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Ceramic Membranes for Separation of Proteins and DNA through In Situ Growth of Alumina Nanofibres inside Porous Substrates

Xue Bin Ke,^a Ren Fu Shao,^b Huai Yong Zhu,^{*a} Yong Yuan,^a Dong Jang Yang,^a Kyle R. Ratinac,^c and Xue Ping Gao^{*d}

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Ceramic membranes were fabricated by *in situ* synthesis of alumina nanofibres in the pores of alumina support as a separation layer, and exhibited a high permeation selectivity of bovine serum albumin relative to bovine hemoglobin (over 60 times) and can effectively retain DNA molecules at high fluxes..

Separation processes that use filtration membranes offer substantial economic, environmental and safety benefits.^{1,2} Ceramic separation membranes are of particular interest in many industrial processes for several reasons: they have excellent durability under extreme conditions and chemical and thermal stability; they also function efficiently within organic and biological systems, and at high temperatures; and they can be cleaned (or sterilized) readily by steam-treatment, while maintaining a long operational life.¹⁻³

However, conventional ceramic membranes currently suffer from a dramatic decline in flux when pore sizes are reduced to enhance selectivity. Such a trade-off is intrinsic to the structure of conventional membranes, which are formed from aggregates of particles. This structure has less than 36% porosity and inevitably has dead-end pores that make no contribution to the flux.³ In contrast, several recent studies have confirmed that the mesh-like structure formed from threads or fibres represents the most efficient structure for pressure-driven membrane filtration. Such structures are able to achieve high selectivity while retaining very high filtration rates.^{4,5} In a previous study, we found that a separation layer of ceramic nanofibres had porosity of over 70% of its volume,⁵ and thus permitted flow rates orders of magnitude greater than conventional membranes. The sizes of the voids in the separation layer of ceramic nanofibres range from several nanometers to tens of nanometers, making them suitable for the separation of large bio-molecules. Large-scale efficient separation of bio-species, such as DNA and protein molecules is needed for the development of gene therapies and DNA-based vaccines.^{6,7} This is a topic of profound significance for bioscience and biotechnology. However, the current membrane systems have not been developed specifically to meet the requirements to attain high flux with high selectivity.

The ceramic membranes constructed with ceramic nanofibres have an additional superior property to those formed with polymers. Metal-oxide surfaces carry surface charges derived from hydroxyl groups, the sign and magnitude of which change when the pH of the surrounding fluid varies. This property can be exploited to enhance the separation performance of ceramic membranes when the species to be

separated carry electrical charges, like many biological substances.^{8,9} In the present study, a superior separation layer of alumina nanofibres was fabricated in the pores of the alumina support by *in situ* transformation of aluminum hydroxide. Figure 1 is the schematical comparison between the proposed new membrane structure and the structure of the conventional ceramic membranes.

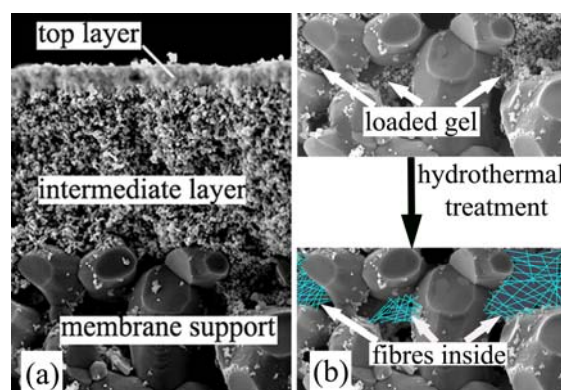


Fig. 1 Profiles of the nanofibre membranes. (a) Conventional technique by sol-gel coating; and (b) schematic process of *in situ* formation of the nanofibres in the pores of the support.

Aluminum-hydroxide precipitate was obtained by adding tetraethylammonium hydroxide to an $\text{Al}(\text{NO}_3)_3$ solution. To ensure boehmite fibres formed from the precipitate in the subsequent hydrothermal reaction, it was crucial to adjust the pH of the precipitate to 5.0. The precipitate was loaded into the micron-scale pores of a porous alumina support by dip coating (top panel of Fig. 1b). The excess precipitate on the top surface was removed simply by scraping and flushing. Then the precipitate-loaded support was put into an autoclave with a Teflon liner, physically separated from 2 mL of water in the bottom of the vessel (Fig. S1), at 170 °C for 72 h. During this hydrothermal treatment, the amorphous precipitate transformed to boehmite (AlOOH) fibres in the pores of the support (Fig. S2) via a steam-assisted conversion process.¹⁰ The resultant membranes were washed with de-ionized water and air dried at 80 °C. Since the volume of the hydrated, amorphous precipitate is much larger than the crystalline boehmite, the transformation from the hydroxide gel to boehmite nanofibres is accompanied by a large decrease in volume, which leads to pores well-dispersed among the boehmite nanofibres (Fig. 1b, lower panel).

