Journal of Colloid and Interface Science 404 (2013) 161–168

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

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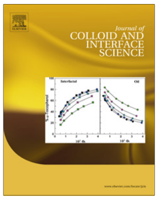

ZnO nanoparticle interactions with phospholipid monolayers

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ARTICLE INFO

Article history:
Received 12 February 2013
Accepted 4 May 2013
Available online 16 May 2013

Keywords:
Zinc oxide nanoparticles
Phospholipid monolayers
Rapid cyclic voltammetry
Particle size
Particle aggregation

ABSTRACT

Aqueous ZnO nanoparticle dispersions interaction with a dioleoyl phosphatidylcholine (DOPC) monolayer is reported in this paper. ZnO-DOPC interactions were investigated using rapid cyclic voltammetry (RCV) by focusing on the effect of the interactions on the characteristics of the capacitance current peaks representing two potential induced phase transitions. Results showed: – (1) The order of interaction of common commercially sourced nanoparticles with DOPC coated Hg electrodes was *NanoTek* > *NanoShield* > *metals basis*. This extent of interaction was inversely related to the ZnO particle size where the *metals basis* nanoparticles were strongly aggregated. The contribution of the non-ionic dispersant added by manufacturer to the *NanoTek* and *NanoShield* interaction was uncertain. (2) Freshly prepared aqueous *Nanosun* ZnO nanoparticle (~25 nm) dispersions interacted with and penetrated DOPC coated Hg electrodes. Aggregation of the nanoparticles, coating of the ZnO with phosphate and coating of the ZnO with fulvic acid minimised ZnO–DOPC interaction. (3) *In-house* synthesised ZnO nanoparticles of lower primary particle size (~6 nm) than *Nanosun* ZnO nanoparticles interacted strongly with DOPC coated Hg electrodes with no evidence of penetration of the nanoparticle in the DOPC monolayer. Even after considerable aggregation of the particle to between 1 and 10 μm, a strong interaction of the *in-house* synthesised ZnO with DOPC was observed.

1. Introduction

The increasing use of engineered nanoparticles has given rise to heightened concern for their biological activity and environmental impact [1]. Nanoparticles in aqueous dispersions have dimensions and properties in between those of micrometer-sized particles and dissolved compounds [2]. Their thermodynamics, transport, mechanical and chemical properties are dependent on their dimensions, and their large surface area generates considerable surface activity. Because of this, nanoparticles become highly active even if they are made of an inert material. In addition, the biological impact of nanoparticles depends on their size and structure in the solution environment as well as on their functionality because a small cluster of metal atoms can have a different chemical potential than the bulk solid and is more easily dissolved [3]. Another possible consequence of the large surface to volume ratio of nanoparticles is that during their transport, they release a concentration of adsorbed surface species which itself may be toxic. This, coupled with the putative ability of nanoparticles to cross cell membranes and enter cells because of their small size [4], will enable them to release dissolved toxic species into the cell interior. Taking into account the full complexity of a nanoparticle's toxicity, an initial step in the study of their biological activity can involve an investigation into the interaction of the particles with and/or permeation into the cell membrane as the primary interface of a biological organism with the surrounding environment.

A study of the interaction of nanoparticles with cell membranes can be carried out on cell cultures or microorganisms, but in these systems, there are more variables that can lead to a more elaborate analysis of the mechanisms involved. Another strategy in carrying out nanoparticle/cell membrane interaction studies is with model membrane systems based on the phospholipid bilayer backbone of the cell membrane such as liposomes. In fact a membrane model is used in the present study and is more manipulable than living cells in facilitating a rapid and controlled study of nanoparticle interaction with the phospholipid assemblies. In this article, the nanoparticle/biomembrane interaction was studied using a phospholipid monolayer on a mercury (Hg) film electrode. This is a powerful membrane model [5] that has been developed for *online* high-throughput application [6] and is custom-designed for investigating phospholipid layer interactions. The system of

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0021-9797/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2013.05.010

phospholipids (usually dioleoyl phosphatidylcholine, DOPC) on a Hg electrode is an established biomembrane model used by leading workers in the field [7–10]. This model has shown similar results with peptide–phospholipid [11] and cholesterol–phospholipid [12,13] interactions to those obtained using the classical biomembrane models of free-standing bilayers and vesicles. Moreover, the monomolecular gramicidin channel in the monolayer on Hg [14,15] was shown to function in a very similar way to the bimolecular gramicidin channel in phospholipid bilayers. The indications are therefore that Hg has a minimal effect on the fluidity of the phospholipids, and hence, the lipid layer's properties are very similar to those of the outer leaflet of free-standing bilayers and vesicles. The main difference in this system with respect to the phospholipid structure in a biological membrane is that the phospholipids form a monolayer on the Hg surface in the absence of an electric field, whereas the phospholipid assembly in a biological membrane is always a bilayer. However, any biological membrane activity is initiated from the interaction at the phospholipid/water interface, which is the same in both a phospholipid monolayer and a bilayer system. The studied model draws its unique properties from the atomically smooth nature of the Hg surface, which has a minimal influence on the phospholipid layer's properties compared to phospholipid layers on solid electrodes, which have a significant effect on the layer's properties.

