Carbohydrate Polymers 96 (2013) 487–494

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Evaluation of anisotropic chitosan hydrogels using analytical Mueller matrix method and scanned laser pico-projector☆

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ARTICLE INFO

Article history:
Received 24 January 2013
Received in revised form 2 April 2013
Accepted 12 April 2013
Available online 22 April 2013

Keywords:
Chitosan
Anisotropic material
Gel
Mueller matrix
Speckle
Scanned laser pico-projector

ABSTRACT

Chitosan has excellent biodegradable, biocompatible and bio-absorbable properties and has been found increasing use in the biomedical field in recent decades. The linear birefringence (LB), linear diattenuation (LD), circular birefringence (CB), circular diattenuation (CD), and depolarization properties of chitosan hydrogel films crosslinked in citrate acid buffer solution (CBS) are extracted using an analytical Mueller matrix method. It is shown that the optical phase retardance property of the hydrogel films provides a reliable indication of both the chitosan concentration of the film and the pH value of the CBS crosslinking environment. In addition, chitosan hydrogel suspension with low-concentration crosslinked in CBS environments with various pH values are studied by the speckle contrast of the projected images obtained when illuminating the suspension with a scanned laser pico-projector (SLPP). It is found that for the samples crosslinked in an acidic environment, the speckle contrast decreases with an increasing pH value. By contrast, for the samples crosslinked in an alkaline CBS environment, the speckle contrast increases as the pH value increases. It is concluded that both the phase retardance and the speckle contrast enable the pH value of the CBS crosslinking solution to be reliably determined. However, of the two methods, the SLPP method yields improved measurement sensitivity. Overall, the results presented in this study show that the analytical Mueller matrix method and SLPP method provide an effective means of characterizing the optical properties, concentration and crosslinking environment of chitosan hydrogel films and suspensions.

1. Introduction

Chitin, a naturally-occurring linear polysaccharide found in arthropods such as crabs, shrimps and lobsters, is the main source of production for chitosan, which has many applications in the agricultural, industrial and biomedical fields (Carreira, Gonçalves, Mendonça, Gil, & Coelho, 2010). Chitin deacetylated under heterogeneous conditions to a degree of deacetylation (DD) of 60% or more is soluble in dilute aqueous acids, while chitin deacetylated under homogeneous conditions to a DD of approximately 50% is soluble in water. Min et al. (2004) fabricated a chitin nano-fibrous matrix for wound dressings using an electro-spinning method and then treated the as-spun matrix with a 40% aqueous NaOH solution to form a chitosan matrix with a DD of 85%.

Chitosan has many favorable biodegradable, biocompatible and mechanical properties and has been found to improve cell adhesion, proliferation and function (Huang, Liao, Yang, Chang, Ju, & Lin, 2009; Naahidi, Jafari, Edalat, Raymond, Khademhosseini, & Chen, 2013). As a result, it is widely used in the biomedical field for such applications as wound healing and controlled drug delivery (Bernkop-Schnürch & Dünnhaupt, 2012). Furthermore, chitosan has a homeostatic property and is therefore an ideal medium for tissue regeneration or cell culture growth. (Giri et al., 2012) For example, Jebahi et al. (2012) used bioglass-chitosan as a pharmaceutical drug to repair bone defect and indicated that bioglass-chitosan seemed to promote an excellent structural response in terms of bone integration. Crompton, Forsythe, Horne, Finkelstein, and Knott (2009) showed that for chitosan hydrogels with a chitosan concentration of 0.25–1.5 w/v%, the chitosan chains are arranged into polymer-rich aggregates with a diameter of 1–2 μm. Berger, Reist, Mayer, Felt, Peppas, and Gurny (2004) showed that ionically crosslinked chitosan hydrogels have a higher swelling sensitivity to pH changes than covalently crosslinked chitosan hydrogels and are therefore better suited to pH-controlled

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http://dx.doi.org/10.1016/j.carbpol.2013.04.032

drug delivery applications. Additionally, the studies (Fen, Yunus, & Yusof, 2012; Montembault, Viton, & Domard, 2005) showed that the physico-chemical properties of chitosan render chitosan an ideal immobilization agent for the active layer in surface plasmon resonance (SPR) sensors. It was found that the extensive interspaces in the resulting polymer network enabled the storage of a large quantity of detecting material, thereby rendering the chitosan gel an ideal immobilization agent for polarimetery-based sensing applications.

The optical characterization for biological materials is advanced inspection and diagnostic applications with non-invasion (Wang, 2002). There is much motivating in the investigation by polarized light according to anisotropic chitosan hydrogels. For example, Dobashi, Tomita, Maki, Chang, and Yamamoto (2011) prepared chitosan hygrogel strings and films with birefringence by immersing a sandwiched chitosan in aqueous acetic acid into aqueous sodium hydroxide. They observed that the photo images of chitosan hygrogel strings and films showed crossed nicols due to an orientation of domains such as polymer aggregation. Also, Csoka, Appel, Eitner, Jirikowski, and Makovitzky (2013) showed that insect chitosan is characterized by birefringence; with the result that its structure is amenable to analysis via polarization microscopy.

In performing a quantitative analysis of an anisotropic sample, effective optical parameters are of interest, namely the linear birefringence (LB), the linear diattenuation (LD), the circular birefringence (CB), the circular diattenuation (CD), and the depolarization index. In recent studies (Pham & Lo, 2012a, 2012b), the current group presented a decoupled analytical technique based on the Mueller matrix method and Stokes polarimetry for extracting the effective optical parameters. The proposed method not only yields high measurement accuracy due to its decoupled nature, but also provides the means to extract the parameters of optical samples having only LB, CB, LD or CD properties without the need for any form of compensation on system or pretreatment on samples. Recently, the current group (Chuang, Sung, Huang, & Lo, 2012) presented a method for measuring the two-dimensional (2-D) concentration of solid and liquid solutions via an inspection of the speckle contrast of the images projected by a scanned laser pico-projector (SLPP). The feasibility of the proposed approach was demonstrated by measuring collagen concentrations (Collagen type I from calf skin was used for measurements and dissolved in 0.1 M acetic acid solution) ranging from 0.025 to 0.125%.

