Physicochemical and Colligative Investigation of α (Shrimp Shell)- and β (Squid Pen)-Chitosan Membranes: Concentration-Gradient-Driven Water Flux and Ion Transport for Salinity Gradient Power and Separation Process Operations

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ABSTRACT: Chitin, and its derivative chitosan, is a naturally occurring biopolymer and an abundant polysaccharide containing acetylated units of N-acetyl-D-glucosamine. Chitosan membranes produced from shrimp shell (α) and squid pen (β) biowaste were prepared by solvent-casting, after which water flux and ionic transport diffusion experiments were conducted using a side-by-side concentration test cell under differing salinity concentration gradients. Physicochemical and experimental investigations were conducted, which confirmed that β-chitin possesses differing and enhanced performance characteristics than α-chitin with respect to diffusive water flux and ionic transport capabilities. In addition, novel colligative investigations through osmotic equilibrium were conducted to determine electrochemical characteristics for the evaluation of salinity gradient power generation suitability. Electrochemical test results under a salinity gradient revealed extremely low energy density values, thereby limiting consideration for commercial utility-scale salinity gradient power renewable energy operations. However, the tested membranes possessed high water and ion flux permeability characteristics that could find use in industrial separation process operations such as those used in the extraction of economically valuable materials from seawater or highly saline industrial fluids, or reduction in the saline content of mining fluids during dewatering and hazardous waste treatment and disposal operations, thereby potentially fostering new market developments, which will drive continued improvements in the responsible biowaste management of this valuable marine bioresource.

1. INTRODUCTION

1.1. General Background. Most of the carbohydrates found in nature occur as polysaccharides, polymers of high molecular weight. Chitin is a naturally occurring biopolymer with great potential for industrial use because of its high amine content and polycatonic nature.1 Chitin is a linear homopolymer (a polymeric carbohydrate molecule with repeating units of a single monomeric unit), containing residues of the monosaccharide N-acetyl-D-glucosamine (GlcNAc) joined by β (1 → 4) linkage. Chitin occurs mainly as the principal element in the hard exoskeletons, inner shell or cell wall of invertebrates, fungi, and yeasts2,3 and is the second most abundant naturally occurring polymer on earth after cellulose. Few biological polymers possess as high a number of amino groups as chitin, which lead to increased strength of the chitin−polymer matrix, increased hydrogen bonding between adjacent polymer layers, and high adsorption properties leading to effective ion-exchange capabilities.4,5 Serving as a natural structural biopolymer,6 chitin and its derivative chitosan possess many interesting properties including unique crystalline structures, multidimensional properties, and non-toxicity and biodegradability in both the solution and solid-state phases.7 Like cellulose, chitin is indigestible by vertebrate animals and forms extended fibers.

With the recognition of chitosan’s uniqueness comes a long history of investigating potential uses in varying forms. For example, in 1936, GW Rigby was granted U.S. patent number 2,040,880 for making chitosan membrane films along with a second patent for making fibers from chitosan.8 Other industrial operational uses include separation membrane technologies (aqueous and gas); flocculation of proteinaceous solids and chelation of metal ions in wastewater treatment, microalgal biomass dewatering, beer/beverage clarification; treatment of wounds and burns by incorporation into healing-
accelerating sutures and antibacterial surgical dressings; and as a feed and food processing additive.1,9

Chitin is not soluble in ordinary solvents. As shown in Figure 1, chitin differs from cellulose within the glucose unit where one hydroxyl group (−OH) is replaced at the C-2 position with one acetylated amino group (NHCOCH₃). Chitosan, derived from chitin by deacetylation, differs from chitin by the converted amine group (free −NH₂), which imparts a hydrophilic and polycationic nature to the chitosan product, enabling its solubility in dilute organic acidic solutions where the pH is <6.6.16−18 Chitin exists in three different crystalline structural/polymorphic forms, referred to as α-, β-, and γ-chitin, which number of chitin chains per cell.19 α-Chitin, the most common polymorphic form found in commercial chitin and chitosans, is frequently obtained from a large amount of available low-cost marine crustacean (e.g., lobsters, crabs, and shrimp) biowaste. β-Chitin is available in reduced quantities from marine cephalopod (squid pen) biowaste but can be obtained from other marine sources such as the crystalline fibrils of some microalgae (diatoms) and the tubes of vestimentiferans [giant undersea tube worms].14,20,21 γ-Chitin is usually obtained from fungi and yeasts with the crystalline structure being a combination of the α- and β-forms.

1.2. Issue Recognition. Chitin is highly acid-resistant, and chitosan is highly alkaline-resistant; characteristics that depend upon the end-use application can lend themselves well for use in separation membrane applications.22 The −OH and −NH₂ functional groups in chitosan facilitate an adsorbent function, which has lent itself to numerous investigations as an adsorbent for the treatment of wastewater and the removal of heavy metals from liquid effluents and natural water by biosorption.23 The sorption capacity of chitin and chitosan materials depends on the origin of the polysaccharide, molecular weight (Mₘ), degree of N-acetylation, and solution properties and varies with crystallinity, affinity for water, and amino group content.24

α-Chitin has a very stable unit cell intrachain, intrasheet, and intersheet hydrogen bonds forming from antiparallel crystalline sheets, whereas the β-chitin unit cell consists of parallel sheets with weaker hydrogen bonds between two intersheets and reduced intrasheet attraction.25−32 These structural changes lead to higher solubility, reactivity, and swelling ability toward solvents of β-chitin than α-chitin after alkali treatments, which could alter the chitosan solution conformations and impact their antibacterial activity.33 Owing to its unique crystalline structural arrangement, β-chitin has been reported to more readily accept intercalated water molecules than α-chitin.34 With the intracrystalline swelling of β-chitin strongly anisotropic without modifications to the β-chitin sheets that are maintained by strong N−H···O=C intermolecular hydrogen bonds.35,36 Considering the reported intercalation of water molecules within its crystalline lattice and weaker intermolecular hydrogen bonding between sheets of parallel chains, β-chitin may possess differing and enhanced performance characteristics than α-chitin with respect to diffusive water flux and ionic transport capabilities.

