Investigating the feasibility of using Polysulfone-Montmorillonite Composite Membranes for Protein Adsorption

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Abstract

The feasibility of immobilisation of montmorillonite (MMT) in polysulfone (PSf) to form mixed matrix membrane (PSf/MMT) to serve as the adsorbent for BSA proteins from a model white wine solution was investigated. Pristine PSf and modified PSf/MMT membranes were synthesized using the phase inversion method and characterized using various surface techniques. Addition of MMT particles in the polysulfone matrix enhanced the hydrophilicity of the membrane surface and promoted the formation of a more porous structure in the PSf/MMT membrane, resulting in greater permeance but lower rejection in comparison to the PSf membrane. In addition, imaging analysis demonstrated recognition of protein adsorption on the adsorptive areas of the MMT particles within the PSf/MMT membrane matrix which confirmed the hydrophobic interactions between the MMT particles and BSA protein molecules. The finding is a significant step for subsequent research to examine the possible applications of clay-filled polymers in selectively removing protein from wine.

Keywords: XPS imaging; protein separation; adsorption; montmorillonite, polysulfone membrane, mixed matrix membrane.

Abbreviations

BSA	bovine serum albumin
MMT	montmorillonite
NMP	N-methyl-2-pyrrolidone
PSf	polysulfone membrane
PSf/MMT	polysufone montmorillonite composite membrane
PSf_F	PSf membrane after filtration test with model wine solution
PSf/MMT_F	PSf/MMT membrane after filtration test with model wine solution
SEM	Scanning Electron Microscope
XPS	X-ray Photoelectron Spectroscopy

1. Introduction

Bentonite, comprising predominantly montmorillonite (MMT, a member of the smectite group), has been extensively used to prevent the protein haze-forming in white wines for more than 70 years (Ferreira et al., 2001; Hsu and Heatherbell, 1987). The process, known as fining, is primarily driven by hydrophobic interactions where positively charged proteins are agglomerated onto the surface of negatively charged clay particles. Studies have shown that bentonite significantly swells and behaves like a series of small plates upon agitation in water. This results in a very large surface area which can adsorb as much as several times as in its dry condition (Sarmento et al., 2000a; Siddiqui, 1968). However, because of its enormous availability, low cost, and the lack of available commercial processes for its separation/ regeneration from the wine, bentonite is usually used once and then discarded into the environment. This results in significant losses of wine captured in the slurry, and a high impact on the environment (Blade and Boulton, 1988; Ferreira et al., 2001; Hsu and Heatherbell, 1987; Salazar et al., 2006a; Sarmento et al., 2000a; Siddiqui, 1968; Waters et al., 2005). A recent article reported that the estimated total cost to the world wine industry of bentonite fining in its current form exceeds US$1 billion (Majewski et al., 2011).

Recently many researchers have looked for alternative methods to remove proteins from white wine. Different techniques have been employed, such as ultrafiltration (Flores et al., 1990; Hsu and Heatherbell, 1987; Hsu et al., 1987), proteolytic enzymes (Waters et al., 1992), flash pasteurization (Pocock et al., 1998), or using polymers or metal oxides (Pashova et al., 2004a; Pashova et al., 2004b; Salazar et al., 2006b; Salazar et al., 2007; Sarmento et al., 2000a; Sarmento et al., 2000b). Ueda et al. (Ueda et al., 1995) and Lit et al. (Liu et al., 2008) used polymer membranes for protein recovery. Avramescu et al. (Avramescu et al., 2003a; Avramescu et al., 2003b; Avramescu et al., 2003c) developed new protein adsorber membranes by the incorporation of various types of ion exchange resins into the polymer membranes.

Researchers examining separations other than protein have reported the application of clay-filled polymer membranes in adsorption processes for gas separation, pervaporation and wastewater treatment (Adoor et al., 2006; Anadão et al., 2010; Choudalakis and Gotsis, 2009; Defontaine et al., 2010; Kim et al., 2006; Picard et al., 2007; Villaluenga et al., 2007; Wang et al., 2004). It has been reported that the incorporation of inorganic particles in the polymer membranes suppresses the formation of macro-voids and enhances the formation of micro-pores. This results in increased permeance and enhances the mechanical and thermal stabilities of the membrane. In addition, the composite membrane (commonly referred to as a mixed matrix membrane) can be reused in multiple adsorption/desorption cycles, thus reducing the amount of waste and the environmental impact compared to conventional clay based adsorption processes (Aerts et al., 2000a; Aerts et al., 2000b; Bottino et al., 2001; Clarizia et al., 2004; Nagarale et al., 2005; Tezuka et al., 2006; Uragami et al., 2005; Vankelecom et al., 1997; Yang et al., 2008; Yang et al., 2007).

These studies reported the ***physical*** improvements of using clay-polymer membranes over pristine polymer membranes, and their uses in different applications, but to date there has been no study to examine the application of clay-polymer membranes for protein adsorption. Thus, the main focus of our work is investigating the feasibility of

the immobilisation of clay particles, in particular MMT, in polymeric materials to form mixed matrix membranes to serve as the adsorbent for BSA proteins from the model white wine solutions. The membrane was used to act as a support substrate for the MMT particles slowing the adsorption and intercalation within the MMT to proceed without using a bentonite slurry. Polysulfone (PSf) was chosen as the membrane polymer due to its thermal, biological, and chemical stablility (Charcosset, 1998). An evaluation of membrane morphology, membrane protein rejection and changes of the membrane surface chemistry with the adsorption process was combined to clarify the key question of this research: "How does addition of MMT particles in the PSf membrane affect membrane structure and BSA protein adsorption"

2. Materials and Methods

2.1 Materials

Polysulfone (PSf) in pellet form with M_w = 35,000, N-methyl-2-pyrrolidone (NMP, >99.5%), potassium D-tartrate monobasic and ethanol were all supplied by Sigma Aldrich, MO, USA. Bovine serum albumin (BSA, Fraction V lgG free) was supplied by Gibco, NY, USA. All chemicals were used as received without further purification. The inorganic particles dispersed in PSf polymer were sodium montmorillonite from Sigma Aldrich, MO, USA. As shown in Fig. 1, the MMT particles were approximately spherical and had an average particle size below 30 μm.