The optical characteristics of chitosan hydrogels are generally examined qualitatively by means of polarization microscopy. By contrast, in the present study, a quantitative examination of the optical characteristics of chitosan hydrogels is performed. Specifically, the analytical Mueller matrix method is applied to extract the effective optical parameters of chitosan hydrogel films with various chitosan concentrations crosslinking in acidic or alkaline citrate acid buffer solutions (CBS). Moreover, SLPP method is also applied to measure the chitosan hydrogel suspensions under the various pH value of the CBS crosslinking environment.

2. Materials and methods

2.1. Extraction of effective optical parameters of anisotropic chitosan hydrogel films using Mueller matrix method

2.1.1. Experimental methodology

According to the studies (Pham & Lo, 2012a, 2012b), the Mueller matrix for a LB material with principal axis angle (α) and phase retardance (β) can be expressed as

$$M_{lb} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(4\alpha)\sin^2(\beta/2)+\cos^2(\beta/2) & \sin(4\alpha)\sin^2(\beta/2) & -\sin(2\alpha)\sin(\beta) \\ 0 & \sin(4\alpha)\sin^2(\beta/2) & -\cos(4\alpha)\sin^2(\beta/2)+\cos^2(\beta/2) & \cos(2\alpha)\sin(\beta) \\ 0 & \sin(2\alpha)\sin(\beta) & -\cos(2\alpha)\sin(\beta) & \cos(\beta) \end{pmatrix} \quad (1)$$

Meanwhile, the Mueller matrix for a LD material with diattenuation axis angle (θ_d) and diattenuation (D) has the form

$$M_{ld} = \begin{pmatrix} \frac{1}{2}\left(1+\frac{1-D}{1+D}\right) & \frac{1}{2}\cos(2\theta_d)\left(1-\frac{1-D}{1+D}\right) & \frac{1}{2}\sin(2\theta_d)\left(1-\frac{1-D}{1+D}\right) & 0 \\ \frac{1}{2}\cos(2\theta_d)\left(1-\frac{1-D}{1+D}\right) & \frac{1}{4}\left(\left(1+\sqrt{\frac{1-D}{1+D}}\right)^2+\cos(4\theta_d)\left(1-\sqrt{\frac{1-D}{1+D}}\right)^2\right) & \frac{1}{4}\sin(4\theta_d)\left(1-\sqrt{\frac{1-D}{1+D}}\right)^2 & 0 \\ \frac{1}{2}\sin(2\theta_d)\left(1-\frac{1-D}{1+D}\right) & \frac{1}{4}\sin(4\theta_d)\left(1-\sqrt{\frac{1-D}{1+D}}\right)^2 & \frac{1}{4}\left(\left(1+\sqrt{\frac{1-D}{1+D}}\right)^2-\cos(4\theta_d)\left(1-\sqrt{\frac{1-D}{1+D}}\right)^2\right) & 0 \\ 0 & 0 & 0 & \sqrt{\frac{1-D}{1+D}} \end{pmatrix} \quad (2)$$

The Mueller matrix for a CB material with optical rotation (γ) is given as

$$M_{cb} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(2\gamma) & \sin(2\gamma) & 0 \\ 0 & -\sin(2\gamma) & \cos(2\gamma) & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \quad (3)$$

The Mueller matrix for a CD material with circular diattenuation (R) can be expressed as

$$M_{cd} = \begin{bmatrix} 1+R^2 & 0 & 0 & 2R \\ 0 & 1-R^2 & 0 & 0 \\ 0 & 0 & 1-R^2 & 0 \\ 2R & 0 & 0 & 1+R^2 \end{bmatrix} \quad (4)$$

Finally, the Mueller matrix for a non-uniform depolarizing material has the form

$$M_{\Delta} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ p_1 & e_1 & 0 & 0 \\ p_2 & 0 & e_2 & 0 \\ p_3 & 0 & 0 & e_3 \end{bmatrix} \quad \text{and} \quad |e1|, |e2|, |e3| \le 1 \quad (5)$$

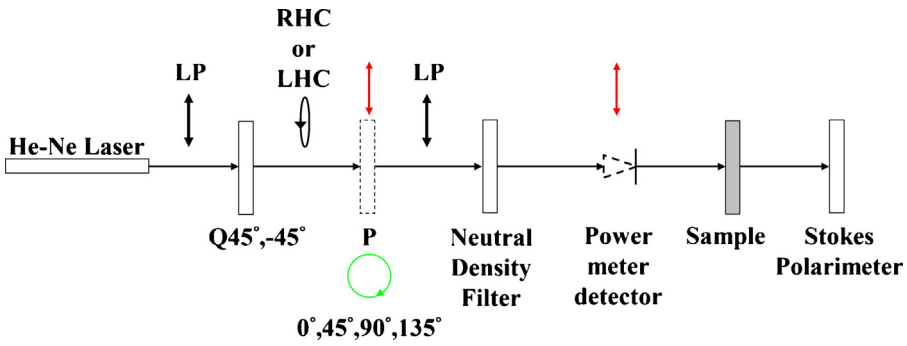

Fig. 1. Schematic illustration of experimental system used to measure effective optical parameters of anisotropic optical samples.

where p_1, p_2 and p_3 are the elements of the polarization vector; e_1 and e_2 are the degrees of linear depolarization (L-Dep); and e_3 is the degree of circular depolarization. Having determined the values of e_1, e_2 and e_3, the depolarization index Δ is computed as

$$\Delta = 1 - \sqrt{\frac{e_1^2 + e_2^2 + e_3^2}{3}} \quad \text{and} \quad 0 \le D \le 1 \tag{6}$$

