Studies on the Cure Kinetics of Chitosan-Glutamic Acid Using Glutaraldehyde as Crosslinker Through Differential Scanning Calorimeter

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ABSTRACT: The curing of chitosan-glutamic acid with glutaraldehyde as curing agent in the presence of chlorpheniramine maleate (CPM) is carried out with the help of differential scanning calorimeter (DSC). The effect of concentration of chitosan and percentage of crosslinker on the curing of chitosan-glutamic acid is studied at a heating rate of 5°C/min. Cure kinetics are measured by the DSC using scans from 25 to 220°C at four different heating rates (3, 5, 7, and 10°C/min) and it is observed that the crosslinking of chitosan-glutamic acid is an exothermic process which results in a positive peak in the DSC thermograms. The activation energy (E_α) is determined by Flynn, Wall, and Ozawa method for curing of the samples. An increase in activation energy (E_α) is observed with the extent of conversion. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 681–688, 2008

Key words: chitosan; crosslinker; IPNs; curing; thermal analysis

INTRODUCTION

Over the last several decades, many different kinds of polymeric systems are proposed as drug carriers. One of these systems is chitosan [(1→4)-2-amino-2-deoxy, β-D-glucan], a natural carbohydrate biopolymer derived by deacetylation (DA) of chitin. Chitin [(1→4)-linked-2-acetamido-2-deoxy-β-D-glucan] is a major component of the shells of crustacea such as crab, shrimp, and crawfish. After cellulose, chitin is the second most abundant natural biopolymer found in nature.[1] Because chitosan is easily soluble in acid, crosslinking of chitosan to form a network is the only way to prepare chitosan based interpenetrating polymer networks (IPNs).

Crosslinking is the process of physically or chemically combining a portion of a molecule to another molecule or portion of itself. This process has many effects on the physiochemical properties of an IPN. Various substances have been used to cure chitosan,[2–6] the most usual compounds are glutaraldehyde, formaldehyde, and other dialdehydes. Curing agents act as spacers and ties between the chains of IPNs, creating rigid chemical crosslinks, which affect the polymeric structure. Transport of drug release medium through the IPN depends upon the rigidity and extent of its crosslinking ability. At high temperatures, crosslinking occurs at a much faster rate.

Differential scanning calorimeter (DSC) measures the temperatures and heat flow associated with transitions in material as a function of time and temperature. DSC technique provides qualitative and quantitative information about physical and chemical changes that give out or take in heat as well as changes in heat capacity using a small amount of sample. Heat evolved during curing can be related to the degree of cure in the polymeric materials. It has many advantages including fast analysis time, easy sample preparation, applicability to both liquids and solids, a wide range of temperature applicability, and excellent quantitative capability.

Authors have synthesized semi-IPN by crosslinking of chitosan and glutamic acid with glutaraldehyde. The prepared semi-IPN is characterized for swelling and release studies of CPM drug. In the drug release study, glutamic acid is used to modulate the drug release from the chitosan based semi-IPN. To form semi-interpenetrating polymer network, two chitosan polymer chains are crosslinked by glutaraldehyde. Amino groups of chitosan and glutamic acid can be reacted with glutaraldehyde resulting in the attachment of glutamic acid in pendent form (Scheme 1). Thus, glutamic acid acts as a spacer between two chains of chitosan and this space between the chains can be varied according to the variation of concentration of glutamic acid in the semi-IPN. The biocompatibility

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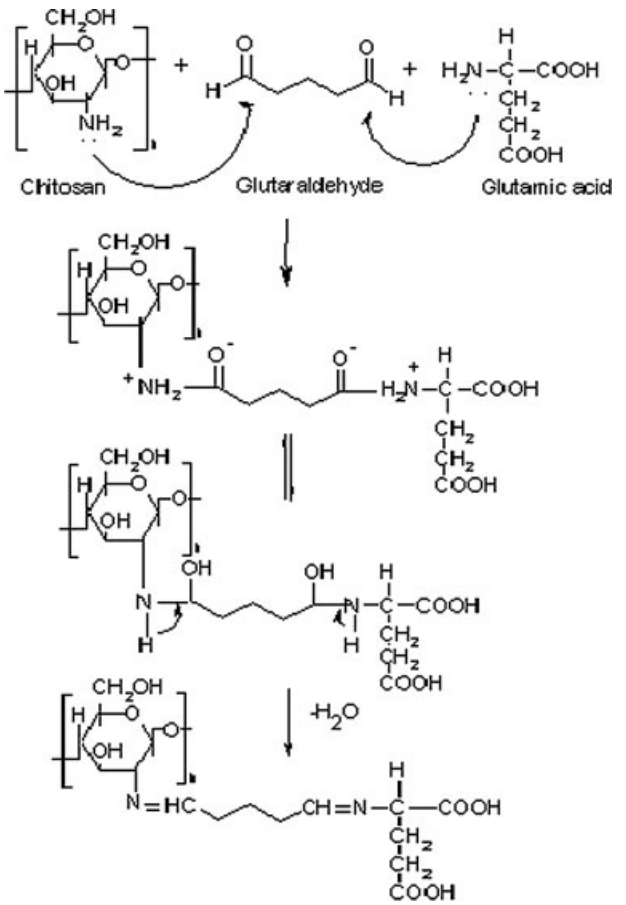

