Alumina nanoporous membrane bioactivation by dip coating technique

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The modification of membranes surfaces is an important field of research in order to improve their performance for a specific application. Surface modification can decrease pore size, change membrane-ions (or charged particles) electrical interactions or enhance membrane-fluid biocompatibility [1]. Different techniques depending on both the support structure and the modifying agent can be used. In the case of medical/pharmaceutical applications such as controlled diffusion or sensing systems, membrane surface modification by dip coating technique (membrane immersion in an appropriated solution) can be a simple and easy way for membrane surface biocompatibilization or bioactivation [2], as well as for pore size/porosity reduction. Nanoporous alumina membranes (NPAMs) synthesized via electrochemical anodization of aluminum are being commonly used in drug delivery devices due to their regular nanoporous structure, practically formed by parallel aligned cylindrical (or honey-combs) pores, with pore radius ranging between 10 nm and 200 nm, and narrow pore distribution [3].

In this work, a commercial nanoporous alumina membrane, Anopore® from Whatmann (sample ANP), with the following morphological parameters (given by supplier),:pore size d_p = 20 nm, thickness Δx = 60 μm and porosity, Θ, ranging between 25 and 50 % (more probable porosity value $<\Theta>$ = 30 % [4]), was used as support, while a bioactive compound, oligo(ethylene glycol)-alkene theophylline substituted (Theophylline **1** derivative or Theo **1**) was selected as modifying agent due to its pharmaceutical interest [5]. ANP samples were immersed in a dichloromethane solution of Theo **1** and slowly stirred for half an hour; the membranes were then removed, washed with dichloromethane and dried at room temperature for 24 h in a disecator to obtain the modified ANP+Theo **1** samples.

Modification of the (external) membrane surface was determined by XPS, using a Physical Electronics PHI ESCA 5701 spectrometer with a non-monochromatic Mg K_α radiation (300 W, 15 kV, 1253.6 eV) as the excitation source [6], while changes in the NaCl diffusive permeability were determined in order to detect Theo **1** coverage of the nanopore walls (internal membrane surfaces modification), by measuring time evolution of NaCl concentration in the solutions at both membranes sides due to the solute flow associated to a concentration gradient [7]. Moreover, electrochemical impedance spectroscopy (EIS) measurements with NaCl solutions were also performed with a Frequency Response Analyzer (FRA Solartron 1260, England) for frequency ranging between 1 Hz and 10 MHz (10 mV maximum voltage), to get information on changes in membrane/solution interface ($f \leq 1$ kHz) and bulk membrane ($f \geq 1$ kHz), for normal membrane working conditions, that is, in contact with solutions [8].

Atomic concentration percentage, AC (%), of the elements present on original and modified membranes were obtained from XPS spectra, and the results show a reduction of around 30 % in the detected AC (%) of aluminium (ANP sample characteristic element) for ANP+Theo **1** membrane when compared with ANP one, as well as an increase in the nitrogen AC (%) (Theo **1** characteristic element) in the case of the ANP+Theo **1** membrane; these results seem to indicate the partial external-surface coverage of the original alumina membrane by the bioactive Theo **1** compound.

The presence of Theo **1** on the pore-wall of the alumina support nanopores of the ANP+Theo **1** membrane was considered by analyzing changes in the NaCl diffusion as can be seen in Fig. 1 (a), where the different slopes of the straight lines obtained for ANP and ANP+Theo **1** membranes, under the same NaCl gradient, are associated to pore size/porosity reduction in the case of the modified membrane and, consequently, to a decrease in the NaCl diffusive permeability. EIS results (Nyquist plots) are shown in Fig. 1 (b) and (c), and they also indicate differences between ANP and ANP+Theo **1** membranes related with both external and internal surfaces deposition of Theo **1** compound; this effect is clearly evident at low frequencies (interfacial effect associated to external surface changes), but it also can be observed at high frequencies (membrane phase). On the other hand, the stability of the ANP+Theo **1** membrane under solution flow measurements should also be remarked, since it can be taken as an indication of rather strong link between the bioactive compound and the alumina

support. Moreover, use of ANP+Theo **1** membrane for protein streptavidine linkage, and consequently, its possible use as a biosensor is under study.

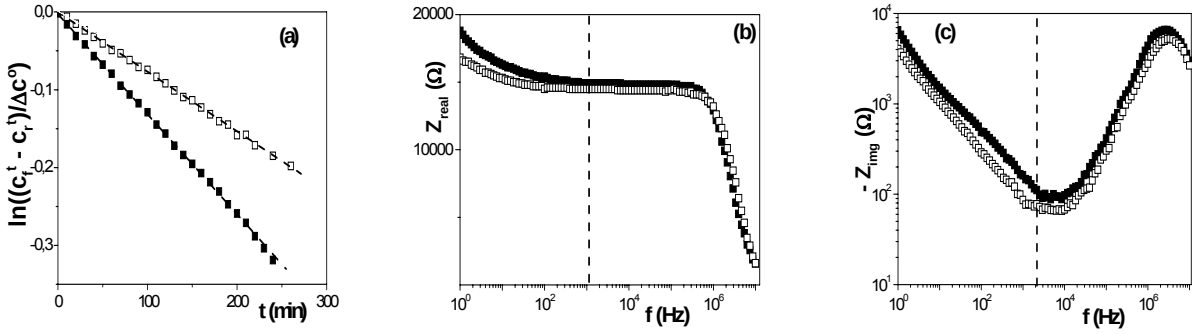

Fig. 1: (a) Time dependence of normalized concentration difference beetwen feed and receiving solutions. Electrochemical impedance spectroscopy plots: (b) Z_{real} versus frequency and (c) – Z_{img} versus frequency. (□) ANP membrane, (■) ANP+Theo **1** membrane.

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