Scrutinizing the Nanostructural and Nanomechanical Features of Regenerated Cellulose Ultrafiltration Membranes

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Abstract: Ultrafiltration (UF) membranes have been widely used for many separation processes in which high performance is required. Commercial regenerated cellulose UF membranes with variable molecular weight cut-offs were characterized by high performance atomic force microscopy (AFM) using the novel quantitative nanomechanical mapping mode and the versatility of its signal channels towards nanoscale features elucidation of the materials surface. In addition, Raman spectroscopy was applied in order to investigate some possible chemical behavior changes associated with the UF membranes' cut-offs. Overall, the results showed that the proposed AFM method was reliable to gain qualitative and quantitative data at unprecedented nanoscale resolution and such information can be used to distinguish UF membranes according to their specific molecular weight cut-offs and properties even on situations in which the molecular behavior were not influenced by the UF membrane' cut-off. This approach can be useful on quality control procedures of researchers and manufacturers producing or modifying these polymeric materials.

Keywords: Ultrafiltration membranes, Atomic force microscopy, Quantitative nanomechanical mapping, Raman spectroscopy, Nanostructural properties, Nanomechanical properties, Molecular weight cut-off.

INTRODUCTION

Ultrafiltration (UF) is a technique commonly applied for the pressure-driven membrane separation of structures up to 100 nm in diameter [1]. In addition, desalting, concentration, and buffer exchange of dilute solutions and suspensions of compounds/ nanostructures are possible using UF membranes [2]. Several materials are in common use for UF procedures including manmade and natural polymers such as polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE), nylon, and cellulose [3]. Regenerated cellulose is a universal hydrophilic solvent resistant and a low protein-binding material typically chosen as a first-line and gold standard membrane towards biomolecules ultrafiltration [4].

In a recent report of our research group on polymeric porous membranes [5], we observed the nanoscale behaviors of microfiltration membranes (0.45 µm porosity filters) formed by different polymers and evaluated by atomic force microscopy (AFM), single-point force spectroscopy, and chemical investigation approaches. That study successfully showed the potential application of AFM and related nanomechanical techniques as alternative tools for quality control of polymeric membranes [5]. However, a major limitation of that study was that only some few spectra could be obtained using the force spectroscopy approach towards the acquisition of nanomechanical data. Current developments in the field allow the rapid and reliable true quantitative nanomechanical mapping of entire AFM scanned areas with superior performance [6].

The present study aims to investigate the use of high performance AFM operated under quantitative nanomechanical mapping mode to investigate the topographical and mechanical surface of regenerated cellulose UF membranes with molecular cut-offs ranging from 3 to 100 kDa. Raman spectroscopy was also used to study the chemical behavior of the UF membranes.

MATERIALS AND METHODS

Amicon Ultra 0.5 mL centrifugal filter devices with molecular weight cut-offs of 3, 10, 30, and 100 kDa were purchased from Millipore and unmounted to get the two Ultracel™ regenerated cellulose membranes. The dissected membranes were mounted onto round shaped metal disks (1 cm diameter) using double-sided adhesive tape. The samples were imaged on Peak Force Quantitative Nanomechanical Mapping (PF-QNM) with ScanAsyst-air mode using the Icon head of a Dimension FastScan/Icon atomic force microscope commercial system (Bruker, USA). AFM images (10 × 10 μm^2) were acquired as 512 × 512 lines in air (22°C) at a probe scan rate up to 1.0 Hz. All the images (retraces) were flattened and plane fitted to remove the