2.2 Membrane preparation

In this paper, two types of membranes, namely polysulfone (PSf) and polysufone montmorillonite composite (PSf/MMT), were prepared via the phase inversion by the immersion precipitation method. The ratio of polysulfone, solvent and MMT powder used is summarised in Table 1. A homogeneous polymer solution consisting of PSf and NMP with suitable weight ratio (Table 1) was initially prepared by continuous stirring for several hours at room temperature until all polysulfone pellets were completely dissolved. The required amount of MMT powders was added into the polymer solution and then stirred for at least six hours at 400 rpm until the solution became visually homogeneous. The casting solution was degassed for 5 min at room temperature and then poured onto a smooth glass plate and spread to a thin film with an Elcometer 3700 Doctor Blade doctor blade with reservoir. Membrane casting was performed using an Elcometer 4330 Basic Motorised Film Applicator (Elcometer Limited, Manchester, United Kingdom), with casting speed and film thickness set at 6 $cm.s^{-1}$ and 250 μm respectively. After casting, the coated glass plate was immersed for coagulation in a de-ionised water bath at room temperature until the membrane detached from the glass surface. The resulting membranes were washed with de-ionised water several times to remove all solvents and left to dry in a fume hood at room temperature.

2.3 Filtration tests

Protein rejection tests were carried out using a dead end pressure filtration cell (Steriltech^{TM} HP4750 Stirred Cell, Steriltech, WA, USA) at room temperature. Model wine solution with 600 $mg.L^{-1}$ bovine serum albumin, 120 $ml.L^{-1}$ ethanol, 2 $g.L^{-1}$ potassium tartrate buffer, and deionised water with pH 3.8 was used to measure the permeance (membrane flux divided by filtration pressure) and rejection of the membrane. This has characteristics and pH comparable to model wine solutions used by previous researchers (Sarmento et al., 2000a; Slatner et al., 1999) which is based on the

work of Blade and Boulton (Blade and Boulton, 1988). Before each measurement, the circular membrane samples with the diameter of 49 mm were cut from the membranes and immersed in de-ionised water overnight. This allows for the MMT to swell to the maximum extent, which can increase the adsorption ability of the protein (Blade and Boulton, 1988). The membrane coupon was installed in the cell and firstly was run with 100 ml of de-ionised water to precondition the membrane to a steady state compaction and removed any weakly attached particles. Model white wine solution was then poured into the cell magnetically stirred at 500 rpm. The pressure was initially kept constant at 5 bar. Time for the liquid to permeate 5 ml was recorded starting from 10 ml (the first clear reading on the measuring cylinder) to 30 ml in order to determine the membrane permeance. For each type of membrane, three different membrane samples were used for filtration test. Thus, the values reported in this paper are average values. The samples of PSf and PSf/MMT membranes after they have been used in filtration with model wine solution were named PSf_F and PSf/MMT_F respectively.

The concentration of BSA protein in permeate and feed solution was measured using an Agilent 8453 UV-vis spectrophotometer (Agilent Technologies, CA, USA) at the absorbance of 280 nm. The absorbance-concentration standard curve was initially developed from 10 standard concentrations from 50 $mg.L^{-1}$ to 700 $mg.L^{-1}$. The protein rejection (R, %) is defined as $(1-(C_p/C_f))$ x 100% where C_p and C_f denote the BSA concentrations of the permeation and the initial feed solution respectively.

2.4 Membrane characterisation

The morphology of the membrane surfaces and the distribution of the MMT particles within the polymer structure were characterised using a Philips XL30 S-FEG Scanning Electron Microscope (SEM) (FEI, Eindhoven, The Netherlands). Cross-sections of the membranes were prepared by breaking the membranes in liquid nitrogen and then coating with platinum using a sputter coater Polaron SC 7640 (Quorum Technologies, East Sussex, UK) at 1.1 kV for 180 s. The topography of the membranes was studied using Atomic Force Microscopy (AFM, Digital Instruments NanoScope IIIa,Veeco, NY, USA) in contact mode with a scan size of 60 µm x 60 µm. The topography was evaluated using two parameters: the average surface roughness (R_a) and skewness (S_k). The average surface roughness represents the average distance between the surface and a mean centreline, whereas the skewness describes the degree of asymmetry of the distribution. A negative skewness value indicates that the sample has more valleys than peaks, and the reverse for a positive skewness value (Thomas, 1999). Roughness data were obtained from a minimum of two samples with 4 different regions on each sample. The values reported are the averages of these measurements.

The hydrophobicity of the membranes were characterised based on their wettability which was evaluated by contact angle data. Contact angle measurements were made using the sessile drop method using a KSV CAM 101 instrument (KSV Instruments Ltd., CT, USA). A droplet of deionised water was placed on the membrane surface which was fixed flat on a glass slide using double-sided carbon tape at room temperature. For each PSf or PSf/MMT membrane, three different specimens were prepared at several regions for each specimen with measurements taken from both sides of the droplet. The measurements were immediately taken after the droplet was on the

membrane surface, and then at 5 s intervals until the water had completely absorbed/permeated into the membrane.

