Preparation and characterization of biopolymers comprising chitosan-grafted-ENR via acid-induced reaction of ENR50 with chitosan

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Abstract. This paper describes the first detailed tailored-approach for the preparation of biopolymers comprising chitosan (CTS) grafted onto the backbone of epoxidized natural rubber (CTS-g-ENR). In a typical experiment, appropriate amount of CTS and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added to a specified amount of ENR50 (ENR with about 50% epoxy content) dissolved in a dual-solvent consisting of 1,4-dioxane and water (97.5:2.5% v/v) and the resulting mixture refluxed with continuous stirring for 6 hours. Nuclear magnetic resonance (NMR) spectral analysis of a biocomposite, CTS-g-ENR-P1, revealed that its epoxy content is 22.36% which is considerably lower than 44.93% as determined for ENR50-control (ENR50 derivative obtained under similar experimental condition but in the absence of CTS). This means that the grafting of CTS onto the backbone of ENR had occurred. The revelation is affirmed by the presence of the characteristic absorption bands of CTS and ENR, and the appearance of new bands at 1219, 902 and 733 cm^{-1} in the Fourier transform infrared (FTIR) spectrum of CTS-g-ENR-P1. Further evidence that CTS had been successfully grafted onto the backbone of ENR can be deduced and described in this paper from the data obtained by means of Differential Scanning Calorimetric analysis, Thermogravimetric analysis and Variable Pressure Scanning Electron Microscopy.

Keywords: biopolymers, chitosan, epoxidized natural rubber

1. Introduction

Chitin is a linear biopolymer and the second most abundant natural polysaccharide after cellulose [1]. The usual commercially available chitin is typically extracted from crab and shrimp shells. Chitosan is derived from chitin [2]. It is composed of predominantly $\alpha(1\rightarrow4)$ -linked 2-amino-2-deoxy- β -D-glucopyranose with some amount of, 2-acetamido-2-deoxy-D-glucopyranose sugars, depending on the degree of N-deacetylation [3–5]. Due to the presence of the primary amino (C-2), primary (C-6) and secondary (C-3) hydroxyl groups on each repeat unit, chitosan is indeed a multinucleophilic material that may undergo specific reaction with other materials

that contain electrophilic sites. Chitosan is being used in a wide range of applications ranging from biomedical engineering, pharmaceutical and cosmetic products to water treatment and plant protection [6]. Being a natural polysaccharide, chitosan exhibits high biodegradability but have poor mechanical properties. As such, modification of polymers that possess excellent thermal and mechanical parameters with chitosan may result in the production of a new class of polymeric materials exhibiting not only desired biodegradability but also good physicochemical and mechanical properties [7]. Incorporation of chitosan in the matrices of elastomers is of interest to researchers in areas that

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involve the utilization of natural rubber or its derivatives and chitosan [8–16]. Of special interest to our current work, Lertwattanaseri *et al.* [13] reported the preparation of a novel model case of bionanocomposites comprising nanofibrous chitosan bonded to the backbone of epoxidized natural rubber (ENR) via both amino and hydroxyl groups. Confirmation of the proposed structure for the ENR–chitosan bionanocomposite was done by qualitative and quantitative FTIR techniques. Considering the nature of the reactivity of the hydroxyl and amino groups of chitosan, we believe the proposed structure is inconclusive. Very recently, Riyajan and Sukhlaaied reported the preparation of ENR-g-chitosan involving the reaction of chitosan radicals with ENR molecules using potassium persulphate ($K_2S_2O_8$) as an initiator [16]. The chemical structure of the ENR-g-chitosan, specifically the presence of ether linkage in the biopolymers, was confirmed by 1H -NMR and ATR-FTIR.

