PREPARATION AND CHARACTERISTICS OF POLYSULFONE DIALYSIS COMPOSITE MEMBRANES MODIFIED WITH NANOCRYSTALLINE CELLULOSE

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Composite membranes for dialysis were prepared by a Loeb-Sourirajan (L-S) phase inversion process. After adding nanocrystalline cellulose (NCC), the ultrafiltration coefficient of the dialysis membrane could reach 48.37 $L•m^{-2}•h^{-1}•mmHg^{-1}$. The clearance of lysozyme and urea could reach 70.25 % and 90.38 %, respectively. Simultaneously, the retention ratio of BSA could remain over 96%. Afterwards, in order to judge the hydrophilic nature of the dialysis membrane, the contact angle and surface energy were tested and calculated. And then the tensile strength and elongation ratio were measured to reflect the mechanical properties. The membranes were also observed with transmission electron microscopy (TEM) and atomic force microscopy (AFM) to reveal the state of dispersion and dimensions of NCC. The porous structures of dialysis membrane were researched with both scanning electron microscopy (SEM) and AFM.

Key words: Nanocrystalline cellulose (NCC), Dialysis composite membrane, Polysulfone

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INTRODUCTION

High-flux dialysis membranes are composed of the materials containing hydrophobic groups, including polyacrylonitrile, polysulfone, polyether sulfone, and polyethyleneimine, along with various hydrophilic components (Fang and Zhao 1998; Lin et al. 2004; Yamamoto et al. 2010). Compared with conventional dialysis membranes, high-flux dialysis membranes have bigger pore sizes and greater permeability of dialysis, which can further enhance the capacity with respect to passing substances of intermediate molecular weight. Recently, an increasing number of published studies have focused on broadening the pore sizes of the membranes while sharpening their molecular weight cutoff, so as to maximize the removal of middle and low molecular weight proteins (Li 2007). Specifically, the goal is to enable to passage of uremic toxins such as β_2-microglobulin (β_2-MG), complement factor D, leptin, adrenomedullin, etc., while simultaneously minimizing the loss of albumin through the membrane (Mann et al. 2003). Thus, the complications to dialysis patients caused by those substances above can be minimized effectively.

Polysulfone (PS) is useful as a membrane separation engineering material due to its excellent properties such as high tensile strength, good chemical stability, durability when heated, pressure resistance, and so on (Guo et al. 2010; Homayoonfal and Akbari 2011). Moreover, with the help of polyvinylpyrrolidone (PVP) or polyethylene glycol

(PEG), the pore size of the polysulfone membrane can be controlled in order to adjust the water flux. So PS has been a preferred material for developing high-flux dialysis membranes. PS has gradually drawn the medical profession's attention, especially in the blood purification field (Bowry and Sudhir 2002; Mann et al. 2003; Huang et al. 2011). It can be made into dialysis membranes, blood filter membranes, plasma membranes, and so on (Ronco and Bowry 2001; Kim and Kim 2006).

Cellulose is one of the most abundant renewable natural polymers in the world. Nanocrystalline cellulose maintains characteristics of natural cellulose including hydrophilicity, high strength and tensile modulus, biodegradability, and renewability (Henriksson and Berglund 2007; Auad et al. 2010). Noornai et al. (2006, 2007) have made cellulose nanocrystals (CNXLs) from cotton and incorporated them into polysulfone films for ultrafiltration. In the present study, the NCC was made from wood and was processed with a high-pressure homogenizer, so that the concentration of the acid during hydrolysis was reduced and the properties of the natural cellulose could be retained. Based on the medical application for dialysis, the investigation focused on NCC in polysulfone as porous membranes. The hydrophilicity of dialysis composite membrane was well improved by the incorporation of NCC, owing to its huge surface areas and many exposed hydroxyl groups. When the blood contacts with the surface of the dialysis membrane, these hydrophilic groups absorb more water and the membrane filters the material faster. Therefore, the defect of polysulfone, which is its hydrophobic character, can be well amended so that the dialysis efficiency of the composite membrane can be augmented.