Chitin is the most abundant natural polymer in the ocean and thereby provides an enormous reservoir of organic carbon and nitrogen to draw from.37−39 Global fisheries contribute significantly to satisfying the world’s need for protein; however, crustacean and cephalopod seafood processing can generate between 35 and 75% biowaste by weight consisting of the shell.
head, and viscera.30 With increasing demands in both the human population and protein needs, continued improvement in sustainable seafood biowaste management through new market development is essential. The twofold research objective of this α- and β-chitin comparison effort was to

- investigate and report on select physicochemical, colligative, and microstructural characteristics needed to substantiate this hypothesis of differing diffusive ion transport and osmotic flow capabilities;
- advance the multifaceted synergistic goal of biowaste management improvement and new market development by extending the consideration of possible chitosan biopolymer membrane uses to developing and sustainable technologies such as salinity gradient power (SGP) generation and industrial separation process operations.

2. RESULTS AND DISCUSSION

2.1. Background. Chitosan’s physicochemical, rheological, and physical properties vary significantly as a function of its molecular weight characterization.41,42 The analytical technique frequently cited in the literature for the determination of chitosan’s molar mass distributions, number-average molecular weight (\(M_n\)), weight-average molecular weight (\(M_w\)) distribution, and polydispersity (\(P_D = M_w/M_n\)) is aqueous gel permeation chromatography (GPC)—size exclusion chromatography (SEC).16,37,43–45 Knowledge of \(M_n\) is important for thermal properties (e.g., glass transition, \(T_g\)) and \(M_w\) for tensile strength and impact resistance (i.e., mechanical properties).

Chitin is insoluble in water and common organic solvents and is usually converted to chitosan (deacetylated form of chitin) for use, with the extractability and degree of deacetylation (DDA, %) dependent upon the conversion process used. When the %DDA approaches 50%, chitin becomes soluble in aqueous acidic solution through the protonation of the NH₂ group and becomes chitosan.46 The presence of both amino and hydroxyl groups provides the chitosan macromolecule unique properties, including being easily dissolved in aqueous acetic acid of low concentrations and possessing a hydrophilic property, which lends to solvent stability and water swelling.47 Chitosan biopolymer membranes were prepared from shrimp shell (α-chitin) and squid pen (β-chitin) chitosan powder by solvent-casting, after which physicochemical testing and colligative water flux and ionic transport diffusion experiments were conducted using synthetic seawater in a side-by-side concentration cell. Diffusion is the spontaneous, net movement of molecules of a substance from a region of high concentration to one of low concentration. Since the molecules are in thermal random motion, there will be more molecules moving from the high-concentration region to the low-concentration region than in the opposite direction. There is no special force on the individual molecules; diffusion is purely a consequence of statistics.

2.2. %DDA and Crystalline Fourier transform infrared (FTIR) Discussion. The functionality of linear polymers, such as chitosan, is highly affected by the %DDA and polymer size obtained during the conversion process.48 Typically, chitosan is obtained by the partial deacetylation of chitin in hot concentrated aqueous alkali (typically 40–50% NaOH for several hours) at 100–160°C for α-chitosan and at 80°C for β-chitosan.24 This hydrolysis step removes some of the acetyl groups resulting in differing amounts of acetylated units of N-acetyl-d-glucosamine (GlcNAc) and deacetylated units of D-glucosamine (GlcN). The %DDA is defined as the molar fraction of GlcN units in the copolymer (chitosan), which is composed of GlcNAc and GlcN units.49 When the majority of GlcNAc units are converted to GlcN units (high %DDA), the polymer becomes highly soluble in dilute acids.

Examination of the measured FTIR spectra can provide useful insight into the molecular characteristics, as well as reveal any observed changes in the chemical bonds.50 FTIR spectra for the α- and β-chitosan samples tested, illustrating both CH stretching and intrasheet or intersheet hydrogen bond characteristics, can be found in Figure 2 (dataset can be found here). %DDA was computed from the measured spectral data using eq 1 and compared to the vendor-supplied data, where \(A_{1655}\) and \(A_{3450}\) were the measured absorbance at 1655 cm⁻¹ (amine group) and 3450 cm⁻¹ (hydroxyl [OH] group), respectively.

\[
\text{%DDA} = \frac{A_{1655}}{A_{3450}} \times 115
\]  

Using eq 3, the computed FTIR %DDA values vs vendor-supplied values are

Figure 2. FTIR spectra comparison of α (shrimp) and β (squid) 2% chitosan membranes.
both the α and β-chitosan sample corresponding to the vibration of CH stretching, assuming the band at 1375 cm$^{-1}$ corresponds to the vibration of CH stretching, indicating that the hydrogen bonds of α- and β-chitosan spectra between 2300 and 2380 cm$^{-1}$ is unknown.

2.3. Molecular Weight Characterization by GPC/SEC Analytical Technique. GPC/SEC is a liquid chromatography technique that separates macromolecules by their size in solution. Aqueous GPC–SEC separation is based upon differential migration between the stationary and mobile phases and governed by the hydrodynamic size and shape of the polymer chains relative to the size and shape of the porous pores within the column packing material. Summary data obtained from the aqueous GPC–SEC testing effort are presented in Table 1.

2.4. Chitosan Membranes Prepared and Tested. In accordance with the procedure outlined in Section 4.4, α- and β-chitosan membrane test samples were made with the following casting constituents:

- Shrimp (α): deionized (DI) = 100 mL; glacial acetic acid = 1 mL; glycerol = 0.7 g; chitosan = 2.02 g.
- Squid (β): DI = 100 mL; glacial acetic acid = 1 mL; glycerol = 0.7 g; chitosan = 2.02 g.

Resulting in a % chitosan composition of nominally 2% (1.95%) α- and β-membranes prepared and evaluated.

2.5. Salinity Gradient Concentration Cell Test Configurations. Instant ocean synthetic sea salt was used throughout this testing effort to provide a suitable medium without the deleterious effects of marine biofouling. The concentrated ionic test solution (full brine) was made from 300 g of instant ocean sea salt dissolved in enough DI water to make 1 L of the total solution. The dilute ionic test solution (1:10 brine) was made by serially diluting 100 mL of the full brine solution to 1 L with DI water. Runs consisted of either full brine or a 1:10 brine solution in the concentrated test cell chamber side and DI water in the dilute test cell chamber side. A summary of test configurations for each test solution type used in this analysis along with the measured electrically loaded cell potential voltage at osmotic equilibrium is presented in Table 2.