Let $[M_\Delta]$, $[M_{lb}]$, $[M_{ld}]$, $[M_{cb}]$ and $[M_{cd}]$ be the Mueller matrices corresponding to the depolarization, LB, LD, CB and CD properties of the anisotropic sample, respectively. According to the studies (Pham & Lo, 2012a, 2012b),

$$S_c = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix}_c = [M_\Delta][M_{lb}][M_{cb}][M_{ld}][M_{cd}]\hat{S}_c = \begin{pmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix} \begin{pmatrix} \hat{S}_0 \\ \hat{S}_1 \\ \hat{S}_2 \\ \hat{S}_3 \end{pmatrix}_c \tag{7}$$

where S_c and $\hat{S}_C$ are the Stokes vectors of the output and input light beams respectively, and m_{11}–m_{44} are the elements of a 4×4 Mueller matrix. It is found that the effective LB, LD, CB, CD and depolarization properties of the anisotropic sample are extracted using six different input polarization lights, namely four linear polarization lights (i.e., $\hat{S}_{0^\circ} = [1, 1, 0, 0]^T$, $\hat{S}_{45^\circ} = [1, 0, 1, 0]^T$, $\hat{S}_{90^\circ} = [1, -1, 0, 0]^T$, and $\hat{S}_{135^\circ} = [1, 0, -1, 0]^T$) and two circular polarization lights (i.e., right-handed $\hat{S}_{RHC} = [1, 0, 0, 1]^T$ and left-handed $\hat{S}_{LHC} = [1, 0, 0, -1]^T$). The proposed method allows nine effective parameters to be obtained, namely the principal axis angle (α), the phase retardance (β), the diattenuation axis angle (θ_d), the diattenuation (D), the optical rotation (γ), the circular diattenuation (R), the degrees of L-Dep (e_1 and e_2), and the degree of circular depolarization (e_3).

Fig. 1 presents a schematic illustration of the experimental setup proposed in (Pham & Lo, 2012a, 2012b) for measuring the effective parameters of an anisotropic sample. As shown, the input light is provided by a frequency-stable He–Ne laser (SL 02/2, SIOS Co.) with the 633 nm wavelength. Meanwhile, the four linear polarization lights (0°, 45°, 90° and 135°) and two circular polarization lights (right-handed and left-handed) are produced using a polarizer (GTH5M, Thorlabs Co.) and quarter-wave plate (QWP0-633-04-4-R10, CVI Co.). Finally, a neutral density filter (NDC-100-2, ONSET Co.) and power meter detector (8842A, OPHIT Co.) are used to ensure that each of the six input polarization lights has an equal intensity prior to entering the sample. The output Stokes parameters were computed from the intensity measurements obtained using a commercial Stokes polarimeter (PAX5710, Thorlabs Co.) at a sampling rate of 30 samples per second. A minimum of 1024 data points was obtained for the effective parameters of each sample. Of these data points, 100 points were then chosen in order to calculate the mean value of each parameter. A series of simulations were performed in (Pham & Lo, 2012a, 2012b) to evaluate the accuracy of the results obtained from the proposed method given errors of ±0.005 in the values of the output Stokes parameters. (Note that the error range defined here is consistent with the measurement precision of a typical commercial Stokes polarimeter (PAX5710, Thorlabs Co.).) Overall, the ability of the proposed method (Pham & Lo, 2012a, 2012b) to extract the orientation angle of LB of samples with a low degree of birefringence can be reliable when retardance is larger than 3°. Moreover, the values of orientation angle of LD can be reliable when linear dichroism is larger than 0.05. The ability of the proposed method to extract the optical parameters of samples with CB larger than 0.1° or CD larger than 0.01 are reliable.

2.1.2. Sample preparation

Chitosan hydrogel films were fabricated using the sandwich gel forming process described in Dobashi, Tomita, Maki, Chang, and Yamamoto (2011). Chitosan solutions were prepared by dissolving chitosan powder (Aldrich 417963, DD ≥75%) in 0.1 M acetic acid solution (Fluka) under moderate magnetic stirring for 3 h at room temperature. (Note that solutions with concentrations of 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 g chitosan/100 ml 0.1 M acetic acid solution were prepared.) Chitosan solutions were then treated by ultrasonic bath for 1 h to keep solutions homogeneous with no phase separation or precipitation. Subsequently, 50 μl of chitosan solution was dropped on a quartz holder and allowed to infiltrate a gap with a thickness of 130 μm between the holder and a circular glass cover slide (diameter: 15 mm). Note that a circular cover slide was deliberately chosen in order to ensure a uniform filling of the gap under the effects of capillary forces. Once the solution had completely filled the gap between the holder and the slide, the resulting chitosan sandwich structure was immersed in 0.1 M NaOH (Merck) (pH = 12) for 3 h at room temperature in order to form a chitosan hydrogel film. Finally, the chitosan hydrogel film was washed by DI water to remove the residual reagent and then blown dry in air.

To evaluate the effects of the pH value of the crosslinking environment on the effective optical parameters, a stock chitosan solution with a concentration of 2.0 g chitosan/100 ml 0.1 M acetic acid solution was prepared. Sandwich structures were produced using the method described above and were then immersed in CBS solutions with pH values of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, respectively,