MATERIALS AND METHODS

Materials

Cellulose pulp was made from the eucalyptus wood and was purchased from Shandong Huatai Paper Mill (Shandong Province, China). Polysulfone (PS, degree of polymerization = 1500) was purchased from Shanghai Shuguang Chemical Plant (Shanghai, China). Urea was purchased from Tianjin Beifangtianyi Chemical Reagent Factory (Tianjin, China). Bovine serum albumin (BSA) and lysozyme were purchased from Beijing Aoboxing Biological Technology Co., Ltd. (Beijing, China). PEG (molecular weight 600, chemically pure), H_2SO_4 (98 wt%), and N,N-dimethylacetamide (DMAC) were purchased from Beijing Chemical Plant (Beijing,China).

Preparation of the Blood-Mimicking Fluid

This study focused on improving the clearance efficiency of middle molecular proteins represented by β_2-MG, so the lysozyme, which was similar to β_2-MG in the following aspects, was selected as a model compound for the present study (Xia and Lv 2007). The molecular weights of these two substances are in parallel to those of lysozyme (14,000 Daltons) and β_2-MG (11,800 Daltons). They both belong to the globulin and their molecules are positively charged. In addition, their isoelectric points are similar to each other, i.e. 4.6 and 5.7. Moreover, BSA's molecular weight is 67,000, so that it can be considered as a high molecular weight protein. It is very close to the molecular weight of

human serum albumin, which is 69,000 Daltons. Therefore, the retention ratio of BSA can be used to characterize the retention ratio of the human serum albumin. For small molecular toxins, urea was selected as the representative.

Because the dialysis process had no chemical reactions, in order to facilitate the research, the blood-mimicking fluid was mixed with urea (45 mmol/L), lysozyme (30 mg/L), BSA (1000 mg/L), and pure water. As the experiment was still in the simulation stage before clinical trials, pure water could be used instead of dialyzate, regardless of electrolyte and acid-base balance.

Preparation of NCC

Cellulose pulp was immersed in H_2SO_4 (15 wt%) solution and reacted at 90 °C for 4 hours by mixing sufficiently with an electric blender (Z89-1, Great Wall Industrial Foreign Trade, Ltd., Zhengzhou, China). At the end of the reaction, the pH value of the solution was regulated, using deionized water, until it was neutral. After sieving and drying with freeze drier (Modulyod-230, Thermo Fisher Scientific, USA), the solids were submerged into DMAC and were homogenized with a high-pressure homogenizer (NS1001S2K, GEA Niro Soavi Co., Italy). Subsequently, a colloidal suspension of NCC was obtained. With the mass of the casting solution as the total amount, the colloidal suspension was then diluted to varying NCC concentrations as: 0, 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 wt%, to check the effects of the NCC on the dialysis membrane performances.

Preparation of the Dialysis Composite Membranes

A quantity of PS (18 wt%) was dissolved in the prepared NCC colloidal suspension (in different concentrations) and added to PEG (3 wt%). The casting solution was obtained after it was swayed at 37 °C in the table concentrator for 24 h (constant-temperature table concentrator, SHK-99-II, Beijing North TZ-Biotech Develop Co., China). Bubbles were purged from the solution under a vacuum degree of 0.1 MPa. Afterwards, a small portion of the casting solution was poured onto a clean glass board and was scraped to a lamella with a homemade drawknife. The scraped lamella, vaporized in air for 10 s, was immersed into water to gel, and the dialysis membrane was obtained, which was soaked in distilled water at least 24 h before testing. This is the whole process of the L-S phase-inversion method for preparing the dialysis composite membrane.

Ultrafiltration Coefficient

The dialysis membrane was washed clean and enclosed into a self-made dialyzer. Then the dialyzer was operated within a certain time at normal temperature. The volume of filtered water [V (m^3)] was obtained in some portions of the membrane under a working time [t (h)]. And then the ultrafiltration coefficient [K_{uf}($L \cdot m^{-2} \cdot h^{-1} \cdot mmHg^{-1}$)] was calculated according to Eq. 1,

$$K_{uf} = V / (A \cdot t \cdot \Delta P) \quad (1)$$

where A is the membrane area (m^2) and ΔP is the osmotic pressure between both sides of the dialysis membrane.