Subsequent calcination at 500 °C converted the boehmite to the γ -alumina phase, as shown by the XRD patterns (Fig. S3),

while the fibril morphology was maintained. The pore volume of the fibre layer was further increased by calcination, owing to the loss of structural water during the transformation from boehmite to γ -Al₂O₃. The γ -Al₂O₃ fibres are about 100-300 nm long and about 10 nm thick (Fig. S3). Pre-treatment of the porous alumina support with a nitric acid solution cleaned the surface of the pore walls in the support and enhanced the contact of the precursor precipitate with the support so that the boehmite nanofibres formed subsequently could bond solidly the support.

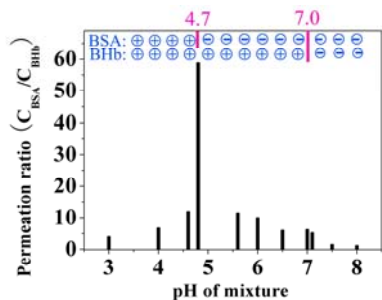


Fig. 2 Permeation selectivity and surface charge of BSA and BHb as a function of pH. The ratio of BSA to BHb in the initial solution was adjusted to 1.

The alumina carries positive surface charges at pH below its isoelectric points (IEP) and the surface charge of the oxide fibres can be used to enhance the separation of charged proteins by the membranes. For instance, it is difficult to separate bovine serum albumin (BSA) and bovine hemoglobin (BHb) by size sieving because BSA is a globular prolate ellipsoid with dimensions of 14×3.8×3.8 nm, and BHb, though more spherical, has similar dimensions of 6.4×5.5×5 nm. However, they are charged proteins; the IEPs of BSA and BHb are 4.7 and 7.0, respectively,¹¹ which allows us to separate them effectively by regulating the pH of the solution containing the two proteins. Figure 2 shows the permeation ratio of a mixture of BSA and BHb at different pH values; the ratio reflects the difference in permeation between the two molecules. At a pH of 4.7, the permeation ratio is about 60, which indicates that the BSA species passed the membrane readily but most of the BHb species were excluded by the membrane. As the pH was increased to about 7.0, the permeation ratio decreased substantially. The IEP of alumina is about 8-9,⁹ and in the filtration tests the alumina nanofibres in the separation layer of the membrane were positively charged. When the filtration tests were conducted at pH 4.7, which is the IEP of BSA, the BSA molecules had no surface charge, while BHb molecules carried positive charges. Thus, the BHb species were electrostatically repelled from the positively charged alumina fibres, resulting in low permeation through the membrane and excellent selectivity. At pH 7 in contrast, the BHb molecules were neutral and passed through the membranes without being repelled, while BSA species carried negative surface charges which allow a relative high flux (after surface adsorption) through the membrane because of the electrostatic attraction. The high permeations of both BHb and BSA species at pH 7 lead to a low filtration selectivity. The nanofibres have a large specific surface area

(160 m²·g⁻¹) and most of the surface area is exposed to the fluid to be filtered. This significantly magnifies the effect of the surface charges, so that tuning surface properties of the alumina nanofibres is an effective means to further tailor the performance of these new membranes.¹²

Another important advantage of these membranes is that they permit high flux, and can work under low operating pressures. Serious drawbacks of the existing processes for separation of bio-substances using size-exclusion and ion-exchange chromatography are time-consuming and high expense.⁶ Studies with polymer membranes have demonstrated the potential of membrane-based filtration process for purification of plasmid DNA and proteins.^{6,7,13} The membranes with a separation layer of nanofibres may permit a high flux, having good potential for large-scale separation of biosubstances. To test the performance of these new membranes, we carried out filtrations with two important bio-molecules. Initially, a 0.1% aqueous solution of ferritin, a protein about 12 nm in size,¹⁴ was used to test the protein-separation ability. We found that the membrane prepared in this study retained 93% of ferritin protein at a high flux of 95.4 L·m⁻²·h⁻¹. The size-separation effects are prominent, which is consistent with the pore size distribution of the resultant membranes (10-20 nm). In contrast, the ultra-filtration flux achieved with hollow-fibre membranes for biological separation was reported to be less than two-thirds of this at 60 L·m⁻²·h⁻¹ or below.⁶

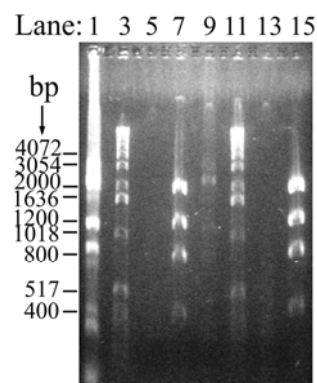


Fig. 3 The agarose-gel electrophoresis results. Lane 1: 20 µL DNA sample before filtration; Lane 3 and Lane 11: 2 µL DNA Molecular-Weight Marker X (Roche); Lane 5: 20 µL DNA sample after filtration; Lane 7 and Lane 15: 2 µL Low-Mass Ladder DNA marker (Invitrogen); Lane 9: a diluted sample (2 µL DNA sample in Lane 1 was diluted to 20 µL with water) before filtration; Lane 13: the diluted sample after filtration; other lanes: blank.