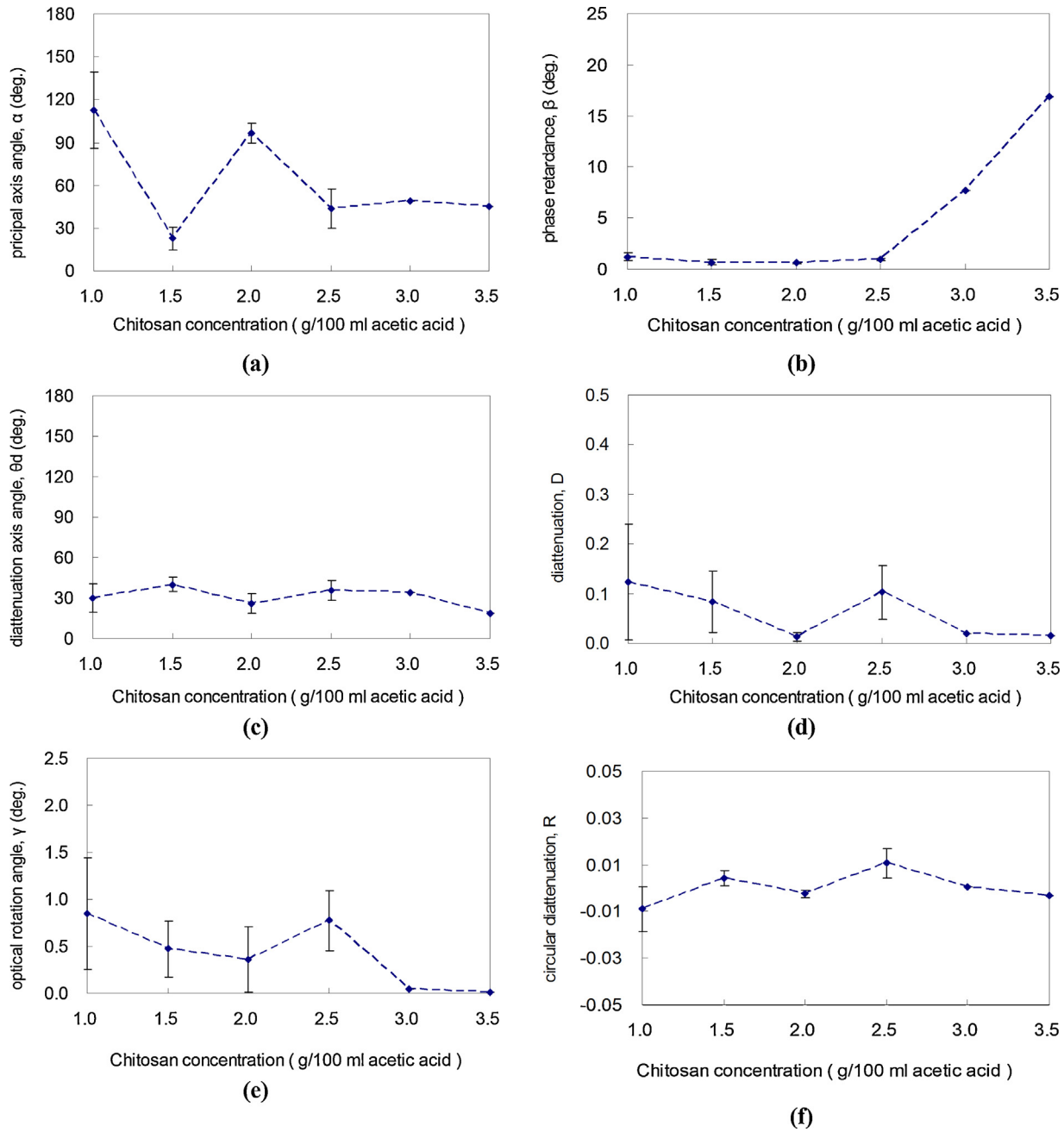

Fig. 2. Variations of (a) principal axis angle α, (b) phase retardance β, (c) diattenuation axis angle θ_d, (d) diattenuation D, (e) optical rotation γ, and (f) circular diattenuation R of chitosan hydrogel films with various chitosan concentrations.

for 3 h at room temperature. The resulting hydrogel films were washed in DI water, blown dry in air and then set aside for further testing.

Speckle contrast measurement using scanning laser pico-projector (SLPP)

3. Experimental methodology

A speckle pattern is typically quantified via the speckle contrast C, which is given by the standard deviation of all the intensity values σ_I divided by the mean intensity I (Goodman & Socorro, 2007; Goodman, 1976), i.e.

$$C = \frac{\sigma_I}{I} \tag{8}$$

It was shown in Chuang, Sung, Huang, and Lo (2012) that the perceived speckle produced by backscattered laser light is highly dependent on the characteristics of the reflecting surface. In the present study, this characteristic is exploited to determine the pH value of the CBS crosslinking agent used in the preparation of chitosan hydrogel suspension with low-concentration. The detail process of the experimental methodology can be found in our previous study (Chuang, Sung, Huang, & Lo, 2012). The measurement system was based on a commercial scanning laser pico-projector (SLPP) (MicroVision; Model: SHOWWX^{TM}; Resolution: WVGA (848 × 480); Aspect Ratio: 16:9 Widescreen; Contrast Ratio: >5000:1; Image Size: from 150 mm to 2500 mm).

Four different projected images of the chitosan samples were obtained using the red laser, green laser, blue laser, and white laser (all SLPP lasers on), respectively. In every case, the projected

image had a size of 200 mm × 112.5 mm. The images were projected onto a sheet of copypaper (Double A, 80 g/m^2) with a thickness of approximately 100 μm. The speckle images (3136 × 2352 pixels) were captured by a digital camera (Olympus, Model E330) under the following settings: F-number: F/20, focal length: 37 mm, exposure time: 1/3 s, and ISO speed: 200. Each speckle image was partitioned into sub-images with a size of 150 × 150 pixels and the two-dimensional speckle contrast of each sub-image was then analyzed using a self-written Matlab program.

3.1.1. Sample preparation

Chitosan hydrogel suspensions were prepared using CBS with pH values ranging from 3.0 to 8.0. The preparation process was commenced by mixing 1.0 g chitosan powder/100 ml 0.1 M acetic acid solution under moderate magnetic stirring for 3 h at room temperature. The resulting solution was homogeneous, with no phase separation or precipitation. The stock solution was then diluted to form a 0.01% chitosan solution (i.e., 0.01 g chitosan/100 ml 0.1 M acetic acid solution). The resulting solution was then mixed with CBS with pH values of 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 for 3 h at room temperature in order to form a homogenous suspension. Finally, the chitosan hydrogel suspensions were placed in quartz containers (45 mm × 27.5 mm × 7.5 mm ($h \times d \times w$); capacity, 5 ml) and set aside for further testing.