2.6. Ion Migration across the Membrane. Samples obtained from the concentration test cell were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) instrumentation. To verify the applicability of the ICP-MS

Table 1. Average GPC Sample Analysis of Sampled α- and β-Chitosan

| sample    | $M_n$ (kDa)$^a$ | $P_d$ ($M_p/M_n$)$^a$ | RFI$^a$ | $\eta$ (dL/g)$^b$ | $R_g$ (nm)$^c$ | $\alpha$ exponent$^d$ | log K constant$^e$ |
|-----------|-----------------|-----------------------|--------|-------------------|---------------|-----------------------|-------------------|
| shrimp (α) | 171.556         | 2.939                 | 0.126  | 2.8718            | 23.85         | 0.558                 | −2.509            |
| squid (β)  | 66.950          | 2.840                 | 0.170  | 3.2905            | 19.27         | 0.722                 | −3.218            |

$^a$Number-average molecular weight measured by gel permeation chromatography (GPC) using a mixture of 0.1 M acetic acid and 0.3 M sodium nitrate in high-performance liquid chromatography (HPLC)-grade water as the mobile phase at 35 °C. $^b$Polydispersity ($M_p/M_n$). $^c$Refractive index (RI) increment (RII) (dn/dc) where values were calculated by assuming a 100% mass injection recovery of the triplicate injections. $^d$Intrinsic viscosity. $^e$Hydrodynamic radius. $^f$Mark–Houwink.

Table 2. Salinity Gradient Concentration Cell Test Run Summary

| test sample number | test sample name | membrane type and nominally loaded voltage potential | test solution type |
|--------------------|-----------------|-----------------------------------------------------|-------------------|
| 1                   | A4-2            | α-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 2                   | A5-3            | α-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, dilute test side |
| 3                   | A5-4            | α-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, concentrated test side |
| 4                   | A7-1            | α-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 5                   | A7-2            | α-chitosan (V = 0.6 mV)                              | full brine/DI, concentrated test side |
| 6                   | B1-3            | β-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 7                   | B1-6            | β-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, dilute test side |
| 8                   | B1-8            | β-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, dilute test side |
| 9                   | B1-9            | β-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, concentrated test side |
| 10                  | B1-10           | β-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 11                  | B1-11           | β-chitosan (V = 0.6 mV)                              | full brine/DI, concentrated test side |
| 12                  | B1-14           | β-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 13                  | B1-16           | β-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 14                  | B1-17           | β-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 15                  | B2-1            | β-chitosan (V = 0.6 mV)                              | full brine/DI, dilute test side |
| 16                  | B2-3            | β-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, dilute test side |
| 17                  | B2-8            | β-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, dilute test side |
| 18                  | B2-11           | β-chitosan (V = 0.6 mV)                              | 1:10 brine/DI, dilute test side |
| 19                  | B1-2            |                                                    | standard, full brine standard |
| 20                  | B1-5            |                                                    | 1:10 brine standard |
| 21                  | A1              |                                                    | standard, DI |
| 22                  | BP-1            | BPM (V = 1.14 mV)                                   | full brine/DI, concentrated test side |
| 23                  | BP-2            | BPM (V = 1.14 mV)                                   | full brine/DI, concentrated test side |
| 24                  | BP-5            | BPM (V = 0.6 mV)                                    | 1:10 brine/DI, dilute test side |
| 25                  | BP-6            | BPM (V = 0.6 mV)                                    | 1:10 brine/DI, concentrated test side |
anion measurements for future use in the calculation of total osmotic pressure, a separate Cl\(^{-}\) ion titration was done on each of the solution test samples for comparison. Discussion of both is presented herein.

2.6.1. ICP-MS. Seawater contains dissolved salts at a total ionic concentration of approximately 1.12 mol/L (M) and a computed osmotic pressure at 25 °C of 27.2 atm. A salt is an electrically neutral ionic compound composed of two oppositely charged ions: cations and anions. When a salt dissolves in water, it dissociates into its individual cations and anions. Seawater is nominally 86% sodium chloride (NaCl) and 14% other ions, with Na\(^+\) and Cl\(^-\) almost completely dissociated. Every naturally occurring element that can be found on earth has one part in a million (1 ppm or 1 mg/kg) has been found dissolved in seawater. However, although present in measurable concentrations, there is a great variation in the concentration magnitudes of the ions present. According to chemical oceanographic convention, a concentration criterion of one part in a million (1 ppm or 1 mg/kg) has been established as the separation point. Elements with higher concentrations to be easily measurable by ICP-MS and are of frequent interest in seawater and seawater brine element recovery studies. Measured ICP-MS sample test results are presented in Table 3 (dataset along with measurement standard deviations (SDs) available at https://usf.box.com/s/54t71bo9v63xulf23ms2vo0mc4q07ay).

2.6.2. Cl\(^-\) Ion Titration. Backup concentration cell water sample Cl\(^-\) ion titration testing began by serially diluting 1 mL of test sample to 100 mL with DI water and then adding it to a 250 mL beaker with magnetic stirring. Both electrodes were immersed in the solution, and agitation began. Silver nitrate (AgNO\(_3\)) was then added in 0.5 mL increments, both the differential potential (V) test sample volume (V) and the original molarity present (C) Cl\(^-\) (AgNO\(_3\)) was then added in 0.5 mL increments, both the differential potential (V) test sample volume (V) and the original molarity present (C) were plotted to determine the titration end point and recorded on a spreadsheet after each addition. Using the data from the spreadsheet, a second-order differential potential curve was plotted to determine the titration end point and corresponding total volume of AgNO\(_3\) (V\(_{AgNO3}\)) was used along with the molarity (molar concentration) of the AgNO\(_3\) titrant (C\(_{AgNO3}\)) (0.1 N for full-brine-based samples or 0.01 N for 1:10 brine-based samples or less) to calculate the desired Cl\(^-\) molarity present (C\(_{Cl^-}\)) in the test sample according to eq 2.

\[
C_{Cl^-} = \frac{(C_{AgNO3} \times V_{AgNO3})}{V_{Cl^-}}
\]
Data obtained from both the Cl\(^-\) ion potentiometric titration \((C_{\text{Cl}^-})\) and the ICP-MS testing are presented in Table 4 with the results displayed in Figure 3.

