Preparation and benchmarking of highly hydrophilic polyaniline poly(2-acrylamido-2-methyl-1-propanesulfonic acid) PANI PAMPSA membranes in the separation of sterols and proteins from fruit juice

Gavino Puggioni[1], Nurul Hainiza Abd-Razak[1,3], Ida Francesca Amura[1,2], Michael R. Bird[1,2], Emma A.C Emanuelsson[1] and Salman Shahid*[1,2]

[1] Department of Chemical Engineering, University of Bath, Bath, United Kingdom, BA2 7AY.

[2] Centre for Advanced Separations Engineering, University of Bath, Bath, United Kingdom, BA2 7AY

[3] Rubber Research Institute of Malaysia, Malaysian Rubber Board, PO Box 10150, 50908 Kuala Lumpur, Malaysia

*Corresponding author. Email address: ss2840@bath.ac.uk

Abstract

A straightforward approach is presented to prepare highly hydrophilic ultrafiltration polyaniline poly(2-acrylamido-2-methyl-1-propanesulfonic acid (PANI PAMPSA) membranes. Their application in the fractionation of phytosterols and proteins from fruit juice is described. The poly(2-acrylamido-2-methyl-1-propanesulfonic (PAMPSA) is added to the aniline during the polymer synthesis and the membrane is prepared via phase inversion forming a highly hydrophilic and mechanically stable ultrafiltration membrane of 200 µm thickness and pure water flux of 126 LMH at 1 bar. The membrane so produced is benchmarked against a hydrophilic commercial regenerated cellulose acetate membrane (RCA) for the separation of phytosterols and proteins from orange juice. Cross-flow filtration experiments show comparable protein separation efficiency of the membranes but better rejection of phytosterols for the commercial RCA membrane. Both commercial and lab prepared membranes are subject to fouling, with the PANI PAMPSA membrane showing higher irreversible fouling. Nevertheless, the PANI PAMPSA membrane showed a good cleaning efficiency of 74 % after three fouling-cleaning cycles. Overall, this work has demonstrated the possibility of use PANI PAMPSA for ultrafiltration application and provided a better understanding of its fouling ability when compared to a commercial membrane in a multicomponent system.

Keywords: biocompounds, sterol, fruit juice, polyaniline, ultrafiltration, fouling

1.0 Introduction

Membrane separation has recently and increasingly become a key unit operation in many industries, with the food and water sectors representing the main areas where membranes have showed the greatest impact. An emerging area for membrane applications is the recovery of bioactive compounds that are found in foods and have specific metabolic and physiological actions that are relevant to the nutraceutical, pharmaceutical and food industries. Unlikely other energy-intensive techniques, membrane separation offers an energy-efficient alternative for the recovery of biocompounds from plants and by-products of agro-industrial application[1]. The current extraction and recovery methods include soxhlet, maceration and hydrodistillation and make use of organic solvents, agitation and high temperatures [2].The extensive use of solvents and the prolonged extraction times is not economically viable and the instability of some of the bioactive compounds to the high temperatures represent a further challenge. Other relatively greener cold extraction techniques include extraction using supercritical fluid, pressurised liquids, ultrasound [3], microwave radiations etc [3,4]. Nevertheless, these techniques have limited application in manufacturing because their scaling-up is challenging and have high operational and maintenance cost [5].

In previous work we have demonstrated the principle of using ultrafiltration to separate phytosterols from proteins in orange juice [7] with RCA membranes displaying the highest permeate flux, the highest transmission of phytosterols from orange juice, and the highest fouling index and cleaning efficiency, when compared to the polyethersulfone (PES) membrane and polyvinylidene fluoride (PVDF) material. RCA membranes are widely used in fruit juice processing to separate bioactive compounds such as phenolics and proteins from fruit juice such as kiwi [8] , apple [9] and pomegranate juice [6]. RCA membranes are relatively cheap and are categorized as hydrophilic membranes, which means that they are able to provid a good resistance to fouling [10]. The surface science of membrane fouling and cleaning

processes was a focus of our previous work [11], whilst optimizing the ultrafiltration process to fractionate the targeted sterol compounds. Despite the great potential of membrane technology in fruit juice processing for the recovery of active biocompounds using commercially available membranes [12,13] the widespread use of this technology is still limited because of the tendency of these membrane to foul. Hence, in this work we focused on investigating the ultrafiltration process for the fractionation of sterols from protein in orange juice using a novel developed membrane material – PANI PAMPSA- as it is believed that the development of more fouling resistant materials in food processing is worthy of investigation. Fouling resistant membrane materials to facilitate the widespread use of membrane technology are the focus of many research areas [14,15]. Among these, polyaniline is a conducting polymer that has been extensively used in membrane fabrication for its versatility, redox chemistry and charge switchability of the membrane surface. PANI doped with organic acids such as PAMPSA, polystyrenesulfonic acid PSSA etc. have shown improved hydrophilicity, making them excellent candidates for antifouling membranes[16]. In addition, the membranes are easy to prepare and possess good chemical stability[17]. Recent work has reported the post-modification of this membrane for solvent resistant nanofiltration [16,17] and investigated its electrical responsive behaviour [18]. However these studies have been carried out in pure solvents and there is a lack of data on the membrane performance in complex systems that mimic real case scenarios as well as the fouling behaviour of the membrane.

Hence, in this study, we exploited the key improvement by incorporating hydrophilic PAMPSA on the PANI backbone to prepare a low fouling ultrafiltration PANI PAMPSA membrane. We believe this to be the first study reported in the literature to investigate polyaniline based membrane performance for the recovery of phytosterols and proteins from fruit juice. The fabrication method offers a simple and straightforward approach to prepare a

highly hydrophilic membrane that is benchmarked against a commercial RCA membrane to assess filtration and membrane fouling performance.

2.0 Experimental Methods

2.1 Materials

Aniline, ammonium persulfate (APS), hydrochloric acid (HCl), HPLC grade acetone, DMF, DMAc, Toluene, N-methyl-2-pyrrolidone (NMP) and 4-methyl piperidine (4-MP) were purchased from Sigma-Aldrich (UK). Poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPSA) was purchased from Sigma Aldrich (Merck, UK) and has average molecular weight of 2,000,000 Da. PET/PBT backing layer- Novatexx 2484 (120 µm) was supplied by Freudenberg Filter technologies (Germany). All solutions were prepared with deionised (DI) water produced from an ELGA deioniser (PURELAB Option). Acetic anhydride, sulphuric acid, chloroform and methanol were purchased from *Merck*, UK. Standards for characterisation such as stigmasterol and butylated hydroxytoluene (BHT) were purchased from *Sigma Aldrich*, UK. The cleaning was carried out using 0.5 % (*w/w*) *P3-Ultrasil 11* from *Henkel Ecolab*, US, a commercial cleaning agent which widely used in food processing using membrane filtration. Orange juice not from concentrate (NFC) was sourced from *Cobell*, UK. The phytosterols and protein concentration of the orange juice used in this study is 0.2 – 0.3 mg/mL and 0.8 – 1.0 mg/mL respectively, as described previously in [7]. These values are in agreement with previous studies [19-21].

2.2 Synthesis of PANI PAMPSA powder

PANI-PAMPSA powder was synthesised by oxidative polymerisation of aniline in PAMPSA using a procedure developed in our research group [17]. Two solutions were made: solution 1 made with the concentration of 0.2 M aniline and 0.05 M PAMPSA and solution 2 with the

concentration of 0,2 M APS, solution 2 was added to the solution 1 slowly in 24 h The obtained dark green PANI-PAMPSA product was filtered and washed firstly with DI water 3 times and then with acetone 3 times until the pH of the filtrate became neutral. The procedure allowed for the removal of impurities, unreacted material and PANI oligomers. The obtained cake layer was then dried in a vacuum oven at 65 °C for 24 h. A dark green powder was obtained. As a control PANI was synthesised using HCl as dopant following the recipe from our previous work [17]. The average molecular weight was determined by Gel permeation chromatography (GPC) as 49,975 g mol^{-1} with a polydispersity of 1.65.

2.3 Membrane fabrication and characterisation techniques

The powder (20% wt) was dissolved in a mixture of NMP, 4-MP and THF (10% of the total solvent) and the solution was left stirred for overnight. All membranes were cast on a bench top laboratory caster. The Novatexx 2484 membrane backing layer was secured using scotch tape on a flat glass plate. An adjustable casting knife was used to cast 200 µm thick films using an adjustable film applicator (Elcometer 4340 automatic film applicator, Elcometer, UK). Evaporation time of 30 s was used before immersing the casted membrane solution into a DI water coagulation bath (Fig S1). The membrane was kept immersed in DI water at room temperature for at least 24 h before suing it for characterisation and filtration experiments.

