**Cellulose Nanofibers Reinforced Sodium Alginate-Polyvinyl Alcohol
Hydrogels: Core-Shell Structure Formation and Property Characterization**

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Abstract

Core-shell structured hydrogels consisting of a flexible interpenetrating polymer network (IPN) core and a rigid semi-IPN shell were prepared through chemical crosslinking of polyvinyl alcohol (PVA) and sodium alginate (SA) with Ca^{2+} and glutaraldehyde. Short cellulose nanofibers (CNFs) extracted from energycane bagasse were incorporated in the hydrogel. The shell was micro-porous and the core was macro-porous. The hydrogels could be used in multiple adsorption–desorption cycles for dyes, and the maximum methyl blue adsorption capacity had a 10 % increase after incorporating CNFs. The homogeneous distribution of CNFs in PVA-SA matrix generated additional hydrogen bonds among the polymer molecular chains, resulting in enhanced density, viscoelasticity, and mechanical strength for the hydrogel. Specifically, the compressive strength of the hydrogel reached 79.5kPa, 3.2 times higher than that of neat hydrogel.

Keywords: Core-shell structure, Hydrogel, Interpenetrating polymer network, Nanoparticles, absorption

1 Introduction

Hydrogels, a network of polymer chains which can swell in aqueous solutions, hold a large amount of water, permeate to oxygen and nutrients, can be used in the fields of implantable artificial organs, drug delivery, cell encapsulation, biosensors, toxic ion removal and water purification (Cushing and Anseth, 2007; Thakur and Thakur, 2014b; Perez et al., 2015; Thakur and Thakur, 2015). However, most hydrogels are soft. Their low mechanical strength and variable dimensions with water content changes severely restrict their range of applications (Sun et al., 2012; Zhang et al., 2015). Increased attention has thus been paid to novel hydrogel architectures including core-shell and interpenetrating network (IPN) structures that offer improved mechanical performance and smaller swelling ratios (Yang et al., 2014; Zhang et al., 2015). In recent years, core-shell or IPN structured hydrogels have found use as biomaterials and adsorbents (Perez et al., 2015; Zhang et al., 2015). IPNs can be classified as semi-IPNs (SIPN) and full-IPNs. SIPNs are cross-linked linear polymer structures and IPNs consist of at least two polymers, in which one polymer network is cross-linked in the presence of the other polymer network (Dragan, 2014; Berrebi et al., 2015; Zhang et al., 2015). It has been shown that the IPN and SIPN structures can improve the mechanical performance and dimensional stability of functional hydrogels (Yang et al., 2014; Berrebi et al., 2015).

Sodium alginate (SA) is a linear polymer comprising 1,4-linked- β -D-mannuronic acid (M) and α -L-guluronic acid (G) units, combined in blocks of M-M, G-G and M-G (Lee and Mooney, 2012; Bidarra et al., 2014). Due to its aqueous-solubility, biocompatibility, non-toxicity, non-immunogenicity, biodegradability and acceptance by human body (Yang et al., 2014; Zhang et al., 2015), SA based core-shell structured polymers are particularly attractive in the textile

industry (as a base for dyes), foods, biomedicines, pharmaceuticals and encapsulation materials, such as drug, gene, cell, enzymes, and lipids delivery (Dalmoro et al., 2012a; Lee and Mooney, 2012; Bidarra et al., 2014; Dragan, 2014; Dalmoro et al., 2016). SA is hydrophilic, absorbing water quickly. Its -COOH and -OH groups participate in hydrogen bonding and van der Waals force; therefore, it is an excellent natural adsorbent and is commonly used for removal of water pollutants, such as heavy metal ions, dyes, fertilizers, microorganisms and enzymes (Aftab et al., 2014; Mohammed et al., 2016). At a pH level of 4, the maximum amounts of -COOH in G units are deprotonated to -COO^- , which permits facile cross-linking by multivalent cations, such as Ca^{2+} , to form SIPN by chelation (Sun et al., 2012; Bidarra et al., 2014; Dragan, 2014; Samanta and Ray, 2014). Usually, the SA-Ca^{2+} structure is formed only in the shells of hydrogels (Dragan, 2014). Compared to the rigid SA, PVA, with one -OH group in each repeating unit, is more flexible (Cho et al., 2009). Therefore, PVA can be blended with SA to improve the flexibility of SA matrixes. PVA/SA blends exhibit good compatibility in aqueous solutions (Cho et al., 2009), and they can be chemically crosslinked with glutaraldehyde (GA), a commonly used crosslinking agent, to form IPN (Jao et al., 2009).

Although core-shell structures can significantly enhance the toughness of hydrogels by three-dimensional physical and/or chemical cross-linkages (Cushing and Anseth, 2007; Perez et al., 2015), the mechanical behavior of most hydrogels is still not satisfactory for some applications. To further improve performance, adsorption capability and dimensional stability, cellulose nanofibers (CNFs) extracted from energycane bagasse were used to reinforce hydrogels (Thakur and Thakur, 2014a). Compared to other nanofillers, such as resin, metallic, or silica nanoparticles, as well as carbon nanotubes, CNFs are attractive, due to the characteristics of biocompatibility, availability, low density, non-toxicity, hydrophilicity, biodegradability and

high crystallinity (Dash et al., 2013; Ummartyotin and Manuspiya, 2015). Because of the hydrophilic nature of PVA and SA, the CNFs could crosslink with the hydrogen bond of the PVA/SA matrix to stabilize the hydrogel (Ummartyotin and Manuspiya, 2015). Moreover, CNFs with high tensile modulus can act as decelerators of propagating cracks and retard the failure of the hydrogel (Shin et al., 2009). Several researchers have studied the cellulose nanocrystal based hydrogel. Mohammed et al. (2016) reported nanocrystal-alginate hydrogel beads used in wastewater treatments. Han et al. (2013) investigated nanoparticle reinforced PVA-borax hydrogels and stated that the compressive stress of the hydrogel at the 90 % strain level could achieve 20 kPa.

