Effects of Reaction Conditions on Intercalation between Gelatin and Montmorillonite: Thermodynamical Impact

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ABSTRACT: To investigate the effects of reaction conditions on a intercalation between gelatin and montmorillonite (MMT) and thermodynamical impact of the intercalation, a series of gelatin/MMT hybrids were prepared by changing the reaction conditions (weight ratio of gelatin and MMT, pH, concentration of gelatin solution and MMT suspension, and reaction temperature).The hybrids were characterized by X-ray diffraction, transmission electron microscopy, atomic absorption spectroscopy, and thermogravimetric analysis. The results showed the intercalation between gelatin and MMT, which was a faster process than the melt intercalation of some synthetic macromolecules such as polystyrene, achieved a thermodynamically stable state in 5 min. All the other reaction conditions, except reaction temperature, affected the intercalation between gelatin and MMT. The reaction was driven by enthalpy. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

INTRODUCTION

Polymer/layered silicate hybrid nanocomposites have attracted notable interests in the past years. In 1993, the Toyota Central Research and Development Labs reported the synthesis of nylon 6/clay hybrid for the first time.[1] From then on, most of the synthetic polymers have been intercalated into the interlayer of layered silicates, and the structure and properties of these polymer/layered silicate nanocomposites were studied.[2–5] There are four principal methods for preparing polymer/layered silicate nanocomposites: (1) *in situ* template synthesis; (2) intercalation of polymer or prepolymer from solution; (3) *in situ* intercalative polymerization; and (4) melt intercalation.[6] For most of engineering polymers, both *in situ* intercalative polymerization and intercalation from solution are limited because neither a suitable monomer nor a compatible polymer-silicate solvent is always available.[7] Polymer melt intercalation is appealing because of its versatility, its compatibility with current polymer processing techniques, and its environmentally benign character due to the absence of solvent.[8] The kinetics of polystyrene melt intercalation in organically modified mica-type silicates were studied systematically by Giannelis.[8–10] They found that the hybrid formation was limited by mass transport into the primary particles of the host silicate and not specifically by diffusion of polymer chains within the silicate galleries. Interplay of entropy and enthalpy factors determined the outcome of polymer inter-

calation. From then on, many investigations such as the intercalation kinetics of long polymers in 2 nm confinements[11] and the phase behavior of polymer/clay nanocomposites in melt intercalation[12] were made and great progress has been achieved.

With the rapid development of bioengineering and biomaterials, macromolecular drug carrier[13] and gene vector[14] have received much attention. Recently, new hybrid materials involving the interaction between biomacromolcules and inorganic clays have been widely investigated because of the unique physicochemical properties and potential applications. The development of layered silicate and biomacromolecule hybrids including protein,[15,16] DNA,[17] chitosan,[18] and so on caused new materials with biological roles. For example, the bovine serum albumin substitution allowed the embedding of the protein into the layered clay galleries in an uncompressed conformation.[16] Clay protected the DNA from degradation.[17] Chitosan/clay hybrids became suitable for anions detection.[18]

In our previous work, a novel biomaterials, gelatin/montmorillonite (MMT) hybrid nanocomposite, was reported for the first time. The mechanical and thermal properties of gelatin were improved significantly by intercalation,[19] then the swelling behavior of the nanocomposite and the influences were described.[20] In addition, the interaction between gelatin and MMT was studied.[21] As a thermosensitive macromolecule, biomacromolecule could be denatured and deactivated in a higher

Table I. Details of the Preparation of Gelatin/MMT hybrids

Sample	Gelatin (g)	MMT (g)	pH	Temperature (°C)	Reaction time (min)
1	3	5	5	60	5
2	3	5	5	60	60
3	3	5	5	60	120
4	3	5	5	60	240
5	1	10	5	60	60
6	1	6	5	60	60
7	1	2	5	60	60
8	1	1	5	60	60
9	2	1	5	60	60
10	4	1	5	60	60
11	10	1	5	60	60
12	3	5	4	60	60
13	3	5	5	60	60
14	3	5	7	60	60
15	3	5	5	60	60
16	0.06	0.1	5	60	60
17	3	5	5	50	60
18	3	5	5	70	60

temperature. The intercalated hybrids should be prepared in aqueous solutions, because most of biomacromolecules are hydrophilic. Therefore, the biomacromolecule intercalation is various from melt intercalation. However, to the best of our knowledge, there is never any report about the investigation on thermodynamical impact of the intercalation between biomacromolecule and layered silicate.

