Tailoring Degree of Esterification and Branching of Poly(glycerol sebacate) by Energy Efficient Microwave Irradiation

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ABSTRACT

Poly(glycerol sebacate) (PGS) is known as an exciting biomaterial owing to its tunable mechanical properties and controllable degradation rate. However, it is always challenging to control these properties. In this study, we have proposed a solvent-based system to provide a better control of reaction temperature in microwave which can minimize the evaporation of monomers and water was collected to control the degree of esterification. Pre-PGSs with varied degree of esterification were prepared using both single mode and multimode microwave irradiation (MI) in this solvent-based reaction system. For similar degree of esterification of pre-PGS, the reaction time was almost halved with a better control on mechanical property by single mode MI compared to multimode MI. Furthermore the single mode MI approach was compared with the conventional heating approach (CH). The mechanical properties and degradation rate of PGSs can be controlled readily by using single mode MI approach compared with CH, which are crucial for its application as a biomaterial. It has been found that the single mode MI not only accelerates the pre-polymerisation process rate by six times, but also speeds up the curing time to the same extend. The Young's Modulus of PGSs prepared by single mode MI is increased from 0.77 to 3.14 MPa when

the degree of esterification is 66.82%, which is 50% higher than that reported in literature. Furthermore, PGS using highly branched pre-PGS prepared by single mode MI method can easily tune the flexibility. It can achieve much higher Young's Modulus than that obtained by CH with a short curing time (< 10 hours). In addition, the residual mass of PGSs prepared by single mode MI is varied from 78.54 % to 92.96 % compared to CH method that ranging from 84.24 % to 93.31 %. Thus, these highly branched PGSs produced by single mode MI also show a wider degradation window (approximately 59 % higher degree of flexibility than CH method), which is found to be highly dependent on the degree of esterification and curing time of pre-polymer, and controlled by branching.

INTRODUCTION

Poly(glycerol sebacate) [PGS] has been studied intensively after the first report by Langer's group in 2002 due to its unique properties.[1] For instance, its rapid degradation kinetic is compatible with the healing rate of tissues (*i.e.* 6-12 weeks), modulated degradation rate favors for drug carrier, and tailored mechanical properties suits as a nerve guide material.[2–4] The cytotoxicity of a biopolymer is always a concern before any clinical application, but both reactants of PGS are endogenous monomers that can be naturally found in human metabolites; glycerol involves in synthesis of phospholipids and sebacic acid is a metabolite in fatty acid oxidation.[5,6] From the previous studies, PGS that already showed less inflammation is favourable in tissue engineering than poly(lactide-co-glycolide), a currently widely utilized biomaterial.[4] In addition, the cytotoxicity of PGS can be further reduced by adding filler into the system. The percentage of dead cells decreased significantly as the crosslink level of PGS increased (*e.g.* by adding Bioglass).[7,8] Furthermore, highly toughened PGS showed excellent cytocompatibility compared to a soft PGS.[9] This is probably due to the rapid degradation of soft PGS which induces an acidic and cellular toxicity environment. In contrast, controllable degradation of highly crosslinked PGS maintains a

low concentration of potentially toxic degraded sebacate in the medium,[2,9,10] which is high preferable but very challenging.

The bio-application of PGS is thus widely known to be determined by its physical properties (e.g. degree of crosslinking and degradation rate) that can be manipulated by synthesis approach. Synthesis of PGS by a conventional approach is very time and energy consuming, which normally requires pre-polymerisation under inert gas condition to prevent oxidation of reactant for 24 h and curing under vacuum condition for 48 h.[1] Microwave irradiation (MI) has recently shown a great potential to replace conventional heating (CH), either for organic or inorganic synthesis.[11,12] It offers a homogeneous and fast heating due to volumetric and selective interaction between microwave and polar molecules, often leading to better crystallinity of the prepared materials. Furthermore, MI with remarkably increased reaction rates and highly reproducibility can readily tune the product selectivity and yields.[13–15] There are a few reports discussing effect of microwave irradiation to the pre-polymerization of PGS,[16,17] in which a household multimode microwave oven was used, may result in an ineffective control of the temperature rise and reaction rate. Uncontrolled temperature rise could both trigger the evaporation of the monomer (e.g. glycerol due to a low boiling point of 290 ˚C) and change the stoichiometric ratio of the reactants that potentially altered the physical and chemical properties of the resulting PGS.[18] In addition, multimode MI provides much weaker energy density than single mode MI,[19] which can influence the degree of crosslinking of a polymer.

Given such attractive potentials of a single mode MI with controllable reaction rate to biopolymer synthesis, to the best of our knowledge there is no report studying PGS synthesis using a single mode MI approach, benefiting from both well-controlled heating power and temperature rise. In this study, a finely-tuned polyesterification of sebacic acid and glycerol to prepare PGS by single mode MI was carried out. To avoid change of the reactants ratio during MI synthesis, we carried out the reactions in a solvent (i.e. toluene with lower boiling point 110˚C) to protect the evaporation of glycerol (boiling point at 290 ˚C) and further set the maximum reaction temperature

of 130 ˚C. All these have ensured that all precursors were remained in the reaction medium throughout the microwave irradiation with well-controlled of reaction temperature. The degree of esterification (DE) was thoroughly investigated in order to control mechanical properties and degradation rate of PGS. For comparison, PGS synthesis was also conducted using CH. The mechanism underlying PGS formation by MI was also discussed.

EXPERIMENTAL

General procedure for synthesis of Poly(glycerol sebacate)

Equimolar of glycerol (99%, Sigma Aldrich) and sebacic acid (> 99% Sigma Aldrich) were measured and pre-mixed in a round bottom flask before adding dry toluene (30 mL) into the mixture. Concentrated H_2SO_4 (3 μL) was then added into the mixture. The reactor was connected to a Drechsel bottle for water collection. This mixture was then ramped under nitrogen gas with Dynamic mode (150 W) in CEM Discover SP system (single mode) for 3 minutes (one cycle) with a temperature limit of 130 ˚C. It was then cooled to room temperature. The cycle was repeated to get 12 and 27 minutes samples. The abovementioned experimental procedures were repeated using CEM MARS system (multimode microwave) under similar microwave conditions (150W, 3 mins/cycle). The cycle was repeated to get a total 21 minute preparation time. Condensed water was collected for calculating the degree of esterification. The pre-PGSs were purified by removing the unreacted glycerol and then were dried using the pump and left in the fumehood overnight at room temperature. The dried prepolymer was then cured in the vacuum oven at 120 ˚C for 2-48 h. PGS were then cooled to room temperature under vacuum condition. For the control experiments, the pre-polymerisation step was carried out by conventional heating method. The reactor was connected with a Dean-Stark for water collection. Similar to the previous method, water was collected while toluene was used as solvent. The curing procedure was remained unchanged.

Characterisations

Fourier Transform Infrared Spectroscopy

The chemical bonds were confirmed using Perkin-Elmer 1605 FT-IR spectrometer in attenuated total reflection (ATR) mode with the frequency range 400-4000 cm^{-1} at 4 cm^{-1} resolution.

Scanning Electron Microscope

For observing the morphology of crosslinked PGS, the samples were fractured into small pieces and coated with Au. The SEM images were taken using JEOL JSM-7410F field emission-scanning electron microscope operating at 2-3 kV.