However, to the best of our knowledge, we are unaware of any work on the crosslinking PVA and SA using GA and calcium sulfate with the presence of CNFs based on core-shell and IPN structure. In the present study, novel hydrogels with core-shell structures were fabricated by casting PVA/SA-GA complexes (IPN core) in CaSO_4 solution to form robust Ca^{2+} -SA shells. The objective was to determine the structure and performance of the hydrogels with and without CNFs to learn their effects on the characteristics of the hydrogels. These hydrogels exhibited promising properties, including high mechanical strength and excellent adsorption-desorption capacity. Specifically, the effects of CNFs on the morphology, light transmittance, and dye adsorption-desorption behavior were studied. Insights into the viscoelastic changes and compression modulus that occurred after incorporating CNFs were obtained from oscillation rheology and uniaxial compression tests. A gelation mechanism for the hydrogel was also proposed for a better understanding of the core-shell 3D network structure. The designed structure of a good ductility core and a high strength shell greatly improved the performance of

the hydrogel, could open the door for interesting applications in biomedicine and sanitary applications.

2 Experimental Section

2.1 Materials

Poly (vinyl alcohol) (PVA) (average M_w 30,000-70,000), methyl blue (MB) and sodium alginate (SA) extracted from brown algae (with reported molecular weight 396000 g/mole and M/G ratio of 1.49) (Dalmoro et al., 2012b) were obtained from Sigma-Aldrich Inc (St. Louis, MO). Glutaraldehyde (25 wt%) (GA) and sodium hydroxide were purchased from Fisher Scientific Inc (Fair Lawn, NJ). Calcium sulfate and sodium phosphate were supplied by EMD Chemicals (Billerica, MA).

Cellulose I fibers isolation and preparation of cellulose nanofibers (CNFs) were as described in our previous work (Yue et al., 2012; Yue et al., 2015a; Yue et al., 2015b). The dried bagasse fibers were pretreated in hot water at 70 °C for 2 h. The resultant material was further treated with 10 wt% NaOH solution at 98 °C for 90 min and reacted with a NaClO₂/glacial CH₃COOH mixture at 75 °C for 2 h. The final cellulose fibers were dried at 40 °C for 48 h. The obtained fibers were then dispersed in 48 wt% sulfuric acid at 45 °C for 1 h and processed through a high-pressure homogenizer for 10 passes to obtain nanofibers (CNFs).

2.2 Preparation of core-shell structure hydrogels

The core-shell structured hydrogels were prepared by crosslinking PVA and SA using GA and calcium sulfate with or without the presence of CNFs (Figure 1). A weighted PVA powder (2.0 wt%) was dissolved in 1.0 wt% CNFs aqueous suspension at 20 °C. Subsequently, dried 2.0

wt% of SA powder was gently added on the surface of the prepared aqueous blend. After 4h of vigorous mechanical stirring at a speed of 300 rpm/min, homogeneous suspension was obtained. The resultant materials were further cross-linked with GA (3.0 v/v%) in nitrogen atmosphere to form the hydrogel core. For achieving the crosslinking between –OH in PVA and aldehyde groups in GA, H₂SO₄ (20 wt%) was added to the suspension until pH = 4.0. After being reacted for 2 days, the semi-fluid core material was obtained. The blend was then transferred to a hollow column mold placed in a glass beaker. The experiments for suitable shell thickness were performed, varying the concentrations of the reaction agent and the immersion time. To obtain a hydrogel with good ductility and solid external surface, an aqueous solution mixture with CaSO₄ (0.015 mol/L) and anhydrous Na₂HPO₄ (0.015 mol/L) was poured into the beaker for 30 min to form the shell. The obtained hydrogel was designated as “Core-Shell-CNF”. The core, crosslinking of PVA/SA with GA, was designated as PSG, whereas the shell, chelating of calcium ions with alginate, was designated as CA. Therefore, PSG-CA-CNF is used as a generic designation for hydrogel with CNFs in comparison with the hydrogel without CNFs (PSG-CA). Dried PVA (2.0 wt%) and SA powder (2.0 wt%) were dissolved in distilled water to produce hydrogel PSG-CA, and all the treatment processes were the same as those applied for PSG-CA-CNF. For purposes of analysis, shells were separated from the core-shell hydrogels by cutting open the hydrogel and rinsing out the core. For separate core samples, some hydrogel was selected prior to the step of placing in the mold and adding CaSO₄. The cores and shells were then freeze-dried using a freeze-dryer for further analysis.

Figure 1. Scheme for preparation of 3D core-shell structured PSG-CA-CNFs hydrogel

2.4 Characterization

2.4.1 Morphology and functional groups of CNFs and hydrogels

TEM with an accelerating voltage of 80 kV was used to investigate the morphology of CNFs. The dimensions of captured CNFs were measured using Image J. 1.45k software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, <http://imagej.nih.gov/ij/>, 1997–2011). The morphology of the freeze-dried hydrogels was characterized using an ESEM at 5 kV. All the freeze-dried samples were characterized by a Bruker FTIR analyzer with an ATR mode with a resolution of 4 cm^{-1} .

2.4.2 Dynamic compression tests and oscillation rheology measurements

Compression tests were performed using an AR-2000EX rheometer. The samples, 40 mm in diameter and 20 mm in height, were placed on the stationary peltier plate, and a uniaxial parallel plate with a diameter of 40 mm was used to press against the sample at a cross head speed of 20 $\mu\text{m/s}$ and 25 °C. The normal force was set at 50 N to make sure that the measurements were ended when the maximum loading reached 50 N. Compressive stress and strain were obtained from measured force and displacement based on initial height of the hydrogels. Before the viscoelastic measurements, dynamic strain sweep was run using parallel-plate geometry (40 mm diameter) to determine the linear viscoelastic region (LVR). The sweep of strain (ϵ) from 0.05 to 100 % was selected for the tests. The frequency sweep was carried out to measure shear storage modulus (G') and loss modulus (G'') as a function of angular frequency (ω) over the range of 1 to 100 rad/s. To determine the viscosity (η) for PSG-CA-CNF cores and the 1.0 wt% CNF colloidal suspensions, dynamic flow sweep was investigated with the shear rate ($\dot{\gamma}$) from 0.1 to 100 s^{-1} using cone-plate geometry (40 mm diameter, 1°59'42" cone angle, truncation cap 52 μm). All the experiments were carried out at 25° C and all samples were equilibrated for 2 min before measurements.

