Chitosan-Based Nanocomposite Membranes With Improved Properties: Effect of Cellulose Acetate Blending and TiO₂ Nanoparticles Incorporation

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The aim of this work was to synthesize nanocomposite membranes based on chitosan (CS) and cellulose acetate (CA) blends containing titanium dioxide (TiO₂) nanoparticles and investigate their morphological, structural, mechanical, and thermal properties along with dye affinity ligand content for using potentially as bioadsorption applications. The samples were characterized by means of scanning electronic microscopy (SEM), energy dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR), tensile, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and water contact angle (WCA). The SEM micrograph images showed a uniform and microporous morphology of the prepared membranes. FTIR analysis confirmed the presence of CA and TiO₂ nanoparticles through the CS matrix in which the components used had good compatibility. The mechanical and thermal studies emphasized that strong interactions occurred between CS, CA, and TiO₂ nanoparticles. Blending CS with CA and the addition of TiO₂ nanoparticles enhanced the hydrophilic property of CS membranes, significantly. The CS/CA based nanocomposite containing 1.5 wt% of TiO₂ nanoparticles showed the remarkable improvements in ultimate tensile strength values and an increase in stiffness more than three times compared to the neat CS membrane sample. Finally, considering the results of tensile test and immobilized Cibacron Blue F3G-A (CB) content measurement, the nanocomposite samples denoted by (50/50) CS/CA/TiO₂ (1.5%) were nominated as the promising material used for potentially bioadsorptive membrane.

INTRODUCTION

Biopolymers are mostly complex compounds produced from renewable resources such as living organisms and plants. These highly attractive and applicable materials participate in biocycle systems eventually allowing for degradation and reabsorption in the nature [1]. The widespread biopolymers can be classified based upon polysaccharides family such as cellulose, starch, chitin, chitosan, dextran, etc. Among these biopolymers, chitosan (CS) has special chemical and biological characteristics including nontoxicity, biocompatibility, antibacterial properties, heavy metal ion chelation ability, hydrophilicity, excellent film-forming ability, and remarkable affinity for proteins. These considerable properties have led to extensive uses of CS in many industrial and biomedical applications, among which is membrane-based processes [2–4]. Nowadays, polymeric porous membranes are of great interest as efficient tools possessing a series of functional groups onto their surfaces which can be used as coupling or adsorptive sites for separation and/or adsorption in many applications [5–7]. Furthermore, CS is predominantly used as an adsorptive media (membranes or beads), mainly because of its hydrophilicity and reactivity. Generally, this biopolymer needs no necessary treatment to increase the number of reactive groups onto its surface consisting of reactive hydroxyl (—OH) and amine (—NH₂) groups. This self-functionality causes CS to be readily modified using different ligands, thus it easily adsorbs heavy metal ions and also couples with biomolecules [1, 3]. Although CS has been abundantly considered in researches for preparing membranes for adsorptive separation purposes, the use of pure CS membranes has been severely limited due to their poor mechanical strength and chemical stability in a variety of biological environments [8, 9].

Various studies have been carried out to improve the mechanical strength of CS membranes. Among them, the following methods have been mostly investigated: (1) preparation of composite membranes by coating CS on some polymeric supports such as polyethersulfone (PES) and cellulose membranes or cellulose filter paper [10–12],

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(2) blending CS with other polymers including cellulose acetate (CA) and poly(vinyl alcohol) (PVA) [7, 13–17], and (3) incorporation of nanoparticles into CS membranes [6, 18–20]. The coating method, however, has encountered some problems including detachment of the coated CS film or incomplete and nonuniform coverage of the support membranes. In contrast to coating, the blending CS with other polymers has been found to be an effective technique to overcome the shortcomings of CS. In adsorptive membranes prepared through blending, CS provides the functionalities required for adsorption of chemical substances, in which the blended polymer is considered as the membrane backbone. Since CS has modifiable functional groups, the stability of its blend enhances with other polymeric materials such as cellulose. To modify CS membrane’s mechanical strength, many researchers have worked on the blend of CS and CA [4, 17, 21]. Khalil et al. [22] studied CS/cellulose blend and reported that the stability of CS-based materials can be improved by blending them with other compatible biopolymers in particular cellulose. CA has natural and renewable characteristics as well as a superior film-forming ability and good mechanical strength to be used as the polymer matrix in fabrication of membranes. Also, the similar chemical structure of CA with that of CS provides the possibility for the formation of miscible blends with CS appropriately [7, 22].

In addition to CS blending with other polymers, incorporation of nanoparticles into the membrane substrates is also a common method for mechanical improvement as far as the development of biohybrid membranes based on CS, which has become of great interest [23]. The amino and hydroxyl functional groups present on CS molecular chains can be linked chemically or physically to inorganic reinforcing networks and trap them strongly in the hybrid preparation [24]. Different types of inorganic fillers such as hydroxyapatite (HAP) [25], calcium phosphate cements [26], and clay [27] as well as some nanoparticles including carbon nanotubes (CNTs) [18], titanium dioxide (TiO2) [21, 28, 29], zinc oxide (ZnO) [30], and alumina (Al2O3) [6, 31] have been frequently used for reinforcing the CS matrix. For example, Salehi et al. [6] used CNTs to modify CS-PVA porous membranes in heavy metal ions adsorption. Wang et al. [18] reported that by the addition of only 0.8 wt% of multiwalled CNTs (MWCNTs), the Young’s modulus, and tensile strength of the CS-based nanocomposites greatly increased up to 93% and 99%, respectively compared to the composites consisting of neat CS. Moreover, Al-Sagheer and Merchant [32] showed that by dispersing TiO2 in the CS matrix, glass transition temperature (T_g) and Young’s modulus of the matrix could be improved. Similarly, Al-Sagheer and Muslim [24] prepared CS-silica (SiO2) hybrids through sol-gel process, exhibiting that the thermal and mechanical properties of the samples were improved by adding SiO2 as a reinforcing inorganic filler in the CS matrix with respect to the pure ones. TiO2 has been chosen extensively as a reinforcing filler due to its low price, chemical stability, antibacterial properties, etc. [23, 32]. TiO2 nanoparticles are used in human food, drugs, cosmetics, and food contact materials owing to nontoxicity based on American Food and Drug Administration (FDA) approval [33]. CS-TiO2 complex film has been reported due to its good surface properties and antibacterial activities [33, 34]. Moreover, it was shown that combining CS with TiO2 nanoparticles into composites could result in films and hydrogel materials with increased tensile strength [32, 35]. Archana et al. observed that there were significant differences in tensile strengths of CS-poly(N-vinylpyrrolidone) (CS-PVP) films with and without TiO2 nanoparticles [33]. High polymer–particle interactions between CS and TiO2 nanoparticles resulted in positive effects on the CS based membrane matrix and enhancement of its mechanical strength owing to hydrogen bonding [32, 36].

