Interpenetrating polymer networks (IPN) based on gelatin/poly(ethylene glycol) dimethacrylate/clay nanocomposites: Structure–properties relationship

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ABSTRACT

The effects of cross-linking sequence (simultaneous or sequential) and incorporation of exfoliated sodium-montmorillonite (Na⁺-MMT) nanoclay on the structure and properties of interpenetrating polymer networks (IPNs) based on gelatin/poly(ethylene glycol) dimethacrylate were studied by means of different complementary techniques. Gelatin and PEGdma phases were cross-linked via chemical and in-situ UV curing, respectively. 2,2-dimethoxy-2-phenylacetophenone (DMPA) (1.5% w/w) was used as photoinitiator to cross-link PEGdma. The results showed that the incorporation of small amount of Na⁺-MMT nanoplatelets accelerates the kinetics of chemical cross-linking of gelatin by glutaraldehyde (1.0% w/w). This led to a new hypothesis concerning the tuning structural evolution of the IPNs by the Na⁺-MMT content. In the case of simultaneous IPNs, in which both phases cross-linked at the same time, the accelerated cross-linking of gelatin in the presence of exfoliated sodium-montmorillonite led to increased structural homogeneity, improved mechanical and thermal properties. Incorporation of nanoclay did not show any significant effect on the structure and properties of the IPNs synthesized via sequential method in which gelatin and PEGdma phases were cross-linked separately. For the semi-IPNs, however, Na⁺-MMT induced macroscopic phase separation and resulted in lower mechanical properties. These results might shed light on the mechanisms underlying structure–property relationship in biohybrid IPNs based on gelatin as promising candidates for tissue engineering and drug delivery applications.

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1. Introduction

Gelatin is a polypeptide derived from thermal hydrolysis of collagen, an abundant protein in animal tissues. It is widely used in food, photography, pharmaceutical and medical applications mainly because of its biocompatibility, nontoxicity and biodegradability [1–3]. Specifically, gelatin can be combined with other macromolecules to produce a special class of polymer blends known as interpenetrating polymer networks (IPN) [4]. IPN can be defined as a non-bonded but inseparable combination of two polymers, each in network form. From the morphology point of view, IPNs are co-continuous in mesoscale i.e., both phases span over the entire sample [5].

Recently, gelatin based IPNs have attracted much attention due to potential applications in tissue engineering scaffolds [6], bioadhesive glues [7], wound healing [8,9], drug delivery [10], subcutaneous delivery of cells and the sustained release of proteins and other bioactive agents [11]. Gelatin has been combined with poly(acrylic acid) [12], polysaccharide [13], poly(1-vinyl-2-pyrrolidinone) [14], dextran [6,15], polymeric sodium alginate [16], poly(2-hydroxyethyl methacrylate) [17], sodium carboxymethyl cellulose [18], poly(N-isopropylacrylamide) [19], polyurethane [20], silk fibroin [21], and polyethylene glycol [11,22–25] to create bio-erodible IPNs for the aforementioned applications.

Gelatin based IPN-structures can be generated via chemical cross-linking of gelatin phase by a variety of chemicals e.g., low molecular weight aldehydes, water soluble carboximidines/N-hydroxysuccinimide mixtures [26], genipin [27], transglutaminase [28], and bis(vinylsulfonyl)methane [29]. Furthermore, UV or gamma radiation allows for in-situ conversion of photo-reactive solutions into gels or solid networks. For example, synthesis of gelatin/poly(ethylene glycol)diacrylate (PEGda) IPNs by radiation cross-linking of PEGda in gelatin/PEGda mixture followed by treatment of the resulted film with glutaraldehyde, have been reported by Kao and his coworkers [11,22–25,30].