Pores on the membrane surfaces were analysed using SEM and ImageJ imaging software (National Institute of Health, Washington DC, USA) to obtain quantitative and qualitative information about pore sizes and morphologies. About 15 SEM images at magnification of 10,000x were acquired at random locations for each sample. From these images, a minimum of 10-15 random areas with around 1,000 pores were analysed using ImageJ. The obtained information included Feret diameter, area, circularity and perimeter of individual pores. The Feret diameter is the longest distance between any two points on the boundary of the pore.

To examine the surface chemical composition of the collected membranes, in particular the protein adsorption onto its surfaces, the membranes were analyzed with X-ray Photoelectron Spectroscopy (XPS) using a Kratos Ultra Axis DLD (Shimadzu, Manchester, UK). The excitation source in use was Al K_α (1486.6 eV). The pressure during analysis was between $1x10^{-9}$ and $1x10^{-8}$ Torr. To prepare the samples for the XPS examination, small pieces of the top and bottom surfaces of each membrane were mounted on a sample bar using double sided carbon conductive adhesive tape. MMT powders were pressed onto the double side carbon tape which was glued on the bar, and then shaking to remove loose material. The cross section of each membrane was tightly sandwiched between two aluminium sheets and installed in the sample bar for the chemical analysis of the exposed cross sections. To obtain the surface chemical composition of the membranes, and corresponding images of all elements of interest, the analysis included the survey scans and narrow scans and then imaging as described in Table 2.

In the current study, elemental images were obtained for carbon (C 1s), aluminium (Al 2p), silicon (Si 2p), nitrogen (N 1s) and oxygen (O 1s). The binding energy was chosen from the narrow scans to include a specific photoelectron peak. The step size was chosen at 0.2 eV. At each step size of binding energy, image with high resolution of 256x256 pixels was taken and was subsequently processed to obtain elemental maps using CasaXPS version 2.3.12. More detail of the processing procedures of the data set can be found in the CasaXPS manual (Casa Software Ltd., 2009).

3. Results and discussion

3.1 Membrane morphology

The morphology of the membrane surfaces and the distribution of the MMT particles in the membrane structure were characterized using SEM. Figure 2 shows the typical morphologies of the top and the bottom surfaces of PSf and PSf/MMT membranes. As typical for phase inversion membranes, the top surface is defined as the side that is exposed to the air during casting whereas the bottom surface is in contact with the glass plate. It was found that pore formation varied between the top and bottom surfaces of each membrane. High densities of pores were observed on the top surfaces of both PSf and PSf/MMT membranes whereas few pores were found at the bottom of the membranes. The formation of a large number of pores at the top surface might relate to the membrane casting process. It is suspected that further optimisation of the membrane casting procedure is required to cast an optimal membrane for fining operations and is

therefore the focus of future work. This would include increasing the immersion time in the water bath (to provide further time for non-solvent penetration and macropore formation in the lower surfaces) and further degassing of the solvent (air may have either produced defects and/or escaped through the membrane film after casting and promoted the formation of surface pores as observed). Optimisation of the membrane was not the aim of this work however, and for this preliminary investigation all membranes were cast in a consistent manner and therefore changes produced by the presence and absence of clay particles can be compared relative to each other and robust conclusions that can be translated to optimised membranes (which are the subject of future work) can be made on this basis. .

The pore-size distributions of the PSf and PSf/MMT top surfaces were characterized using ImageJ and shown in Fig. 3a. Pores were found with a quite regular distribution all over the top surface of the membranes. Also the proportion of pores with the diameter from 0.3 to 1 μm on the PSf top surface (64.2%) was higher than on the PSf/MMT top surface (47.3%). For the case of larger pores with the diameter from 1 to 3 μm, the pore proportion on the PSf surface was about 35.5% relative to PSf/MMT surface. It was also observed that there was no pore with the diameter larger 3.5 μm on the PSf top surface whereas approximately 8% of this pore type was found on the PSf/MMT surface. It appears that that addition of MMT into the polymer solution weakens the interaction among polymer molecules and expands the spacing between them in the solutions, thus enhancing the diffusion rate of the solvent (Yan et al., 2007). This resulted in larger pores on the PSf/MMT top surface compared to the PSf one. The appearance of larger pores on the PSf/MMT top surface had an effect on the number of pores per unit area. It can be seen from Fig. 3b that the average number of pores on the PSf and PSf/MMT surfaces per square micron was 0.12 and 0.1 respectively. However this difference is not statistically significant within the errors of the data collected.

To further examine the membrane structure, several cross sections of the membranes were prepared and studied. The typical cross section of PSf membrane is shown in Fig. 4. In general, the membrane had an asymmetric structure across the cross-section. Four distinct layers were observed across the cross section: a thin dense skin layer (1) at the top surface, supported by an irregular micro-tubular pores layer (2) with an open macro-void structure (3), and porous sponge-like layer (4) at the bottom of the cross section. Incorporation of MMT particles in polysulfone solutions resulted in significant changes in the membrane structure as can be seen in Fig. 5. The MMT particles were distributed across the membrane thickness and tightly held within the porous polymer matrix. The PSf/MMT presented an interconnected porous structure, without evidence of macro-voids in the open pore structure (large holes (4) observed in Fig. 5a were not macro-voids, but were likely formed as the particles detached off the membrane network during fracturing the membrane for SEM observation). It has been reported that the formation of macro-voids relates to the diffusion rate of different phases in the casting solution (Smolders et al., 1992; Vandezande et al., 2009). In particular, the difference in concentration between NMP solvent and polymer in the casting solution resulted in the difference in their diffusion rates and hence promoted the formation of macro-voids in the PSf membrane (Vandezande et al., 2009). In contrast, introduction of solid particles into the polymer solution increased the viscosity of the casting solution and promoted the formation of nuclei in solution resulting in delayed diffusion which suppressed the

growth of macro-voids formation in the PSf/MMT membrane (Vandezande et al., 2009).