Retention Ratio and Clearance

Retention ratio of a dialysis membrane, reflecting the retention capacity of the composite membrane for a given solute in solution, means that the amount of specific solute retained with the dialysis membrane as a percentage of total amount in the solution. The retention ratio of the BSA was measured within a certain time at 37 °C, and the absorbance of the blood mimicking fluid and the filtered solutions were tested at 280 nm with an ultraviolet–visible spectrophotometer (UV-9100, Third Analysis Apparatus Co., Shanghai, China) (Sivakumar et al. 2007). The retention ratio was calculated with Eq. 2,

$$R = [\ 1 - (A_f / A_i\ )\ ] \text{ x } 100\% \quad (2)$$

where R is the retention ratio (%) and A_i and A_t are the absorbencies of the initial and filtered solution, respectively.

The passage or clearance of lysozyme was tested at the same temperature, above which the absorbance of the blood-mimicking fluid was detected at 230 nm with the UV spectrophotometer. Similarly, the clearance of urea was measured in the same way, except that the test condition was at 199 nm. The clearance was calculated with Eq. 3,

$$C = [\ (A_o - A_t\ ) / A_o\ ] \text{ x } 100\% \quad (3)$$

where C is the clearance (%) and A_0 and A_t are the absorbencies of the initial and sampling time solution, respectively.

Contact Angle and Surface Energy

The contact angle (θ) values of the composite membranes with different NCC contents were tested with a JGW-360a contact-angle-testing instrument (Chengde Testing Machines Co., Ltd., Hebei, China) (Ke et al. 2005).

Mechanical Properties

The dialysis composite membranes were cut up to strips with 100 mm length and 15 mm width with half of them dried at 100 °C in the drying oven. Afterwards, the strips were tested by a computer-controlled tensile testing machine (DCP-KZ300, Changjiang Papermaking Instruments Co., Ltd., Sichuan, China) with a testing speed of 20 mm/min and clipping distance of 50 mm.

Surface Topography of the Dialysis Composite Membranes and NCC

The topographic structure of NCC was scanned with a transmission electron microscope (TEM, H-7500, Hitachi, Japan) (Azeredo et al. 2010). The dialysis membranes were broken in liquid nitrogen, then cross-sectioned. The bottom surfaces and the cross-section of the membrane, sprayed with gold, were observed with a scanning electron microscope (SEM, S-3000n, Hitachi, Japan) (Raguime et al. 2007; Qiu et al. 2009).

The membrane surface layers and its roughness could be evaluated from the atomic force microscope images (AFM, SPn9000, SHIMADZU, Hitachi, Japan) (Cheng et al. 2009).

RESULTS AND DISCUSSION

TEM Observations of NCC

The TEM images showed that the nanocrystalline celluloses were well-distributed in organic solution (Fig.1). They had lengths measuring about 150 to 500 hundred nanometers and nanosized diameters ranging from 20 up to 50 nanometers. Being treated under the high pressure homogenization, the nanocrystalline cellulose particles were distributed in the form of a single or multiple winding nanofibers, with a small part of them agglomerating as irregular shapes. Based on this dispersion condition, the important feature of NCC, which was the large surface areas, could be fully utilized. Thus, after mixing with the polysulfone, the hydroxyl groups on the surface of NCC could increase the hydrophilic character of composite membranes