4. Results and discussion

4.1. Effective optical parameters of chitosan hydrogel films

4.1.1. Chitosan hydrogel films with different chitosan concentrations

Due to the macro pK_a of chitosan is 6.3, chitosan is soluble in acid solution when pH value is under 6.3 (Shu, Zhu, & Song, 2001). Thus, chitosan was prepared in an alkaline solution with 0.1 M NaOH (pH value was about 12) and formed as hydrogels with different concentrations. Chitosan hydrogel films with the thicknesses as 130 μm and pH value as 12 for the environment of gelation process were all remained for the test. Fig. 2 illustrates the experimental results obtained for the effective LB, LD, CB and CD properties of the chitosan hydrogel films with different chitosan concentrations. Note that in implementing the proposed Mueller matrix method, 1024 data points were obtained for the effective parameters (α, β, θ_d, D, γ, R, e_1, e_2, and e_3) of each sample and 100 points were then chosen in order to calculate the mean value of each parameter.

It is seen in Fig. 2(a) that the principal axis angle (α) varies randomly in the range of 30–120° given an increasing chitosan concentration from 1 to 2.5 g/100 ml acetic acid. Also, the corresponding phase retardations all close to zero are found in Fig. 2(b). Peppas and Lucht (1986) reported that hydrogels comprise a macro-molecular network-like structure when swollen in water or biological fluids. In other words, the chitosan polymer chains crosslink randomly and form a three-dimensional (3-D) network; giving rise to a random principal axis angle. As the chitosan concentration increases from 2.5 to 3.5 g/100 ml acetic acid, the phase retardance in Fig. 2(b) increases from 0° to 16°. In Fig. 2(a), the corresponding the principal axis angles are all close to 45° and it can be explained that the chitosan chains were arranged much orderly with the higher chitosan concentrations. Berger, Reist, Mayer, Felt, Peppas, and Gurny (2004) showed that in chitosan hydrogels, the chitosan polymer chains may crosslink with one another covalently to form a 3-D network. In the chitosan hydrogel films prepared in the present study, the number of polymeric chains per unit volume of sample increases with an increasing chitosan concentration. As a consequence, the density of the 3-D network also increases and gives rise to a corresponding increase in the phase retardance (β).

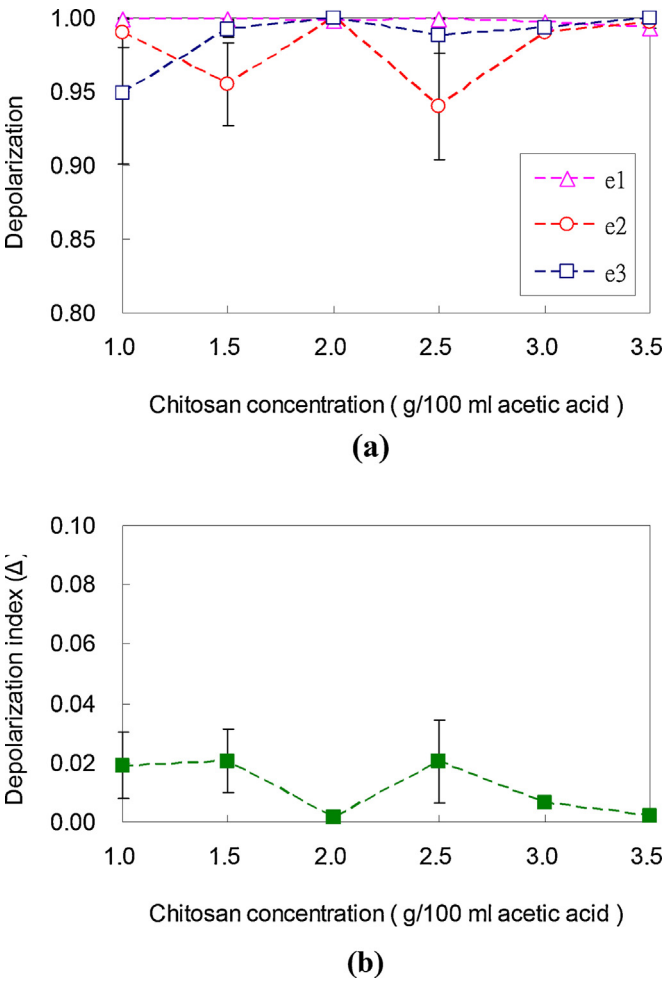

Fig. 3. (a) The degrees of linear depolarization (e_1, and e_2) and the degree of circular depolarization (e_3) and (b) the depolarization index (Δ) of chitosan hydrogel films with various chitosan concentrations.

As shown in Fig. 2(d), the diattenuation of the present chitosan hydrogel films is less than 0.15 and the diattenuation axis angle (θ_d) varies randomly between 15° and 45° (see Fig. 2(c)) as the chitosan concentration is increased. As a result, chitosan hydrogels have much less LD property. Moreover, Fig. 2(e) shows that irrespective of the chitosan concentration, the optical rotation angle of the chitosan hydrogel films is less than 1.5°. In other words, the chitosan hydrogel films have no CB property. Similarly, in Fig. 2(f), the circular diattenuation (CD) has a value of less than 0.01 for all considered values of the chitosan concentration. Thus, it is implied that the chitosan hydrogel films also have no CD property.

Additionally, the degrees of linear depolarization (e_1 and e_2) and the degree of circular depolarization (e_3) of various chitosan hydrogel films (chitosan concentrations increased from 1.0 to 3.5 g/100 ml acetic acid solution) were shown in Fig. 3(a). Thus, the depolarization index (Δ) of chitosan hydrogel films with various chitosan concentrations were shown in Fig. 3(b). It is noted that Pham and Lo (2012a, 2012b) used a quartz containers (45 mm × 10 mm × 10 mm ($h \times d \times w$); capacity, 4.5 ml) to measure the depolarization index (Δ) of the turbid media named an aqueous suspension of polystyrene beads (diameter of poly styrene beads = 5 μm) and its depolarization index (Δ) was about 0.35. In our study, the light cannot penetrate to chitosan hydrogel film when the thickness was about 10 mm; therefore, the thickness about 130 μm is chosen for measuring the depolarization index. As a result, the data displayed that e_1, e_2 and e_3 were all close to 1 and

