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PHYSICO-CHEMICAL STUDIES OF SUCROSE THIN FILMS

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Sucrose is a natural osmolyte accumulated in cells of organisms as they adapt to environmental stress. In vitro sucrose increases protein stability and forces partially unfolded structures to refold. Thin films of sucrose ($C_{12}H_{22}O_{11}$) were deposited on thin cut glass substrates by thermal evaporation technique (p~ 10^{-5} torr). Characteristics of thin films were putted into evidence by Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM) and differential thermal analysis and thermal gravimetric analysis (TG/DTA). The experimental results confirm a uniform deposition of an adherent layer.

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1. Introduction

The preparation of uniform adherent thin films, deposited on different substrates, and having a good biological activity is representing now an important aim in the field of biochemical research. The difference between biomaterials and passive materials (dielectrics) is mainly consisting in their specific biochemical function. The biomaterial transfer requires the preserve of molecular function [1-3].

Sucrose is a compatible osmolyte, belonging to a class of low molecular weight compounds ($C_{12}H_{22}O_{11}$) produced by both prokaryotic and eukaryotic cells to protect proteins against the deleterious effects of harsh environmental conditions of water, cold and heat stress [4]. In peculiar, the study of sucrose-protein interactions has attracted considerable attention for the importance of sucrose in biochemical science and in the pharmaceutical industry, where it is commonly used as an additive to protein formulations, to protect labile proteins from the harmful effects of high temperature, freezing and drying [4]. In the cell metabolism, membrane resistance under sucrose is very low because of a large negative surface potential [5]. It is suggested that certain carbohydrates may stabilize membranes at low-water activities [6]. The organization of water at a lipid/membrane interface is a matter of interest, because it determines important functional properties of bio-membranes such as the excluded volume, hydratation forces and the dipole potential [7]. Different techniques as sucrose gap technique for assessing membrane potentials of nerve axons with extracellular electrodes were extended and in peculiar the gap technique is useful for measuring pharmacological modifications of channel properties that are irreversible [5]. The sucrose gap technique has also seen widespread application to multicellular muscle tissue. As it was stated [6] the effects of sugar on the physical properties of phospholipids have been examined using a variety of physical techniques. It has been reported that in the presence of carbohydrates (monosaccharides) as pentoses and hexoses and dissaccaharides as well as trisaccaharides the temperature (T_m) of maximal excess apparent specific heat ($C_{max)}$ of 1,2-DPPC (1,2- dipalmitoyl-3-sn-phosphatidylcholine) is "essentially unchanged" and the calorimetric enthalpy (ΔH_{cal}) is decreased. Surface pressure-area and surface potential-area measurements obtained from monolayers of 1,2-DPPC indicate that at low-surface pressures (10-30 mNm^{-1})

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sucrose (≥ 1.5 M) causes the monolayer to become more liquid-expanded i.e. increases the molecular area, which makes the film more liquid in character [6]. As presented [6], during the phase transition process there is a melting of "ice-like" water that reflects the difference in the amount of icelike water around the hydrocarbon tails between gel and liquid crystalline phases attributed to a smaller difference in ice-like water in the two phases in the presence of carbohydrate [6]. Mechanisms proposed for the effect of sucrose on protein [8] may help to explain the effect of sucrose on aqueous suspension of 1,2 –DPPC liposomes. We remark the propensity of sugar solutions, for example, to harden into amorphous glassy solids thus forms the basis for the worldwide sugar confection industry as well as the ability of organisms to endure severe desiccation [9]. Knowledge of details regarding the structure and dynamics of the membrane influenced by the surrounding medium give an insight view into the mechanisms of the protective action of the different saccharides for membranes under stress and into the number of water molecules at the hydratation layer around the chemical group of phospholipids [9]. For its protective properties sucrose as well as iron oxide doped dextran is used as biological probes in Magnetic Resonance Imaging (MRI).

In this paper we present the physic-chemical characteristics of sucrose thin films deposited on glass in medium vacuum conditions.