2.4.3 Adsorption and desorption of methyl blue (MB)

Adsorption experiments were carried out in 100 ml MB solution with concentration ranging from 0.05 to 0.15 g/L at 25 °C. Hydrogel (0.1g-0.4g) was added into the MB solution and removed after reaching absorbent equilibrium. The adsorption capacity of PSG-CA-CNFs and PSG-CA was determined by UV-vis spectrophotometer at the wavelength of 664 nm. The

amount of MB adsorbed per unit hydrogel mass at equilibrium q_e (mg/g) was calculated from the following mass balance equation.

$$q_e = (C_0 - C_e) \times V/m \quad (3)$$

Where C_0 and C_e (mg/L) are the initial and equilibrium MB concentrations, respectively; V (L) is the volume of the MB solution, and m (g) is the mass of hydrogel.

Desorption was effected by washing the hydrogel surface with distilled water at room temperature for 10 min.

3 Results and Discussion

3.1 Morphology of CNF and hydrogel

The average lengths of the CNFs were measured to be 485 ± 168 and their widths varied from 7 to 15 nm. The CNF lengths were shorter than those of reported CNFs obtained from wood (Han et al., 2013b), suggesting that the size of our CNFs was affected by material sources and/or treatment conditions. In this study, CNFs were obtained by a combination of NaOH, NaClO_2 , H_2SO_4 and mechanical treatments (Figure 1 (step 1)) and these treatment conditions contributed to the smaller CNF dimensions.

The CNF-reinforced hydrogels were successfully prepared (Figure 1 (step 2 and 3)). Figures 2a and 2b show that the hydrogels possessed a micro-porous shell and a macro-porous core. Pores with diameters ranging from 5 to 40 μm were formed in the core due to high water content ($\geq 92\%$, Figure S1) in the cores (Figure 2c), demonstrating that PVA-SA possessed comparatively large surface area and porous structure (Thankam et al., 2013). The shell is a compact membrane wrapping the core. Figure 2d displays the rough and crazed shell surface.

192 The thickness of the shell was $60 \pm 11 \mu\text{m}$ and pores in the cross-section of the shell were around
193 $2 \pm 0.8 \mu\text{m}$ (Figure 2e). Apparently, the morphology of the core-shell structure was strongly
194 affected by the formation of the chemical bonds. The egg box junctions between Ca^{2+} and –
195 COO^-/OH^- resulted in a compact, dense, micro-porous structure in the shell and the covalent
196 bonding through PVA-SA and GA led to a looser, macro-porous structure in the core. To further
197 illustrate the core-shell structure, the hydrogel was immersed in methyl blue for 10 min and then
198 washed with distilled water several times. Because of the compactness of the shell, incomplete
199 washing could not fully desorb the dye from the shell. However, the dye in the core was more
200 easily rinsed out owing to its macro-porous and loose nature, resulting in the stained shell and
201 uncolored core (Figure 2f).

Figure 2. The morphology of hydrogels: (a) SEM images of freeze-dried core- shell structured hydrogel, (b-e) enlarged view of the marked areas in (a); and (f) optical micrograph of a cross

section of core-shell structured hydrogel after dyeing and rinsing; (g) SEM pictures of hydrogel core with 1.0 wt% CNFs and (h) amplified view of the marked regions in (g).

The morphology of the PSG-CA-CNF core is detailed in Figures 2g and 2h. The CNFs are agglomerated into micro-sized bundles by strong hydrogen bonding, and these CNF bundles are well dispersed in the PVA/SA matrix, resulting in significantly improved mechanical performance. Moreover, the porous macro-structure significantly enlarged the surface area in the core, and it facilitated the movement of dye particles and metal ions within the core network. This resulted in a high adsorption capability.

3.2 Formation mechanism and PVA/SA complex structure

Figure 3 shows the FTIR spectra of the PSG-CA shell, PSG-CA core and PSG-CA-CNF core. The chemical structure change in shell was basically unnoticeable by FTIR with the incorporation of small amount of CNFs, leading to similar FTIR spectra for both prepared shells. Therefore, only one curve for the shell is exhibited in Figure 3. The bands around $3000\text{--}3600\text{ cm}^{-1}$ were assigned to --OH vibration for the PSG-CA shell, PSG-CA and PSG-CA-CNF cores. The intensities of these peaks for the shell were smaller than those for the core. A possible reason was that although the number of free --OH groups in the core of PSG-CA and PSG-CA-CNF were reduced by the crosslinking, the number of --OH groups in the core still larger than those in the shell, resulting in the increased intensity of the peaks in core. In addition, the band at 2928 cm^{-1} was attributed to C-H stretching and the bands at 1093 cm^{-1} and 1020 cm^{-1} were assigned to C-O stretching for both core and shell (Kahya et al., 2010; Thankam et al., 2013). Compared to the core, a small peak at 2928 cm^{-1} and the small peaks at 1093 cm^{-1} and 1020 cm^{-1} evidenced the small number of C-H and C-O groups in SA (Jao et al., 2009; Thankam et al., 2013; Bekin et al.,

2014). For better understanding the FTIR result, the FTIR analysis of each polymer (PVA, SA and CNFs) present in Figure S2.