Based on the mentioned studies, it can be concluded that a focus on CS-based hybrid systems in terms of blending with CA and TiO2 incorporation simultaneously has not been considered so far. Therefore, by taking into account the individual features of CS, CA, and TiO2 nanoparticles and their proper interaction, it seems that the combination of them can result in a promising material for fabrication of adsorptive CS-based membranes.

For many years, dye ligands particularly Cibacron Blue F3G-A (CB), which can be used as affinity ligands, are the most widely used materials in protein separation and purification. In the literature, the dye affinity ligand CB has been applied for extensive purifications of a variety of enzymes, such as kinases, phosphatases, and dehydrogenases and used for albumin separation and purification from various sources including human serum [37–40]. CB is an inexpensive commercially available dye ligand with a good stability and group specificity which can be easily immobilized in matrices, especially bearing hydroxyl and amine groups (such as CS) [37, 41]. In many studies, CB has been employed as ligand affinity and immobilized in different matrices to isolate and remove albumin [42–44].

The aim of this research is to prepare CS-based nanocomposite membranes with a desirable mechanical strength and highly immobilized dye ligand content. In order to improve the chemical and mechanical resistances of these membrane samples, CS is blended with CA by the addition of TiO2 nanoparticles to fabricate a biohybrid membrane of CS/CA/TiO2 via solvent evaporation method. Accordingly, the following four main steps are considered: (1) fabrication of neat CS, CS/CA (50/50) blended membranes, CS/TiO2, and CS/CA/TiO2 nanocomposite membranes, (2) comparison and investigation of the physical, mechanical, and thermal properties of the prepared membrane samples using a series of tests in terms of SEM, water contact angle (WCA), permeability, porosity, tensile test, thermogravimetric analysis (TGA), and DSC, (3) preparation of affinity adsorptive CS-based membranes.
nanocomposite membranes via immobilization of Cibacron Blue F3G-A (CB) onto the surface of the prepared membrane samples, and (4) measurement of the dye ligand content on the prepared membrane samples via elemental analysis to evaluate their potential use as dye affinity adsorptive membranes.

EXPERIMENTAL

Materials

Chitosan (CS) (medium molecular weight, degree of deacetylation 85%–90% and viscosity 200 cP for the solution concentration 0.5 wt% in acetic acid 0.5% at 20 °C) was obtained from Chitotech Co., Iran, and used as received. Cellulose acetate (CA) (biodegradable, molecular weight 37 kDa and acetyl content 40%) was purchased from Sigma-Aldrich Co., Pilsburg, The Netherlands. Polyethylene glycol (PEG) (porogen, with a molecular weight of 20 kDa) was provided from Merck Co., Germany. Titanium dioxide (TiO2) nanoparticles (AEROSIL, EVONIK Industries. Cibacron Blue F3G-A (CB) (a dye affinity ligand) was purchased from Sigma-Aldrich Co., Pilsburg, The Netherlands (Scheme 1). All the other chemicals were analytical reagent grades and used without further purification.

Samples Preparation

Preparation of Porous CS/CA Blend Membranes.

Weighed CS powder was dissolved in acetic acid solution (60 wt%) and stirred at 60°C for 2 h to obtain a homogenous polymer solution with a concentration of 5 wt%. Then, weighed CA powder was added to the CS solution and stirred for 3 h to attain the CS/CA blend solutions with two ratios of 50/50 and 50/100 (weight by weight). Subsequently, the predetermined weight of PEG powder (PEG/CS ratio of 100/50) as a porogen, was incorporated in the blend mixtures for producing CS/CA-based membrane samples with microporous structures [45]. Subsequently, the resulting polymeric solutions were degassed in an ultrasonic bath during 1 h and then allowed to be steady for 12 h for air bubble removal, whereby uniform solutions were obtained. The prepared solutions were casted in a Petri dish (10 cm in diameter) until the solvent was evaporated completely at room temperature for 48 h. Moreover, in order to prepare the porous CS/CA membranes, the CS/CA/PEG casting film samples were immersed into sodium hydroxide (NaOH) solution (1 M) for 24 h until dissolution of PEG molecules. Eventually, the porous membrane samples were created. To increase the chemical resistance of these prepared membranes, an epichlorohydrin (ECH) solution was used as a crosslinker. Regarding crosslinking process, 1 g of each wet CS-based membrane was immersed into the ECH solution (0.01 M prepared in 0.067 M of NaOH solution at 60°C) under continuous agitation for 2 h [46]. After this treatment, the blend membrane samples were rinsed three times with deionized water to remove unreacted ECH.

Preparation of CS-Based Nanocomposite Membranes.

According to the procedure for the preparation of CS/CA blend membranes, the fabrication of CS-based nanocomposite membranes was formulated by the addition of TiO2 nanoparticles in the preliminary blend solution. Briefly, weighed TiO2 nanoparticles powder was well-dispersed in acetic acid solution (60 wt%) with a ultrasonic bath for 3 h at 60°C to attain homogenous mixtures (TiO2 concentrations of 1.5% and 3% per CS weight). Then, the CS and CA were slowly added little by little to the mixtures, under constant stirring. The other steps were the same as those mentioned in the previous section. The CS/TiO2 nanocomposite membrane was also prepared. The prepared nanocomposite membranes were measured 540 ± 14 μm in thickness.

Preparation of Dye Affinity Adsorptive CS-Based Nanocomposite Membranes.

CB was immobilized in the surfaces of samples as dye affinity ligand to evaluate their possible performance with regards to biosorption process. CB was covalently coupled on the membranes’ surfaces through nucleophilic reaction involving –Cl atoms within CB chemical structure and hydroxyl/amine groups (–OH/–NH2) onto the surfaces of CS-based membranes. To facilitate this reaction, it was conducted at a high temperature (80°C) under basic condition [0.2 M of sodium carbonate (Na2CO3)]. Specifically, the dye solution with a concentration of 1 mg mL–1 was first prepared and then the samples’ pieces were immersed into 20 mL of the dye solution, then the mixture was shaken at 60°C for 30 min. Thereafter, sodium chloride (NaCl) (1 M) was added to salt out the dye. This procedure caused the dye molecules to deposit as a solid layer.
on the samples’ surfaces. After 30 min, the solution temperature was increased up to 80°C and then a few amounts of Na₂CO₃ (0.2 M) were added dropwise to the suspensions, where the pH of the medium increased up to 10 owing to acceleration of the covalent coupling reaction of the dye [7]. The coupling reaction was allowed for 2 h and finally the dye-functionalized samples were successfully washed using a hot mixture consisting of water/methanol/NaCl (2 M)/potassium hydrogen phosphate (K₂HPO₄) at pH 7 until no dye could be detected for the prevention of any dye leakage. The resulting samples functionalized with CB dye were stored in a deionized water containing 0.02 wt% of sodium azide (NaN₃) at 4°C for further evaluation.