Swelling and drug release kinetics of these IPNs revealed that the degradation kinetics can be varied and tailored for a specific tissue engineering and drug delivery needs [21]. Their IPN films showed an enhanced elasticity and strength as well as longer degradation time when compared with glutaraldehyde-fixed gelatin hydrogels [11]. They also showed that modifying the gelatin backbone with PEG-monoacatester ester and/or polyanions decreased the IPN elasticity at ambient temperature [11,22]. Moreover, semi-IPNs of gelatin/PEGda were shown to be effective matrices for the delivery of drugs and growth factors and interestingly could mimic extracellular matrix and transport the therapeutic molecules and nutrients to the proliferating cells during wound healing [25,30].

Furthermore, biocompatible IPN-structured hydrogels based on gelatin and dextran have demonstrated to show improved mechanical properties and controllable enzymatic degradation [6,15]. When synthesized from chemically modified dextran and cross-linked by UV, these IPN polymers can promote adhesion of endothelial cells in 2-dimensional culture and effectively encapsulate vascular smooth muscle cells in 3-dimensional scaffolds as well as support their migration and proliferation [6].

Despite biodegradability, biocompatibility and low production cost, poor mechanical properties of gelatin limits applications of this biopolymer in bioengineered systems [11,22–32]. By emerging the research area of polymer nanocomposites [33], however, gelatin based nanocomposites with improved properties have been prepared. For instance, a relatively small amount of nanoclay can cause significant improvements in the mechanical and thermal properties of gelatin [34–40]. Rao reported a Young’s modulus of 8.3 GPa, almost three times that of neat sample, by dispersing 10 wt.% of exfoliated montmorillonite clay into gelatin [37].

Nonetheless, specific study on the nanocomposites of gelatin based IPNs has not been reported yet. Specifically, the effect of nanoclay on the microstructure and properties of these materials has remained elusive. Therefore, the main objective of this work was to explore how exfoliated sodium-montmorillonite (Na+–MMT) can alter the structural evolution, phase morphology and application properties of gelatin/poly(ethylene glycol) dimethacrylate (PEGdMA) semi and full-IPNs. Furthermore, the effect of cross-linking protocol, i.e., simultaneous versus sequential was investigated. The properties of the IPN systems were then characterized and related to the structures developed during different synthesis protocols. Here, it is hoped to shed light on the mechanisms underlying the development of morphology and final properties in gelatin based biohybrid IPNs as promising materials for drug delivery and tissue engineering.

2. Experimental section

2.1. Materials

Gelatin (Type B, 80–120 bloom, pH 5 ± 0.5) and glutaraldehyde (25% (w/w) solution in water) were purchased from Merck. Poly(ethylene glycol)dimethacrylate (PEGdMA, 750 Da, liquid in room temperature) was obtained from Sigma–Aldrich. 2,2-dimethoxy-2-phenylacetophenone (DMPA), UV initiator for cross-linking of PEGdMA, and Na+–MMT (Cloisite® Na+) were purchased from ACROS and Southern Clay Products Inc., respectively. All chemicals were of reagent grade and except glutaraldehyde, were used without further purification. Monomeric glutaraldehyde was purified from polymeric glutaraldehyde by treatment with charcoal (5% w/v) for 3–4 times [41]. Deionized water was used in all experiments.

2.2. Gelatin/nanoclay dispersion

2.2.1. Preparation

Gelatin (0.2 g mL−1) was dissolved in deionized water at 60 °C under stirring for 4 h. Separately, a 3 wt.% Na+–MMT dispersion (0.03 g mL−1) was prepared by dispersing clay in deionized water via magnetic stirring for 24 h and then ultrasonication (LABSONIC® P, B. Braun, Germany) for 10 min at room temperature. Clay dispersion was slowly added to the gelatin solution at 50 °C and the mixture was stirred using a magnetic stirrer for 2 h at 400 rpm. The samples were prepared in three different nanoclay to gelatin weight ratio (0, 1.5, 5% w/wgelatin). To do so, 0, 1.5, and 5 mL of the 3 wt.% nanoclay dispersion were added to 15 mL of the 20 wt.% gelation solution. By adding deionized water, the final gelatin concentration and volume of gelatin/clay mixtures were adjusted at 10 wt.% and 30 mL, respectively. Final concentrations of clay in the mixtures were 0, 0.0015, and 0.005 g mL−1 for three different weight percent ratios of nanoclay to gelatin, respectively. Finally, for chemical crosslinking of gelatin, 0.12 mL glutaraldehyde (1% w/wgelatin) was added to gelatin/nanoclay mixtures and vortexed thoroughly for 1 min. The mixtures were immediately transferred to rheometer to measure the chemical gelation kinetics of gelatin in the presence of nanoclay. All prepared samples had a pH of around 5.2.