Table 4. Salinity Gradient Concentration Cell Test Run Summary

| run  | membrane type | test solution type | ICP-MS Cl \(^-\) (M) | titration Cl \(^-\) (M) | measurement difference between % |
|------|---------------|--------------------|-----------------------|------------------------|--------------------------------|
| A4-2 | α-chitosan     | full brine/DI       | 2.140                 | 2.293                  | −7                              |
| A5-3 | α-chitosan     | 1:10 brine/DI       | 0.166                 | 0.154                  | 7                               |
| A7-1 | α-chitosan     | full brine/DI       | 2.145                 | 2.352                  | −10                             |
| B1-3 | β-chitosan     | full brine/DI       | 2.306                 | 2.568                  | −11                             |
| B1-6 | β-chitosan     | 1:10 brine/DI       | 0.227                 | 0.210                  | 8                               |
| B1-8 | β-chitosan     | 1:10 brine/DI       | 0.212                 | 0.201                  | 5                               |
| B1-10| β-chitosan     | full brine/DI       | 2.440                 | 2.650                  | −9                              |
| B1-14| β-chitosan     | full brine/DI       | 2.306                 | 2.654                  | −15                             |
| B1-16| β-chitosan     | full brine/DI       | 2.455                 | 2.489                  | −1                              |
| B1-17| β-chitosan     | full brine/DI       | 2.455                 | 2.551                  | −4                              |
| B2-1 | β-chitosan     | full brine/DI       | 2.319                 | 2.502                  | −8                              |
| B2-3 | β-chitosan     | 1:10 brine/DI       | 0.194                 | 0.188                  | 3                               |
| B2-8 | β-chitosan     | 1:10 brine/DI       | 0.195                 | 0.198                  | −2                              |
| B2-11| β-chitosan     | 1:10 brine/DI       | 0.268                 | 0.259                  | 3                               |
| B1-2 | BPM            | full brine          | 3.766                 | 3.606                  | 4                               |
| B1-5 | BPM            | 1:10 brine/DI       | 0.425                 | 0.394                  | 7                               |
| BP-1 | BPM            | 1:100 brine         | 0.058                 | 0.055                  | 6                               |

Figure 3. ICP-MS vs potentiometric titration-chloride ion testing method comparison.

Close examination of the Figure 3 results showed that in general, above a Cl\(^-\) ion concentration of ~1 M, the % difference from the Cl\(^-\) titration produced values slightly higher than the ICP-MS (runs A5-3, B1-6, B1-8, B2-3, B2-8, B2-11; median = 4.32%, standard deviation = 3.23%, and variance \(s^2\) = 0.10%). The variances from the two sample concentration regions were compared using a two-tailed F-test and along with a 95% confidence null hypothesis \((H_0)\) that no significant difference in the variances exists \((\text{eqs 3 and 4})\)

\[
F_{\text{exp}} = \frac{s_A^2}{s_B^2} \tag{3}
\]

\[
H_0: s_A^2 = s_B^2 \tag{4}
\]

Pursuant to this, the value for \(F_{\text{exp}} = (0.0016)^2/(0.0010)^2 = 2.2945\), and the critical value for \(F (0.05, 7, 5)\) is 6.853. Because \(F_{\text{exp}}\) is less than \(F (0.05, 7, 5)\), we retain the null hypothesis and have no evidence for a significant difference between \(s_A\) and \(s_B\), which means that the standard deviations of the 14 test samples can be pooled.

Visual examination of the corresponding measurement closeness presented in the Figure 3 data reveals no significant observed determinate error affecting the results. To statistically substantiate this observation, a paired two-tailed \(t\)-test method statistical analysis was used with the entire 17 (14 tests and 3 standard) % difference sample dataset (covering a broad Cl\(^-\) concentration range between 0.058 M and 3.766 M; median \((X)\) of ~1.35% and a 7.27% standard deviation \((SD)\)) along with a 95% confidence null hypothesis that the overall median is not significantly different from 0 \((\text{eqs 5 and 6})\)

\[
t_{\text{exp}} = \mu - X/\sqrt{n/SD} \tag{5}
\]

\[
H_0: X = \mu \tag{6}
\]

Pursuant to this, the value for \(t_{\text{exp}} = (0.0 - (-0.0135))/\sqrt{17}/0.0727 = 0.765\), and the critical value for \(t (0.05, 16)\) is 2.120. Because \(t_{\text{exp}}\) is less than \(t (0.05, 16)\), we retain the null hypothesis and have no evidence for a significant difference between \(X = \mu\). Statistically based results from both two-tailed \(F\)-test and Student’s \(t\) test support the use of the ICP-MS measurements for Cl\(^-\) and Br\(^-\) and the effectiveness of the ICP-MS methodology steps taken to minimize any carryover measurement effects.

2.7. Osmotic Pressure, Water and Cl\(^-\) Ion Transport, Multi-Ion Transport, and Osmotic Equilibrium Discussion. Discussions on water and ionic transport diffusion and how it relates to osmotic pressure and osmotic equilibrium are presented herein.

2.7.1. Osmotic Pressure. The osmotic pressure, \(\pi\), of a solution containing \(n\) moles of solute particles in a solution of volume \(V\) can be determined in rough approximation under dilute (ideal) conditions using the van’t Hoff equation, which obeys a form like the ideal-gas law

\[
\pi V = nRT \tag{7}
\]

where \(V\) is the volume of the solution, \(n\) is the number of moles of solute, \(R\) is the ideal-gas constant, and \(T\) is the temperature on the Kelvin scale. Equation 7 can be rewritten as

\[
\pi = (n/V)RT = MRT \tag{8}
\]

where \(M\) is the molarity of the solution, expressed as the number of moles of solute per liter of solution, and the units of \(\pi\) are in atmospheres (atm). The ICP-MS provides individual ion concentrations in units of parts per billion, which are converted to molarity and then summed together to determine
the total solution molarity. According to the van’t Hoff equation, an ideal solution containing 1 mol of dissolved particles per liter of solvent (1 M) at 25 °C will have an osmotic pressure of 22.2 atm.

2.7.2. Water and Cl⁻ Ion Transport. Typical Cl⁻ ion and water transport diffusion measurements across a casted nominal 2% β-chitosan membrane under an equalizing full brine/DI concentration gradient are presented in Figure 4. The shape of the Cl⁻ ion diffusion across the membrane under test from the concentrated test chamber side into the dilute test chamber side was determined using a chloride-ion-selective electrode immersed in the dilute test chamber. Data postprocessing included normalizing each measured Cl⁻ ion concentration data point to the maximum value measured once equilibrium was reached (t > 20 h). Water transport from the dilute test chamber into the concentrated test chamber was determined through periodic observations of rising water within a manometer emanating from the concentration test chamber side against a vertically mounted measurement tape with discrete data points measured and a fitted polynomial presented for comparison.