Characterization

Scanning Electron Microscopy Studies. In general, the morphological observations of CS-based membrane and nanocomposite membrane samples in two aspects of surface and cross-sectional area were investigated. In this way, the micrograph images were taken by using a scanning electron microscopy (SEM) (model XL-30, Philips, The Netherlands). Prior to SEM test, a small piece of each sample (with dense and porous structures) was cut and coated with a thin layer of gold to attain a high resolution. On the other hand, the samples were cryo-fractured under liquid nitrogen in order to obtain a sharp and brittle-fractured surface for better observation of the cross-sectional areas. Also, the SEM device was equipped with energy dispersive X-ray (EDX) accessory and was used to analyze quantitatively the element compositions onto the surfaces of the prepared nanocomposite membrane samples.

Porosity Measurements. Since the CS-based samples were prepared for using them potentially bioadsorptive applications, the amount of porosity which has a proportional relation to available active sites was measured. In this study, the relative porosities of the membrane samples based on CS and CS/CA blends in the presence and absence of TiO₂ nanoparticles were calculated by using Eq. 1 as follows [47]:

\[
e = \left(1 - \frac{\rho_d}{\rho_p}\right) \times 100, \tag{1}
\]

where “e” is the porosity, and “ρₚ” and “ρₐ” are the densities of porous and dense membrane samples, respectively. The amount of “ρₚ” was calculated by the division of “W” as the dry weight at 50°C to “Vₚ” as the volume of the porous membrane samples. The volume of membrane samples (Vₚ) was calculated according to Eq. 2 as follows:

\[
V_p = a \times b \times z, \tag{2}
\]

where a, b, and z are the length, width, and thickness of the cut piece of samples (mm), respectively. Also, ρₚ was obtained by the same method calculated ρₚ with replacing the porous membrane with the dense membrane samples. For minimizing the errors and calculating the standard deviation (SD) of data, the number of five samples were used for each dense and porous membrane sample.

WCA Measurements. WCA values of the surfaces of membrane samples based on CS and CS/CA blends with and without TiO₂ nanoparticles were measured by using a sessile drop method by means of an automatic contact angle meter (model G10, Kruss, Germany) to determine the extent of surface hydrophilicity of the samples. Therefore, a 0.5 to 1 μL drop of pure water was placed on the dried membranes’ surfaces with a microsyringe, and the WCA value between the air–water and surface of each sample was measured within 6 s. The WCA measurements were performed in triplicate using separate pieces of the membranes.

Water Permeability. The flux measurements of pure water through the membranes were carried out in a dead-end system with a 100 mL stirred filtration cell. The surface area of membrane in the filtration cell was 7.5 cm². The required pressure was supplied by a nitrogen gas cylinder. Permeate was collected and weighed during the experiments by an analytical balance which connected to a computer to save the data.

Fourier Transform Infrared Spectroscopy Analysis. For qualitative investigations regarding the extent of miscibility and different groups’ interactions probably happen between CA and CS in the blend membrane samples with and without TiO₂ nanoparticles into the CS matrix, an attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy (model EQUINOX 55, Bruker) was utilized. The ATR-FTIR spectra of neat CS, CA/CS membranes and CA/CS/TiO₂ nanocomposite membranes were investigated in the wavenumbers ranging from 400 to 4,000 cm⁻¹ with a resolution of 4 cm⁻¹.

Tensile Test. Mechanical properties of the samples based on CS were measured according to ASTM D 882 by using a tensile universal machine (model 5566, Instron, UK) at room temperature and relative humidity of 55% with a crosshead speed of 5 mm min⁻¹. The samples were cut into dumbbell-shaped strips with 60 mm × 10 mm dimensions and five strips were measured for each sample.

Thermogravimetric Analysis. TGA was performed using an apparatus (model Q500, TA Instruments) with a simultaneous utilization of derivative TGA (DTGA) thermal analyzer system. For all measurements, a precise weighed membrane samples (5 mg) was put on an aluminum pan under a dynamic nitrogen atmosphere with a flow rate of 100 mL min⁻¹ and then the chamber temperature was increased from ambient to 600°C with a heating
rate of $10^\circ C \text{min}^{-1}$. For each degradation or weight loss step for the samples, the temperature at which the degradation starts ($T_{\text{onset}}$), temperature at maximum degradation rate ($T_{\text{max}}$), and percentage of weight loss ($W\%$) were calculated from TGA and DTGA curves.

**Differential Scanning Calorimetry Analysis.** DSC analysis was performed with an apparatus (Model Q2000, TA Instruments, DuPont Co.) equipped with a liquid nitrogen cooling system. After a complete drying of the membrane samples, 15 mg of each sample was precisely weighed and put on an aluminum pan, then the sample was heated from room temperature to 400°C with a heating rate of $10^\circ C \text{min}^{-1}$ under 4 mL min$^{-1}$ of nitrogen flow rate. All the represented DSC thermograms have been obtained from first heating of the prepared membrane samples.

**Elemental Analysis.** The amount of CB immobilized in the prepared CS-based membrane samples was measured by using an elemental analysis instrument (Eager 300, Thermo Finnigan) by considering the sulfur stoichiometry. The elemental analysis results were obtained based on the weight of sulfur (S) per 100 weight unit of the samples. Accordingly, in order to measure the immobilized CB content in the samples, the obtained values from the instrument were divided to molecular weight of sulfur (32 g mol$^{-1}$) to calculate the amount of sulfur molar content consumed; subsequently, the one third of resulting values were considered as the answer because each CB molecule contains three sulfur elements (see Scheme 1) to obtain the dye ligand content onto the surfaces of samples with unit of $\mu$mol g$^{-1}$.

**Statistical Analysis.** It has been tried to consider minimum three replications to represent the data throughout the experiments for the samples. Comparison between the prepared CS-based membrane and nanocomposite samples was conducted in Sigma Plot 11.0 software. Statistical analyses were performed for the ultimate tensile strength, the elongation at break point and the immobilized CB content results using one-way analysis of variance (ANOVA) ($P < 0.05$). In order to determine which results have a meaningful difference with others, least significant difference (LSD) test with a 95% confidence interval was conducted for every binary combination of the prepared samples.