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slope due to sample tilting prior quantitative analyses. The parameters consisted of measure the average and root mean square of the height sensor, peak force error, Derjaguin-Muller-Toporov (DMT) modulus, Log DMT modulus, adhesion, deformation, dissipation and height channels. The quantitative results of six sampled areas of different membranes were expressed as the mean ± standard error of the mean (SEM) and any possible statistically significant differences among the samples were evaluated for multiple comparisons by one-way analysis of variance (ANOVA) method using the Tukey's pairwise test available on Past 3.0 statistics Pack. The same samples were also investigated by Raman spectroscopy using a WITec Alpha 300 RA confocal Raman microscope coupled with a charge-coupled device (CCD) detector and a XTRA II single frequency diode laser at 785 nm (Power < 400 mW). Raman spectra were recorded in spectroscopy mode using a high numerical air Zeiss EC Epiplan Neofluar 100× objective (NA = 0.9 DIC). The integration time was 0.5 s and 100 acquisitions were performed in six replicates for each sample with only spectra background subtraction.

RESULTS AND DISCUSSION

Figures **1-3** show representative AFM images with the topographical and mechanical properties of the UF membranes. Eight signal channels were

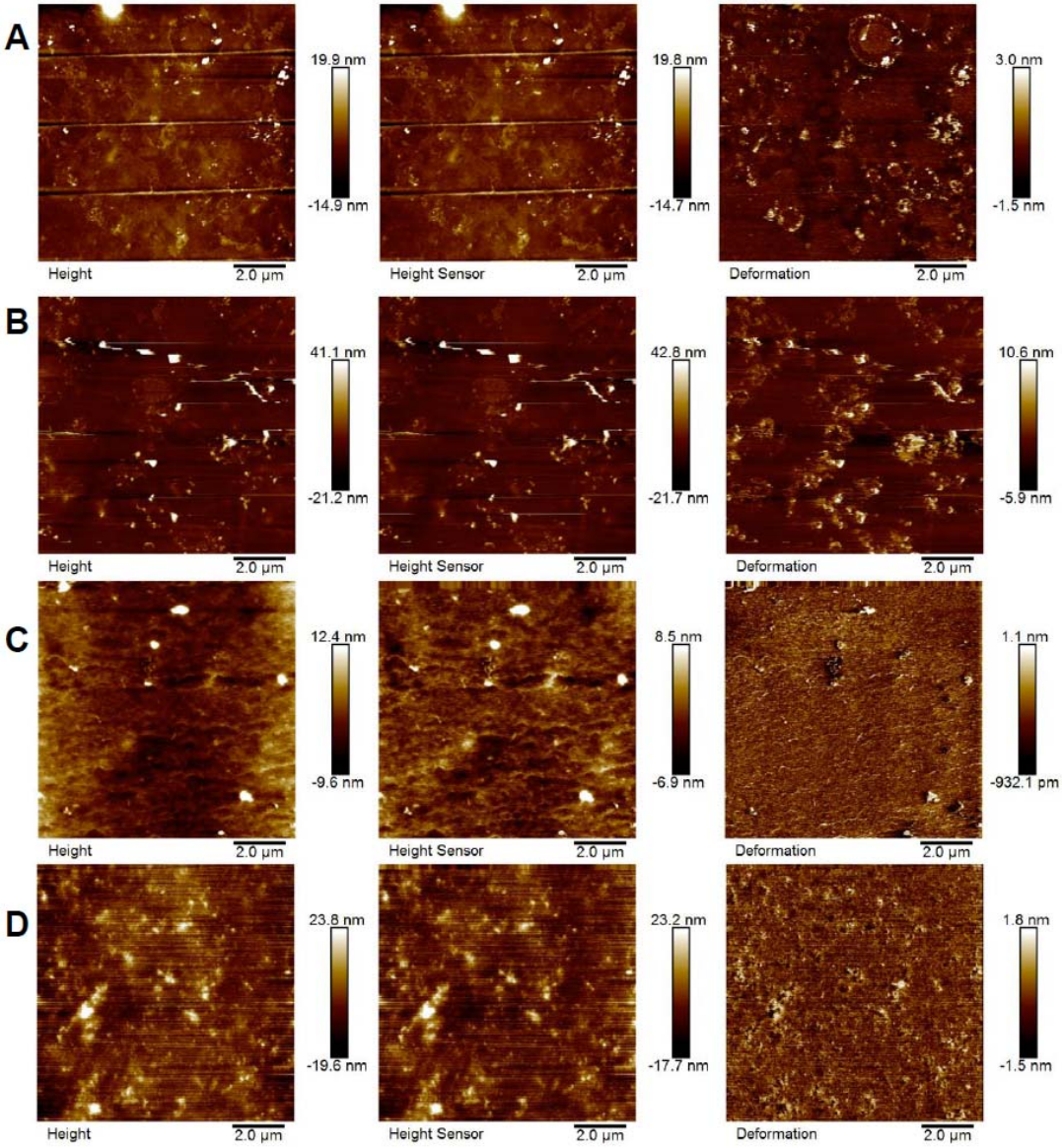

Figure 1: Representative AFM images of regenerated cellulose ultrafiltration membranes obtained by peak force quantitative nanomechanical mapping mode under height, height sensor, and deformation signal channels. (**A**) 3 kDa cut-off membrane; (**B**) 10 kDa cut-off membrane; (**C**) 30 kDa cut-off membrane; (**D**) 100 kDa cut-off membrane.

