Colloids and Surfaces B: Biointerfaces 140 (2016) 135–141

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Interaction of polyhedral oligomeric silsesquioxane containing epoxycyclohexyl groups with cholesterol at the air/water interface

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ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form
26 November 2015
Accepted 1 December 2015
Available online 23 December 2015

Keywords:
Cholesterol
Langmuir monolayer
Polyhedral oligomeric
Silsesquioxanes

ABSTRACT

Binary mixtures of cholesterol and fully-condensed octakis[{2-(3,4-epoxycyclohexyl) etyl}dimethylsilyloxy]octasilsesquioxane (OE-POSS) were characterized using Langmuir trough for obtaining surface pressure-area isotherms. The most characteristic feature of the mixed films is the presence of two collapse points on the isotherms. The first one is attributed to the collapse of less stable OE-POSS and it occurs at similar surface pressures for all compositions, while the second one corresponds to cholesterol collapse. Brewster angle microscopy observations confirmed the collapse behavior of the mixed film. Strong condensing effect was observed for the mean molecular areas dependence on cholesterol content in the film. Moreover, formation of microdomains of each component in the matrix of the other one was confirmed by BAM images. For the reasons of molecular structures and interactions a true mixed and homogenous film did not form in the systems considered. Phase separation was observed for all the compositions experimented. The lack of the interactions of OE-POSS with biomembrane components represented by cholesterol is beneficial for applications of OE-POSS in biomedical devices.

1. Introduction

Silsesquioxanes are hybrid compounds consisting of silicon-oxygen skeleton and different substituents. These molecules assume the random, ladder, cage or partial cage structure described by the general formula $RSiO_{1,5}$, where R is a hydrogen atom or an organic functional group. In the last few years, much attention has been focused on silsesquioxanes with cage structure, called polyhedral oligomeric silsesquioxanes (POSS). POSS moieties due to nano-sized structure are excellent nanofillers that efficiently improve mechanical properties of polymers, endowing them with greater temperature stability and oxidation resistance, surface hardening as well as reduction in flammability [1]. Over the last several years, the studies of polyhedral oligomeric silsesquioxanes incorporated into polymers have intensified, revealing their new possible applications in biomaterials. It has been confirmed that POSS cages, as nanoscale building blocks, can be incorporated into other polymers and improve their mechanical and viscoelastic properties. Thus, POSS-containing functional materials have been suggested to be used in tissue engineering and biomedical devices.

The biomaterials presently available for clinical applications in bypass, stents etc. are mostly made of poly(ethyleneterephthalate) or polytetrafluoroethylene. In the last years polyurethanes have also been widely investigated as potential components of biomedical devices. A number of interesting strategies have been developed including POSS-related polymers. Thanks to large potential in functionalization, POSS can be incorporated into various polymers through grafting, blending or copolymerization. For instance, poly(carbonate-silsesquioxane-bridge-urea) urethane has been developed for cardiovascular devices and confirmed as potential scaffold for tissue engineering [2]. A novel inorganic-organic hybrid composites, based on acrylic materials and POSS have been proposed for denture materials [3]. In both cases, POSS moieties have been recognized as non-toxic and cytocompatible. POSS are also promising materials for use in scaffolds for drug delivery, as imaging reagents and in combinatorial drug development [1]. Moreover, the family of POSS nanostructures offers new performance possibilities in cosmetic formulations [4].

The most important criteria for the selection of polymers for medical or biological use are biostability and cytocompatibility. Higher toxicity of polymers for living organism compared with ceramics and metals, forces intensive research in this area and development of new, safe materials. Cytocompatibility of materials can be investigated by testing their impact on different tissues. However, these effects are first of all a result of material interaction with a cell and its membrane. Therefore, it is essential to characterize the behavior of all polymer additives being in contact with living cell and obtain the information at molecular level.

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http://dx.doi.org/10.1016/j.colsurfb.2015.12.002
0927-7765/

The recent approaches to study biomembrane interactions with drugs and other compounds involve Langmuir monolayer technique as this monolayer has been recognized mimicking cell membrane [5]. In this method, model biological membrane is formed using phospholipids and/or sterols in the form of a monolayer at the air/water interface. Although the biomembrane has bilayer structure, there are a lot of similarities between mono- and bilayers formed by lipids. Although lipid monolayers are much less complex than real biological membranes, they have been useful in modelling such interactions for several years [6–8]. In the Langmuir technique, the drug or other substance is inserted into the monolayer by co-spreading or injection below the monolayer into the subphase, if the drug is water-soluble. Several effects may be observed as a result of additional component insertion: changes in membrane rigidity, morphology or viscoelastic properties, phase separation, stabilization of electric charges as well as ordering of the molecules at the interface [9]. Thus, the Langmuir monolayer method is useful in analysis of the interactions of antibiotics [10], cyclodextrines [11], antifungal substances [12] or anticancer drugs [13,14] with model biological membrane at molecular level. Moreover, the increasing production and use of nanomaterials made the study of their potential impact on the biological environment extremely interesting. Guzman et al. have found that hydrophilic silica nanoparticles may have significant influence on mixed DPPC-cholesterol Langmuir monolayers [15]. However, the effect of silsesquioxanes on biological membrane has not been studied yet, despite numerous possible applications of these materials in biomedicine.