**RESULTS AND DISCUSSION**

**Blending CS With Both CA and TiO$_2$ Nanoparticles in the Presence of PEG and ECH**

Despite some reports on the common films and nanocomposites based on CS, the simultaneous use of CA biopolymer and TiO$_2$ nanoparticles blended with CS matrix has not been studied to investigate their characteristics especially the mechanical properties and dye ligand content for potential use as an affinity adsorptive membrane. The use of ECH as crosslinker and PEG as porogen in the CS-based membrane samples was also considered in this work for achieving the chemical resistance and porosity formation. In this way, ECH could easily react with both $\text{NH}_2$ and $\text{OH}$ functional groups on the CS-based membranes. Moreover, the $\text{NH}_2$ groups within CS molecular structure have a greater activity compared to $\text{OH}$ groups, which is related to their electronegativity; however, in the presence of NaOH solution, the $\text{OH}$ groups are allowed to react with ECH by attacking its chlorine groups [11, 22, 46]; as a result, crosslinking between the hydroxyl groups in the CS molecules (and also in the CA molecules) occurs by ECH. On the basis of this reaction, the active $\text{NH}_2$ groups have still remained for a later coupling reaction with other molecules such as the dye ligand. To improve the mechanical strength of CS-based membranes for biosorption applications, blending CS with CA was carried out along with incorporation of TiO$_2$ nanoparticles as a reinforcing filler to fabricate the nanocomposite membranes of interest. Accordingly, the CS-based samples have the following composition: CS films (without porogen), CS membranes (PEG/CS = 2), blended CS/CA membranes (PEG/CS = 100/50, CS/CA = 50/50 and 50/100), CS nanocomposite membranes (PEG/CS = 100/50, TiO$_2$ = 1.5 and 3 wt% of CS), CS/CA blend nanocomposite membranes (PEG/CS = 100/50, CS/CA = 50/50 and 50/100, TiO$_2$ = 1.5 and 3 wt% of CS). To fabricate the samples with high adsorption capacity, preferably the maximum CS concentration of 5 wt% was used; the higher concentration led to a considerable increase in the viscosity of CS in acetic acid solution, preventing the CS solution from membrane formation. Moreover, TiO$_2$ nanoparticles with two concentrations of 1.5 and 3 wt% were considered to avoid rising of the solution viscosity, leading to an appropriate homogeneity with the casting solution. TiO$_2$ nanoparticles with amount of higher than 3 wt% resulted in an increase in the viscosity of blend solutions which caused the formation of nonuniform membranes [23].

**Morphology Observations**

The SEM micrograph images of the neat CS film, the neat CS membrane, CS/TiO$_2$, and CS/CA/TiO$_2$ nanocomposite membrane samples are illustrated in Fig. 1 from their cross-sectional areas. As can be observed, all the samples showed a symmetric structure in the cross-sectional fraction because of their processing technique. In general, the neat CS film has a dense structure, while the neat CS membrane possesses a porous structure. The weak interactions between the CS chains and PEG molecules cause the porogen to be easily extracted [48] and thus, a porous structure is generated. This will result in an increase in the internal capacity and specific surface area of the CS-based membranes for adsorption.
applications [45]. As can be deduced from Fig. 1, although the addition of TiO$_2$ nanoparticles to the CS membrane sample led to the formation of a homogenous structure owing to the strong interactions between CS chains and TiO$_2$, a few TiO$_2$ agglomerations were formed in the membrane matrices. The cross-sectional micrographs of the CS/CA/TiO$_2$ nanocomposite membrane showed a rough interlinked network. It seems that incorporation of both CA and TiO$_2$ nanoparticles into the CS membrane solution resulted in such structure due to the interactions of CS, CA, and TiO$_2$ nanoparticles which could lead to the transformation of inorganic/organic blend to a material with an interlinked network structure. The same observation was reported by Li et al. [49] for morphology structure of CA/ZnO nanocomposite membranes with interlinked network and loose nodule structures.

Figure 2 depicts the SEM micrograph images from the surfaces of the neat CS membrane and CS/TiO$_2$ as well as CS/CA/TiO$_2$ nanocomposite along with EDX patterns of CS/TiO$_2$ and CS/CA/TiO$_2$ nanocomposite membrane samples. As is evident in this figure, the surface of CS/TiO$_2$ sample was smoother than that of the CS membrane and CS/CA/TiO$_2$ nanocomposite membrane samples. The similar results were reported by Li et al. [49] for PES/TiO$_2$ nanocomposite membranes, where the surface became smoother by the addition of nanoparticles. Also, it seems that the CS/CA/TiO$_2$ sample has the roughest surface which might be due to the rough interlinked network structure of its matrix. As is shown in EDX patterns, they revealed presence of the Ti element in CS/TiO$_2$ and CS/CA/TiO$_2$ nanocomposite membranes. The two energy levels of this element namely Ti (L$_\alpha$) and Ti (K$_\alpha$) have been detected on the surfaces of the samples as the peak intensity of the CA/CS/TiO$_2$ nanocomposite membrane was 20 times larger than that of CS/TiO$_2$ sample, which was associated with the migration of the nanoparticles from the bulk to the surface and some TiO$_2$ nanoparticles agglomerates formation followed by greater roughness [50].

**ATR-FTIR Analysis**

In order to investigate the CS and CA blending and also their interactions with TiO$_2$ nanoparticles through CS-based membrane and nanocomposite samples, FTIR
technique was utilized. Figure 3 indicates the FTIR characteristic peaks of the prepared samples including the CS membrane and also other CS-based membrane samples blended with CA and/or TiO₂ nanoparticles within the wavenumber range of 600–4,000 cm⁻¹. In this figure, the spectra of membrane samples containing 3 wt% of TiO₂ have not been shown because of their negligible differences compared to the peak intensities of those samples with 1.5 wt% TiO₂ content. Typically, the main peak around 3,450 cm⁻¹ was assigned to AOH bands overlapping with the stretching of primary amine through the carbohydrate ring of CS structure [51]. This band was found to shift at a lower frequency especially for blended CS with other polymers and nanocomposites because of the increase in either H-bonding in the matrix or the hydrated structure of CS structure which might indicate the interaction of —OH groups on CA with NH₂ or OH groups on CS [52]. Also, the peak at 2,900 cm⁻¹ in CS spectra was corresponded to the stretching carbon-hydrogen (C–H), while the strong vibrations at 1,650 and 1,600 cm⁻¹ were related to the stretching amide types I and II, respectively. The peaks at 1,420 cm⁻¹ and 1,050 cm⁻¹ were corresponded to the flexural vibration of CH₂ and stretching vibration of C—O, respectively [9, 33, 48]. The type of hydrogen bonding within CS, CA, and TiO₂ nanoparticles might be complicated due to several groups that could form hydrogen bonds in CS. The chemical structure of CS contains amine, residual amide, hydroxyl groups which can form hydrogen bonds with the hydroxyl groups of CA, and TiO₂ nanoparticles. Therefore, the evidence of hydrogen-bonding interactions can be found from the change of amide carbonyl band at 1,645 cm⁻¹ [48]. Comparing the FTIR spectra of CS and CS/CA blending membranes for investigation of interaction between CS and CA revealed the shift of carbonyl band at 1,645 cm⁻¹ for CS membrane sample to 1,641 cm⁻¹ for CS/CA membrane sample, indicating the

FIG. 2. The SEM micrograph images of the surfaces of the neat CS membrane, CS/TiO₂ (1.5 wt%), and CS/CA/TiO₂ (1.5 wt%) nanocomposite membrane samples (the scale bars are 5 and 50 microns) along with the EDX patterns.