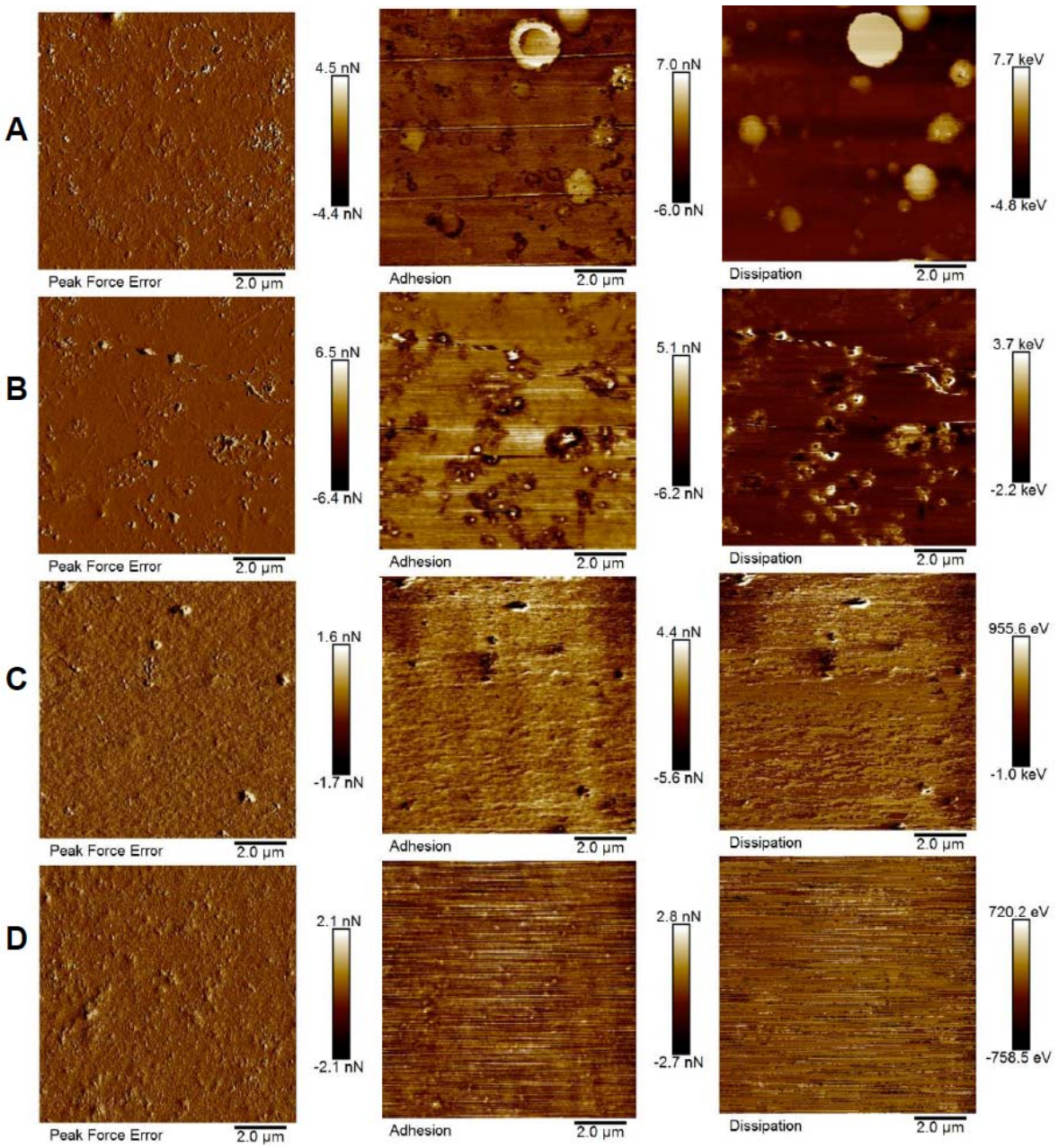

Figure 2: Representative AFM images of regenerated cellulose ultrafiltration membranes obtained by peak force quantitative nanomechanical mapping mode under peak force error, adhesion, and energy dissipation signal channels. (**A**) 3 kDa cut-off membrane; (**B**) 10 kDa cut-off membrane; (**C**) 30 kDa cut-off membrane; (**D**) 100 kDa cut-off membrane.

simultaneously acquired in order to perform the imaging of the samples' surface.

Height and height sensor images showed nanostructured surfaces for membranes of all evaluated molecular cut-offs (Figure **1**, left and center). The images showed the presence of small or large agglomerates with geometries ranging from quasi-spherical to completely amorphous. For some membranes (i.e. Figure **1A**), it was also observed the presence of some non-artifact transversal lines along the images that could be associated with some manufacturing process since they were a recurrent and periodic event (after each 2.5 µm). Deformation images of the UF membranes' surface elicited a relative stiffness with no significant deformation associated with the penetration of the tip, except for the distortion observed in the agglomerates (Figure **1**, right).

Peak force error images were provided only to show the finer details of the UF membranes' nanostructure and confirmed the homogeneity of the surfaces (Figure **2**, left). Adhesion images of the membranes showed the presence of unexpected adhesive structures

(Figure **2**, center). In fact, this acquisition mode is commonly described to show details beyond surface topography. This fact is intriguing and very important when considering the application of this type of device during UF procedures. Similar results were observed from energy dissipation images that reinforced the contrast among the diversity of regions in the samples but there was no clear tendency of mechanical properties associated with the membrane cut-off (Figure **2**, right).

DMT modulus and Log DMT modulus images displayed the surface rigidity/elasticity of the membrane samples as derived from fitting with the DMT model from contact mechanics (Figure **3**). It is surprisingly the level of nanomechanical details gained through this acquisition channel which could interfere with UF procedures and this acquisition channel can be considered fundamental during AFM investigation of membrane' surfaces.

It is noteworthy that qualitative AFM data are subject to a personal interpretation and thus must be considered with caution. In addition, it is virtually impossible to display all the acquired images, typically hundreds, for comparative purposes. Therefore, the use of quantitative information from AFM data is crucial and even from images obtained from channels other than the typical topography. Figure **4** show the average and the RMS values of the parameters measured from the multiple AFM images available from PF- QNM mode of the UF membranes.