Figure 3. FTIR spectra of PSG-CA-CNF core, PSG-CA core and PSG-CA shell

A schematic representation of crosslinking reaction in core and shell is shown in Figure 4. The shell of PSG-CA possessed SIPN structure, which was achieved through crosslinking of G units in alginate chains through Ca^{2+} cations in the presence of PVA (Sun et al., 2012; Thankam et al., 2013; Samanta and Ray, 2014). During this process, Na_2HPO_4 , working as a crosslinking retardation agent, was used to slow gelation rates to form a uniform outer layer, resulting in dense and compact egg box junctions in a hydrogel shell (Cho et al., 2009; Dragan, 2014). The peaks around 1591 and 1415 cm^{-1} represented the anti-symmetric and symmetric stretching vibrations of -COO^- groups in the shell, confirming the formation of SIPN (Kahya et al., 2010; Bekin et al., 2014).

The core contained PVA and SA, and these two polymers reacted with GA independently. The IPN structure was a “double network”, generated by crosslinking -CHO groups in GA with –

242 OH groups in SA and PVA to form acetals and ether linkages. This conclusion was supported by
243 absorbance bands at 1371, 1250 and 950 cm^{-1} (Yeom and Lee, 1996; Kim et al., 2003). Besides
244 the IPN structure, PVA and SA could be cross-linked through hydrogen bonding and van der
245 Waals force, contributing to a physical entanglement and chemically crosslinked 3D network in
246 the core. After crosslinking of PVA-SA and GA, the characteristic band at 1591 cm^{-1}
247 corresponding to -COO^- groups in SA was shifted to 1600 cm^{-1} , which might be caused by the
248 formation of IPN structure (Kahya et al., 2010). Apart from these peaks, the peak located at 1715
249 cm^{-1} arose from the stretching vibration of the C=O group in excess of GA (Yeom and Lee,
250 1996). The band at 829 cm^{-1} corresponded to the presence of Na-O bonds in sodium alginate
251 (Bekin et al., 2014). After incorporation of CNFs, the intensity of hydrogen bonding in PSG-CA-
252 CNF was larger than those in PSG-CA (Figure 3), indicating inter/intra hydrogen bonding among
253 the CNFs and between PVA-SA and CNFs.

Figure 4. A schematic representation of the core-shell structured hydrogels

Compared to the egg box junctions in the shells, the crosslinking of PVA-SA with GA can be controlled more easily, and a longer crosslink length can be obtained (Bekin et al., 2014). Owing to the loose and porous structure of the core, its strength was lower than that of the shell. However, the viscoelastic core can adsorb and distribute applied force under deformation, providing inner support for the hydrogels. Therefore, the combination of ductile core and rigid shell gives the hydrogels an ideal structure that can sustain high stress (Figure 5).

3.3 Compression property of hydrogels and viscoelasticity of IPN core

The stress–strain curves of core-shell structured hydrogels before shell rupture are presented in Figure 5a. To compare the compressive strength of the hydrogels with/without CNFs, the stresses at the 20 %, 35 %, and 45 % strain levels were evaluated (Figure 5b).

In the first stage, the two hydrogels exhibited a typical linear stress–strain behavior. This indicated that the core and shell changed from a relaxed state to a stressed state and the hydrogels deformed elastically to store energy for resisting applied stresses (Zhao et al., 2015). In the subsequent stage, the stress slightly deviated from linear response, and the energy-dissipation happened inside the network to resist the compression stress (Gyarmati et al., 2015; Zhao et al., 2015). In the final stage, a sharp increase in stress with an extremely large deformation was presented, suggesting that the network interactions for shell almost reached its full extent. The ultimate compressive strength for hydrogel was reached at a strain around 47 %. After shell rupture, the core might still be able to bear some compression stresses.

Figure 5. Compression property of hydrogels and viscoelastic performance of IPN core: (a) compression stress–strain curves of hydrogels with insert picture showing compression process; (b) a comparison of compressive stress and elastic modulus at different strain levels; (c) strain dependence of G' for CNF suspension, PSG-CA and PSG-CA-CNF cores measured at $\omega = 1.0$ Hz; (d) frequency dependence of G' and G'' for 1.0 wt% CNFs, PSG-CA and PSG-CA-CNF

cores; (e) shear rate dependence of viscosity for CNF suspension, PSG-CA and PSG-CA-CNF cores; (f) mechanism of deformation and reformation of hydrogen bonding in hydrogels.

The compressive stress of PSG-CA at the 45 % strain level was 15.67 ± 0.33 kPa (Figure 5b). Compared with other virgin hydrogels with the compression stress (at the strain level of 80 %) in the range of 3-5 kPa (Sun et al., 2015), the observed high compressive load revealed the stiff nature of the current core-shell structure hydrogel. This was attributed to the existence of SA in both the core and the shell and all of the having many –OH groups. When CNFs (average length of 485 nm) were incorporated into the core-shell structured hydrogel, the compressive stress of PSG-CA-CNF at the 45 % strain level was 50.51 ± 1.09 kPa and PSG-CA-CNF could sustain a pressure of 79.5 ± 2.35 kPa, which was 3.2 times higher than that of the hydrogel without CNFs, (Figure 5b) (Hou et al., 2015). In addition, the hydrogel without CNFs (PSG-CA) had a low elastic modulus ($E\epsilon$) (Figure 5a, b), (determined from the slope of the linear portion of the stress–strain curve), indicative of a fairly soft hydrogel with a relatively weak network (Han et al., 2013a; Sun et al., 2015). The stress-strain curve for PSG-CA-CNF had a greater slope than that of the PSG-CA, further demonstrating that PSG-CA-CNF with a larger $E\epsilon$ can resist much higher pressure than PSG-CA. It was reported that hydrogel mechanical properties were highly related to the crystalline structure of CNFs, the distribution of CNFs in IPNs and the degree of cross-linking within the polymer matrix (Liu et al., 2011; Hou et al., 2015). In Figure S3, CNFs presented a typical cellulose I structure and its crystallinity index, d-spacing and crystal size of CNFs were 53.4 %, 0.386 nm and 2.57 nm, respectively (Yue et al., 2015b). In addition, PSG-CA-CNFs manifested high stability and superior homogeneity at visible wavelength range of 300–800 nm (Figure S4), suggesting that CNFs were well-dispersed in the core and shell matrixes without any visible aggregation. The homogeneous dispersion state of CNFs was