It was expected that the differences in the morphologies between the PSf and PSf/MMT membranes, would contribute to the permeance and rejection ability of these membranes with BSA model wine solution. The formation of larger size of the pores at the PSf/MMT surfaces could enhance the flow of the model wine solution, thus causing a higher BSA pemeance over the pristine PSf membrane, as discussed in more detail later. In addition, the absence of the macrovoids within the PSf/MMT membrane may enhance the mechanical strength of the membrane during high pressure applications.

3.2 Membrane topography

The topography of the membrane surfaces was characterized using AFM. 3-D imaging of typical topographies of the PSf and PSf/MMT membranes are shown in Fig. 6 and the corresponding surface roughness values are presented in Fig. 7. The results revealed that the bottom surfaces of the PSf and PSf/MMT membranes were macroscopically smooth with the average surface roughness of 35.2 ± 13.8 μm and 108.62 ± 8.3 μm respectively. In contrast, the presence of a significant number of pores on the top surfaces of these membranes unduly affected the average surface roughness measurements, and resulting in the negative skewness values (Fig. 7b). The average surface roughness of PSf and PSf/MMT top surface were 350.2 ± 36.5 nm and 965.8 ± 192.3 nm respectively. It was also found that that the larger pores were observed on the PSf/MMT top surface which is consistent with the SEM observations.

The smoother PSf top surface resulted in its smaller absolute surface area. The data analysis shows that the surface area of PSf and PSf/MMT membranes were 4,688 ± 156 μm^2 and 6,142 ± 915 μm^2 respectively in the same projected area of 3,600 μm^2. The increment of the surface area and the surface roughness value of the PSf/MMT membrane compared to the PSf one indicates that the addition of MMT particles enhances the effective filtration area and probably the hydrophobic property, and thus improving the permeation through the modified PSf/MMT membrane.

3.3 Contact angle measurements

The hydrophobic nature of the PSf and PSf/MMT membranes was characterised by recording contact angle measurements. The contact angle was immediately taken within one second after the water droplet was on the membrane surface. The water contact angle on the PSf top surface was 89.4 ± 2.3º, indicating the hydrophobic nature of the surface. In contrast, the PSf/MMT surface showed a much more hydrophilic surface with a much lower contact angle of 70.4 ± 3.2º. The changes of water contact angle with time are subsequently observed until the water droplet adsorbed completely into the membrane and shown in Fig. 8. It was observed that the droplet spread out quickly upon impact. The contact angle decreased rapidly within the first 30 s and subsequently reduced linearly. The water droplet was completely adsorbed on the PSf membrane in 35 ± 3 min whereas it took only 12 ± 1 min on the PSf/MMT membrane top surface. This indicated that addition of MMT particles enhanced the hydrophilicity of the PSf/MMT membrane, and it would contribute to lower BSA protein adsorption because of the reduced hydrophobic interaction between membrane surface and protein.

3.4 Flux and rejection measurements

Filtration tests of the model white wine solution through the PSf and PSf/MMT membranes were performed to evaluate their permeate permeance and BSA protein rejection. Figure 9 represents the corresponding permeance of the PSf and PSf/MMT membranes respectively. It was observed that model wine solution was not permeable through the PSf membrane at 5 bar, but can easily flow through the PSf/MMT membrane at the same pressure. The pristine PSf membrane instead required an operating pressure of 20 bar. It was observed that the PSf membrane permeance was initially at 5.1 $L.m^{-2}.h^{-1}.bar^{-1}$ and subsequently declined to zero after approximately half an hour. The permeance through the PSf/MMT membrane decreased from 77.8 $L.m^{-2}.h^{-1}.bar^{-1}$ at the beginning to 15.3 $L.m^{-2}.h^{-1}.bar^{-1}$ at the end of the filtration test, which was significantly higher than that of the PSf membrane. The differences in permeance induced the BSA protein rejection between PSf and PSf/MMt membranes. It was found that the rejection of the PSf/MMT membrane at 5 bar was 33.8 ± 5.2 %, whilst the PSf rejection was 68.5% ± 17.9 at much higher operation pressure of 20 bar.

The differences in operating pressure, permeance and rejection ability of PSf and PSf/MMT membranes with model wine solution were expected to be linked to their surface properties. Schneider et al. (Schneider et al., 1988) reported that the pressure required to push the flow through the microporous hydrophobic membranes is inversely proportional to the pore radius. As such, the formation of the denser skin layer, at the PSf top surfaces hindered the flow of wine solution, and thus required higher pressure and longer filtration times resulting in lower BSA permeance. The denser structure at the PSf surface also rejected protein molecules. In addition, the hydrophobic nature of the PSf membrane enhanced the hydrophobic interaction between the membrane surface and protein, causing concentration polarisation and probably fouling, and correspondingly a higher rejection of BSA protein molecules as observed. In contrast, the porous structures of the PSf/MMT membrane surface, its higher surface area and its hydrophilic nature induced a higher permeance but lower protein rejection.