FIG. 3. The ATR-FTIR spectra of the prepared CS-based membrane and nanocomposite membrane samples consisting of CA and TiO₂ nanoparticles. [Color figure can be viewed at wileyonlinelibrary.com]
hydrogen bonding between CS and CA. Moreover, the intensity of $\text{OH}$, $3'\text{OH}$, and $5'\text{OH}$ groups (at 3,350, 1,030, and 1,080 cm$^{-1}$, respectively) of CS/CA samples became markedly sharper than that of CS samples due to existence of these groups in CA molecule chains [7]. Compared with CS membrane, a new broad absorption band around 900 cm$^{-1}$ could be found in the FTIR spectra of CS/TiO$_2$ and CS/CA/TiO$_2$ nanocomposite membranes, which could be ascribed to evidence of interphase compatibility in the nanocomposite membrane samples showing the interaction of Ti Lewis sites with the $-\text{NH}_2$ groups from CS chain (asymmetric stretching mode of Ti$\equiv$O) [32, 51]. Also the shift of amid carbonyl band at 1,645 cm$^{-1}$ for CS to 1,641 cm$^{-1}$ and 1,640 cm$^{-1}$ for CS/TiO$_2$ (1.5%) and CS/CA/TiO$_2$ (1.5%), respectively, indicated hydrogen interaction between TiO$_2$ nanoparticles and NH$_2$ and OH groups of CS [32].

### Porosity, WCA, and Pure Water Flux Measurements

The porosity, WCA, and pure water flux measurements of the samples including CS and CS/CA membranes and also CS and CS/CA (50/50 and 50/100) nanocomposite membranes with two different concentrations of TiO$_2$ nanoparticles (1.5 and 3 wt%) were studied and their results represented in Table 1. All the samples have a porosity in the range between 61% and 76%, suggesting enough physical active sites for adsorption purposes. Considering porosity percentage of the samples, it could be observed that the CS-based membrane samples include of CA (with both ratio of 50/50 and 50/100) showed lower porosity compared to the neat CS membrane sample and further addition of TiO$_2$ did not seem to have any significant effect on the porosity. The porosity of 74.3% for the neat CS was reduced to 61.86%, 61.65%, and 61.51%, for (50/50) CS/CA, (50/50) CS/CA/TiO$_2$ (1.5%), and (50/50) CS/CA/TiO$_2$ (3%), respectively (similar trends have been obtained for the membrane samples with CS/CA ratio of 50/100). Also it is indicated that although based on the SEM micrographs, the CS/CA-base membrane samples showed a loose nodule structure with microvoids, the CS/TiO$_2$ nanocomposite membrane samples had a higher porosity. This might be due to the increase of casting solution viscosity after CA blending, causing reduction of solvent evaporation rate and PEG removal, leading to lower porosity in the bulk of the membranes [45]. Moreover, it might be attributed to the electrostatic interaction between $-\text{NH}$ groups of CS and $-\text{OH}$ groups of CA leaving less room for pore formation [52]. On the other hand, it seemed that addition of TiO$_2$ nanoparticles did not bring about significant effects on the porosities of CS-based membrane samples because of probably its less influence on the viscosity increase of the casting solution in comparison with CA addition.

WCA is an appropriate criterion for hydrophilicity/hydrophobicity of the membrane surfaces. In this regard, the WCA amounts decreased from 60° in the neat CS membrane to 43.2° and 39.32° corresponding to CS/CA blended samples with the ratios of 50/50 and 50/100, respectively. Consequently, blending CS with CA significantly enhanced the hydrophilicity of the CS membrane. For CS nanocomposite membranes containing 1.5 wt% and 3 wt% of TiO$_2$ nanoparticles, WCAs were 35.6° and 23.6°, respectively showing the effect of nanoparticle incorporation on elevation of hydrophilicity of the neat CS membrane. This trend was also observed in CS/CA membrane after incorporation of TiO$_2$ nanoparticles from 43.2° to 32.5° and 24.5° for 1.5 wt% and 3 wt%, respectively. This significant reduction in the WCA degree clearly indicated the development of hydrophilic surfaces for the membrane samples using TiO$_2$ nanoparticles [53, 54]. The exposed hydroxyl groups at the surface of TiO$_2$ nanoparticles with high specific surface area and surface energy actually results in increase of the hydrophilicity of the prepared nanocomposite membranes [55].

Predictably, lower WCA values resulted in a higher hydrophilicity of the samples containing TiO$_2$ nanoparticles, which allowed to have promising materials with lower nonspecific adsorption used for potential biosorption processes.

The pure water flux content of membranes is associated with some major factors including bulk porosity, morphology, hydrophilicity, etc. Hence, the addition of CA and TiO$_2$ nanoparticles to the CS-based membrane...
could influence the pure water flux via the following alternatives: on the one hand, CA and TiO₂ nanoparticles might make the membrane more hydrophilic, which could enhance the pure water flux, while on the other hand, the permeation properties could be affected by addition of CA and TiO₂ nanoparticles, exerting its influence through the morphology of the membrane. The results of pure water flux of the prepared CS-based membranes are shown in Table 1. In general, all the CS-based membranes treated with CA and TiO₂ nanoparticles have higher water flux than neat CS membrane, which could be attributed to greater hydrophilic properties of them compared to CS membrane without treatment. The (50/100) CS/CA/TiO₂ (1.5 wt%) nanocomposite membrane showed the highest water flux (~233 L/m²·h) among other prepared membranes. With regards to (50/50) and (50/100) CS/CA blended nanocomposite membranes, it was observed that by increasing TiO₂ nanoparticles from 1.5 to 3 wt%, a decrease was seen in pure water flux from ~140.87 to ~116.15 L/m²·h and ~233 to 180 L/m²·h, respectively. It can be said that the growth of the solution viscosity of the CS/CA/TiO₂ blend system due to TiO₂ incorporation [56] resulted in more compact and rough interlinked network structure of the membrane. This caused higher resistivity of PEG molecules extraction to form micro interconnected pores within the membrane matrices, which led to water flux decrease. Similar findings were reported by Salehi and Madaeni for CS/PVA membranes [45]. For CS/TiO₂ blend system the increase of TiO₂ did not increase the viscosity of the casting solution so that to prevent the easy extraction of PEG molecules. Therefore, the extraction of the porogen led to formation of interconnected pores within the CS/TiO₂ membrane. Moreover, more amount of TiO₂ nanoparticles effectively hydrophilized the CS/TiO₂ (3%) membrane surface and resulted in the flux enhancement.