2.3.1 Fourier transform infrared spectroscopy FTIR

The chemical structure and the incorporation of the sulfonic groups of the PAMPSA to the PANI backbone was studied by FTIR. The FTIR spectra of dry PANI PAMPSA (both powder and membranes) were obtained using a Spectrum 100™ – FTIR Spectrometer (PerkinElmer, USA) fitted with an attenuated total reflectance (ATR) detector. A background scan was run prior to sample testing and spectra were recorded from 4000 to 650 cm^{-1} in transmission mode with a spectral resolution of 4 cm^{-1} and 64 scans.

2.3.2 Field emission scanning electron microscopy FSEM

Membrane morphology was studied using FSEM (JSM-6301F, JEOL, Germany). Lab made membranes and commercial RCA membranes were prepared by freeze fracturing them in liquid nitrogen and drying them in vacuum overnight. Before the analysis was performed the samples were coated in chromium using a sputter coater (Q150T S, Quorum) under argon for 5 min.

2.3.3 Dynamic contact angle

PANI PAMPSA and PANI membrane hydrophilicity was studied by dynamic contact angle analysis. (Contact Angle System OCA 15Pro, Dataphysics, Germany). The instrument consists in an automatic dispenser system equipped with a long needle glass syringe which dispense a small drop of liquid, and a mobile platform where the membrane was fitted. Once the drop leaves the needle the instrument starts to measure the variation of angle with time. A double side tape glued to the support layer was used to keep the membrane flat on the platform. The analysis was performed using sessile drop technique (4 µL) and data were recorded for 60 s and repeated 2 times. Water was used as liquid. the technique gives an important data to measure the wetting characteristic of the membrane

2.3.4 Zeta potential

Membrane surface charge measurement was carried out by using Zetasizer nano series model ZS, Malvern-Panalytical, UK. Zeta potential planar cell (ZEN 1020) along with tracer particles (Latex beads, polystyrene 0.3 µm mean particle size). Both the magnitude of the particle electrophoresis and the electro-osmosis generated by the wall zeta potential were used to calculate the zeta potential at the wall surface.

2.3.5 Dynamic mechanical analysis DMA

The mechanical properties of the membrane were studied using a dynamic mechanical analyser (Mettler-Toledo, DMA1, STAR System) up to a temperature of 100°C with a heating rate of 1K min^{-1}. The membranes were cut into strips of 20 mm (L) x 5.0 mm (W) and secure on a clamp in dual cantilever mode.

2.4 Evaluation of membrane performance

Two polymeric membranes were used; (1) A commercial flat-sheet regenerated cellulose acetate (RCA) membrane (RC70PP) with 10 kDa MWCO supplied by Alfa Laval, Denmark and (2) A lab synthesised PANI PAMPSA membrane (MWCO ~ 10 kDa, Fig S3). The MWCO of PANI PAMPSA membrane was determined following the HPLC characterisation procedure as detailed in authors previous works (please see SI) and [18, 28]. The RCA 10 kDa commercial membrane was conditioned with deionised water (DI) water at 60 °C to remove glycerol preservative applied by the manufacturer. The PANI PAMPSA membrane was conditioned with DI water at 20 °C to ensure wetting of the membrane. Filtration experiments were carried out on each of the membranes using a cross-flow membrane filtration system *LabStak M10* manufactured by *DSS* (now *Alfa Laval*), Denmark. A schematic design of the M10 filtration system applied in this study is illustrated in Fig. 1 [11]. The ultrafiltration steps have been described in detail by Abd-Razak et al.[7]. Pure water flux (PWF) measurements were determined for each membrane using DI water prior to fouling (before filtration), after fouling (after filtration) and after chemical cleaning.

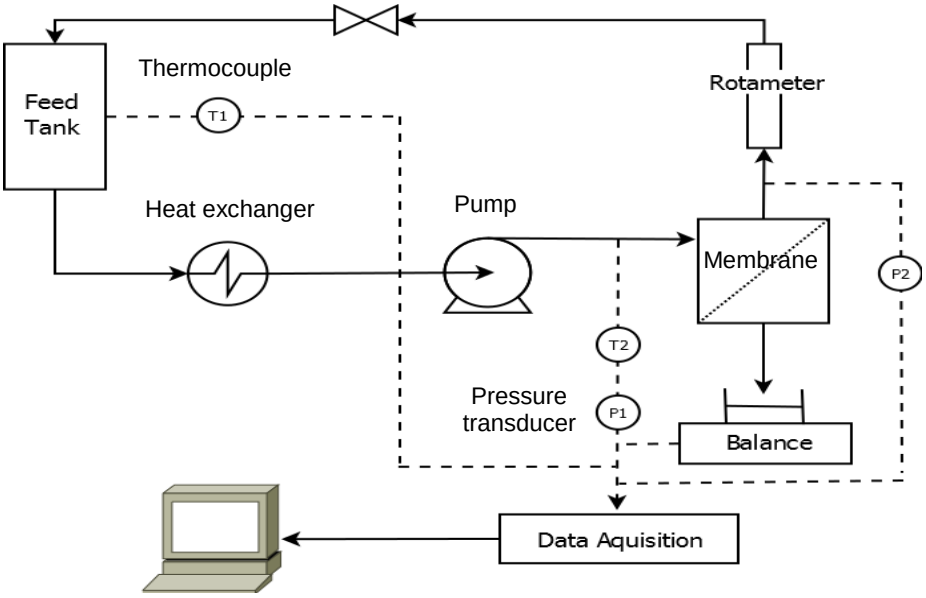


Fig. 1: A schematic diagram of the M10 filtration system

2.4.1 Pure water flux and permeate flux analysis

During all the filtration tests a pre-conditioning step was performed for the permeate flux to reach steady state. Pure water flux (Equation 1) is defined as follows:

$$Jw = \frac{V}{A \times \Delta t} \quad (1)$$

Where V (L) is the permeate volume; A (m^2) is the membrane effective filtration area and Δt (h) is the filtration time. The general equation (2) was used to calculate the permeate flux through a membrane.

$$J = \frac{\Delta P}{\mu R} \quad (2)$$

where J is the flux through the membrane (m s^{-1}), ΔP (Pa) is the transmembrane pressure (TMP), μ is the dynamic viscosity (Pa s) and R represents the total resistance (m^{-1}). A membrane displays resistances when fouled and these can be characterised by the resistance (J) in series model as shown in equation (3) and (4) [22].

$$J = \frac{\Delta P}{\mu\,(R_m + R_{cp} + R_f)} \tag{3}$$

$$R_f = R_{ir} + R_r \tag{4}$$

where R_m is the conditioned virgin membrane resistance, R_{cp} is the resistance due to concentration polarisation, R_f is the fouling resistance, R_{ir} is the irreversible fouling resistance and R_r is the reversible fouling resistance. The rejection (R) of total phytosterols and protein during filtration were calculated by equation (5).

$$R = (1 - \frac{C_p}{C_r}) \times 100 \tag{5}$$

where C_p is the solute concentration in the permeate and C_r is the solute concentration in the retentate. In this case, the retentate was recycled back into the feed tank. The cleaning efficiency (CE) is calculated according to equation (6) [5].

$$CE = (\frac{WP_1}{WP_0}) \times 100\% \tag{6}$$

where WP_0 is the pure water permeability (LMH) of the virgin membrane and WP_1 is the pure water permeability after the cleaning. The pH of orange juice was found to be pH 3.45. Orange juice contains 0.2 – 0.3 mg/mL phytosterols and 0.8 – 1.0 mg/mL protein.

2.4.2 Analyses of compounds

2.4.2.1 Total phytosterol

The amount of total phytosterols in all filtration samples was determined based on the Liebermann-Buchard (LB) method using stigmasterol as standard and a spectrophotometer (Cary 100, *Agilent,* USA) as described in detail by Abd-Razak et al. [11]. The total phytosterol content (TPC) was calculated using the standard photometric formula in equation (7) [23,24]:

$$\text{TPC} = C_s \times \frac{A_u}{A_s} \quad (7)$$

where C_s = standard concentration, A_u = Absorbance of the sample, A_s = Absorbance of the standard. All measurements were carried out in triplicate and the results were averaged.

2.4.2.2 Proteins

Protein concentration was analysed by the Bradford method [25, 26] using bovine serum albumin (BSA) as standard and a spectrophotometer (Cary 100, *Agilent,* USA) as described previously by Abd-Razak et al. [11]. The assay is based on the binding of the acidic dye solution Coomassie Brilliant Blue G-250 to protein at maximum absorbance from 465 to 595 nm [26].

3.0 Results and discussion

3.1 Characterisation of PANI PAMPSA membrane

3.1.1 FT-IR

Fig. 2 shows the FT-IR spectrum of PANI PAMPSA membrane. The vertically dashed lines represent peak widths. The absorption bands at approximately 1225-1113 and 1038 cm^{-1} correspond to the asymmetric and symmetric stretching of –SO2– in the PAMPSA respectively [16, 17]. The peak at 1166 cm^{-1} could be assigned to the vibrational band of the nitrogen quinone on the PANI [17] (Fig. S2). The PANI PAMPSA spectrum showed characteristic peaks at 1497 cm^{-1} and 1590 cm^{-1} corresponding to the benzenoid and quinoid form of PANI. Finally, the methyl groups of PAMPSA give rise to absorption bands at 1420 and 1382 cm^{-1}. These results are consistent with previous FTIR results [16, 17] and confirm that the obtained polymer is PANI PAMPSA.