Chitosan is soluble in acidic aqueous media [17, 18] whereas ENR, being hydrophobic in nature, is not [19]. This is the likely reason why there is very limited number of reports in the literature on homogeneous (liquid-liquid phase) or semi-heterogeneous (liquid-solid phase) reaction condition involving chitosan and ENR. Furthermore, ENR is known to undergo ring-opening and double bond cleavage reactions [20–25]. To date, there is no report on the stability of the microstructure of ENR in conditions with different concentrations of acid. In view of this finding and as part of our interest to gain further insights on the physicochemical and mechanical properties of natural rubber latex films that were subjected to acid and/or base treatments [26–27], we have carried out the study on the effect of acidity on the stability of the molecular structure of ENR50. We found by means of NMR spectroscopy that the epoxidized isoprene units of ENR50 did not undergo significant ring-opening reaction in 1,4-dioxane acidified by the addition of dilute HCl or $AlCl_3 \cdot 6H_2O$ solution. This observation prompted us to investigate and hereby report our findings on the acid-induced reaction of ENR50 with chitosan.

2. Experimental

2.1. Materials

Epoxidized natural rubber with about 50% epoxy content (designated as ENR50) and with relative

M_w of $3.8 \cdot 10^5$ g/mol was supplied by Malaysian Rubber Board, Kuala Lumpur, Malaysia. Chitosan (CTS) with relative M_w of $1.0 \cdot 10^5$ g/mol was provided by Advanced Materials Research Centre at Kulim, Kedah, Malaysia. The degree deacetylation of the CTS was determined by a titration method [28] and found to be 96%. HCl and $AlCl_3 \cdot 6H_2O$ (purchased from Merck, Darmstadt, Germany), 1,4-dioxane (Fisher, Malaysia) and NaOH (System Chemar) were used as received. Deuterated chloroform (Merck, 99.8% with 0.03% TMS) was used as the NMR solvent.

2.2. Instruments

1H -NMR spectra of samples dissolved in $CDCl_3$ were recorded on a Bruker Avance-400 NMR spectrometer operating at 400.13 MHz. Tetramethylsiloxane (TMS) was used as the internal reference. Infrared spectra were recorded using a Perkin-Elmer 2000 Infrared Spectrometer with 16 scans at 4 cm^{-1} resolution. Solid ENR50 and CTS-g-ENR biopolymers were analysed directly using an attenuated total reflection (ATR) technique within the wave number range of $4000\text{--}650\text{ cm}^{-1}$. CTS was analyzed in the form of KBr pellet (KBr: CTS = 98:2) within the wave number range of $4000\text{ to }400\text{ cm}^{-1}$. Differential Scanning Calorimetry (DSC) analysis was carried out using Perkin-Elmer Pyris-1 DSC, equipped with an internal cooler 2P-cooling accessory and calibrated using n-decane and indium. Samples (5–10 mg) were examined within an atmosphere of dry nitrogen gas maintained at a flow rate of 50 mL/min. All samples were annealed at a heating rate of $20^\circ\text{C}/\text{min}$ (for both heating and quenching). For the first scan, samples were heated from -50°C up to at least 100°C and hold for about 2 minutes before cooling (at $20^\circ\text{C}/\text{min}$) back to -50°C . The second scan was performed after 2–5 minutes of waiting time. Thermal weight loss measurements were made using Perkin-Elmer TGA-7 thermogravimetric analyser. Testing was carried out under a stream of dry nitrogen gas (flow rate = 30 mL/min) at temperature range of 40 to 600°C with heating rate of $10^\circ\text{C}/\text{min}$. Weight of samples used was within 4–10 mg. Data of Variable Pressure Scanning Electron Microscopy (VPSEM) was obtained with the use of Carl Zeiss EVO LS 10 Scanning Electron Microscope. The morphology of the ENR50, CTS and CTS-g-ENR biopolymers were studied.

2.3. Methods

2.3.1. Exposure of ENR50 to different concentrations of HCl or $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$

ENR50 (1.0 g) was dissolved in 95 mL 1,4-dioxane at room temperature inside a 250 mL two-necked round bottom flask equipped with a magnetic stirrer and a reflux condenser. Then 5 mL of 0.0001 M HCl was added dropwise and the resulting solution stirred for 4 hours. A sample of the solution (10 mL) was isolated every hour and placed in about 10 mL of chilled 0.0001 M sodium hydroxide to neutralize and precipitate the resulting ENR50 derivative. This derivative was isolated and washed with ample amount (>200 mL) of distilled water. Then it was placed in an oven with the temperature maintained at 50°C for 24 hours. The procedure was repeated but with the use of 0.001 M, 1.0 M and 3.0 M HCl. All resulting polymeric materials were analysed by NMR spectroscopy.