Scheme 1 Mechanism of curing of chitosan and glutamic acid with glutaraldehyde.

is another reason behind the use of glutamic acid along with chitosan.

In the present work, the curing kinetics of chitosan-glutamic acid with glutaraldehyde is studied by nonisothermal differential scanning calorimetry (DSC). The different heating rates of 3, 5, 7, and 10°C/min are applied to measure the heat flow of dynamic curing processes. The data are fitted by means of the Flynn, Wall, and Ozawa method to find out the activation energy as a function of conversion of the crosslinking process.

EXPERIMENTAL

Materials

Chitosan (total nitrogen: 7% minimum, percentage of deacetylation 80%; ignition residue (sulfate): < 2% and loss on drying <15%) was supplied by Tokyo Kasei Organic Chemicals, Japan and used as received. Chlorpheniramine maleate (CPM) ($C_{16}H_{19}ClN_2C_4\ H_4O_4$) was received as a gift sample from Japson Pharmaceuticals, Sangrur, India. L-glutamic acid ($C_5H_9\ NO_4$) (MW = 147.13), glutaraldehyde ($C_5H_8O_2$) (MW=100.11) 25% aqueous, and acetic acid were purchased from CDH (New Delhi, India) and were of analytical grade.

Technique

Calorimetric measurements were carried out by differential scanning calorimetry (DSC). The samples were prepared by mixing the known quantities of chitosan, glutamic acid, drug, and glutaraldehyde at room temperature. The glutaraldehyde was added just to prepare the uniform mixture of the constituents. The composition and designation of the samples are given in Tables II–IV. The prepared samples were transferred immediately to aluminum pan, weighed from 3 to 5 mg and placed in DSC for thermal analysis.

All DSC studies of curing behavior were performed with a DSC L63 LINSEIL differential scanning calorimeter. Prior to DSC runs, the temperature and heat were calibrated using indium and zinc standards. The sealed aluminum pan containing the sample was placed in DSC cell and heated according to the program of constant heating rate. The measurements were conducted under nitrogen atmosphere. Tests were performed in a dynamic mode at various heating rates over a temperature range of 25 to 220°C. The heating rates used in the different scans were 3, 5, 7, and 10°C/min. The heat of cure was determined from the peak area under the cure exotherm.

Experimental design

In the first type of scanning, the DSC experiments were carried out to study the effect of concentration of chitosan on the curing of chitosan and glutamic acid at heating rate of 5°C/min. A second type of scanning run was performed on the samples having equal composition of chitosan and glutamic acid with a varying concentration of crosslinker to study the effect of concentration of crosslinker on curing at a constant heating rate of 5°C/min. In the third type of scanning, samples having fixed concentration of crosslinker with varying proportions of chitosan/glutamic acid

TABLE I
DSC Results Showing the Effect of Bound Water on Curing at a Constant Heating Rate of 5°C/min

Designation	Chitosan (g)	Glutamic acid (g)	Drug (mg)	Distilled water (g)	Maxi. peak temp.(°C)	Enthalpy of transition (J g^{-1})
W1	1.0	–	–	3.7	71.1	711.74
W2	–	1.0	–	3.7	60.1	220.47
W3	0.5	0.5	100	3.7	67.1	429.14

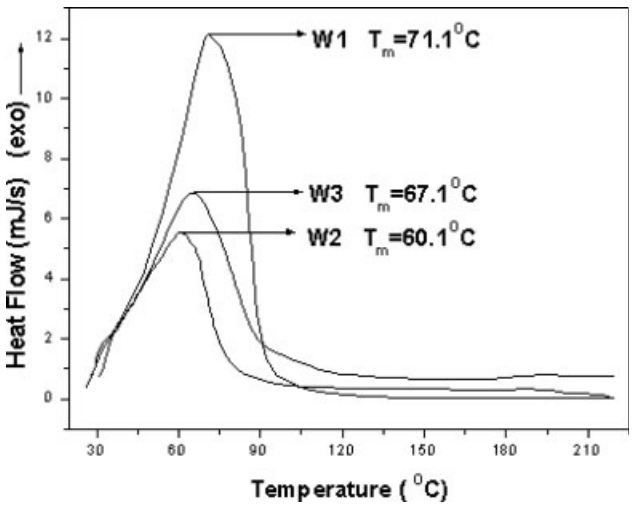

Figure 1 DSC plots showing the effect of presence of water during curing at a heating rate of 5°C/min (W1, chitosan-water; W2, glutamic acid-water; and W3, chitosan-glutamic acid-drug-water).