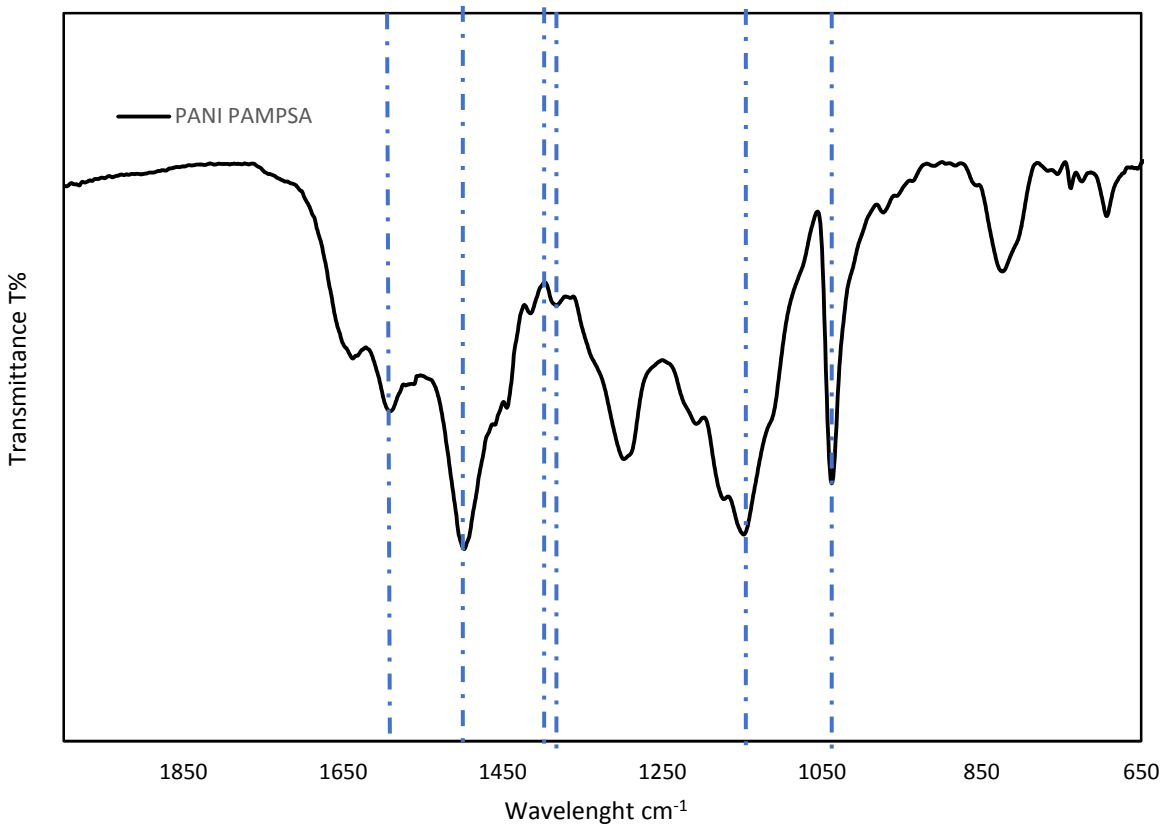


Fig. 2: FT IR spectrum of PANI PAMPSA membrane

3.1.2 Characterisation of membrane morphology

Fig. 3 reports the morphologies of the lab synthesised PANI PAMPSA membrane at different magnification. The surface appears smooth with no defects. The cross-sectional images of membrane show a typical morphology of a phase inversion membrane: a denser skin layer, a transition region and a relatively porous layer. The backing layer has been removed in SEM analysis. The use of larger acid like PAMSA could produce a greater intermolecular spacing between the PANI chains and therefore expanded the membrane pore structures, resulting in the formation of a loose membrane topology with higher porosity and larger pore sizes. It is important to note that no macrovoids were formed in the membrane. The membrane microstructure is influenced by the viscosity of the membrane solution and de-mixing kinetics. A greater viscous hindrance slows down the de-mixing rate and favours formation of 'sponge-

like' substructures while inhibiting the formation of large macrovoids. The RCA 10 kDa membrane consists of a dense cellulosic top layer and a porous support layer as shown in Fig. 3 (e). The support layer of RCA 10 was prepared from polypropylene (PP). It can be seen that the top layer was easily detached from the support layer during the SEM analysis. However, the membrane was not affected during the ultrafiltration process.

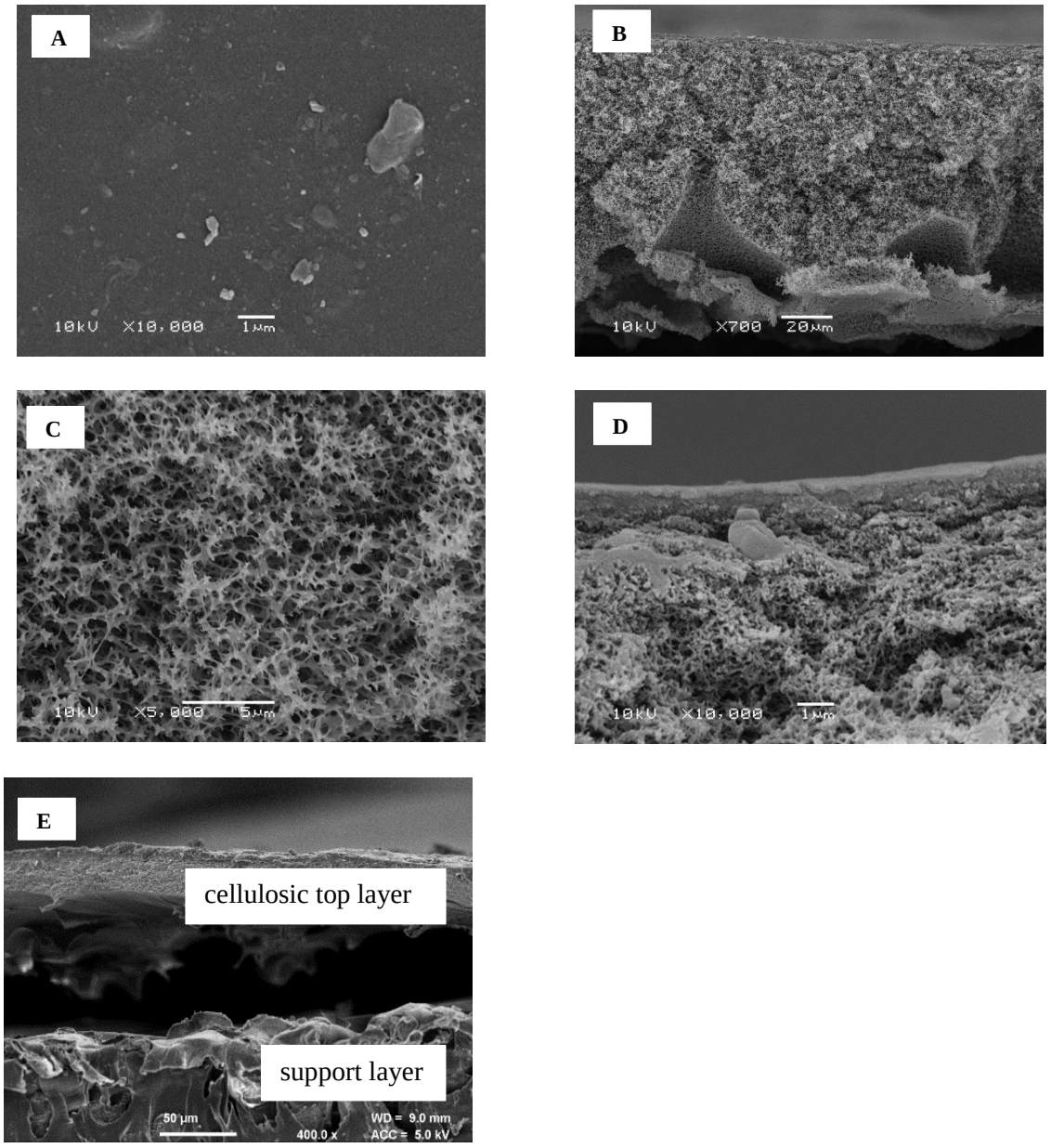


Fig. 3: SEM images of PANI PAMSA membrane (A) surface and (B-D) cross-sections and RCA membrane (E) cross-section.