Examination of Figure 4 reveals a similar curve shape across the β-chitosan membrane for the Cl⁻ ion transport under both high (full brine) and low (1:10 brine) test cell solution conditions and water transport via net osmotic flow (dataset available at https://usf.box.com/s/5471bo9vv633xuf23ms2vo0mc4q07ay). Extended and varying temporal observations of water transport measurements made for the B1-17 full brine run are presented along with the automated B1-17 Cl⁻ measurement run data. Also included in Figure 4 is a normalized 5-run average obtained from the dilute side of a 1:10 brine concentration/DI test configuration for comparison to the B1-17 full brine run data. The smaller fluctuation observed in the five-run average plot is a function of the five-run averaging. Select measured values for Cl⁻ ion migration and water transport across both α- and β-chitosan membranes for various conditions are presented in Table 5.

Examination of the Table 5 results reveals

- An increase in the observed water level heights within the manometer with increased initial Cl⁻ concentration (osmotic pressure) under the full brine/DI test condition for both the α- and β-chitosan membranes.
- A significant increase in the observed water level height for the β-chitosan membrane as compared to that for the α-chitosan membrane (2.4 times) over the same DI/full brine solution test condition.

Table 5. Select Cl⁻ Ion and Water Transport Values across α- and β-Chitosan Membranes

| membrane type | test cell solution condition | ICP-MS Cl⁻ ion concentration (M) | average maximum water level height (cm) | run configurations |
|---------------|------------------------------|----------------------------------|----------------------------------------|-------------------|
| α-chitosan    | full brine/DI                | 2.142 ± 0.002                    | 22.225                                 | A4-2, A7-1        |
| β-chitosan    | full brine/DI                | 2.371 ± 0.062                    | 53.550                                 | B1-3, B1-10, B1-14, B1-16, B1-17, B2-1 |
| α-chitosan    | 1:10 brine/DI                | 0.166 ± 0.007                    | 0 (none observed)                      | A5-3             |
| β-chitosan    | 1:10 brine/DI                | 0.219 ± 0.027                    | 5.080                                  | B1-6, B1-8, B2-3, B2-8, B2-11 |

"ICP-MS SD used for single measurement.

2.7.3. α- and β-Chitosan Membrane Multi-Ion Transport. Using select data from the Table 2 run summary and Table 3 measured ICP-MS solution data, cross α- and β-chitosan membrane ion transport concentrations for Na, Cl, K, Mg, Ca, B, Br, Li, Sr, and Rb were calculated and are presented in Figure 5a–c for full brine/DI and Figure 5d–f for 1:10 brine/DI. Specific runs used are

- Full brine/DI; for α-chitosan, the average of runs A4-2, A7-1, and for β-chitosan, the average of runs B1-3, B1-10, B1-14, B1-16, B1-17, B2-1.
- 1:10 Brine/DI; for α-chitosan, run A5-3, and for β-chitosan, the average of runs B1-6, B1-8, B2-3, B2-8, B2-11.

Close examination reveals greater transport in the β-chitosan membrane over the α-chitosan membrane for monovalent Na, Cl, and K ions for both solution concentrations examined. These ions have larger crystal radii and weaker hydration shells, which may enable easier detachment from their hydration layer while passing across the β-chitosan membrane with its weaker intermolecular hydrogen bonding between the sheets of parallel chains. Cross-membrane ion transport also occurred for the remaining ions examined. However, with the exception of greater transport observed in the α-chitosan membrane over the β-chitosan membrane for the divalent Mg ion in the full brine solution, the closeness and SD overlap prevent an accurate prediction of any overall trend determination. Therefore, it was not possible to determine from the measured data if greater transport would also occur in the α-chitosan membrane over the β-chitosan membrane for the other divalent and trivalent ions tested. However, enough information was obtained to confirm the initial reports in the literature, which postulated that β-chitin may possess differing and enhanced performance characteristics than α-chitin with respect to diffusive water flux and ionic transport capabilities.

2.7.4. Osmotic Equilibrium. Using eq 8 and measured ICP-MS solution data obtained from Table 3, an example calculation of the osmotic pressure and resulting osmotic equilibrium after t > 20 h is presented in Table 6.

2.8. Osmotic-Pressure-Driven Market Opportunities. Salinity gradient power (SGP) generation and/or separation process operations are possible market areas discussed herein for consideration. Although numerous SGP solutions have been discussed in the literature, the most often cited
technologies focus on variations of two existing water desalting membrane processes: pressure-retarded osmosis (PRO) and reverse electrodialysis (RED), where a significant driving force in the industrial development of membranes already exists. PRO and RED utilize the electrochemical properties of solutions of differing saline concentrations (salinity) separated...
by charged semipermeable ion-exchange membranes.54–56 In PRO, the osmotic process increases the volumetric flow of the high-pressure solution and is the energy-transfer mechanism with the gross energy gain per unit membrane area equal to the product of the pressure difference multiplied by the volume flow of freshwater through the membrane.57 Key to PRO is the cost-effective manufacture of semipermeable membranes with high water flux permeability and high salt retention (low salt flux). In RED, anion exchange membranes (AEMs) and cation exchange membranes (CEMs) are alternately arranged to form a repeating unit called a cell. The basic RED stack consists of several hundred AEM/CEM cell pairs bound together between end electrodes (anode and cathode) with the driving electromotive force (EMF) in RED provided solely by the salinity concentration gradient. Voltages are generated across each membrane generated from the differences in chemical potentials of the salt ions found in the concentrated and dilute solutions with the back EMF of the transmembrane voltages’ additive. Key to RED, also known as a dialytic battery, is the cost-effective manufacture of semipermeable ion-exchange membranes with high permselectivity (highly permeable for counterions but impermeable to co-ions).

To examine the suitability of chitosan membranes for PRO and/or RED operations, we need to examine both the water flux and ion transport characteristics from an osmotic process perspective. Examination of Figure 4 revealed the presence of an osmotic transport flux, which leveled off to zero when osmotic equilibrium was reached, and examination of Figures 5a–f revealed the occurrence of cross-membrane ion transport. From this, enough detail is available to determine that neither membrane possessed the necessary high salt retention (low salt flux) or high permselectivity to either anions or cations required for PRO/RED operations. Therefore, based on the above findings, the use of chitosan membranes would likely not be a good fit for commercial-scale PRO or RED SGP operations.