(3/7, 5/5, and 7/3) were cured. The varying heating rates from 3 to 10°C/min were applied to the samples to see the effect of rate of heating on curing and in that process to calculate the activation energy of curing. In all the DSC experiments, a fixed amount of drug (100 mg) was added to utilize the resultant information for controlled drug release studies through semi-IPN.

Mechanism of crosslinking reaction

Crosslinking is the process of chemically joining of two molecules by a covalent bond. Glutaraldehyde has two aldehyde groups, separated by a flexible chain of three methylene bridges (OHC—$(CH_2)_3$—CHO). The mechanism of crosslinking of chitosan and glutamic acid through glutaraldehyde is shown in Scheme 1. The curing reaction among three components, chitosan-glutaraldehyde-glutamic acid can be described by a two step kinetic model. Imine linkages (—C=N—) are formed among chitosan–chitosan and chitosan-glutamic acid molecules through glutaraldehyde.

RESULTS AND DISCUSSION

Differential scanning calorimeter (DSC) is used to study the kinetics/thermal characteristics of curing of chitosan and glutamic acid by glutaraldehyde. It is observed that the crosslinking of chitosan-glutamic acid is an exothermic process, which results in a positive peak in the DSC curve. These curves are used to calculate the enthalpy of cure by integrating the peak corresponding to a transition. The characteristic points of DSC curves are summarized in Tables II–IV.

Influence of presence of bound water in samples

Glutaraldehyde in water solution is used for crosslinking of chitosan and glutamic acid. So, all the samples with glutaraldehyde contain more than 50% of water. Thus, it is required to assess the interaction between water and drug carrier. Thermal analysis is performed to assess the interaction of water with chitosan and glutamic acid at a heating rate of 5°C/min. DSC scans of the samples as per Table I are shown in Figure 1. DSC analysis of chitosan-water (W1) shows a significant transition band in the range between 30 and 96°C, which is due to dehydration of loosely bound water molecules. In DSC graph of glutamic acid-water (sample W2), the transition band due to loss of water molecule is towards lower temperature range, i.e., 25–79.4°C. This may be due to the fact that the water molecule is more strongly bound to chitosan in comparison to glutamic acid. The water retention property of chitosan is more because of its hydrophilic nature. This interaction of chitosan-water molecules is reduced in sample W3, due to the presence of equal amount of glutamic acid. More over, glutamic acid is a low molecular weight compound as compared to chitosan and has a higher C : O ratio, as a result of which, the water evaporation peak appeared at 60.1°C (sample W2). When both of chitosan and glutamic acid along with drug are used (sample W3), the dehydration peak is observed at an intermediate temperature of 67.1°C. Although, the same amount of water (3.7 g) is present in all of the samples, the observed variation in peak area is believed to be due to water association with chitosan and glutamic acid. When glutaraldehyde is used for the preparation of semi-

TABLE II
DSC Results Showing the Effect of Concentration of Chitosan on Curing of Chitosan-Glutamic Acid at a Constant Heating Rate of 5°C/min

Designation	Chitosan (g)	Glutamic acid (g)	Drug (mg)	Glutaraldehyde (25%) (g)	Maxi. peak temp. (°C)	Enthalpy of curing peak (J g^{-1})
G1	1.0	0.0	100	3.7	75.1	1637.6
G2	0.7	0.3	100	3.7	74.2	680.32
G3	0.5	0.5	100	3.7	65.4	551.94
G4	0.3	0.7	100	3.7	62.3	494.36
G5	0.0	1.0	100	3.7	54.8	264.82

