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Wetting and electrokinetic properties of cholesterol—Revisited

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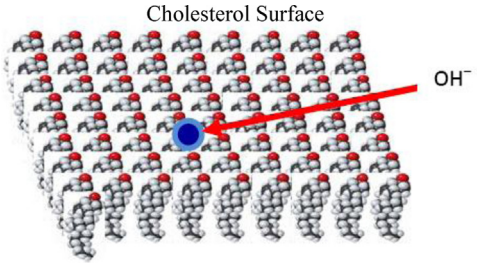
HIGHLIGHTS

- Surface free energy of cholesterol pellets and layers deposited on glass differ significantly.
- The surface structures, pictures and images from optical and confocal microscope, SEM and profilometer, are presented.
- The pellets structure are not uniform even at a large deposited amount, what reflects in their surface free energy.
- The zeta potentials of silica with deposited cholesterol versus pH lie between those of bare silica and bare cholesterol.
- The electrokinetic charge at pH 11 and zeta potential −50 mV of bare cholesterol is very low 0.596 μC/cm^2.

GRAPHICAL ABSTRACT

The electrokinetic charge at slip plane in 0.001 M NaCl, pH 11, $\zeta = -50$ mV, $\sigma ek = 0.596$ μC/cm^2.



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ABSTRACT

The important role of cholesterol in human body is well known. Therefore its properties have been investigated in many aspects. However, still some of its surface physicochemical properties are not well understood. Among them are wetting and electrokinetic properties. Therefore the aim of this paper was to study these properties and to compare them with the published literature data. For this purpose the advancing and receding contact angles of water, formamide and diiodomethane on the surface of cholesterol pellets and on its layers deposited on glass from its solution in chloroform were measured and then the surface free energy γ_S of the layers was calculated from van Oss' (LWAB) and Chibowski's (CAH) models. Also the images of the surfaces from SEM, confocal and optical microscopes, and profilometer were obtained. It was found that the surface of cholesterol pellets is more hydrophobic than this of the deposited layers, even if up to 200 statistical monolayers have been deposited on glass surface. From the images it was seen that the deposited layers were not uniform and at a larger coverage the needle-like cholesterol crystals were formed on the surface. From the zeta potential of cholesterol as a function of pH in 10^{-3} M NaCl was found that cholesterol shows the isoelectric point at pH 2.65, which is within the range of the literature data 1.8–3. The hydroxyl ions OH^- are the potential determining and probably adsorb on —OH groups of cholesterol molecules. In the pH range 3–6 the literature and our results of the negative zeta potential of cholesterol are convergent, except for those measured on a cholesterol suspension precipitated in water from its solution in 1-propanol. The zeta potential of silica particles covered with statistical monolayer of cholesterol lie between those of pure silica and cholesterol suspensions. The calculated electrokinetic charge at the cholesterol surface is very low, e.g. at pH 11, where $\zeta = -50$ mV, it amounts one excess negative charge (OH^- ion) on the surface of 73 cholesterol molecules ($\sigma^d = 0.422$ μC/cm^2). The results obtained in this paper partially confirm those obtained previously and bring new insight into the structures of cholesterol layers deposited on glass.

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1. Introduction

The important role of cholesterol in a human body is well known. Therefore its properties have been investigated for several decades in many aspects [1–13]. Chemically cholesterol is a lipid which is produced by the liver in living organisms and then transported in the bloodstream and it is recycled. A person having a weight of ca.70 kg synthesizes daily about 1 g of cholesterol, and total body content of it is about 35 g. In the liver, cholesterol is converted to bile, which is then stored in the gallbladder and excreted via the bile into the digestive tract. However, about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the bloodstream. Moreover, in small amounts cholesterol is also found in plants and fungi. The molecular structure of cholesterol is following:

The name 'cholesterol' originates from the Greek *chole* (bile) and *stereos* (solid) with the added suffix *-ol* for an alcohol. The name 'cholesterine' was used for the first time by chemist Eugène Chevreul in 1815, although it was identified much earlier in 1769 in a solid form by François Poulletier de la Salle in gallstones.

In living cell membranes cholesterol is responsible for their fluidity and permeability. Through the interaction with the phospholipid fatty acid chains in the membranes, cholesterol increases their packing thus reducing the membrane fluidity. Simultaneously, the polar headgroups of phospholipids and sphingolipids interact with the hydroxyl group of cholesterol molecules. As a result, cholesterol plays an important structuring role regulating the membrane permeability too.