In this article, gelatin/MMT hybrid was chosen as a model of biomacromolecule/layered silicate nanocomposite. Gelatin, a protein, is an amphoteric polyelectrolyte with an isoelectric point (pI) of 5.05. Gelatin's macromolecules exhibit different conformations and charge distributions with different pH. MMT is a natural layered silicate. Each layer consists of two silica tetrahedral sheets and one aluminum octahedral sheet. Cations such as Na^+, Ca^{2+}, and Mg^{2+} occupy the interlayer galleries of MMT. These cations can be exchanged by some organic cations.[4,5,8] In this work, thermodynamical impact of the intercalation between gelatin and MMT were investigated by changing the weight ratio of gelatin and MMT, pH, concentration of gelatin solution and MMT suspension, and reaction temperature. We think this work will offer an insight into developing protein drug delivery, gene vector, and so on.

EXPERIMENTAL

Materials
Gelatin (Type B, extracted from bovine skin) was purchased from Sigma Chemical (St. Louis, MO). Sodium MMT was supplied by Huate Chemical (Zhejiang, China). All other reagents were all of analytical grade.

Characterization
X-ray diffraction (XRD) patterns were recorded at 2°/min on a Rigaku DMAX-RC diffractometer (Japan) using CuKα radiation

($\lambda = 0.154$ nm) at a generator voltage of 50 kV and a generator current of 180 mA. The nanostructure features were characterized by Hitachi H-800 transmission electron microscopy (TEM) with 200 kV accelerating voltage. The thermogravimetric analysis (TGA) measurements were performed on a Rigaku TA-50 instrument (Japan) under nitrogen atmosphere over the temperature range 50–800°C at a heating rate of 10°C /min. A Pgeneral (China) TAS-990 atomic absorption spectrometry (AAS) was used to measure the concentration of Na^+ in the solution. Samples were diluted to 50 mL with l mL reactant.

Preparation of Gelatin/MMT Hybrids
Gelatin powder was soaked in 150 mL distilled water then heated at 60°C to gain a homogeneous solution under vigorous stirring (pH was adjusted with HCl or NaOH solution, respectively). MMT was ultrasonically dispersed in 100 mL distilled water at room temperature to obtain a suspension. The gelatin solutions were added dropwise into the MMT suspensions under vigorous stirring in a certain condition (reaction temperature and time). Then, those obtained samples were frozen in a freezer (Sanyo, MDF-382) at −80°C for 12 h. The frozen samples were lyophilized within a freeze-dryer (Chaist, Alpha-2-4) for 48 h. The gelatin/MMT hybrid powders were obtained through grinding and sieving. In the above process, the amount of gelatin and MMT, pH, reaction temperature, and time were all variable, which were given in Table I.