2.2.2. Rheological measurements

Anton Paar Physica MCR 300 stress-controlled rheometer in the oscillatory mode was used. First, to determine the linear viscoelastic region of each sample, a strain sweep test was performed in the strain range of 0.01–100% at 30 °C and at a constant frequency of 1 Hz. Then, the main measurements were performed at a constant strain of 1% in the linear viscoelastic regime, and at constant
angular frequency of 6.28 rad s⁻¹ (1 Hz) at 30 °C. A solution volume of 20 mL was used for the measurements in a Couette cell (cup diameter 14.46 mm, bob diameter 13.33 mm). During gelation of gelatin/Na⁺-MMT/GA solutions, storage modulus, \( G' \), and loss modulus, \( G'' \), were recorded as a function of time. The gelation point was determined from the crossover point of storage and loss moduli.

2.3. Semi and full IPNs nanocomposite films preparation

DMPA as photo initiator (0.0075 g, 1.5% w/wPEGdmA) was added to 0.5 g PEGdmA at room temperature and vortexed to prepare a transparent and homogeneous solution. Then, the gelatin/nanoclay mixture, prepared according to 2.2, was added to this solution at 50 °C and stirred using a magnetic stirrer for 15 min at 250 rpm. Finally, glutaraldehyde (1% w/wgelatin) was added to gelatin/Na⁺-MMT/PEGdmA/DMPA mixture and vortexed thoroughly for 1 min. Then, 5 mL of the final mixture was cast into a Petri dish (50 mm in diameter) and radiated by a UV source (300 W) at a distance of 40 cm for 15 min to simultaneously cross-link PEGdmA and gelatin. The samples A1 (0% w/wpolymer clay), B1 (1.5% w/wpolymer clay) and C1 (5% w/wpolymer clay) were prepared by simultaneous cross-linking method.

For the synthesis of sequential IPNs, the gelatin/Na⁺-MMT/PEGdmA/DMPA mixtures were treated with GA (1% w/wgelatin) and was stirred for 1 min at 300 rpm. The mixture was then allowed to stand in the Petri dish for 30 min at 30 °C until the chemical cross-linking of gelatin is complete. The semi-crosslinked film was then irradiated under UV for 15 min to cross-link PEGdmA in the gelatin network matrix. The samples A2 (0% w/wpolymer clay), B2 (1.5% w/wpolymer clay) and C2 (5% w/wpolymer clay) were prepared via sequential protocol. In addition, for the synthesis of semi-IPNs, the gelatin/Na⁺-MMT/PEGdmA/DMPA mixtures were irradiated under UV for 15 min but were not treated with GA. The samples A3 (0% w/wpolymer clay), B3 (1.5% w/wpolymer clay) and C3 (5% w/wpolymer clay) were prepared by this method. Gelatin to PEGdmA weight ratio was the same for all samples (0.5/0.5 g).

All samples were slowly dried at room temperature for 2 weeks and in a vacuum oven for 48 h to form films of about 300 μm in thickness.

2.4. Atomic force microscopy studies

2.4.1. Nano-indentation tests

A Hysitron Triboscope with a Berkovich-type indentation tip, in conjunction with a Digital Instruments Nanoscope III atomic force microscope (AFM) were used for both nano-indentation and topography images of samples at room temperature.