Tensile Tests

Tensile strength tests were carried out on the crosslinked PGS samples by cutting into rectangular strips (4-5 mm x 15 mm x 1-2 mm of thickness) with Perkin-Elmer Dynamic Mechanical Analyser (DMA 7e, Perkin-Elmer Instruments, USA) at room temperature. All the measured dimensions of the samples were counted during the test. The initial load was set at 1 mN and increased to 6000 mN with a rate of 200mN min^{-1}. Six or more repeats were carried out for each sample and final results were average of these measurements. The Young's Modulus of each specimen was calculated from the gradient of stress-strain curve using the Pyris™ software.

Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF)

Mass spectrometric studies were used to identify molecular weight of the oligomers and were acquired in UCL Chemistry Mass Spectrometry Facility MALDI. It was operated in positive Reflectron mode using the mass range of m/z 500 to 5000 with 100 shots/spectrum. Pre-PGS samples were dissolved in THF to prepare concentration of 9-10 mg mL^{-1}. Then, samples were then prepared by 1:1 dilution with matrix (α-cyano-4-hydroxy-cinnamic acid) in water-acetonitrile (2:8, v/v), 0.5 % formic acid. 3 μL of the resulting sample was spotted onto the MALDI target plate and allowed it to dry. Samples were analysed on using a Waters MALDI micro MX (Waters, UK) with a nitrogen laser in reflectron mode using delayed extraction (500 nsec) and an accelerating voltage

of 120V, pulse 2500, and detector 2000. The simulated isotopic peak patterns were constructed using online software MoIE- Molecular Mass Calculator v2.02 (http://mods.rna.albany.edu/masspec/MoIE).

NMR spectroscopy

NMR spectra were recorded using a Bruker Avance III 600 MHz NMR spectrometer equipped with a 5 mm cryoprobe (^{1}H 600.13 MHz). NMR spectra of the pre-polymers (pre-PGS) were obtained in acetone-d6. All chemical shifts (δ) were given in ppm, where the residual C_3H_6O peak was used as an internal reference for ^{1}H NMR (δ_H = 2.09 ppm), and the C_3H_6O peak for ^{13}C NMR (δ_C = 206.68 ppm). The pre-PGS samples were pre-treated by washing with ethyl acetate to remove the unreacted sebacic acid. The filtrate was then dried under vacuum before the NMR analysis. The resulting data were processed and analysed using ACD/NMR Processor software.

Degradation study

For each sample, five or more polymer specimens were cut into dimensions of 5x5 mm^2 before storing in the standard phosphate buffer saline (PBS, 1X) at 37 ˚C for 28 days. The initial weigh of the PGS samples were recorded before putting into the PBS solution. During the degradation test, the PBS solution was refreshed daily. At pre-set time (after 7, 14, 21, 28 days), these degraded specimens were dried in a drying oven at 60 ˚C for 12 h and residue mass of the specimens was recorded.

RESULTS AND DISCUSSION

Synthesis of poly(glycerol sebacate) pre-polymer [pre-PGS] using single mode and multimode microwave irradiation (MI)

Pre-PGSs has been prepared by multimode domestic microwave,[16,17] thus the influence of single mode and multimode microwave irradiation (MI) on pre-PGSs synthesis was firstly studied here. Single mode MI was found to be more energy efficient than multimode MI to synthesize

viscous pre-PGS with similar degree of esterification. For instance, 12 mins of microwave treatment is needed to synthesize the pre-PGS with ca. 70% DE by single mode MI, which not achieved until 21 mins irradiation using multimode while the pre-PGSs prepared by both methods show a characteristic ester linkage (C=O bond) at 1734 cm^{-1} and 1730 cm^{-1}, respectively (see ***Supporting Information, Figure S1***). The rapid reaction time is attributed to the highly intensity in single mode microwave.[19,20] Other than reaction time, the mechanical properties of the crosslinked PGSs, obtained by 8 h curing of pre-PGSs synthesized by both microwave methods, were also studied (see ***Figure 1***). Based on **Figure 1a**, Young's modulus of the PGSs synthesized by single mode MI (2.60 ± 0.34 MPa) is smaller than the PGSs synthesized by multimode MI (3.31 ± 0.30 MPa), while the ultimate tensile strength of the PGSs by single mode is somewhat higher than that by multimode MI (approximately 0.46 ± 0.06 MPa for single mode MI and 0.41 ± 0.15 MPa for multimode MI-***see Supporting Information, S2***). The elongation at break for PGSs is 27.73 ± 1.87 % and 17.00 ± 7.77%, for single mode and multimode, respectively (**Figure 1b**). In short, PGS synthesized by single mode MI has 20 % smaller Young's modulus than that prepared by multimode MI. This can be readily increased by increasing microwave power or reaction time. Simultaneously elongation at break can also be tuned, therefore the degree of flexibility to tune PGSs properties can be easily controlled by single mode MI. In addition, PGSs fabricated by single mode MI have slightly higher ultimate tensile strength. Owing to the potential to tune PGSs properties with higher degree of flexibility, faster reaction rate and higher energy efficiency, the experimental conditions were then optimised using single mode MI and further compared with CH.

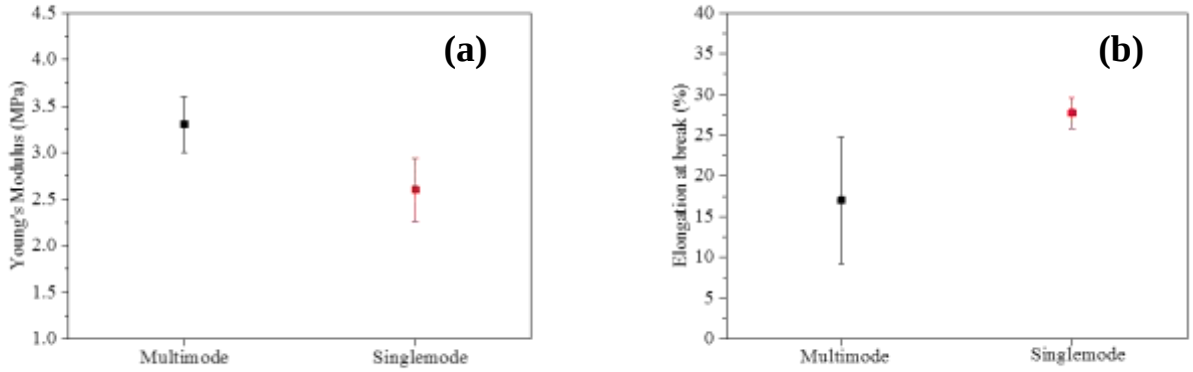


Figure 1: (a) Young's modulus and (b) elongation at break of PGSs prepared by 12 min single mode MI and 21 min multimode MI, followed by 8 h curing.

Preparation of poly(glycerol sebacate) pre-polymer [pre-PGS] using single mode MI and CH

A two-step preparation procedure was applied to synthesise all PGSs. The first step was pre-polymerisation under MI or CH conditions and the second was curing of pre-PGSs in the vacuum oven. In a typical procedure, a mixture of sebacic acid and glycerol in dry toluene was heated to reflux temperature to prepare pre-PGSs and the condensed water was collected as a measure of degree of esterification (DE) - as illustrated in ***Supporting Information, Figure S3***.[21] In this mixture, glycerol interacts much stronger with microwave than others. This is because the loss tangent of glycerol (tan σ = 0.651) is higher than toluene (tan σ = 0.040) due to multi-hydroxide groups attached, and the long chain of polar molecules (i.e. sebacic acid) always has weaker interaction than short chain with microwave.[22,23] The pre-PGSs were then purified by only removing unreacted glycerol and further characterised.