Although the use of the chitosan membranes tested herein would likely not be a good fit for either PRO or RED SGP operations, consideration was given for possible use in an electrochemical concentration fuel cell158 as part of a fuel cell membrane electrode assembly. Chitosan-based membrane electrolyte has been considered as an alternate candidate in the production of economical fuel cells.159,160 As shown in Table 3, both chitosan membranes revealed a nominally loaded membrane voltage potential of 0.6 mV under osmotic equilibrium conditions for either test solution concentration amount @100% relative humidity, corresponding to a power density of ∼1.5 nW/cm² as tested. This contrasts to almost an order of magnitude lower than the ∼8.5 nW/cm² power density previously measured by the author using bipolar ion-exchange membranes in the same electrochemical test cell.144–56 To put these results into perspective for comparison purposes, according to the European Commission (EC) salinity power estimates,54 the first commercial 10 MW PRO SGP generation plants would need membranes capable of production of at least 0.6 mW/cm².

A review of the recent literature revealed chitin films prepared with crab-shell-derived purified chitin using a group of enzymes obtained from *Streptomyces griseus*. The purified chitin slurry was dispersed in DI water with chitin sheets prepared by suction filtration.56 The proton conductivity of these films was examined in a traditional H₂/air fuel cell with the findings that the chitin becomes the electrolyte of the fuel cell in the humidified condition with a typical power density of 1.35 mW/cm² at a 100% relative humidity. Kawabata deduced that the relation between the chitin hydrated structure and the proton conduction path formed by the hydrogen bond with the water molecule is significantly important and that these water molecules form hydrogen bonds between the hydroxyl and amino-acetyl groups.

It is important to mention that the electrical conduction method of the traditional H₂/air fuel cell using proton-conducting cation permselective chitin sheets is different from that of the ∼90% DDA nonion-selective chitosan membranes in an electrochemical fuel cell considered herein. In addition, the partial deacetylation process used to convert chitin to chitosan removes some of the amino-acetyl groups, which may contribute to the low energy density observed in the ∼90% DDA chitosan membranes evaluated herein. While it is possible that the energy density output of the electrochemical fuel cell will improve slightly upon using chitosan membranes with a lower % DDA value, it is unlikely that the necessary large-scale improvements in energy density from nW/cm² to mW/cm² for utility-scale generation purposes will be realized going from ∼90% DDA to the ∼50% DDA lower limits for chitin/chitosan conversion.

### 2.9. Dry Membrane Thickness and Gel Swelling Index (GSI) Discussion

Typical measurements of dry thickness and % gel swelling index (GSI) for new/used membranes (using eq 10 of Section 4.9) are presented as follows:

- **Shrimp (α)** new: thickness = 0.07112 mm; wet weight = 350 g; dry weight = 150 g; computed % GSI = 1.33.
- **Shrimp (α)** used: thickness = 0.06858 mm; wet weight = 1,350 g; dry weight = 530 g; computed % GSI = 1.55.
- **Squid (β)** new: thickness = 0.10160 mm; wet weight = 720 g; dry weight = 320 g; computed % GSI = 1.25.
- **Squid (β)** used: thickness = 0.05388 mm; wet weight = 660 g; dry weight = 280 g; computed % GSI = 1.36.

Examination reveals that although the dry thickness of the new β-chitosan membrane was more than that for the new α-chitosan membrane, the %GSI value for the new β-chitosan membrane was less than that for the new α-chitosan membrane. Compare that to the used β-chitosan membranes, which was lower in both dry thickness and %GSI than that of a used α-chitosan membrane. This result is also supported by visual/textual observations in which the used β-chitosan membrane physically felt thinner than and was not as stiff as when new.

### Table 6. Sample Osmotic Equilibrium Value

| run | test solution type | total solution molarity (M) | ideal-gas constant | temp (%K) | computed osmotic pressure (atm) | computed osmotic pressure of max. difference (%) |
|-----|-------------------|-----------------------------|-------------------|-----------|-------------------------------|-----------------------------------------------|
| B1-17 | full brine/DI | 4.7097 | 0.08205783 | 296.6 | 114.6 | 60 |
| B1-11 | full brine/DI | 4.6499 | 0.08205783 | 296.6 | 113.2 | 60 |

*Full brine/DI maximum difference between runs A5-1 and B1-2 = 192.5 atm. Slight variation from the expected nominal 50% due to previously mentioned deviation from ideal concentration conditions of the highly concentrated full brine.*
2.10. Scanning Electron Microscopy (SEM) Membrane Discussion. SEM 10,000× (low-resolution) and 50,000× (high-resolution) images from a new piece of \( \alpha \)-chitosan membrane are presented in Figure 6a,b, respectively. SEM images from a new piece of \( \beta \)-chitosan membrane under magnifications of 10,000× and 50,000× are presented in Figure 6c,d, respectively. Examination of the 10,000× images reveals general surface cracking present in both images with the \( \alpha \)-chitosan membrane exhibiting more. Examination of the higher 50,000× resolution images reveals more detail of the crack structure and patterns. In neither case was a pore-like structure observed at either magnification in either imaged membrane sample. Although not examined directly, the increased diffusive water flux and ionic transport capabilities noted herein for the \( \beta \)-chitosan membrane exhibiting more. Examination of the higher 50,000× resolution images reveals more detail of the crack structure and patterns. In neither case was a pore-like structure observed at either magnification in either imaged membrane sample. Although not examined directly, the increased diffusive water flux and ionic transport capabilities noted herein for the \( \beta \)-chitosan membrane could be attributed to weaker intermolecular hydrogen bonding found in \( \beta \)-chitosan between the sheets of parallel chains.

The 50,000× \( \alpha \)-chitosan image with the larger cracks displayed was very unstable under the electron beam with the crack expansion occurring during observation. It is conjectured that the thinner \( \alpha \)-membrane is breaking up under the hot electron beam as evidenced by the cracks being wider in the image center where the beam is more concentrated. This crack expansion was not observed on the 50,000× \( \beta \)-chitosan sample when imaged. No similar expansion was noted during observation on either of the 10,000× samples but any minor expansion would be harder to see at 10,000× vs 50,000×; so, it is difficult to determine if the differences in the width of the crack between the two 10,000× images are real differences or reflect an instability problem (heating) caused by the differences in the membrane thickness.

3. CONCLUSIONS

Physicochemical and novel colligative investigations of \( \alpha \)- and \( \beta \)-chitosan membranes were conducted, which confirmed literature discussions that \( \beta \)-chitin may possess differing and enhanced performance characteristics than \( \alpha \)-chitin with respect to diffusive water flux and ionic transport capabilities. Electrochemical test results under a salinity gradient revealed extremely low energy density values across either membrane, thereby limiting future consideration in commercial utility-scale salinity gradient power energy generation operations. However, the tested membranes possessed high water and ion flux permeability characteristics that could foster new market developments into separation process operations such as those used in the extraction of economically valuable materials from seawater or highly saline industrial fluids, the reduction in the saline content of mining fluids during dewatering, or during hazardous waste treatment and disposal operations.