Nano-indentation test was performed at constant loading and unloading rates of 25 μN s⁻¹ and to a maximum load \( P_{\text{max}} \) of 500 μN. During each test, the maximum load was held for 10 s and then unloading started. The reduced modulus, \( E_r \) and hardness, \( H \), were calculated from the unloading curves following the method of Oliver and Pharr [42,43]. The reduced modulus is related to Young’s modulus, \( E \), by \( 1/E_r = (1 - v^2)/E_1 + (1 - v^2)/E_2 \) where subscripts 1 refers to the indenter material, subscripts 2 refers to the indented material, and \( v \) is Poisson’s ratio. The hardness (\( H \)) of sample is defined as: \( H = P_{\text{max}}/A_r \) where \( P_{\text{max}} \) and \( A_r \) are the peak indentation load and residual area.

2.4.2. Homogeneity index

Due to nano-metric scale of the nano-indentation test, the same loading and unloading responses to the indentation at different regions of the sample may be attributed to the structural homogeneity. For this purpose, a Homogeneity Index (HI) was calculated from the normalized standard deviation of the displacement data at a given force applied to the different regions of each sample. The HI varies between zero and 1 for fully heterogeneous and homogeneous structures, respectively.

2.4.3. Phase contrast images

Topology and phase contrast images were obtained by AFM in the phase-contrast mode to illustrate the homogeneity of the phases.

2.5. Dynamic mechanical thermal analysis (DMTA)

Dynamic mechanical analysis was performed in a Diamond Dynamic Mechanical Analyzer (Perkin–Elmer) operated in tensile mode. Samples with the dimension of 50 × 13 × 0.3 mm³ were cooled to −50 °C and then ramped to 300 °C at the heating rate of 3 °C min⁻¹ while oscillated at a frequency of 1 Hz.

2.6. Mechanical tests

Tensile strength (TS) at break was determined via a microtensile tester (MECMESIN, USA). The samples were cut in the size of 1 cm × 4 cm strips. Tensile strength at break was calculated via the force divided to cross section of the samples. At least 3 specimens were tested for each system. All data were expressed as the mean ± standard deviation (SD) for \( n = 3 \).

2.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetry measurements were carried out using a TA Instrument, DSC Q100 (USA). The heat flow thermograms for A3 and B3 samples were taken from −80 °C to 260 °C, and for other samples from room temperature to 260 °C at a heating rate of 10 °C min⁻¹. The glass transition temperature was defined as the midpoint between the onset and endpoint of the baseline shift.

2.8. Scanning electron microscopy (SEM)

The morphological feature of the semi-IPN samples was investigated by a scanning electron microscope (SEM, DSM-960 A ZEISS, Germany) operated at 10 kV. To extract the gelatin phase, the samples were washed with deionized water for a weak and then dried. Subsequently, the samples were vacuum dehydrated and spattered with gold film.

2.9. X-ray diffraction

X-ray diffraction (XRD) studies on the sequential IPN samples (A2, B2, C2) were carried out using XPert Philips diffractometer (Netherlands) by using CuKα radiation (\( \lambda = 0.154 \text{ nm} \)) at a generator voltage of 40 kV and a generator current of 40 mA. The samples were scanned from the start angle of 1° to stop angle of 11° at the rate of 0.02° s⁻¹.

3. Results and discussion

The gelation behavior of the IPN components has a crucial role in the development of final morphology. Assuming complete miscibility before gelation occurs, the phase separation triggers immediately after gelation of one of the components. It has been accepted that as the gelation times of the components are closer, the less difference in the compositions of the phase separated IPN is occurred and when the gelation of both components starts simultaneously, a single phase IPN forms [44]. In the case of gelatin/
PEGdMA IPNs, gelatin is cross-linked via chemical cross-linking, and PEGdMA is cross-linked by UV radiation, which allows for the in-situ conversion of the photo-reactive solution into gel. The rate of chemical gelation of the components in the aforementioned system can be easily altered by several parameters such as temperature, solution concentration, cross-linking agent concentration, pH, UV radiation intensity, etc. However, in this study, it has been mainly concentrated on the role of cross-linking sequence on the structure, morphology and mechanical properties of the resulted IPNs. Furthermore, the effect of Na\(^+\)-MMT on the gelation behavior of gelatin and its consequences on the development of the IPN morphology is investigated.