Table 1: Water collected and degree of esterification (DE) by MI and CH methods.

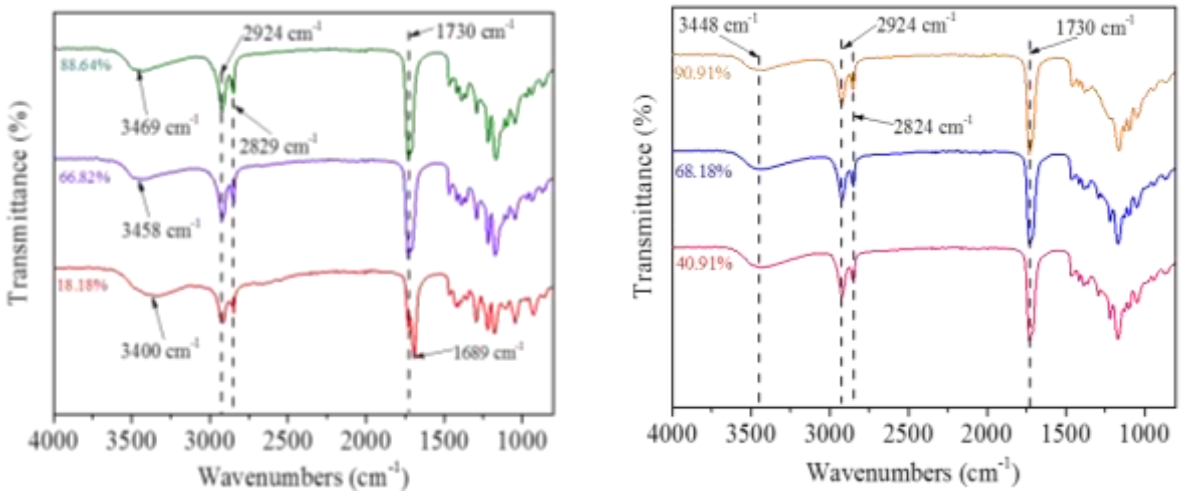
Methods	Heating Time (mins)	Volume of Water (mL)	DE (%)
MI	3	0.40	18.18
	12	1.47	66.82
	27	1.95	88.64
CH	50	0.90	40.91
	77	1.50	68.18
	166	2.00	90.91

Pre-PGSs, prepared by both methods, with different DE are listed in **Table 1**. One can see that MI provides at least a six-fold faster reaction rate than CH method by comparing the amount of water collected. According to water collection profile (***see Supporting Information, Figure S3***), MI almost starts the esterification process when MI is turned on. However, it takes more than 30 minutes to collect water droplets by CH. MI (dielectric heating) speeds up the reaction rate significantly by generating the heat homogeneously in bulk solution via dipole rotation where the polar species (e.g. glycerol molecules) align themselves with rapidly changing electrical field of the microwave as such the reactants could be activated selectively. More importantly, MI provides the heat internally and tends to eliminate the 'thermal wall effect'.[24,25]Hence, the condensed water molecules are evaporated faster in microwave due to large dielectric loss of water molecules, which further shifts the reaction towards polymer formation.[26–28]

DE can be used as a measure of the polymerisation degree and more importantly for predicting the unreacted alcohol groups after the pre-polymerisation process. In this study, the DE is calculated using the **Equation 1** stated as below:

$$DE(\%) = \frac{Amount\ of\ water\ collected\ (mL)}{Theoretical\ amount\ of\ water\ formed\ (mL)}\ x100\% \quad \textbf{Equation (1)}$$

The results of DE are shown in **Table 1**. We also observed that pre-PGSs prepared by MI method with 18.18 % DE and 66.82% DE were in wax and viscous liquid form, respectively, comparable with the previous reports.[17] On the other hand, the DE is increased by longer reaction time in both heating methods, however, this increment is drastic by MI. For instance, a minimum six-fold increase in reaction rate can be obtained by MI compared to by CH (ca. at similar DE 70%).



(a) **(b)**

Figure 2. ATR-FTIR spectra of pre-PGS prepared at different DE by (a) MI and (b) CH methods. These pre-polymer showed strong signal of ester bond (C=O) at 1730 cm^{-1} and hydroxyl bond (—OH) around 3400-3469 cm^{-1} when using MI method. Similarly, the pre-polymer that prepared via CH also showed C=O signal at 1730 cm^{-1} and —OH signal at 3448 cm^{-1}.

ATR-FTIR was used to confirm the formation of ester functional groups (**Figure 2**). A sharp peak is found at 1730 cm^{-1} which corresponds to the carbonyl (C=O) stretching mode of the ester linkage, and the bands appeared at 2924, 2829 and 2824 cm^{-1} are attributed to C—H stretching of polymer backbone.[16,17] Relative intensity of characteristic ester carbonyl increases as reaction time increases, suggesting high degree of polymerisation.[17] The peak at 1689 cm^{-1} is referred to the carbonyl stretching of the remaining free sebacic acid after pre-polymer formation. The pre-PGSs prepared by CH and MI show a broad peak around 3448 cm^{-1} and 3400-3469 cm^{-1}, respectively, attributed to hydroxyl (alcohol group) stretching of pre-polymer. As the MI time increases, the right shift of the alcohol group is observed. The precursor glycerol contains two alcohol groups. One is primary and the other is secondary alcohol group, which have a mixed peak around 3290 cm^{-1} (***Supporting Information, Figure S4***). The primary alcohol group has higher frequency compared to secondary alcohol.[29] The observed continuous right shift may indicate the pre-PGS prepared by MI contains more primary than secondary alcohol groups. In other words, MI approach uses more secondary alcohol group than CH approach.