The experiment was repeated with the use of 0.75 and 0.30 g of aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) in lieu of HCl. ENR50 derivatives obtained after been exposed to $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ for 2, 4 and 24 hours were analysed by NMR spectroscopy.

2.3.2. Reaction of chitosan with ENR50 in acidified 1,4-dioxane

ENR50 (1.0 g) was dissolved in a dual-solvent consisting of water and 1,4-dioxane (2.5:97.5% v/v) inside a 250 mL two-necked round bottom flask equipped with a magnetic stirrer and a reflux condenser. Then CTS (1.0 g) and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (0.75 g) were added and the content of the flask was refluxed with continuous stirring for 6 hours. Subsequently, the resulting mixture was cooled and filtered. The filtrate containing the product was concentrated under reduced pressure using a rotary evaporator and cast on a glass plate. The product (CTS-g-ENR-P1) was dried in an oven (50°C) for 24 hours.

The experiment was repeated with the use of a lower concentration (i.e. 0.30 g) of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. The product obtained is designated as CTS-g-ENR-P2. For the purpose of obtaining a reference sample (ENR50-control), ENR50 was subjected to similar reaction condition with the use of 0.75 g of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ but in the absence of CTS. All resulting polymeric materials were characterized by means of FT-NMR, FT-IR, SEM, DSC and TGA techniques.

4. Results and discussion

4.1. Effect of acidity on the stability of the molecular structure of ENR50

^1H -NMR spectra of ENR50 and ENR50 derivatives that were obtained after ENR50 been exposed to 0.0001, 0.001, 1.0 and 3.0 M of HCl for 1 hour are depicted in Figure 1. The epoxy group of an epoxidized natural rubber is known to be randomly distributed on the backbone of the polymer. Nonetheless, a fairly comprehensive characterization of the structure of liquid epoxidized natural rubber by means of 1D-NMR and 2D-NMR spectroscopy is available in the literature [29]. For simplicity, the proton resonances of ENR50 as observed in Figure 1 are assigned to a simplified structure depicted in Figure 2 as follows: methyl protons bonded to the epoxidized isoprene unit **a** ($-\text{CH}_2-\text{CH}_3\text{COCH}-\text{CH}_2-$) = 1.29 ppm; methylene protons bonded to the epoxidized isoprene unit **b** ($-\text{CH}_2-\text{CH}_3\text{COCH}-\text{CH}_2-$) = 1.55 ppm, methyl protons bonded to the isoprene unit **c**

Figure 1. ^1H -NMR spectra of (i) ENR50 and its derivatives obtained after ENR50 has been exposed (ii) 0.0001 M, (iii) 0.001 M, (iv) 1.0 M and (v) 3.0 M of HCl for 1 hour

Figure 2. A simplified structure of ENR50

($-\text{CH}_2-\text{CH}_3\text{C}=\text{CH}-\text{CH}_2-$) = 1.67 ppm, methylene protons bonded to the isoprene unit **d** ($-\text{CH}_2-\text{CH}_3\text{COCH}-\text{CH}_2-$) overlapped with the methylene protons bonded to the epoxidized isoprene unit **e** ($-\text{CH}_2-\text{CH}_3\text{COCH}-\text{CH}_2-$) = 2.04 ppm; methylene protons bonded to the isoprene unit **f** ($-\text{CH}_2-\text{CH}_3\text{COCH}-\text{CH}_2-$) = 2.15 ppm; methine proton bonded to the epoxidized isoprene unit **g** ($-\text{CH}_2-\text{CH}_3\text{COCH}-\text{CH}_2-$) = 2.70 ppm; methine proton bonded to the isoprene unit **h** ($-\text{CH}_2-\text{CH}_3\text{C}=\text{CH}-\text{CH}_2-$) = 5.10 ppm. The presence of residual amount of 1,4-dioxane and chloroform can be observed at 7.16 and 3.70 ppm. The epoxy content [30] can be determined with the use of Equation (1):

$$\text{Epoxy content} = \frac{A_{2.7}}{A_{2.7} + A_{5.1}} \cdot 100\% \quad (1)$$