Two earlier studies have investigated the activity of polymeric [16] and SiO_2 [17] nanoparticles towards the phospholipid monolayer on Hg system. The activity of SiO_2 nanoparticles on the monolayer as an example of inorganic nanoparticles is inversely related to their particle size and the data could be explained as related to the effectiveness of the SiO_2 packing enabling maximum contact between the SiO_2 particle and phospholipid surface to be obtained. This reported study extends the investigation to ZnO nanoparticles which possess interesting properties enabling studies of their activity to lipid layers particularly challenging. In contrast to SiO_2 which is amorphous, ZnO is crystalline and polar [18]. ZnO shows a tendency to dissolve in aqueous dispersion and release free Zn^{2+} ion [19]. Finally, ZnO nanoparticles have a strong tendency to aggregate [20,21].

ZnO is a semiconductor material which has many applications in industry. This has led to ZnO nanomaterials being used in the production of sunscreens [22], solar cells [23–25], electroluminescent devices [26], electrochromic windows [27] and chemical sensors [28–30] and for functional coating formulations to protect wood, plastics and textiles from UV and microbial degradation [31,32]. This increased use of ZnO nanoparticles results in increased perceived health and environmental risks. The small size of nanoparticles leads to both greater mobility and higher activity to biological membranes [33], which includes stronger binding to [34], and permeability within [35] the biological membrane. The biological activity of ZnO nanoparticles has been confirmed by a number of research articles [36,37]. At present, an understanding of the factors in the toxicity of ZnO nanoparticles is rather ambiguous. It is not clear whether the toxicity of the ZnO arises from the Zn^{2+} which is released from ZnO [36] and known to be toxic and/or from the ZnO particle itself. A study into the affinity of ZnO particles for representative biological molecules would help explain whether the ZnO particle is directly implicated in any toxic event. To attempt to answer this, the current paper reports a study on the influence of ZnO nanoparticles on the phospholipid (DOPC) monolayer coated Hg film electrode. The ability of the nanoparticles to adsorb on and penetrate the phospholipid monolayer was studied and the effect of the size and functionality of the nanoparticles on the interaction was investigated. The DOPC monolayers are interrogated electrochemically using rapid cyclic voltammetry (RCV) comprised of a fast negative or cathodic going voltage ramp followed by a fast positive or anodic going voltage ramp both traversing a voltage excursion. The recorded current is directly proportional to the capacitance of the layers. Interaction of the nanoparticles with the layers is shown by a depression in two consecutive current peaks which are representative of two consecutive negative potential induced phospholipid phase transitions respectively [16,17]. Penetration of the DOPC layers by the nanoparticles gives rise to an increase in the capacitance current value of the monolayer [16].

2. Experimental

2.1. Materials

All ZnO nanoparticle concentrations are expressed as weight per volume except where stated. The ZnO nanoparticles used are displayed in Table 1. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was obtained from Avanti Polar Lipids (Alabaster, US) and was of >99% purity. All other reagents were of analytical grade and purchased from Sigma–Aldrich.

2.2. Methods

2.2.1. Electrochemical flow system setup

The flow system has been described previously [17] and consisted of: (i) a peristaltic pump (Cole-Parmer instrument co. Cat N. 7554-20), (ii) two universal valve switching modules (Anachem Ltd.), (iii) a 10 dm^3 electrolyte reservoir, (iv) a 25 cm^3 sample cell, (v) two magnetic stirrers for stirring of solution in electrolyte reservoir and sample cell, and (vi) a flow cell. All parts were connected to each other by teflon and silicon tubing. The flow cell was made from plexiglass with silicon rubber sealing. A REF201 Red Rod Ag/AgCl 3.0 mol dm^{-3} KCl reference electrode (VWR international Ltd.) was fitted into the cell, and all potentials in this paper unless otherwise stated are quoted versus the potential of this electrode. Silicon wafer-based microfabricated Pt electrodes (Tyndall National Institute, Ireland) were inserted into the flow cell. These electrodes consisted of eight Pt discs with diameter of 1 mm and two Pt rectangles of 8.3 mm long and 1.8 mm wide, embedded on a 28 by 28 mm^2 diced silicon wafer substrate possessing a surface of 0.2 μm dry silicon oxide. Each Pt area was connected to respective contact pads by a 0.5 mm thick Pt trace interconnect which was insulated with approximately 1.5 mm of Si_3N_4 deposited by plasma enhanced chemical vapour deposition (PECVD). The microfabricated Pt electrode was connected to an Autolab potentiostat, PGSTAT 30 (Ecochemie, Utrecht, Netherlands) interfaced to a Powerlab 4/30 signal generator (AD Instruments Ltd.) controlled by Scope™ software. The volume of the flow cell was 0.15 cm^3. The flow system was used in four different settings depending on the positions of valves; these were as follows:

1. Electrolyte was drawn from an electrolyte reservoir and discharged to waste after passing through flow cell.
2. Electrolyte was drawn from electrolyte reservoir and filled the sample cell.
3. Electrolyte was drawn from sample cell and discharged to waste after passing through flow cell.
4. Electrolyte was drawn from sample cell, passed through flow cell and re-entered into the sample cell by cycling. All solutions were constantly purged with argon (Air Products) during all electrochemical experiments.

A schematic of the flow system is depicted in Fig. 1 which allowed for DOPC deposition and removal and RCV measurements to be carried out with electrolyte from the electrolyte cell and exposure of DOPC to the sample from sample cell by a simple