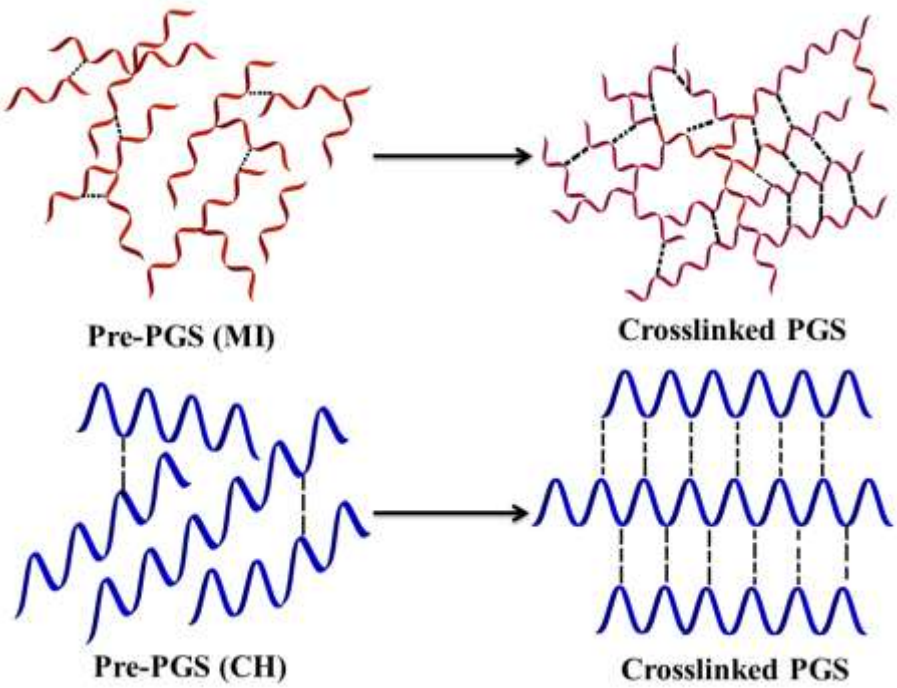
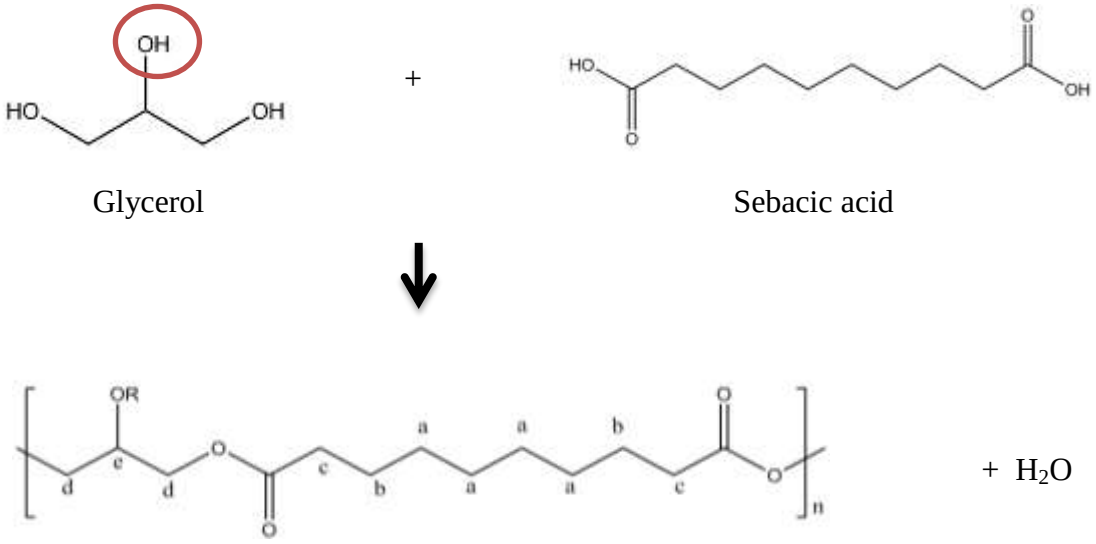


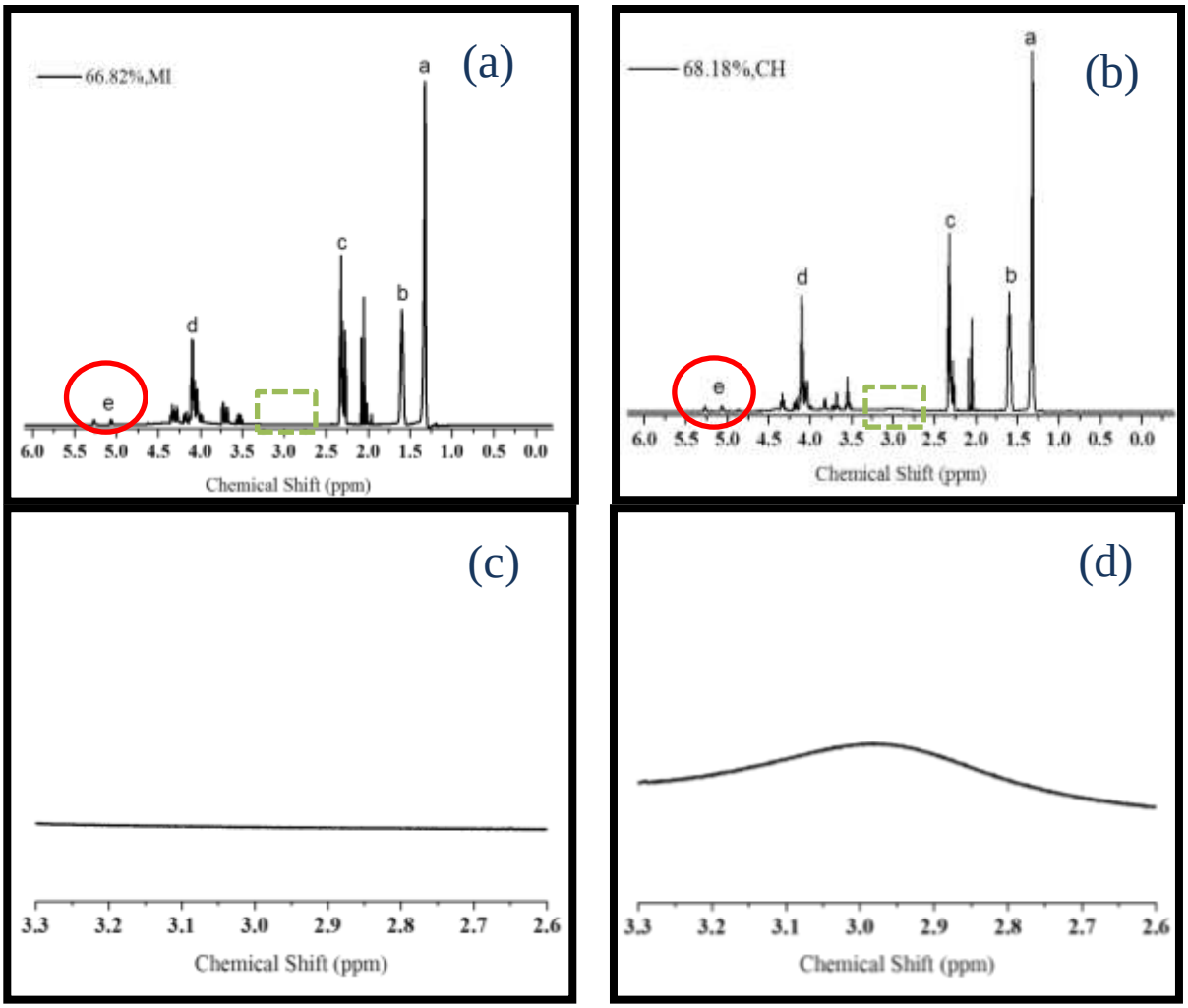
Figure 3. Proposed scheme for the possible structure of pre-PGS that prepolymerised via MI and CH methods, and crosslinked PGS after the curing process. The dotted line in the figure indicates the cross-linking.



Scheme 1. Polycondensation of PGS using equimolar glycerol and sebacic acid where R can refer to hydrogen or a branched chain. The red circled region is referred to the secondary alcohol group of glycerol.