RESULTS AND DISCUSSION

Intercalation between Gelatin and MMT
The XRD patterns of MMT and the gelatin/MMT nanocomposites prepared with different reaction time (sample 1–4 in Table I) are shown in Figure 1. MMT exhibits the (001) reflection at $2\theta = 7.05°$, while the gelatin/MMT nanocomposites
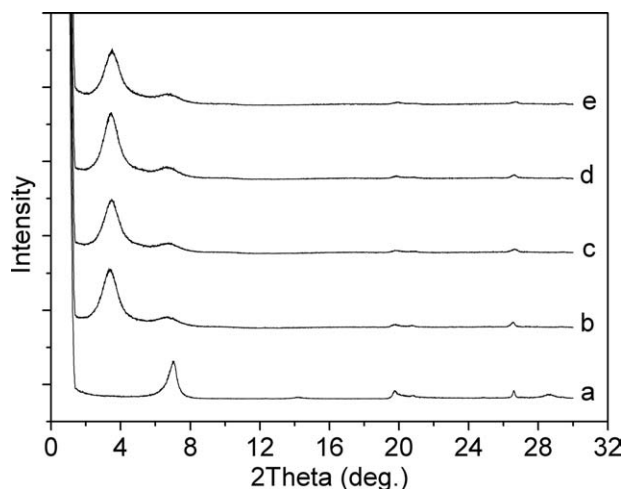
Figure 1. XRD patterns of MMT and gelatin/MMT nanocomposites prepared at different reaction times. (a) MMT; (b) 5 min; (c) 60 min; (d) 120 min; (e) 240 min.

exhibit the (001) reflections at 2θ of about $3.45°$, which are much lower than the (001) diffraction peak of MMT. Intercalated structures were formed in the gelatin/MMT nanocomposites.

The basal spacing of MMT and the MMT in gelatin/MMT nanocomposites is shown in Table II. The basal spacing of MMT in the nanocomposites increases to about 2.56 nm, which is much larger than that of MMT, due to the insertion of gelatin molecules into the interlayer of MMT. On the other hand, with the increasing reaction time, the basal spacing of the MMT in the nanocomposites almost keeps constant. The intercalated structures were formed and the intercalation reaction achieved the thermodynamically stable state in 5 min.

Vaia reported a 3×10^4 molecular weight polystyrene (PS30)/octadecylammonium-exchanged fluorohectorite (F18) mixture annealed *in situ* at 160°C in vacuum.[8] The XRD results showed that during the anneal, the intensity of the diffraction peaks corresponding to the pristine silicate is progressively reduced while a set of new peaks appears corresponding to the basal spacing of PS30/F18 intercalated hybrid. The whole intercalation reaction lasted more than 400 min. However, the intercalation reaction between gelatin and MMT was much different from that between PS30 and F18. The former was a quicker process than the latter and the system of gelatin and MMT achieved the thermodynamically stable state in 5 min.

Table II. XRD Data of MMT and Thegelatin/MMT Nanocomposites Prepared with Different Reaction Time

| Reaction time (min)	2θ (°)	d_{001} (nm)
MMT	7.05	1.25
5	3.36	2.63
6	3.50	2.52
120	3.43	2.57
240	3.50	2.52
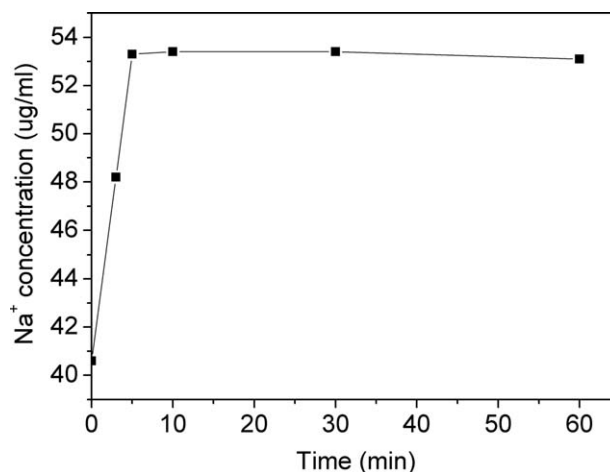
**Figure 2.** Free Na^+ concentration in the solution at different reaction times.

Na^+ in the interlayer of MMT can be easily replaced by other cations.[4,5,8] The formation of gelatin/MMT nanocomposite was accompanied by the replacement of Na^+ by $-NH_3^+$ on gelatin, and the concentration of Na^+ in the solution was changed. To make a quantitative analysis of the amount of intercalated gelatin, AAS was used to measure the concentration of Na^+ in the solution.