Nevertheless extensive studies for many years upon cholesterol role and its properties [1–13], it is still an object of investigations [14–18] and some of its surface physicochemical properties are not well understood, among them are the wetting and electrokinetic ones. Looking at the chemical formula of cholesterol (Fig. 1), it can be seen that there is only one polar —OH group, one double bond (π electron), and a large apolar sterol ring. Nevertheless it behaves as visibly polar substance and can orient with the polar —OH group to a polar solid substrate [3]. Also its electrokinetic potential depends on the solution pH in which it is dispersed [6–9]. To our knowledge, so far there is no published paper in which both surface energy and electrokinetic potential were compared for the same sample of cholesterol. These two parameters may play crucial role in cholesterol particles adhesion to the blood vessels. Therefore it seemed to us interesting to conduct such investigations and revisit so far published results. Hopefully they would shed new light on surface properties of this biologically important compound which, unfortunately, among other things, blockes the blood vessels.

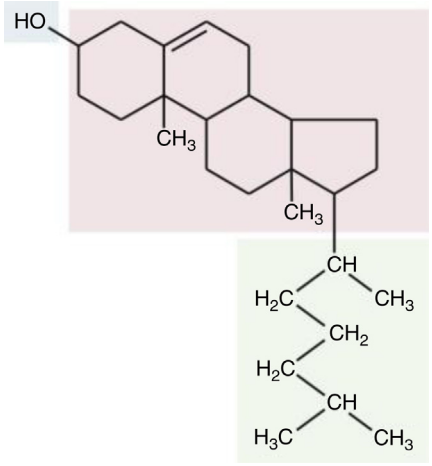

Fig. 1. Chemical structure of cholesterol.

2. Experimental

2.1. Materials

The cholesterol (>99%; Sigma) was used without further purification. The chloroform (POCH S.A. Poland) was used as a solvent to deposit of the cholesterol layers. As the solid support microscope glass slides 38 mm × 26 mm (Comex, Poland) were used. The probe liquids for contact angle measurements were water (18.2 MΩ cm, Millipore, from a Milli-Q System), formamide (98%; Aldrich) and diiodomethane (99%; Aldrich). The silica gel (purity p. a.) powder was purchased from Fluka A.G. The specific surface area of the silica gel determined by Brunauer, Emmet, and Teller (BET) thermal desorption of nitrogen was 7.1 m^2/g.

2.2. Methods

2.2.1. Deposition of cholesterol (Chol) on glass slides or silica powder from the chloroformic solutions

The glass slides were first cleaned in methanol in an ultrasonic bath for 30 min, then in Milli-Q water ultrasonic bath, and dried in a desiccator at 100 °C. The cholesterol layers were prepared by pouring progressively 0.5 mL of the chloroformic solution on the glass slides and waiting until the solvent has evaporated. To obtain one-statistical-monolayer coverage 1.7 μL of Chol solutions, containing 1 mg/mL, was added to 0.5 mL of the chloroform. The statistical monolayer was calculated from the geometric surface area of the glass slides and the cross section of the lipid molecule which was determined from π–surface *A* isotherm 38 $Å^2$ for Chol [4,19]. The consecutive layer was formed on the first one, already dried, by pouring the same volume of the solution. The powdered samples of $SiO_2 \cdot xH_2O$ of known specific surface area were precovered with calculated statistical mono- (ML) or bi- (BL) layer of Chol. Then, the obtained solutions were mixed with 10 mg of silica placed in a beaker and then put under high vacuum in a vacuum dryer for 24 h, in order to remove the chloroform.

2.2.2. Contact angle measurements

The contact angles of water, formamide and diiodomethane were measured by sessile drop method using contact angle meter (GBX Co., France) equipped with a video camera and computer software (WinDrop+++). They were calculated by fitting a mathematical expression to the shape of the drop. The advancing contact angles were measured for 6 μL droplets settled on the surface with a help of an automatic deposition system and the receding one after sucking into the syringe 2 μL of the liquid from the droplet. The receding contact angle (which is smaller than the advancing one) appears if the original volume of the droplet (here 6 μL) is reduced. The reason for this phenomenon is the surface roughness and/or the liquid film left behind the retreated three-phase contact line of the droplet. The readings of contact angle were taken for both sides of droplet for all three probe liquids at 20 ± 1 °C.

2.2.3. Surface topography investigations

Surface topography of the studied samples was imagined by using: optical microscope (Eclipse E600 POL, Nikon), confocal microscope Inverted Metallurgical Microscope (MA 200M, Nikon), Scanning Electron Microscope–Focused Ion Beam (SEM–FIB) (Quanta™ 3D FEG). The MA200 Metallurgical Microscope combines captured images with data on their observation settings for more comprehensive documentation. Nikon's DS-U2 Camera Control Unit and NIS-Elements Imaging Software allow the users to perform everything from basic image capture to the measurement, analysis, and management of captured images. The Quanta™ 3D FEG is the most versatile high-resolution, low-vacuum SEM/FIB for 2D and 3D material characterization and analysis which gives clear