From these observations, it can be concluded that the permeance increased with the decrease in rejection of the membrane, which was coincident with the hydrophilic nature and increasing size of pores on the surfaces as would be expected from a porous membrane separating a large protein like BSA, where separation is mainly via a size exclusion (porous flow) mechanism. However a size exclusion mechanism is not selective towards the removal of haze causing proteins – other similar or larger sized components important to the quality of the wine (e.g. taste, colour and mouth feel) would be non-selectively removed as well. Consequently ensuring that the membranes separate components mainly via the more selective adsorption mechanism is crucial in ensuring that wine proteins are selectively removed. Note that BSA has adsorption properties (i.e. isoelectric point of around 4.3) within the range of wine haze proteins (Slatner et al., 1999) and so is a suitable model compound to investigate wine fining by adsorption. As a result of having the potential for separation via both adsorption and size exclusion, the separation mechanism of the more open (porous) PSf/MMT mixed matrix membrane is more complicated. Therefore the selectivity mechanism was explored further by XPS analysis.

3.5 Surface chemistry of the membranes.

To examine the surface chemical composition of the collected membranes, the membrane surfaces were analyzed with XPS. In addition, high spatial resolution XPS imaging was used to examine where the BSA protein was on both the membrane surfaces and within the membrane cross-section. Therefore, the top and bottom surfaces and cross section of each membrane were analyzed. In addition, the chemical composition of MMT particles was also analyzed. Typical survey scans of the MMT particles, the top surfaces of PSf and PSf_F membranes are shown in Fig. 10. From the survey scans, the surface chemical compositions of each sample were calculated and presented in Table 3.

As can be seen in Fig. 10a and Table 3, the compositions of the MMT powders include silicon (Si 2p), aluminium (Al 2p), oxygen (O 1s), calcium (Ca 2p), sodium (Na 1s), and adventitious carbon (C 1s) which were in agreement with literature (Barr et al., 1995). The survey spectra of the top, bottom surfaces and cross section of the PSf membrane showed three single peaks of C 1s, O 1s and sulphur (S 2p) at the binding energy of 284.5 eV, 532.5 eV and 167.5 eV respectively. For the polysulfone membranes after they have been used in filtrations with the model wine solution (PSf_F as defined in 'Materials and Methods' section), a fourth peak appeared at the binding energy of 399.5 eV which is the proteinaceous N 1s peak (Fig. 10c). A trace of nitrogen was found on both the top and bottom surfaces of the PSf_F membrane, but at a different concentration. The nitrogen proportion at the top surface (5.8%) was much smaller than that at the bottom surface (12.7%) of the PSf_F membrane. In addition, typical XPS images of C 1s, O 1s and N 1s at the PSf_F bottom surface in Fig. 11 confirmed very good adsorption of protein on the PSf surface.

Evidence of nitrogen was found on both the top and bottom surface of the PSf_F membrane. However, chemical analysis, and N 1s imaging of its cross section, revealed that there was no detectable trace of nitrogen across the membrane thickness. From these observations, it can be suggested that the separation of BSA by the PSf membrane mainly relates to its morphology, particularly the number of pores and pore size on the membrane surface (Fig. 2). The smaller the pores were at the bottom surface, the higher protein selectivity was achieved. In addition, hydrophobic nature of the PSf membrane may also contribute to the adsorbed protein on the membrane surface. However, the observation of limited protein adsorption across the thickness of the membrane where its structure mainly composed of open pores and macro-voids suggested that morphology is more important factor than the hydrophobicity in protein separation membrane.

It is noted that the changes in surface chemistry observed here are only for the 10 nm outermost layer within the XPS depth of analysis (Briggs and Seah, 1990). Thus, the evidence of Si 2p and Al 2p peaks in the surface compositions of PSf/MMT and PSf/MMT_F membranes (Table 3) confirmed the presence of MMT particles within 10 nm at the top and bottom surfaces of the membrane. Like the PSf_F membrane, nitrogen was found on both the top and bottom surfaces of the PSf/MMT_F membrane, but in a lesser amount. There were 1.8% and 5.2% of nitrogen on the top and bottom surfaces of the PSf/MMT_F membrane. The differences in the nitrogen proportion detected on the surfaces between PSf_F and PSf/MMT_F membranes were again in

relation to the amount and size of pores distributed on the membrane surface as well as the hydrophobicity of the surface, as discussed in the previous paragraph.

In contrast to the PSf_F cross section (with no detectable nitrogen), 2.2% of nitrogen was found in the PSf/MMT_F cross section. That indicated that the BSA protein was probably trapped inside the pore network in the PSf/MMT_F membrane. To further understand whether the adsorption was due to the membrane structure or the hydrophobic interactions between the active sites on the MMT particles and BSA protein, XPS imaging of C 1s, O 1s, N 1s, Al 2p and Si 2p were obtained. Figure 12 shows the images taken from an area within the cross section of the PSf/MMT_F membrane. It was observed that the analysis area of 100 x 100 µm was completely covered with carbon (Fig. 12a). The presence of Al 2p (Fig. 12d) and Si 2p (Fig. 12e) indicated the presence of MMT particles within the cross section surface in an island-like formation (corresponding to MMT particles). It was also found that the MMT surface was covered by nitrogen, as can be seen in Fig. 12c-f. This evidence indicated that the protein adsorption mechanism of the PSf/MMT_F cross section did not relate solely to its morphology, but occurred on the functional adsorptive sites of the MMT particles.

The difference in chemical analysis between the PSf and PSf/MMT membranes was again in good agreement with the morphology observations, hydrophobicity and the measurements of the permeance and rejection. The hydrophobic nature along with the denser structure with lower porosity at the top and bottom surfaces of the PSf membrane resisted flow, but enhanced the protein selectivity at these surfaces. In contrast, the porous sublayer along the membrane thickness had no effect on the sepration characteristics of the PSf membrane. Thus, the separation of the protein on the PSf membrane was solely related to their hydrophobic nature and dense struture at the top and bottom surfaces.