where $A_{2.7}$ and $A_{5.1}$ refer to the integral area of **g** = 2.7 and **h** = 5.1, respectively. From Figure 1(i), the actual epoxy content of ENR50 is determined to be 51.05% whereas that of the ENR50 derivatives (Figure 1(ii)–1(v)) obtained after ENR50 having been exposed to 0.0001, 0.001, 1.0 and 3.0 M HCl is 46.98, 46.12, 14.09 and 10.55%, respectively. The effect of acidity on the stability of the molecular structure of ENR50 becomes clear when we consider the degree of reduction of its epoxy content. The difference in epoxy content of ENR50 and that of the ENR50 derivative obtained after having been exposed to 1.0 and 3.0 M HCl is 36.96 and 40.50%, respectively. However, when in 0.0001 and 0.001 M the difference is only 4.07 and 4.93%, respectively. This means the molecular structure of ENR50 remained fairly intact at low acid concentration ($[\text{H}^+] \leq 0.001 \text{ M}$) even though its epoxidized isoprene units were likely to be fully protonated and susceptible to undergo ring-opening reaction with nucleophiles.

4.2. Effect of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ on the epoxidized isoprene unit of ENR50

A solution containing $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ is mildly acidic (pH = 4.5) due to the formation of hydronium ion as illustrated by the Brønsted-Lowry acid-base reac-

Figure 3. Acid hydrolysis of aluminium ion

tion in Figure 3. When $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ dissolved in water, the aluminium ion will be surrounded with six water molecules. The Al^{3+} aqua complex ion, $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$, is a weak Brønsted-Lowry acid that donates a proton, H^+ , forming the Al^{2+} aqua complex ion, $[\text{Al}(\text{H}_2\text{O})_5\text{OH}]^{2+}$, whereas the water molecule acts as a weak Brønsted-Lowry base that accepts the proton forming the hydronium ion, H_3O^+ .

^1H -NMR spectra of ENR50 derivatives that were obtained after ENR50 having been exposed to $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ for 2, 4 and 24 hours are depicted in Figure 4. Their corresponding epoxy content is determined to be 44.93, 44.04 and 38.84%, respectively. In keeping with the above finding wherein diluted HCl was used, the epoxidized isoprene units of

Figure 4. ^1H -NMR spectra of ENR50 derivatives obtained after ENR50 has been exposed to $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ for (i) 2 hr, (ii) 4 hr and (iii) 24 hr

ENR50 did not undergo significant ring-opening reaction even though being likely fully protonated in a solution containing $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$.

4.3. Nuclear magnetic resonance spectral analysis

Figure 5 depicts the fourier transformed ^1H -NMR spectra of ENR50-control and CTS-g-ENR-P1. The epoxy content of ENR50-control and CTS-g-ENR-P1 is determined to be 44.93 and 22.36%, respectively. It is therefore evident that ENR50 underwent a substantial reduction in epoxy content only in the presence of CTS. As such, it is reasonable to deduce that the grafting of CTS onto the backbone ENR50 had likely occurred via ring-opening reaction of protonated epoxidized isoprene units. The plausible reaction pathways leading to the formation of CTS-g-ENR biopolymers is discussed later in the text.

4.4. Infrared spectral analysis

Figure 6 shows the Fourier transform infrared spectra of ENR50, CTS and CTS-g-ENR-P1. The characteristic vibrational absorption bands of ENR50: 2961, 2923 and 2856 cm^{-1} are due to C–H stretchings of the $-\text{CH}_3-$ and $-\text{CH}_2-$ groups, 1660 cm^{-1} is due the C=C stretching, 1448 and 1376 cm^{-1} are due to C–H bendings of the $-\text{CH}_2-$ and $-\text{CH}_3-$ groups, 1259 and 1018 cm^{-1} are due to C–O–C stretching and bending of the epoxy group, 873 cm^{-1} is due to C–H bending attached to the epoxy group