3.1.3 Membrane surface hydrophilicity

The hydrophilicity of the prepared PANI PAMPSA membrane was investigated via dynamic contact angle technique and compared with hydrophilicity of the unmodified PANI membrane. The incorporation of the big polyacid group during synthesis can impart hydrophilic properties to the membrane due to the presence of the sulfonic acid groups. In addition, PAMPSA can also form hydrogen bonding with water, hence an increase in the water permeation rate and rapidly decrease of contact angle should be expected for PANI PAMPSA membrane [27, 28]. Fig. 4 reports the contact angle results for the PANI and PANI PAMSA membranes. PANI PAMPSA shows a rapid decrease of the water contact angle over time with an initial value of 42 ° and a rapid reducing rate of the 77 % after 55 s. In contrast, PANI membrane shows a slower reducing rate and an initial angle of 73 °. Contact angles values rapidly change over time and did not reach a steady value. Membranes that show a contact angle below 90° are considered hydrophilic, however, PANI PAMPSA membrane could be considered highly hydrophilic because of the rapid change over time and its initial angle (below 50 °). It could also be hypothesised that the increased hydrophilicity of PANI PAMPSA will increase its fouling resistance making it a good candidate for a benchmarking against the very hydrophilic commercial RCA membrane which has shown a contact angle of 11 ° as reported in a previous work [11].

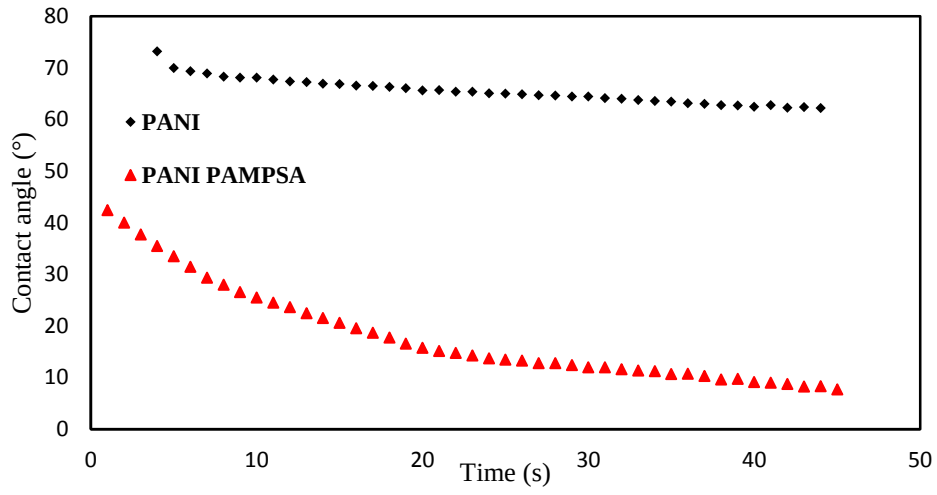


Fig. 4: Contact angle over time for PANI PAMPSA and PANI membrane. Data are average of 2 membrane samples from 2 different batches.

3.1.4 Mechanical stability of PANI PAMPSA membrane

Previous works have reported PANI PAMSA membranes with a greater flexibility and improved mechanical stability due to the ionic bonds and double-stranded network between polymer acids and PANI chains [28, 29]. To further characterise the elastic behaviour of the prepared PANI PAMPSA membrane, the mechanical response of the membrane at different temperature was investigated. The degree of stiffness of the material or storage modulus was measured, and the data are reported in Fig. 5. It is noted that the initial value of the storage modulus at 324 MPa decreases with temperature increases, indicating a decreasing in stiffness of the polymer chains. The hump of the loss module E'' curve can be attributed to an increase in molecular motion, but the absence of an evident step decrease for E' curve does not indicate any transition or physical change. The absence of any transition between 25 and 100 °C gives an indication of the membrane response in that temperature range, confirming the mechanical stability and flexibility of the synthesised membrane and itsapplicability in processes where temperatures higher than ambient values arerequired, or cleaning steps are performed with hot solutions.

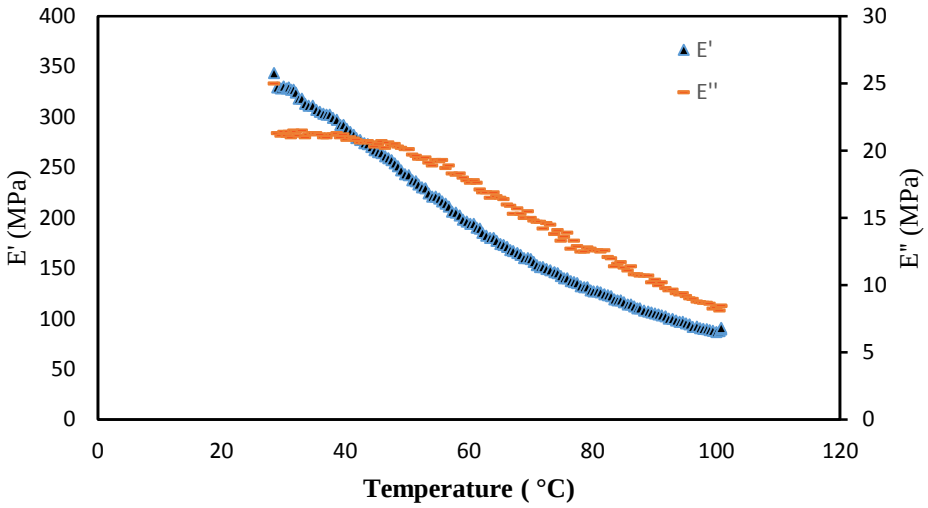


Fig. 5: Dynamic mechanical analysis of PANI PAMPSA membrane. Data are average of 2 membrane samples from 2 different batches.

3.2 Permeate flux analysis

Fig. 6 shows the time course of permeate flux for the ultrafiltration of orange juice using PANI PAMPSA and RCA membranes. The ultrafiltration was stopped at 60 min. It can be seen that the lab made membrane and the commercial RCA have comparable permeate fluxes of 31 ± 2 L m^{-2} h^{-1} and 29 ± 1 L m^{-2} h^{-1} at the beginning of the filtration. The initial permeate flux continued to decline gradually with filtration time until it reached a steady-state value at *ca*. 22 L m^{-2} h^{-1}. The permeate flux of PANI PAMPSA and RCA membranes dropped to 22 L m^{-2} h^{-1}, indicating a flux decline of 29 % and 24 % respectively. The decrease of permeate flux can be described by the effect of membrane fouling phenomena [5, 25].

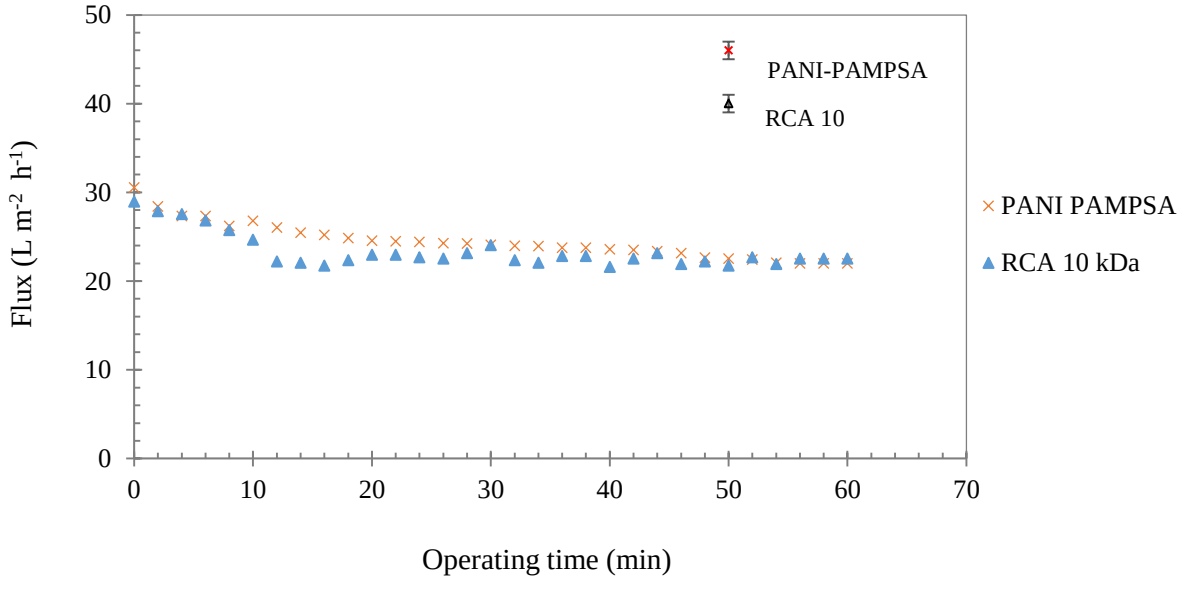


Fig. 6: Time course of permeate flux using PANI PAMPSA and RCA membranes. The largest error for this dataset is ± 2 L m^{-2} h^{-1}.