In spite of a clear tendency towards higher values of average and RMS according to the membrane cut-off increase, there was no significant difference ($P > 0.05$)

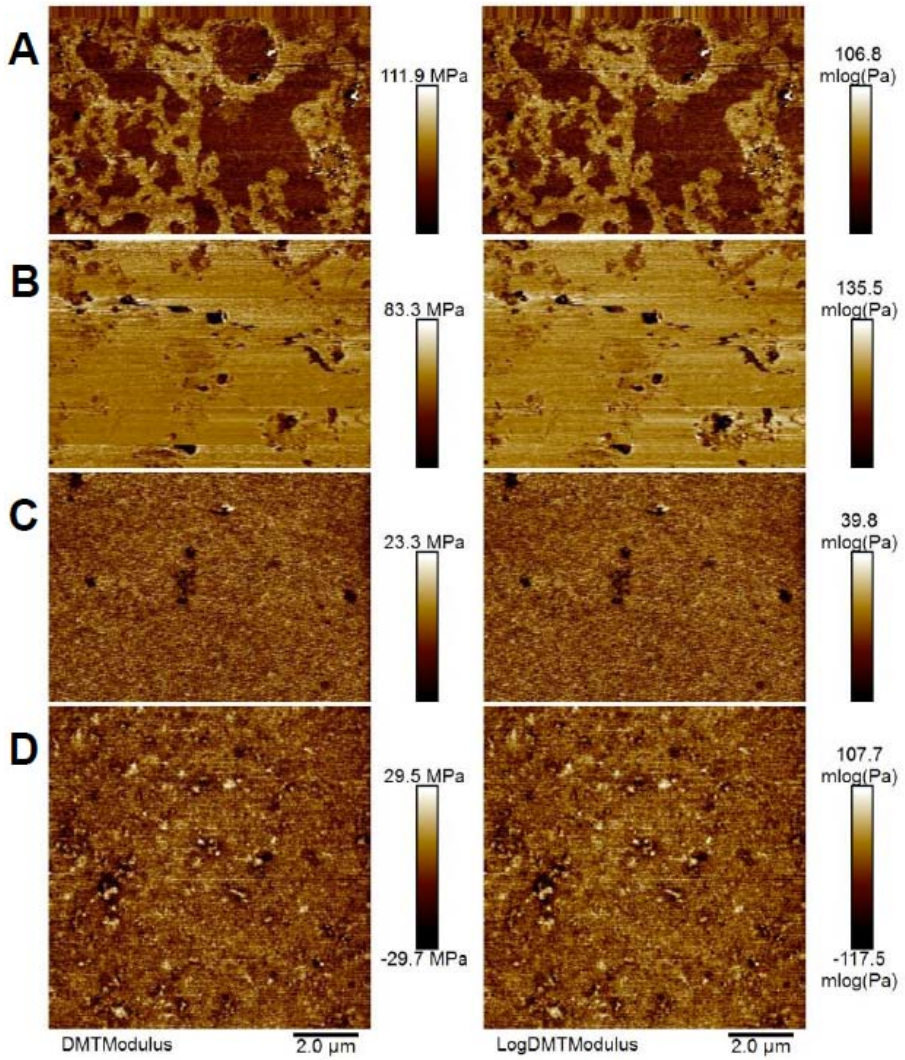

Figure 3: Representative AFM images of regenerated cellulose ultrafiltration membranes obtained by peak force quantitative nanomechanical mapping mode under DMT modulus and Log DMT modulus signal channels. (**A**) 3 kDa cut-off membrane; (**B**) 10 kDa cut-off membrane; (**C**) 30 kDa cut-off membrane; (**D**) 100 kDa cut-off membrane.