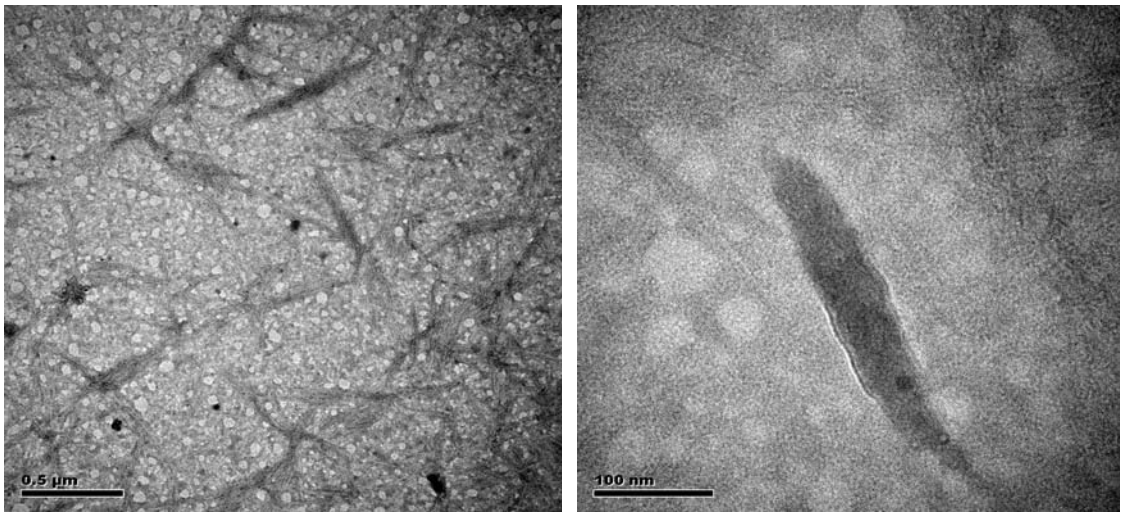

Fig. 1. TEM image of NCC

Effects of the NCC Contents on the Permeability of the Dialysis Membranes

The ultrafiltration coefficient of the dialysis membrane was boosted significantly with an increase in the NCC content (Fig. 2). When the NCC content reached 0.3 wt%, compared with the pure PS membrane, the ultrafiltration coefficient rose up from 24.0 $L \cdot m^{-2} \cdot h^{-1} \cdot mmHg^{-1}$ to 48.4 $L \cdot m^{-2} \cdot h^{-1} \cdot mmHg^{-1}$, and the clearance of lysozyme increased from 42.7% to 70.3%, while the clearance of urea increased from 62.9% to 90.4%. Then the incremental gains tended to disappear when NCC continued to be added. Simultaneously, the retention ratio of BSA remained at a high level, above 96%.

Due to a myriad of hydroxyl groups in cellulose molecules, the strong hydrogen bonds could be constituted both between diverse molecules and within the molecules. NCC had a huge surface area and a high proportion of exposed hydroxide radicals relative to its tiny dimensions. So it had a very good moisture-absorption ability so as to accelerate the velocity of water diffusing into the casting solution and finish the phase-inversion process. In this case, the dialysis membranes obtained a porous and more loosened structure, thereby increasing the ultrafiltration coefficient.

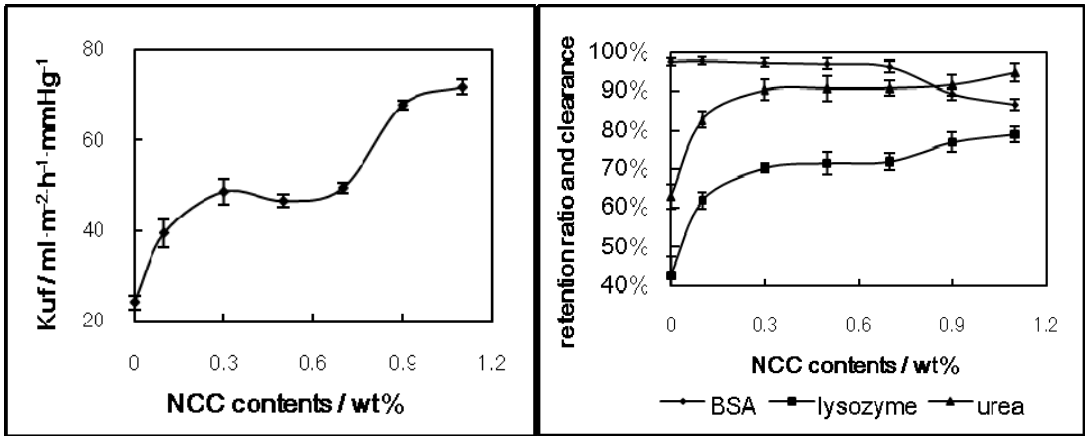

Fig. 2. Effects of the NCC contents on the permeability of the dialysis membranes. The error bars represent ± one standard deviation of the data.

However, when the NCC content was above 0.7 wt%, the ultrafiltration coefficient soared again, in concert with an augmentation in the clearance of lysozyme and urea. Unfortunately, there was a big fall of the retention ratio of BSA to below 90%. NCC colloidal suspension, in itself, was viscous. Once the concentration exceeded a certain extent, it could cause the casting solution to be overly viscous, resulting in difficulty of preparation and even giving rise to pore defects. Moreover, the larger was the content of NCC solution, the stronger were the intermolecular forces. Therefore, the NCC granules were not well dispersed, and they even were prone to conglomeration, which made them lose their advantage. Thus, 0.3 wt% of NCC content was a suitable amount.