Figure 2 shows the concentration of Na^+ in the solution with different reaction time. The concentration increases rapidly with the reaction time in the first 5 min, indicating the gelatin started to insert into the interlayer of MMT. With the increasing reaction time from 5 min, the concentration never changed anymore, which meant the ion-exchange reaction reached equilibrium and the amount of intercalated gelatin kept constant.

TGA curves of gelatin and the gelatin/MMT nanocomposites prepared with different reaction time are shown in Figure 3. The onset thermal decomposed temperature of the nanocomposites is higher than that of gelatin, and the thermal decomposed
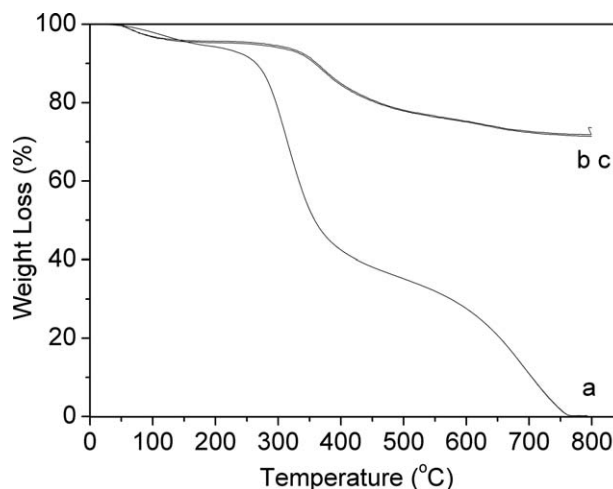
Figure 3. TGA curves of gelatin and gelatin/MMT nanocomposites at different reaction times.
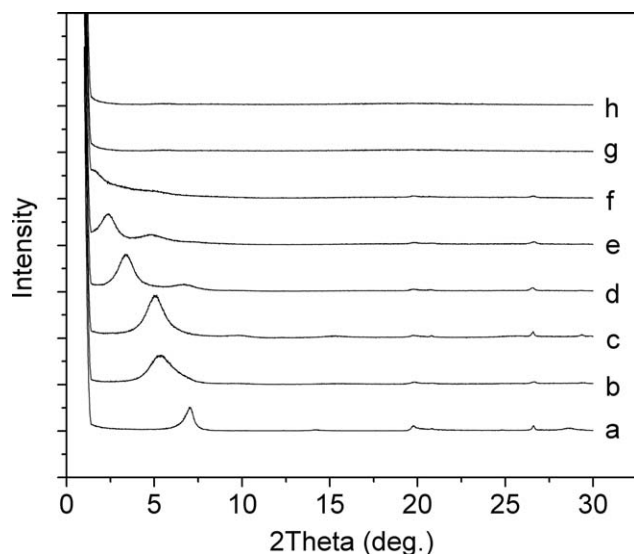
Figure 4. XRD patterns of MMT and gelatin/MMT nanocomposites prepared at different weight ratio (gelatin : MMT).

rate of the nanocomposites is obviously reduced. Intercalation with MMT significantly inhibited the weight loss of gelatin. In addition, the curve of gelatin shows two sharp weight losses. The first weight loss occurs at about 265°C and the second one at 600°C. The curves of gelatin/MMT nanocomposites just have one weight loss at about 330°C due to the gelatin chains interacted strongly with MMT. The curve of gelatin/MMT hybrids with reaction time of 5 min is much similar to that of 60 min. The amount of intercalated gelatin in the hybrids with reaction time of 5 and 60 min was almost equal.

All the above results revealed that the system achieved the thermodynamically stable state in 5 min and the intercalation reaction between gelatin and MMT was a very quick process, which was the most notable characteristic of intercalation between gelatin and MMT.