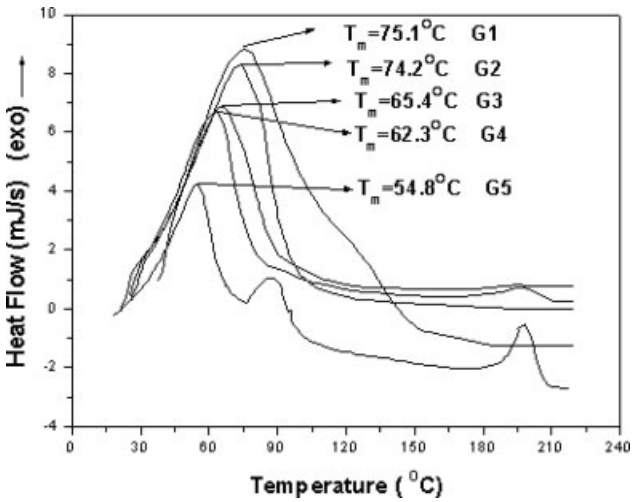

Figure 2 DSC plots indicating the effect of concentration of chitosan on crosslinking of chitosan and glutamic acid (wt % of chitosan in mixture: 100% (G1); 70% (G2); 50%, (G3); 30% (G4); and 0% (G5)).

IPNs, similar effect of bound water is expected to be present in subsequent DSC runs.

Influence of concentration of chitosan

In Table II, DSC results regarding the influence of concentration of chitosan for a fixed concentration of glutaraldehyde are reported. Typical nonisothermal DSC spectra of chitosan-glutamic acid samples along with curatives in the temperature range of 25–220°C are illustrated in Figure 2.

The DSC thermograms of curing of chitosan and glutamic acid with glutaraldehyde reflect the curing process. The curing peaks in chitosan-glutamic acid thermograms are superimposed with the dehydration peaks. As the same concentration of glutaraldehyde is present in these samples, the dehydration peak area will be equal. Thus, one can discuss comparative curing amongst these samples. It is observed that the curing starts from the beginning of the scanning in all the samples. The curing of chitosan and glutamic acid with glutaraldehyde is a fast reaction as the process of curing starts immediately after the mixing of the constituents. In case of sample G1, the thermogram line raises to maximum peak temperature 75.1°C and the subsequent fall shows the completion of curing reaction.

As one moves from G1 to G5 in Figure 2, the relative concentration of chitosan decreases. The height and width of curing peak is observed to be decreasing with decrease in chitosan concentration and the maximum peak temperature shifts towards a lower temperature for the given heating rate of 5°C/min. The kinetics of curing reactions strongly depends on the nature and amount of reactants participating in the reaction.

A week and slightly broad peak is observed for low chitosan/glutamic acid (3/7) ratio at 198.3°C (sample G4). To check the origin of this peak, another experiment is conducted without adding chitosan to the sample and rest of the constituents were kept unchanged (sample G5). It is clear from this test that this small peak is due to the presence of glutamic acid. Earlier, this peak was of negligible height, due to the small proportion of glutamic acid (G1–G3). Aly[7] also observed a similar melting peak at 211.72°C for the analysis of pure L-glutamic acid. The same peak is also observed in Figure 4 for the samples (F1–F4) in which the maximum peak temperature shifts towards higher temperature as the rate of heating increases. Another peak at 87.4°C (sample 5) in Figure 2 might be due to the loss of crystallinity of glutamic acid by the reaction with glutaraldehyde. The disappearance of small peaks at temperatures 87.4 and 198.3°C (in samples G1–G4) shows that as the concentration of chitosan increases, the stability of the matrix increases.

The area under the peak decreases with the shifting of curing temperature towards lower temperatures. This indicates a decrease in the enthalpy of curing with a decrease in chitosan concentration. It is observed that in the absence of glutamic acid as in sample G1, the enthalpy is 1637.56 J g^{-1}, which decreases to 264.82 J g^{-1} for sample G5 having maximum concentration of glutamic acid.

Influence of concentration of crosslinker

To know the effect of concentration of crosslinker on curing of chitosan-glutamic acid, the curing experiments are performed with varying concentration of glutaraldehyde (3.13, 6.25, 12.5, and 25%). The compo-

TABLE III
DSC Results Showing the Effect of Concentration of Curing Agent on Curing of Chitosan-Glutamic Acid at a Constant Heating Rate of 5°C/min

Designation	Chitosan (g)	Glutamic acid (g)	Drug (mg)	Glutaraldehyde (3.7 g) %	Maxi. peak temp. (°C)	Enthalpy of curing peak (J g^{-1})
J1	0.5	0.5	100	25	65.4	551.94
J2	0.5	0.5	100	12.5	66.8	505.73
J3	0.5	0.5	100	6.25	68.1	651.65
J4	0.5	0.5	100	3.13	71.4	795.6