Based on the obtained IR spectra, the highly branched pre-PGS structure by MI approach is proposed as depicts in **Figure 3.** MI interacts strongly with glycerol as mentioned above, leading to activation of both alcohol groups in glycerol which react more efficiently with sebacic acid

compared to that happening in CH approach. In order to justify this hypothesis, the obtained pre-polymers were further characterized by MALDI-TOF and ^{1}H NMR. ^{1}H NMR spectra of the obtained pre-PGSs, synthesised using both heating methods, were recorded using acetone-d6 (δ2.09 ppm) as the deuterated solvent. As depicted in **Scheme 1,** 5 different sets of protons (**H_{a-e})** can be assigned to the repeating units of pre-PGS.[30]



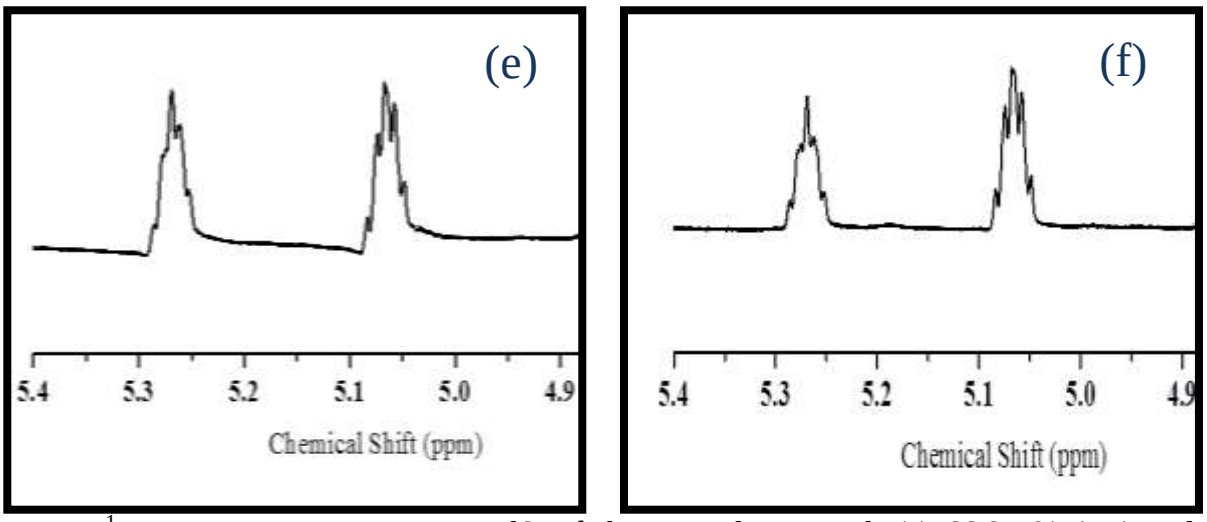


Figure 4. ^{1}H NMR spectra, in acetone-d6, of the pre-polymer with (a) 66.82 % (MI) and (b) 68.18% (CH). The sebacic chain peak in the pre-PGS is illustrated at δ1.32 ppm (H_a), δ1.59 ppm (H_b), δ2.32 ppm (H_c) while H_d and H_e attributed to the glycerol chain in the pre-polymer. The ^{1}H NMR spectra demonstrate the typical molecular structure of pre-PGS where the additional peak at 2.09 ppm is solvent acetone-d6 peak. The next two figures referred to the region of δ 2.65-3.30 ppm for (c) 66.82 % and (d) 68.18% of pre-PGS that synthesized by MI and CH, respectively. The significant peak in (d) suggests the excessive of secondary hydroxyl group (H_e) of glycerol existing in the pre-polymer prepared by CH method. The last two figures are illustrated the region at δ 4.90-5.40 ppm for the pre-PGS with (e) 66.82 %-MI and (f) 68.18%-CH. These two peaks assign to H_e which corresponded to 1,2-diacylglyceride (δ 5.07 ppm) and 1,2,3-triacylglyceride (δ 5.27 ppm).

^{1}H NMR spectra of the pre-PGSs, prepared by MI (66.82 % DE) and CH (68.18 % DE), display the chemical shifts at δ1.32 ppm (H_a), δ1.59 ppm (H_b), δ2.32 ppm (H_c), which are attributed to –CO-CH_2-CH_2-CH_2- group in the pre-polymer from the precursor sebacic chain (**Figure 4a and 4b)**.[31,32] The additional peaks at δ 3.50-5.50 ppm identified in the spectrum are ascribed to the H_d and H_e in PGS molecular chain from glycerol.[33] All these assigned chemical shifts are also presented in ^{1}H NMR spectra of the pre-PGSs with low DE (<41%) by MI and CH method (***Supporting Information, Figure S5***). Consistently, ^{13}C NMR analysis of these samples shows signals: 25-34 ppm for the sebacate methylene carbon, 60-75 ppm for the methylene and methine carbons of glycerol and glyceride units, and 172-174 ppm for the signals of carboxylic acids and esters (***Supporting Information,* Figure S6**).[34]

More importantly, a significant broad signal is observed at δ 2.65-3.30 ppm in the ^{1}H NMR spectrum of the pre-PGS (68.18% DE) prepared using CH method (**Figure 4d**). This signal is assigned to the secondary hydroxyl groups of glycerol (H_e in **Scheme 1**), agreement with the previous reports.[35] In contrast, no such signal is observed in the ^{1}H NMR spectrum of the pre-PGS

(66.82% DE) prepared using MI (**Figure 4c**). Presence of excessive secondary alcohol groups in the pre-PGS prepared by CH suggests that CH method produces linear polymer chains, whereas MI facilitates branching by selectively activating all alcohol groups as indicated in **Figure 3,** which is crucial to control the property of the final polymer.

Further study was carried out to understand MI induced branching by observing the ^{1}H NMR between δ 4.9 and δ 5.3 ppm which corresponded to H_e and more specifically referred to 1,2,3-triacylglyceride (δ 5.27 ppm)- as illustrates in **Figure 4e and 4f**. [34] Relative signal intensity ratios can be used to understand the change in the presence amount of secondary alcohol groups of glycerol. The relative signal intensity ratio of H_b or H_c to H_a is a constant (ca. 0.5). This is because these protons are not involved in the esterification process. When reaching ca. 70% DE, this relative ratio of H_e/H_a is 15% higher under MI (0.0132) than that under CH condition (0.0115). More interestingly, the relative ratio of H_e/H_a corresponding to 1,2,3-triacylglyceride (δ 5.27 ppm) are 0.012 and 0.010 for MI (18.18% DE) and CH (40.91% DE), respectively. Higher the relative H_e/H_a ratio at δ 5.27 ppm, higher esterification of secondary alcohol groups of glycerol. This indicates higher branched pre-polymer achieved by MI method.