In order to check the influence of a representative silsesquioxane on the components of biomembrane, we studied co-spread mixed monolayers of cholesterol and OE-POSS. Cholesterol is located in phospholipid bilayer with its hydroxyl group close to phospholipid heads and its hydrophobic rings and side chain within the fatty acid chains of the membrane interior. The rigid steroid ring interacting with lipid tails make them stiffen. In this way cholesterol has significant impact on bilayer fluidity and permeability [16–18]. It is essential for integrity, organization and function of biomembrane. Eukaryotic animal membranes contain approximately one cholesterol molecule for every two phospholipid molecules, but it is rarely found in bacterial or plant membranes. Thus, a study of the effect of OE-POSS on cholesterol in Langmuir monolayer seems to be reasonable from the practical point of view. Moreover, recently, we have shown that OE-POSS forms stable monolayer itself at the air/water interface [19]. This silsesquioxane was found to form liquid-like monolayer of the average stability at the air/water interface. Some other silsesquioxanes were also recognized as a new class of film-forming materials at the air/water interface [20–22]. This means that the expected results may be also highly valuable from the physicochemical point of view.

2. Experimental

2.1. Materials

Fully-condensed octakis[{2-(3,4-epoxycyclohexyl) etyl}dimethylsilyloxy]octa-silsesquioxane (abbreviated as OE-POSS) was synthesized in two steps according to the methodology presented in [23,24] with slight modification.

Cholesterol (CHOL) (≥99%) was purchased in Sigma–Aldrich. The chemical structures of both compounds are presented in Fig. 1. Both compounds were dissolved in chloroform of spectroscopic grade (Uvasol, Merck) to obtain the mixtures of different molar ratio of components.

2.2. Isotherm experiment

For monolayer preparation the Langmuir trough (KSV Nima) of the surface area 587 cm^2 was used. The trough was filled with ultrapure water (18 MΩ cm, 71.98 ± 0.01 mN/m). Platinum Wilhelmy plate connected with the balance, recorded the surface pressure to a resolution of 4 μN/m. The mean molecular area (A) was recorded in $Å^2$. The $\pi(A)$ isotherm was obtained upon symmetrical compression initiated by movement of two barriers, after chloroform evaporation (20 min). During all measurements the temperature was controlled by a Julabo circulator. Before the experiment, the surface of water was cleaned using a suction pump until the change in the surface pressure after maximum compression was below 0.2 mN/m. All samples were spread on the subphase with a Hamilton microliter syringe. The compression was performed at a constant rate (5 mm/min). Floating optical Table (Standa) under the Langmuir trough and all other devices ensured vibrations-free conditions and a Laminar flow hood (Alpina) surrounding the equipment eliminated dust from the environment. Each isotherm experiment was repeated five times to ensure the reproducibility of the curves up to ±2 $Å^2$.

2.3. Analysis of π(A) isotherms

On the basis of surface pressure (area) isotherms, the parameters of compression were determined. $A_{lift\text{-}up}$ is the mean molecular area that corresponds to the beginning of surface pressure increase during the compression. A_0 is the limiting area, determined by the extrapolation the slope of the isotherm at high surface pressure to $\pi = 0$. The symbols π_{coll1} and A_{coll1} stand for surface pressure and mean molecular area, respectively, corresponding to the first collapse point on the isotherm.

Using $\pi(A)$ isotherms, compression modulus was calculated according to the equation [25]

$$Cs^{-1} = -A\left(\frac{\partial \pi}{\partial A}\right)_t \quad (1)$$

Compression modulus reflects variations in the physical state of monolayers. According to the criterion given by Davies and Rideal [26] for liquid-expanded (LE) films, this parameter ranges from 12.5 to 50 mN/m, while for liquid-condensed (LC) ones it varies from 50 to 250 and for $Cs^{-1} > 250$ mN/m the monolayer is in solid state (S). The higher the value of Cs^{-1}, the lower the interfacial elasticity. Here, Cs^{-1} was calculated and plotted as a function of surface pressure for mixtures with all cholesterol molar ratios.Mean molecular areas of mixed films (A_{12}) were determined directly from $\pi(A)$ isotherms at a given surface pressure for all mixed systems. A_{ex} is the excess molecular area calculated according to the equation:

$$A_{ex} = A_{12} - A_{ideal} = A_{12} - X_1A_1 - X_2A_2, \quad (2)$$

where A_{12} stands for the mean molecular area of the mixture at given surface pressure and A_n is the mean molecular area of the component at a given surface pressure.

2.4. Brewster angle microscopy

Brewster angle microscopy (BAM) was used to visualize the monolayer morphology. Different domain shapes and sizes observed as different reflection density or grey levels indicate the monolayer phases. These morphologies are also related to changes in the monolayer thickness due to formation of three-dimensional aggregates. Here a BAM device (MicroBAM, KSV Nima) was used to watch monolayer morphology. The light source was a laser diode (659 nm). The field of view was 3.6 × 4.0 mm and the resolution was approx. 6 microns per pixel (i.e., better than 12 micron