attributed to the interaction between -OH groups in CNFs with -OH groups in both IPN structured core and SIPN structured shell. Specifically, the number of free intra- and intermolecular -OH groups on CNFs can crosslink with each other and participate in crosslinking of CNFs, PVA and SA through hydrogen bonding and van der Waals force with the presence of water molecules, leading to a physical entanglement among them. Therefore, the formation of hydrogel networks were a comprehensive cooperation of chemically cross-linking effects (-CHO groups in GA with -OH groups in SA and PVA to form acetals and ether linkages) and physical entanglement via hydrogen bonding (El Miri et al., 2015), resulting in a tangled, crosslinked and stable 3D network structure (Han et al., 2013a). Consequently, CNFs which acted as crosslinking agents, could sustain a portion of the compressive load, and contribute to a promising mechanical strength for PSG-CA-CNF hydrogels.

To further investigate the ductility and the level of viscoelasticity increase led by the incorporation of CNFs into the hydrogels, various dynamic oscillation rheology measurements were performed for the IPN core. First of all, dynamic strain sweep was carried out to define the linear viscoelastic region (LVR), in which storage modulus (G') was independent to the applied strain. The end of LVR was defined as the critical strain (ϵ_c) (Benhamou et al., 2014). As shown in Figure 5c, the ϵ_c values for CNFs suspension, PSG-CA-CNF and PSG-CA cores were 3.9, 1.0, and 2.0 %. In general, the shorter the LVR, the more rigid the sample (Han et al., 2014). Therefore, the shorter LVR of PSG-CA-CNF core implied the stiffness of the network. For all of the hydrogels, when ϵ was less than 1.0 %, G' was basically independent of the applied ϵ . A strain level of 1.0 % was then selected in the subsequent tests to ensure that the deformation of each sample was within the LVR.

G' and loss modulus (G'') as a function of frequency (ω) for CNFs suspension, PSG-CA, and PSG-CA-CNF cores are shown in Figure 5d. The CNFs suspension had the higher G' values than G'' values over the frequency range from 1 to 100 rad/s. This observation suggested that the reversible potential energy (elastic properties) were dominant as compared to irreversible consumed energy (viscous characteristics) (Benhamou et al., 2014). As ω increased, G' (rigidity of CNFs) and G'' (viscosity of CNFs) increased at higher frequencies (Figure 5d, e). The phenomenon was caused by the equilibrium state of CNFs being broken at high frequency and an ordered network structure being reestablished by the rearrangement of CNFs (Han et al., 2014). In the low frequency region, G' and G'' were relatively independent of frequency. As ω increased, G'' gradually approached G' , which was a sign of the deformation and re-formation of hydrogen bonding and interactions between PVA and SA (Figure 5f) (Wang and Chen, 2011). For the PSG-CA core, G' and G'' were 45 and 26 Pa, respectively. These relatively high values occurred because the formation of an IPN structure. After incorporation of CNFs, the values of the elastic modulus reached 600 Pa and the viscous modulus achieved 320 Pa for the PSG-CA-CNF core. This favorable mechanical performance was attributed to additional interactions between CNFs and PVA-SA chains (Liu et al., 2011). At high frequency, the inter/intra-molecular hydrogen bonds among CNFs and the interactions between PVA-SA and CNFs were partly broken. Meanwhile, a multitude of disrupted hydrogen bonds reformed and entanglements among CNFs, PVA and SA were rebuilt (Figure 5f).

Figure 5e displays the steady shear viscosity (η) of a CNFs suspension as well as PSG-CA and PSG-CA-CNF cores as a function of shear rate ($\dot{\gamma}$). With the increase of $\dot{\gamma}$, η values of CNFs suspension and PSG-CA, PSG-CA-CNF core complexes decreased. A possible reason for this pronounced shear-thinning behavior was that the inter/intra molecular interaction among CNFs

as well as the entanglement between PVA-SA and CNFs were disrupted at a rotational rate faster than the rate of reformation (El Miri et al., 2015). With incorporation of CNFs, the η was significantly increased. Therefore, the core with high modulus and viscosity along with a rigid shell allowed the hydrogels to achieve at favorable performance and build a solid foundation for future applications.

3.4 Adsorption and desorption performances

The adsorption of methyl blue (MB) on core-shell structured hydrogels at constant temperature (25 °C) was studied and the experimental results are shown in Figure 6. In Figure 6a, at low initial concentration (≤ 5 mg/L), the number of adsorbed molecules increased quickly due to abundant free adsorption sites on the hydrogel pore surfaces. The concentration differential in MB solution and hydrogels also provided a strong driving force (Zheng et al., 2014). As the concentration increased, more active adsorption sites of hydrogels were occupied and the adsorbed MB on hydrogels was increased. Thereafter, the adsorption rate decreased until reaching the maximum adsorption capacity. It was reported that various factors influenced MB adsorption, such as pore volume in sorbent surfaces, sorbent surface chemistries, and the interaction between MB and sorbent (Ghorai et al., 2013). Because of the porous nature, the absorbent nature of SA (active sites of -COOH), and chemical stability of IPN and SIPN structure (Aftab et al., 2014), hydrogel could easily adsorb MB, which was deposited on the highly crosslinked polymer matrix (Bhattacharyya and Ray, 2015). In addition, the PSG-CA-CNF absorbed a greater amount of MB than PSG-CA. A possible reason was that the active sites of -OH, -SO₄ groups on the surfaces of CNFs led to more free energy for adsorbing MB, resulting in a synergistic behavior of CNFs and PSG-CA on adsorption.