THERMODYNAMICS IMPACT OF THE INTERCALATION BETWEEN GELATIN AND MMT

Effect of Weight Ratio of Gelatin and MMT on the Intercalation

XRD patterns of MMT and the gelatin/MMT nanocomposites prepared with different weight ratio (sample 5–11 in Table I) are shown in Figure 4. The gelatin/MMT nanocomposites prepared with weight ratio of 1 : 10, 1 : 6, 1 : 2, 1 : 1, and 2 : 1 exhibit the (001) reflections at 2θ of 5.34°, 5.00°, 3.36°, 2.36°, and 1.48°, corresponding d-values are 1.65, 1.77, 2.63, 3.74, and 5.96 nm, respectively (Table III). The basal spacing of MMT in the gelatin/MMT nanocomposites increased with the increasing amount of gelatin and was larger than 1.26 nm (MMT) due to the insertion.

With the weight ratio increasing to 4 : 1 and 10 : 1, the (001) reflection disappears. As much more gelatin molecules inserted into the interlayer of MMT, the basal spacing became too large to maintain the intercalated structure. The absences of the (001)

reflections revealed the basal spacing of MMT in these samples was more than 8 nm,[22] and the exfoliated structure was formed.

The gelatin/MMT nanocomposites were characterized by TEM, as an addition to XRD. TEM images of the gelatin/MMT nanocomposite with weight ratio of 4 : 1 are shown in Figure 5. The bright field represents the gelatin matrix and the dark field stands for the layers of MMT. The basal spacing of MMT was more than 8 nm, and exfoliated structure was formed. The results of XRD and TEM confirmed the exfoliated structure was obtained in the nanocomposites with weight ratio of 4 : 1 (or higher). The basal spacing of MMT in the gelatin/MMT hybrids increased with the amount of gelatin and caused the formation of exfoliated structure finally. More and more gelatin chains inserted into MMT interlayers made the gelatin a crimp conformation and increased the reactive groups of gelatin chains that interacted with reactive sites on MMT. It was beneficial to intercalation reaction that the enthalpy change of the system increased.

Effect of pH on the Intercalation

As an amphoteric polyelectrolyte (pI = 5.05), gelatin molecules show different conformations and charge distributions in solutions with different pH. Moreover, pH has great influence on the intercalation of gelatin with MMT.

In Figure 6, the nanocomposites prepared with pH of 4, 5, and 7 (sample 12–14 in Table I) exhibit (001) reflections at 2θ of 2.25°, 3.36°, and 3.42°, and the basal spacing are 3.92, 2.63, and 2.49 nm, respectively. The basal spacing increases with the decreasing pH. Especially, when pH < pI, the basal spacing increases obviously, and the gelatin chains with more positive charges were enwrapped by negative charges on MMT layers through static electric interaction. Meanwhile, because of the electrostatic repulsion among positive charges, gelatin molecules were in an extended conformation. There were more gelatin chain segments interacting with MMT. The above two explanations interpreted the reasons of enthalpy changes through the intercalation process. When pH > pI, negative charges of gelatin chains and MMT layers resisted the intercalation reaction, and the basal spacing never decreased. The intercalation reaction was driven by the interaction between hydrophobic moieties on gelatin and MMT, and affected by the interaction between —COO⁻ on gelatin chains and Al³⁺ on MMT layers.[23]

Table III. XRD Data of MMT and the Gelatin/MMT Nanocomposites Prepared with Different Weight Ratio

Weight ratio (gelatin : MMT)	2θ (°)	d_{001} (nm)
MMT	7.03	1.26
1 : 10	5.34	1.65
1 : 6	5.00	1.77
1 : 2	3.36	2.63
1 : 1	2.36	3.74
2 : 1	1.48	5.96
4 : 1	–	–
10 : 1	–	–
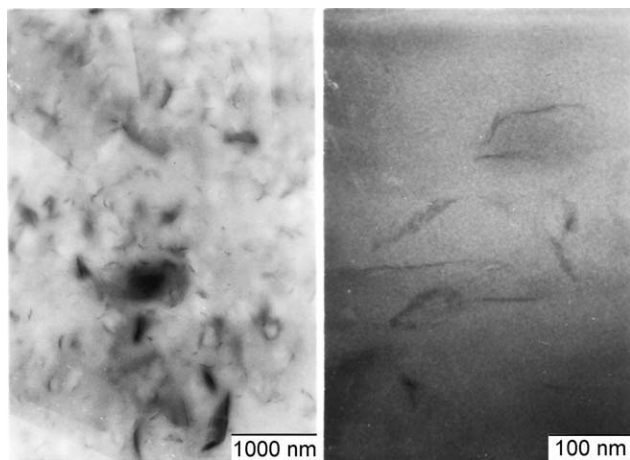
Figure 5. TEM images of gelatin/MMT nanocomposite.