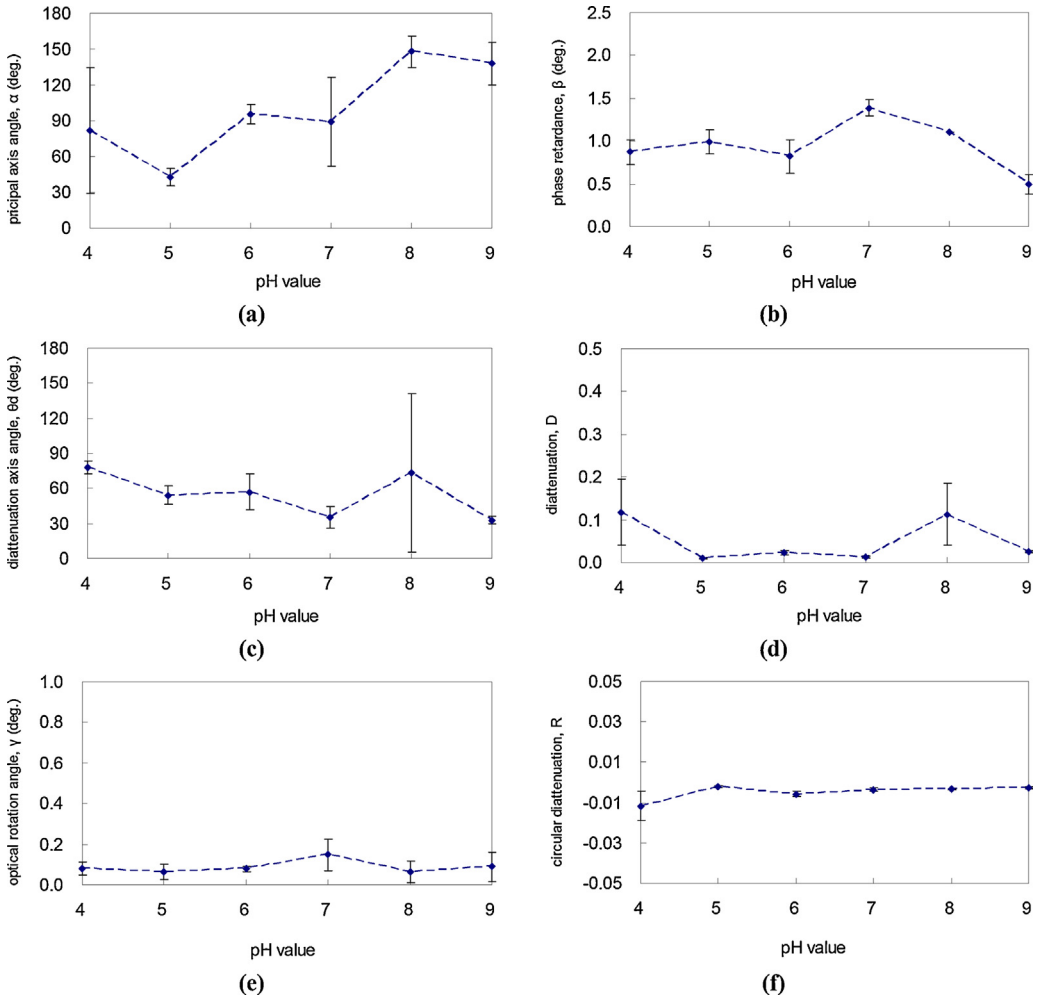

Fig. 4. Variations of (a) principal axis angle α, (b) phase retardance β, (c) diattenuation axis angle θ_d, (d) diattenuation D, (e) optical rotation γ, and (f) circular diattenuation R of chitosan hydrogel films crosslinked in CBS with different pH values.

the depolarization index (Δ) was less than 0.03 with various chitosan concentrations. In other words, no significant depolarization of the light occurs while chitosan concentration is increased.

In order to study the reliability in statics between chitosan concentration and effective optical parameters of anisotropic chitosan hydrogel films, One-Way ANOVA technique is applied to analyze those experimental data. The P value <0.05 was considered to be statistically significant, and P value <0.001 was considered to be highly statistically significant (Schmittgen & Zakrajsek, 2000). Thus, the results displayed that P value of phase retardance (β) in Fig. 2(b) was less than 0.001 so it was considered to be highly statistically significant. As expected that the other P values in Fig. 2 (except Fig. 2(a) and (b)) are all larger than 0.05, thus their corresponding effective optical parameters are statically insignificant to various chitosan concentrations.

4.1.2. Chitosan hydrogel films crosslinked in CBS with different pH values

Chitosan hydrogel films crosslinking by different pH values of CBS were prepared and the thicknesses as 130 μm were all remained for the test. Fig. 4 illustrates the experimental results obtained for the effective LB, LD, CB and CD properties of the chitosan hydrogel films crosslinked in CBS with different pH values. As shown in Fig. 4(a), the principal axis angle (α) displays randomly from 30° to 150° as the pH value increases from pH 4 to pH 9. Chitosan was composited by polymer chains and formed hydrogels, and then polymer chains of chitosan were arranged into polymer-rich aggregates without liquid crystal phase. In the macro observation, polymers chains of chitosan were dispersed randomly so the principal axis angles displayed randomly. According to Berger, Reist, Mayer, Felt, Peppas, and Gurny (2004), chitosan hydrogels may be formed as a result of either covalent bonding between the chitosan polymer chains or ionic bonding between the chitosan polymer chains and negatively charged crosslinkers.