Figure 6. The adsorption and desorption behavior of hydrogels: (a) experimental data of MB adsorption on hydrogels, and the predicted Langmuir and Freundlich isotherms; (b) Langmuir isotherms determined from the linear plot of $1/q_e$ versus $1/C_e$; (c) the hydrogels maximum adsorption capacity for the four cycles of the adsorption–desorption process; (d) adsorption and (e) desorption behavior of MB on hydrogels

Table 1. Comparison of the parameters predicted from Langmuir and Freundlich models with experimental data obtained from hydrogel adsorption of methyl blue

Sample	$q_m(\text{exp})$ (mg/g)	Predicted parameter					
		Langmuir			Freundlich		
		q_m (mg/g)	K_L (L/mg)	R^2	K_F (mg/g)	$1/n$	R^2
PSG-CA	33.6	34.0	0.650	0.995	9.00	0.525	0.802
PSG-CA-CNF	36.3	37.2	0.634	0.996	9.91	0.510	0.813

Langmuir and Freundlich isotherm models (Equations (4) and (5), respectively) were applied to the MB adsorption.

$$q_e = (q_m K_L C_e) / (1 + K_L C_e) \quad (4)$$

Where q_m is the Langmuir constant related to maximum adsorption capacity (mg/g), and K_L is the Langmuir adsorption equilibrium coefficient reflecting affinity of adsorbent toward adsorbate (L/mg).

$$q_e = K_F C_e^{1/n} \quad (5)$$

where K_F is the Freundlich adsorption equilibrium coefficient (L/mg), and $1/n$ is the adsorption intensity, respectively.

These two models can be described in a linear form as Equations (6) and (7). The experimental data satisfying the linearity of the isotherm indicated the adsorption process followed the isotherm. Then the values of isotherm parameters (K_L and q_m for Langmuir, K_F and $1/n$ for Freundlich) could be calculated using slope and intercept. The obtained isotherm parameters are listed in Table 1.

$$1/q_e = (1/K_L q_m)(1/C_e) + 1/q_m \quad (6)$$

$$\log q_e = \frac{1}{n} \log C_e + \log K_F \quad (7)$$

Langmuir isotherm models assume a monolayer adsorption, that the adsorbent surface is homogeneous, and that all active sites have the same affinity to adsorbate (Bhattacharyya and Ray, 2014; Chung et al., 2015). On the other hand, the Freundlich isotherm model applies to multilayer adsorption on heterogeneous surfaces with different adsorption energies (Bhattacharyya and Ray, 2015). As shown in Figure 6b and Table 1, the linear fitting of Langmuir isotherm model and the high value of $R^2 (\geq 0.99)$ indicated experimental data having a favorable mathematical fitting to the Langmuir isotherm, implying that MB adsorbed on hydrogels was monolayer adsorption and the adsorbed MB layer was one molecule depth in thickness (Ghorai et al., 2013; Chung et al., 2015). The calculated maximum adsorption amount of MB (q_m) on PSG-CA and PSG-CA-CNF was 34.0 and 37.2 mg/g, which were very close to the experimental values (33.6 for PSG-CA and 36.3 for PSG-CA-CNF).

The parameter R_L was used to evaluate the adsorption favorability in the Langmuir isotherm. The Langmuir process is unfavorable when $R_L > 1$, favorable when $0 < R_L < 1$, linear when $R_L = 1$ and irreversible when $R_L = 0$ (Ghorai et al., 2013; Bhattacharyya and Ray, 2015).

$$R_L = 1/(1 + C_0 K_L) \quad (8)$$

In this study, the K_L values were about 0.65 and the R_L values were in the range of 0-1, which signify a promising adsorption ability of the hydrogels for MB (Ghorai et al., 2013; Bhattacharyya and Ray, 2014).

As shown in Figure 6c, although there were slight reductions in the adsorption capacities, the hydrogel exhibited a satisfactory adsorption capacity after two to three cycles of the adsorption–desorption process, proving that the hydrogel that offered strong electrostatic interaction energies could be employed multiple cycles (Bhattacharyya and Ray, 2014). Figure 6d shows that after the adsorption process, the MB solution was much lighter in color, further demonstrating that the hydrogel adsorbed most of the MB from aqueous solution. Figure 6e shows that the adsorbed hydrogel, which was thoroughly rinsed with distilled water for 10 minutes, was white without any trace of blue. This high desorption performance is important for achieving multiple adsorption–desorption cycles by the hydrogels.

4 Conclusions

Core-shell structured hydrogels consisting of a flexible **interpenetrating polymer network (IPN)** core and a rigid **semi-IPN** shell were prepared through chelation and covalent bonding of polyvinyl **alcohol-sodium alginate (PVA-SA)** with Ca^{2+} and **glutaraldehyde (GA)**, respectively. **Cellulose nanofibers (CNFs)** were incorporated into the hydrogel system and reinforced the hydrogels via inter/intra-molecular hydrogen bonds. The hydrogels had micro-porous shells and

macro-porous cores. The neat **PSG-CA hydrogels (the hydrogel without CNFs)** with a water content of 93.2 % provided an elastic modulus of 2.91 kPa and a maximum methyl blue (MB) adsorption capacity of 33.6 mg/g. The dynamic oscillation and compression measurements showed that the CNFs dramatically elevated the viscoelasticity of the core and the compressive strength of the hydrogels. Specifically, the storage modulus and compressive strength of **PSG-CA-CNF (the hydrogel with CNFs)** were 13 and 3.2 times higher than those of neat PSG-CA. The incorporation of CNFs into core-shell structured hydrogels also increased the MB adsorption capacity by 10 %. The MB adsorbed on hydrogels in monolayers and multiple adsorption–desorption cycles could be applied to the hydrogels.

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Supporting Information: The water contents and densities of hydrogels; FTIR spectra of PVA, SA and CNFs; X-ray diffraction patterns of CNFs; The transparency of CNF suspension and hydrogels

5 References

Aftab, K., Akhtar, K., Jabbar, A., 2014. Batch and column study for Pb-II remediation from industrial effluents using glutaraldehyde–alginate–fungi biocomposites. Ecological Engineering 73, 319-325.

448 Bekin, S., Sarmad, S., Gürkan, K., Keçeli, G., Gürdağ, G., 2014. Synthesis, characterization and
 449 bending behavior of electroresponsive sodium alginate/poly(acrylic acid) interpenetrating
 450 network films under an electric field stimulus. *Sensors and Actuators B: Chemical* 202,
 451 878-892.