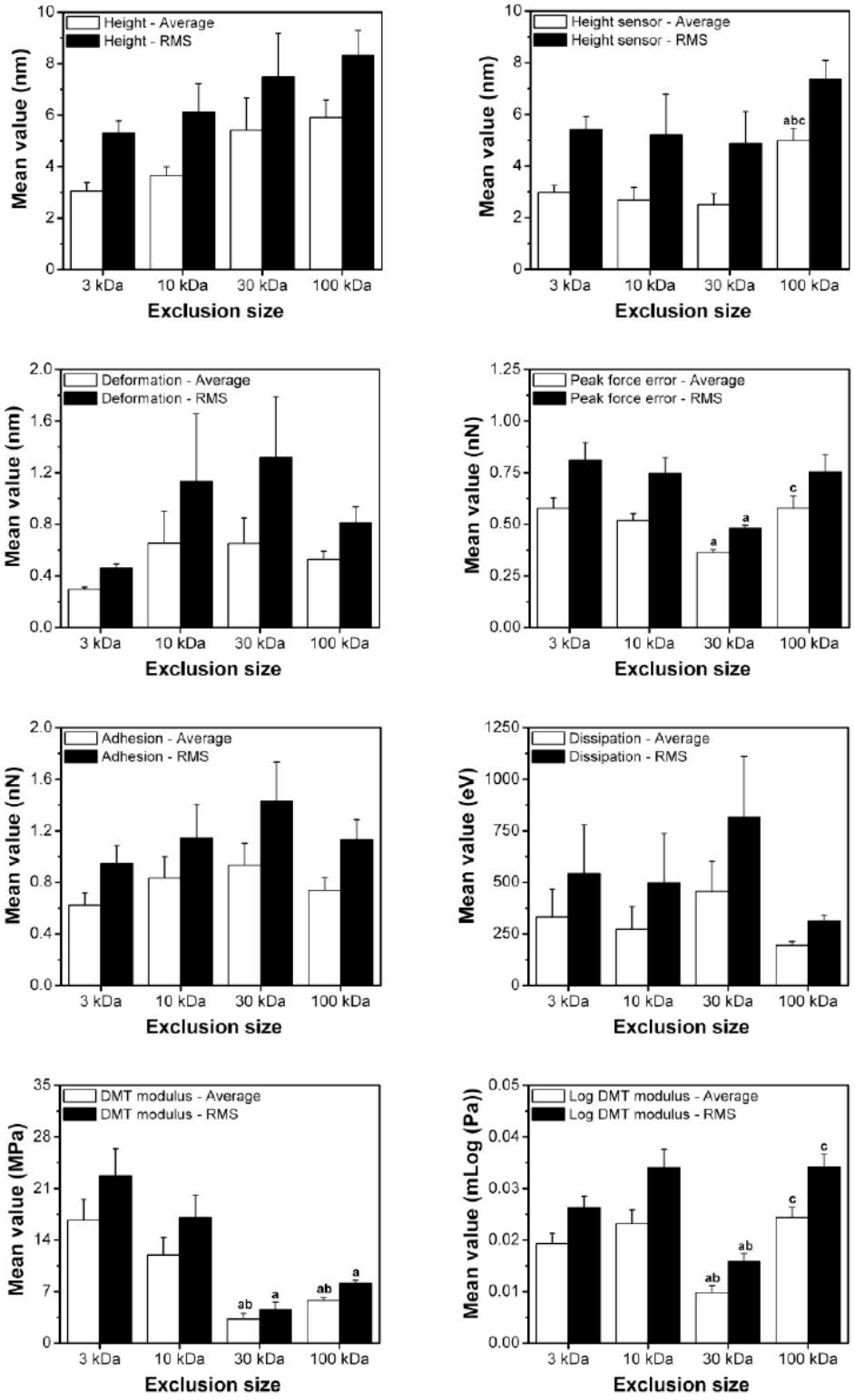

Figure 4: Quantitative AFM data. Letters “a”, “b”, and “c” refer to statistically significant differences ($P < 0.05$) when compared to 3 kDa, 10 kDa, and 30 kDa; respectively. The bars values are expressed as the mean ± SEM.