In Fig. 4(b), the phase retardance (β) roughly increases from 0.87° to 1.39° as the pH value increases from pH 4 to pH 7 (i.e., acidic environment), but reduces from 1.39° to 0.49° as the pH value increases from pH 7 to pH 9 (i.e., alkaline environment). It is noted that the macro pK_a of chitosan is 6.3, and thus chitosan is soluble in acid solutions with a pH value of less than 6.3. In an acidic environment, the amine compound of chitosan is ionized (adopts a positive charge) and bonds with the crosslinkers with a negative charge. Sufficient charge numbers are necessary for anions to cross-link chitosan by electrostatic force. Citrate has three anions and the charge numbers of the anions were mainly controlled by solution pH. With the increase of solution pH from acidic to neutral condition, the charge numbers of citrate increased. Shu, Zhu, and Song (2001) indicated that the low charge density of the citrate at low pH (pH values less than 4) and rising charge numbers led to the increasing pH value. For chitosan, the increase of pH value resulted in the decrease of the ionization degree of amine groups greatly. Furthermore, citrate with $pK_{a1} = 3.138$, $pK_{a2} = 4.76$ and $pK_{a3} = 6.40$ ionized one charge number for pH values less than 4, two charge numbers for pH values less than 5, and three charge numbers for

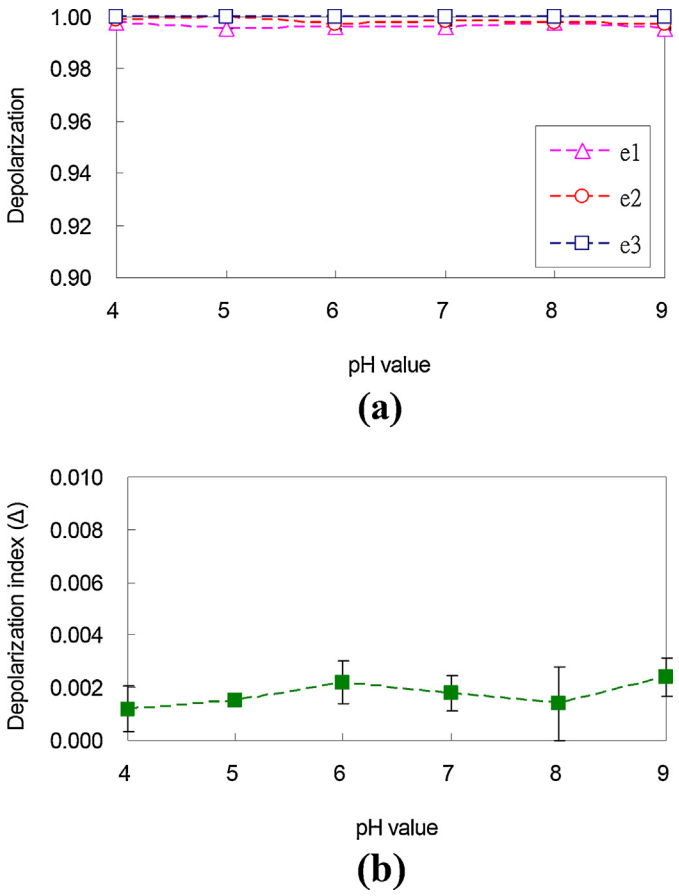

Fig. 5. (a) The degrees of linear depolarization (e_1, and e_2) and the degree of circular depolarization (e_3) and (b) the depolarization index (Δ) of chitosan hydrogel films crosslinked in acidic and alkaline CBS environments.

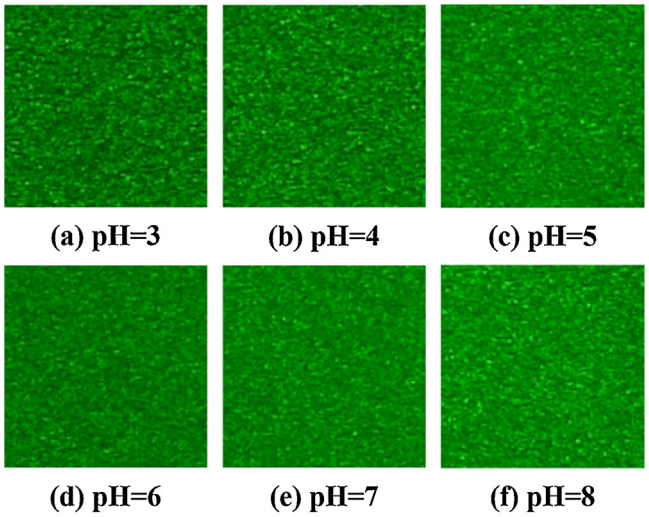

Fig. 6. (a)–(f) Green laser speckle images of chitosan hydrogel suspension crosslinked in CBS with different pH values (image size: 150 × 150 pixels).

pH values less than 7, respectively. In other words, the crosslinking ability of the CBS solution increases with an increasing pH value and gives rise to a greater crosslinking density as a result.

In addition, the crosslinking reaction is influenced (Shu, Zhu, & Song, 2001) by the size of crosslinkers during the crosslinking process. In general, small-scale crosslinkers diffuse more rapidly in the chitosan hydrogel network than large-scale crosslinkers. In alkaline environments, the chitosan polymer chains are neutralized by hydroxyl groups. Moreover, the crosslinkers (i.e., citrate) have a large size, and therefore diffuse more slowly than the hydroxyl groups. As a result, the crosslinking density is reduced in alkaline environments. Consequently, as shown in Fig. 4(b), the phase retardance (β) reduces with an increasing pH value in the alkaline crosslinking environment.

Fig. 4(d) shows that the hydrogel films have a much lower diattenuation (D) as the pH value of the CBS increases from pH 4 to pH 9. Also, it is found that the diattenuation axis angle (θ_d) varies randomly in the range of 0° to 150° as the pH value of the CBS increases, as shown in Fig. 4(c). Fig. 4(e) shows that the optical rotation angle (γ) is less than 0.3° for all the pH values. Similarly, Fig. 4(f) shows that the circular diattenuation (R) has a value of less than 0.01 for all of the considered crosslinking environments. Thus, it is inferred that the chitosan hydrogel films have neither CB nor CD properties.