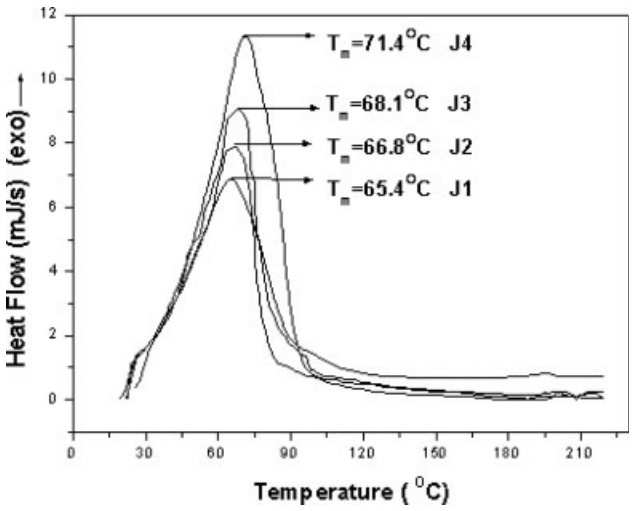

Figure 3 DSC plots indicating the effect of concentration of crosslinker on curing of chitosan and glutamic acid (wt % of crosslinker in mixture: 25% (J1); 12.5% (J2); 6.25% (J3); and 3.13% (J4)).

sition of samples is reported in Table III and the effect of percentage of crosslinker on the curing of chitosan and glutamic acid for the uniform heating rate of 5°C/min is shown in Figure 3. The presence of varied concentration of glutaraldehyde solution (aqueous) will lead to the presence of varied dehydration peak (area). Thus, apparently, comparison of curing peak area with the variation in concentration of curative is difficult. But, the higher enthalpy (peak area) of the sample, J1 compared to sample, W3 indicates the effect of curative. So, one can compare the effect of concentration of curative as the total weight of the crosslinker is fixed (i.e., 3.7 g). The curing peak is observed at a temperature of 65.4°C for the sample J1, having maximum concentration of crosslinker. As the concentration of glutaraldehyde decreases, the number of aldehyde groups available for the crosslinking with chitosan and glutamic acid decreases, leading to reduction in the extent of reaction. Because of this, more time is taken by the samples to be completely cured and the curing peaks shift towards a higher temperature.

As the concentration of glutaraldehyde increases, the exothermic peak shifts to a lower temperature. In sample J4, having lowest concentration of curing agent, the rate of crosslinking is less or it takes more time to be completely cured and hence, the curing peak shifts towards a higher temperature, i.e., 71.4°C. This shift of peak is also due to the fact that as the concentration of crosslinker decreases, the amount of bound water increases in the samples. This increased amount of water results in stronger adhesion forces between chitosan-water molecules and thus shifts the reaction peak towards a higher temperature. The enthalpy of curing peak increases with a decrease in concentration of crosslinker due to the requirement of higher energy to complete the cure reaction.

Influence of rate of heating

DSC thermograms are obtained to know the effect of rate of heating on cuing reaction. The composition and the total enthalpy of cure reaction measured as a function of heating rate is reported in Table IV and DSC plots are illustrated in Figures 4–6. The rate of heating influences the curing process. The reaction maxima for the samples having same composition of constituents (samples F, H and I) shift to a higher temperature with increasing heating rates. Further it is observed that, the enthalpy of curing peak as well as area under the thermogram increases for all the samples having different composition of chitosan.

TABLE IV
DSC Results Showing Maximum Exothermic Peak Temperatures of Different Samples of Chitosan-Glutamic Acid at Various Heating Rates

Designation	Chitosan (g)	Glutamic acid (g)	Drug (mg)	Gluteraldehyde (25%) (g)	Maxi. peak temp. (°C)	Rate of heating (°C/min)	Enthalpy of curing peak ($J\ g^{-1}$)
F1	0.3	0.7	100	3.7	55.3	3	350.08
F2	0.3	0.7	100	3.7	62.3	5	494.36
F3	0.3	0.7	100	3.7	70.5	7	625.14
F4	0.3	0.7	100	3.7	74.8	10	716.22
H1	0.5	0.5	100	3.7	57.6	3	438.78
H2	0.5	0.5	100	3.7	65.4	5	551.94
H3	0.5	0.5	100	3.7	75.5	7	643.38
H4	0.5	0.5	100	3.7	81.5	10	726.28
I1	0.7	0.3	100	3.7	62.3	3	477.28
I2	0.7	0.3	100	3.7	74.2	5	680.32
I3	0.7	0.3	100	3.7	79.1	7	747.26
I4	0.7	0.3	100	3.7	86.1	10	790.05