### 3.1. Rheological measurements

The structural evolution during chemical cross-linking of gelatin can be effectively probed by linear viscoelastic measurements. In this paper, the linear viscoelastic properties of the gelatin/Na\(^+\)-MMT systems during cross-linking were characterized via small-amplitude shear oscillatory measurements in the time domain. Fig. 1 shows the evolution of storage modules (\(G'\)) and loss modulus (\(G''\)) as a function of time for pure gelatin solution and gelatin–Na\(^+\)-MMT nanoplatelet systems containing 1.5 and 5% w/w gelatin nanoclay at 30 °C. As expected, both moduli increased with time due to the formation of chemical cross-links between the gelatin chains. The gelation time of gelatin/Na\(^+\)-MMT/glutaraldehyde systems were calculated from the intersection of the storage and the loss moduli (Table 1). In short, rheological measurements showed that the gelation time decreased with increasing the nanoclay content in gelatin/nanoclay systems, which cross-linked by glutaraldehyde. It seems that Na\(^+\)-MMT nanoplatelets play a pseudo-catalytic role and accelerate the cross-linking kinetics by hastening the formation of chemical connections between the gelatin molecules. It is generally accepted that during cross-linking, aldehyde functional groups of the glutaraldehyde bridges between free amino (–NH\(_2\)) groups of lysine or hydroxylysine through a nucleophilic addition type reaction [45]. The accelerating role of Na\(^+\)-MMT nanoplatelets may be attributed to the electrostatic interactions between negative charges on the Na\(^+\)-MMT and positive charges on the gelatin (NH\(_3\)) [46,47]. As proposed by Bowman et al. [48], the polarization of gelatin molecules by the electric field of Na\(^+\)-MMT platelets, bring the positively charged units closer to the clay surfaces. Under these conditions, the gelatin molecules can attach to the surface of the suspended MMT platelets in which segments containing more negatively charged units extending out into the bulk solution. It seems, adsorption of gelatin chains on the MMT platelets increased the number of effective collisions between the aldehyde and the amino groups and hence led to the faster cross-linking.

### 3.2. General observations

All IPN films (A\(_1\), A\(_2\), C\(_1\), and C\(_2\)) were transparent in the studied clay-loading range (Fig. 2(a)). Semi-IPN films, however, (A\(_3\) and C\(_3\)) were not thoroughly transparent. Transparency of the full IPN films implies nano-meter scale phase separation in these samples. On the other hand, microscopic phase separation (greater than 500 nm) in semi-IPNs resulted in an opaque appearance. The scale of phase separation in semi-IPN samples can be easily investigated by SEM. Fig. 2(b) and (c) shows the fracture surface morphology of cross-PEGdMA-inter-gelatin (A\(_3\) sample) before and after selective extraction of the linear component (gelatin). For extracting of the gelatin phase, the sample was washed with deionized water for a weak. Interpenetrating structure with phase separation in the scale of few microns was obvious in gelatin/PEGdMA semi-IPN (Fig 2(c)).

### 3.3. Assessment of the morphology of the samples by AFM

Nano-indentation and topography of IPN samples were studied by a Hystron Triboscope with a Berkovich-type indentation tip at room temperature. For each sample, a number of force–displacement

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**Table 1**

<table>
<thead>
<tr>
<th>Clay(Na(^+)-MMT) %</th>
<th>Gelation time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>516</td>
</tr>
<tr>
<td>1.5</td>
<td>312</td>
</tr>
<tr>
<td>5</td>
<td>252</td>
</tr>
</tbody>
</table>

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Fig. 1. Gelation behavior of gelatin 10% (w/v) solutions, cross-linked by glutaraldehyde in different percentages of nanoclay. Storage modules (\(G'\)) and loss modulus (\(G''\)) as a function of time.