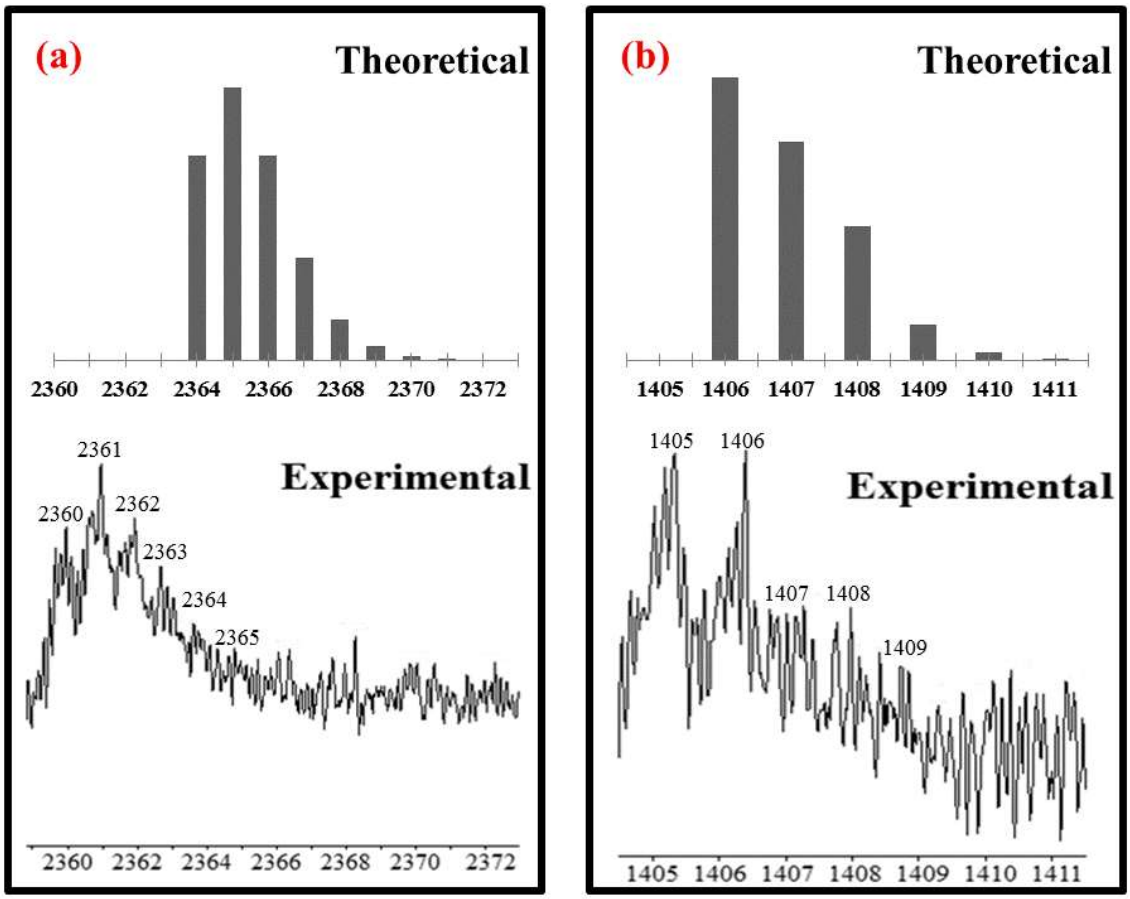


Figure 5. (a)MALDI-TOF spectra of pre-PGS (18.18% DE-MI) show the maximum detected oligomer mass at 2360 m/z. This spectra is slightly left shifted when comparing to the theoretical MALDI spectra, GSGSGSGSGSGSGSGSGS $[C_{117}H_{200}O_{46}Na]^+$.(b) MALDI-TOF spectra of pre-PGS (68.18% DE-CH) depict the maximum oligomers mass at 1405 m/z which is well-fitted with the theoretical spectra that created by the software, i.e. GSGSGSGSGSG, $[C_{68}H_{118}O_{28}Na]^+$.

Other than ^{1}H NMR spectra, the MALDI-TOF mass spectra, recorded from 500 to 5000 *m/z*, of the pre-PGSs synthesized by both methods were also used to verify the formation of oligomeric structures (***Supporting Information,* Figure S7**). The profile of experimental spectra of the samples prepared by either MI or CH is consistent with the theoretical spectra (**Figure 5**), suggesting the validity of the modelling. However, the pre-PGS prepared by MI (DE=18.18% or DE=66.82%) shows monoisotopic mass at *ca.* 2360 *m/z* (**Figure 5a** and ***Supporting Information,* Figure S8**), appearing to be shifted by 4 *m/z* when compared with the theoretical monoisotopic mass at 2364 *m/z* ($[C_{117}H_{200}O_{46}Na]^+$) that calculate using a likely linear PGS with 9 repeating units. The shift (4 *m/z*) is thought to be most likely due to esterification reaction of secondary alcohol groups of glycerol to produce branched PGS, which is in agreement with the NMR spectra. However, the pre-PGSs

prepared by CH (DE=68.18%, **Figure 5b)** shows monoisotopic mass at *ca.* 1405 *m/z* ($[C_{68}H_{118}O_{28}Na]^+$,consisting of a linear PGS with 6 glycerol and 5 sebacic acid units) which is very close to the theoretical monoisotopic mass at *ca.* 1406 *m/z* ($[C_{68}H_{118}O_{28}Na]^+$) (**Figure 5b** -both theoretical and experimental monoisotopic patterns). This comparable monoisotopic mass also suggests that MI produces different prepolymer structure than CH. Similar results can be obtained when DE is equal to 40.91% (***Supporting Information,* Figure S9**).

Previous studies reported that a range of possible oligomers could only be produced from the reactants glycerol and sebacic acid when tuning their ratio.[36] Surprisingly, different PGS structures (*e.g.* branched/heavily crosslinked or linear) can be obtained by only changing the heating method while maintaining a constant stoichiometric ratio of glycerol to sebacic acid (1:1). Overall results obtained from NMR and MALDI-TOF spectra suggest that the microwave promotes the formation of branching oligomers in pre-PGS (detailed structure is represented in **Figure 3**). Naturally, primary alcohol group is likely to react with carboxylic acid group to generate polymer linear chains, while secondary alcohol group facilitates the branching. Microwave can produce polymer with higher molecular weight,[37] thus, it very likely activate both secondary and primary alcohol groups simultaneously due to their similar polarity which couples well with MI. Therefore, at low DE, MI prefers branching in different directions while CH is likely to form shorter chain prior to curing process.

Morphology of Crosslinked PGS Samples

After curing the pre-PGS in the vacuum oven, a significant reduction in the intensity of the hydroxyl band is observed- as seen in ***Supporting Information, Figure S10***. In order to investigate the effect of heating method on the surface morphology, SEM images of both appearance and cross section were obtained (***Supporting Information, Figure S11)***. For the internal structure analysis, the specimens were fractured in the liquid nitrogen instead of cutting with blade to prevent smearing. The SEM images show a smooth topography for surface of either PGS samples and MI does not alter the morphology of PGS since all the crosslinked specimens show similar morphology.

This morphology also suggests that the pre-PGS samples are free of solvent (toluene) due to complete removal of solvent before curing process, which is very crucial prior to use in bio-applications (also justified by the ^{1}H NMR results, **Figure 4**).

Optimization of Curing Time and its Mechanical Properties

To further discover the effect of branched pre-PGS on curing time and mechanical properties, we used similar DE (ca. 70% DE) of pre-PGS and further cured in the vacuum oven from 2-48 h. By reducing the curing time to 2 hr, PGS specimens prepared by MI (DE=66.82%) show faster toughening compared with CH samples (DE=68.18%) – can be seen in ***Supporting Information, Figure S12***. The PGSs samples cured with shorter time were also characterised using IR spectra where the ester linkage is found with a reduction of hydroxyl bond from time to time- as seen in ***Supporting Information, Figure S12***. This may be due to the branched pre-PGS samples that produced by MI require shorter curing time to form stiff polymer. It can be concluded that higher branching of pre-PGS achieved by MI facilitates the formation of crosslinked PGS in a very short curing time.