Mechanical Properties of the Samples

To investigate the influence of blending CA and TiO₂ nanoparticles incorporation into the CS-based membrane matrix on the mechanical properties especially ultimate tensile strength, a series of samples such as CS and CS/CA (50/50) blend membranes as well as their nanocomposite membranes containing 1.5 and 3 wt% TiO₂ nanoparticles and CS/CA (50/100) containing TiO₂ (1.5 wt%) were examined via tensile test. Figure 4 and Table 2 represent the stress–strain curves and their numerical values of those samples, respectively. The stress–strain curves exhibited that all the samples have higher ultimate tensile strength values than the neat CS membrane. Interestingly, the weak tensile strength of CS membrane could be increased from 1.43 to 12.95 MPa by adding 1.5 wt% TiO₂ nanoparticles. The same trends were observed for Young’s modulus and elongation at break increasing from 13.55 to 29.83 MPa and 10.55% to 43.9%, respectively. Also, similar results have been found in the literature, suggesting elevation of the mechanical properties of CS composite samples by the addition of TiO₂ [32, 35, 57]. For example, Archana et al. [33] reported that TiO₂ nanoparticles allowed for increasing the ultimate tensile strength of CS-PVP-based wound dressing materials. This was attributed to the suitable distribution of the TiO₂ particles in the CS matrix because of the interfacial interactions between the basic amine sites (–NH₂) in the biopolymer and the Lewis acidic sites present in the TiO₂ [32]. Moreover, the –OH and –NH₂ groups of the CS molecule chains could form intramolecular and intermolecular hydrogen bonds, seriously hindering the rotation and movement of the molecular chain. When TiO₂ nanoparticles were introduced, the intermolecular hydrogen bonds were weakened and new hydrogen bonds were formed between CS and TiO₂, which made the rotation and movement of molecular chain easier. On the other hand, with the increase of incorporating amount of TiO₂ nanoparticles from 1.5 to 3 wt%, a ductile-brittle transition in the CS nanocomposite membranes occurred where a significant decrease was observed in the ultimate tensile strength (P = 0.027) and elongation at break (P < 0.001). The same report was released by Li et al., for CS-ZnO nanocomposite membranes [30].

Although blending CS with CA resulted in a slight increase in the ultimate tensile strength of the samples, a significant decline happened in the elongation at break point owing to the higher brittle property of CA than that of CS. According to Table 2, by the addition of TiO₂ nanoparticles with the amount of 1.5 wt% to CS/CA (50/50) membrane sample, the ultimate tensile strength, Young’s modulus, and elongation at break favorably increased from 1.73 MPa, 29.83 MPa, and 5.8% to 9.1 MPa, 50.52 MPa, and 18%, respectively. To assess the reinforcement role of CA in the blended nanocomposite membranes, tensile properties were measured for the nanocomposite sample including CS/CA with ratio of 50/
100 (TiO$_2$ = 1.5 wt%). Increasing CA content by twice as large as CS content caused increased Young’s modulus from 50.52 to 60.31 MPa ($P < 0.001$) and elongation at break decline from 18% to 14.16% ($P < 0.001$) compared to (50/50) CS/CA/TiO$_2$ (1.5%); however, this blend ratio has no significant effect on the ultimate tensile strength ($P > 0.05$). Young’s modulus, or in other words, elastic modulus is one of the most important mechanical descriptors of a material and can be used as an index for material stiffness. Therefore, as represented in Table 2, the nanocomposite blended samples including (50/50) CS/CA/TiO$_2$ (1.5%), (50/50) CS/CA/TiO$_2$ (3%), and (50/100) CS/CA/TiO$_2$ (1.5%) showed more than a threefold increase in the stiffness compared to the neat CS membrane sample. Consequently, it revealed that blending CS with CA and incorporation of TiO$_2$ nanoparticles into them could be considered as two simultaneous effective solutions for improvement of stiffness factor in such a prepared membrane.

**Thermal Properties**

**TGA Studies.** In order to investigate the thermal properties of the blended and nanocomposite CS-based membrane samples, thermal gravimetric analysis was used. TGA patterns followed corresponding DTGA curves of the membrane samples including neat CS membrane, CS/TiO$_2$ (1.5 wt%) nanocomposite membrane, (50/50) CS/CA membrane, and (50/50) CS/CA/TiO$_2$ (1.5 wt%) nanocomposite membranes, at temperature in the range of 25°C–600°C have been demonstrated in Fig. 5a–d. All the samples showed two distinct weight losses, with the first one observed at around 100°C, owing to the vaporization of absorbed water and residual solvent through the samples [58, 59]. The second weight loss area, for the neat CS, started at 273°C and reached a maximum at 350°C with a weight loss of 71.4% which corresponded to glucosamine chains decomposition, vaporization, and elimination of volatile products [60]. The second weight loss occurred within the temperature range of 250°C to 350°C which is the region of major weight loss for the CS/CA, CS/TiO$_2$, and CS/CA/TiO$_2$ membrane samples, related to the thermal and oxidative decomposition of the polymeric (CS and CA) membrane matrices [13, 61, 62]. Therefore, the related rapid weight loss of about 63.6%, 69.4%, and 62.7% were obtained for the mentioned samples, respectively.