Fig. 5 shows the experimental results obtained for the depolarization of the chitosan hydrogel films crosslinked in acidic and alkaline CBS solutions. The degrees of linear depolarization (e_1, and e_2) and the degree of circular depolarization (e_3) of the chitosan hydrogel films crosslinked in acidic and alkaline CBS solutions were shown in Fig. 5(a). The depolarization index (Δ) of the chitosan hydrogel films crosslinked in acidic and alkaline CBS solutions was shown in Fig. 5(b). It is observed that Δ has a much low value of less than 0.004 in every case. Again, the lower depolarization index was due to the thinner thickness of measured samples in scattering the light. In other words, none of the chitosan hydrogel films crosslinked in acidic and alkaline CBS solutions causes a depolarization of the light.

Furthermore, the results displayed that P value of phase retardance (β) in Fig. 4(b) was about 0.013 and less than 0.05, so it was considered to be statistically significant. Thus, as shown in Fig. 4(b), the phase retardance (β) increases with an increasing pH value within the acidic crosslinking regime and the phase retardance (β) reduces with an increasing pH value in the alkaline crosslinking regime. As expected that the other P values in Figs. 4 and 5 (except Fig. 4(b)) are all larger than 0.05 and thus their corresponding effective optical parameters are statically insignificant to the chitosan hydrogel films crosslinked in acidic and alkaline CBS solutions.

It is concluded that LB property in phase retardance (β) were obviously responded to the variation of chitosan concentration and crosslinking environment. In general, the results presented above show that the principal axis angle α and diattenuation axis angle θ_d of the chitosan hydrogel films vary randomly with an increasing chitosan concentration or CBS pH value. Moreover, the results show that the chitosan hydrogel films have no obvious properties on LD, CB and CD. However, the results presented in Figs. 2(b) and 4(b) show that the phase retardance β is directly related to both the chitosan concentration and the pH value of the CBS crosslinking solution. In other words, the results confirm that the Mueller matrix method proposed in (Pham & Lo, 2012a, 2012b) provides a feasible means of characterizing both the concentration and the crosslinking environment of chitosan hydrogel films.

4.2. Speckle contrast measurement by SLPP

Fig. 6(a)–(f) presents the green speckle images obtained for the chitosan hydrogel suspension prepared in different crosslinking environments. (Note that in (Chuang, Sung, Huang, & Lo, 2012), it was shown that the green laser provides a greater speckle contrast than the red, blue or white lasers, and thus only the green speckle images are shown here.) Fig. 7 presents the corresponding results for the variation of the speckle contrast with the pH value of the CBS crosslinkers. It is observed that the speckle contrast is directly related to the pH value of the crosslinking environment.

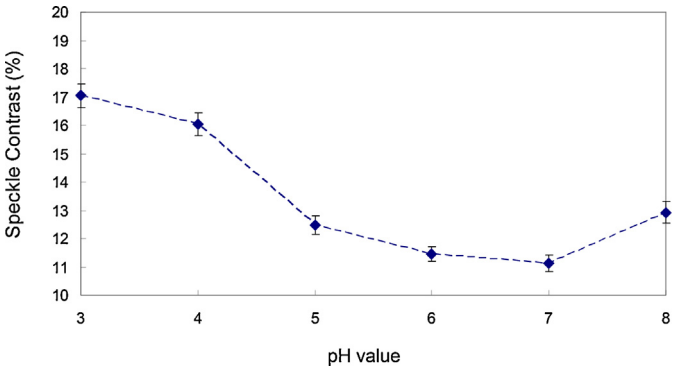

Fig. 7. Variation of speckle contrast with CBS pH value under green laser illumination.

Specifically, the speckle contrast reduces from 17.04% to 11.12% as the pH value of the CBS solution increases from pH = 3–7, but increases up to 12.92% as the pH value further increases to pH = 8. Furthermore, the results displayed that P value of speckle contrast was <0.001, so it was considered to be highly statistically significant. Thus, as shown in Fig. 7, the speckle contrast decreases with an increasing pH value within the acidic crosslinking regime but increases within the alkaline environment.

As described previously, for $pK_{a1} = 3.138$, $pK_{a2} = 4.76$ and $pK_{a3} = 6.40$, the CBS solution ionizes one reactive position for pH values less than 4, two reactive positions for pH values less than 5, and three reactive positions for pH values less than 7. The results presented in Fig. 7 show that for a CBS solution with pH = 3, crosslinking does not occur, and thus a high speckle contrast is obtained. Thereafter, the crosslinking degree increases with an increasing pH value in the acidic range; resulting in a lower speckle contrast, but the crosslinking degree decreases with an increasing pH value in the alkaline range; resulting in a higher speckle contrast.

Overall, the Mueller matrix method and SLPP method both enable the pH value of the CBS crosslinkers to be reliably determined by means of the phase retardance property β and speckle contrast, respectively. However, it is noted that the speckle contrast provides a more sensitive measurement of the CBS crosslinking environment.

5. Conclusions

The LB, CB, LD, CD and depolarization properties of chitosan hydrogel films prepared with different chitosan concentrations and in different crosslinking environments have been evaluated using the analytical Mueller matrix method. The phase retardance is directly related to both the chitosan concentration and the pH value of crosslinking environments. Specifically, the phase retardance increases continuously with an increasing chitosan concentration. Moreover the phase retardance increases with an increasing pH value in the acidic range, but decreases in the alkaline range. Additionally, the crosslinking environment of chitosan hydrogel suspension with low-concentration has been determined by measuring the speckle intensity of SLPP. The speckle intensity reduces as the pH value increased at acid environment, but increases at alkaline environment.

Overall, the present results have demonstrated that the crosslinking environment was reliably determined by analytical Mueller matrix method and SLPP method. However, of the two methods, the speckle contrast method yields improved measurement sensitivity. In general, this study provided effective tools for characterizing the concentration and crosslinking environment with a variety of chitosan hydrogel films and suspension. The two methods are expected to be potential for biomedical, chemical and environmental monitoring applications.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the National Science Council of Taiwan under Grant No. NSC99-2221-E-006-034-MY3. This research received funding from the Headquarters of University Advancement at the National Cheng Kung University, which is sponsored by the Ministry of Education, Taiwan, R.O.C.

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