in the nanoroughness obtained from height images (Figure **4**). Otherwise, the average nanoroughness obtained from the UF membranes was much smaller than those previously observed by our research group when characterizing polymeric microporous membranes [5]. This fact reinforces the idea that the materials described in the present study have much better filtration performance than those from

conventional filtering membranes as previously observed by other authors [3]. Values obtained from height sensor images were slight different from those values obtained from height images since their signal are acquired from the Z piezoelectric position sensor (Figure **4**). Anyway, they corroborated the observation that there was a tendency to increase the height values related the cut-off size increase, and showed statistically significant differences for the mean value observed for the average nanoroughness of the 100 kDa UF membrane (Figure **4**). Deformation is typically an undesired side effect or even artifact of the AFM tip scanning. Otherwise, it can be imaged and measured in order to elicit important mechanical properties of materials. The deformation of the UF membranes was in the range of subnanometer to some few nanometers in mean value 9 Figure **4**). In addition, there was not any statistically significant difference ($P > 0.05$) among the UF membranes of different cut-offs (Figure **4**).

Average and RMS values of peak force error images obtained from PF-QNM mode can be compared to the deflection error from other AFM acquisition modes. It is not easily clear the importance of this internal sensor data, but the 30 kDa cut-off membranes were statistically significant different ($P < 0.05$) from 3 kDa and 100 kDa UF membranes' data (Figure **4**). The relevance of this data will be further explored when comparing a plethora of manmade and natural polymeric materials but some evidence suggest that it could be associated with morphological differences *per se* [7, 8]. Ultrafiltration membranes of different cut-offs showed similar ($P > 0.05$) adhesion and energy dissipation average and RMS values (Figure **4**). It suggests that in spite of different molecular weight cut-offs, they share comparable ultrafiltration capabilities and their interference with the filtered compounds must be analogous. DMT modulus and Log DMT modulus channels are probably the most important parameters when investigating the nanomechanical properties of a polymeric material under quantitative viewpoint. In fact, they express finely the stiffness (rigidity) of a sample. DMT modulus channel is the reduced Young's Modulus obtained by fitting the retraction curve using the DMT model [6]. The log DMT modulus channel is the logarithm of the elastic modulus of the sample based on the DMT model and directly reveals nanoscale differences in elasticity parameters [6]. In general, the regenerated