The membranes prepared in this study were also used to separate linear DNA molecules that ranged from 50 to 3500 base pairs (bp). The concentration and size of DNA molecules were measured by spectrometry and agarose-gel electrophoresis before and after filtration.⁶ The content of DNA in the filtrate was 3 ng·µL⁻¹ (Lane 13 in Fig. 3), reduced from 10 ng·µL⁻¹ in the initial solution (Lane 9 in Fig. 3) by filtering. About 70% of the DNA molecules were retained by the membrane at a flux of 230.4 L·m⁻²·h⁻¹. The high flux we achieved in this study is attributed to the large porosity of the separation layer, which has porosity of over 70% of its

volume, far greater than that of conventional membranes.

It is known that conventional membranes are formed from aggregates of particles and inevitably have dead-end pores that make no contribution to the flux (Fig. 1a). Also, separation layers fabricated by the ship-in-a-bottle approach have been reported to construct a uniform nanopore.^{15,16} In these processes, porous supports were impregnated with carbon nanotubes¹⁶ or mesoporous silica.¹⁷ However, it is still difficult for the resultant materials to achieve high flux and selectivity simultaneously.

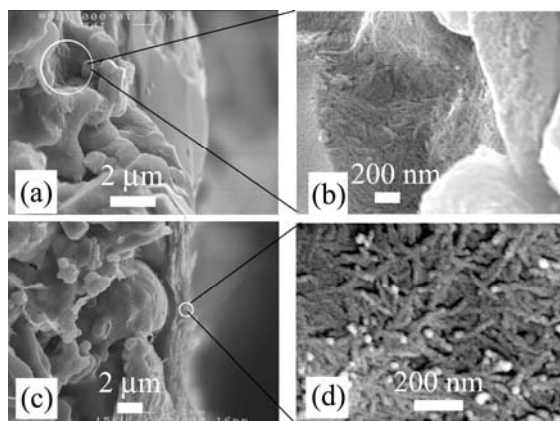


Fig. 4 Scanning electron microscopy (SEM) images of the membranes with alumina nanofibres: (a) cross-section of a membrane prepared via *in situ* formation of nanofibres, (b) top view of the area in (a), (c) cross-section of a membrane prepared by spin-coating, and (d) top view of the area in (c).

As shown in Figure 4, the alumina fibres were formed mainly in a region, of approximately 1 μm thickness, just below the top surface. As a separation layer, this is relatively thin and we expected it would permit a large flux to pass through in use. The randomly oriented alumina fibres filled the pores of the alumina supports, forming a mesh-structure (as shown in Fig. S2), which is unlikely to form pinholes and cracks. Compared with the fibres membrane prepared by spin-coating, the separation layer of nanofibres fabricated by *in situ* formation approach is better protected within the pores of the support and the nanofibres are well anchored to the walls of the supports. The voids in the separation layers of alumina nanofibres range from several nanometers to tens of nanometers, which overlap with the sizes of DNA and protein molecules. These voids are the channels for the filtration flux and the new membranes permit a high flux. This feature highlights the potential of the new membranes for the separation of biological substances and pharmaceuticals.

In general, this new approach for membrane construction has several major benefits over conventional membranes. Firstly, it is particularly suitable for tangential-flow filtration, which effectively relieves surface fouling during filtration and increases ease of cleaning. Secondly, there is no need for the intermediate-layer that usually lies between the porous support and the separation layer in traditional ceramic membranes. Thirdly, the transformation from hydrated amorphous precursor to the nanofibres of a crystalline phase creates homogeneously distributed pores of high volume fraction in the resultant membranes. Finally, the pH-

dependent surface charge of the alumina fibres can be used to enhance and tailor the separation of bio-substances with surface charges. The features of this new approach offer potential for significant improvement in the efficiency of membrane manufacture, for reduction in fabrication costs, and for ready scalability. The resultant membranes exhibit excellent separation performance when it comes to biological substances. This study highlights a new avenue for ceramic separation membranes, both in their fabrication and in their application for separating bio-molecules.

Notes and references

^a School of Physical and Chemical Sciences, Queensland University of Technology, Brisbane, Qld 4001, Australia. Fax: +61 7 3138 1804; Tel: +61 7 3138 1581; E-mail: hy.zhu@qut.edu.au

^b School of Molecular and Microbial Sciences, The University of Queensland, St Lucia, Qld 4072, Australia

^c Australian Key Centre for Microscopy and Microanalysis, The University of Sydney, Sydney NSW 2006, Australia

^d Institute of New Energy Material Chemistry, Nankai University, Tianjin 300071, China. E-mail: xpgao@nankai.edu.cn

† Electronic Supplementary Information (ESI) available: Preparation of boemite nanofibres membrane, separation process of proteins and DNA, and detailed characterisation techniques. See DOI: 10.1039/b000000x/

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