Incorporation of MMT particles in the polysulfone matrix enhanced the hydrophilicity of the surface and promoted the formation of a more porous structure in the PSf/MMT membrane, resulting in greater permeance but lower rejection in comparison to the PSf membrane. However, evidence of protein on the PSf/MMT top, bottom surfaces, and on the MMT particle surfaces within the PSf/MMT cross section confirmed that the protein adsorption mechanism in this case occurred both onto the geometric area and on the functional adsorptive site of the MMT particles.

4. Conclusions

PSf/MMT composite membrane with 10% polysulfone and 10% montmorrillonite was synthesised using the phase change method. The structure and adsorption ability of the PSf/MMT membrane were systematically examined and compared to that of unmodified PSf membrane. It was observed that the PSf membrane posed a denser structure which was mainly linked with the size of pores at the top and bottom surfaces. This in turn required higher pressure to induce the flow transport, and hence lower permeance. However, due to its denser structure and hydrophobic nature, the BSA protein rejection of the PSf membrane was higher and mostly occurred at the membrane surfaces which led to undesired fouling. In addition, the occurrence of macrovoids along

the membrane thickness can contribute to the mechanical failure of the membrane during high pressure applications.

Inclusions of MMT particles inside the polymer solution resulted in the formation of the membrane with a porous structure and a hydrophilic surface, which in turn reduced the hydrophobic interaction at the surface and required a lower pressure to obtain an acceptable permeance. This therefore reduced fouling and enhanced permeability respectively. The MMT particles were found to be well-distributed along the thickness of the membrane which could contribute to the improvement in mechanical stability of the membrane under high applied pressure. Most importantly, it was demonstrated that there was targeted protein adsorption on the adsorptive areas of the MMT particles within the membrane matrix, confirming that there are hydrophobic interactions between the MMT particles and BSA protein molecules. This means that the mixed matrix membrane works as intended - it supports the MMT particles without blocking the adsorption sites within the polymer matrix. This indicates that it is possible to make a membrane with this system that is able to be porous enough to allow the molecules responsible for wine's flavour and texture to pass through whilst removing the unwanted proteins. Therefore, these findings are a significant step to better understanding the fundamental knowledge in adsorption ability of MMT particles and to predict the possible applications of using clay-filled polymer membrane to remove protein from wine in a membrane type module, opening up the possibility of continuous wine fining operations.

Due to the adsorption of BSA protein on the MMT particles, it will be necessary to regenerate the PSf/MMT membrane once the MMT active sites are saturated with BSA proteins. The interactions between the BSA protein and MMT particles at different conditions of pH and ethanol content are currently being investigated to determine the reversibility of the MMT. So far it has been found that changing the pH is an effective way to desorb BSA protein from the MMT particles, and thus regenerate the PSf/MMT membrane. However, the details of the protein-MMT interactions are under study and will be part of a future publication. Also the lifetime/reusueability of these types of membranes in the wine fining application has yet to be assessed, but will be an important aspect in terms of industrial viability and adoption.

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References

Adoor, S.G., Sairam, M., Manjeshwar, L.S., Raju, K.V.S.N., & Aminabhavi, T.M. (2006). Sodium montmorillonite clay loaded novel mixed matrix membranes of poly(vinyl alcohol) for pervaporation dehydration of aqueous mixtures of isopropanol and 1,4-dioxane. *Journal of Membrane Science, 285*(1-2), 182-195.

Aerts, P., Genne, I., Kuypers, S., Leysen, R., Vankelecom, I.F.J., & Jacobs, P.A. (2000a). Polysulfone-aerosil composite membranes Part 2. The influence of the addition of aerosil on the skin characteristics and membrane properties. *Journal of Membrane Science, 178*(1-2), 1-11.

Aerts, P., Van Hoof, E., Leysen, R., Vankelecom, I.F.J., & Jacobs, P.A. (2000b). Polysulfone-Aerosil composite membranes. Part 1. The influence of the addition of Aerosil on the formation process and membrane morphology. *Journal of Membrane Science*, *176*(1), 63-73.

Anadão, P., Sato, L.F., Wiebeck, H., & Valenzuela-Díaz, F.R. (2010). Montmorillonite as a component of polysulfone nanocomposite membranes. *Applied Clay Science*, *48*(1-2), 127-132.

Avramescu, M.-E., Borneman, Z., & Wessling, M. (2003a). Mixed-matrix membrane adsorbers for protein separation. *Journal of Chromatography A*, *1006*(1-2), 171-183.

Avramescu, M.-E., Girones, M., Borneman, Z., & Wessling, M. (2003b). Preparation of mixed matrix adsorber membranes for protein recovery. *Journal of Membrane Science*, *218*(1-2), 219-233.

Avramescu, M.E., Borneman, Z., & Wessling, M. (2003c). Dynamic behavior of adsorber membranes for protein recovery. *Biotechnology and bioengineering*, *84*(5), 564-72.

Barr, T.L., Seal, S., He, H., & Klinowski, J. (1995). X-ray photoelectron spectroscopic studies of kaolinite and montmorillonite. *Vacuum*, *46*(12), 1391-1395.

Blade, W.H.Boulton, R. (1988). Adsorption of protein by bentonite in a model wine solution. *American Journal of Enology and Viticulture*, *39*, 193-199.

Bottino, A., Capannelli, G., D'Asti, V., & Piaggio, P. (2001). Preparation and properties of novel organic-inorganic porous membranes. *Separation and Purification Technology*, *22-23*, 269-275.

Briggs, D.Seah, M.P. (1990). *Practical surface analysis*. Chichester; New York; Aarau: Wiley; Salle + Sauerländer.

Casa Software Ltd., U., *CasaXPS: Processing Software for XPS, AES, SIMS and More*. 2009.

