparticles synthesis. When nanoparticles are introduced in cell culture medium or injected in vivo, adsorption of serum proteins, co-factors, and nutrients is taking place, depending on the particle's size, surface charge, and time [22]. These dynamics cannot be easily studied by the methods presented here, therefore we focus on the interaction of amorphous silica nanoparticles with model membranes. Despite the fact that many studies have been performed, the underlying hemolytic mechanism of SNPs remains unknown.

In previous experiments [23], we were able to design SNPs, which did not induce any oxidative stress in cultured cells and acellular environment. Interestingly, these anionic SNPs with a size of 85 nm caused hemolysis, whereas amine-modified cationic SNPs with comparable size did not induce hemolysis [23]. Following up on this observation, we decided to use these SNPs as a well characterized model system to study mechanisms of silanol-induced hemolysis excluding the compromising factor of ROS generation. Of primary interest was thereby the direct interaction of SNPs with membrane phospholipids. Thus, artificial phospholipid membranes were used to exclude possible contributions of membrane proteins or poorly defined membrane constituents of erythrocytes. We used different biophysical approaches, namely a dye leakage assay, isothermal titration calorimetry (ITC), dynamic light scattering (DLS), and solid state nuclear magnetic resonance (ssNMR) to monitor the interaction of non-porous SNPs with phospholipid model membranes. $^{2}$H NMR using labeled phospholipids offered the possibility to discriminate between molecular interactions at the level of phosphatidylcholine headgroups. $^{31}$P NMR allowed us to study the structure and dynamics of phospholipid bilayers upon their interaction with SNPs.

## 2. Methods

### 2.1. Materials

Chemicals for silica nanoparticles synthesis were obtained from Sigma-Aldrich (Buchs, Switzerland). 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine-1,1,2,2-d4 (d4-POPC), and Sphingomyelin were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Cholesterol was from Fluka (Buchs, Switzerland). 1-Aminonaphthalene-3,6,8-trisulfonic acid, disodium salt (ANTS) was from Molecular Probes (Eugene, Oregon, USA). N,N′-p-xylylenebis(pyridinium bromide) (DPX) was from Sigma Aldrich (Buchs, Switzerland).

### 2.2. Synthesis and characterization of silica nanoparticles (SNPs)

SNPs were synthesized and characterized as described previously [23]. In brief, the reaction solutions had a molar composition of 1 TEOS: 0.087 $NH_3$: 64.3 EtOH: 27.8 $H_2O$. The reaction solution was stirred overnight. Alteration of SNPs net surface charge was carried out by direct incorporation of amine groups through the co-condensation of aminosilanes (3-aminopropyl)triethoxysilane during the synthesis (10 mol% of existing TEOS). The mixture was stirred at room temperature for 3 h (amino-modified). SNPs were collected by centrifugation, washed 3 times with EtOH and dried overnight under vacuum at room temperature. Dry state diameter was measured by transmission electron microscopy (TEM). Zeta potential was obtained by analysis of electrophoretic mobility of particles (Delsa Nano C, Beckman, Nyon, Switzerland).

### 2.3. Preparation of lipid vesicles

POPC in chloroform was dried under a gentle stream of nitrogen followed by high vacuum overnight. The lipid film was resuspended in buffer and vortexed, leading to multilamellar vesicles (MLVs) with a

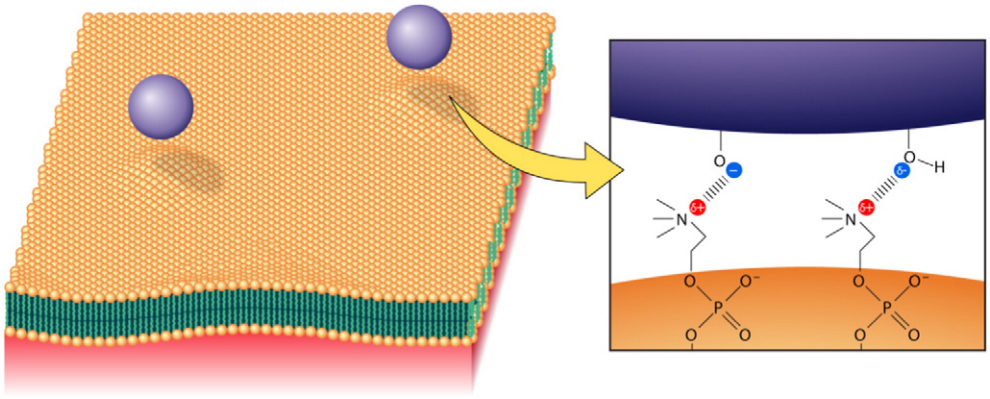


**Fig. 1.** Proposed mechanisms for SNP mediated hemolysis. SNPs mediate hemolysis via electrostatic interaction between negatively charged surface silanols and positively charged quaternary amines on the plasma membrane. Besides undissociated and dissociated silanols, other groups such as isolated silanols (not shown) are present on the particle surface. A more detailed insight on sol-gel silica nanoparticles surface is found in reference [20].