4. EXPERIMENTAL SECTION: MATERIALS AND METHODS

4.1. Materials. Chitosan membranes were prepared from two commercially obtained sources: shrimp shells from Sigma-Aldrich Corporation (CAS 9012-76-4; Sigma-Aldrich P/N C3646-25G), and squid pens from GTC Bio Corporation, Qingdao, China (SGC-2). The shrimp-based product was obtained as a white powder with a vendor-supplied DDA value of 88%, and the squid-based product was obtained as a white powder with a vendor-supplied DDA value of 91.7%. The
viscosity of a solution of 1% chitosan (by weight) in 1% (by volume) aqueous acetic acid was provided by the vendor as 232 cP for the shrimp-based chitosan and ≤300 cP for the squid-based chitosan.

Reagent-grade chemicals obtained and used include glacial acetic acid (C₂H₄O₂), glycerol (C₃H₈O₃), sodium hydroxide (NaOH), nitric acid (HNO₃), potassium bromide (KBr), silver nitrate (AgNO₃), and sodium acetate (NaC₂H₃O₂). Synthetic seawater was prepared using instant ocean sea salt (Spectrum Brands) dissolved in Milli-Q ultrapure (18.2 MΩ cm) water from a Millipore purification system.

4.2. Determination of DDA and Crystalline Characteristics. Fourier transform infrared (FTIR) spectrometry was used to determine the DDA and examine any observed changes in intrasheet or intersheet hydrogen bond characteristics of the α- and β-chitosan samples. %DDA results were then compared to the vendor-supplied values. Chitosan powder was mixed with KBr (1:15) and pressed into a pellet. The spectrum was collected in transmission mode over a 400–4000 cm⁻¹ range by placing the pellet in the beam path of an FTIR spectrometer (Nicolet Magna). A total of 256 scans at a 2 cm⁻¹ resolution were averaged and corrected for background CO₂ and water in a nitrogen-purged compartment.

4.3. Molecular Weight Characterization by Aqueous GPC/SEC Discussion. Aqueous GPC/SEC testing was conducted on a Viscotek TDA305 and GPCmax system, running OmniSEC. 4.6.2 analysis software and configured for GPC/SEC triple detection analysis. The GPC/SEC system was equipped with a temperature-controlled oven housing three detectors: refractive index (RI), right angle and low angle light scattering (RALS/LALS), and a four-capillary differential viscometer. In triple detection SEC/GPC, the RI detector is employed to calculate concentration, refractive index increment (dn/dc), and injection recovery. Light scattering provides absolute molecular weight, while the viscometer delivers intrinsic viscosity (η), hydrodynamic radius (R_h), and conformational and structural parameters. SECs used were

- PolyAnalytik AquaGEL GPC column-206, exclusion limit: >20 × 10⁵ Da PEO
- PolyAnalytik AquaGEL GPC column-204, exclusion limit: >1 × 10⁶ Da PEO.
- PolyAnalytik AquaGEL GPC column-203, exclusion limit: >1 × 10⁶ Da PEO.
- PolyAnalytik AquaGEL GPC column-202.5, exclusion limit: >1 × 10⁵ Da PEO.

Standards at a concentration of 3 mg/mL and a 0.152 mL/g dn/dc consisted of

- Calibration: Pullulan Narrow 50 kDa (PULL 50K, Lot # PATD-PUL 50K-5).
- Verification: Pullulan Broad 30 kDa (PULL 30K, Lot # PATD-PBR 30K).
- Verification: Pullulan Narrow 10 kDa (PULL 10K, Lot # PATD-PUL 10K).

The mobile phase selected for use consisted of 0.1 M acetic acid and 0.3 M sodium nitrate mixture in HPLC-grade water. Chitosan powder samples were dissolved in the mobile phase at a concentration of approximately 4.0 mg/mL (4.40 for shrimp and 4.35 for squid) and filtered through a 0.22 μm poly(ether sulfone) (PES) membrane syringe filter prior to injection. Injection parameters include 100 μL injection, column temperature: 35 °C, flow rate: 0.7 mL/min, run time: 60 min. Run summary consisted of triplicate runs with the verification standards injected at the end of the sample injections to verify detector calibrations.

4.4. Chitosan Membrane Preparation by Casting/Solvent Evaporation. The chitosan membrane solution was prepared by combining DI water, chitosan, and glacial acetic acid (casting solvent) in a 200 mL beaker and placed on a magnetic stirrer plate with moderate stirring for 48 h at room temperature until thoroughly dissolved and clear. The solution was then heated to 60 °C, and glycerol was added as a plasticizer. After mixing for 15 min, the solution was removed from the heat for 30 min, followed by 1 h under a 15 in. Hg vacuum to degas. After setting for 2 h outside the vacuum chamber at ambient laboratory conditions (nominally 20–25 °C, 40–55% RH air, standard pressure), a single membrane was cast by pouring the chitosan/glycerol solution onto a leveled 20.3 × 27.9 cm² (8 ×11 in.) glass plate framed with strips of 0.635 cm (1/4 in.) thick acrylic (methyl methacrylate). Water and acetic acid evaporation occurred under ambient conditions for at least 3 days under a ventilated fume hood, scored in half along the short side, and peeled from the casting surface. Since the as-cast membranes are completely soluble in water at this point, the dried membranes were placed in a 2% NaOH solution (10.05 g of NaOH in 500 mL of DI water) for 30 min and then washed extensively with DI water until neutral pH was obtained. The neutralized and now insoluble membranes were stored in DI water until they were placed inside the concentration cell test fixture at the commencement of the water flux and ionic transport diffusion experiments.