Effects of the NCC Contents on the Hydrophilicity of the Dialysis Membrane

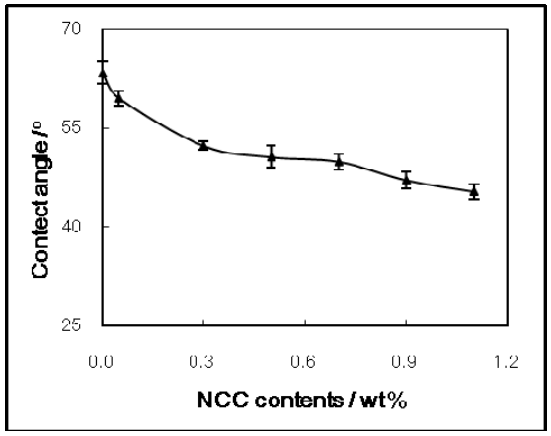

Fig. 3. Effects of the NCC contents on the contact angle. The error bars represent ± one standard deviation of the data.

The contact angle between the membrane surface and pure water can express the hydrophilic character of a membrane. A lower contact angle implies that the membrane has excellent hydrophilic and antifouling characteristics. Figure 3 shows that the contact angle decreased with an increase in the NCC content owing to those many hydrophilic groups in cellulose molecules.

In the process of blending with polysulfone, the NCC was dispersed into the casting solution. When the membrane was formed, lots of NCC were scattered to the internal parts and surface of the membrane. Thus, a large number of hydroxyl groups covered on the membrane surface. This made the polysulfone, which was initially hydrophobic, able to absorb the water more easily. So the NCC could help to decrease the contact angle of the dialysis membrane, indicating an increase in the surface energy. Therefore, the addition of NCC increased the hydrophilic nature of the PS membrane.

Effects of the NCC Contents on the Mechanical Properties

The tensile strength and the elongation ratio of the dialysis composite membrane both showed an escalating trend along with an increase in NCC content (Fig. 4). With the NCC content reaching 0.3 wt% from 0, the tensile strength of the dry membrane rose from 7.3 MPa to 10.0 MPa, increasing by 36.4%; while it rose from 8.9 MPa to 12.1 MPa for the wet membrane, increasing by 35.1%. Analogously, the elongation ratio of the dry membrane went up from 14.1% to 19.8%, increasing by 40.2 %; and for wet membrane, it went up from 17.0% to 23.2%, increasing by 36.5%. When the NCC content continued to increase, the tensile strength and elongation ratio decreased gradually.

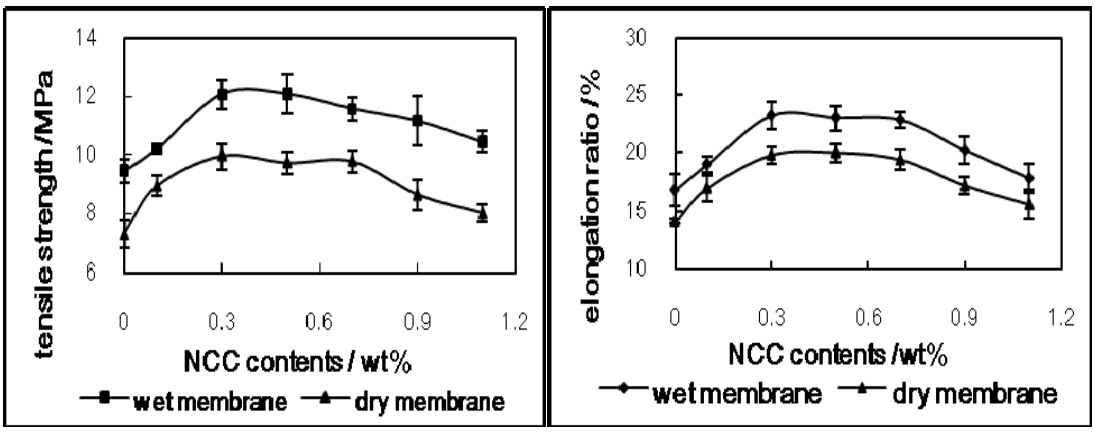

Fig. 4. Effects of the NCC contents on the tensile strength (left) and elongation ratio (right) of the dialysis composite membranes. The error bars represent ± one standard deviation of the data.