Surprisingly, the Young's modulus of the specimens (after 48 h curing) fabricated in this study are at least 50% higher without changing the molar ratio of glycerol and sebacic acid as compared to the previous results.[16,17,38,39] This is probably due to our modified synthetic procedure in which the unreacted glycerol was removed from the bulk pre-PGS prior to the curing process. Consequently, the remaining sebacic acid probably only functions as a crosslinking agent and thus produces a stiffer polymer. This novel preparation approach proposes that PGS with a broad range of stiffness can be produced without additional crosslinking agent, but by simply adjusting the degree of esterification and curing time. This highly toughened PGS may also improve the cytocompatibility of PGS.[9]

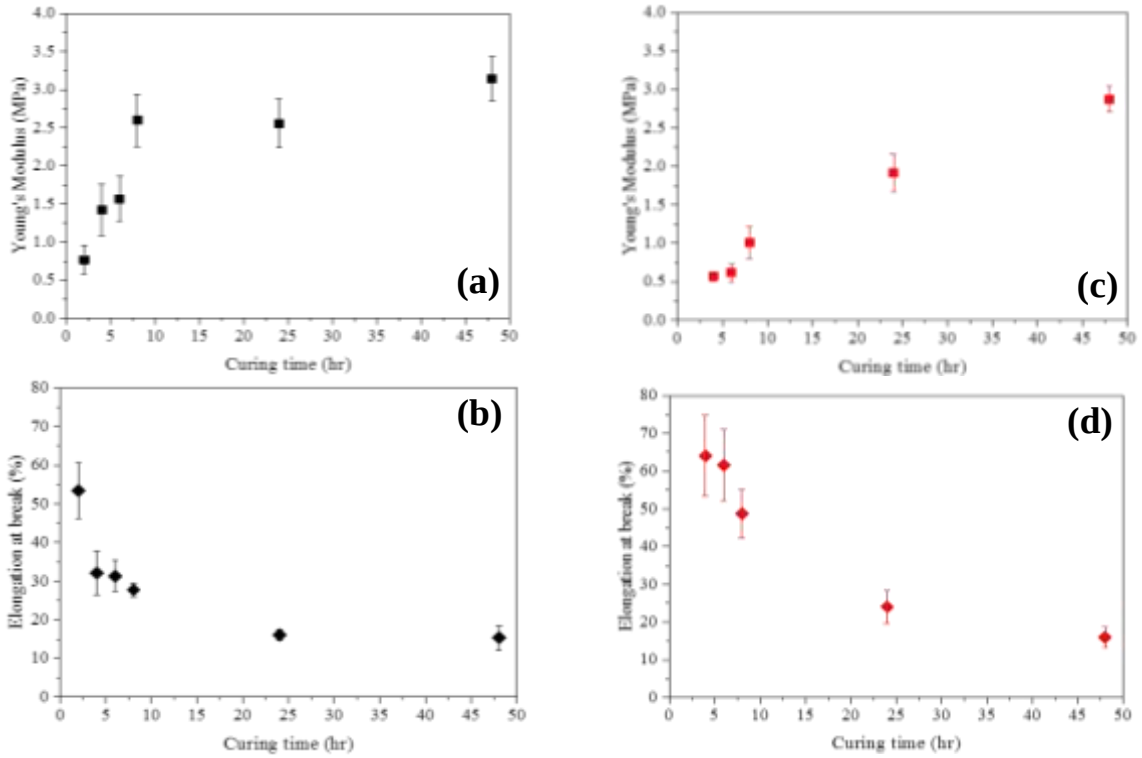


Figure 6. Young's modulus (MPa) and elongation at break of crosslinked PGS (ca. 70% DE) prepared by (a-b) MI and (c-d) CH method, respectively. The pre-PGS samples were pre-polymerised at predetermined degree of esterification before curing for 2-48 h in the vacuum oven.

The mechanical properties of crosslinked PGS **(Figure 6**) that prepared by both methods are clearly different. As the curing time increases, the Young's Modulus of these specimens are increased but at a different step. The Young's modulus of PGSs by MI after 8 h curing is equivalent to that achieved in 48 h by CH, thus MI reduces the curing time by a factor of six, similar results to that observed in pre-polymerisation process. The Young's modulus of PGSs by MI after 8 h curing is increased from 0.77 ± 0.19 to 2.60 ± 0.34 MPa (a window of 1.83 MPa) and elongation at break is reduced from 53.41 ± 7.14 to 27.73 ± 1.87 %. On the other hand, PGSs synthesised by CH at similar curing time have smaller Young's modulus, ranging from 0.57 ± 0.06 MPa to 1.01 ± 0.21 MPa (a window of 0.44 MPa) and elongation at break is from 64.07 ± 10.71 to 48.72 ± 6.51 %. The ultimate tensile strength of these PGS samples fabricated is depicted in ***Supporting Information,*** **Figure S13-** from 0.26 ± 0.07 to 0.46 ± 0.06 MPa for MI's samples and from 0.22 ± 0.03 to 0.35 ± 0.08 MPa for CH's samples. The Young's modulus of these PGS specimens after 48 h of curing time is comparable (i.e. 3.14 ± 0.28 MPa and 2.87± 0.17 MPa for MI and CH, respectively).

As stated in previous studies, the extent of crosslinking affects the properties of a polymer significantly.[40–42] A weak and soft polymer is produced at a low crosslink density. On the contrary, a stronger and stiffer polymer can be formed at higher crosslink density. These polymers would have different bio-applications. In this study, MI speeds up the curing process significantly and CH method can produce PGS with similar mechanical properties only if the curing time is long enough. Therefore, the Young's modulus of PGSs prepared by MI can be tuned easily, where a larger range of Young's modulus can be obtained (i.e. three times wider) with a short curing time as compared to CH. This is because a higher degree of branching pre-PGS is synthesised by MI rather than a linear pre-PGS by CH.

Degradation of PGS

Degradation of PGS is a hydrolytic process where the ester bonds in the polymer chains react with water molecules, resulting into shorter chains via surface erosion process. The degradation occurs within a region near the surface and provides a linear changes in mechanical performance because the surface erosion happens much earlier than in the bulk of the sample.[43] According to Wu *et al.*, initial porosity and pore size affected the degradation rate due to a variation of diffusion rate.[44] However, PGS samples produced herein have similar morphology (see SEM images, ***Supporting Information,* Figure S11**), so it is not a dominating factor for the degradation rate of PGSs in our study.

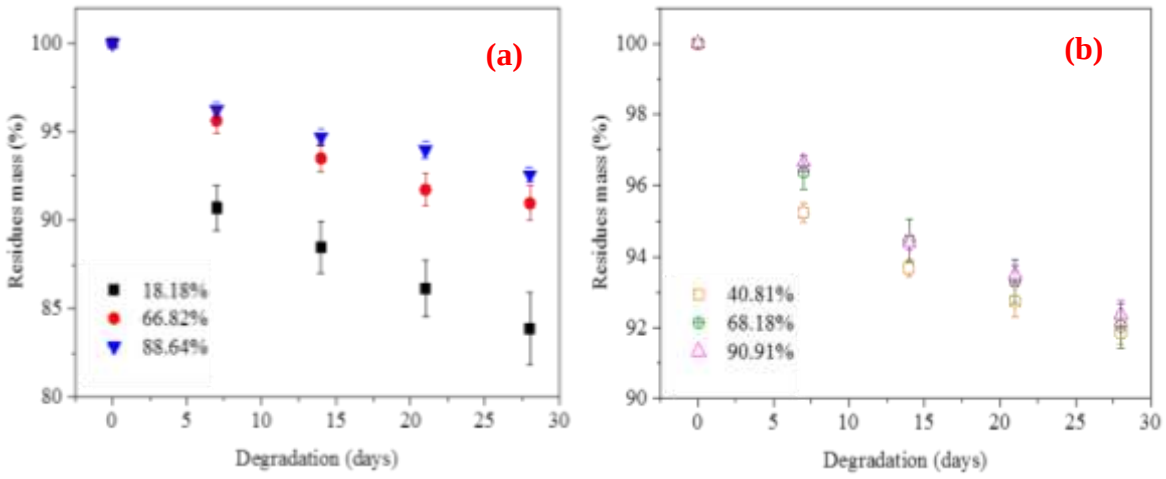


Figure 7: Degradation profile of PGS [pre-polymerised by (a) MI and (b) CH method and then cured for 24 h] in phosphate buffered solution.