Furthermore, with a closer observation of the DTGA curves in Fig. 5a–d, it can be seen that the neat CS membrane sample DTGA revealed a single degradation peak at the temperature range of 220°C–370°C, but the other three samples of CS/CA, CS/TiO$_2$, and CS/CA/TiO$_2$ indicated a doublet degradation peak in this temperature range in their corresponding DTGA curves. The neat CS and CS/TiO$_2$ membranes revealed a thermal event at the maximum temperature of 293°C and 297°C, respectively, associated with the depolymerization of CS chains. Moreover, this degradation profile of CS is in good agreement with the results of Rotta et al. [63], that have detected a maximum degradation rate for CS film by DTGA at 307.3°C. Importantly, the observed small second peak at 284°C for CS/TiO$_2$ membrane sample might be attributed to the introduction of Ti-O network in the CS/TiO$_2$ nanocomposite membrane (based on the FTIR spectrum analysis). Additionally, the mentioned doublet peaks in the DTGA curves exhibited that blending with CA results in exhibition of two maximum degradation rates within the temperature range of 220°C–370°C for CS/CA (at 297°C and 327°C) and CS/CA/TiO$_2$ (at 300°C and 334°C) membrane samples, regarding decomposition of COO$^-$ from the polysaccharides and CS and CA polymeric chains [64]. The same results were reported by Weng et al. [61], for cellulose/CS nanofiltration membranes with a temperature of the maximum degradation rate at 293.3°C and 347.9°C due to CS and CA decomposition, respectively. The results of TGA and DTGA curves are also represented in Table 3. As it is evident, at the first degradation temperature range ($T_{dl}$), the $T_{max}$ of CS/CA and CS/CA/TiO$_2$ membrane samples were decreased compared to the neat CS membrane. Such differences could be attributed to the degree of hydrophilicity and porosity of the various samples. According to the results of WCA and porosity in Table 1, although the CS/CA and CS/CA/TiO$_2$ samples were more hydrophilic than the neat CS, leading to higher interaction with the water molecules, their lower porosity resulted in lower free volume for water adsorption. On the other hand, for the CS/TiO$_2$ membrane sample, incorporation of TiO$_2$ nanoparticles had no a

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ultimate tensile strength (MPa)</th>
<th>Young’s modulus (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat CS membrane</td>
<td>$1.43 \pm 0.07^a$</td>
<td>$13.55 \pm 0.68$</td>
<td>$10.55 \pm 0.53$</td>
</tr>
<tr>
<td>(50/50) CS/CA membrane</td>
<td>$1.73 \pm 0.09^a$</td>
<td>$29.83 \pm 1.49^a$</td>
<td>$5.8 \pm 0.29$</td>
</tr>
<tr>
<td>CS/TiO$_2$ (1.5%) nanocomposite membrane</td>
<td>$12.95 \pm 0.65$</td>
<td>$29.49 \pm 1.47^b$</td>
<td>$43.9 \pm 2.19$</td>
</tr>
<tr>
<td>CS/TiO$_2$ (3%) nanocomposite membrane</td>
<td>$6.9 \pm 0.34^b$</td>
<td>$28.87 \pm 1.44^b$</td>
<td>$23.9 \pm 1.19$</td>
</tr>
<tr>
<td>(50/50) CS/CA/TiO$_2$ (1.5%) nanocomposite membrane</td>
<td>$9.1 \pm 0.45^c$</td>
<td>$50.52 \pm 2.52$</td>
<td>$18 \pm 0.9$</td>
</tr>
<tr>
<td>(50/50) CS/CA/TiO$_2$ (3%) nanocomposite membrane</td>
<td>$6.9 \pm 34^b$</td>
<td>$45.16 \pm 2.26$</td>
<td>$15.28 \pm 0.76^a$</td>
</tr>
<tr>
<td>(50/100) CS/CA/TiO$_2$ (1.5%) nanocomposite membrane</td>
<td>$8.54 \pm 0.43^c$</td>
<td>$60.31 \pm 3.02$</td>
<td>$14.16 \pm 0.71^a$</td>
</tr>
</tbody>
</table>

The same words in each column show that their corresponding data have no significant difference based on ANOVA ($P > 0.05$).
significant effect on $T_{\text{max}}$ compared to the neat CS. This could be interpreted in terms of lower hydrogen bonding interactions of the CS polymer chains with the water molecules owing to their interaction with the incorporated nanoparticles. At the second degradation temperature range ($T_{d2}$), the $T_{\text{onset}}$ values (the temperature at which degradation initiates) were 273°C, 271°C, 275°C, and 279°C for the neat CS, CS/TiO$_2$, CS/CA, and CS/CA/TiO$_2$ membrane samples, respectively. Comparing the $T_{\text{onset}}$ at this stage of DTGA curves for the samples, it could be concluded that blending CS with CA and also incorporation of TiO$_2$ have not influenced the thermal stability of CS-based membranes. Similar reports were published for CS/TiO$_2$ nanocomposite as CS/TiO$_2$ hybrid film [65] and CS/TiO$_2$ nanocomposite films for food packaging [51]. These results confirmed the presence of CA and TiO$_2$ in the CS matrix of the prepared membrane samples and their good miscibility thanks to molecular interaction and hydrogen binding [61, 65, 66]. The residual weight (ash content) of the CS and CS/CA membranes were 7.11% and 14.4%, respectively which could be attributed to the carbon residuals. Further, for the CS/TiO$_2$ and CS/CA/TiO$_2$ nanocomposite membranes, these values were 9.58% and 14.36%, respectively due to residuals of both carbon element and TiO$_2$ inorganic nanoparticles which have not burnt or undergone pyrolysis [60, 65].

**DSC Studies.** In this work, DSC was applied as an efficient tool to investigate the transition temperatures in terms of dehydration and decomposition temperatures of the prepared samples including neat CS and (50/50) CS/CA blend membranes as well as their nanocomposite membranes containing TiO$_2$ nanoparticles (1.5 wt%).

**TABLE 3.** TGA and DTGA results of the prepared CS-based membranes and nanocomposite CS-based membranes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>First degradation temperature ($T_{d1}$)</th>
<th>Second degradation temperature ($T_{d2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{onset}}$ (°C)</td>
<td>$T_{\text{max}}$ (°C)</td>
</tr>
<tr>
<td>Neat CS membrane</td>
<td>47</td>
<td>85.2</td>
</tr>
<tr>
<td>CS/TiO$_2$ (1.5%) nanocomposite membrane</td>
<td>49</td>
<td>85</td>
</tr>
<tr>
<td>(50/50) CS/CA membrane</td>
<td>44</td>
<td>72</td>
</tr>
<tr>
<td>(50/50) CS/CA/TiO$_2$ (1.5%) nanocomposite membrane</td>
<td>46</td>
<td>78</td>
</tr>
</tbody>
</table>
Figure 6 represents the DSC thermograms of these samples in two separate regions. It is known to all that most polysaccharides, for example, CS biopolymer do not melt but degrade upon heating, which undergo thermal decomposition prior to melting at high temperatures [9, 63, 67]. Thus no endothermic melting peaks are observed in the DSC curves of the membrane samples.