Fig. 2. (a) Transparency of the full IPN nanocomposite films and blurriness of the semi-IPN films. SEM images of the semi-IPNs based on cross-linked PEGdMA and linear gelatin (b) before, and (c) after extracting of gelatin phase.
curves were obtained in the area of 9 µm² and under loading and unloading rates of 25 µN s⁻¹ [Fig. 3(a) and (b)].

In the case of simultaneous IPNs, the similarity of the indentation curves, acquired from different areas of the sample, verified the enhancing role of exfoliated montmorillonite in phase homogeneity (Fig. 3(c) and (d)). According to rheological measurements (Section 3.1), the difference between gelation times of two polymers in SIM-IPNs decreased with increasing of Na⁺-MMT content. Therefore, as clay content increased, the compositions of the two phases became closer and homogeneity of the phases improved.

Furthermore, for SEQ-IPNs, Na⁺-MMT content did not significantly influence homogeneity of the phases (Fig. 3(c) and (e)). Because of sequential method of synthesis, the difference between gelation times of two polymers did not considerably change with increasing of Na⁺-MMT content.

On the other hand, the phase homogeneity of semi-IPNs was significantly decreased with increasing of Na⁺-MMT content, whereas a macroscopic phase separation was occurred in the semi-IPN sample containing 5% Na⁺-MMT [Fig. 3(f)].

3.4. Mechanical and nano-mechanical properties

The difference in mechanical behavior of the investigated samples can be explained based on different network structures evolved during the cross-linking process. The effect of Na⁺-MMT and synthesis protocols (SEQ, SIM, and semi-IPN) on the mechanical and nano-mechanical properties, which were respectively measured by atomic force microscopy and micro-tensile, is summarized in Table 2. The average reduced modulus, Eᵣ, and the hardness, H, of full and semi-IPNs were calculated from the unloading force–displacement curves following the method of Oliver and Pharr by atomic force microscopy [49].

<table>
<thead>
<tr>
<th>Samples</th>
<th>Elastic modulus Eᵣ (GPa)</th>
<th>Hardness H (GPa)</th>
<th>Micro-tensile Strength (TS) (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁, SIM-IPN (no clay)</td>
<td>3.44 ± 0.059</td>
<td>0.244 ± 0.018</td>
<td>30.42 ± 0.39</td>
</tr>
<tr>
<td>B₁, SIM-IPN (1.5% clay)</td>
<td>3.90 ± 0.158</td>
<td>0.356 ± 0.015</td>
<td>23.99 ± 0.03</td>
</tr>
<tr>
<td>C₁, SIM-IPN (5% clay)</td>
<td>6.04 ± 0.125</td>
<td>0.396 ± 0.008</td>
<td>33.39 ± 0.125</td>
</tr>
<tr>
<td>A₂, SEQ-IPN (no clay)</td>
<td>4.43 ± 0.28</td>
<td>0.329 ± 0.031</td>
<td>28.74 ± 1.645</td>
</tr>
<tr>
<td>B₂, SEQ-IPN (1.5% clay)</td>
<td>3.81 ± 0.23</td>
<td>0.335 ± 0.012</td>
<td>22.76 ± 3.625</td>
</tr>
<tr>
<td>C₂, SEQ-IPN (5% clay)</td>
<td>3.93 ± 0.03</td>
<td>0.276 ± 0.03</td>
<td>24.4 ± 0.705</td>
</tr>
<tr>
<td>A₃, Semi-IPN (no clay)</td>
<td>3.43 ± 0.07</td>
<td>0.18 ± 0.001</td>
<td>23.48 ± 1.045</td>
</tr>
<tr>
<td>B₃, Semi-IPN (1.5% clay)</td>
<td>2.43 ± 0.2</td>
<td>0.13 ± 0.0004</td>
<td>18.6 ± 1.235</td>
</tr>
<tr>
<td>C₃, Semi-IPN (5% clay)</td>
<td>–</td>
<td>–</td>
<td>17.56 ± 2.065</td>
</tr>
</tbody>
</table>

* Value are given as mean ± SD from four determinations (n = 4).