Figure 7. XRD patterns of gelatin/MMT nanocomposites prepared with different concentrations.

Effect of Concentration of Gelatin Solution and MMT Suspension on the Intercalation

Figure 7 shows XRD patterns of the gelatin/MMT nanocomposites prepared with different concentrations (sample 15 and 16 in Table I). With the decrease of the concentration, the (001) reflection of MMT in the nanocomposites shifts from 3.36° to 5.35° and basal spacing of it declines significantly from 2.63 to 1.65 nm. The activity of gelatin molecules and MMT increased with the decline of the concentration, which resulted in the decrease of the probability of contacting and interacting between them.[24] Therefore, the amount of gelatin absorbed onto MMT layers decreased and most of reactive sites of MMT never interacted with the gelatin. This contributed to the reduction of total enthalpy variation in the system.

Effect of Reaction Temperature on the Intercalation

XRD patterns of the gelatin/MMT nanocomposites prepared at different reaction temperatures (sample 17 and 18 in Table I) are shown in Figure 8. The two XRD patterns of nanocomposites are almost the same, exhibiting the (001) reflection at 2θ
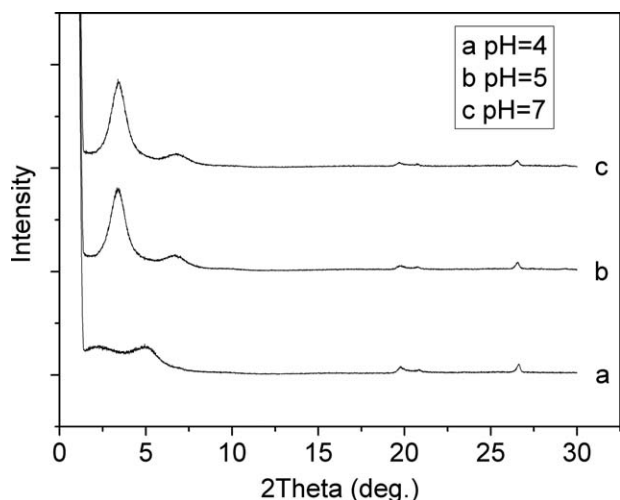
of 3.39°. The reaction temperature had almost no effect on the basal spacing of MMT in the gelatin/MMT nanocomposites. The entropy almost kept constant with the increasing reaction temperature.

According to the Second Law of Thermodynamics, spontaneously proceeding of the intercalation of polymer and layered silicate needs a negative variation in the Gibbs free energy:

$$\Delta G = \Delta H - T\Delta S < 0$$

where ΔG is Gibbs free energy changes, ΔH represents enthalpy changes, T stands for absolute temperature, and ΔS is entropy changes.

Assuming the configurations and interactions of the various constituents are independent, the free energy changes of hybrid