The results indicated that the mechanical properties were improved compared to the pure PS membrane. This effect was mainly attributed to the fact that NCC is a very tiny substance with large surface areas and surface energy. Hydrogen bonds, with great activity, were formed between PS and NCC. So NCC can be well dispersed in this polymer and improve the interfacial bonding strength, enhancing the mechanical properties. Also, during the hydrolysis of cellulose pulp, the amorphous region was broken down and filtered out. So the NCC retained the crystalline region. This ordered

crystalline structure made the NCC had a better rigidity. Therefore, in the macromolecular network, the strength of the NCC was transferred to the composite membrane. But when NCC was added excessively, the agglomeration of NCC resulted in a decrease of the interfacial strength, including pore defects, which led to a decline of mechanical properties.

The tensile strength and elongation ratio of the wet membrane were a little higher than those of the dry membrane. This was because the moisture had a softening effect on the dialysis composite membrane that increased the toughness of the material. However, the dry membrane was more fragile and had weaker intermolecular bonding. Therefore, the dialysis membrane had better mechanical properties while in the wet state.

SEM Observations of the Pure PS and the Dialysis Composite Membranes

SEM images indicated that the size and amount of pores on the bottom surfaces of the dialysis membrane both increased with an augment in NCC content, while the pore size of the support layer had a similar variation trend (Fig. 5). Furthermore, the pores had better connectivity than those of the pure PS membrane by adding appropriate NCC content.

But when the NCC content was too high, the fingerlike pores of the support layer were shaped irregularly and unstably, even forming pore defects on the surface. This was because NCC speeded up the pervasion of water into the casting solution. The surface layer that formed thereafter hindered the solvent from flowing into the water bath and supported proper growing conditions for the liquid phase with a low polymer content, which could grow propitiously and form structures having large pores. Compared with the composite membrane, the pure PS membrane was dissimilar and had a compact surface layer with small pore size. As a result of an increasing amount of low-polymer-phase molecules formed, rather than growing favorably, the pure PS membrane had an obviously shorter and thinner finger-like structure.

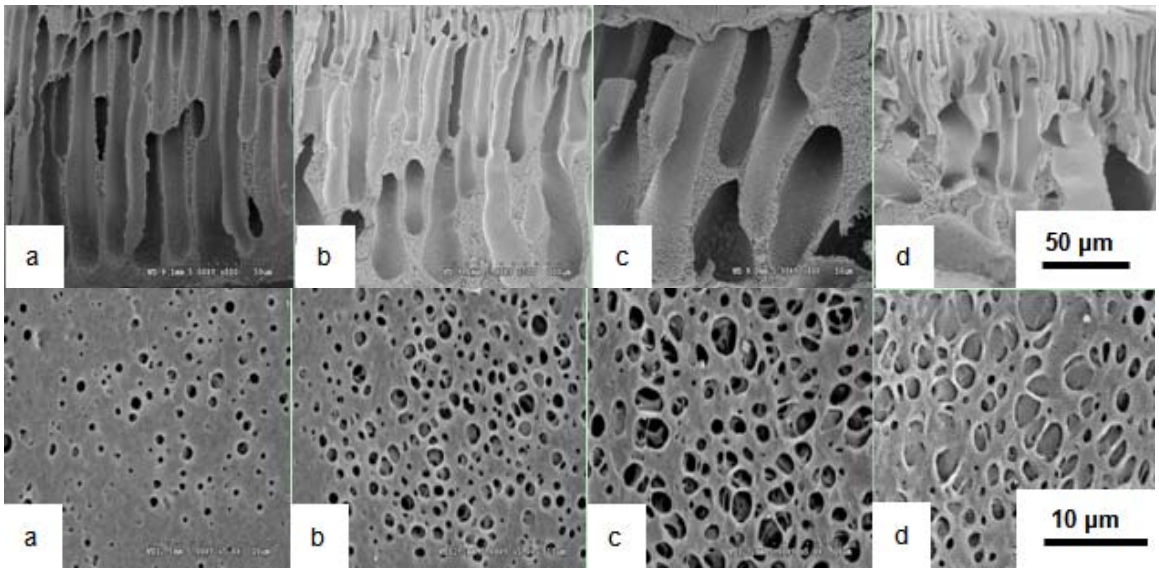

Fig. 5. SEM images of the cross-sections (upper) and bottom surfaces (below) of the pure PS and blend membranes with different NCC contents: (a) 0 %; (b) 0.3%; (c) 0.7%; (d) 1.1%