The surface erosion mechanism undergoes by PGS is ideal for many drug delivery applications due to slow water permeation rate and water-vulnerable drugs can be protected.[45] Thus, degradation properties of PGS specimens were tested in phosphate buffered saline (PBS) solution. Firstly, the PGS specimens with different degree of esterification (ca. 18-90%) but same curing time of 24 h were investigated (**Figure 7**). The degradation rate of PGS samples that prepared by both methods decreases fast initially and then reaches a linear mass loss. With an increment in DE, the degradation rates of all samples are decreased. The residual mass of the PGS specimens (ca. 18.18% DE) after 28 days is 83.88 ± 2.02 % (16.12 % degraded) and the residues mass of PGS with 66.82 % DE is 90.96 ± 0.96 % (9.04 % degraded). For CH method, the residual mass of PGS samples are 91.89 ± 0.32 % for 40.81% DE and 92.56 ± 0.24% for 68.18% DE, where these samples degrade less than 10 %. When reaching ca. 90% DE, the PGS samples degrade slowest (only 6.20-7.42 % degraded) where the residual mass are 92.58 ± 0.40 % and 93.80 ± 0.40 % for MI and CH, respectively.

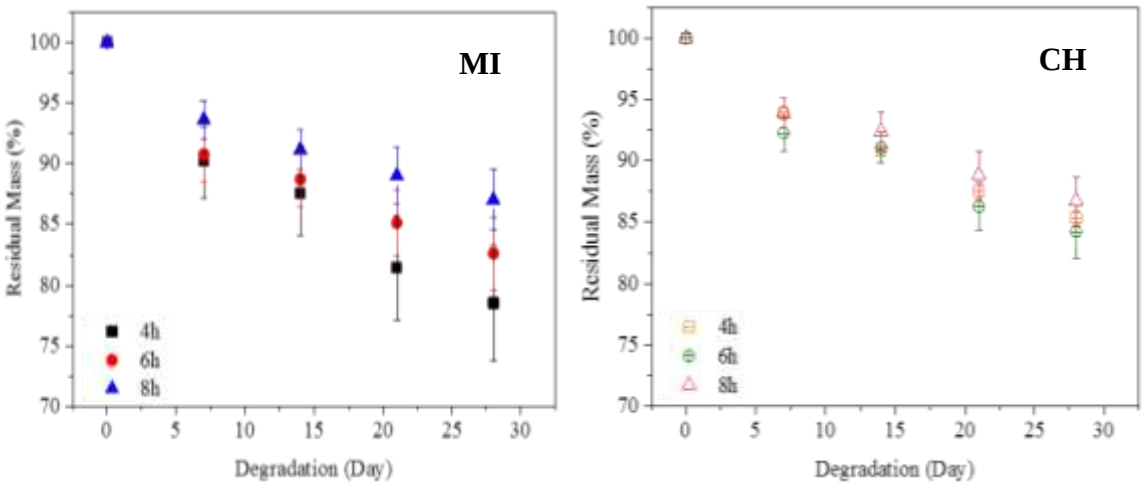


Figure 8: Degradation rate of crosslinked PGS at 70% DE (pre-polymerised by MI and CH method and then cured for 4-8 h) in phosphate buffered solution.

Secondly, the PGS samples with similar DE (ca. 70 %) but different curing time were also tested. As illustrated in **Figure 8**, PGS specimens prepared by MI show a broader range of degradation profile compared to CH samples. After long curing time (≥ 8h), MI samples show

similar degradation profile to the CH samples where both degrade to approximately 87 % (ca. 13% mass loss). Interestingly, when reducing the curing time to 4 and 6 h, the degradation rate of sample prepared by MI changes significantly while only little effects on the CH samples. For instance, after 4 h curing time, the residual mass of MI sample is 78.54 ± 4.76 % (21.46% degraded) compared to 85.37 ± 0.58 % of CH sample (14.63 % degraded). On the other hand, by further increasing the curing time to 24-48h, the PGS samples by both methods show slow and comparable degradation rate while the residual mass is above 90 %- refer ***Supporting Information, Figure S14***.

Based on these degradation results, it can be found that the branched structure of PGS samples prepared by MI method provide a wider degradation rate window compared to the PGS samples prepared by CH with similar DE. In other words, the polymers prepared by MI show a degradation rate that ranged from 78.54 % to 92.96 % (a window of 14.42%), but those by CH are from 84.24 % to 93.31% (a window of 9.07%). Thus, MI provides approximately 59% wider degradation window as compared to CH. The large and controllable degradation rates enable more flexible drug delivery. A branched structure can give rise to a rapid hydrolytic cleavage due to more terminals which are attacked by water during degradation.[46] In both methods, the increment in curing time or DE generally decreases the mass loss of PGS samples. This is because pre-polymerisation and curing process both increase the number of ester bonds needed to be broken via surface erosion. Therefore, PGS with lower DE or shorter curing time degrades faster due to less number of ester bonds to be cleaved. On the other hand, the PGS prepared by both methods at similar DE but longer curing time (> 8h) shows a similar degradation rate (residual mass > 90%). This is due to the DE could reach a saturated point. As a result, the number of ester bonds to be broken is comparable. Therefore, the degradation rate does not alter much at this stage.

CONCLUSIONS

The pre-PGSs have been prepared by single mode MI in comparison with multimode MI and CH approaches. The reaction temperature has also been monitored and controlled with a

solvent with a low boiling point to improve the reproducibility and reduce the evaporation of monomers which is neglected in previous studies using multimode MI. [16,17] It also offers a well-control over temperature, homogeneous temperature medium and an ease to manipulate the degree of esterification. In addition, the degree of esterification of pre-PGS was finely tailored by collecting the condensed water which is crucial to control the mechanical properties and degradation rate.

The single mode MI has been proven to be more energy efficient than multimode MI with a high degree of flexibility to tune PGSs Young's modulus and elongation at break. When comparing single mode MI with CH method, single mode MI dramatically speeds up both pre-polymerisation and curing processes, e.g. by a factor of six, due to fast and selective heating mechanism. The degree of esterification of pre-PGS can also be easily controlled by single mode MI which strongly affects the polymer properties (e.g. degradation rate and mechanical strength). Besides, the highly branched pre-PGSs are fabricated (proven by NMR and MALDI-TOF spectra) using single mode MI method without changing the molar ratio of glycerol and sebacic acid. In addition, by using this proposed strategy, the Young's Modulus of PGS (from 0.77 ± 0.19 to 3.14± 0.28 MPa) increases by 50 % when compared to the reported literature.[16,17,38,39] Moreover, the PGS samples prepared by single mode MI also reduce the curing time where a stiffer polymer is produced after 2 h curing in the vacuum oven. In addition, pre-polymer samples (DE = 66.82 %) of single mode MI method cured for only 8 h shows Young's Modulus comparable to the one prepared by CH method and cured for 48 h. For degradation test, degree of esterification and curing time affect the degradation rate significantly. This is because these two factors strongly control the degradation rate of PGSs due to increment of ester linkage that needed to be cleaved. The highly branched pre-PGS fabricated by single mode MI provide 59% wider window for degradation compared to the linear chain yielded by CH. In total, the new microwave approach provides a higher degree of freedom to tune mechanical properties (threefold) and degradation rate (59%) in order to meet demands of

various applications such as drug delivery vectors which highly depend on the degradation rate of the polymer.

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