In SIM-IPNs, the incorporation of Na⁺-MMT significantly increased the nano-mechanical properties. Incorporation of 5% w/ w clay led to increase of Eᵣ form 3.44 to 6.04 GPa. However, in the case of SEQ-IPNs and semi-IPNs, nanoclay had a detrimental effect on Eᵣ and H. Moreover, the hardness values showed a clear dependence on the cross-linking method and the nanoclay content. The different dependence of the elastic modulus and hardness of the IPNs on nanoclay content may be attributed to the different nature of the two parameters. Elastic modulus is an intrinsic material property and is fundamentally related to the secondary interac- tions. On the other hand, hardness is an engineering property and for some materials, it can be related to the yield strength. In viscoelastic materials, hardness is a time-dependent function where the total deformation can be considered because of viscous, elastic and plastic deformation components [50].

In spite of useful data, in nano-indentation test, the measurements are limited to the areas of few micrometers or even nanometers which may not be the representative of the whole sample. Furthermore, the nano-indentation test only reflects the surface mechanical properties of the IPN films. Therefore, in addition to the nano-mechanical tests, conventional mechanical tests were also performed for better understanding of the relationship between structure and properties. The tensile strength data obtained by tensile measurements are also summarized in Table 2. Generally, the micro-tensile results (tensile strength) are similar to nano-mechanical results (Eᵣ, H). As clay content increased, tensile strength (TS) considerably increased for simultaneous IPN, but it decreased for sequential and semi-IPNs. These results also confirmed previous observations, which indicated the phase separation slowed down by addition of Na⁺-MMT in SIM-IPN and intensified in the semi-IPN. The decrease of the gelation time difference in SIM-IPNs led to restricted phase separation and homogeneity of the phases, which resulted in increasing of the nano-mechanical (Eᵣ, and H) and mechanical (TS) properties. Conversely, the increase of the gelation time difference of the two polymers in SEQ-IPNs led to phase separation in nano-metric scale and decreased Eᵣ, H, and TS. The phases in semi-IPNs are likely to separate with addition of nanoclay because gelatin chains do not efficiently cross-link [Fig. 3(f)].

3.5. Thermal properties

Two mechanisms of phase separation are nucleation and growth, and spinodal decomposition. Differences in the conditions of phase separation predetermine the physical and morphological features of the IPNs. As a rule, simultaneous IPNs are phase-separated by a spinodal mechanism and sequential ones via a...
mechanism of nucleation and growth [44]. It is evident that after the transition of the system from the one-phase state into the metastable and unstable regions happened, the stable chain entanglements do not allow the full separation of network fragments and the system stays in the state of “forced” miscibility (compatibility) [51]. The incomplete phase separation leads to the formation of a transition zone or an interphase between two evolved phases [44]. A way to establish the existence of an interfacial region in phase-separated IPNs might be the application of differential scanning calorimetry (DSC) [52,53].

The DSC of SIM-IPN showed a broad transition between two glass transition temperatures of the separated phases (Fig. 4(a) and (c)). This is a consequence of the presence of an interfacial region, which possesses a locally varying composition. The broad glass transition peak possibly indicated that the IPNs were partially mixed at the molecular level. Glass transitions of gelatin and PEGdmA separated phases were around 210 °C and ~50 °C, respectively (Fig. 4(c)). Increasing of Na⁺-MMT content led to improvement of the interfacial region in SIM-IPNs. Particularly, with addition of 5 wt.% Na⁺-MMT in SIM-IPN, it was observed that the interfacial region between two-glass transition expanded and thus segregation of the phases decreased. In addition, interfacial region was tending to expand toward higher temperatures due to the presence of Na⁺-MMT that acts as a hard segment and restricts the molecular motion. The dynamic mechanical analysis (DMA) confirmed this claim [Fig. 4(b)]. While, two distinct transition were observed for sample A₁ (SIM-IPN, no clay), the sample C₁ (SIM-IPN, 5% clay) showed only one transition at high temperatures (~215 °C). Such a high Tₓ of ~200 °C for dried gelatin films has already been reported by several authors [54–56].