Charcosset, C. (1998). Review: Purification of proteins by membrane chromatography. *Journal of Chemical Technology and Biotechnology*, *71*(2), 95–110.

Choudalakis, G.Gotsis, A.D. (2009). Permeability of polymer/clay nanocomposites: A review. *European Polymer Journal*, *45*(4), 967-984.

Clarizia, G., Algieri, C., & Drioli, E. (2004). Filler-polymer combination: A route to modify gas transport properties of a polymeric membrane. *Polymer*, *45*(16), 5671-5681.

Defontaine, G., Barichard, A., Letaief, S., Feng, C., Matsuura, T., & Detellier, C. (2010). Nanoporous polymer-clay hybrid membranes for gas separation. *Journal of Colloid and Interface Science*, *343*(2), 622-627.

Ferreira, R.B., Piçarra-Pereira, M.A., Monteiro, S., Loureiro, V.B., & Teixeira, A.R. (2001). The wine proteins. *Trends in Food Science & Technology*, *12*(7), 230-239.

Flores, J.H., Heatherbell, D.A., & McDaniel, M.R. (1990). Ultrafiltration of wine: effect of ultrafiltration on white Riesling and Gewürztraminer wine composition and stability. *American Journal of Enology and Viticulture, 41*, 207-214.

Hsu, J.-C.Heatherbell, D.A. (1987). Heat-unstable proteins in wine. I Characterization and removal by Bentonite fining and heat treatment. *American Journal of Enology and Viticulture*, *38*(1), 11-16.

Hsu, J.C., Heatherbell, D.A., Flores, J.H., & Watson, B.T. (1987). Heat-unstable proteins in grape juice and wine. II Characterization and removal by ultrafiltration. *American Journal of Enology and Viticulture, 38*, 17-22.

Kim, Y., Lee, J.S., Rhee, C.H., Kim, H.K., & Chang, H. (2006). Montmorillonite functionalized with perfluorinated sulfonic acid for proton-conducting organic-inorganic composite membranes. *Journal of Power Sources*, *162*(1), 180-185.

Liu, S.X., Kim, J.-T., & Kim, S. (2008). Effect of polymer surface modification on polymer-protein interaction via hydrophilic polymer grafting. *Journal of Food Science, 73*(3), 143-150.

Majewski, P., Barbalet, A., & Waters, E.J. (2011). $1 billion hidden cost of bentonite fining. *The Australian and New Zealand Grapegrower & Winemaker, 569*, 58-62.

Nagarale, R.K., Shahi, V.K., & Rangarajan, R. (2005). Preparation of polyvinyl alcohol-silica hybrid heterogeneous anion-exchange membranes by sol-gel method and their characterization. *Journal of Membrane Science*, *248*(1-2), 37-44.

Pashova, V., Güell, C., & López, F. (2004a). White wine continuous protein stabilization by packed column. *Journal of Agricultural and Food Chemistry 52,* 1558–1563.

Pashova, V., Güell, C., Pueyo, E., López-Barajas, M., Polo, M.C., & López, F. (2004b). White wine protein stabilization by a continuous process using a packed column. *American Journal of Enology and Viticulture, 55*, 195-198.

Picard, E., Vermogen, A., Gérard, J.F., & Espuche, E. (2007). Barrier properties of nylon 6-montmorillonite nanocomposite membranes prepared by melt blending: Influence of the clay content and dispersion state: Consequences on modelling. *Journal of Membrane Science*, *292*(1-2), 133-144.

Pocock, K.F., Hayasaka, Y., Pen, Z., William, P.J., & Waters, E.J. (1998). The effect of mechanical harvesting and long-distance transport on the concentration of haze-forming proteins in grape juice. *Australian Journal of Grape and Wine Research, 4*, 23-29.

Salazar, F., Achaerandio, I., Labb, M., Gell, C., & Lpez, F. (2006a). Comparative study of protein stabilization in white wine using zirconia and bentonite: physicochemical and wine sensory analysis. *Journal of Agricultural and Food Chemistry*, *54*(26), 9955-9958.

Salazar, F.N., Achaerandio, I., Labbé, M.A., Güell, C., & López, F. (2006b). Comparative study of protein stabilisation in white wine using zirconia and bentonite: physiochemical and wine sensory analysis. *Journal of Agricultural and Food Chemistry, 54*, 9955–9958.

Salazar, F.N., de Bruijn, J.P.F., Seminario, L., & Güell, C.L., F (2007). Improvement of wine crossflow microfiltration by a new hybrid process. *Journal of Food Engineering, 79*, 1329-1336.

Sarmento, M.R., Oliveira, J.C., & Boulton, R.B. (2000a). Selection of low swelling materials for protein adsorption from white wines. *International Journal of Food Science & Technology, 35*(1), 41-47.

Sarmento, M.R., Oliveira, J.C., Slatner, M., & Boulton, R.B. (2000b). Influence of intrinsic factors on conventional wine protein stability tests. *Food Control, 11*, 423–432.

Schneider, K., Hölz, W., Wollbeck, R., & Ripperger, S. (1988). Membranes and modules for transmembrane distillation. *Journal of Membrane Science, 39*(1), 25-42.

Siddiqui, M.K.H. (1968). *Bleaching Earths*: Oxford, New York, Pergamon Press.

Slatner, M., Boulton, R.B., Sarmento, M.R., & Oliviera, J.C. (1999). Kinetics of the adsorption of bovine serum albumin contained in a model wine solution by non-swelling ion exchange resins. *Journal of Food Engineering, 39*, 65–71.