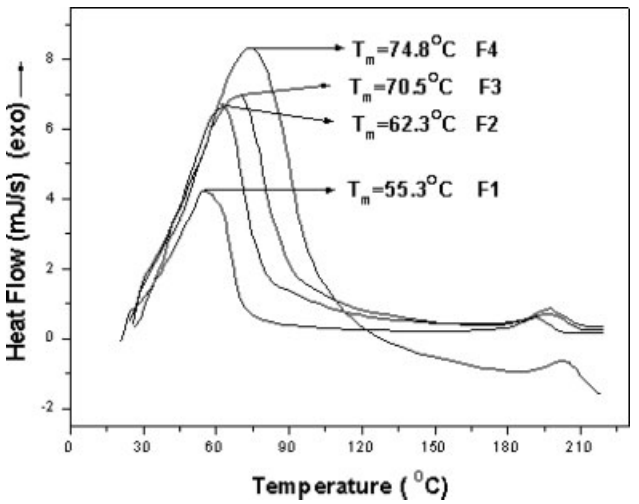

Figure 4 DSC plots of crosslinking of chitosan and glutamic acid (3/7 weight ratio) at various heating rates: (F1) 3°C/min, (F2) 5°C/min, (F3) 7°C/min, and (F4) 10°C/min.

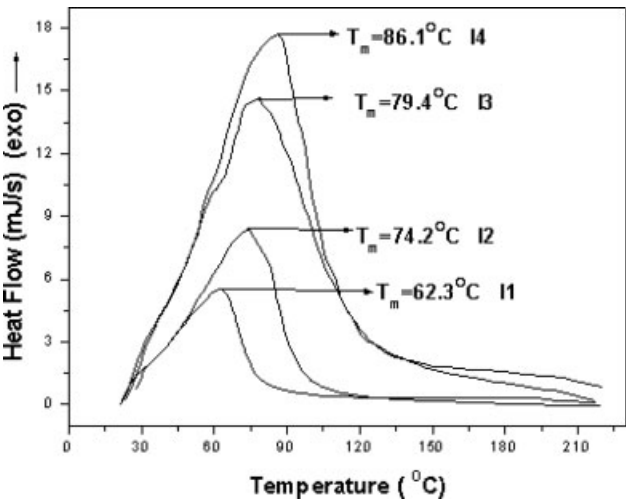

Figure 6 DSC plots of crosslinking of chitosan and glutamic acid (7/3 weight ratio) at various heating rates: (I1) 3°C/min, (I2) 5°C/min, (I3) 7°C/min, and (I4) 10°C/min.

Kinetic analysis

Crosslinking of chitosan and glutamic acid through glutaraldehyde is a complex phenomenon due to simultaneously occurrence of multiple-step curing. It is generally believed that the activation energy (E) and frequency factor (A) remain same for one step reaction; however, it is reported[8–10] that in multistep reactions, these kinetic parameters may vary with the progress of the reaction (α). If the process involves several steps with different activation energies, the relative contribution of these steps to over all reaction rates will vary with both temperature and extent of conversion.[11] This variation can be determined by isoconversional methods. The isoconversional methods employ multiple temperature programs (i.e., different heating rates and temperatures) to obtain data for varying heating rates at a constant extent of conversion.[12]

For nonisothermal conditions, when the temperature is raised at a constant heating rate β, Doyle[13] gives the following relation;

$$\ln(\beta_i) = \text{Const.} - \frac{1.05\ E_\alpha}{RT_{\alpha,i}} \qquad (1)$$

which is used in most popular isoconversional methods of Flynn and Wall[14] and Ozawa.[15] It involves measuring temperatures corresponding to fixed values of conversion, α from experiments at different heating rates, β and plotting $\ln(\beta_i)$ against $1/T_i$. The

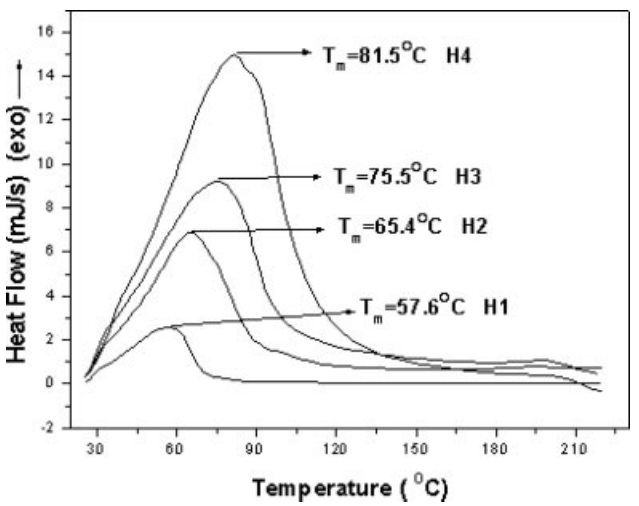

Figure 5 DSC plots of crosslinking of chitosan and glutamic acid (5/5 weight ratio) at various heating rates: (H1) 3°C/min, (H2) 5°C/min, (H3) 7°C/min, and (H4) 10°C/min.