3.2.1 Pure water flux

Pure water flux (PWF) values were measured for both membranes using DI water. The experiments were performed under three different conditions (i) before fouling, (ii) after fouling and (iii) after cleaning over three different cycles. Fig. 7 shows the PWF of tested membranes at a TMP of 1.0 bar and at 20 °C. PANI PAMPSA and RCA membranes presented the pure water flux of 128 - 186 L m-2 h-1 and 77 - 132 L m-2 h-1 respectively, for three different cycles. According to the results, RCA membrane showed lower pure water fluxes than the PANI PAMPSA membrane and the pure water flux before fouling (PWF 1) was reduced after fouling (PWF 2) for RCA membrane in all cycles. For the PANI PAMPSA membrane, a loss in performance was observed only during Cycle I with PWF before fouling (PWF 1 I) reducing after fouling (PWF 2 I). As expected, the ultrafiltration process was affected by the membrane fouling, thus, a cleaning method was required to regenerate the membrane.

The cleaning stage was performed using the commercial cleaning agent named Ultrasil 11 which is widely used in food process research using membrane [30] and has pH 11. Fig. 7 demonstrates that for the RCA membrane, cleaning method was effective in regenerating the membrane with the pure water flux after cleaning higher than that seen after fouling (eg: PWF 3 I > PWF 2 I). However, the PANI PAMPSA membranes behave differently in Cycle I and Cycle II: the fluxes after cleaning were lower than the fluxes after fouling (eg: PWF 3 II < PWF 2 II). This behaviour was not expected but it could be due to the PANI membrane being sensitive to high pH. The different behaviour in cycle II and III showed by PANI PAMPSA can be explained as a response to the variation of pH experienced by the membrane after cleaning with ultrasil-11, at pH 11. It could be hypothesised that PANI PAMPSA undergoes configurational changes leading to variation in the pore dimension and hence the permeability. In a previous work [18], the pH dependence of PANI doped membrane has been highlighted and it was found that exposure to alkaline environment causes the swelling of the membrane

and subsequent pore constriction. However, it is also be considered that with the increase of solution pH, the positive sites of the PANI PAMPSA can be deprotonated and this can further affect the membrane filtration performance After the cleaning step in cycle I, the flux observed after fouling in cycle II and cycle III is higher than the pure water flux suggesting a pore-opening effect of the cleaning agent. The Ultrasil-11 with basic pH does not affect the RCA membrane, however PAMPSA and PANI possess charged functional groups which are affected by the pH of the feed solution [18]. As Ultrasil-11 is a common cleaning agent for restoring membrane flux and de-foul membranes used in food industry, it was selected for the cleaning step and compare the ultrafiltration performed by the two membranes. However the influence of pH on PANI membrane performance during cleaning steps is not fully understood and was not addressed in this work where the focus was on understanding the performance of these novel membranes in complex multicomponent systems.

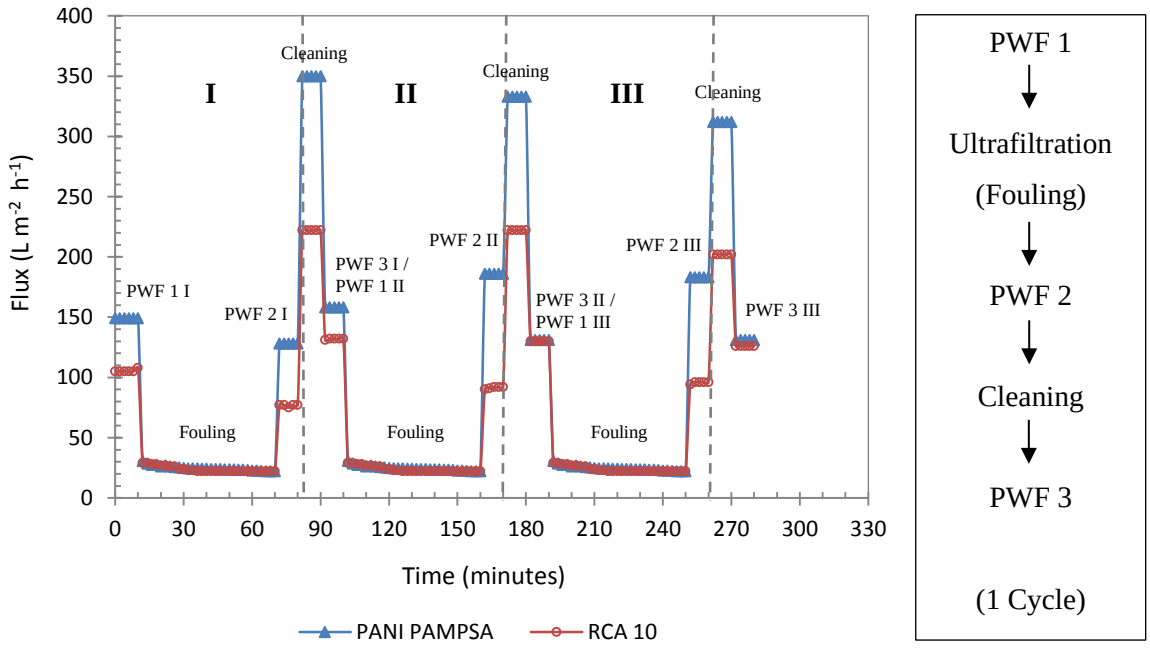
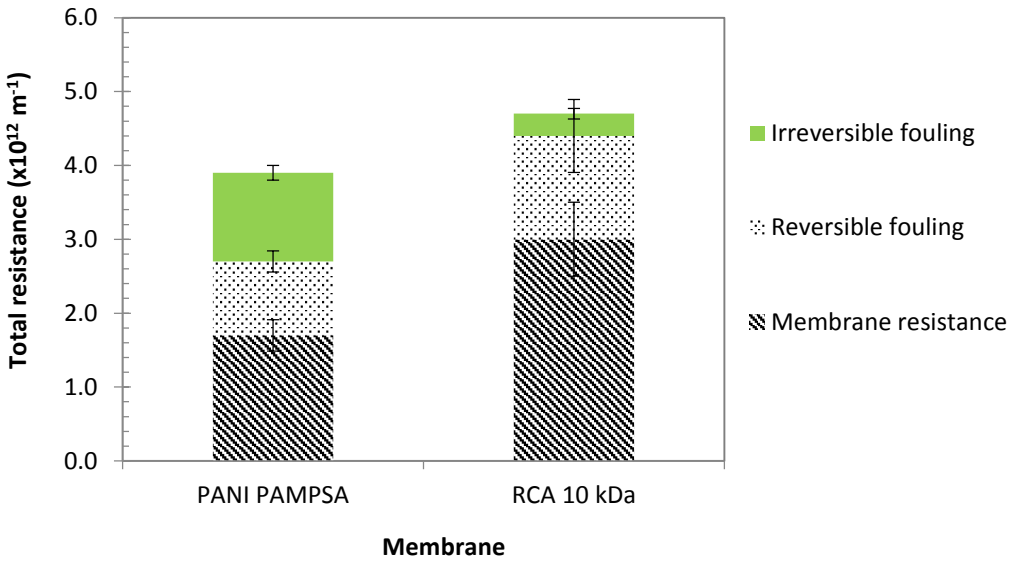


Fig. 7: Pure water fluxes of two membranes tested; PANI PAMPSA and RCA.

The cleaned membranes present a higher permeate flux when compared to the PWFs and this can be explained as a result of cleaning agents such as Ultrasil 11 (used in this study)

362 that can foul the membrane with surfactants. Surfactants may lead to a flux which can
363 occasionally be higher that the pure water flux obtained when using a clean membrane. If
364 surfactants coat a fouled membrane, the flux can still be higher than that seen for a clean
365 membrane. Hence, to have a better indication of surface conditions, other techniques such
366 electron microscopy can be used to determine whether the membrane is physically clean and
367 with no feed related foulants being present. After rinsing and further feed processing, these
368 surfactants desorb from the surface, and flux may then be more representative of the
369 interactions that have occurred between the polymer and the feed materials.