Smolders, C.A., Reuvers, A.J., Boom, R.M., & Wienk, I.M. (1992). Microstructures in phase-inversion membranes. Part I. Formation of macrovoids. *Journal of Membrane Science, 73*(2-3), 259-275.

Tezuka, T., Tadanaga, K., Hayashi, A., & Tatsumisago, M. (2006). Inorganic-organic hybrid membranes with anhydrous proton conduction prepared from 3-aminopropyltriethoxysilane and sulfuric acid by the sol-gel method. *Journal of the American Chemical Society, 128*(51), 16470-16471.

Thomas, T.R. (1999). *Rough surfaces*. London: Imperial College Press.

Ueda, T., Ishihara, K., & Nakabayashi, N. (1995). Adsorption-desorption of proteins on phospholipid polymer surfaces evaluated by dynamic contact angle measurement. *Journal of Biomedical Materials Research, 29*, 381-387.

Uragami, T., Matsugi, H., & Miyata, T. (2005). Pervaporation characteristics of organic-inorganic hybrid membranes composed of poly(vinyl alcohol-co-acrylic acid) and tetraethoxysilane for water/ethanol separation. *Macromolecules, 38*(20), 8440-8446.

Vandezande, P., Li, X., Gevers, L.E.M., & Vankelecom, I.F.J. (2009). High throughput study of phase inversion parameters for polyimide-based SRNF membranes. *Journal of Membrane Science, 330*(1-2), 307-318.

Vankelecom, I.F.J., De Beukelaer, S., & Uytterhoeven, J.B. (1997). Sorption and pervaporation of aroma compounds using zeolite-filled PDMS membranes. *Journal of Physical Chemistry B, 101*(26), 5186-5190.

Villaluenga, J.P.G., Khayet, M., López-Manchado, M.A., Valentin, J.L., Seoane, B., & Mengual, J.I. (2007). Gas transport properties of polypropylene/clay composite membranes. *European Polymer Journal*, *43*(4), 1132-1143.

Wang, Y.-C., Fan, S.-C., Lee, K.-R., Li, C.-L., Huang, S.-H., Tsai, H.-A., & Lai, J.-Y. (2004). Polyamide/SDS-clay hybrid nanocomposite membrane application to water-ethanol mixture pervaporation separation. *Journal of Membrane Science, 239*, 219-226.

Waters, E.J., Alexander, G., Muhlack, R., Pocock, K.F., Colby, C., O'Neill, B.K., Høj, P.B., & Jones, P. (2005). Preventing protein haze in bottled white wine. *Australian Journal of Grape and Wine Research, 11*, 215-225.

Waters, E.J., Wallace, W., & Williams, P.J. (1992). Identification of heat-unstable wine proteins and their resistance to peptidases. *Journal of Agricultural and Food Chemistry 40*, 1514-1519.

Yan, L., Hui, L., Xianda, S., Jianghong, L., & Shuili, Y. (2007). Confocal laser scanning microscope analysis of organic-inorganic microporous membranes. *Desalination*, *217*(1-3), 203-211.

Yang, Y.-n., Jun, W., Qing-zhu, Z., Xue-si, C., & Hui-xuan, Z. (2008). The research of rheology and thermodynamics of organic-inorganic hybrid membrane during the membrane formation. *Journal of Membrane Science*, *311*(1-2), 200-207.

Yang, Y., Zhang, H., Wang, P., Zheng, Q., & Li, J. (2007). The influence of nano-sized TiO2 fillers on the morphologies and properties of PSF UF membrane. *Journal of Membrane Science, 288*(1-2), 231-238.

Fig. 1: Micrograph of MMT particles. The insert image on the bottom right of the image shows the shape of a typical MMT particle.
Fig. 2: Typical SEM images of the (a) top, and (b) bottom surfaces of PSf membrane, (c) top, and (d) bottom surfaces of PSf/MMT membrane.
Fig. 3: (a) Size distribution of pores on the PSf and PSf/MMT top surfaces, and (b) number of pores per square micrometer on the PSf and PSf/MMT top surfaces.
Fig. 4: Typical images of (a) PSf cross section, (b, c) enlargement of selected areas in (a).
Fig. 5: Typical images of (a) PSf/MMT cross section, and (b,c) enlargement of selected areas in (a) showing the distribution of MMT particles within the polymer matrix.
Fig. 6: 3D images of the (a) PSf top surface, (b) PSf bottom surface, (c) PSf/MMT top surface, and (d) PSf/MMT bottom surface. Images are of 60 µm sample square.
Fig. 7: Changes of (a) average surface roughness and (b) skewness resulting from the incorporation of MMT particles in the polysulfone membranes.
Fig. 8: Measurements of contact angle with time. The dotted line indicates the standard deviation of the measurements.
Fig. 9: Average permeance for PSf and PSf/MMT with corresponding pressure.
Fig. 10: Typical XPS survey scans of (a) MMT particles, top surface of (b) PSf membrane, and (c) PSf_F membrane (the arrow indicates the presence of nitrogen peak).
Fig. 11: Photoelectron (a) C 1s, (b) O 1s, and (c) N 1s images acquired from the same area on the PSf_F bottom surface. Bright areas in the images are enriched in PSf_F bottom surface. The analysis area was 110 x 110 µm.
Fig. 12: XPS images from PSf/MMT_F cross section (a) carbon distribution, (b) oxygen distribution, (c) nitrogen distribution, (d) aluminium distribution, (e) silicon distribution, and (f) overlay of silicon and nitrogen showing blend composition. The analysis area was 110 x 110 µm.

Table 1: Membrane preparation conditions
Table 2: Summaries of XPS parameters in this study
Table 3: Surface compositions (atomic %) of the samples following various conditions.