The DSC results for the semi-IPNs showed that the adding of 5% w/w Na⁺-MMT leads to disappearance of the interfacial region due to complete segregation of the phases [Fig. 4(c)]. The appearance of only two relaxation transitions (at ~210 °C and ~50 °C) was the first sign of the heterogeneous two-phase structure in the C₃ sample (semi IPN, 5% nanoclay). In the case of A₃ sample (cross-PEGdmA-inter-gelatin, no Na⁺-MMT), the peak between two relaxation transitions may be attributed to interfacial region and more to the melting of gelatin triple-helices. When a gelatin solution is cooled below 30 °C, a three-dimensional reversible network is formed. This is due to the formation of triple-stranded helices [2,29]. Based on the DSC thermogram of the C₃ sample (cross-PEGdmA-inter-gelatin, 5% w/w Na⁺-MMT), it can be implied that the triple helices formation is suppressed in the presence of Na⁺-MMT.

In sum, incorporation of Na⁺-MMT to SIM-IPN led to restricted phase separation and to improved mechanical, nanomechanical and thermal properties. However, in the semi-IPNs, the nanoclay had a detrimental effect and induced segregation of the phases and decreased the mechanical and thermal properties.

To illustrate morphological homogeneity in full-IPN samples, three-dimensional phase contrast and topography images of SIM and SEQ-IPN are shown in Fig. 5. In nano metric scale, full-IPNs (especially SIM-IPN) did not show any phase contrast with adding 5% w/w Na⁺-MMT. The morphology of SIM-IPNs showed an entirely co-continuous structure of the gelatin and PEGdmA phases, which can be considered as a sign of mixing at molecular-level. SIM-IPN and SEQ-IPN morphological observations agree well with nano-indentation, thermal and rheological results. In sum, IPN samples with 5% Na⁺-MMT did not show phase separation due to the interpenetration of the component polymers. In addition, phase contrast AFM images and transparency of all samples proposed exfoliated structure for the nanocomposites samples. Only clay in C₂ sample (SEQ-IPN with 5% w/w Na⁺-MMT) showed an intermediate structure (intercalated/exfoliated). XRD results confirmed these results (Fig. 6). A similar result on nanoplatelet dispersion in gelatin films has been obtained by Ruseckaite et al. [57] via AFM measurements.

4. Conclusions

The effects of cross-linking sequence and incorporation of sodium-montmorillonite nanoclay on the structure, morphology and properties of gelatin/PEGdmA semi and full-IPNs were investigated. Depending on the type of cross-linking route and the amount of nanoclay, different types of structures can be obtained. Interestingly, kinetics of chemical cross-linking of gelatin and therefore the structural evolution of the IPNs can be manipulated by the nanoclay content. As incorporation of 5% w/w exfoliated sodium-montmorillonite to the gelatin solution decreased the chemical gelation time by a factor of 2. In the case of simultaneous IPNs, this accelerated cross-linking of gelatin in the presence of
nanoclay perfectly matched with the cross-linking process of PEGdma and led to increased homogeneity (less phase separation), improved mechanical and thermal properties. On the other hand, the incorporation of nanoclay to semi-IPNs led to macroscopic phase separation and thus to the reduced interpenetrating of the chains and finally resulted in deterioration of both thermal and mechanical properties. These findings have the potential to alter our understanding of phase separation in biointerpenetrating polymer networks and thereby contribute to novel paradigms for the fabrication of well-defined structures for tissue engineering and drug delivery applications.

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