370 The total resistances were calculated from the flux data. A test for concentration
371 polarisation was carried out and the results showed that concentration polarisation is not an
372 important fouling related resistance in this system. Fig. 8 shows the total resistances including
373 membrane, reversible fouling and irreversible fouling for the membranes tested. The
374 conditioned virgin membrane resistances before fouling for PANI PAMPSA and RCA
375 membranes were 1.7 x 10^{12} m^{-1} and 3.0 x 10^{12} m^{-1} respectively. These values increased after
376 fouling, to 3.9 x 10^{12} m^{-1} and 4.7 x 10^{12} m^{-1} respectively, which were 1.6 and 2.3 times more
377 than those seen before fouling. Thus, it can be concluded that both membranes became fouled
378 during filtration. The RCA membrane displayed higher total membrane resistance and this is
379 reflected in lower pure water flux for RCA in Fig. 7. Table 1 shows the percentages of total
380 resistances including membrane resistance, reversible fouling and irreversible fouling. For
381 RCA 10 kDa membrane, the increase in the total resistance after fouling was mainly due to
382 reversible fouling (30 ± 5%) rather than irreversible fouling (6 ± 2%). Irreversible fouling (31
383 ± 2%) showed higher percentage compared to reversible fouling (26 ± 2%) for PANI PAMPSA
384 membrane as shown in Table 1. It was clear that RCA 10 kDa membrane was easily cleaned
385 compared to PANI PAMPSA, as it showed the lowest percentage of irreversible fouling.
386



387

388 Fig. 8: Total resistances including membrane, reversible fouling and irreversible fouling

389

390 **Table 1** Percentages of the breakdown of total resistances

	PANI PAMPSA	**RCA 10kDa**
Membrane resistance (%)	44 ± 4	64 ± 5
Reversible fouling (%)	26 ± 2	30 ± 5
Irreversible fouling (%)	31 ± 2	6 ± 2

391

392

393 The cleaning efficiency was calculated by comparing the pure water permeability
394 before and after cleaning [6]. RCA membrane exhibited higher cleaning efficiencies with 98 ±
395 1 % compared to PANI PAMPSA membrane (74 ± 11 %) (Fig. S4). This may suggest that the
396 fouling resistance was removed by the cleaning agent for the fouled membranes [30] . From
397 this result, it can be noted that the chemical cleaning method using 0.5 wt % Ultrasil-11 was
398 highly effective in regenerating RCA membrane, but less effective in regenerating PANI
399 PAMPSA membrane. This may suggest that the PANI membrane is pH sensitive at high pH of
400 cleaning process which affected the membrane surface charge.

3.2.2 Rejection of key compounds

Ultrafiltration was used to enable the separation of phytosterols from protein in orange juice. The separation efficiency and the effect of membrane fouling were studied by measuring the rejection of key compounds such as phytosterols and proteins content. Samples from the feed, retentate and permeate streams were collected and characterised for both compounds. Fig. 9 illustrates the rejection of compounds by PANI PAMPSA and RCA membranes. As previously reported, the RCA membrane presented good separation efficiency with 32 ± 4 % rejection towards phytosterols [11]. The lowest rejection of phytosterols by the tested membrane indicates the best separation efficiency. It can be seen in Fig. 9, PANI PAMPSA membrane showed higher rejection of phytosterols with 58 ± 2 %. Meanwhile for the protein content, both membranes showed higher rejection of protein of 97 ± 1 %. The molecular weight of proteins in orange juice were in the range 12 kDa to 71 kDa [31]. Thus, the higher molecular weight compounds were rejected by smaller pore size membrane and this increased the fouling layer [32]. It is possible that the membrane was fouled by protein-based compounds or other hydrophilic sub micelles [33, 37]. This data was supported by the flux declining results in Fig. 6 showing that the membrane has been fouled during the filtration. From this rejection results, it can be concluded that the protein can be removed from the sterols stream by using both PANI PAMSA and RCA membrane but PANI membrane showed a good result in term of the protein rejection which is comparable to the commercial RCA membrane. As reported in our previous study [7], cake fouling was the dominant mechanism for RCA 10 kDa membrane, as proteins were highly rejected by the 10 kDa membrane. As the PANI PAMPSA membrane has a similar MWCO of 10 kDa (Fig. S3), it is postulated that the PANI-PAMPSA membrane was also fouled with a cake of proteins as both membranes showed higher rejection of protein of 97 ± 1 % (Fig. 9).

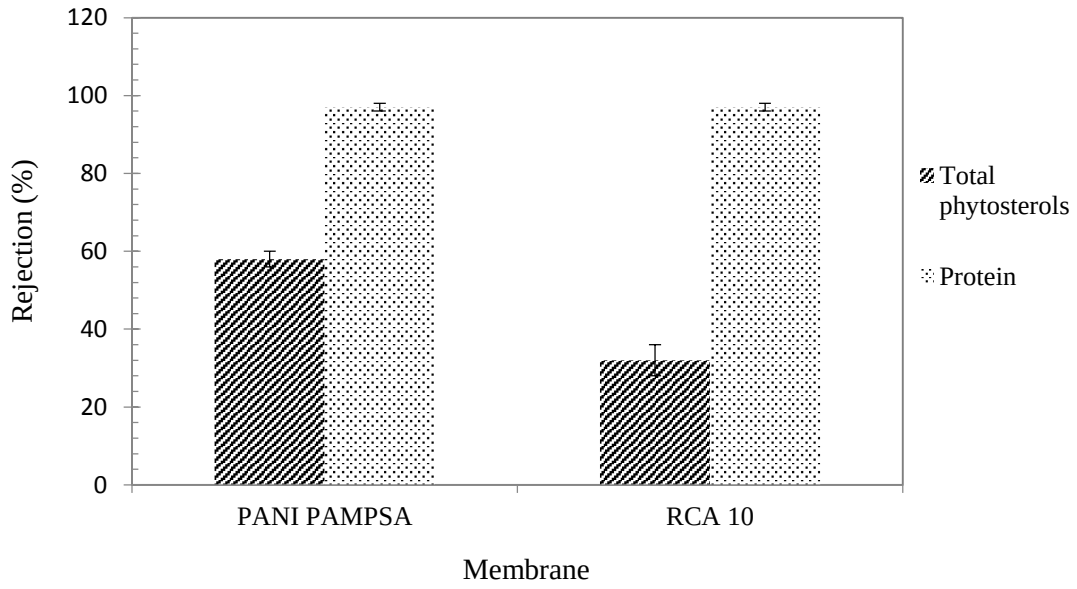


Fig. 9: Rejection of phytosterols and protein by PANI PAMPSA and RCA membranes.

3.2.3 Mass balance of key compounds

Table 2 illustrates a mass balance for the ultrafiltration of total phytosterols and protein using PANI PAMPSA and RCA membranes. The initial volume of the orange juice for the ultrafiltration was 3000 mL. The total phytosterols present in feed solution were 259 ± 11 mg/L. The yields of total phytosterols in the permeate for PANI PAMPSA and RCA membranes were 23 ± 2 mg/L and 43 ± 2 mg/L respectively. The mass concentration ratio of sterol to protein was increased from feed to permeate streams for both membranes. For PANI membrane, the mass concentration ratio of sterol to protein changed from 0.27 in the feed to 3.00 in the permeate. The mass concentration ratio of sterol to protein increased from 0.27 in the feed to 5.00 in the permeate for the RCA membrane. The permeate from the RCA membrane showed the higher ratio of sterols to protein compared to PANI membrane. The 18 % loss of phytosterols in the system for PANI membrane and 21 % loss for RCA membrane were presumably due to the fouling effect during the filtration [25]. It is hypothesised that the sterols were trapped by the fouling layer and did not pass through the membrane. The protein mass in the feed solution was 947 ± 23 mg/L. The yields of proteins in the permeate for PANI PAMPSA

443 and RCA membranes were 8 ± 2 mg/L and 9 ± 2 mg/L respectively. The losses of the feed
444 proteins for both membranes were presumably due to the adsorption of protein solute inside
445 the membrane pores or on the membrane surface [25]. It can be noted that the highest recovery
446 of phytosterols in the permeate (43 ± 2 mg/L) was obtained by using RCA membrane.
447
448 Table 2: Mass balance for total phytosterols and protein by UF process of orange juice with
449 different membranes; (a) PANI PAMPSA and (b) RCA 10.

(a) PANI PAMPSA	**Feed**	**Final retentate**		**Total permeate**		**Total (%)**
Volume (mL)	3000	2300	77%	700	23%	100
Total sterols (mg)	742	539	73%	69	9%	82
Protein (mg)	2772	2212	80%	23	1%	81
Mass concentration ratio (sterols to protein)	0.27			3.00		

450

(b) **RCA 10**	**Feed**	**Final retentate**		**Total permeate**		**Total (%)**
Volume (mL)	3000	2150	72%	850	28%	100
Total sterols (mg)	810	504	62%	135	17%	79
Protein (mg)	2910	2408	83%	26	1%	84
Mass concentration ratio (sterols to protein)	0.27			5.00		

451

3.3 Surface charge of PANI PAMPSA membrane

The PANI PAMPSA membrane's fouling tendency was further studied via surface charge analysis. A surface exposed to an aqueous environment assumes an electric surface charge which arises either from dissociation or protonation of surface functional groups or from selective adsorption of ions. The pristine PANI PAMPSA membrane showed a negative Z potential of -16 mV. It is usually accepted that the negative charges of PAMPSA are balanced by the positive charges of the PANI backbone, however it has been reported that the polymer matrix is negatively charged due to the dissociation of the macromolecular acid with a pKa of 0.87 [34]. After fouling and decrease in the permeate flux, the PANI PAMPSA membrane was extensively cleaned with a solution at pH 11.7 and then rinsed with water increasing the z potential to -11 mV. The minor change in the net surface charge towards less negative value could be attribute to the buffer effects of the sulfonic and carboxylic groups of the PAMPSA and the irreversible adsorption of charged compounds onto the membrane surface [35].