and 837 cm^{-1} is due to C–H bending attached to the unsaturated carbon [31]. The characteristic bands of CTS: broad band at 3600–3100 cm^{-1} are due to a combination of O–H and N–H stretchings of the hydroxyl and primary amine groups, 2956 cm^{-1} is due to the C–H stretching of the $-\text{CH}_2-$ group, 1638 and 1560 cm^{-1} (overlapped) are due to the N–H bendings of the amide and primary amine groups, broad band at 1080–1033 cm^{-1} is due the skeletal vibration involving C–O stretching, 896 cm^{-1} is due the stretching of C–O–C linkage [32]. For CTS-g-ENR-P1 (Figure 6(iii)), the normally broad band due to the absorption of hydroxyl and amino groups became narrower and shifted to a lower frequency, centered at about 3322 cm^{-1} , indicating that chemical reaction had taken place predominantly at the primary amine rather than at the hydroxyl groups. The considerable reduction of the intensity of the band at 873 cm^{-1} suggests that the epoxy group was involved in the reaction. The presence of the characteristic absorption band at 1645 cm^{-1} due to the N–H bending suggests that the primary amine group of chitosan was involved in the reaction. Furthermore, the appearance of additional bands at 733, 902 and 1219 cm^{-1} assignable to N–H wag, C–N bond and saccharide structure of chitosan, respectively [33], affirms that CTS-g-ENR-P1 was produced likely via the acid-induced attack of the primary amine of the former on the epoxide ring of the latter.

Figure 5. ^1H -NMR spectra of (i) ENR50-control and (ii) CTS-g-ENR-P1

Figure 6. FTIR spectra of (i) ENR50, (ii) CTS and (iii) CTS-g-ENR-P1

4.5. Differential scanning calorimetric analysis

The thermograms (DSC second run curves) of CTS/ENR50-control, CTS-g-ENR-P1 and CTS-g-ENR-P2 are illustrated in Figure 7. Two cycles of heating and cooling runs were carried out in order to eliminate the moisture effect, and the temperature region was taken into consideration to avoid thermal degradation in the first heating. The T_g of CTS/ENR50-control, sample prepared by physically mixing appropriate amounts of CTS and ENR50, was found to be -27.24°C . This T_g is very close to that of ENR50 (-23.90°C), indicating no reaction took place under this condition. The T_g of CTS is not observed in Figure 7 because it occurred at a higher temperature, 203°C [34], i.e., outside the temperature range indicated. The T_g of CTS-g-ENR-P2, the biocomposite obtained with the use of a lower concentration of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, is 2.88°C . The fact that only one T_g was detected for CTS-g-ENR-P2 exemplify a new product that had formed arising from the grafting of CTS onto the backbone ENR50. The T_g of CTS-g-ENR-P1, the biocomposite obtained with the use of a higher concentration of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, is also not

Figure 7. DSC curves of the CTS/ENR50-control, CTS-g-ENR-P1 and CTS-g-ENR-P2

observed in Figure 7 because it is closer to that of CTS which occurred outside the temperature range indicated.

4.6. Thermogravimetric analysis

The thermogravimetric (TGA) and derivative thermogravimetric (DTG) curves of ENR50, CTS, CTS-g-ENR-P1 and CTS-g-ENR-P2 are depicted in Figure 8 and Figure 9, respectively. The DTG curve of ENR50 exhibits one-step degradation process with rapid weight loss of 48% at 418°C . The major mass loss occurred within 310 and 570°C is 96% due to the total pyrolysis (decomposition of the hydrocarbon in nitrogen atmosphere) of the rubber matrices. The amount of carbonized residue deduced from the TGA curve of ENR50 beyond 600°C is about 2.5%. DTG curve of CTS exhibits mass loss at two stages. The initial mass loss of 12% occurred within 40 and 240°C due to loss of solvent molecules (water and acetic acid). The second stage shows a mass loss of 52% within 240 and 400°C due to degradation (via chain scission) of CTS [35]. The amount of carbonized residue deduced from the TGA curve of CTS beyond 600°C is about 33.8% which is quite substantial compared to that of ENR50. However, from the view point of thermal stability it is of interest to note that within 270 and 340°C only about 1% of ENR50 degraded whereas CTS degraded by about 38%. Therefore, ENR50 is thermally more stable than CTS.