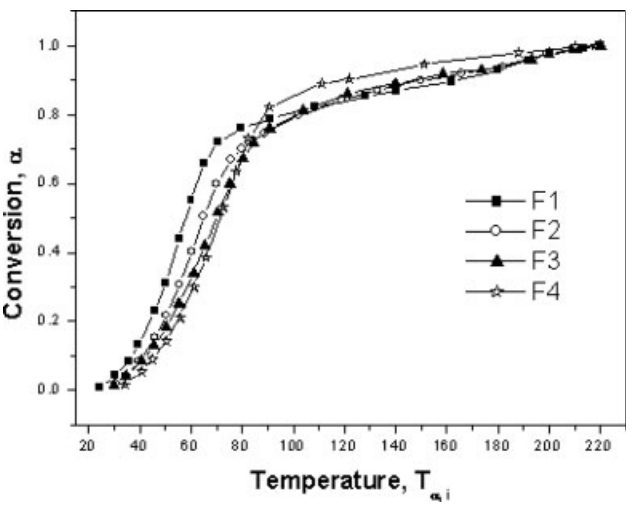

Figure 7 The conversion-temperature curves for curing of chitosan-glutamic acid (3/7 weight ratio) by glutaraldehyde, obtained at various heating rates. For the details of the heating rates, see Figure 4.

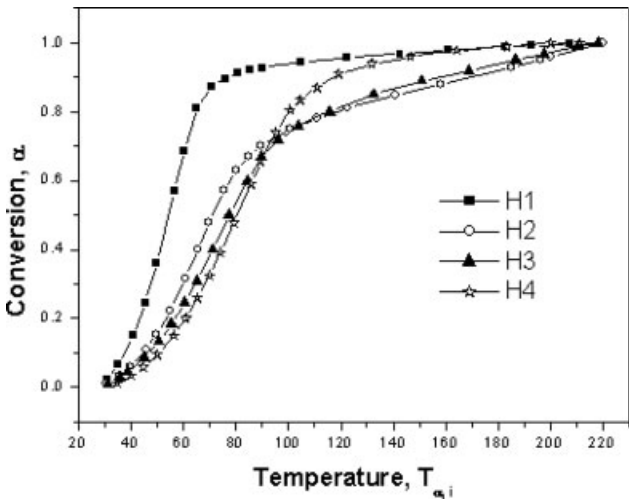

Figure 8 The conversion-temperature curves for curing of chitosan-glutamic acid (5/5 weight ratio) by glutaraldehyde, obtained at various heating rates. For the details of the heating rates, see Figure 5.

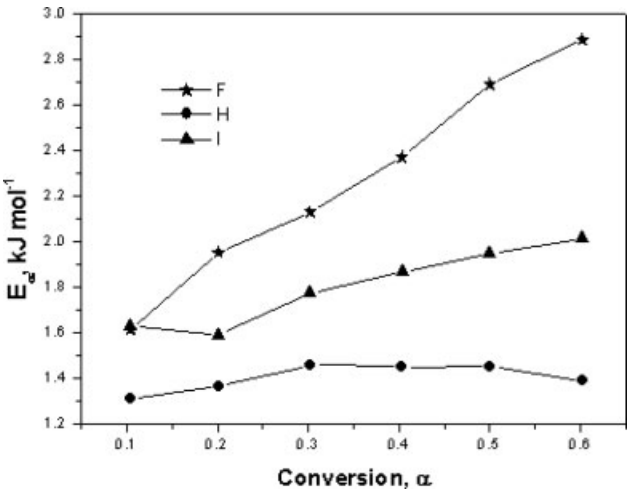

Figure 10 The E_α dependencies obtained for curing of chitosan-glutamic acid by glutaraldehyde for different compositions of chitosan/glutamic acid (F: 3/7, H: 5/5, and I: 7/3) by isoconversional method [eq. (1)].

slopes of such plots give $-E_\alpha/R$, where R is the gas constant and E_α is the activation energy at a particular value of extent of conversion, α.