3.4 Membrane fouling: visual study

To assess membrane fouling and evaluate the effectiveness of the cleaning we carried out a visual study of the PANI PAMPSA membrane and the RCA membrane surface using SEM. Fig. 10 and Fig. display i) the surface of the pristine membrane ii) fouled membrane and iii) the cleaned membrane.

As reported in Section 3.1, the cleaning efficiency was superior for the RCA membrane whereas the PANI PAMPSA membrane showed a higher irreversible fouling value. From SEM it was possible to characterise the membrane before and after cleaning. The fouled membrane shows the presence of a dispersed layer on the top of the membrane surface which appeared rougher when compared with the pristine membrane. Interestingly, the cleaned membrane SEM image shows a greater similarity to that of the pristine membrane, and shows no sign of the

dispersed layer. This result confirms the effectiveness of cleaning the PANI PAMPSA membrane using 0.5 wt % Ultrasil-11. This formulation was able to remove the adsorbed matter from the top surface.

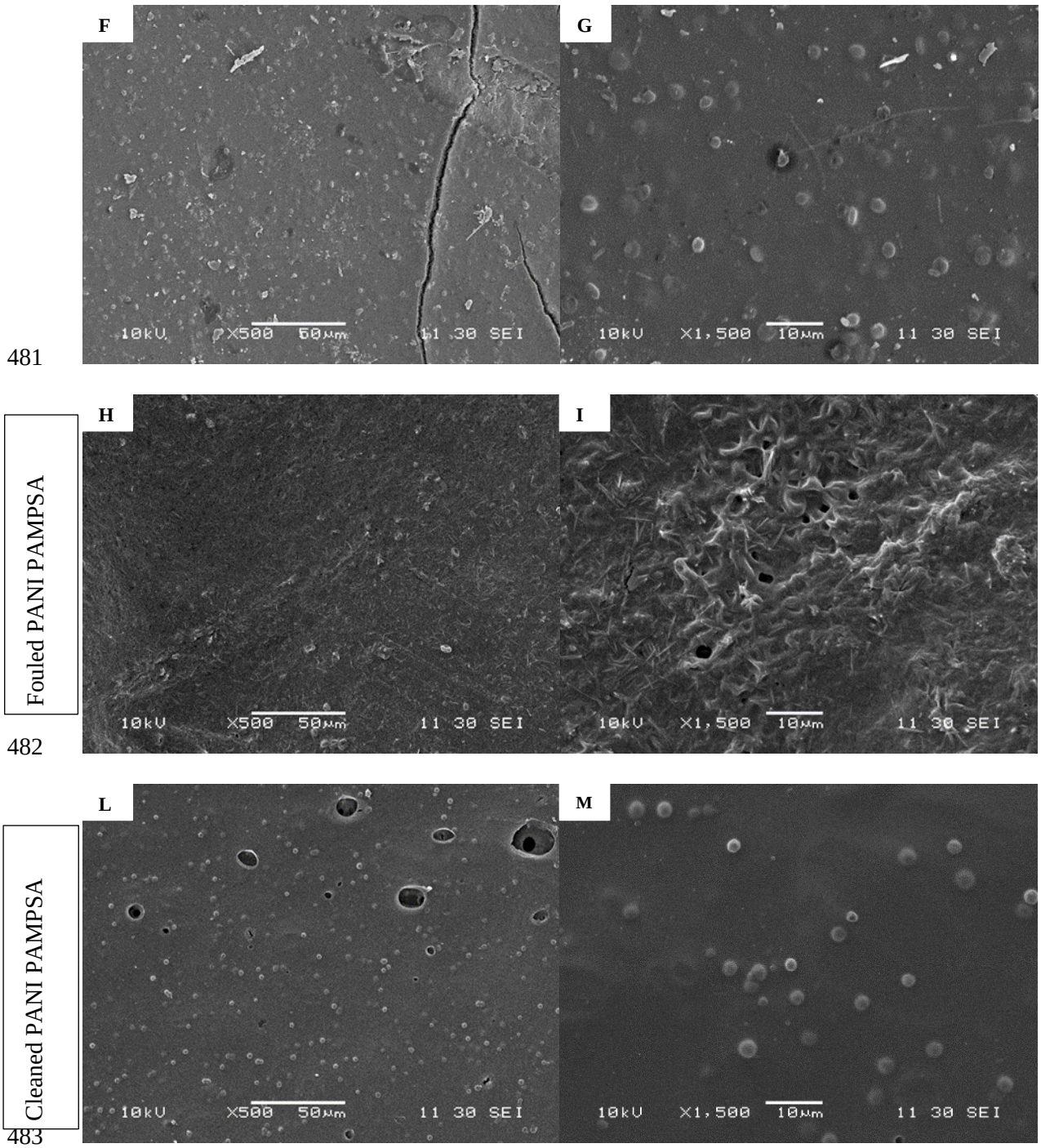


Fig. 10: SEM images of membrane surfaces of (F,G) pristine PANI PAMPSA; (H,I) Fouled PANI PAMPSA and (L,M) cleaned PANI PAMPSA.

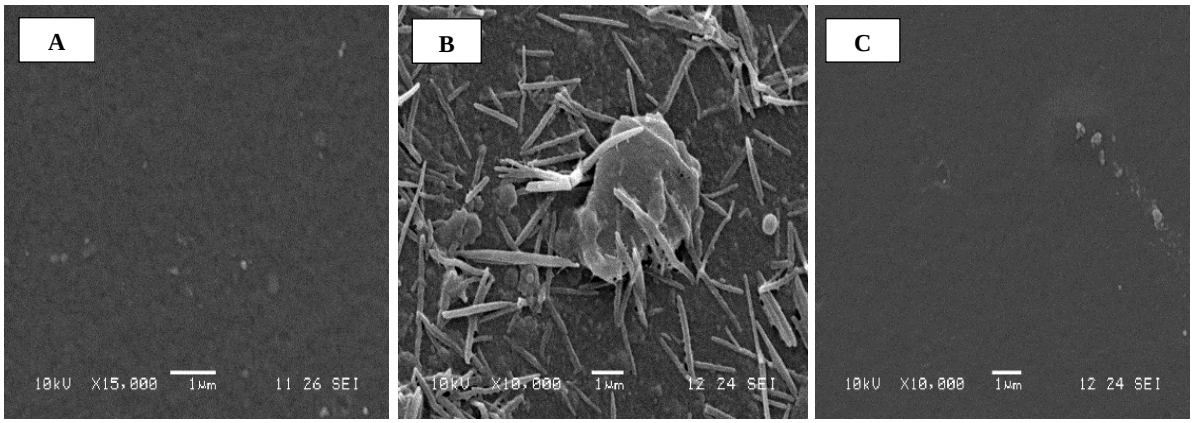


Fig. 11: SEM images of RCA membrane surfaces (A) conditioned membrane, (B) fouled membrane, and (C) cleaned membrane

4.0 Conclusion

In this work, the lab made PANI PAMPSA membrane was benchmarked against a commercial RCA membrane. The separation of phytosterols from protein in orange juice were investigated, focussing on both filtration performance and membrane fouling. The PANI PAMPSA membrane was synthesised via phase inversion in water from a solution of PAMPSA 20 wt% in NMP, 4-MP and THF. Physical and chemical characterisation showed that the presence of acid sulfonic groups imparted hydrophilicity to the PANI backbone, resulting in a decreased contact angle value of 77% after 60 s. The PANI PAMPSA membrane of 200 μm thickness showed a pure water flux of 126 $Lm^{-2}h^{-1}$. Cross-flow ultrafiltration of orange juice showed that fouling occurs for both membranes with the PANI PAMPSA membrane showing slightly higher irreversible fouling than the RCA membrane. The cleaning efficiency was high for both membranes, with PANI PAMPSA membrane showing a value of 74% after 3 fouling-cleaning cycles. This study is the first reported in the literature to evaluate the PANI PAMPSA

membrane in a complex model system and benchmarks it against a commercially available membrane.

Acknowledgments

This research was made possible by the financial support of the European Research Council (ERC) through Consolidator grant TUNEMEM (Project reference: 646769; funded under H2020-EU.1.1.-EXCELLENT SCIENCE). We also thank the *Malaysian Rubber Board* for providing a PhD studentship for Nurul Hainiza Abd-Razak. The authors also thank Dr. Haofei Guo of *Alfa Laval*, Denmark for kindly supplying the commercial membranes used in this study.