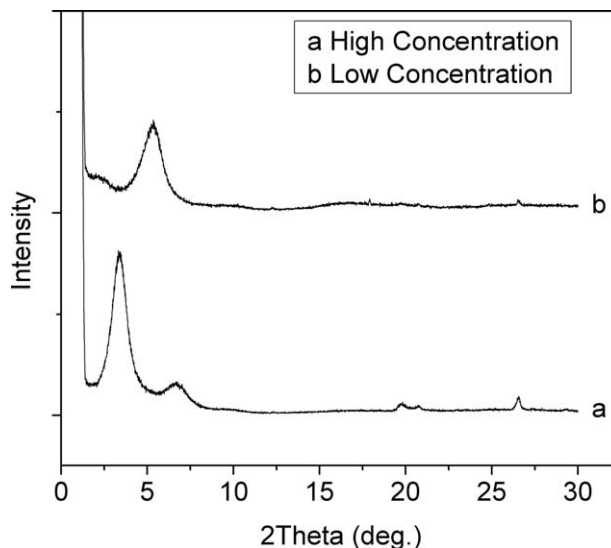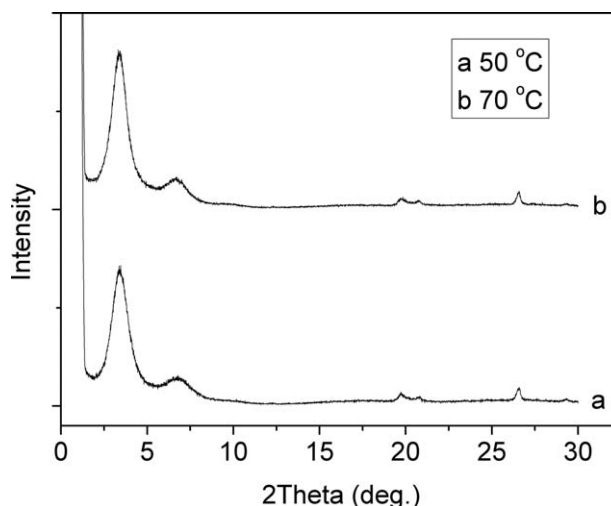**Figure 6.** XRD patterns of gelatin/MMT nanocomposites prepared at different pH. (a) pH = 4; (b) pH = 5; (c) pH = 7.

Figure 8. XRD patterns of gelatin/MMT nanocomposites prepared at different reaction temperatures.

formation are separable into independent enthalpic and entropic terms.[9] The entropic term is the sum of the conformational changes associated with the polymer and the hydrated Na^+ between the interlayers of MMT. The movement of gelatin molecules inserted into interlayer of MMT was confined by the lamella of MMT. Although the confinement of the polymer chains inside the silicate galleries results in a decrease in the overall entropy of the macromolecular chains, this entropic penalty may be compensated by the increase in the freedom of the hydrated Na^+. Therefore, the total ΔS of the intercalation was negative[6] and the process obeyed the relation of $\Delta H < T\Delta S < 0$. The increasing of the absolute value of ΔH and ΔS were all beneficial to the intercalation. Meanwhile, because of our results that the temperature could not affect the intercalation reaction, the item of $T\Delta S$ was rarely changed with the increasing reaction temperature. Therefore, the absolute value of ΔS was too small to be a determinant role of the thermodynamic state of the intercalation. Except reaction temperature, all the other reaction conditions affected the intercalation. Strong interactions between gelatin and MMT made the reaction to get a negative enthalpy change, which caused a negative variation in the Gibbs free energy. The intercalation was driven by enthalpy.

CONCLUSIONS

As a model of biomacromolecule/layered silicate nanocomposite, gelatin/MMT nanocomposites were prepared and characterized. Results revealed that the intercalation between gelatin and MMT was a quick process and achieved the thermodynamically stable state in 5 min, which was a typical characteristic of intercalation between gelatin and MMT. The intercalation reaction was affected by the reaction conditions (weight ratio of gelatin and MMT, pH, and concentration of gelatin solution and MMT suspension). When the weight ratio (gelatin : MMT) was relatively small (2 : 1 or smaller), the intercalated structure was obtained. With the increasing of weight ratio to 4 : 1 or larger, the exfoliated structure was formed. The basal spacing of MMT in the gelatin/MMT hybrids increased with the decrease of pH. A higher concentration of gelatin solution and MMT suspension made a larger basal spacing of MMT in the gelatin/MMT hybrids. Because of strong interaction between gelatin and MMT, the reaction system had a negative enthalpy change, which contributed to a negative variation in the Gibbs free energy. The intercalation was driven by enthalpy.

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