The relationships between the extent of conversion and temperature for different heating rates and with varying composition of chitosan are obtained from Figures 4–6 and are shown in Figures 7–9. The dependence of activation energy on the extent of conversion is determined by eq. (1) and is reported in Figure 10. It is observed that the activation energy, E_α increases with extent of conversion, α for the samples F and I. As the reaction progresses the molecular weight of the crosslinked polymer increases. The stepwise crosslinking process results in a decrease in molecular mobility and hence the curing kinetics is controlled by diffusion of reactants at latter stage. Because of the decrease in the mobility of the crosslinked chains, the penetration of the crosslinker becomes difficult and therefore, higher amount of energy is required for further curing of the reactants. The increase in activation energy indicates that the rate of crosslinking can be limited not by diffusion, but by the mobility of longer polymer chains. In the latter case, diffusion encounters a larger energy barrier resulting in an increase in activation energy. However, there is not much change in the E_α for the sample H as depicted in Figure 10.

Moreover, the information about variation in enthalpy of curing (ΔH) and activation energy (E_α) is useful to study the thermal stability of any drug carrier. The magnitude of the E_α is expected to reflect the extent of crosslinking of the constituents. The value of E_α is more for lesser concentration of chitosan (sample F). In other words, lower activation energy is required for curing of samples having higher concentration of chitosan (sample I). Hence, from the knowledge of E_α and the extent of conversion (α), one can crosslink the material to the desired extent and the drug release rate from the polymer matrix can be modulated.

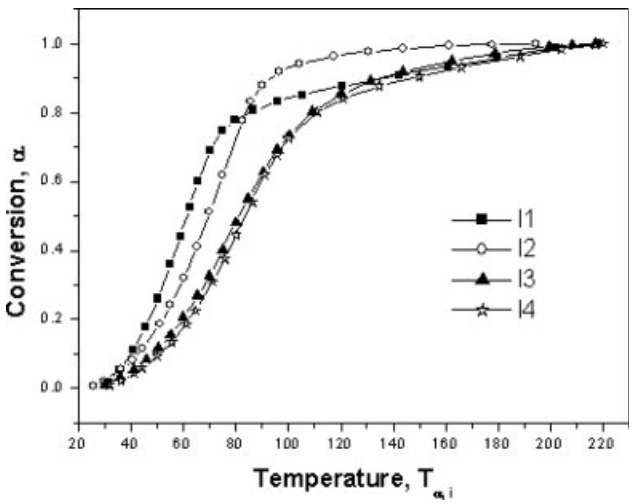

Figure 9 The conversion-temperature curves for curing of chitosan-glutamic acid (7/3 weight ratio) by glutaraldehyde, obtained at various heating rates. For the details of the heating rates, see Figure 6.

CONCLUSIONS

Thermodynamic properties of curing of chitosan and glutamic acid with glutaraldehyde having various proportionate mixtures are studied through DSC. The concentration of chitosan and glutaraldehyde has

enormous effect on the cure kinetics. The total enthalpy of cure reaction increases with an increase in concentration of chitosan and a decrease in a concentration of glutaraldehyde. Experiments are performed to check the effect of rate of heating on cure kinetics at four different heating rates (3, 5, 7, and 10°C/min). The increasing rate of heating increases the enthalpy of curing (peak area). The activation energy as a function of extent of conversion is determined by Flynn, Wall and Ozawa analysis. The application of isoconversional method to curing reaction resulted in an increase in activation energy (E_α) with extent of conversion.

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References

1. No, H. K.; Meyers, S. P. J Agric Food Chem 1989, 37, 580.
2. Draget, K. I.; Varum, K. M.; Moen, E.; Gynnild, H.; Smidsrod, O. Biomaterials 1992, 13, 635.
3. Hirano, S.; Yamaguchi, R.; Matsuda, N.; Miura, O.; Kondo, Y. Agric Biol Chem 1977, 41, 1547.
4. Lin-Gibson, S.; Walls, H. J.; Kennedy, S. B.; Welsh, E. R. Carbohydr Polym 2003, 54, 193.
5. Kumar, M. N. V. R.; Gupta, K. C. J Appl Polym Sci 2000, 76, 672.
6. Kumari, K.; Kundu, P. P. J Appl Polym Sci 2007, 103, 3751.
7. Aly, A. S. Die Angew Makromol Chem 1998, 259, 13.
8. Vyazovkin, S.; Wight, C. A. Annu Rev Phys Chem 1997, 48, 125.
9. Vyazovkin, S. New J Chem 2000, 24, 913.
10. Vyazovkin, S. Int Rev Phys Chem 2000, 19, 45.
11. Vyazovkin, S. Thermochim Acta 2000, 355, 155.
12. Vyazovkin, S.; Sbirrazzuoli, N. Macromol Rapid Commun 2006, 27, 1515.
13. Doyle, C. D. J Appl Polym Sci 1962, 6, 639.
14. Flynn, J. H.; Wall, L. A. J Res Natl Bur Stand Sect A 1966, 70, 487.
15. Ozawa, T. Bull Chem Soc Jpn 1965, 38, 1881.