Both DTG curves of CTS-g-ENR-P1 and CTS-g-ENR-P2 show approximately similar three degradation stages. The first mass loss of 3% occurred within 40 and 150°C attributable to solvent molecules (water and 1,4-dioxane) imbedded in the biopolymers. The second mass loss of 15% occurred within 140 and 300°C due to the destruction of amino

Figure 8. TGA curves for ENR50, CTS, CTS-g-ENR-P1 and CTS-g-ENR-P2

Figure 9. DTG curves for ENR50, CTS, CTS-g-ENR50-P1 and CTS-g-ENR50-P2

groups of CTS [9]. The third is the major mass loss of 56% occurred within 300 and 594°C due to a combination of chain scission of the grafted CTS and total decomposition of the rubber matrices. The amount of carbonized residue deduced from the TGA curves of CTS-g-ENR-P1 and CTS-g-ENR-P2 beyond 600°C is 24 and 9%, respectively. Therefore, it is apparent that lesser amount of CTS incorporated (grafted) onto the rubber matrices resulted in a lower amount of carbonized residue beyond 600°C. The thermal stabilities of CTS-g-ENR-P1 and CTS-

g-ENR-P2 are quite similar and likely due to a combination of that of CTS and ENR50, i.e., higher than that of CTS but lower than that of ENR50.

4.7. Morphology study

SEM micrographs of CTS, ENR50 and CTS-g-ENR-P1 are shown in Figure 10. The chitosan has a flaky and uneven shape with smooth surface but the ENR50 displays a rough and groove like structure with some debris. Micrographs of the biocomposite show a one-phase morphology with a reduced gap between the chitosan and the rubber matrices suggesting not only good interfacial interactions but also very likely that CTS has been successfully grafted onto the backbone of the ENR.

4.8. Reaction pathways for the formation of CTS-g-ENR biopolymers

The plausible reaction pathways for the formation of CTS-g-ENR biopolymers are proposed in Figure 11. In a mildly acidic condition such as in solution containing $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (pH = 4.5), the epoxidized isoprene units of ENR50 would likely be protonated and susceptible to attack by reactive nucleophiles. How-

Figure 10. SEM micrographs of (i) CTS, (ii) ENR50, (iii) CTS-g-ENR-P1 at magnification of 42×, (iv) CTS-g-ENR-P1 biopolymers at magnification of 500×

Figure 11. Reaction pathways for the formation of CTS-g-ENR biopolymers

ever under the similar mildly acidic condition, CTS being a multinucleophilic material would be partially protonated. The unhindered primary (C-6) hydroxyl group of CTS would be protonated rendering it non-reactive, whereas some of the sterically hindered primary amino (C-2) and secondary (C-3) hydroxyl groups would be free, i.e., remain as reactive nucleophiles. A primary amino group is much more reactive than a secondary hydroxyl group. As such, the grafting of CTS onto the backbone of ENR50 would involve solely the attack of the primary amino (C-2) of CTS on the protonated epoxidized isoprene units of ENR50. The attack may occur via sterically favored pathway (a) and sterically disfavored pathway (b) as illustrated in Figure 11. However, considering that CTS is a bulky polymeric entity and the fact that ENR50 remained structurally stable in the mildly acidic condition, we are of the opinion that the acid-induced reaction of ENR50 with CTS occurred preferentially or regioselectively via the sterically favored pathway. This notion is further supported by the infrared spectral analysis described above.

5. Conclusions

The effect of acidity on the stability of the molecular structure of ENR50 was investigated. It is hitherto elucidated by means of NMR spectroscopy that ENR50 remained structurally stable in 1,4-dioxane acidified by the addition of dilute HCl or

$AlCl_3 \cdot 6H_2O$ solution. Based on this finding, the acid-induced reaction of ENR50 with CTS in a dual-solvent consisting of 1,4-dioxane and water (97.5:2.5% v/v) was explored. Two type of biopolymers CTS-g-ENR-P1 and CTS-g-ENR-P2 consisting of higher and lower amount CTS, respectively, grafted onto the backbone on the natural rubber derivative were prepared. The structural and physicochemical properties of the products as compared to that of the starting materials were characterized by means of FT-NMR, FT-IR, SEM, DSC and TGA techniques. The regioselectivity of the acid-induced reaction is discussed and it is concluded that the biopolymers were formed via sterically favored pathway involving the attack of the primary amino (C-2) of CTS on the protonated epoxidized isoprene units of ENR50.

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