As can be seen from Fig. 6, all the samples showed broad endothermic peaks between ambient temperature and 270°C. The same results were represented by El-Hefian et al., [68], and by Ghaee et al., [69] for CS/PVA blended films and CS/CA composite membranes, respectively. Considering the CS structure which contains hydroxyl and amine groups, water molecules can interact with both mentioned polar groups in this macromolecule [58, 59]. Thus the prepared CS-based membrane samples inevitably contain a quantity of water which may act as plasticizer, affecting glass transition temperature (Tg) to lower values [70] and also may overlap with other thermal events like Tg [59, 71]. Therefore, the observed endothermic peaks of CS-based membranes, often identified as dehydration temperature (Td) due to the evaporation of residual water, are associated with the hydrophilic groups of the polymers [67]. Accordingly, neat CS, CS/CA, CS/TiO2, and CS/CA/TiO2 samples have Td of 128.5°C, 144.6°C, 134.7°C, and 125.5°C, respectively. Pierógi et al. [72], also observed an endothermic temperature between 130°C and 160°C from DSC thermograms of dual cross-linked CS hydrogels. The Td differences between the neat CS membrane and the other prepared CS-based membranes can be attributed to the degree of interactions between water molecules and the various samples. Regarding the literature, the interaction of water molecules with amine groups is weaker than with hydroxyl groups of CS membranes [58]. Consequently, the increase of Td for CS/CA and CS/TiO2 samples compared to the neat CS membrane might be related to greater hydrophilic characteristics of those samples leading to strong interactions of water molecules with the membrane matrices. On the other hand, after introduction of CA and TiO2 to the CS matrix, the number of NH2 groups decreased in the molecular structures of the membranes samples due to interactions with hydroxyl groups on CA chains and TiO2 nanoparticles, respectively [58, 65]. Therefore, some water molecules that would be bound to amine groups, they have now be bound to hydroxyl groups, resulting in water release at higher temperature (TD) for the CS/CA and CS/TiO2 membrane samples. In contrast to CS/CA and CS/TiO2 samples, the CS/CA/TiO2 nanocomposite membrane has a Td lower than CS sample, which could be attributed to the rough interlinked network of this sample compared to the others, leading to lower resistance for water evaporation. This is in good agreement with the results of Neto et al. [58], for CS-polyethylene oxide blended films. This kind of structure of CS/CA/TiO2 nanocomposite membrane sample was also confirmed by the SEM micrograph images. The sharp exothermic peaks of the samples were also observed in DSC thermograms of the samples. These exothermic peaks belonged to the temperatures at which the samples were decomposed. Clearly, the decomposition temperatures were observed at 293.2°C for the neat CS membrane and 302°C corresponding to the other samples containing CA and TiO2 nanoparticles [69]. On the other hand, the second sharp exothermic peaks appeared in the thermograms of the CS/TiO2, CS/CA, and CS/CA/TiO2 samples around 280°C which might be attributed to the physical and molecular structure changes of these polymers owing to the interactions of the molecular chains and among the components together [32, 48]. The observation of DSC thermograms of the membranes samples were in good agreement with the results of TGA curves.

The Immobilized CB Dye Ligand Contents in the Membrane Samples

As mentioned earlier, the aim of this work was preparation of an affinity adsorptive CS-based membrane with improved mechanical strength and a high CB F3G-A dye ligand content. Dye content is one of the several factors that affects the adsorption performance of affinity membranes [73, 74]. According to the measurement of elemental analysis assay, the CB contents immobilized in the CS-based membrane samples are shown in the diagram of Fig. 7. In Fig. 7 (down), the prepared CS-based membranes were categorized with regard to samples with different TiO2 nanoparticles contents, where in each category the ratio of CS/CA was also variable. As can be seen, the neat CS membrane revealed the highest CB content (77 μmol g−1 per membrane weight) compared to the other samples (P <0.001). It is observed that the addition of CA resulted in a slight reduction in the CB content of the CS-based membrane. This was attributed to lower porosities through the membrane samples after introduction of CA, which has been
proven and shown in Table 1. This phenomenon caused a decrease in the amounts of available active sites for immobilization of CB molecules. On the other hand, regarding the neat CS, CS/CA (50/50), and CS/CA (50/100), it was observed that by increasing the TiO2 nanoparticles content from 1.5 to 3 wt%, the immobilized CB contents diminished. This might be related to the formation of strong interactions between CS functional groups and TiO2 nanoparticles, resulting in diminished active site for CB immobilization in the CS-based nanocomposite membrane samples.

Considering the results of tensile test and immobilized CB F3G-A content measurements, the CS-based nanocomposite samples including 1.5 wt% TiO2 were the desirable prepared affinity membranes. CS/TiO2 (1.5%), (50/50) CS/CA/TiO2 (1.5%), and (50/100) CS/CA/TiO2 (1.5%) nanocomposite membrane samples provided satisfying mechanical stiffness as well as elevated immobilized CB F3G-A content. As shown in Fig. 7 (up), among these three samples, (50/50) CS/CA/TiO2 (1.5%) nanocomposite sample with higher immobilized CB F3G-A content (P < 0.001) than (50/100) CS/CA/TiO2 sample and higher Young’s modulus than CS/TiO2 (1.5%) sample would be a promising material to be used as a bioadsorptive membrane.

CONCLUSIONS

The CS/CA blend nanocomposite membrane samples containing TiO2 nanoparticles were successfully prepared used for potential bioadsorption applications. In this way, to overcome the shortcomings of neat CS membrane sample in terms of low mechanical and hydrophilic/hydrophobic properties, two different techniques including blending CS with CA as well as the addition of TiO2 nanoparticles were simultaneously applied. The SEM micrograph images showed symmetric and microporous structures of the samples. The EDX patterns revealed that the samples consisted of C, O, and Ti elements. The porosity measurements for all of the prepared nanocomposite membranes showed that they had a desired porous structure to provide enough active sites for the adsorption purpose. In addition, the WCA values showed an increase in the hydrophilicity of the blended nanocomposite membranes in relation to the neat CS samples suggesting that the blending of CS with CA and also the incorporation of TiO2 nanoparticles could improve the surface hydrophilicity of the samples. The mechanical and thermal studies emphasized that strong interactions occurred between CS, CA, and TiO2 nanoparticles. Moreover, the CS/TiO2 (1.5%), (50/50) CS/CA/TiO2 (1.5%), and (50/100) CS/CA/TiO2 (1.5%) nanocomposite membranes showed a remarkable improvement in ultimate tensile strength, with their values increased more than three times compared to the neat CS membrane sample. Finally, the elemental analysis of the samples indicated that addition of CA and TiO2 nanoparticles led to a slight reduction in the immobilized CB content with regards to the neat CS membrane. Regarding the aim of this research for preparation of an affinity CS-based membrane with improved mechanical strength for bioadsorptive application, considering the results of tensile test and immobilized CB F3G-A content measurement, the nanocomposite membrane sample denoted by CS/CA (50/50)/TiO2 (1.5%) was the selected sample and the promising material used for potentially bioadsorptive membrane.

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