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Investigation of the action of acetyl groups of chitosan on phospholipid membrane models

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Abstract – Chitosan was produced by applying the ultrasound-assisted deacetylation process, USAD Process, to beta-chitin [1]. Two samples with different average degrees of acetylation, $\overline{DA}$, 5.6% and 27.6%, were obtained and characterized. Langmuir films of phospholipid dimirystoyl phosphatidic acid (DMPA) were fabricated to mimic a cell membrane model. The chitosan samples obtained with the USAD Process were used to produce mixed Langmuir films with phospholipids aiming at investigating the influence from the acetyl groups of chitosan on cell membrane models. The films were characterized by surface pressure and surface potential measurements.

Chitosan, a cationic polylectrolyte when dissolved in aqueous acid media, is used biomedical, pharmaceutical and biotechnological applications, due to its special properties such as bioactivity, biocompatibility and biodegradability [2]. Due to its cationic character, chitosan interacts with the negatively charged surface of the cell membranes. Thus, the investigation on such interactions is a main issue concerning the chitosan applications in human beings. Therefore, this study is focused on the interactions between chitosan and cell membrane models to evaluate the importance of the content of acetyl groups of chitosan units in such interactions.

Chitosan was prepared by applying the ultrasound-assisted deacetylation, referred to as USAD process, an innovative process recently developed to promote deacetylation of chitin [1]. The reaction was carried out in a jacketed glass reactor coupled to a thermostatic bath, the reaction time and the ultrasound irradiation amplitude being adjusted to result in two different chitosan samples: Sample Chi5, possessing $\overline{DA}$ = 5.6 % and Sample Chi27, with $\overline{DA}$ = 27.6%. The sample Chi5 was prepared after two consecutive reactions in which a medium amplitude and long irradiation duration were used, while the sample Chi27 resulted from a single step reaction employing high amplitude and short irradiation duration. Beta-chitin extracted from squid pens (*Loligo sp.*) was used as raw material and both reactions were carried out at 60ºC. The chitosan samples were characterized by capillary viscosity, ^{1}H NMR and infrared spectroscopy and titrimetry, which allowed for its structural characterization.

Langmuir films of DMPA phospholipid mixed with Chi5 and Chi27 were produced in a KSV Langmuir trough 5000 housed in a class 10,000 clean room. The temperature was controlled at 23ºC ± 1. To obtain the Langmuir monolayers, 150 µL of a 0.5 mg. mL^{-1} phospholipid solution prepared in chloroform was spread on the top of a buffer subphase containing the samples Chi5 and Chi27. The buffer used in the subphase is a Theorell-Stenhagen buffer, pH 3.5. The trough is equipped with a surface sensor (Wilhelmy) and a Kelvin probe to measure the surface potential. The mixed films obtained with DMPA and both chitosan samples (Chi5 and Chi27) were characterized by the surface pressure and potential and the results were compared to evaluate the effects on the membrane model.

[1] UNIVERSIDADE DE SÃO PAULO. Instituto de Química de São Carlos. Sérgio Paulo Campana-Filho, et.al. **Processo para preparação de quitosana por meio de desacetilação de quitina**. Protocolo nº 018090022335, 4 maio 2009.
[2] Rinaudo, M., *Chitin and chitosan: Properties and applications,* **Progress in Polymer Science**, 31, 603 (2006).