Nomenclature

Abbreviation

APS	Ammonium persulfate
LB	Liebermann-Buchard
MWCO	molecular weight cut-off
NMP	N-methyl-2-pyrrolidone
PANI	Polyaniline
PAMPSA	Poly(2-acrylamido-2-methyl-1-propanesulfonic acid)
PWF	pure water flux
R	rejection ratio
RCA	regenerated cellulose acetate
TPC	total phytosterol content
UF	ultrafiltration

Symbols

A	absorbance	nm
C_p	solute concentration in the permeate	mg mL^{-1}

C_r	solute concentration in retentate	mg mL^{-1}
ΔP	transmembrane pressure	bar or Pa
J	flux	L m^{-2} h^{-1}
P	pressure	bar or Pa
R	rejection ratio	%
R_{cp}	concentration polarisation resistance	m^{-1}
R_f	fouling resistance	m^{-1}
R_{ir}	irreversible fouling resistance	m^{-1}
R_m	membrane resistance	m^{-1}
R_r	reversible fouling resistance	m^{-1}
R_{tot}	total resistance	m^{-1}
T	temperature	°C
t	time	sec or min or hr

Greek symbols

ξ	zeta potential	mV
θ	contact angle	°
ρ	fluid density	kg m^{-3}
μ	dynamic viscosity of fluid	Pa s

Reference

[1] M. Almanasrah, L.B. Roseiro, R. Bogel-Lukasik, F. Carvalheiro, C. Brazinha, J. Crespo, M. Kallioinen, M. Mänttäri, L.C. Duarte, Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction, Industrial Crops and Products, 70 (2015) 443-450.

[2] S. Armenta, S. Garrigues, M. de la Guardia, The role of green extraction techniques in Green Analytical Chemistry, TrAC Trends in Analytical Chemistry, 71 (2015) 2-8.

[3] B.K. Tiwari, Ultrasound: A clean, green extraction technology, TrAC Trends in Analytical Chemistry, 71 (2015) 100-109.

[5] C. Rodríguez-Pérez, R. Quirantes-Piné, A. Fernández-Gutiérrez, A. Segura-Carretero, Optimization of extraction method to obtain a phenolic compounds-rich extract from Moringa oleifera Lam leaves, Industrial Crops and Products, 66 (2015) 246-254.

[6] C. Conidi, A. Cassano, F. Caiazzo, E. Drioli, Separation and purification of phenolic compounds from pomegranate juice by ultrafiltration and nanofiltration membranes, Journal of Food Engineering, 195 (2017) 1-13.

[7] N.H. Abd-Razak, M.N. Zairossani, Y.M.J. Chew, M.R. Bird, Fouling Analysis and the Recovery of Phytosterols from Orange Juice Using Regenerated Cellulose Ultrafiltration Membranes, Food and Bioprocess Technology, 13 (2020) 2012-2028.

[8] A. Cassano, L. Donato, C. Conidi, E. Drioli, Recovery of bioactive compounds in kiwifruit juice by ultrafiltration, Innovative Food Science & Emerging Technologies, 9 (2008) 556-562.

[9] Gulec, H.A., Bagci, P.O., Bagci, U., 2017. Clarification of Apple Juice Using Polymeric Ultrafiltration Membranes: a Comparative Evaluation of Membrane Fouling and Juice Quality. Food and Bioprocess Technology 10, 875-885.

[10] Bai, H., Zhou, Y., Wang, X., Zhang, L., 2012. The Permeability and Mechanical Properties of Cellulose Acetate Membranes Blended with Polyethylene glycol 600 for Treatment of Municipal Sewage. Procedia Environmental Sciences 16, 346-351.

[11] N.H. Abd-Razak, Y.M.J. Chew, M.R. Bird, Membrane fouling during the fractionation of phytosterols isolated from orange juice, Food and Bioproducts Processing, 113 (2019) 10-21.

[12] S.A. Ilame, S. V. Singh, Application of Membrane Separation in Fruit and Vegetable Juice Processing: A Review, Critical Reviews in Food Science and Nutrition, 55 (2015) 964-987.

[13] A.P. Echavarría, C. Torras, J. Pagán, A. Ibarz, Fruit Juice Processing and Membrane Technology Application, Food Engineering Reviews, 3 (2011) 136-158.

[14] D. Rana, T. Matsuura, Surface Modifications for Antifouling Membranes, Chemical Reviews, 110 (2010) 2448-2471.

[15] W. Guo, H.-H. Ngo, J. Li, A mini-review on membrane fouling, Bioresource Technology, 122 (2012) 27-34.

[16] J. Shen, S. Shahid, A. Sarihan, D.A. Patterson, E.A.C. Emanuelsson, Effect of polyacid dopants on the performance of polyaniline membranes in organic solvent nanofiltration, Separation and Purification Technology, 204 (2018) 336-344.

[17] A. Sarihan, S. Shahid, J. Shen, I. Amura, D.A. Patterson, E.A.C. Emanuelsson, Exploiting the electrical conductivity of poly-acid doped polyaniline membranes with enhanced durability for organic solvent nanofiltration, Journal of Membrane Science, 579 (2019) 11-21.

[18] L.L. Xu, S. Shahid, D.A. Patterson, E.A.C. Emanuelsson, Flexible electro-responsive in-situ polymer acid doped polyaniline membranes for permeation enhancement and membrane fouling removal, Journal of Membrane Science, (2018).

[19] V. Piironen, J. Toivo, R. Puupponen-Pimiä, A.-M. Lampi, Plant sterols in vegetables, fruits and berries, Journal of the Science of Food and Agriculture, 83 (2003) 330-337.

[20] A. Jiménez-Escrig, A.B. Santos-Hidalgo, F. Saura-Calixto, Common Sources and Estimated Intake of Plant Sterols in the Spanish Diet, Journal of Agricultural and Food Chemistry, 54 (2006) 3462-3471.

[21] Cobell, Orange Juice Not From Concentrate (NFC), in, Cobell, Exeter, United Kingdom, 2016.

[22] R. Jiraratananon, A. Chanachai, A study of fouling in the ultrafiltration of passion fruit juice, Journal of Membrane Science, 111 (1996) 39-48.

[23] E. Kim, M. Goldberg, Serum cholesterol assay using a stable Liebermann-Burchard reagent, Clinical chemistry, 15 (1969) 1171-1179.

[24] L.B.D.C. Araújo, S.L. Silva, M.A.M. Galvão, M.R.A. Ferreira, E.L. Araújo, K.P. Randau, L.A.L. Soares, Total phytosterol content in drug materials and extracts from roots of Acanthospermum hispidum by UV-VIS spectrophotometry, Revista Brasileira de Farmacognosia, 23 (2013) 736-742.

[25] N.J. Kruger, The Bradford method for protein quantitation, Methods in molecular biology (Clifton, N.J.), 32 (1994) 9-15.

[26] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem, 72 (1976) 248-254.

[27] J.E. Yoo, J.L. Cross, T.L. Bucholz, K.S. Lee, M.P. Espe, Y.-L. Loo, Improving the electrical conductivity of polymer acid-doped polyaniline by controlling the template molecular weight, Journal of Materials Chemistry, 17 (2007) 1268-1275.

[28] L. Xu, S. Shahid, A.K. Holda, E.A.C. Emanuelsson, D.A. Patterson, Stimuli responsive conductive polyaniline membrane: In-filtration electrical tuneability of flux and MWCO, Journal of Membrane Science, 552 (2018) 153-166.

[29] B.D. Malhotra, S. Ghosh, R. Chandra, Polyaniline/Polymeric acid composite, a novel conducting rubber, Journal of Applied Polymer Science, 40 (1990) 1049-1052.

[30] D. Wu, M.R. Bird, The Fouling and Cleaning of Ultrafiltration Membranes During The Filtration of Model Tea Component Solutions, Journal of Food Process Engineering, 30 (2007) 293-323.

[31] A. Sass-Kiss, M. Sass, Immunoanalytical method for quality control of orange juice products, J Agric Food Chem, 48 (2000) 4027-4031.

628 [32] P.J. Evans, M.R. Bird, A. Pihlajamäki, M. Nyström, The influence of hydrophobicity,
629 roughness and charge upon ultrafiltration membranes for black tea liquor clarification, Journal
630 of Membrane Science, 313 (2008) 250-262.
631 [33] I.S. Argyle, A. Pihlajamäki, M.R. Bird, Black tea liquor ultrafiltration: Effect of ethanol
632 pre-treatment upon fouling and cleaning characteristics, Food and Bioproducts Processing, 93
633 (2015) 289-297.
634 [34] J. Shen, S. Shahid, I. Amura, A. Sarihan, M. Tian, E.A.C. Emanuelsson, Enhanced
635 adsorption of cationic and anionic dyes from aqueous solutions by polyacid doped polyaniline,
636 Synthetic Metals, 245 (2018) 151-159.
637 [35] H. Cui, Y. Qian, H. An, C. Sun, J. Zhai, Q. Li, Electrochemical removal of fluoride from
638 water by PAOA-modified carbon felt electrodes in a continuous flow reactor, Water Research,
639 46 (2012) 3943-3950.
640