4.5. Water Cl⁻ Ion Migration across the Membrane. The laboratory test apparatus consisted of a single, side-by-side concentration cell of cubic design with nominal outer dimensions of 10 cm × 10 cm × 7 cm, connected to a Vernier LabPro sensor interface for remote data collection using Logger Pro 3 data collection software. The test cell consists of end plates, electrodes made from #40 wire silver (Ag) mesh, two symmetrical test chambers (a concentrated solution side and a dilute solution side), and a single chitosan membrane under test, all separated by gaskets for sealing the solution side and a dilute solution side), and a single chitosan membrane under test, all separated by gaskets for sealing the liquid within. Inner test chamber nominal dimensions were 7 cm × 7 cm × 2.5 cm. A detailed description of the concentration test cell operation can be found in Merz. During each run, "loaded" cell potential voltage measurements were logged across the electrodes and a 500 Ω (nom.) resistor was connected in parallel. Cumulative multispecies ion transfer across the membrane was determined using ICP-MS analysis of samples selectively removed from the test cell at run completion. Visual evidence of the migration of water moving across the membrane from the dilute test chamber to the concentrated test chamber under direct osmotic flow was observed at ambient laboratory conditions. To accomplish this, a manometer, constructed out of a piece of 0.635 cm (1/4 in.) ID Tygon tubing, was attached to the exit port of the concentrated solution side of the test chamber and secured vertically above the test cell with the other end of the tube open to ambient laboratory conditions. The exit port tube was examined for the presence of water, and when observed, its incremental height change was recorded as it rose vertically in the tube until it stopped when isotonic (equal osmotic pressure) conditions were obtained in each chamber side.
4.6. ICP-MS Discussion. Aqueous samples obtained from the concentration cell were diluted by a factor of 10−1000 with 2% nitric acid, except where concentrations were below the lowest calibration standard, in which case no dilution was performed. A small amount of internal standard solution containing Be, Sc, Ge, and Y was added to each sample to correct for instrumental drift during analysis. Prepared samples were analyzed with an Agilent 7500cx ICP-MS equipped with a concentric micromist nebulizer, a double-pass quartz spray chamber, and a high matrix introduction (HMI) accessory. Samples were introduced into the ICP-MS via Tygon tubing using an ASX-500 autosampler. An external six-point calibration curve was used to determine elemental concentrations. A 2% nitric acid solution was used as a blank and to rinse the instrument between samples. Samples were analyzed for Li, Na, Mg, K, Ca, Rb, and Sr at lower dilution factors (10 or 100). Because of their potential for carryover, Cl, B, and Br were analyzed separately at higher dilution factors (100 or 1000), along with an extended rinse time using both a 5% nitric acid solution and a 2% nitric acid solution. Additionally, a blank was analyzed after every standard and sample to minimize any carryover. Anions Cl− and Br− are typically not measured with the ICP-MS but because of their relative importance as major seawater constituents, Cl− > 5.06% and Br− > 0.173% total salt in seawater55, their inclusion was important to this analysis. Evaluation of the published literature revealed the use of ICP-MS for the determination of Br− and analysis of Cl− from sweat for use in the diagnosis of cystic fibrosis.67,68 Because of the high concentrations of Cl− present in the samples, a separate Cl− ion titration was made on each test sample for ICP-MS comparison to ensure the validity of the ICP-MS Cl− measurement method and resulting values.

4.7. Cl− Ion Titration Discussion. Chloride ion concentrations were determined by potentiometric titration69,70 with silver nitrate (AgNO3) using a converted dual-electrode pH meter with the agitation of the immersed electrodes achieved using a 120S Fisher Scientific magnetic stirrer. During titration, a Fluke 8062A True RMS multimeter was used to detect the change in potential between a Thermo Orion 9416BN silver/sulfide half-cell electrode and a Thermo Orion 900200 double-junction reference electrode. During the titration, the two electrodes were connected to the pH meter via the terminals used for the glass electrode and calomel electrode normally used in pH measurements.

When AgNO3 is slowly added to the Cl− ion containing synthetic seawater test sample, an insoluble precipitate of silver chloride (AgCl) forms according to eq 9

\[ \text{Ag}^+ (aq) + \text{Cl}^- (aq) \rightarrow \text{AgCl}(s) \]  

(9)

The end point of the titration occurs when all of the chloride ions are precipitated and is determined by the multimeter reading at which the greatest change in voltage has occurred for a small and equal added increment of AgNO3.

4.8. Osmotic Pressure. Certain materials, including many biological membranes, are semipermeable, meaning that when they come in contact with a solution, they selectively allow the passage of certain molecules or ions to cross the membrane while blocking others. Osmosis or osmotic flow refers to the net diffusional movement of solvent molecules across a semipermeable membrane under the effect of a concentration gradient toward the solution with the higher solute concentration. The only way to stop osmosis is to raise the hydrostatic pressure on the concentrated solution side of the membrane and achieve osmotic equilibrium. This can be done through the application of a suitable amount of external pressure, by letting the pressure build up via osmotic flow into an enclosed region, or in the case of our test chamber through the pressure difference resulting from the unequal vertical liquid height in the concentrated side exit port tube. The pressure required to achieve osmotic equilibrium and stop the net osmotic flow is known as the osmotic pressure.71,72

Osmotic pressure along with boiling point elevation, freezing point depression, and vapor pressure depression are known as colligative properties that arise solely from the dilution of a solvent by nonvolatile solutes. The word colligative comes from the Latin colligatus meaning to bind together. Colligative properties are physical properties of solutions that depend almost entirely on the total concentration of the dissolved species (ions or molecules) and not on the nature or identity of the species present55.

4.9. Dry Membrane Thickness and Gel Swelling Index (GSI) Determination. Dry membrane thickness and gel swelling index (GSI) measurements were obtained from new/used pieces of the cast α- and β-chitosan membranes. Because of limited source availability, these pieces were obtained from the same manufactured batch but were not the same piece. The “used” pieces were the actual membranes used in the concentration test cell, subject to both transmembrane water and ion transport, whereas the “new” pieces were batch remnants that were only exposed to DI water. New/used dry membrane thickness and GSI measurements were initiated by placing samples of each membrane into a desiccator and weighing them daily until there was no measurable change in weight as compared to the prior measurement, after which a final weight was recorded, as well as a dry thickness measurement using a dial caliper. The samples were then placed in DI water for 24 h, after which they were removed, wiped with a dry tissue, and weighed. This procedure continued until there was no measurable change in weight as compared to the prior measurement, after which a final weight was recorded and the GSI computed using eq 10

\[ \text{GSI} = \left( \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \right) \times 100 \]  

(10)

Comparable visual evidence of surface deformation and overall shrinkage was present after drying, especially in the “used” pieces.

4.10. SEM Membrane Examination. A small sample from each of the cast membranes was mounted on an aluminum stub and coated with a thin layer of gold/palladium metal. It was then imaged at two different magnifications (10 000× and 50 000×) using a Hitachi S-3500N variable pressure scanning electron microscope with a resolution of 3 nm. Images were adjusted to match the contrast and brightness of each other for comparison.

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