AFM Analysis of the Dialysis Composite Membranes

There were many pores of different sizes on the rough surface of the dialysis composite membrane (Fig. 6). Compared with the bottom surface with loosened configuration and larger pore sizes, the surface layer was bestrewed with pores of minute sizes distributed in the range 5 to 15 nm, which was much smaller. Thus, the composite membrane had a typical asymmetrical porous structure.

Compared with pure PS membrane, NCC was clearly distributed on the surface of the dialysis composite membrane. Just as was shown in the TEM images, NCC had a length of nearly 150 nm and a 20 to 50 nm width in the polymer. AFM images demonstrated that NCC still maintained its own surface topography in the finished polymer product. Hence, NCC could play an important role in the dialysis composite membrane to improve the hydrophilicity, mechanical strength, and some other properties.

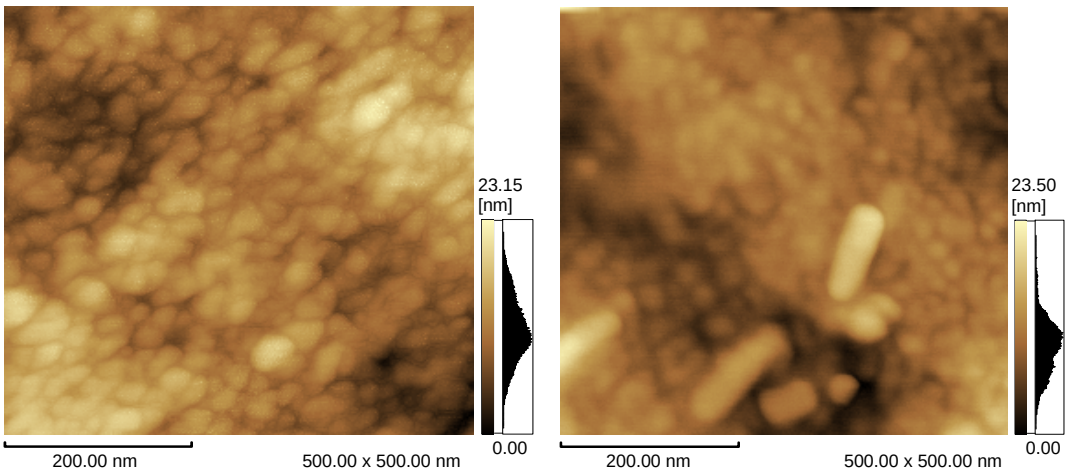

Fig. 6. AFM images of the pure PS (left) and the composite membrane (right)

CONCLUSIONS

A dialysis composite membrane was successfully prepared by the Loeb-Sourirajan phase-inversion method. According to permeability tests, when the NCC content was 0.3 wt%, the ultrafiltration coefficient of the dialysis membrane reached 48.4 $L \cdot m^{-2} \cdot h^{-1} \cdot mmHg^{-1}$. The clearance of lysozyme and urea reached 70.3% and 90.4%, respectively. Simultaneously, the retention ratio of BSA remained over 96%. The decreasing contact angle with increasing NCC content indicated an improvement in the hydrophilicity of the dialysis membrane. The tensile strength and the elongation ratio of the dry dialysis membrane increased by 36.4% and 40.2%, respectively, while mechanical properties could be better maintained in the wet state. But excessive NCC content could cause agglomeration, which resulted in a decline in all aspects of the membrane. Both TEM and AFM images showed that NCC was well-distributed and had nearly 150 nm length and 20 to 50 nm width. According to observations of SEM and AFM, the composite membrane had a clear asymmetrical structure. The NCC changed the structure of the composite membrane and thereby improved its properties.

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