452 Benhamou, K., Dufresne, A., Magnin, A., Mortha, G., Kaddami, H., 2014. Control of size and
 453 viscoelastic properties of nanofibrillated cellulose from palm tree by varying the TEMPO-
 454 mediated oxidation time. *Carbohydrate Polymers* 99, 74-83.

455 Berrebi, M., Fabre-Francke, I., Lavédrine, B., Fichet, O., 2015. Development of organic glass
 456 using Interpenetrating Polymer Networks with enhanced resistance towards scratches and
 457 solvents. *European Polymer Journal* 63, 132-140.

458 Bhattacharyya, R., Ray, S.K., 2014. Enhanced adsorption of synthetic dyes from aqueous
 459 solution by a semi-interpenetrating network hydrogel based on starch. *Journal of Industrial*
 460 *and Engineering Chemistry* 20, 3714-3725.

461 Bhattacharyya, R., Ray, S.K., 2015. Adsorption of industrial dyes by semi-IPN hydrogels of
 462 Acrylic copolymers and sodium alginate. *Journal of Industrial and Engineering Chemistry*
 463 22, 92-102.

464 Bidarra, S.J., Barrias, C.C., Granja, P.L., 2014. Injectable alginate hydrogels for cell delivery in
 465 tissue engineering. *Acta Biomaterialia* 10, 1646-1662.

466 Cho, S.H., Lim, S.M., Han, D.K., Yuk, S.H., Lm, G.I., Lee, J.H., 2009. Time-Dependent
 467 Alginate/Polyvinyl Alcohol Hydrogels as Injectable Cell Carriers. *Journal of Biomaterials*
 468 *Science -- Polymer Edition* 20, 863-876.

469 Chung, H.K., Kim, W.H., Park, J., Cho, J., Jeong, T.Y., Park, P.K., 2015. Application of
 470 Langmuir and Freundlich isotherms to predict adsorbate removal efficiency or required
 471 amount of adsorbent. *Journal of Industrial and Engineering Chemistry* 28, 241-246.
 472 Cushing, M.C., Anseth, K.S., 2007. Hydrogel Cell Cultures. *Science* 316, 1133-1134.
 473 Dalmoro, A., Barba, A.A., Lamberti, G., d'Amore, M., 2012a. Intensifying the
 474 microencapsulation process: Ultrasonic atomization as an innovative approach. *European*
 475 *Journal of Pharmaceutics and Biopharmaceutics* 80, 471-477.
 476 Dalmoro, A., Barba, A.A., Lamberti, G., Grassi, M., d'Amore, M., 2012b. Pharmaceutical
 477 applications of biocompatible polymer blends containing sodium alginate. *Advances in*
 478 *Polymer Technology* 31, 219-230.
 479 Dalmoro, A., Sitenkov, A.Y., Lamberti, G., Barba, A.A., Moustafine, R.I., 2016. Ultrasonic
 480 atomization and polyelectrolyte complexation to produce gastroresistant shell-core
 481 microparticles. *Journal of Applied Polymer Science* 133, n/a-n/a.
 482 Dash, R., Foston, M., Ragauskas, A.J., 2013. Improving the mechanical and thermal properties
 483 of gelatin hydrogels cross-linked by cellulose nanowhiskers. *Carbohydrate Polymers* 91,
 484 638-645.
 485 Dragan, E.S., 2014. Design and applications of interpenetrating polymer network hydrogels. A
 486 review. *Chemical Engineering Journal* 243, 572-590.
 487 El Miri, N., Abdelouandi, K., Barakat, A., Zahouily, M., Fihri, A., Solhy, A., El Achaby, M.,
 488 2015. Bio-nanocomposite films reinforced with cellulose nanocrystals: Rheology of film-
 489 forming solutions, transparency, water vapor barrier and tensile properties of films.
 490 *Carbohydrate Polymers* 129, 156-167.

491 Ghorai, S., Sarkar, A.K., Panda, A.B., Pal, S., 2013. Effective removal of Congo red dye from
 492 aqueous solution using modified xanthan gum/silica hybrid nanocomposite as adsorbent.
 493 Bioresource Technology 144, 485-491.

494 Gyarmati, B., Meszar, E.Z., Kiss, L., Deli, M.A., Laszlo, K., Szilagyi, A., 2015.
 495 Supramacroporous chemically cross-linked poly(aspartic acid) hydrogels. Acta
 496 Biomaterialia 22, 32-38.

497 Han, J., Lei, T., Wu, Q., 2013a. Facile preparation of mouldable polyvinyl alcohol-borax
 498 hydrogels reinforced by well-dispersed cellulose nanoparticles: physical, viscoelastic and
 499 mechanical properties. Cellulose 20, 2947-2958.

500 Han, J., Lei, T., Wu, Q., 2014. High-water-content mouldable polyvinyl alcohol-borax hydrogels
 501 reinforced by well-dispersed cellulose nanoparticles: Dynamic rheological properties and
 502 hydrogel formation mechanism. Carbohydrate Polymers 102, 306-316.

503 Han, J., Zhou, C., Wu, Y., Liu, F., Wu, Q., 2013b. Self-Assembling Behavior of Cellulose
 504 Nanoparticles during Freeze-Drying: Effect of Suspension Concentration, Particle Size,
 505 Crystal Structure, and Surface Charge. Biomacromolecules 14, 1529-1540.

506 Hou, R.X., Nie, L., Du, G.L., Xiong, X.P., Fu, J., 2015. Natural polysaccharides promote
 507 chondrocyte adhesion and proliferation on magnetic nanoparticle/PVA composite
 508 hydrogels. Colloids and Surfaces B-Biointerfaces 132, 146-154.

509 Jao, W., Chen, H., Lin, C., Yang, M., 2009. The controlled release behavior and pH- and thermo-
 510 sensitivity of alginate/poly(vinyl alcohol) blended hydrogels. Polymers for Advanced
 511 Technologies 20, 680-688.