2. Experimental method

2.1 Sample preparation

Powder of sucrose was supplied by Merck Company at 99, 99 % purity. The powder was in a specific quantity for vacuum deposition. The sucrose thin films were deposited by thermal evaporation using a HOCH VAKUUM Dresden system. The thin films were deposited on glass substrates. For evaporation in medium vacuum ($p\sim 8 \times 10^{-6}$ torr) it was used a wolfram boat, and the intensity of the maximum current through boat was $I^{max} \sim 40$ A for $t \sim 5$ sec. The thickness of the sucrose thin films is ~449 nm from S1. The sucrose thin films were characterized by different techniques namely: infrared Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and differential thermal analysis and thermal gravimetric analysis (TG/DTA).

2.2 Sample characterization

IR spectroscopic studies were performed in the range 1800-400 cm^{-1} using a FTIR Spectrum BX spectrometer (4000-350 cm^{-1}) in transmission mode with the resolution 8 cm^{-1}. The surface morphology and growth mode of the deposited Sucrose thin films were investigated by scanning electron microscopy (SEM) in a XL-30-ESEM TMP system. For the elemental analysis the electron microscope was equipped with an energy dispersive X-ray (EDS) attachment.

On the powder, Differential thermal Analysis and Thermal Gravimetric Analysis were performed using the Shimatzu DTG-TA-50 and DTA 50 analyzer in the 25-800^{0}C temperature range, air environment.

3. Results and discussion

We present in Fig. 1 (sample S and sample S1) the SEM micrographs for Sucrose powder (sample S) and Sucrose thin film deposited on glass (sample S1). In order to record a SEM image the Sucrose powder was deposited on a double adhesive carbon band and afterwards was deposited a fine layer of gold (the sample S a disordered aspect with scratches and valleys on a uniform background specific for non-crystalline samples). For the Sucrose thin film (sample S1 with a thickness of 149 nm) we remark an ordered aspect of droplets. For the sucrose thin film (sample S1 with a thickness of 149 nm) we remark an ordered aspect of droplets in a uniform matrix.

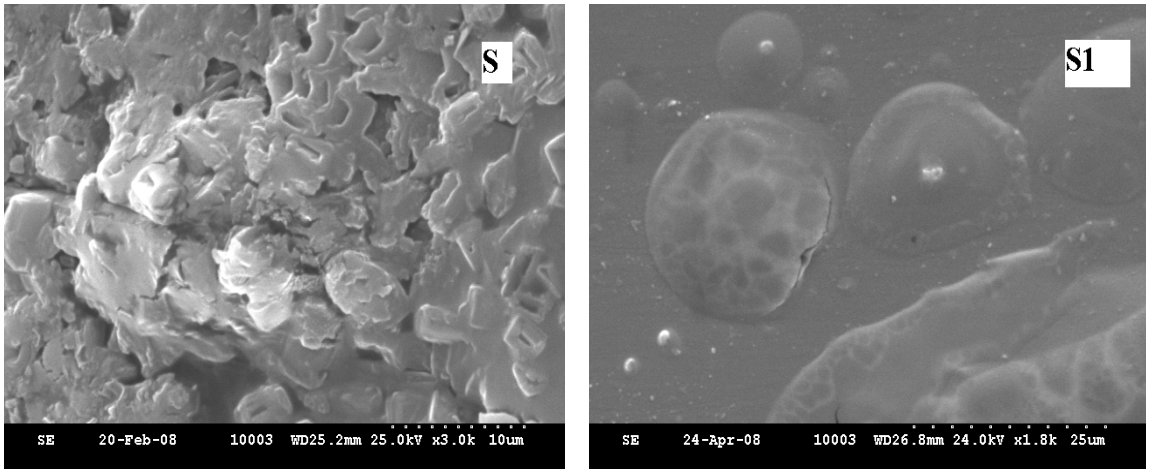

Fig. 1. The SEM images of sucrose powder (sample S) and thin films (sample S1).

The thermal profiles of the sucrose powder material are shown in Figure 2. A slow weight loss can be observed during the heating process until around 300^0C. The DTA curve (curve b) has three endothermic peaks, at around 195^0C, 226^0C and at around 400^0C respectively, resulting from heat induced decomposition. In previous thermogravimetry studies, correlated with mass spectral analyses, the slow weight loss until about 100 °C was attributed to detachment of adsorbed water molecules from the sucrose surface [13-14]. The thermal decomposition of sucrose was consodered to start at temperature higher than 195°C. Sucrose decomposes as:

$$C_{12}H_{22}O_{11} \rightarrow 12C + 11H_2O$$

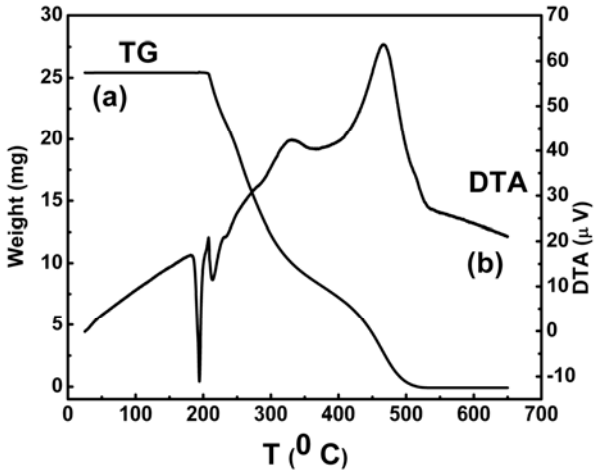

Fig. 2. The DTA/TGA evolution curves for sucrose powder.

The deposited thin films of Sucrose were investigated by FTIR spectrometry with the aim to obtain first information about their molecular structure as compared to the powder material (sample S) used for the targets preparation (Fig. 3).

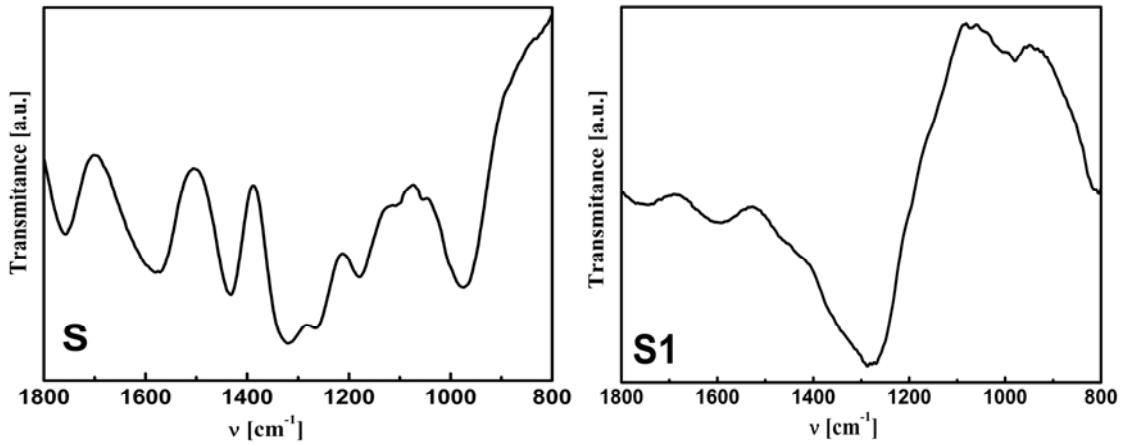

Fig. 3. The FT-IR spectra of sucrose powder (sample S) and thin films (sample S1).

The IR spectra for S and S1 samples show the vibration modes of sucrose. In these spectra the characteristic bands of sucrose are observed in the 1250-800 cm^{-1} range both for S and S1. From this point of view the exposed spectra are similar [14]. As presented in literature [15] the most suitable region for the IR measurements of sucrose has been found to be the 1250-800 cm^{-1} region. Namely, the shoulder at 800 cm^{-1} of sample S can be related to CH_2 group and the range 950-1300 cm^{-1} is related to vibration mode C-O-C group [16-17]. The vibration range related to hydrogen bonded water molecules adsorbed on the surface is present in the region 1600 cm^{-1} for the sample S (as can be observed in Figure 3). The band at 1750-1850 cm^{-1} are related to a C=O bond [18]. The difference in intensity of different transmission peaks are related to the thickness of sucrose thin films as the measured exposed volume decreases.

4. Conclusions

Sucrose biomolecular thin films were grown by vacuum deposition techniques. The sucrose thin films vacuum deposited on glass presented a good adhesion. The thickness of the sucrose thin films is ~449 nm. The surface morphology, the chemical composition were investigated. This method resulted in the deposition of uniform thin films, with chemical composition and molecular structure identical to those of the starting biomaterial used for the target preparation. The in vitro studies concerning cell morphology and viability displayed by the osteoblasts cell line hFOB 1.19 were realised.

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