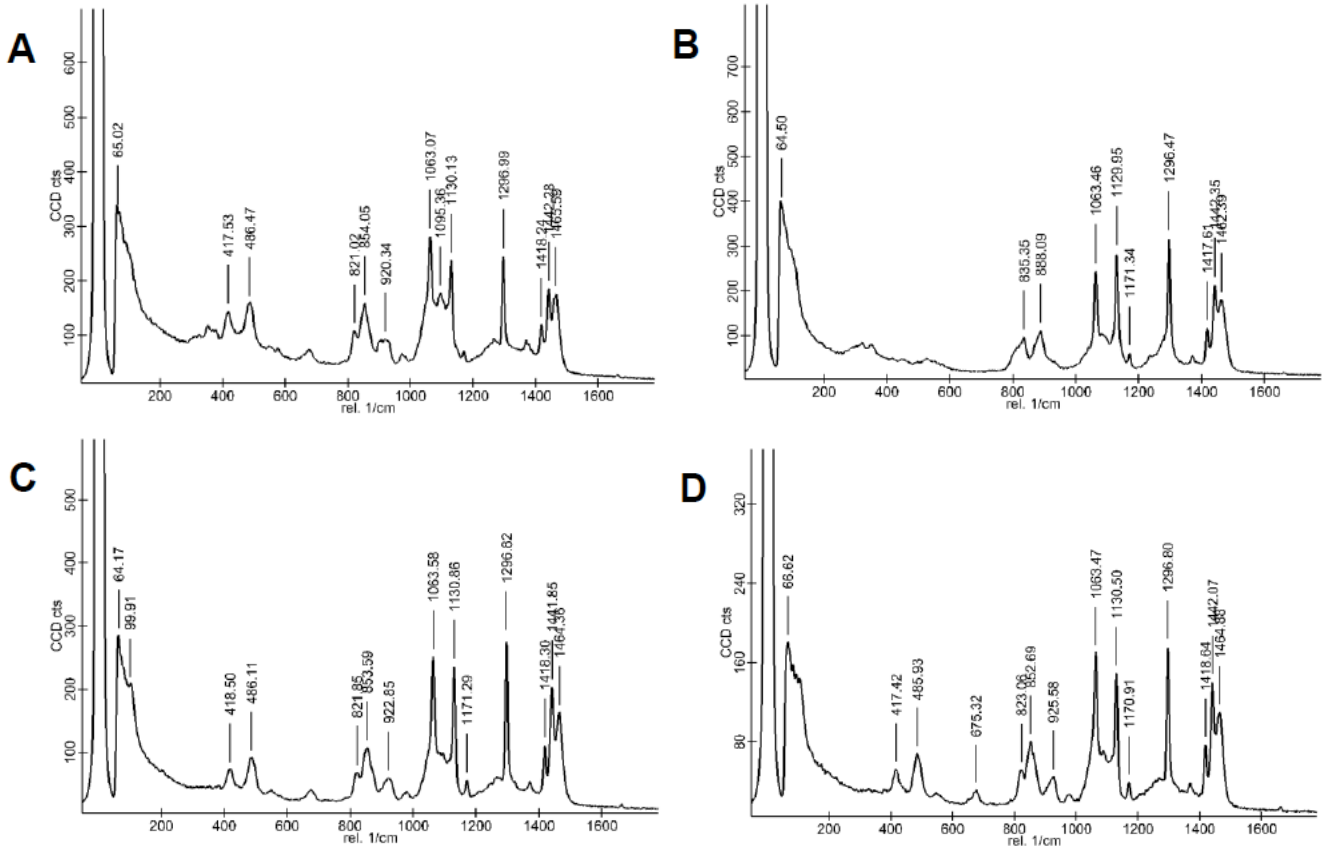

Figure 5: Raman spectra for ultrafiltration membranes constituted of regenerated cellulose. (**A**) 3 kDa cut-off membrane; (**B**) 10 kDa cut-off membrane; (**C**) 30 kDa cut-off membrane; (**D**) 100 kDa cut-off membrane.

cellulose UF membranes with 30 and 100 kDa cut-offs showed lower average DMT modulus values than those observed for the membranes with 3 and 10 kDa cut-offs (Figure **4**). In the case of the log DMT modulus values, the UF membranes with 30 kDa values followed the same general tendency of the DMT modulus parameter but in this case the values were also statistically significant different from those observed for UF membranes with 100 kDa cut-offs (Figure **4**, $P < 0.05$). Overall, the differences on quantitative nanomechanical parameters observed to the regenerated cellulose UF membranes could be related to the filtration performance according to specific cut-offs.

Taking into account the differences observed in the nanostructural and nanomechanical properties of the UF membranes with different cut-offs, the last question to be considered was the occurrence of any possible difference on the chemical behavior of the membranes that could be detectable by Raman spectroscopy. Typical peaks and bands of regenerated cellulose [9], no significant shift, and only small signal-to-noise differences were detected among membranes exhibiting different cut-offs (Figure **5**).

The present study showed that nanomechanical (e.g. DMT modulus and Log DMT modulus) more than nanostructural (e.g. height) parameters could be used in order to distinguish UF membranes according to their molecular weight cut-offs. It was also observed that these changes were possible without significantly modify the chemical behavior of the regenerated cellulose in the membranes' surface. Novel AFM acquisition modes and their outstanding signal channels showed to be useful and reliable in the evaluation of UF membranes and these data can be appropriate in the future during quality control strategies since these parameters can be quantified and used to define the structural and mechanical properties of these polymeric materials. Similar approaches can be successfully applied to the surfaces of other types of polymeric materials.

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