512 Kahya, S., Solak, E.K., Sanli, O., 2010. Sodium alginate/poly(vinyl alcohol) alloy membranes
 513 for the pervaporation, vapour permeation and vapour permeation with temperature

difference separation of dimethylformamide/water mixtures: A comparative study. Vacuum
84, 1092-1102.

Kim, J.H., Jegal, J., Lee, K.H., Lee, Y., 2003. Enantioselective permeation of alpha-amino acid
optical isomers through crosslinked sodium alginate membranes. Journal of Applied
Polymer Science 89, 3046-3051.

Lee, K.Y., Mooney, D.J., 2012. Alginate: Properties and biomedical applications. Progress in
Polymer Science 37, 106-126.

Liu, D., Chen, X., Yue, Y., Chen, M., Wu, Q., 2011. Structure and rheology of nanocrystalline
cellulose. Carbohydrate Polymers 84, 316-322.

Mohammed, N., Grishkewich, N., Waeijen, H.A., Berry, R.M., Tam, K.C., 2016. Continuous
flow adsorption of methylene blue by cellulose nanocrystal-alginate hydrogel beads in
fixed bed columns. Carbohydrate Polymers 136, 1194-1202.

Perez, R.A., Kim, J.-H., Buitrago, J.O., Wall, I.B., Kim, H.-W., 2015. Novel therapeutic core–
shell hydrogel scaffolds with sequential delivery of cobalt and bone morphogenetic protein-
2 for synergistic bone regeneration. Acta Biomaterialia 23, 295-308.

Samanta, H.S., Ray, S.K., 2014. Synthesis, characterization, swelling and drug release behavior
of semi-interpenetrating network hydrogels of sodium alginate and polyacrylamide.
Carbohydrate Polymers 99, 666-678.

Shin, M.K., Spinks, G.M., Shin, S.R., Kim, S.I., Kim, S.J., 2009. Nanocomposite Hydrogel with
High Toughness for Bioactuators. Advanced Materials 21, 1712-1715.

Sun, F., Lin, M., Dong, Z., Zhang, J., Wang, C., Wang, S., Song, F., 2015. Nanosilica-induced
high mechanical strength of nanocomposite hydrogel for killing fluids. Journal of Colloid
and Interface Science 458, 45-52.

537 Sun, J.-Y., Zhao, X., Illeperuma, W.R.K., Chaudhuri, O., Oh, K.H., Mooney, D.J., Vlassak, J.J.,
 538 Suo, Z., 2012. Highly stretchable and tough hydrogels. *Nature* 489, 133-136.
 539 Thakur, V.K., Thakur, M.K., 2014a. Processing and characterization of natural cellulose
 540 fibers/thermoset polymer composites. *Carbohydrate Polymers* 109, 102-117.
 541 Thakur, V.K., Thakur, M.K., 2014b. Recent trends in hydrogels based on psyllium
 542 polysaccharide: a review. *Journal of Cleaner Production* 82, 1-15.
 543 Thakur, V.K., Thakur, M.K., 2015. Recent advances in green hydrogels from lignin: a review.
 544 *International Journal of Biological Macromolecules* 72, 834-847.
 545 Thankam, F.G., Muthu, J., Sankar, V., Gopal, R.K., 2013. Growth and survival of cells in
 546 biosynthetic poly vinyl alcohol-alginate IPN hydrogels for cardiac applications. *Colloids*
 547 *and Surfaces B-Biointerfaces* 107, 137-145.
 548 Ummartyotin, S., Manuspiya, H., 2015. A critical review on cellulose: From fundamental to an
 549 approach on sensor technology. *Renewable and Sustainable Energy Reviews* 41, 402-412.
 550 Wang, Y., Chen, L., 2011. Impacts of nanowhisker on formation kinetics and properties of all-
 551 cellulose composite gels. *Carbohydrate Polymers* 83, 1937-1946.
 552 Yang, J.-M., Wang, N.-C., Chiu, H.-C., 2014. Preparation and characterization of poly(vinyl
 553 alcohol)/sodium alginate blended membrane for alkaline solid polymer electrolytes
 554 membrane. *Journal of Membrane Science* 457, 139-148.
 555 Yeom, C.K., Lee, K.H., 1996. Pervaporation separation of water-acetic acid mixtures through
 556 poly(vinyl alcohol) membranes crosslinked with glutaraldehyde. *Journal of Membrane*
 557 *Science* 109, 257-265.

- Yue, Y., Han, J., Han, G., Aita, G.M., Wu, Q., 2015a. Cellulose fibers isolated from energycane bagasse using alkaline and sodium chlorite treatments: Structural, chemical and thermal properties. *Industrial Crops and Products* 76, 355-363.
- Yue, Y., Han, J., Han, G., Zhang, Q., French, A.D., Wu, Q., 2015b. Characterization of cellulose I/II hybrid fibers isolated from energycane bagasse during the delignification process: Morphology, crystallinity and percentage estimation. *Carbohydrate Polymers* 133, 438-447.
- Yue, Y., Zhou, C., French, A.D., Xia, G., Han, G., Wang, Q., Wu, Q., 2012. Comparative properties of cellulose nano-crystals from native and mercerized cotton fibers. *Cellulose* 19, 1173-1187.
- Zhang, Y., Liu, J., Huang, L., Wang, Z., Wang, L., 2015. Design and performance of a sericin-alginate interpenetrating network hydrogel for cell and drug delivery. *Scientific Reports* 5, 12374.
- Zhao, W., Shi, Z., Chen, X., Yang, G., Lenardi, C., Liu, C., 2015. Microstructural and mechanical characteristics of PHEMA-based nanofibre-reinforced hydrogel under compression. *Composites Part B-Engineering* 76, 292-299.
- Zheng, Y., Zhu, Y., Wang, A., 2014. Highly efficient and selective adsorption of malachite green onto granular composite hydrogel. *Chemical Engineering Journal* 257, 66-73.